Dielectrophoresis-Assisted Deterministic Lateral Displacement for Particle and Cell Sorting

BY

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THESIS

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AC	Alternating current
BSA	Bovine serum albumin
CTCs	Circulating tumor cells
DC	Direct current
DEP	Dielectrophoresis
DI	Deionized
DLD	Deterministic lateral displacement
DMEM	Dulbecco's Modified Eagle's Medium
EpCAM	Epithelial adhesion molecules
FACS	Fluorescent activated cell sorting
FBS	Fetal Bovine Serum
FDA	Food and Drug Administration
FFF	Field flow fractionation
LOC	Lab on a chip
NCF	Nanotechnology Core Facility
nDEP	Negative DEP
PBS	Phosphate buffered saline
pDEP)	Positive DEP
PDMS	Polydimethylsiloxane
PVC	Polyvinyl Chloride
RBC	Red blood cell
RPMI	Roswell Park Memorial Institute medium

SDS	Sodium dodecyl sulfate
WBC	White blood cell
WSU	Washington State University

SUMMARY

This work presents an experimental proof of concept and preliminary findings for a microfluidic device to separate micro-particles and cancer cells. Cancer is one of the major reasons of human deaths now a-days and need to be addressed with more efficient techniques to reduce the death trolls such as early diagnosis which can help save huge number of lives. Cancer tumor in a metastasis state release cancer cells into the blood stream and these cells can land onto other organs of human body resulting in a secondary tumor and the process can go on. Based on research studies, these circulating tumor cells (CTC), if captured from blood stream, can be helpful to study the behavior of cancer tumor, especially early detection of cancer presence and patient-specific response to treatments. This thesis focuses on the experimental study of a micro-particle/cell separation technique based on di-electrophoresis (DEP) and deterministic lateral displacement (DLD) in a microfluidic channel. Our collaborator at Washington State University Vancouver simulated the idea of this technique using COMSOL. For the purpose of experimental studies, multiple devices were fabricated to test the idea of di-electrophoresis assisted deterministic lateral displacement structure. First, the position of electrode to generate vertical electric field in DLD flow was finalized after trying multiple designs and fabrication challenges. Second, the novel idea of vertical electrode with DLD was tested with micro-particles and the results were compared with published work of DLD having parallel electric field. Lastly, experiments were conducted with cancer cells A549 independently and results were studied with simulation findings. The experimental results produced and tested so far are promising for the further studies to develop a device capable of separating circulating tumor cells from blood samples drawn from patients.

ABSTRACT

Deterministic lateral displacement (DLD) and di-electrophoresis (DEP) have been proved as promising tools in cell sorting for circulating tumor cell (CTC) separation. However, both methods have their drawbacks. For example, DLD lacks the ability to separate white blood cells and cancer cells with similar sizes, and DEP separation is often based on trapping mechanism, which suffers from low throughput. In this presentation, we report a microfluidic device based on the combination of DLD and DEP, which is enabled by a novel contactless electrode design. These electrodes help generate a non-uniform electric field around the DLD pillars, which then induces a DEP force to the cells that are passing through the microchannel. The device can perform cell sorting with overlapping sizes and with high throughput at the same time. We studied and characterized the device with both experimental and simulation work. For A549 lung tumor cells with 14-micron size, the device can control its trajectory from bumped mode to zig zag mode. Cell viability testing showed that more than 95% of the cells were viable with 30mins flow rate at 0.5ml/hr at applied voltage of 800V. These results demonstrate our method as a promising tool for label-free CTC separation for cancer diagnosis.

CHAPTER 1

INTRODUCTION

Microfluidics is a fast growing technology in recent era, which has a large range of application in fields of bioengineering, medical sciences, medicine, drug delivery, reagent/ liquid sample analysis, electronics, healthcare and defense [1] . Lab on a chip (LOC) is one of the most interesting and useful point of care diagnostic concept capitalized on microfluidics. It involves the lab testing techniques such as sample mixing with chemicals but on microscale, which involves the transporting of sample from one micro-chamber to another and separation methods to purify biological material for downstream studies. Other use of separation methods in microfluidics devices is sorting of objects in a sample based on sizes which has applications in food processing industry, chemical and biological research [2-4]. Bacterial activity need to be monitored in food processing by using separation of micro-size objects in a food sample to keep it away from hazardous bacteria. In biological application, living cells and healthy cells need separation from dead and infectious cells for diagnosis, prevention and monitoring of diseases [5, 6]. Cell sorting bases on manipulating different characteristics such as physical and chemical properties i.e stiffness, size, electric/ magnetic conductivities etc [5, 7]. For instance, infected malaria cells are 50 times stiffer than healthy cells and are unable to deform enough to pass through capillaries [8]. Many tumor cells are larger in size as compared to red blood cells [5].

Cancer metastasis is responsible for more than 90% cancer deaths as well as organ damage in human body, whereas the main reason of cancer metastases is circulating tumor cells (CTCs) [9, 10]. CTCs are the harmful cells, in very scarce number with ratio of 1 CTC to (1x10^6) blood cells, found circulating in bloodstream due to dissemination from first tumor somewhere in an organ of a cancer patient [11]. Due to their circulation in blood, CTCs usually find certain favorable environment to grow and produce secondary tumor in distant organs [12]. CTCs contains the physical and biological properties of primary tumor and hence can be utilized as biomarker for cancer prognosis, diagnostics and helpful tool for the development of new therapies. For clinical studies blood samples are mostly drawn from the patient, in which the CTCs serves as liquid biopsy to study the response of new drugs for tumor cure and monitoring [13, 14]. This approach, CTCs screening from blood, provides a support to develop a treatment based not only on primary tumor characteristic but also CTCs molecular characteristics to deal metastasis. However, the challenge associated with this approach of cancer study is the heterogenic nature of CTCs. CTCs differ their shape and genes detail although disseminated from one same tumor. Handling is another issue as most of CTCs have very short life of 1 hour in process of transferring from cell culture to device. So, a rapid technology for enrichment is required which can separate CTCs from blood sample with high recovery and viability for analysis [15].

Many techniques are present for CTCs screening but none of these are able to meet the challenges described above. Cell Search TM (Verdex, USA) is an only United States Food and Drug Administration (FDA) approved device for batch processing CTCs separation

and enrichment for diagnostic analysis. It utilizes an immune-magnetic technique, in which CTCs with epithelial adhesion molecules (EpCAM) bind with magnetic particles coating using antibody (anti-EpCAM) and capture from blood sample using magnetic attraction. Although Cell Search is FDA approved, it possesses drawbacks such as cell loss due to sample handling with pipetting and chemical use for magnetics particle biding. In general, the batch process involves steps including chemical treatment, pipetting, sample transferring to multiple containers. Hence it increases the chance of CTCs losing and badly affects cells viability. Based on such limitations, many researchers in the area of targeted cell separation and analytical study have been focusing on microfluidics Lab on a Chips (LOC) platforms. In the past decade, a great number of research articles have been reporting on microfluidics design for CTCs isolation [16].

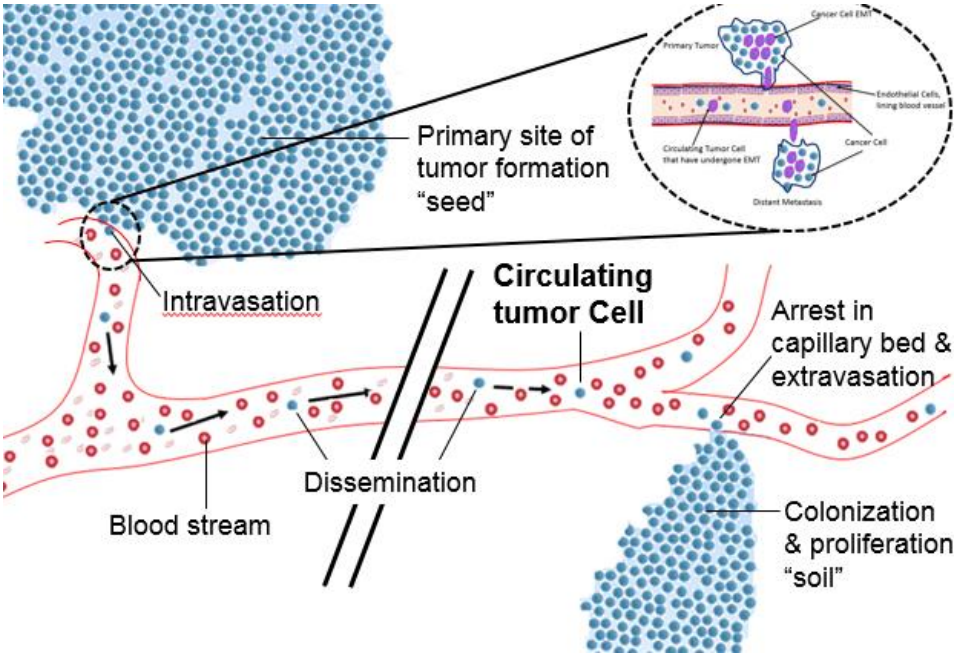


Figure 1: Illustration of circulating cancer cells causing secondary tumor in metastasis stage

In past recent years, efforts have been made by our group to come up with novel ideas in the field of CTCs separation. The major part of published work done is mostly consist of numerical and simulation studies including deformability based microfiltration for CTCs separation based on shear forces acting on cells with variable stiffness [17], effects of entry droplet [18] and 3D geometry for the CTCs enrichments [19]. In this experimental work, we are presenting a combination of two separation techniques to enhance CTCs separation. Before moving to our new work, it is necessary here to talk about potential and marvelous ideas of microfluidics separation.

1.1 Microfluidics separation

Microfluidics devices are miniaturize fluidic systems which manipulates small volume of sample and reagents for multiple purposes including bio-sensing, mimicking biological organs and economical chemical based testing to replace need of huge lab equipment's. Devices are considered as micro if either the volume of fluid used is in range less than micro liters or one of device dimensions is in range of less than 100 micrometers. The working principle utilizes general physical laws of fluid mechanics and electromechanical systems and exhibits a large range of advantages over macro systems. The primary benefits of microfluidics include low cost, high throughput, high resolution and sensitivity, easy to combine with other system to enhance performance and final results.

Additionally, microfluidics can provide the user with continuity for the entire process (i.e., a one-step process of loading sample–separation–identification), resulting in the reduction of cell loss. For these reasons, microfluidics is now applied in CTC research. As the cells may be lost during transportation of the sample from the macro-components to the micro-components of microfluidic devices, causing considerable deterioration in the cell detection sensitivity. This intrinsic cell loss has been investigated and can be effectively minimized through the following measures. (a) Increasing the tubing diameter of the device, connecting the sample storage and the micro-device. (b) Applying a hydrodynamic focusing approach for sample delivery in order to reduce cells contacting and adhering to the walls of the micro-channel and chip inlet. (c) Optimizing the filter design with a zigzag arrangement of the pillars to prolong the effective filter length and (e) the use of diamond-shaped pillars instead of the traditionally used rectangular shape, so as to reduce the gap length between any two given pillars (i.e., pressure drop) at the filter region[1, 20].

Before moving to the idea purposed in this thesis for particles and CTCs sorting, it is necessary to discuss most recent development in microfluidics separation to develop an understanding about the advancement of field. The separation principles can be classified in three sections: 1) Florescent label-based 2) Bead-based and 3) Label-free method. Each category contains subsections based on the physical and chemical techniques used independently as well as in combination for sorting tasks.

1.1.1 Fluorescent label-based cell sorting:

This technique uses cell staining and fluorescent probes to differentiate the cell type in a given sample. In contrast to traditional fluorescent activated cell sorting (FACS), cells stained with fluorescence label are arranged in a laminar flow while a laser beam focused on cells get scattered on to the detector based on different cells in presence. The difference in scattered intensities generate a specific signal corresponding to distinct cell type. But in FACS each cell is transferred to charged droplets and screened on the basis of static affinity [21]. Since cells are organized discretely in the flow, this technique yields high efficiencies as well as based on easily available immunostaining and reliable time saving preparations reduce the experimental error. On the basis of these benefit, researchers always prefers fluorescent-label sorting over bead base labeling and have coupled it with other physical principles to use in microchip sorting devices.

1.4.1 Electrokinetics Mechanisms

Electrokinetics is an effect in form of the movement of particles or cell due to applied electric field [22, 23]. As mentioned earlier electrostatic are used to sort charged aerosol containing the discreet cells in FACS, electrokinetics are also useful to directly influence cell movement in the fluid. Electro-kinetics are mostly further differentiate into three types 1) electrophoresis, 2) di-electrophoresis and 3) electroosmotic flow; which are good enough to provide force for cell displacement with the length of microfluidic chips.

