

Dielectrophoresis(DEP) and travelling wave
dielectrophoresis(TWDEP) based cell manipulation using
microfluidics

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Indian Institute of Technology Hyderabad
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The Degree of Master of Technology



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June, 2015

Declaration

I declare that this written submission represents my ideas in my own words, and where others' ideas or words have been included, I have adequately cited and referenced the original sources. I also declare that I have adhered to all principles of academic honesty and integrity and have not misrepresented or fabricated or falsified any idea/data/fact/source in my submission. I understand that any violation of the above will be a cause for disciplinary action by the Institute and can also evoke penal action from the sources that have thus not been properly cited, or from whom proper permission has not been taken when needed.



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Approval Sheet

This thesis entitled Dielectrophoresis(DEP) and travelling wave dielectrophoresis(TWDEP) based cell manipulation using microfluidics by Karhade Pallavi is approved for the degree of Master of Technology from IIT Hyderabad.



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Dedicated to

My parents

Abstract

Microfluidics is a new trend in medical instrumentation to miniaturize the size of medical devices used in laboratories. Bacterial cell manipulation can be done using “Travelling wave dielectrophoresis(TwDEP)” technique on a Lab on Chip device. In traditional DEP method, polarized cells get either attracted or repelled from electrodes depending on their dielectric properties and size, hence, get separated vertically. In order to collect these separated cells at the outlet hydraulic drag force is required. Whereas in the case of TwDEP cells get vertically separated like normal DEP method and along with it they also get pumped at output, horizontally due to travelling wave. This travelling wave is generated by applying voltage with phase difference at each electrode. Such voltage creates non-uniform electric field with phase shift and hence has force on cells on both the axes. Such devices can be connected to external electric circuits and can be used as bio-sensors.

TwDEP is preferred over traditional DEP method so that accurate cell separation as well as pumping is achieved. The objective of this project is to manipulate the bacterial cells using travelling wave DEP technique using the dielectric properties of the cell and to see the effect of various electrode geometries such as Interdigitated electrodes and spiral electrodes on cell separation

capabilities. Modeling and simulation is done using COMSOL multiphysics software so that experimental and simulation results can be compared at the later stages. For this project, electrodes used are ITO coated glass electrodes and channels are formed on PDMS surface with the help silicon mold. Interdigitated and spiral electrodes are to be designed and their effectiveness should be studied on cells.

Table of Contents

1	Background	1
1.1	Introduction to Microfluidics	1
1.2	Introduction to Dielectrophoresis and Travelling Wave Dielectrophoresis	2
1.3	Introduction to COMSOL	5
1.3.1	Steps to form a basic template in COMSOL	6
2	Literature survey	9
2.1	Submicron particle trapping using Travelling Wave Dielectrophoresis	9
2.2	3D Dielectrophoretic chips : Trapping and separation of cell population	9
2.3	Microfluidic pumping based on Travelling Wave Dielectrophoresis	10
2.4	Large area Travelling Wave Dielectrophoresis particle separator ...	11
2.5	Counterflow DeP for trypanosome enrichment and detection in blood	11
3	Project Modeling	14
3.1	Introduction	14
3.2	Working principle	14
3.3	COMSOL modules and governing equations	15
4	Simulations and Results	20
4.1	Matlab plots	20
4.2	Geometry in COMSOL-2D and 3D	22

4.3	Meshing.....	24
4.4	COMSOL modules (simulations)	25
5	Summary and future work	44
	References	46

List of Figures

Figure-1: Anisotropic particle model.....	2
Figure 2(a): $\text{Re}(f_{\text{CM}})$ for an insulator	3
Figure 2(b): $\text{Re}(f_{\text{CM}})$ for isotropic particle.....	4
Fig. 2(c): $\text{Re}(f_{\text{CM}})$ for an anisotropic particle	4
Fig. 3: DEP force in channel	4
Fig. 4: Two types of DEP forces.....	5
Figure 5: Approach to design microfluidic chip.....	7
Figure 6: Flow chart for simulation	17
Figure 7(a): $\text{Re}(f_{\text{CM}})$ for cryptosporidium with water as a medium.....	20
Figure 7(b): $\text{Re}(f_{\text{CM}})$ for cryptosporidium with PBS as a medium	21
Figure 8(a): F_{DEP} for cryptosporidium with water as a medium	21
Figure 8(b): F_{DEP} for cryptosporidium with PBS as a medium.....	22
Figure 9: COMSOL 2D design(channel and electrodes)	23
Figure 10: COMSOL 3D design(channel and electrodes)	23
Figure 11(a) : 2-D free triangular meshing.....	24
Figure 11(b): 3-D free tetrahedral meshing.....	25
Figure 12: Voltage at each electrode.....	25
Figure 13(a): Electric potential at $t=0$ s.....	27
Figure 13(b): Electric potential at $t=1.5$ s.....	27
Figure 13(c): Electric potential at $t=2.5$ s	28
Figure -13(d): Electric potential at $t=3$ s	28
Figure 14(a): Normalized electric field at $t=0$ s	29
Figure 14(b): Normalized electric field at $t=1.5$ s	29
Figure 14(c): Normalized electric field at $t=2.5$ s	30
Figure 14(d): Normalized electric field at $t=3$ s.....	30
Figure 15(a): $\nabla E ^2$ at $t=0$ s.....	31
Figure 15(b): $\nabla E ^2$ at $t=1.5$ s.....	31
Figure 15(c): $\nabla E ^2$ at $t=2.7$ s	32

Figure 15(d): $\nabla E ^2$ at $t=3s$	32
Figure 16(a): F_{DEP} at $t=0$ s.....	33
Figure 16(b): F_{DEP} at $t=1.7$ s.....	33
Figure 16(c): F_{DEP} at $t=2.5$ s.....	34
Fig. 16(d): F_{DEP} at $t=3$ s.....	34
Figure 17(a): Electric potential at $t=0.1$ s.....	35
Figure 17(b): Electric potential at $t=0.3$ s.....	35
Figure 17(c): Electric potential at $t=0.6$ s.....	36
Figure 17(d): Electric potential at $t=1$ s.....	36
Figure 18: Velocity profile in 2D.....	38
Figure 19: Velocity profile in 3D.....	38
Figure 20: Dimensionless model for a small portion of the channel.....	39
Figure. 21(a): Concentration profile at $V=2V$	39
Figure. 21(b): Concentration profile at $V=4V$	40
Figure. 22(a): Particle trajectory at $t=0.1$ s.....	41
Figure. 22(b): Particle trajectory at $t=1.2$ s.....	41
Figure. 22(c): Particle trajectory at $t=2$ s.....	42
Figure. 22(d): Particle trajectory at $t=2.8$ s.....	42

List of Tables:

Table -1 : Dimensions of channel and electrode	23
Table-2: Properties of Cryptosporidium.....	26
Table 3 : Settings in Creeping flow Module	37

Chapter 1

Background

1.1 Introduction to Microfluidics

Lab on a chip is a technology where in several large laboratory experiments are shrunk in a single micro chip. It is a part of the multidisciplinary field known as Microfluidics. The microfluidic chip, designed to handle minute amount of fluids is fabricated on silicon, glass or polymer interface. It takes minor amount of fluid and performs various operations on it like mixing, pumping and sorting etc. The construction, design and the system behavior differ from that of macro scale devices. Microfluidics techniques also have applications in places like laser ablation, blood-cell separation, drug screening, cell manipulation [1][2] , PCR amplification[3], [4] etc.

Cell manipulation can be achieved using various methods in microfluidics such as :

- 1) Acoustic method
- 2)Magnetic method
- 3) Electrokinetic method

Electrokinetic method is where the particle's electric properties such as dielectric constant, conductivity etc are considered to perform various operations on them[5].

The subdivisions of Electrokinetic methods[6] are as follows :

- a) Electro-osmosis
- b) Electrophoresis
- c) Induced charge electro-osmosis
- d) Dielectrophoresis

1.2 Introduction to Dielectrophoresis and Travelling Wave Dielectrophoresis

In dielectrophoresis process, when a dielectric particle is subjected to non-uniform electric field, dipolar moment is induced within the particle and the resultant force experienced by the particle is called as the DEP force. This resultant force depends on various factors such as, size of the particle, relative permittivity of the particle(ϵ_p) and of the medium of suspension(ϵ_m), conductivity of both(σ_p , σ_m) and the electric field applied(E)[6]–[8][9][10]. A derived term called Clausius-Mossotti factor (f_{CM}) is used for calculation of DEP force and for spherical particle it is represented as given in 1.1.

$$f_{CM} = \frac{\epsilon_p^* - \epsilon_m^*}{\epsilon_p^* + 2\epsilon_m^*} \quad --(1.1)$$

$$\text{Where, } \epsilon^* = \epsilon - i \frac{\sigma}{\omega} \text{ and } \omega = 2\pi f$$

The above equation is in the case of an isotropic particle. But for an anisotropic case the equation would be different. The anisotropic particle model is as shown in fig. 1 below,

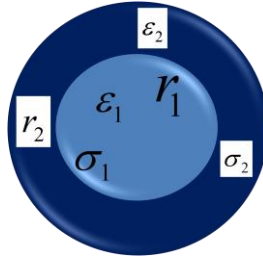


Figure-1: Anisotropic particle model

Where r_1 and r_2 are inner and outer radius respectively. Hence for such a model f_{CM} is given as follows,

$$f_{CM} = \frac{\epsilon_{eff}^* - \epsilon_m^*}{\epsilon_{eff}^* + 2\epsilon_m^*} \quad --(1.2)$$

Where, ϵ_{eff} is the overall dielectric constant of two shell module which is calculated as follows,

$$\varepsilon_{eff}^* = (\varepsilon_2^*) \frac{\left(\frac{r_2}{r_1}\right)^3 + 2\left(\frac{\varepsilon_1^* - \varepsilon_2^*}{\varepsilon_1^* + 2\varepsilon_2^*}\right)}{\left(\frac{r_2}{r_1}\right)^3 - \left(\frac{\varepsilon_1^* - \varepsilon_2^*}{\varepsilon_1^* + 2\varepsilon_2^*}\right)} \quad --(1.3)$$

The DEP force is given as follows,

$$Force_{DEP} = 2\pi a^3 \varepsilon_m \text{Re}(f_{CM}) \nabla |E|^2 \quad --(1.4)$$

Clausius-Mossotti factor plays an important role for calculating DEP force. Change in real part of Clausius-Mossotti factor changes the amount of force. Figure-2(a,b,c) shows how f_{CM} responds to frequency changes for an insulator, an isotropic particle and to an anisotropic particle. Which shows that isotropic particle has only single cross over frequency which indicates that f_{CM} changes its sign at that particular frequency hence the DEP force. And for anisotropic particle there are two cross over frequencies. We can also see that at very high or very low frequency values f_{CM} almost saturates due to dominance of real part and imaginary part respectively.