Electrophoresis. It is the movement of oppositely charged particle or cells under the influence of uniform electric field of direct current (DC). Most of the cells are charged with negative affinity due to chemical group presences on the membrane, and applied force is directly related to charge density. Takahashi *et al.* utilized fluorescence labeled cell sorting with electrophoresis to perform downstream sorting with upstream laser detector [12]. Yao *et al.* coupled similar device with gravity force to perform cell screening with no convective flow in upside position [24]. Recently Guo *et al.* presented high throughput cell sorting by using electrophoretic force with continuous flow oil-water droplets [25]. The cells were prefocused using fluorescence label method and encapsulated in droplet while devices screened the droplet with only single cells from other droplets[26].

Dielectrophoresis. Unlike electrophoresis, particles and cells are exposed to a non-uniform electric field and they experience a force, dielectrophoresis (DEP) force, due to their polarizability compared to containing fluid. Cells/particles do not require surface charge to move under the influence DEP force, an alternating current (AC) is enough to polarize them [12]. In non-uniform AC electric field, cells/ particles with high permeability than containing fluid/buffer, will move towards maximum field region and experience positive DEP (pDEP) [27]. In case of higher buffer permeability of fluid than cell, it will experience negative DEP (nDEP) which is preferable for cell sorting as it has less adverse effect on viability. Wang *et al.* developed microfluidics device with lateral electrode to microfluidics channel to generate the repulsive force on cell flow to sort in five outlet channels [28]. Many groups have reported the microsystems for sorting of cells

encapsulated in droplets using DEP for downstream analysis of continuous genomic and proteomic [29-31]. Mazutis *et al.* presented an idea to proportionalized the cells with beads coated with marker antibodies into emulsify droplets and utilized for the analysis of antibody secretion from cell for fluorescent label downstream screening with DEP (Fig. 2)

Electroosmotic flow. In this principle, unlike cells/particles movement with electrophoresis and dielectrophoresis in stationary fluid, fluid itself move under electric field due to charged solvated ions hence transport the suspend cells/particles in medium (Fig 2) [32]. In literature, many group reported the use of electroosmotic flow to sort cells in microfabricated FACS [33-35]One down side of electroosmotic flow is electrolysis of electrolyte at electrodes generating bubbles for flow hindrance and chemical such as hydrogen peroxide. Both of the phenomena are harmful for cell viability, device operation

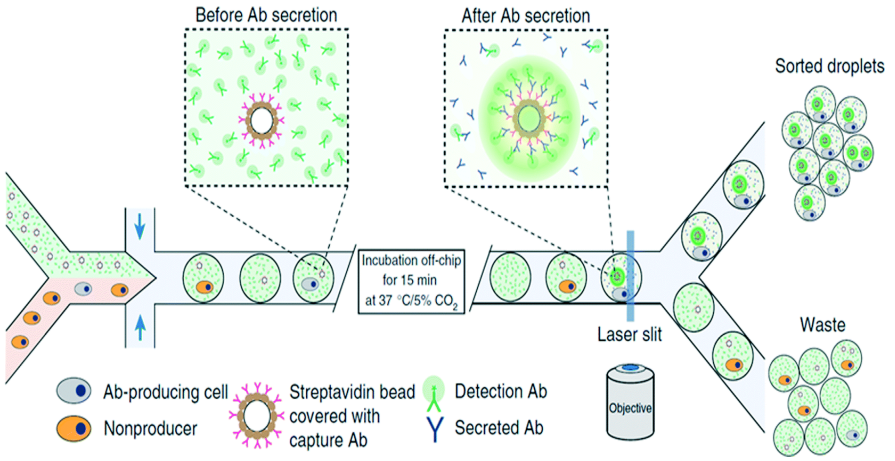


Figure 2: Droplet-based microrea-ctor and cell sorter using DEP. Reproduced with permission from [28]. Copyright 2013 Nature Publishing Group

and control [36]. Puttaswamy *et al.* reduced these effects by using nDEP and AC electrophoresis to perform cell sorting [37].

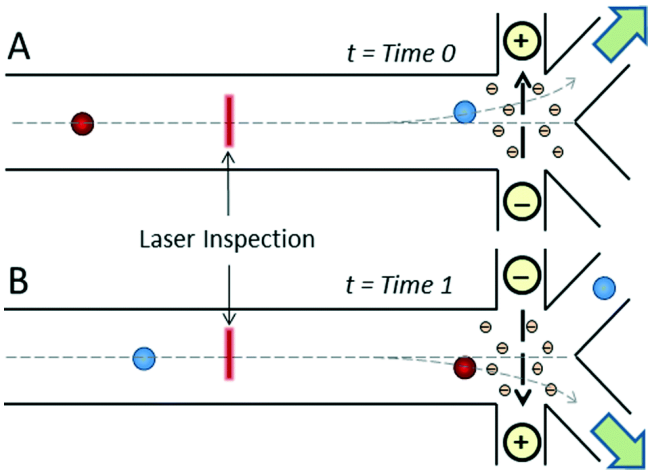


Figure 3: Direct current (DC) electroosmotic cell sorting. Reproduced with permission fro [34]. Copyright 2014 American Chemical Society.

1.4.2 Acoustophoresis

Acoustic pressure waves are able to move an object in fluid medium, a phenomenon called acoustophoresis [38]. Recently acousticfluidics has provided many diverse methodologies to develop analytical flow cytometry [24, 39, 40]. Major benefit of using acoustic waves in microfluidics for cell sorting is that it yields the best viability [41-43]. There are mainly three type of waves: traveling [44], bulk standing [45] and standing surface acoustic [46].

Bulk acoustic standing waves are generated by exciting microchannel with ultrasound to resonance frequency such that wavelengths are in order of microfluidics device dimension.

The particles suspended in medium experience radiation forces which is given as following expression [47, 48].

$$F_{\mathrm{R}} = -\frac{2}{3}\left(\frac{\beta_{\mathrm{f}}\left(\pi p_0\right)}{\lambda}\right)\phi\left(\beta_{\mathrm{c,f}}\rho_{\mathrm{c,f}}\right)\sin\left(2kx\right)$$

Acoustic constant factor ϕ, determines the direction of the forces acting on cells/ particles, depends on parameters such as density of fluid and cell compressibility. Cells move towards the antinode and node of acoustic waves if the ϕ is negative and positive respectively (Fig 3) [49, 50]. Johanssaon *et al.* was the first one who used the bulk acoustic waves with fluorescent label cell sorting, the cells in normal stream line were moved to pressure node and then manipulated in to different trajectories based on their densities and compressibility with respect to buffer [43]. Jakobsson *et al.* recently fabricated a

microfluidics device with acoustic waves moving cells on one side of device fluorescence label based screening and separation [51].[52]

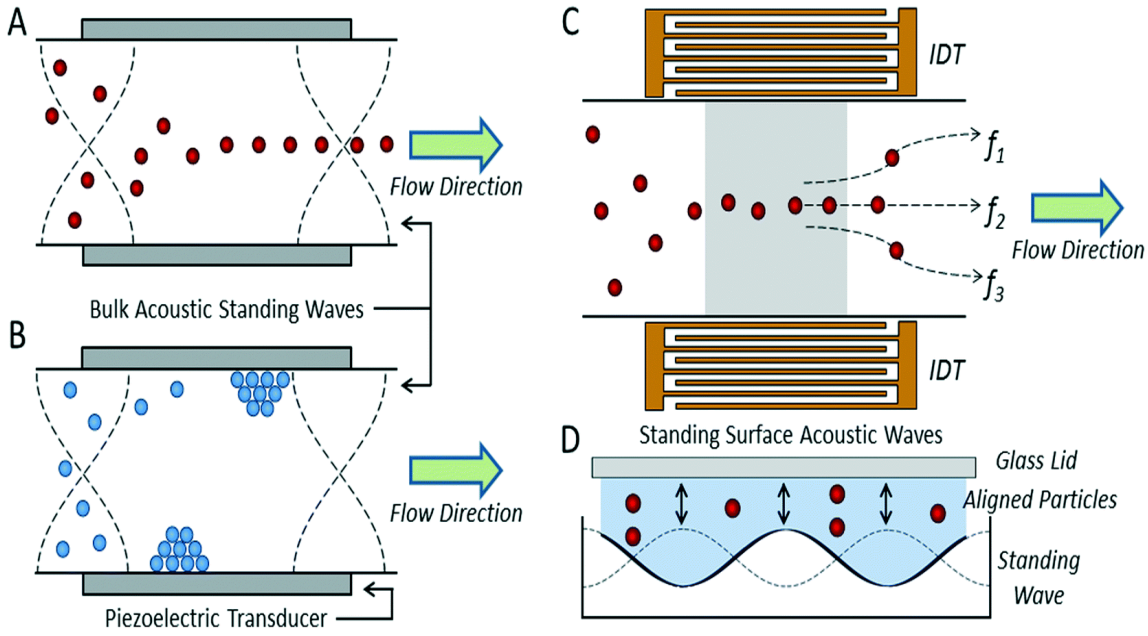


Figure 4: Acoustofluidic manipulation of cells and particles. Reproduced with permission from [48]. Copyright 2008 Annual Reviews

1.4.3 Optical Manipulations

Highly focused beams of light has also been used to exert displacement forces on cells in sample flow similar to pressure and electric forces [53]. These focused beams have advantages over electrical and pressure forces as it provide more control in space to handle atomic level objects and does not affect cell behaviors or damages [54]. Focused light on cells create two type of forces scattering and gradient due to different between refractive indices of cell and fluid (fig. 5A).

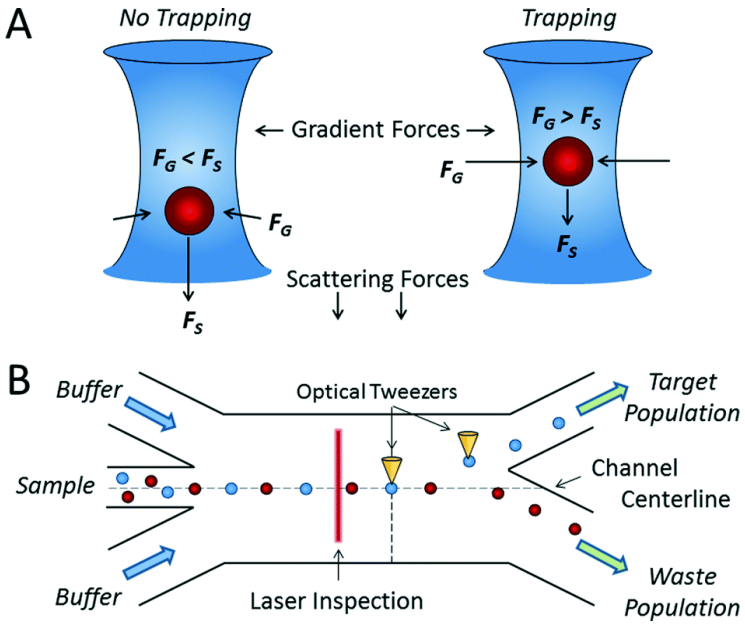


Figure 5: Cell sorting by optical force switching. A) When scattering forces (FS) exceed gradient forces Reproduced with permission from [51]. Copyright 2008 Annual Reviews. B) A hydrodynamically focused stream of cells detected by laser action are captured and displaced by optical tweezers for sorting. Reproduced with permission from [58]. Copyright Royal Society of Chemistry

These forces trap cells and act as optical tweezer while this technique helped in analysis of molecules and cells in sample [55]. Ashkin [56] used this principle to manipulate particles motion in the fluid. This technique has also been utilized to sort cells but it yields less throughput [57] although recent development in micro optical switches has enabled this technique easy to use as fluorescent label based sorting [58, 59]. Many research groups have used this concept to use a focus beam to generate an opposite radiation force to sort

cells (Fig. 5B) [59-62]. Wu *et al.* utilized bubbles generated locally by pulsed laser to deflect cells for sorting at a rate of 20,000 cell per second [63, 64].

1.4.4 Mechanical Sorting

In mechanical sorting no external electric, magnetic and optical fields are present. It performs micro object sorting with the use of mechanical forces. Krüger *et al.* made the use of change in hydrodynamic flow with the help of an external rotary valve to sort cells [65]. Whereas Fu *et al* and E. coli *et al* made similar device and coupled it with a trapping system to enhance the working of device [66]. In addition, the same design was used to separate beads from blood cells [67]. *Chen et al.* change the flow of cell with negative

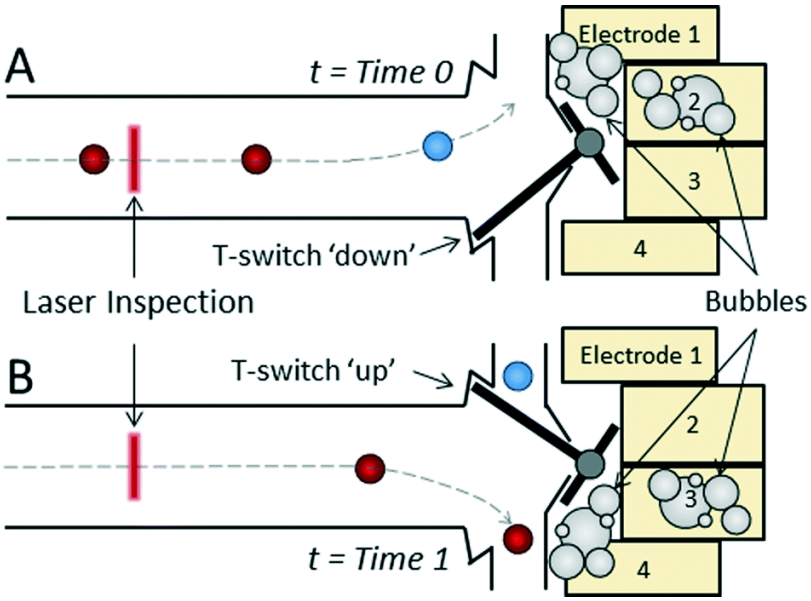


Figure 6: Mechanical beam system for sorting. Reproduced with permission from [65]. Copyright 2005 Royal Society of Chemistry

pressure by drawing fluid out of the device using valves [68]. *Ho et al.* made a mechanical micro T-switch operated bubbles in microfluidics devices for sorting (fig.) [69].

1.1.2 Bead-based cell sorting

In this type, small particles are bonded to the cells and are sorted with response to external fields. The sorting depends on the size, material and adhesion of particles to the cell surface. It can be used to debulk cells as well as sorting in bio-fluids samples [70]. Miltenyi *et al.* was the first to present magnetic sorting of cells labeled with beads, which was later miniaturized to microchip using magnetophoresis (MAP) [71-73]

Magnetophoresis

Cells are sorted with the help of magnetic forces acting on beads adhered to the surface. A permanent magnet or magnetic coil is used to generate magnetic field and beads are mostly of iron in form of ferrous fluid whereas force acting on cell depends on the magnetic moiety due to beads sizes [74]. *Adams et al.* presented sorting of cell using different sizes of iron beads for different cell type [75]. *Carr et al.* made a sorter which separate the sample based on the magnetic moments magnitude across beads [76]. In case of rare cell such as CTCs, the separation is very challenging in flow [77]. *Hoshino et al.* succeeded in CTCs separation from blood using MAP [78] (fig. 7A) with macro-magnets to generate electric

filed. Tumor cells in a blood sample were covered with iron oxide nanoparticles using antibody and epithelial cell adhesion molecules (EpCAM). Cells with ferric particles were

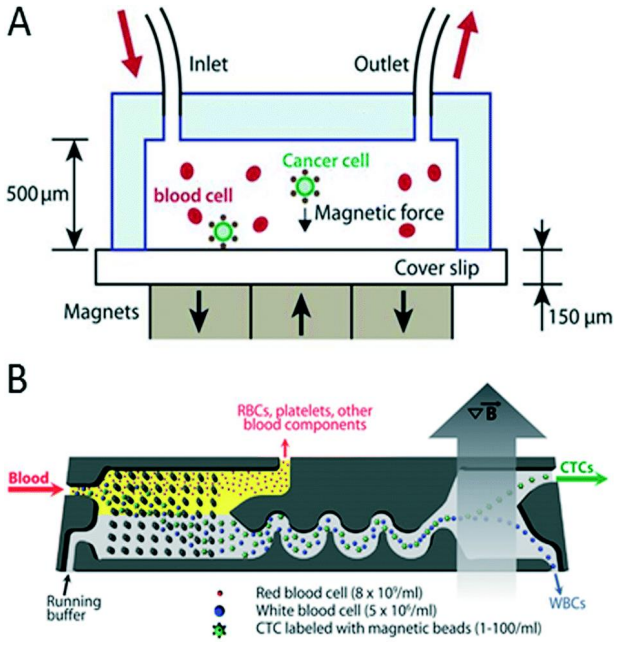


Figure 7: Magnetic Bead based label separation of CTCs [74] Copyright 2011 Royal Society of Chemistry. B) Magnetic Bead based label separation of CTCs with DLD and inertial focusing [79]. Copyright 2013 The American Association for Advancement of Science.

sticked to the surface of chip due to external magnetic field of permanent magnet. *Darabi et al.* used micro- magnet in grid pattern on the chip surface for same technique [79]. *Kang et al.* used perpendicular channel parallel to main channel for magnetic adhesion [80] while other group reported the use of magnetic compartment for the pairing of magnetic labeled cells [81].

Researcher developed a three-stage CTC separation chip using deterministic lateral displacement to separate leukocyte and CTCs from blood; inertial focusing to align them before separating bead labeled white blood cells from CTCs using magnetic field Fig B [82, 83].

Electrokinetic mechanisms:

Hu et al. demonstrated the dielectrophoresis phenomena on bead label cells under alternating current, the cells with beads experienced more DEP force compared to unlabeled cells [84]. In this system, polystyrene beads were used to separate three bacteria types exhibiting different DEP response to bacteria covered with different sizes of polystyrene [85]. To enhance the separation, a magnetic component was introduced in the

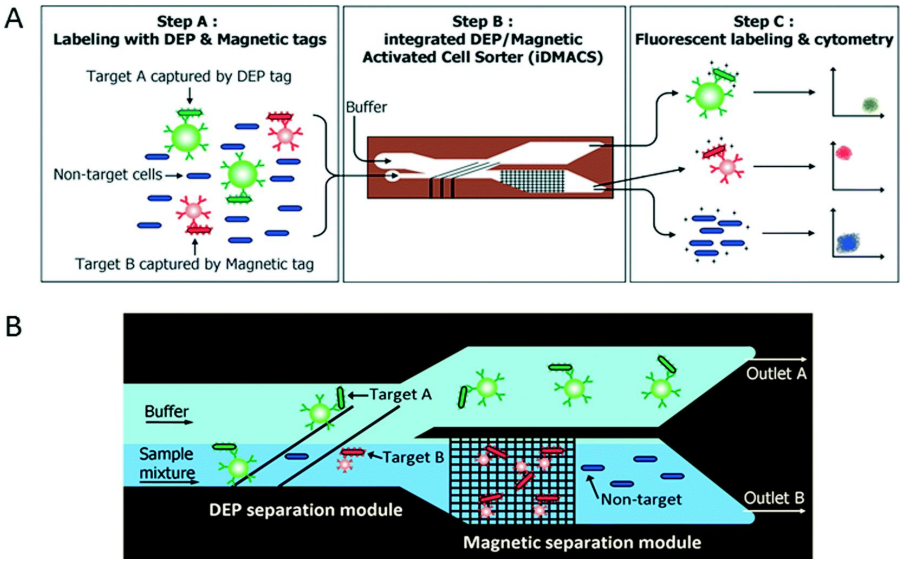


Figure 8: Multi-target cell sorting using DEP and magnetic trapping. Reproduced with permission from [82, 82]. Copyrights 2009 Royal Society of Chemistry

same system to trap cells Fig. 8B [86]. *Cheng et al.* used tilted electrodes on chip surface to sort bacteria in two channels based on labeled or unlabeled with polystyrene [87].

1.1.3 Label-free cell sorting

Label free sorting deals with the physical properties of cells to manipulate them for separations. It is one of the most studied and preferred sorting methods because it reduces the sample preparation time and provides high throughput. Deterministic lateral displacement is a technique that separate micro objects based on their sizes without using any labeling [88]. Inertial focusing in curved channels uses inertial forces, cell densities, compressibility and boundary effect of flow in fluid stream to sort micro particles [89]. Passive label free sorting methods also utilize hydrodynamic spreading, filtration and immobilization [90]. It also utilizes DEP to separate cells as explained earlier in introduction. *Hung et al.* was the first to exhibit sorting of cell lines with DEP principle [91]. Field flow fractionation (FFF) is a technique that utilized the magnetic, centrifugal, electric and thermal methods to control the position of micro object in parabolic flow through channel [92-94]. In one DEP FFF system reported, the microelectrode on the surface of flow channel provide the DEP force against the gravity force Fig.9A [95].

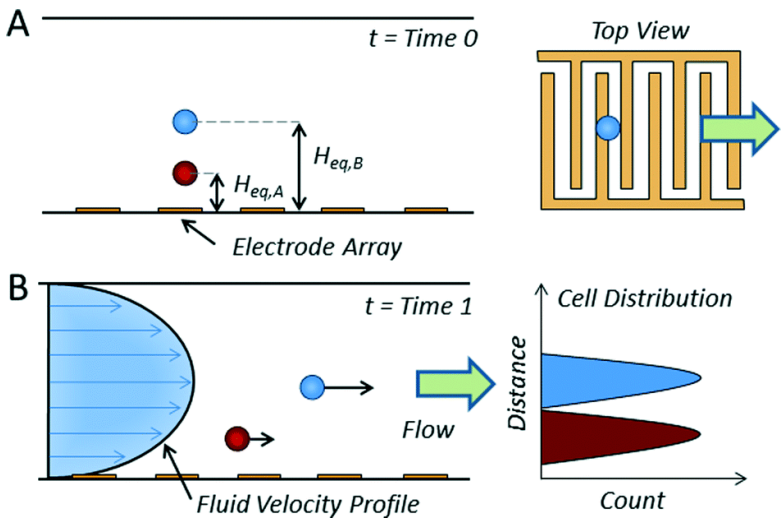


Figure 9: Sorting by nDEP-assisted field-flow fractionation. Reproduced with permission from [91]. Copyrights 2008 Royal Society of Chemistry

For label free cell sorting, the combination of DLD and DEP is used in this work for high throughput cell and particles sorting. The project primarily provides an experimental study to proof contactless DEP assisted DLD principle using lateral electrode along DLD pillar arrays. That is why it is needed here to review DLD design and DEP principle.

1.2 THEORY

1.2.1 DLD DESIGN

Deterministic lateral displacement technology is a restriction in fluid channels in form of pillar arrays. Pillars are arranged in rows, which are shifted laterally at fixed distance from preceding rows. The gap and angle between consecutive pillars controls the flow of fluid lamina through the channel and separate particles based on their sizes as compare to critical size of DLD. The mechanism of DLD depends on the size of particle in streamline flowing through pillar constriction and width of each streamline. If the center of particle is out of the streamline width, it will collide with pillar and will be deflected to another streamline. If the size of particle is smaller than the critical size of DLD, the center of particle will remain in one streamline throughout the flow (Fig. 10). The process continues each time a

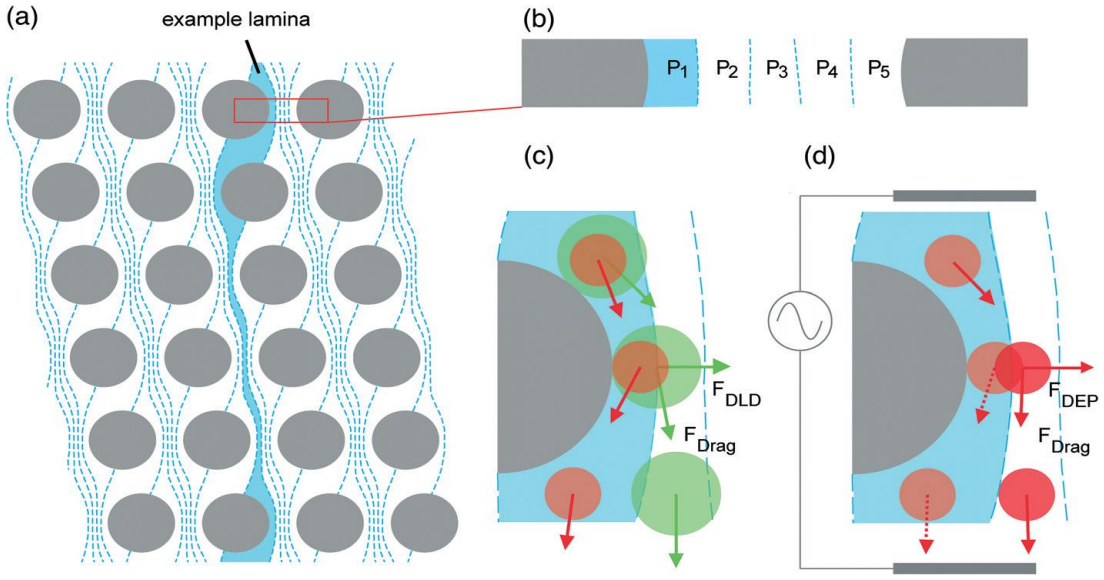


Figure 10: a) Lamina flow through DLD pillar array. (b) Streamlines between two pillars. (c) Forces acting on particle flowing through DLD. (d) Forces acting on particle under Electric field

particle passes by the pillar and separate based on particles size compare to designed critical

size. Before we proceed to the designing theory of DLD, there are some basic fluidic notion that should be considered for fluidic flow in micro-channels.