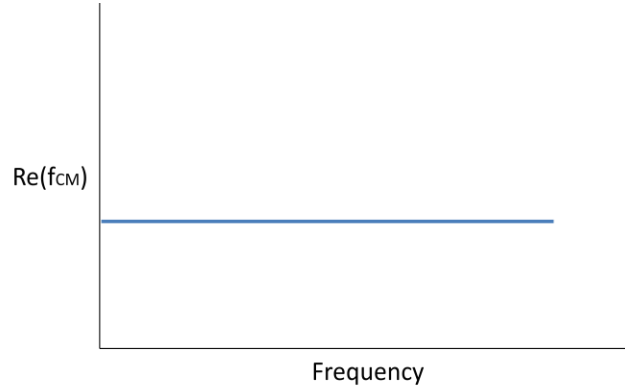


Figure 2(a): $\text{Re}(f_{CM})$ for an insulator

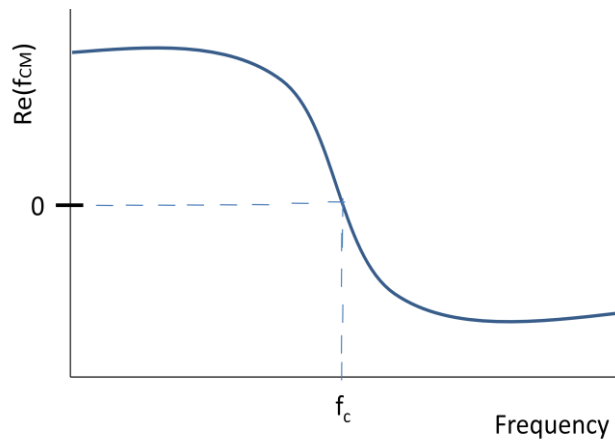


Figure 2(b): $\text{Re}(f_{CM})$ for isotropic particle

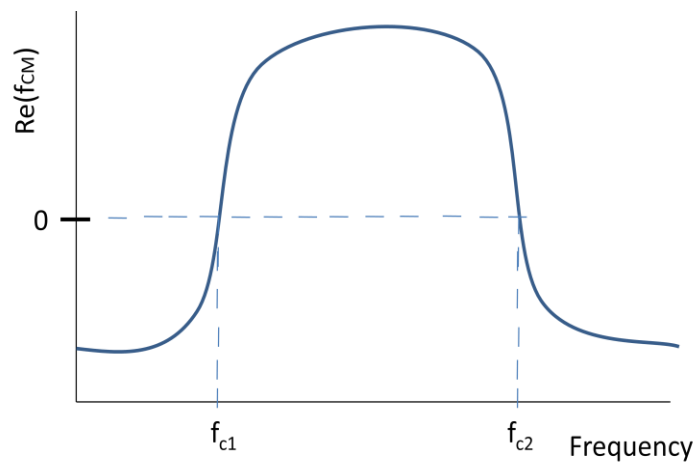


Fig. 2(c): $\text{Re}(f_{CM})$ for an anisotropic particle

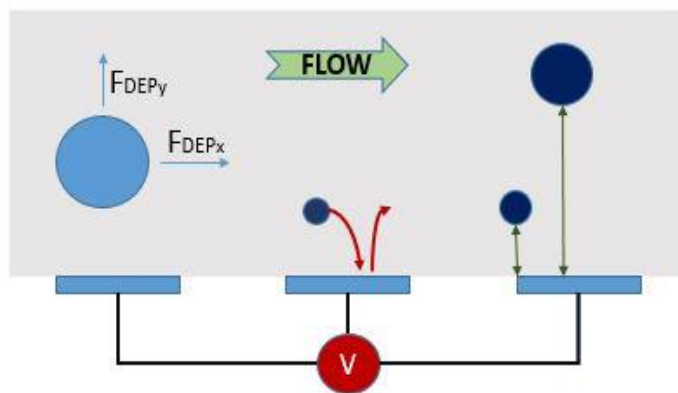


Fig. 3: DEP force in channel

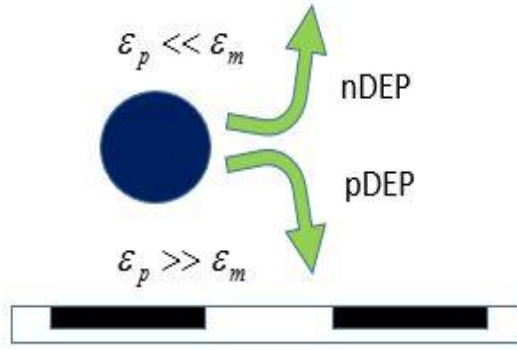


Fig. 4: Two types of DEP forces

As shown in above figure-3, when a non-uniform electric voltage is applied to the electrodes, depending on the size of the dielectric particle, levitation occurs and due to external force or flow force of medium(fluid) particles are carried forward. The particles are either attracted or repelled from electrodes depending on relative permittivity of particle and that of the fluid. If particle's relative permittivity is lesser than that of medium then particle gets repelled from electrodes and it is called as negative DEP (nDEP) and vice versa for positive DEP (pDEP) as per figure-4. In similar way if instead of DC non-uniform voltage, AC non-uniform voltage is applied then the electric field varies spatially as well as with respect to time. where phase difference (ϕ) plays an important role. This AC voltage then adds up to the normal DEP force and is called as travelling wave DEP(TwDEP)[8]. Hence, the total DEP force is given as follows,

$$F_{DEP(total)} = \pi a^3 \epsilon_m \text{Re}(f_{CM}) \nabla |E|^2 + 2\pi a^3 \epsilon_m \text{Im}(f_{CM}) [E_x^2 \nabla \phi_x + E_y^2 \nabla \phi_y + E_z^2 \nabla \phi_z] \quad --(1.5)$$

1.3 Introduction to COMSOL

For designing and simulating the microfluidics chip, COMSOL Multiphysics software is a helpful tool[11], [12]. Based on advanced numerical method, COMSOL is a platform which is used to simulate or/and design physics based problems. As the name suggests 'Multiphysics' it is also used for coupled phenomena[11], [13]. AC/DC,CFD, Heat Transfer, Fluid Flow are few examples of modules in COMSOL. Following are the features of COMSOL :

- Simulation is done virtually for physics based problems.

- In all the modules we can get various models for reference.
- Coupling of two modules is possible. In few new versions of COMSOL, some of the modules have in-built coupling.
- User defined functions and boundary conditions are possible in COMSOL.
- There are more than thirty add-ons available.
- There is a facility to include our own equations.
- Along with built-in interfaces to Matlab and ECAD etc. there is a provision for user-defined interfaces.

1.3.1 Steps to form a basic template in COMSOL

In order to start with the new project in COMSOL, the following steps can be followed

- Select the model wizard option or a blank model.
- If model wizard is chosen then select space dimension to be worked in i.e; either 0D, 1D, 2D or 3D with or without axis symmetry.
- Required physics should be selected once space dimension is chosen. More than one physics can be added at a time.
- After Physics is added, go to Study and select the required one. Available Study options are Stationary, Time dependent, Frequency Domain, Small Signal Analysis, Eigen Value and Eigen Frequency etc. Once Study is selected the basic template is formed in tree format where geometry can be formed.

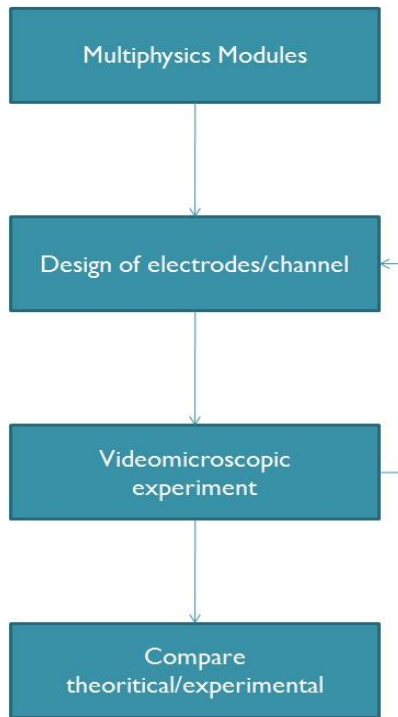


Figure 5: Approach to design microfluidic chip

After forming the basic template, forming the required geometry and assigning the required condition, simulate the model for results. Once the results match to the expectations then we can proceed for designing it practically. The designed chip then can be used to test its functionality. If its functionality is correct then the theoretical and practical results are tallied or else we need to design it again practically. Hence this overall design and testing flow can be shown in fig. 5.

Chapter 2

Literature survey

2.1 Submicron particle trapping using Travelling Wave Dielectrophoresis

Suspended in different viscous and dense fluid media, submicron particles are analyzed in this paper by utilizing the combination of dielectrophoresis (DEP) travelling wave dielectrophoresis (TwDEP) technique[7]. Finite element method is used for numerical simulation to get the results and FreeFEM++ is used for simulation[14]. The geometry consists of a rectangular chamber with an array of parallel and planar electrodes. For analysis, nDEP is considered where DEP force is used to concentrate the particles in the median plane and TwDEP is used as a drag force. Dimensionless form of Laplace equation for potential is solved for the case where the width of all electrodes, distance between them, characteristics length and height of the chamber are all equal to $50\mu\text{m}$. A particle of diameter $200\mu\text{m}$ is used in water. The analysis shows how an optimized nano-scale device can be designed and also helps us understanding physics and DEP behavior. It also shows how by varying electric field and frequency, the particle trajectory can be controlled which is a tough job to do in nano scale devices. Such devices are useful in enhancing performance of filtering devices, extraction from fluid waste etc.