TECHNICAL ASPECTS OF FLUID FLOW IN DLD

DLD deals with the fluid flow in microscale [96], corresponding to size range of cells and bacteria. Due to small scale, the phenomena such as diffusion, flow resistance and laminar flow is more dominant as compare to macro-flows and affects the control of microfluidics system [97, 98]. These phenomena in microscale are really important and influential to DLD [99].

Laminar Flow

As we scale down the size of channel to micro level, the viscous forces are more dominant than inertial and the flow remains in laminar range [98]. The Naiver-Stokes equation for incompressible fluid is given as follow:

$$\rho\left(\frac{\partial v}{\partial t} + v.\nabla v\right) = -\nabla p + \eta\nabla^2 v \qquad \ldots\ldots\ldots (1)$$

As the inertial forces in fluid flow through miniature channels are having negligible effect [44], the above equation can be rewrite as follow;

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

Reynold number is a dimensionless densities ratio of inertial force and viscous force in fluid flow and determine the nature of flow in channels;

$$R_e = \frac{\rho \cdot vD_H}{\eta}$$

Where D_H is a hydraulic diameter provided as [24];

$$D_H = 2\omega h \,/\, (w + h)$$

Due to very small dimension of microfluidics channels, the hydraulic diameter is very small and that is why the flow in such miniature channels are always in laminar regime. Which means two or more fluids are made to flow through micro channels, fluids will flow in lamina next to each other without mixing immediately. The only possible mixing in this case is due to diffusion but there is approximately no convective mixing [99][100].

Diffusion

As mentioned earlier, due to laminar flow the main possibly mixing is due to diffusion in micro-channels. In DLD, the larger particles in range of micrometer are rarely effected by diffusion but smaller particle can mix from one lamina to other due to diffusion. The mixing of smaller particles can affect the efficiency of DLD but mixing can be reduced by increasing fluid flow velocity.

Peclet number is a ratio of the rates of convection and diffusion, when a particle moves a distance due axial convection and radial diffusion respectively;

$$P_e \equiv vw/D = \frac{diffuission\ time}{convection\ time}$$

Where *D* is a diffusion coefficient which is calculated from naiver-strokes equation for spherical particles [99].

$$D = \frac{kT}{6\pi\eta\alpha}$$

Where *k* is a Boltzmann constant, *T* is the temperature and α is the hydrodynamic radius.

Fluidic resistance

As dimension of channel decreases the friction between fluid flow and the walls of channel increases and hence increases the resistance to flow. Channel with complex geometry and high surface to volume ratio have higher resistance to flow. The relationship between resistance and flow rate for pressure driven flow is given by;

$$Q = \frac{\Delta p}{R}$$

Where, Δp is the pressure difference across the channel. If the microchannel is of rectangle shape in cross section with height h, length l and width w. The resistance for high aspect ratio height is larger than width and length is also considered, is provided as [97];

$$R = \frac{12\eta l}{wh^3}$$

In case of low aspect ratios i.e w=h, resistance are given as;

$$R = \frac{12\eta l}{wh^3}\left[1 - \frac{h}{w}\left(\frac{192}{\pi^5}\Sigma \sum_{n=1,3,5}^{\infty} \frac{1}{n^5}\tan h\left(\frac{n\pi w}{2h}\right)\right)\right]^{-1}$$

In the DLD, aspect ratio is high i.e. the gap between pillars is too smaller than height of the pillar, the parabolic profile of fluid flow is dominant in in between gaps and given as;

$$R = \frac{12\eta l}{hw^3}$$

DLD Principle

The DLD pillar array is in form of square in which rows and columns are perpendicular to each other but the array itself is tilted at an angel θ to the fluid entering the device. There is another way in which the rows are perpendicular to the fluid flow but each row is shifted by $\Delta\lambda$ to its successive row. The distance between two pillar is called gap *G*, the distance between center of post/pillar to other pillar in same row or column is given by λ, the

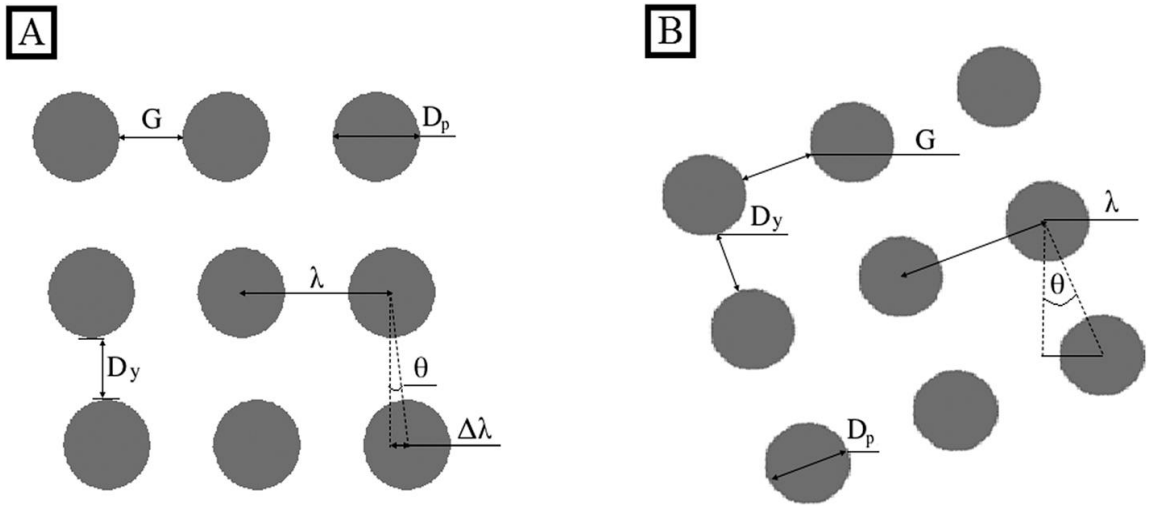


Figure 11: Illustration of DLD design parameters (a) tilted rows (b) pillar array tilted to flow

diameter of pillar is $D_{p,}$ the distance between two post in tilted position is D_y and the angle of tilt is given as θ as shown in Fig 11.

When the pillars of N+1 row of the DLD is at the same position of first row, then N is called Period of DLD and related to tilt angle as follow;

$$\lambda \tan\theta = \frac{\lambda}{N}$$

N is also related to distance between post centers in same row and difference of post center in tilted rows as;

$$N = \lambda/\Delta\lambda$$

The inverse of period N is called row shift fraction and equal to the tangent of tilt angle;

$$\varepsilon = \frac{\Delta\lambda}{\lambda} = \frac{1}{N} = tan\theta$$

The critical diameter of the device is given as in term of shift fraction below and the particles bigger than this diameter will go in displacement mode i.e it will change it streamlines while passing across pillar;

$$D_C = 2\beta = 2\alpha.G\varepsilon$$

Davis [101] presented an empirical formula based on his experimental results with devices having different range of gap size for the DLD design of critical diameter D_C.

$$D_C = 1.4\ G\varepsilon^{0.8}$$

1.2.2 DEP

If the size of particle is greater than 1 micrometer, Brownian motion is negligible for the particle flowing through non-uniform electric field. The Dielectric force generated on micro particle in microfluidics flow is given as following formula [102-104];

$$\boldsymbol{F}_{DEP} = 2\pi\varepsilon r_{cell}^3 Re(K_{CM})\nabla(\boldsymbol{E}\cdot\boldsymbol{E}),$$

With assumptions that particles are of spherical shape, no oscillations, approximately dipole and homogenous. In the equation, r_{cell} represents the radius of particles or cell, ε is the permittivity of suspending medium, $\nabla(\boldsymbol{E}\text{^}\boldsymbol{2})$ is the local electric field gradient due to AC voltage, whereas $Re(K_{CM})$ is called the real portion of Clausius–Mossotti. This factor is one main driving parameter to manipulate the force acting on the particles / cells. It has the range of value from -0.5 to 1.0. It defines the nature of DEP i.e. nDEP for -0.5 to 0 and pDEP for the range 0 – 1.0. It depends entirely on the permittivity of the suspending medium and particles / cells.

In case of micro particles, mostly used for experiment are spherical micro-particles, the Clausius–Mossotti is given by:

$$K_{CM} = \left(\bar{\varepsilon}_{particle} - \bar{\varepsilon}\right)/\left(\bar{\varepsilon}_{particle} + 2\bar{\varepsilon}\right).$$

$\bar{\varepsilon}_{particle}$ is permittivity of particles, $\bar{\varepsilon}$ is complex permittivity of medium containing particle or sometime also referred as buffer. $\bar{\varepsilon}$ is given as;

$$\bar{\varepsilon} = \varepsilon - \frac{j\sigma}{\omega}$$

Whereas ε is the permittivity of medium, σ is the conductivity of medium and ω is the angular frequency.

As the cell is not identical to solid micro-particles polystyrene, due to biological structures of cytoplasm, cell membrane and other fluid inside.

The approximate permittivity of cell is given as follow, while depending upon the permittivity of medium and cytoplasm.

$$\bar{\varepsilon}_{cell} = \bar{\varepsilon}_{mem}\,\gamma^3 + 2\left(\frac{\bar{\varepsilon}_{cyt} - \bar{\varepsilon}_{mem}}{\bar{\varepsilon}_{cyt} + 2\bar{\varepsilon}_{mem}}\right) \Big/ \left(\gamma^3 - \left(\frac{\bar{\varepsilon}_{cyt} - \bar{\varepsilon}_{mem}}{\bar{\varepsilon}_{cyt} + 2\bar{\varepsilon}_{mem}}\right)\right)$$

and

$$\gamma = \frac{r}{r - d}$$

Where d is the thickness of cell membrane, r is the radius of cell, $\bar{\varepsilon}_{cyt}$ is the permetivity of cytoplasm of cell and $\bar{\varepsilon}_{mem}$ is for medium.[105-108]

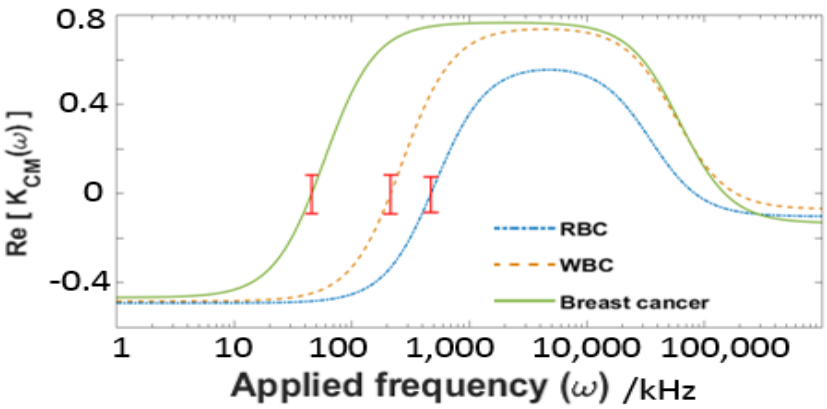


Figure 12: Plot of Clausius–Mossotti for different cells

Fig. 12 is the plot of Clausius–Mossotti for different cells with respect to applied frequencies range using the equations discussed. Based on different size, permittivity and conductivity, these cell exhibit different DEP responses to applied frequency.

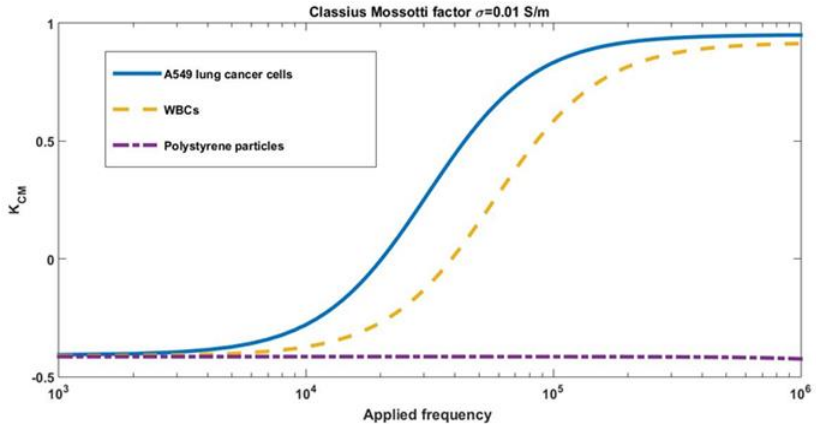


Figure 13: Plot of Clausius–Mossotti for cells and polystyrene particles

Similarly, fig. 13 plotted the Clausius-Mossotti for lung cancer cell, WBC and polystyrene particles to represent their responses to applied electric field frequencies.