2.2 3D Dielectrophoretic chips : Trapping and separation of cell population

The given paper presents the experimental results of trapping yeast cells and method for separation of two cell population for two different 3D electrode structures[15]. The 3D electrode structures considered are DEP chip with 3D silicon electrodes and DEP chip with asymmetric electrodes[16]. The structure of the device is such that a conductive layer of silicon is sandwiched between two layers of glass wafer. The intermediate layer forms the microfluidic channel and two DEP

electrodes. These electrodes are then connected through via holes to metallization layer on bottom glass surface. In case of asymmetric electrode structure, a special configuration of electrodes which is bulk silicon electrode and a thin electrode is used so that vertically an asymmetric distribution of the electric field is achieved. The experiment is performed on yeast cells.

In the first configuration of 3D electrodes, drive signal is a sinusoidal wave from function generator which was increased from 0V to 25V gradually with frequency in between 20kHz to 100kHz. At 13V P-P cells started experiencing pDEP force and as the voltage increased cell started moving faster and at 25V a stable equilibrium concentration pattern is seen for 10 seconds[15].

Where as in the second configuration the same testing methodologies is used and it is observed that more force is experienced at 3D electrode than that of planar at low distances from the floor of channel. This force decreases as the distance increases.

2.3 Microfluidic pumping based on Travelling Wave Dielectrophoresis

Here the microfluidic pumping is carried using travelling wave dielectrophoresis process. Numerical and experimental results are then compared. Electrodes used have width and uniform spacing of 20 μ m and 180 μ m respectively and are 90mm long[17]. Between the gaps of electrodes and to the wall, Neumann boundary condition is applied. Whereas for input and output periodic boundary is condition is considered. Numerical result shows distribution of the electric field as well as DEP force. It also shows how hydrodynamic interaction between neighboring particles can enhance the induced flow and how velocity depends on particle concentration. FLUENT software package is used for simulation. Whereas in experimental result shows that at lower frequency pDEP occurs and at higher frequency nDEP is experienced by particles[18]. The experimental setup has array of interdigitated electrodes of 100nm thickness. There are 10 electrodes of 20 μ m width and 180 μ m gap between each of them. The electrodes are covered by a thin layer of parylene C to avoid corrosion and electrolysis. Applied voltage is varied from 10V to 30V and

frequency between 1 to 1000kHz. The comparison of both the results shows the measurement uncertainty in flow velocity is $5.14\mu\text{m/s}$.

2.4 Large area Travelling Wave Dielectrophoresis particle separator

This paper shows how using multilayering technique 100 different microelectrode are designed constructed and how TWD technique separates bio-particle quickly and with better resolution. Each electrode in the array is $10\mu\text{m}$ wide and in single layer there are 10 electrodes each connected to a bonding pad[19]. Components of whole rabbit blood is separated using this set-up. When a four phase signal of 10V peak to peak with frequency of 500kHz is applied it shows the difference between velocity of blood component. Ultimately the result shows mean velocity of $32\mu\text{m/s}$ and $20\mu\text{m/s}$ for erythrocytes and leukocytes respectively. These two particles were separated in two bands with a path difference of 7.5mm. But, in case of purposes like diagnostic where small amount must be separated in small bands which is possible using TWD arrays as the efficiency of separation depends on the length of the array.

2.5 Counterflow Dielectrophoresis for trypanosome enrichment and detection in blood

Sleeping sickness is caused by a single-celled protozoan parasites. In order to separate them from blood dielectrophoresis technique is used in the given paper[20]. In this the cell counterflow technique is used, that is, opposed bidirectional movement is used to separate Trypanosomes from blood. The electrodes used here are four arm spiral microelectrode array with each electrode phase shifted by 90° [21]. When one type of cell gets repelled from array the other type of cell gets attracted and concentrates at the centre of the array. The blood cells experience nDEP force and hence gets repelled from electrodes whereas the larger cells including trypanosomes experience pDEP force therefore gets attracted towards them. The four equally spaced electrodes have width and inter electrode spacing of $30\mu\text{m}$ and thickness of 200nm. The total width of spiral electrode is 2.9mm.

The above mentioned papers along with few more other papers have been studied for the project purpose such that TwDEP method can be used in microfluidic chip and the numerical simulation can be performed on it.

Chapter 3

Project Modeling

3.1 Introduction

Cryptosporidium are one of the protozoan parasites. These parasites produce oocysts in their life cycle whose minor quantity in human body can lead to diarrhea[22], [23] . Hence it is needed to have a technique to identify and manipulate them. This can be achieved using microfluidics chip. Using dielectrophoresis (DEP) and travelling wave dielectrophoresis (TWDEP) manipulation of cryptosporidium cell is possible[24]. This project is one such model where the simulation is performed to achieve the aggregation of Cryptosporidium cells concentrated in bulk at various places in given channel. This concentration of Cryptosporidium is in response to the DEP and TWDEP force exerted on them.

3.2 Working principle

A microfluidic channel having rectangular electrodes at its bottom surface, is excited with AC voltage such that channel experiences non-uniform electric field. When Cryptosporidium along with de-ionized water as the media is passed through it, due to exposure to electric field the dipole is formed on the outer surface and because of non-uniformity in the field cells experience force which is the total DEP force(DEP+TWDEP)given in equation 1.5[2]. There is a contrast between electric properties of cell and the medium which is proportional to the DEP force experienced by cells. Since they are almost spherical in shape the given equation is used to calculate its Clausius-Mossotti factor. The size of oocysts is generally 2-5 μm [25].

3.3 COMSOL modules and governing equations

Simulation of the project is done using COMSOL multiphysics software. For concentration profile, Matlab linked with COMSOL is used. As we mentioned in chapter 1, there are many modules available in COMSOL. Out of which Electric Current module, Creeping Flow module, Particle Trajectory module and Co-efficient form PDE module are used in our project. As mentioned in chapter-1, the total DEP force depends on dielectric constant of medium and cell, conductivity of medium and cell, size of the cell gets affected due to the velocity profile of media. Therefore if we wish to see concentration of Cryptosporidium in a channel we need to consider all factors. Laplace equation, Navier-Stokes equation and Mass transport equation are the three governing equations in this project.

When Electric current module is computed, COMSOL solves Laplace equation to give electric field value(E) for given exciting voltage (ϕ). From electric field value then gradient of square of electric field can be calculated($\nabla|E|^2$) which is useful in calculating total DEP force on Cryptosporidium[26]. The Laplace equation is given [27] in equation 3.1,

$$-\nabla\phi = E \quad \text{--(3.1)}$$

In case of only DEP force Electric field module is solved as a steady state/Stationary problem but in case of TWDEP it is solved as a Time Dependent problem. For only DEP force the exciting voltage is non-uniform DC voltage whereas in the case of TWDEP it is non-uniform alternating voltage (successive electrodes are given phase shifts) such that a travelling wave is formed along the length of the channel.

Creeping flow module is studied for steady state. The velocity profile of the medium(u) is calculated by COMSOL by solving the Navier-Stokes equation. This depends on the factors like density of medium(ρ), Dynamic viscosity of medium(μ), pressure(P) and initial inlet velocity of the fluid(u_0). The Navier-Stokes equation is given [28] as shown in eq. 3.2

$$\rho\left(\frac{\partial\vec{u}}{\partial t} + (\nabla\cdot\vec{u})\vec{u}\right) = -\nabla P + \mu\nabla^2\vec{u} \quad \text{--(3.2)}$$

First term in equation 3.2 on LHS is a Transient term which leads to time dependent behavior of velocity. The second term in LHS is Inertial term. On RHS the terms are Pressure term and viscous term respectively. In case of creeping flow module where velocity values are in micron range and Reynolds' number is lesser than 0.1, the viscous term is neglected.

In Co-efficient form PDE module we solve Mass transport equation to get the concentration of Cryptosporidium cells(C). This equation is given as given [26] in equation 3.3 ,

$$\frac{\partial C}{\partial t} + \nabla \cdot (-D \nabla C + \vec{v} \cdot C + C(u F_{DEP})) = 0 \quad --(3.3)$$

First term in the above equation (eq. 3.3) is transient. The second one is a diffusive term which shows the effect of diffusion on the concentration of cells. Diffusion coefficient (D) contributes to the diffusive term which is a function of particle mobility (u), absolute temperature (T) and Boltzmann constant (k_b). Third term is a convective term where vector v is the velocity profile obtained by solving Navier-Stokes equation from Creeping flow module. And the last term is for external force on cells which in this case is the total DEP force(F_{DEP}) and (u) is particle mobility which depends on cell size (a) and dynamic viscosity of the medium(μ_m). Diffusion constant (D) and particle mobility (u) is given by the following equation (3.4)[26],

$$D = u K_b T \quad --(3.4)$$

$$u = \frac{1}{6\pi a \mu_b} \quad --(3.5)$$

Whereas in our case we have considered steady state Mass transport equation for an interdigitated electrode structure for which gradient of electric field is given as equation (3.6)[29],

$$\nabla E_y^2 = -\frac{\pi}{d} \left(V \frac{\pi}{d}\right)^2 \cdot \frac{1}{k(\cos(\frac{c}{2}))^2} \cdot e^{-\frac{x}{d}} \quad --(3.6)$$

Where $c = \frac{\pi d_1}{2d}$ and $d = \frac{d_1 + d_2}{2}$

Where d_1 is width of the electrode, d_2 is spacing between electrodes and V is applied voltage.

For solving Mass transport equation using above formula we have used Matlab linked with COMSOL to get the concentration profile. Therefore we can see that coupling of modules is done in order to get the concentration of Cryptosporidium. Particle Trajectory module is used to see visually how an individual cell or a group of cells are moving in a channel under the influence of multiple forces acting upon it. In this case we have DEP force acting. Hence the overall multiphysics flow can be represented as,

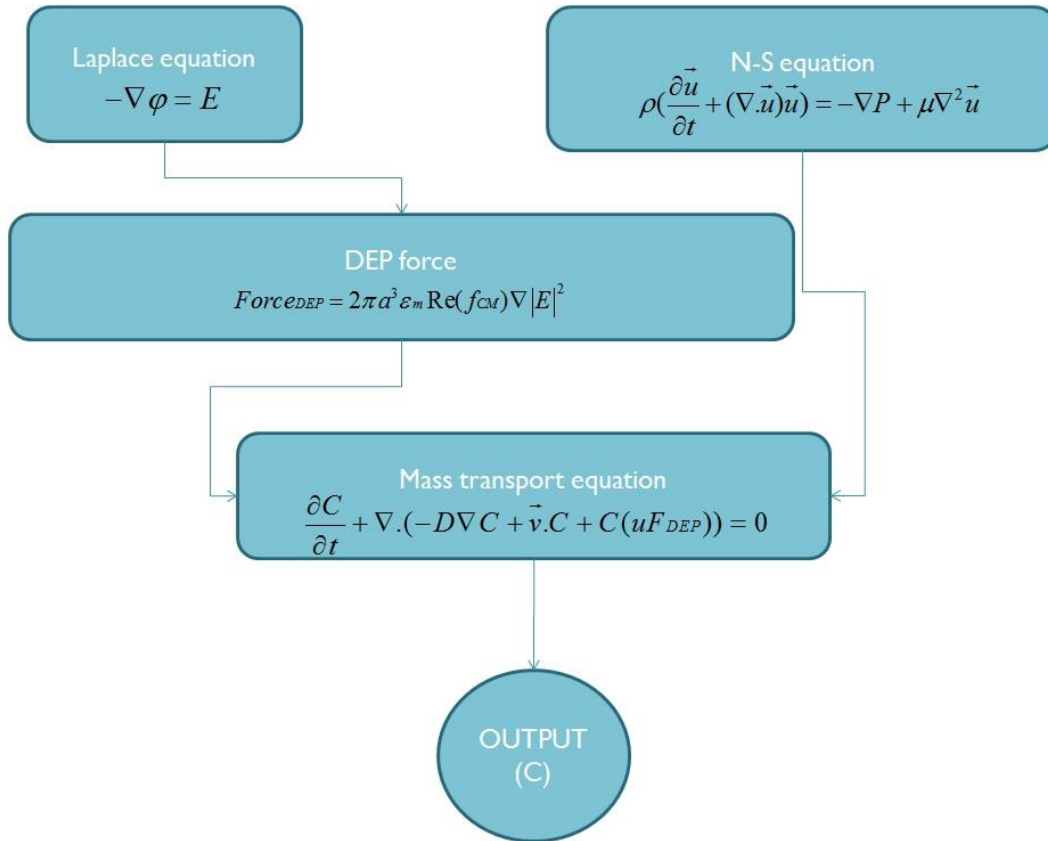


Figure 6: Flow chart for simulation

As shown in fig. 6, by solving Laplace equation the resultant electric field value is used for calculating DEP force which then is inserted as external force quantity in mass transport equation. This is a part where either direct DEP force value or electric field value is inserted in co-efficient form PDE by mentioning the particular name of that quantity given by COMSOL(for example, electric field in x-direction in Electric Current module is mentioned as ec.Ex). Similar way once the creeping flow module is simulated the resultant velocity profile (spf.U) is used in mass transport equation. Hence the value of concentration obtained by solving the mass transport equation coupled with Navier-Stokes and Laplace equation, is the required output(C).

Chapter 4

Simulations and Results

4.1 Matlab plots

Apart from COMSOL, Matlab is used to plot real part of Clausius-Mossotti factor against frequency and the DEP force against frequency shown in figure-19(a,b) and figure-20(a,b) respectively. It is plotted considering DI-water as medium and Phosphate buffered saline(PBS) as medium second time.

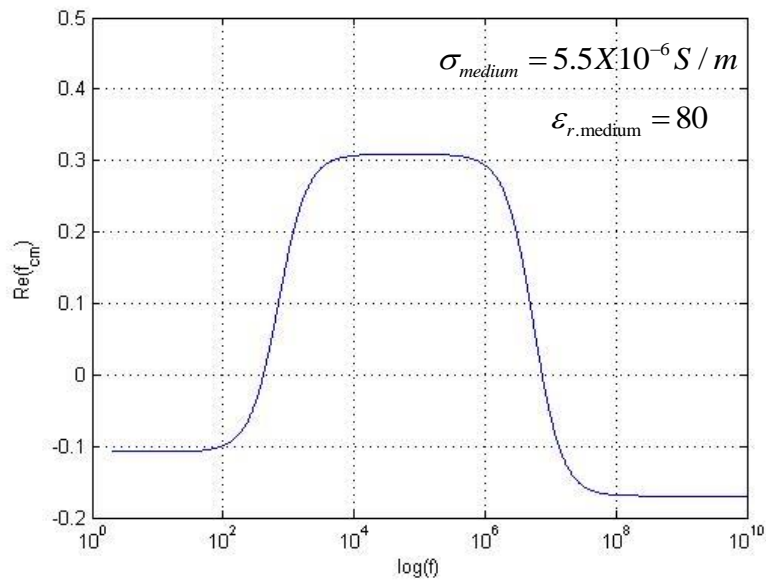


Figure 7(a): $\text{Re}(f_{\text{CM}})$ for cryptosporidium with water as a medium

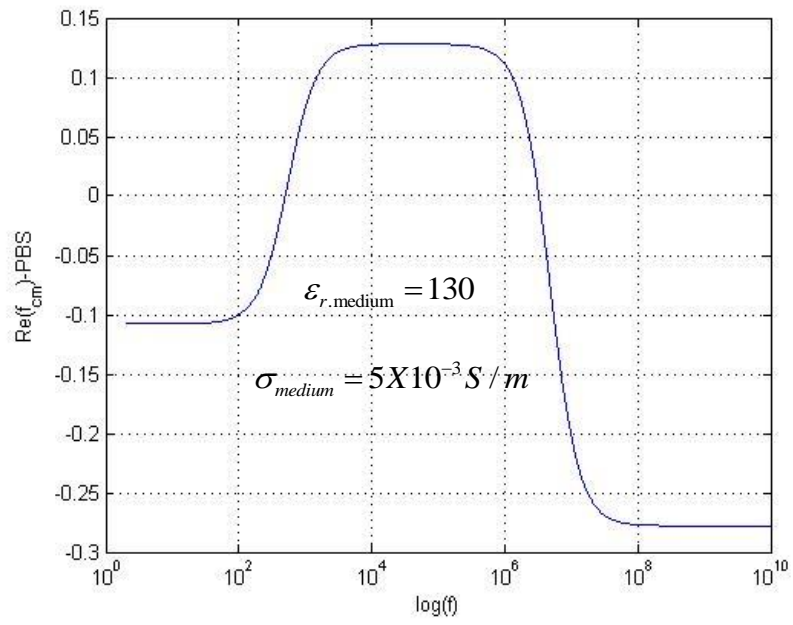


Figure 7(b): $\text{Re}(f_{CM})$ for cryptosporidium with PBS as a medium

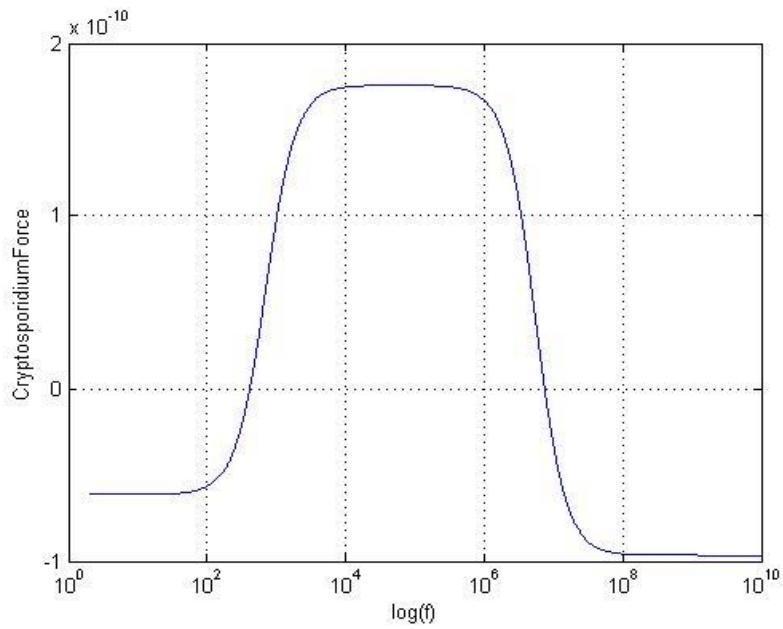


Figure 8(a): F_{DEP} for cryptosporidium with water as a medium

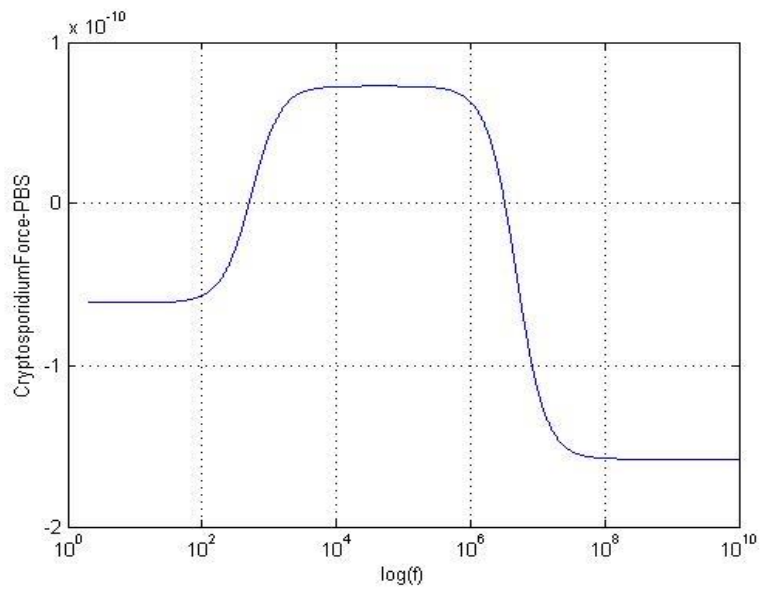


Figure 8(b): F_{DEP} for cryptosporidium with PBS as a medium

As you can see from the graphs, at different frequencies for PBS and DI water, the DEP force becomes zero. For PBS medium the value of cross over frequencies are almost 300Hz and 3MHz where as for DI water medium the values are almost 400Hz and 3.6MHz.