The central idea of our work is to achieve a frequency in the microfluidic DEP assisted DLD device where CTCs experience no DEP force while the WBC and RBC experience nDEP. This DEP force on blood cells would make them to go displacement mode while cancer cells will go zigzag through DLD as shown in Figure 17.

CHAPTER 2

EXPERIMENTATION SETUP

The purpose of this thesis is to validate the concept of DLD coupled with DEP for microfluidics sorting. Our collaborator at Washington State University (WSU) through the simulation carried out the primary proof of idea. To make the experimental evidence of this new method, the work plan was arranged in multiple steps including; device design development for DEP coupled DLD, fabrication and experimentation. This chapter of thesis comprises on the details of experimentation procedures, sample and methodology of testing parameters.

DLD is a technique to separate cells due to difference of the size with high throughput, but unable to work on same size. Many of the cells that need to be sorted are sometime of same sizes. The benefit of DEP method is that it can differentiate size cells such as WBC and lung cells based on their electrical properties as shown in Figure 12 and 13. Whereas DEP lacks in high throughput and affects cell viability in low flow rates.

The idea of combining two techniques is considered to reduce the inability of each individual techniques and will provide high through put of same size cell separation.

2.1 DEP-assisted DLD Device Design:

Based on theory presented in introduction, the DLD design is a technique to separate the particles/ cells based on different sizes but the dilemma with this technique is that it is unable to separate same size particles. While describing DEP, we can separate the particles based on their polarizability and nature regardless of their size specifically. The idea suggested here is to couple DLD design with DEP. It has been tried already to tune the size of DLD with DEP force [109]; the authors used the electric field parallel to the flow of fluid by simply contacting the electrode with inlet and outlet of the device. Nevertheless, the concept has never been tested on cells, and the DLD with vertical electric field has never been reported yet to separate the particles or to tune the DLD. In vertical orientation the electrode were placed along the DLD pillar array on the both side.

The orientation of electric field has one of the vital role in this work. If the electrode are placed in outlet and inlet of fluid as in [109], the electric field will be parallel to flow as shown in the fig 14A. This idea is good for the particles as they observe only pDEP as shown in plot fig. 13. But in this parallel electric field the cells will experience nDEP but would not be able to change lamina because of high intensity electric field region between DLD pillars. So, parallel electric field will yield trapping of cells by not aiding the sorting or separation. While in case of perpendicular electric field orientation, in which the cells encounter low electric field region instead high field as in parallel orientation. Hence cells

under influence of DEP force will easily go through with lamina of buffer flowing without trapping as shown in figure 16-17.

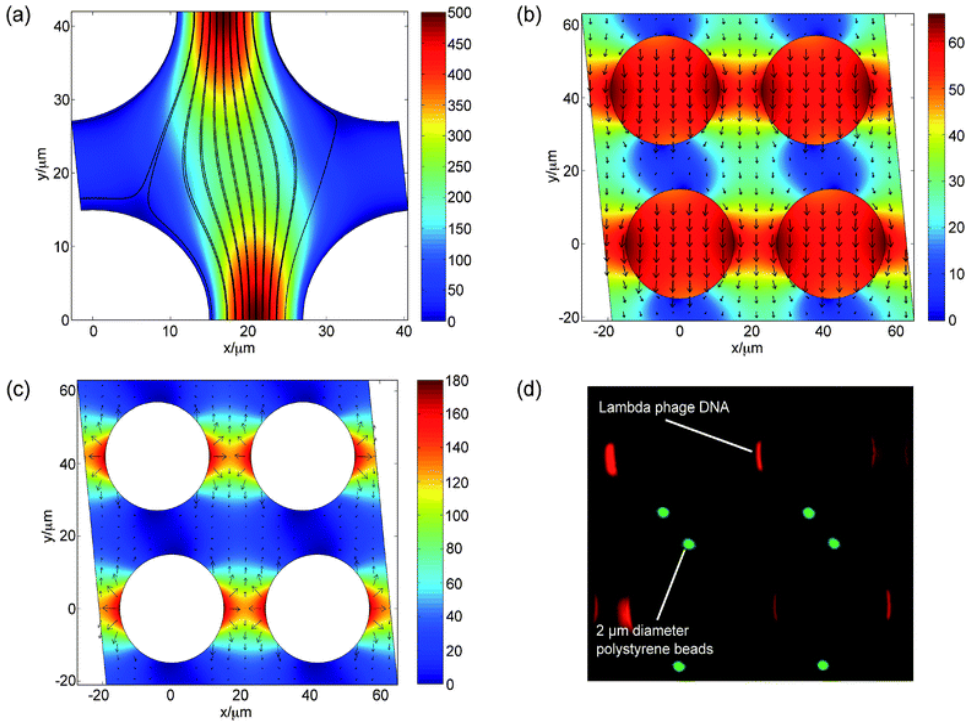


Figure 14A; Simulation of Parallel insulator based DEP electric field through DLD. Reprinted with permission from [109] a) Lamina flow between pillar region b) Electric field intensities region in DLD. c) Energy work done by DEP in DLD region. d) trapping of biological objects in high intensity E region.

So, the idea being tested in this thesis is to use vertical electric field in DLD. Our collaborator through the simulation in COMSOL Multiphysics developed the primary idea. Below are some simulation results. The simulation were carried out to separate circulating

tumor cells from blood cells while using the non-uniform electric field in DLD. The electrode were adjusted on the side of DLD pillar array hence the direction of electric field was perpendicular to the flow of liquid.

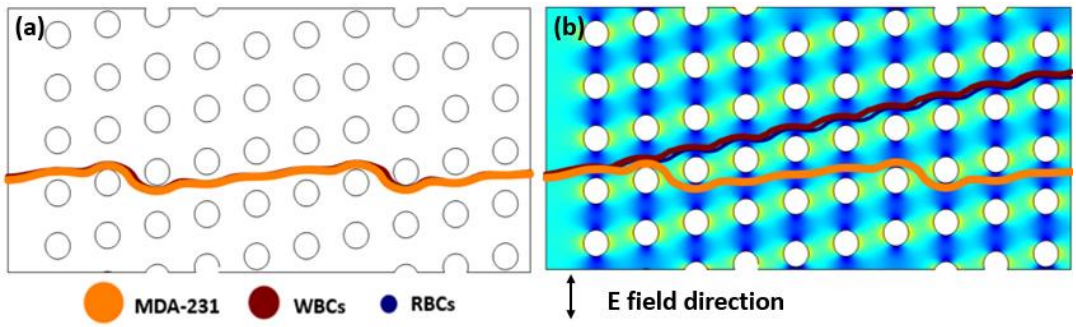


Figure 14B: COMSOL Simulation for breast cell separation from blood using DEP assisted DLD. Credit from Mohammad Agahmoo

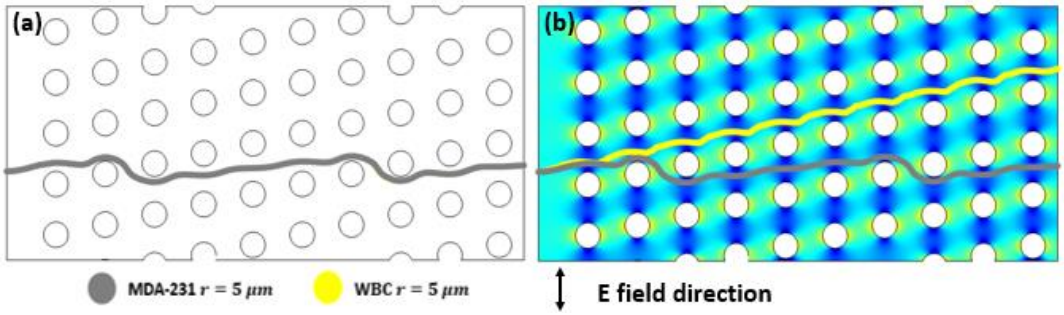


Figure 15: COMSOL Simulation for breast cell separation from white blood cell using DEP assisted DLD. Credit from Mohammad Agahmoo

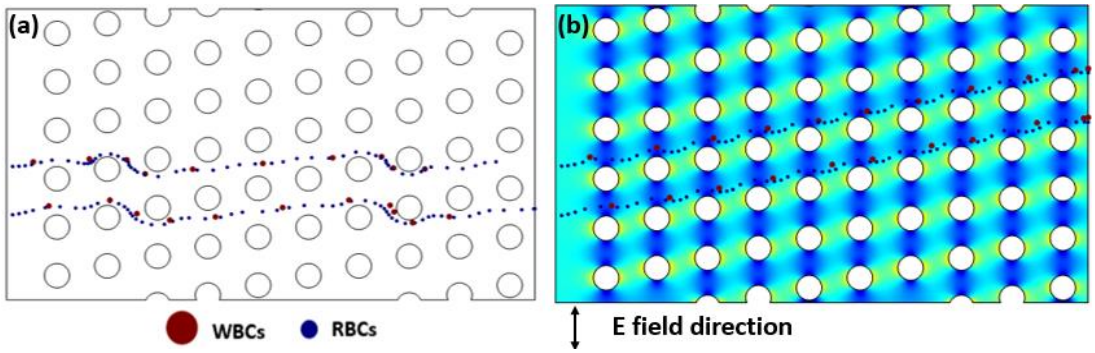


Figure 16: COMSOL Simulation for blood cell flowing through DEP assisted DLD. Credit from Mohammad Agahmoo

Simulations shows the separation of breast cancer cells and WBC in DEP assisted DLD in Figure 15 while Figure 14 is presenting the separation of cancer cells from whole blood cells.

2.2 Methods

2.2.1 Device Design:

To perform the experimental run, the first challenge was to make a practical device. DLD design and DEP principle were already presented but the incorporation of electric field across the DLD were never presented before. To deal with this challenge, we tried multiple design before reaching ultimate selection presented below. In this design, sideways lateral channel were made to serve as electrode channel. The separation between DLD and electrode channel was just 20 microns of PDMS. While the details design of devices are presented in table below. Electrode channels were filled with conducting material such as

PBS 1X or some time with liquid metal galinstan. The electrodes were connected to an electrical source to generate the electric filled inside. The equivalence electric resistance can be described as in figure 18 design idea inspired and printed with permission of [110].

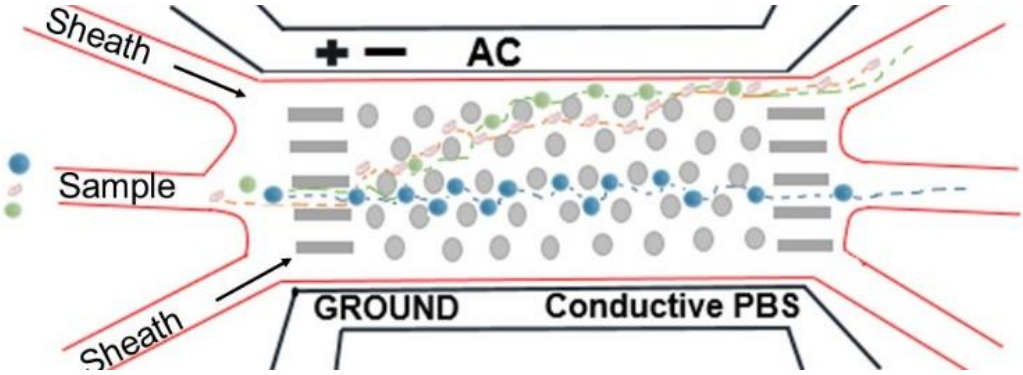


Figure 17: Illustration of DEP assisted DLD device design

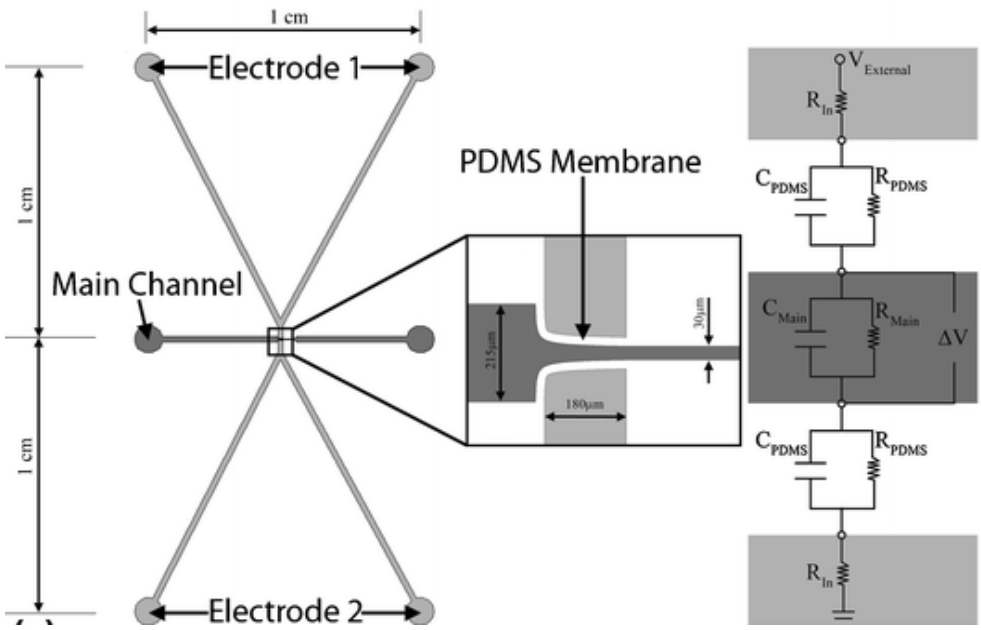


Figure 18: Illustration of equivalence electric resistance of electrode PDMS wall. Reprinted with the permission from [104]. Copyrights

Table 1: Designs details for DEP assisted DLD devices

	Design 1	**Design 2**	**Design 3**
Critical Size	20 micron	6 micron	14 micron
Post Diameter	40 micron	30 micron	40 micron
Tilted angle	7.13º	6º	11.46º
Post Gap	60 micron	12 micron	25 micron
Width of Electrode PDMS Capacitive wall	20 micron	25 micron	25 micron

2.2.2 Device Fabrication:

Once the design selection for the practical demonstration of simulate idea was done. The AutoCAD file was sent to vendor for the mask printing. To generate the master stamp, Photoresist SU-8 2050 was used onto 4-inch silicon wafer <100>. The depth of device was approximately 40 micrometer, the photoresist was dispensed on to silicon wafer with rate of 1 milliliter (ml) per 25mm and spun up to 4500 rpm using spin coater in clean room of UIC Nanotechnology Core Facility (NCF). Photoresist was soft baked at 65oC for 3 mins and 95 ^{o}C for 6 minutes before UV Exposure. UV light exposure of 12 seconds was shed to the photoresist to provide energy of 240 MJ/cm^2 using 20MJ/sec. cm^2 Mask aligner to get desire depth. SU-8 2050 coat was further post baked at 65oC for 1 minute and 95oC for 6 minutes. Finally, the silicon wafer SU-8 2050 coat was placed in the developer for 6 minutes to etch the exposed photoresist. Wafer was cleaned using Isopropyl Alcohol (IPA) and deionized (DI) water. Final inspection of mold fabrication was carried out under microscope and prefect SU-8 2050 stamp molds were stored in dust free containers.

After the master stamp fabrication, the Polydimethylsiloxane (PDMS) was used to make the replica of stamp mold and to use it as experimental device. The PDMS sylgard 184 from Dow conining was used in this regard and the ratio of 10:1 was for elastomer to hardener. The elastomer and hardener was mixed properly until it is saturated with large number of bubbles. To remove bubbles PDMS inside plastic conical tubing was spun in the 6 inch radius centrifuge for 5minutes at 5000rpm. Once the bubbles were removed, the

PDMS was poured onto the SU-8 2050 mold master stamp slowly such that it cover all the pattern and filled the mold container up to 1 cm. The poured PDMS was left for one day in room temperature for hardening. After the solidification, the PDMS casting with stamp replica was removed carefully and thorough holes punched using holes puncher from TED PELLA, INC. This holes serves as inlet and outlet of the device to connect to the pumps via tubing.

PDMS casting with microfluidics channels, DLD micro-pillar arrays, side electrode channel with wall and holes for connections was covered with glass slides using plasma treatment for permanent bonding. Micro-glass slides 3x2 inches from corning dow were cleaned with methanol followed by IPA and DI water. PDMS casting bonding surface was cleaned using scotch tape. Both PDMS casting and glass slide were placed inside Herrick plasma etcher and treated with oxygen plasma generated at 350atm at high frequency

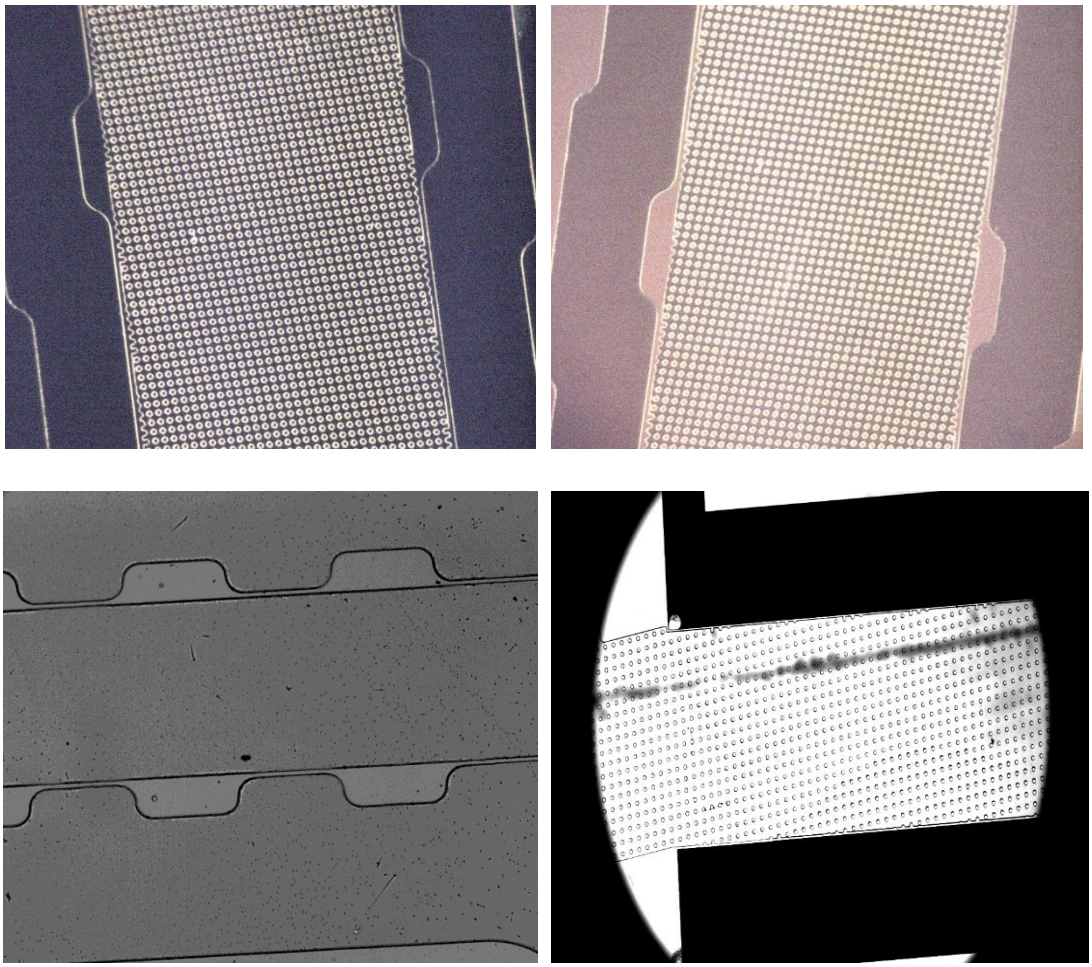


Figure 18: Fabrication Steps A-B) SU-8 micro-mold on silicon wafer substrate. C-D) Final devices with PDMS casting sealed with glass slide

setting of microwave for 30 seconds. After plasma treatment, glass and PDMS was bond permanently together.

2.2.3 Device Setup

To setup device for experimentation, first step is to filled electrode channels with conductive fluid. For this purpose PBS 1X and liquid metal glainstan was use separately.

One side of electrode channel was connected with Polyvinyl Chloride (PVC) fitting to serve as minute head of fluid on one side of electrode channel for natural flow through channel. Conducting fluid was filled in fitting and a pipe fitting with empty syringe attached was connected to other side of the channel. The syringe was used to suck fluid through electrode channel by moving plunger outward in syringe. This method avoids bubble accumulation in the channel and provide uniform electrode. Once the flow is established, pipe with syringe is removed and the hole was closed with scotch tape while the other end with PVC fitting was left open for electric wire connection. Next step is to make inlet and outlet connection for fluidic flow through DLD. The input pipping was connected to syringes and made full with sample with no bubbles inside. The other end of pipes were connected to the inlet of device. The syringes were adjusted onto Harvard pumps for control flow. For outlets, pipes were connected to PDMS outlet holes and were left open in to container. The device is placed on to inverted Nikon microscope attached with Canon high-speed camera for flow visualization through device and image capturing for analysis. Then electrical wires from high voltage amplifier Terk were dipped into PVC fitting attached to electrode channel.

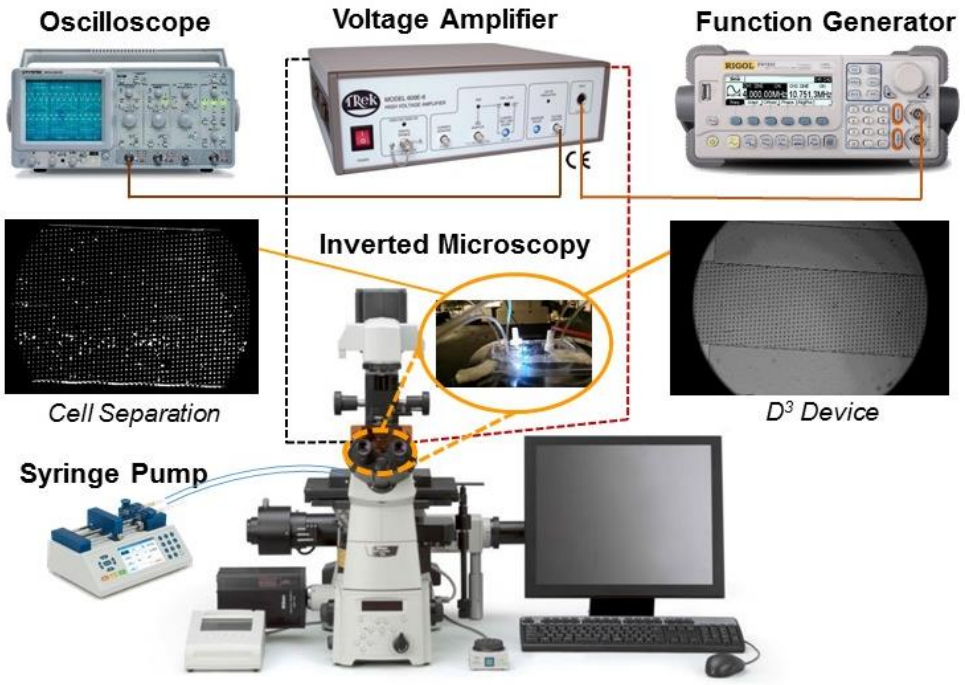


Figure 19: Illustration of experimental setup

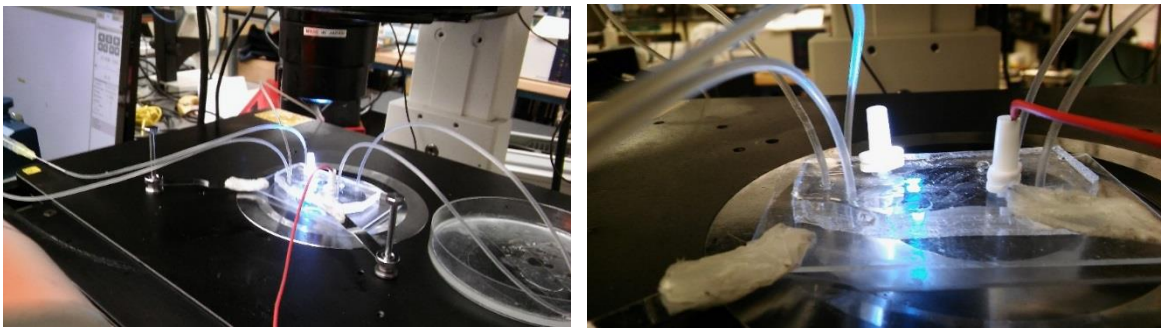


Figure 20: Device setup A-B)

2.3 Apparatus

2.3.1 Particles

The micro spherical particles were used in the size ranges of .1, 1, 5 and 10 micrometers.