4.2 Geometry in COMSOL-2D and 3D

A rectangular channel with 4 rectangular electrodes at the bottom surface is created in COMSOL. The geometry is as shown in fig. 9& fig. 10 and the dimensions of both are as mentioned in table-1



Figure 9: COMSOL 2D design(channel and electrodes)

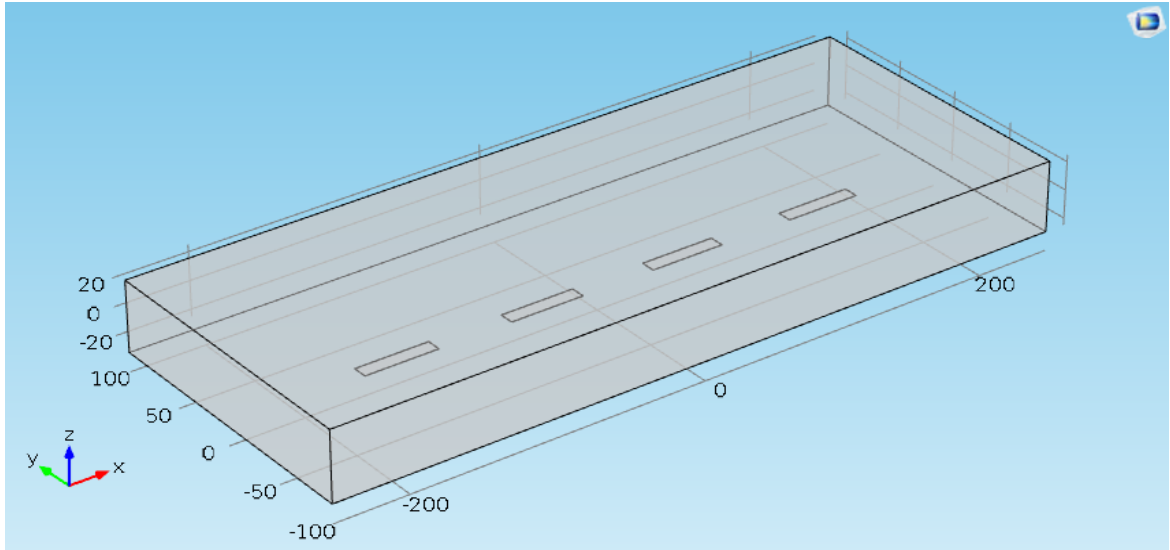


Figure 10: COMSOL 3D design(channel and electrodes)

Table -1 : Dimensions of channel and electrode

Frequency	3MHz
Height of channel	50 μ m
Length of channel	500 μ m
Width of channel(Only for 3D)	200 μ m
Width of the electrode	10 μ m
Height of the electrode	200nm
Length of the electrode	50 μ m
Spacing between electrodes	50 μ m

4.3 Meshing

In order for COMSOL to solve the given equations for a given geometry by finite element method, it is necessary to mesh the given geometry according to our need. In our case it is required to have dense mesh at electrodes due to availability of more electric field in the nearby areas hence as shown in fig.11(a,b) free triangular mesh is used for geometry for 2-D and for 3-D free tetrahedral is used. It is also clearly visible that electrode areas are highly dense compared to other places. Specially in the case of 3-D structure where height of the electrodes is very small it is followed that to get appropriate solution the corners of such small geometry is meshed dense.

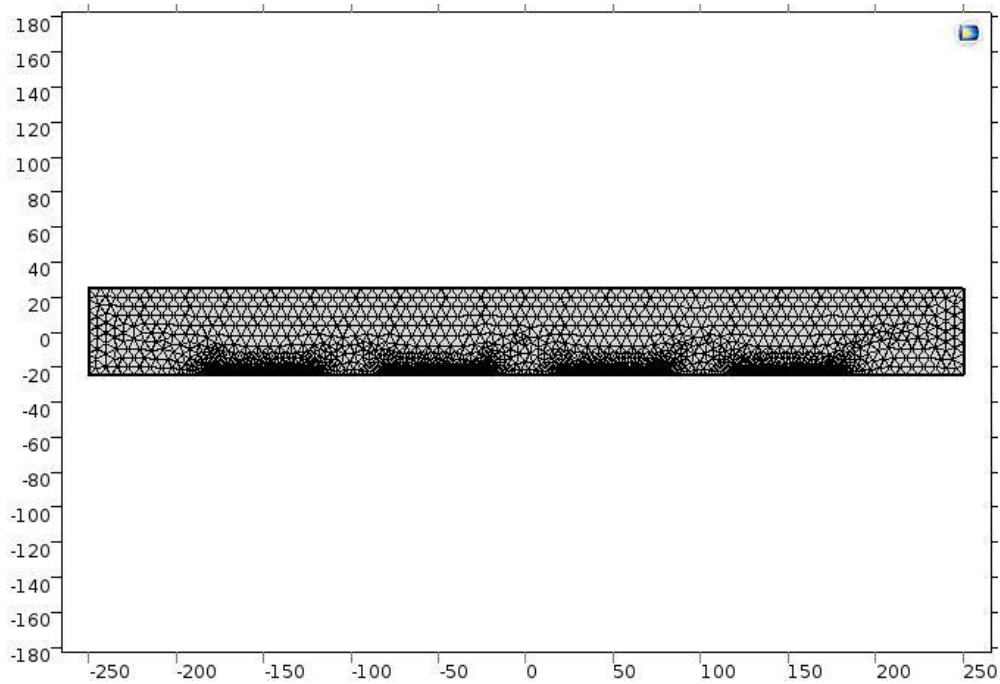


Figure 11(a) : 2-D free triangular meshing

(Shows the variation in meshing density. It is dense near electrode regions)

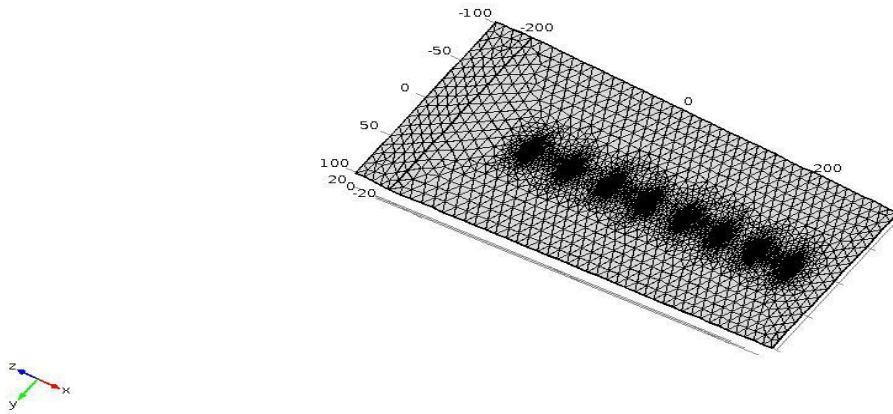


Figure 11(b): 3-D free tetrahedral meshing
(Dense near the corner regions of electrodes)

4.4 COMSOL modules (simulations)

In Electric Current module, all the four electrodes are provided with AC voltage with 90° phase shift at a frequency (f) of 3MHz as shown in fig. 12

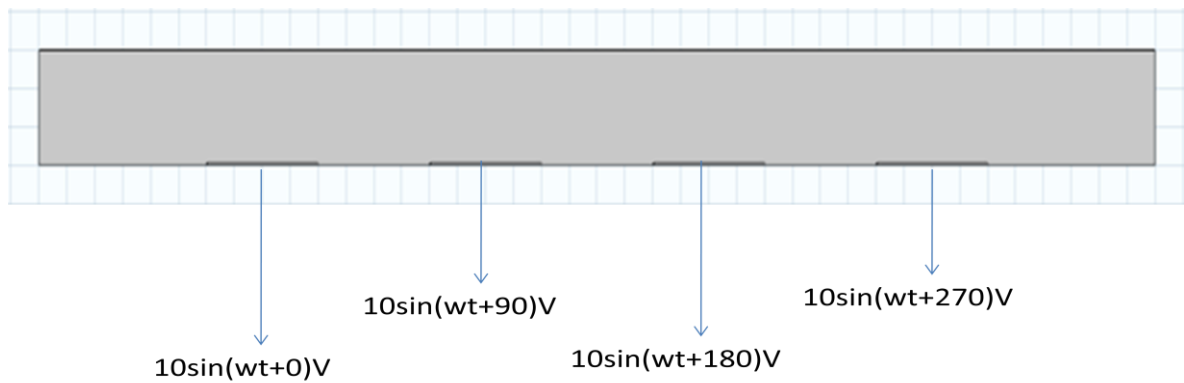


Figure 12: Voltage at each electrode

After solving it as a time dependent module we can see potential variation in channel which looks like a wave travelling along the length as shown in figure-13(a,b,c,d). In similar way we can see varying electric field in figure-14(a,b,c,d). Since the variation in the value of $\nabla|E|^2$ is negligible, this change can be seen after

zooming in nearby one of the electrodes area. Hence we can see this change near the edge of the second electrode in figure -15(a,b,c,d) . Due to smaller variation in gradient of electric field and as the constant parameters in calculating total DEP force has very lesser value, the total DEP force is in the order of 10^{-9} N. Since even this variation in force is invisible in total channel, the zoomed version near the edge of second electrode is shown in Figure -16(a,b,c,d). For the calculation of DEP force Cryptosporidium properties as mentioned in table - 2 are used [8].