2.3.2 Cell Culturing

The cells were first thaw at 37°C from frozen storage. The culture was kept at 37C with 5% CO2 environment incubator. A549 cell were grown in T25 culture flask in total volume of 7ml. The medium used was DMEM with 10% FBS and 1% Penicillin streptomycin. The cells were grown for four days until 100% confluency

Cell Sample preparation: To produce cell sample, medium was first removed from culture. 2ml 0.5% trypsin solution was injected to flask for 5mins at 37°C to detach cells from glass surface. The spherical detached cells in trypsin solution were then mixed with 5ml of Phosphate Buffered Saline. The mixture was centrifuged at 3000rpm for 5mins to separate cell at bottom. The solution was then replaced with fresh PBS and mixed cells with micropipette. This cell washing process was repeated thrice. The final mixture of cells in PBS was used for experiments.

2.3.3 Buffers

For the experimentation purpose two different type of buffers were used with same fluidic conductivity value but different composition depending on the type of sorting under consideration. In the case of experimentation with particles, PBS 1X was diluted with the DI water unless the fluidic conductivity was reached to 100 microsimens and pH was maintained to 6.7. The sample with cancer cells A549 was used with buffer made out of sucrose .85[w/v], 0.0003% glucose [w/v], 0.00725 RPMI [v/v] [111]. So that cells remains alive in the buffer for long time during experiment.

2.3.4 Cell Sample Preparation:

To create cell sample for experimentation, first of all T70 flask were removed from incubator and place in biological hood. Cell culture medium was removed using pipets and then culture was treated with 3ml of Trypsin 0.1X solution to detach cell from flask surface. The flask was placed in incubator again for 30 minutes to speed up cell membrane cutting process. After 30 minutes, flask was taken out and vibrated by tapping on cell surface of flask with fingers gently. The cell would start separating from the flask surface and settled down in bottom corner of flask when put it in vertical direction. Added 7 ml of medium again to cell-trypsin solution and mixed by flowing fluid in out of pipette. Then solution was transferred to conical tube from flask and centrifuged at 500g for minutes to settle down cells in bottom and medium was removed using pippete. The cell were then filled with cell buffer and repeated the process with centrifuge to wash cell from medium and trypsin. After the cell was washed, the sample was desired to make with 3×10^6 cell/ml.

2.3.5 Electrical setup:

A functional arbitrary waveform generator commercially available by RIGOL DG 1022A was used to produce AC voltage signal with frequency range of 25 MHz. As the capacitance and resistance of DLD structure is very huge between PDMS wall of electrode channels, a powerful voltage amplifier by TREK model 609E-6 was used for the desire range of voltage up to 1500 volts. To provide make sure proper monitoring of waveform signal an oscilloscope by Tektronics TDS 3054 was connected with amplifier. Amplified

signal (1000:1) output from voltage amplifier and ground wire dipped in the PVC fitting attached to electrode channel, to make contact with conductive fluid to complete electric circuit across the flow through DLD pillars.

To provide electric voltage to device, caution was taken while turning amplifier. To avoid high voltage surge and device breakdown, a proper stable voltage at lower value was maintained before inserting amplifier wire into conducting liquid. First, small value for voltage was set as V_{rms} = 0.1 at the frequency of 100 Hz. Then signal was forwarded to amplifier by pushing channel on button on signal generator. The output from amplifier was monitored through oscilloscope and connections were made with device electrode channels. Once the electric circuit is established in the device, the value of V_{rms} and frequency was increase incrementally by observing flow through DLD under microscope.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Experimental Run with particle Design 1:

Validation of vertical Electrical Field in DLD: Polystyrene microsphere particles of size 4&1 micron were utilized through the DLD-vertical DEP device**.** Particles experienced negative dep force and moved within it stream but bumped along displacement when electric field was applied **(b)** & **(c)**. Without electric field particles were zigzagging and distributed all along stream of sample following **(a).** Upon applied Electric field, Particles were deflected down side along pillars angle.

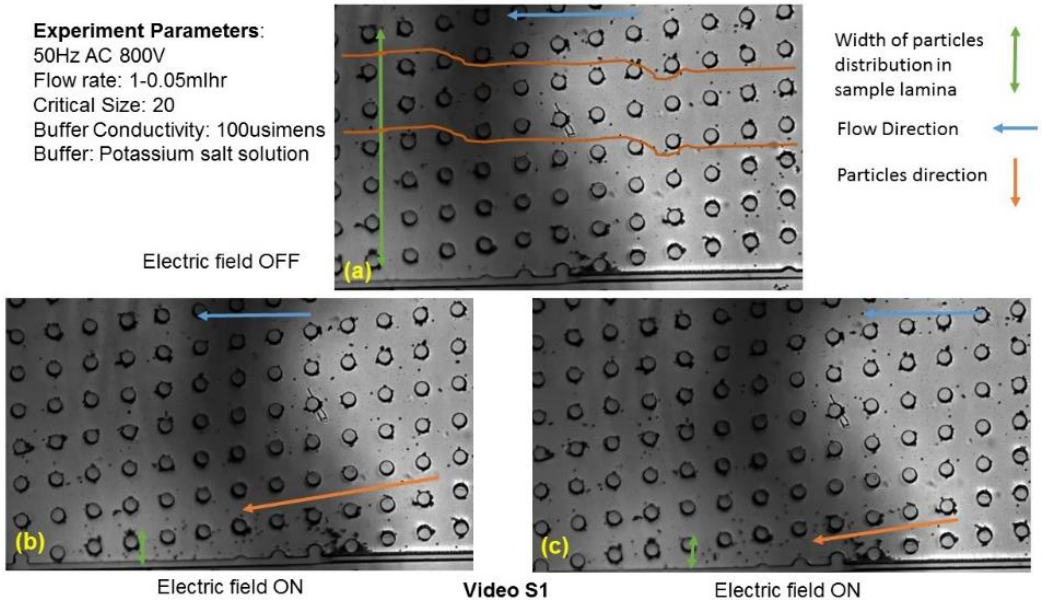


Figure 22: pDEP changing the direction of micro particles of polystyrene

3.2 Experimental Run with particle Design 2:

Tuning of DLD Critical size with Dep due to non-uniform Vertical Electrical Field:

Particles experienced negative dep force and moved across the Dep buffer lamina. Bumped along displacement when electric field was applied. Without applied field, the particle were zigzagging on lower side of device. With applied field, the particles moved to upper

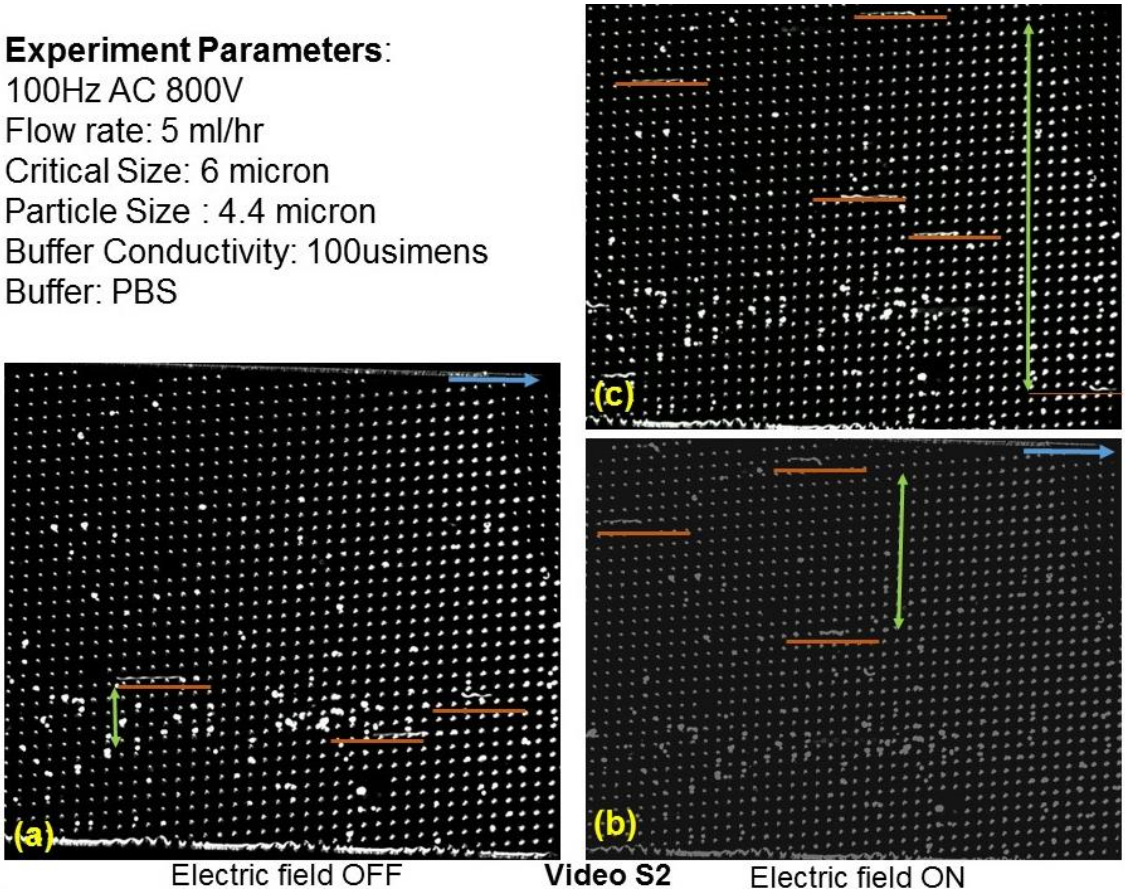


Figure 23: Tuning the critical size with parallel DEP assisted DLD

laminas and followed displacement mode. The results are comparable with published idea of DLD tuning with parallel electric field. Application of vertical non-uniform field tune the critical size of device from 6 to 4micron.

3.3 Experimental Run with Cells Design 3:

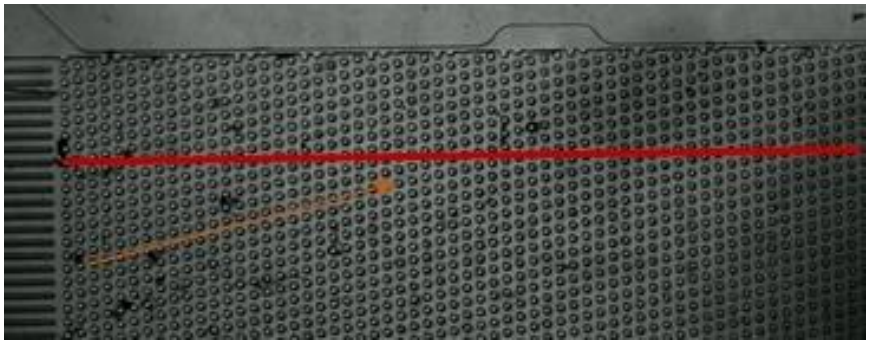
A549 lung epithelial Cancer cell line: The cells were passed through a DLD with Critical size of 14micron. The cancer cells A549 are 10 micron size ideally and went zigzag through DLD without electric field **(Video S3).** As for the experiment, most of Cell were clogged to each other and oversized the 14-micron limit including some Cells individually bigger enough 15microns approx. These clogged Cells went through the DLD in bumped mode without application of any AC voltage frequency **(Videos S3a)**. When the AC frequency in range of 40khz to 500khz applied, the cell experience the +ve Dep force due to non-uniform vertical electric field which made the Cancer cell of size 10mciron **(Video 3b)** and even bigger than critical size to go zigzag **(Video S3c).** +ve Dep force kept oversize clogged cells in zigzag and opposite to DLD angle. The concept of our idea earlier presented is to keep CTCs in zigzag mode. The figures video S3, S3b and S3c are the trajectory indication of single Cell by combining multiple pictures, at constant interval, into one.

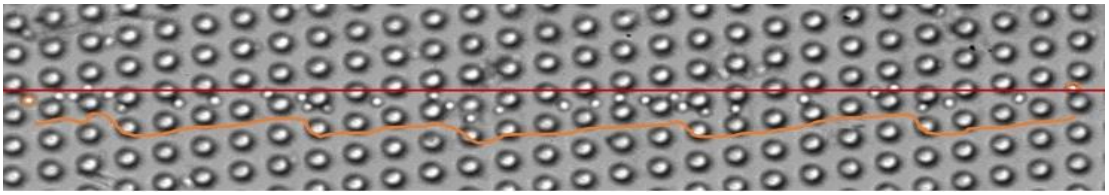
Experiment Parameters:

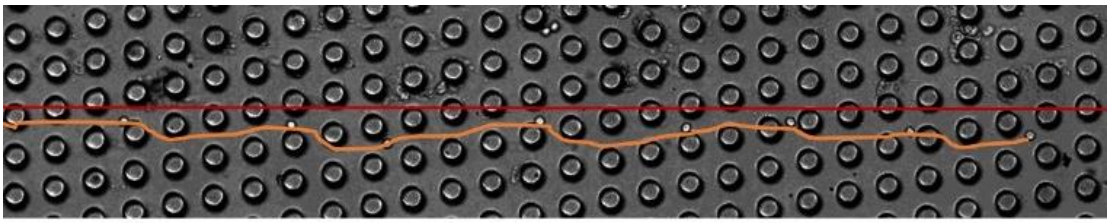
Cell Sample flow rate: 1.5 ml/hr
Buffer flow rate : 1ml/hr
Critical Size: 14 micron
Buffer Conductivity: 100usimens
Buffer: PBS
Voltage: 800V

Reference Horizontal line
Trajectory line for single cell

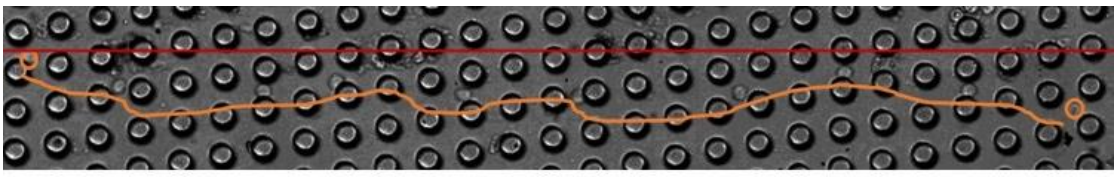
Frequencies
Video S3b and S3c = 10, 30, 50 khz







Video S3b



Video S3c

Figure 24: pDEP force changing the A549 cells from bumping to zigzag mode

3.4 Cell Viability

The cell samples were run through the device at different voltage with speed of 2ml/hr until 30mins to measure the cell survival. The Cell viability of the sample were conducted before experiment and then three runs were made with different voltages. The cells survival rate was decreased with increase in the voltage. But the viability for the optimal device operation at 800 volts were 93% which is still promising for the further cell analysis.

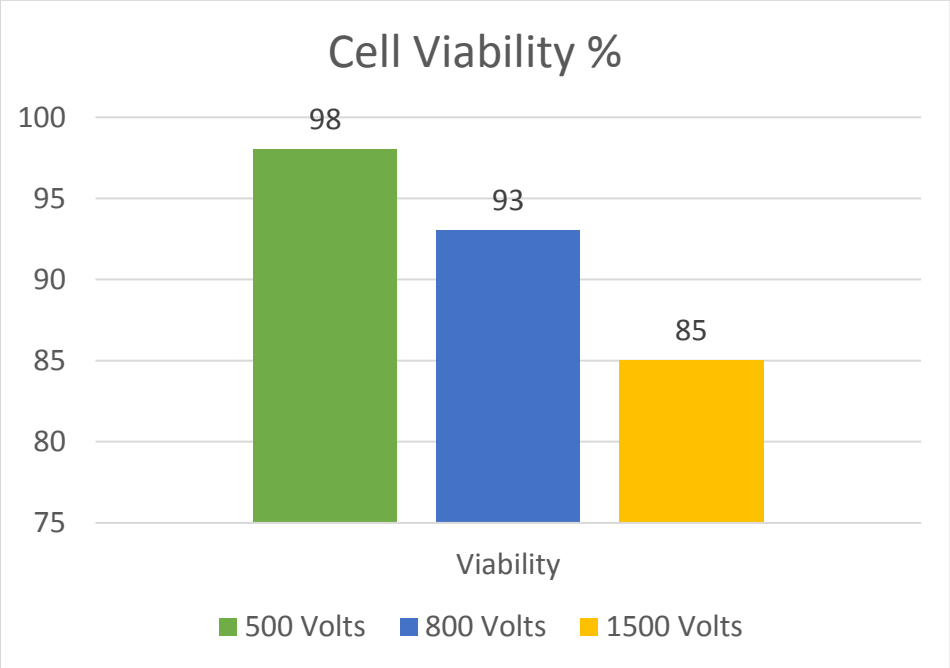


Table 2: Post experiment cell viability results

2ml/hr, 30mins	**Before**	**500 volts**	**800 volts**	**1500 volts**
% Cell Viability	99.99%	98%	93%	85%

Viable Cell per Square	109.5	24.25	27.2	9.5
Dilution Factor	2	4	4	4
Cell Count /ml	2.2x10^6	9.8x10^5	1.08x10^6	3.8x10^5

There was a big reduction in cell count after experiment. There are two main reason for this. First, the increase in dilution due to two-sheath flow along sample in the device and second is the cell adhesion inside device.

CHAPTER 4

CONCLUSION

The objective and goals of this project were presented in chapter 1 along with the background of cancer research and microfluidics contribution in cancer cell separation. The project is primarily consisting of design, fabrication of microfluidics device and results discussion about simulation finding. The chapter 2 described the methods and design parameters for device fabrication as well as procedures required to carry out cell culturing and sample preparations. In chapter 3 we presented the discussion on results and compared it with already published paper as well as with our expectation based on simulation with COMSOL.

The experimental setup is presented with three categories, such as first section deals with proof of concept to generate electric field in DLD. This section is quite successful in proving our novel idea of combining DLD with lateral electrode. The electric fields behaved as expected based on simulation. This experiment result set a starting base for further experimentation. Second round of experiments were conducted to compare the ability of our idea competence with already published idea of tuning DLD with Dep in parallel direction of flow. The results we produced are exactly in accordance to their finding. We enabled the change in polystyrene particles motion from straight to displacement with application of electric field. The electric field due to lateral electrode was successful to produce positive DEP (pDEP) force on particles and hence tuning critical

size of DLD but this time with vertical electric field. Based on previous promising results, last attempt was made with the cancer tumor cells A549. The device designed for this trial was based on critical size of 14 micrometers but due to irregularity of cell sizes the experiment was conduct a little bit different. The cells went displacement mode initially and were made to change their direction upon DEP force application. For the same cell experiment results, the viability of cells was counted with different flow rates and electric field strength. The viability results were in the range of 85 to 97% which is quite promising.

The work of this project is quite promising for us as it provides experimental proof of concept of our novel idea of label free tumor cell sorting. Although the results with cells were not exactly in accordance with simulation results but still provide a direction to move next. Definitely, it teach to reconsider the conductivity and permeability relation between cells and buffer. The results differs a little bit with simulation but still promising to continue our project. The work presented in this thesis is a small step toward the separation of tumor cells from blood sample using DEP coupled with DLD but provide a strong base to move on.

FUTURE WORK AND GOALS

The ultimate goal of this project is to separate cancer tumor cells in the blood as presented in simulation figure 16-17. However, the results produced so far are simple, promising and a first proof of concept we presented to separate CTCs. Still there is a lot of work required to reach final destination. The next steps to be taken in this regards include the study of experimental runs with A549 with particles, WBC with cancer cells and ultimately whole blood spiked with CTCs.

There is also a consideration for study of joule heating effect on cell viability and devices stability due use of high operating voltage in range of 800-1500 V.

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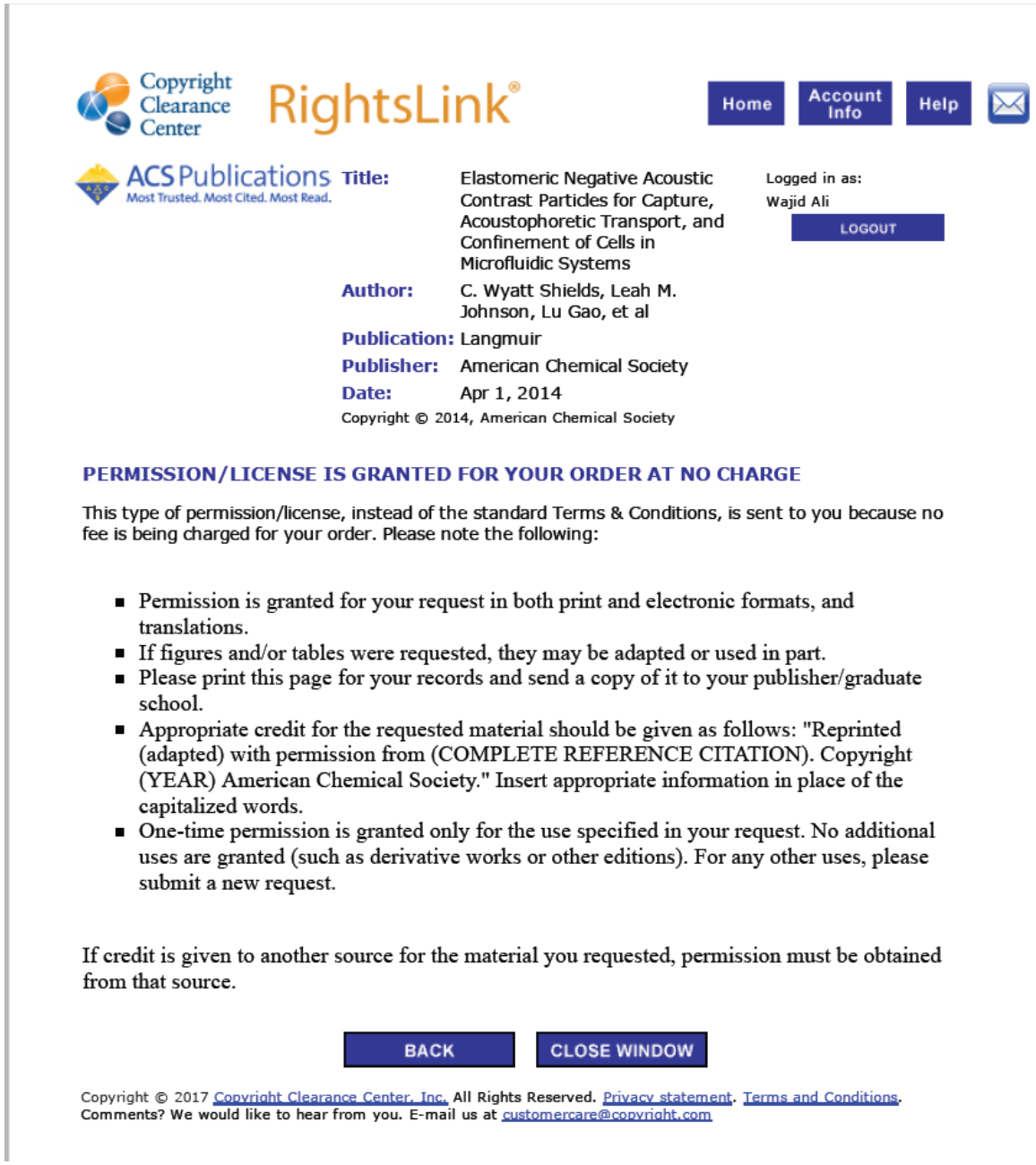

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VITAE

WAJID ALI

Education

University of Illinois at Chicago (UIC) Spring 2017
Master of Science in Mechanical Engineering, CGPA 3.83
Recipient of Graduate College Full Time BOT Tuition Waiver – Spring '17, Spring '16, Fall '16

University of Engineering & Technology (UET), Lahore August 2013
Bachelor of Science in Mechanical Engineering, CGPA 3.42

Research Experience

UIC Xu Microfluidics Lab *Research Assistant* Aug 2015 - Present
Developed a Microfluidic device for separation of micro-particles/cells Utilized SOLIDWORKS, AutoCAD, CAMBAM, 3D printer, CNC Micro-Milling Machine and PDMS casting for rapid prototyping and design for experiment in lab
Served as a Technical Advisor to undergraduate Senior Design Project: "Liquid Metal 3D Printer –Product Development"

UIC Nanotechnology Core Facility *Student User* Dec 2015 - Present
Worked in cleanroom facility for characterization and manufacturing of MEMS/Nano devices.
Studied and developed understanding with characterization techniques such as STM, AFM, Raman spectroscopy, LEED, TEM, X-rays techniques, etc.

Internships Experience

Pakistan Oilfields Limited *Meyal Oilfield, Attock* August 2012
Observed and studied mechanical systems involve in oil well construction and production
Posted in rotations for study and observation of Meyal gas purification plant, LPG plant, and POL gas storage & distribution system

WARTSILA *200 MW HFO Fired, CC IPP NCPL* July 2012-Aug 2012
Worked and prepared a detailed report on studies of Plant Operations & Maintenance, Wartsila 18V46 engine, HRSGs
Hands on experience of 12k Maintenance of Wartsila 18V46 engine's fuel injectors and heavy furnace oil (HFO) separator

Pakistan Elektron Limited *Development Department* June 2011-July 2011
Experienced product development methods and industrial design for manufacturing at plant.
Analyzed the manufacturing processes involved in the production at the plant including thermo-vacuum forming, foaming, extruding, injection molding and metal body fabrication

Leadership

ASME UET Lahore Chapter; Member, Board of Governor 2012-13

ASME UET Lahore Chapter; Event Manager for presentation competition “Future Fantasy” in MECHNO’12 (Mechanical National Olympiad) 2012

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