Table-2: Properties of Cryptosporidium

Size of the particle	2 μ m
Cytoplasm Relative permittivity	61.35
cell membrane relative permittivity	9.84
Medium relative permittivity	80
Conductivity of cytoplasm	0.047 S/m
Conductivity of membrane	186x10 ⁻⁹ S/m
Conductivity of medium	5x10 ⁻⁶ S/m
ϵ_0	8.85x10 ⁻¹² F/m

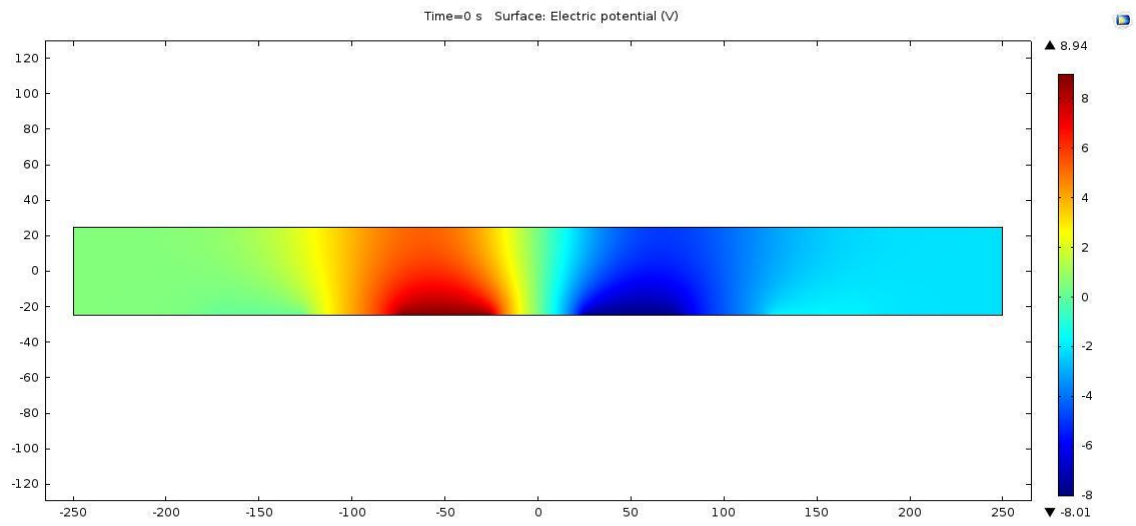


Figure 13(a): Electric potential at $t=0$ s

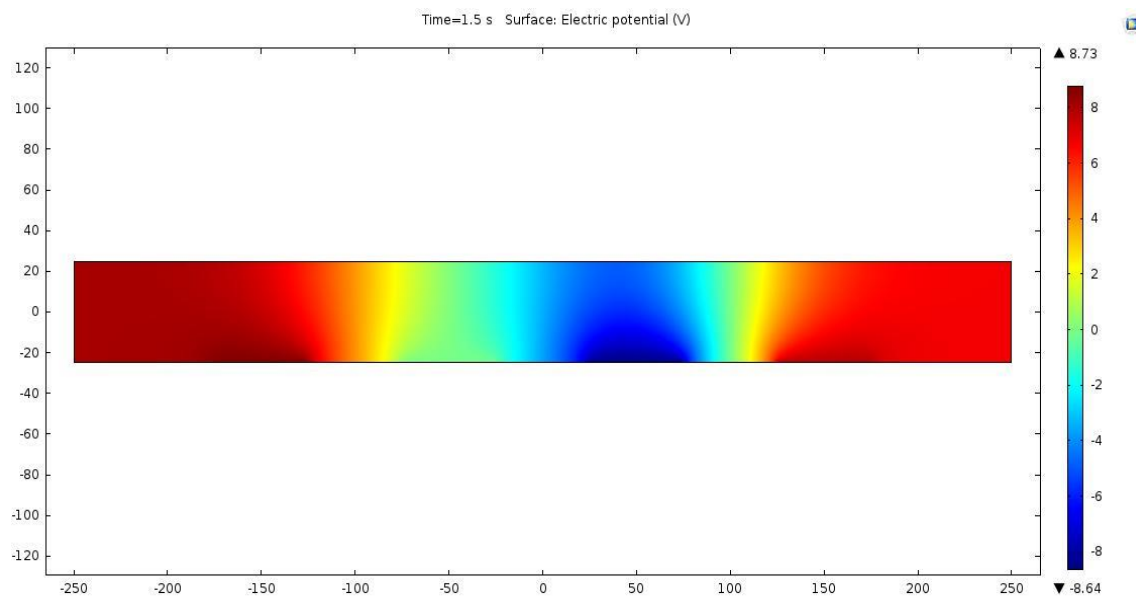


Figure 13(b): Electric potential at $t=1.5$ s

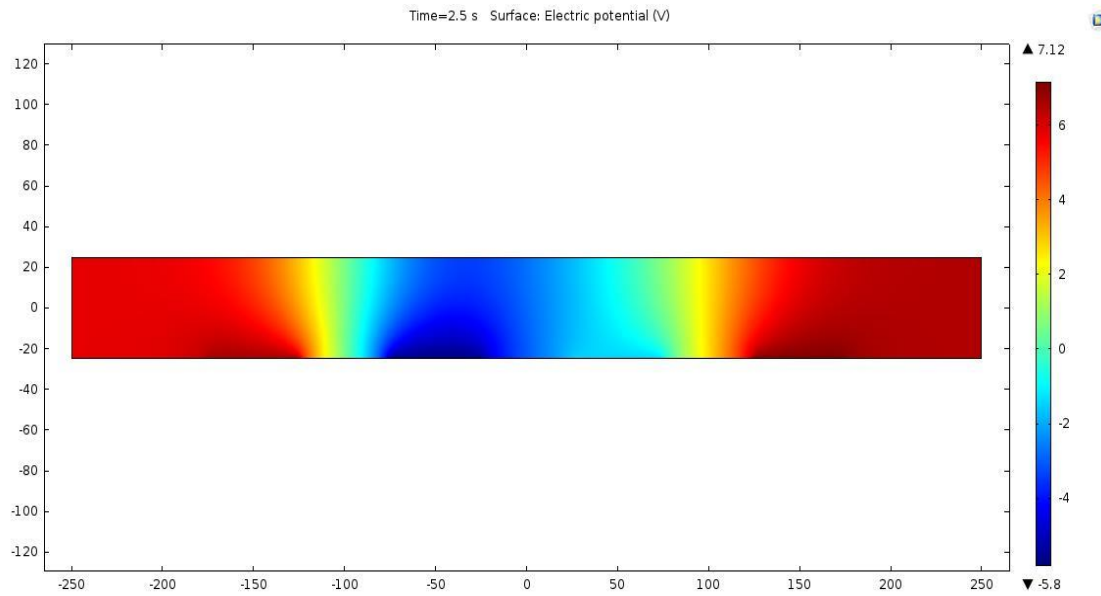


Figure 13(c): Electric potential at $t=2.5$ s

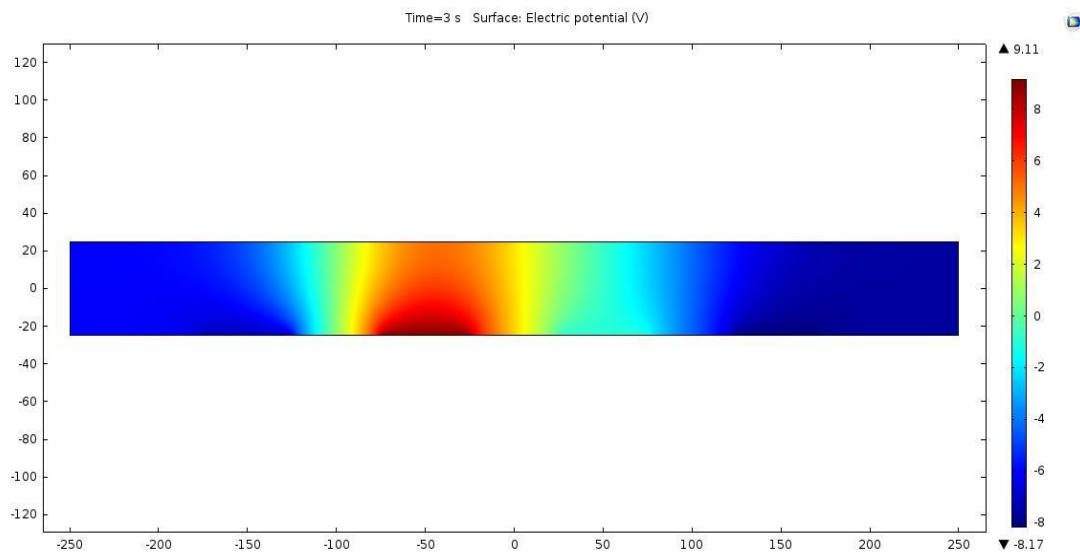


Figure -13(d): Electric potential at $t=3$ s

From electric potential figure as shown in fig. 13(a,b,c,d) we can visually see how wave looks like travelling towards the right side in the channel. Due to phase difference there are different voltages at each electrode at a given time which then changes with respect to time.

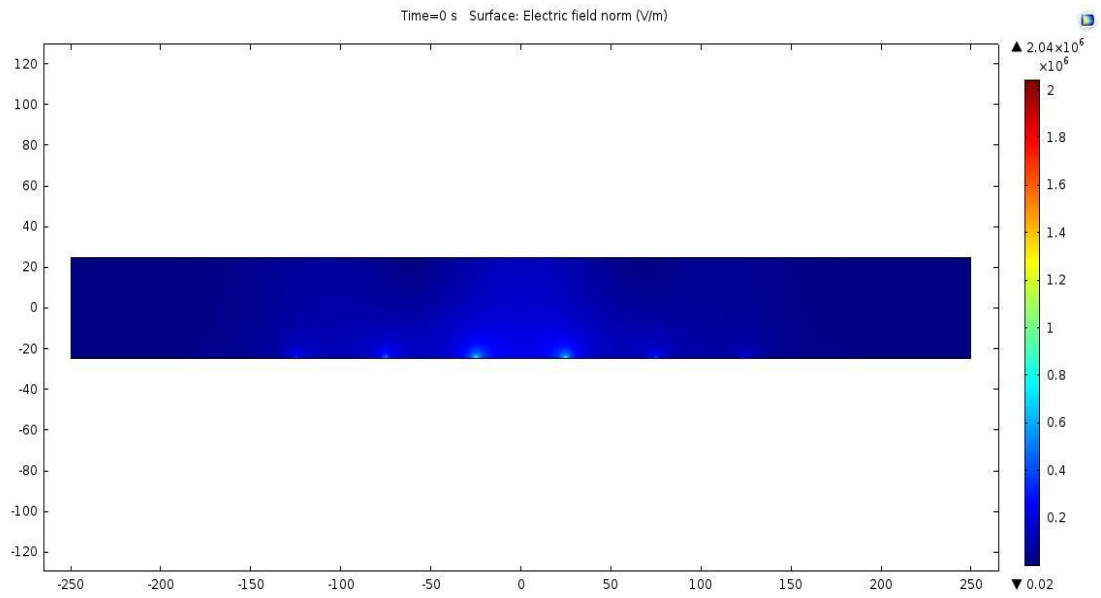


Figure 14(a): Normalized electric field at $t=0$ s

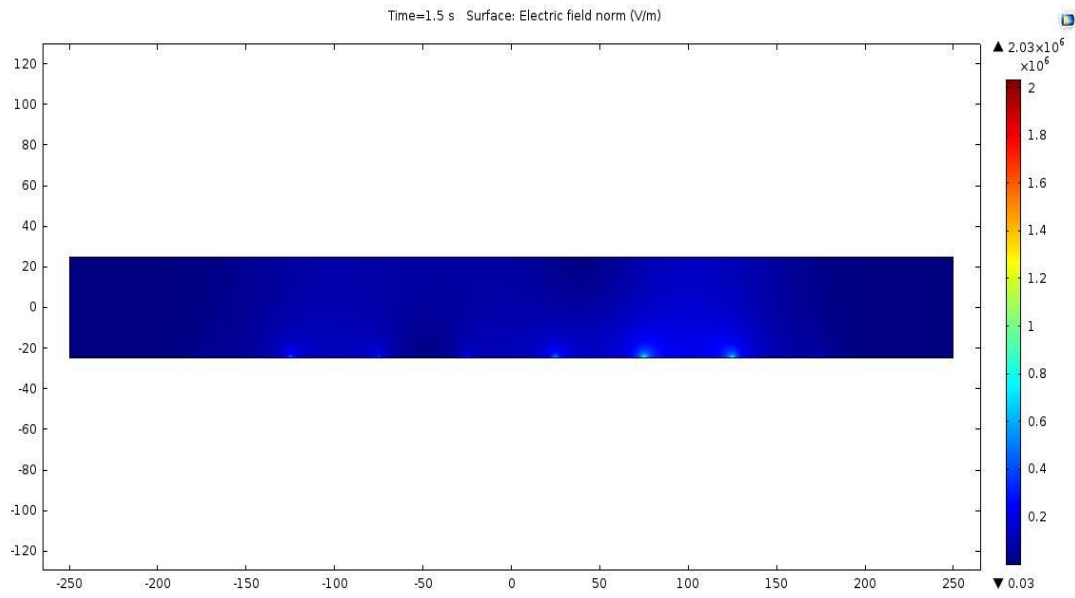


Figure 14(b): Normalized electric field at $t=1.5$ s

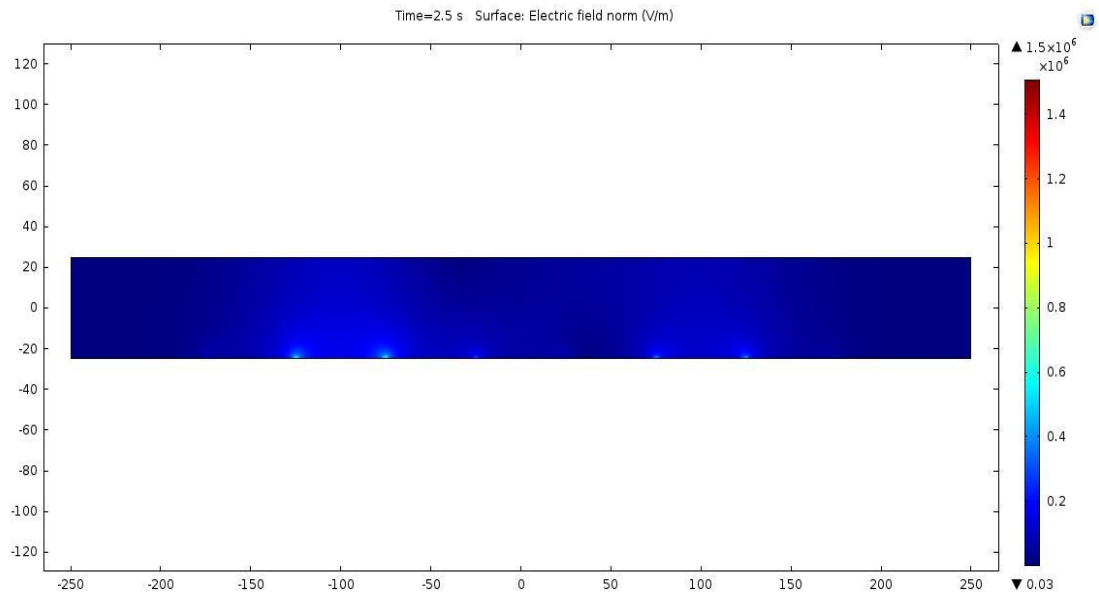


Figure 14(c): Normalized electric field at $t=2.5$ s

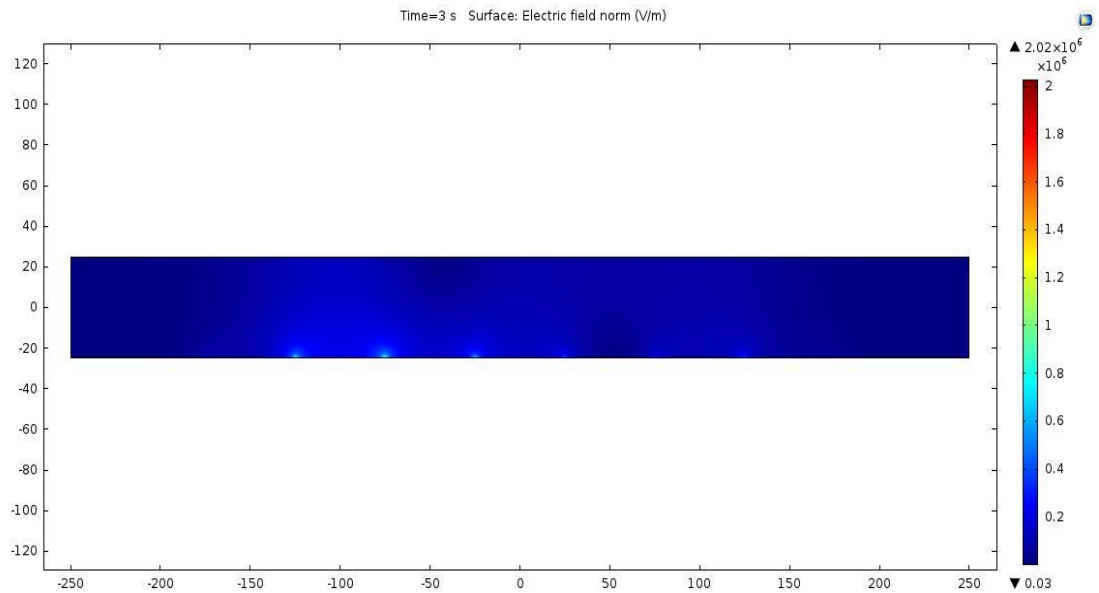


Figure 14(d): Normalized electric field at $t=3$ s

Electric field images (as in Fig. 14(a,b,c,d)) makes it clear that the field is strongest near the edges of the electrodes therefore small bright variation is visible near the electrode ending regions. Due to this we can see maximum values of gradient of square of electric field and DEP force near the electrode edges as shown in Fig. 15(a,b,c,d) and Fig. 16(a,b,c,d)

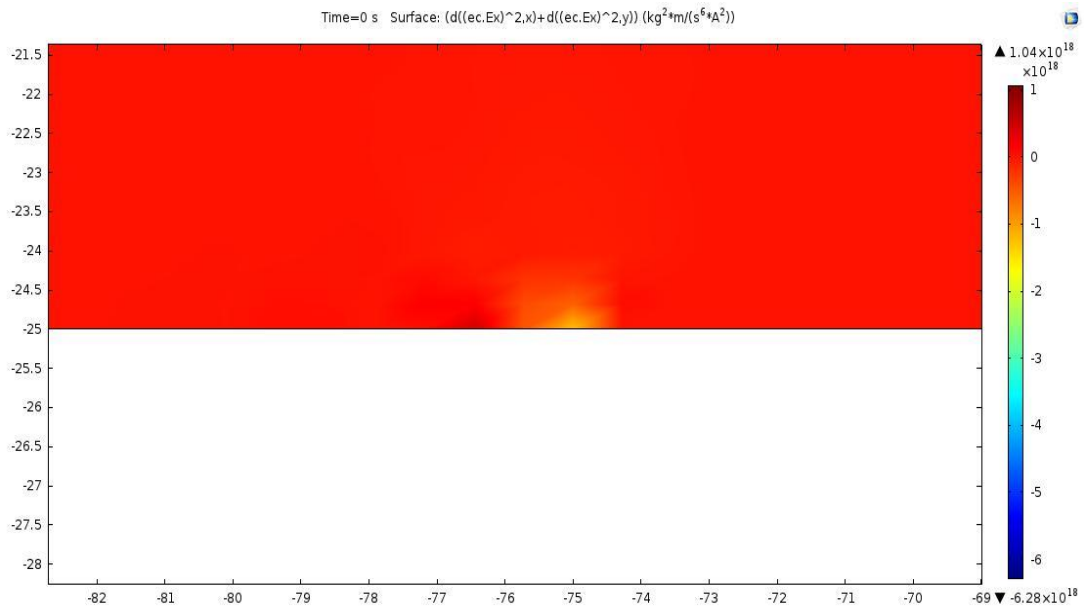


Figure 15(a): $\nabla|E|^2$ at $t=0$ s

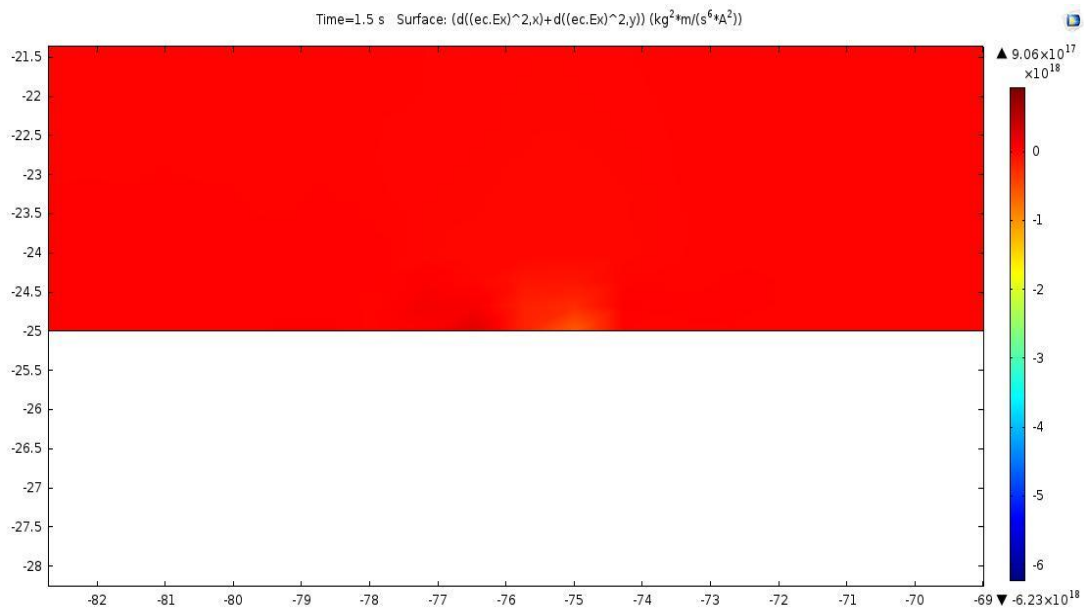


Figure 15(b): $\nabla|E|^2$ at $t=1.5$ s

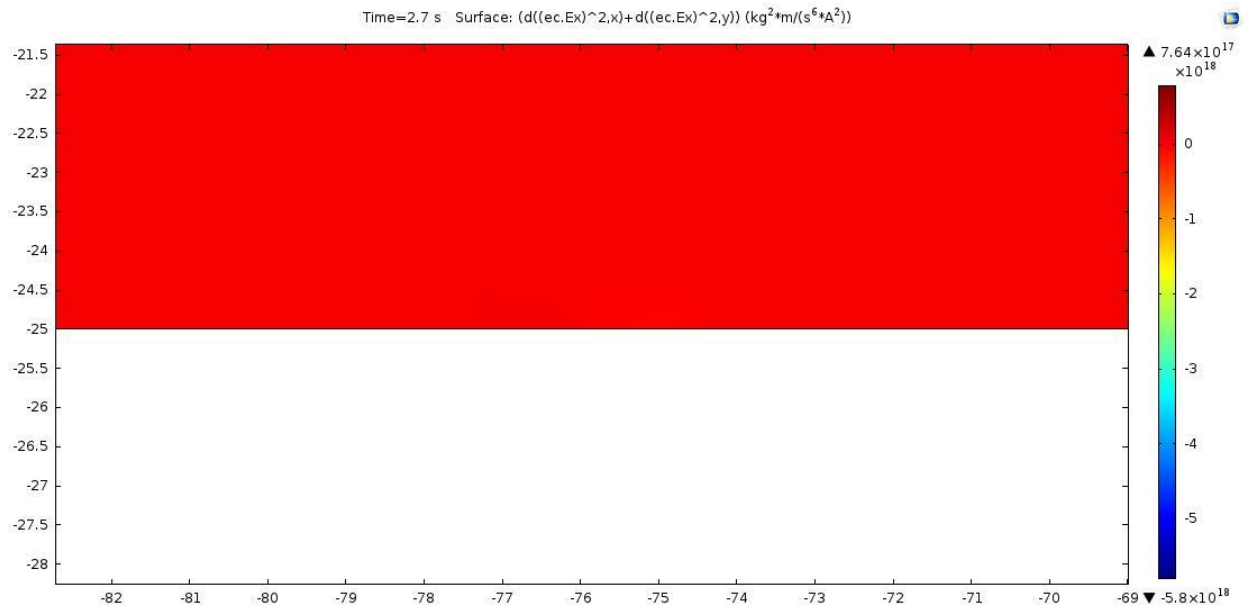


Figure 15(c): $\nabla|E|^2$ at $t=2.7s$

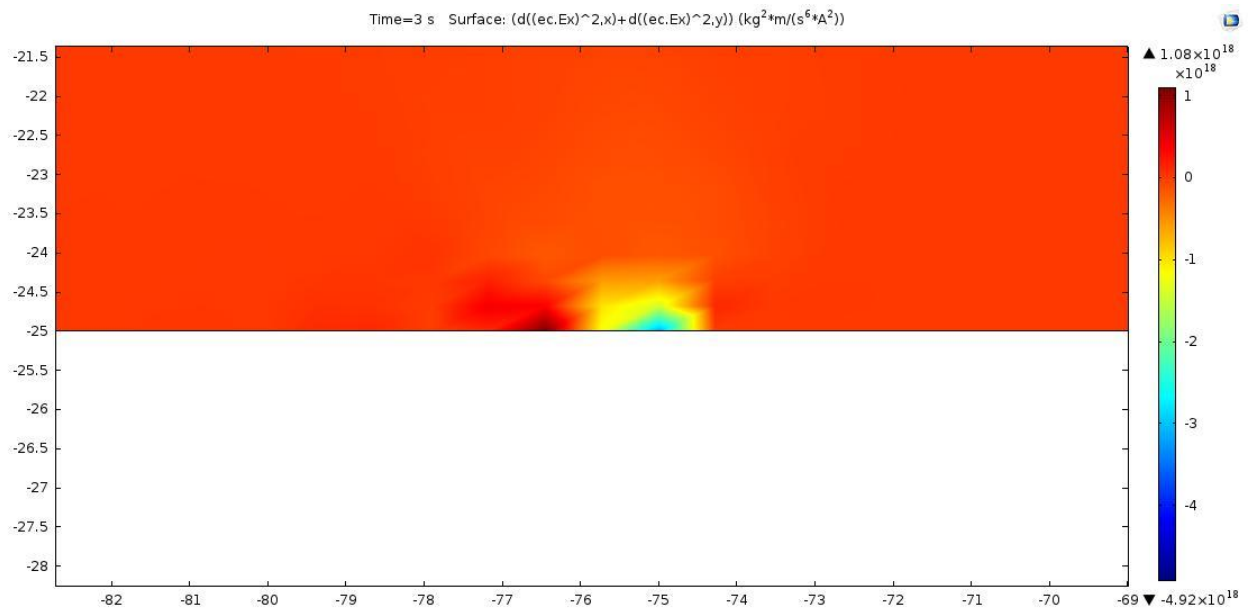


Figure 15(d): $\nabla|E|^2$ at $t=3s$

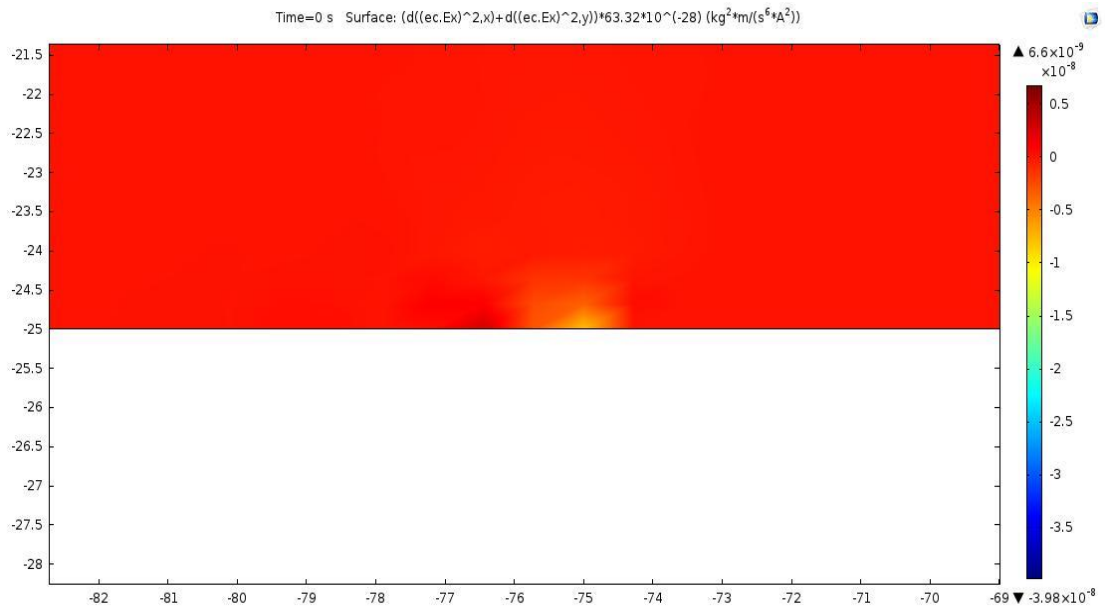


Figure 16(a): F_{DEP} at $t=0$ s

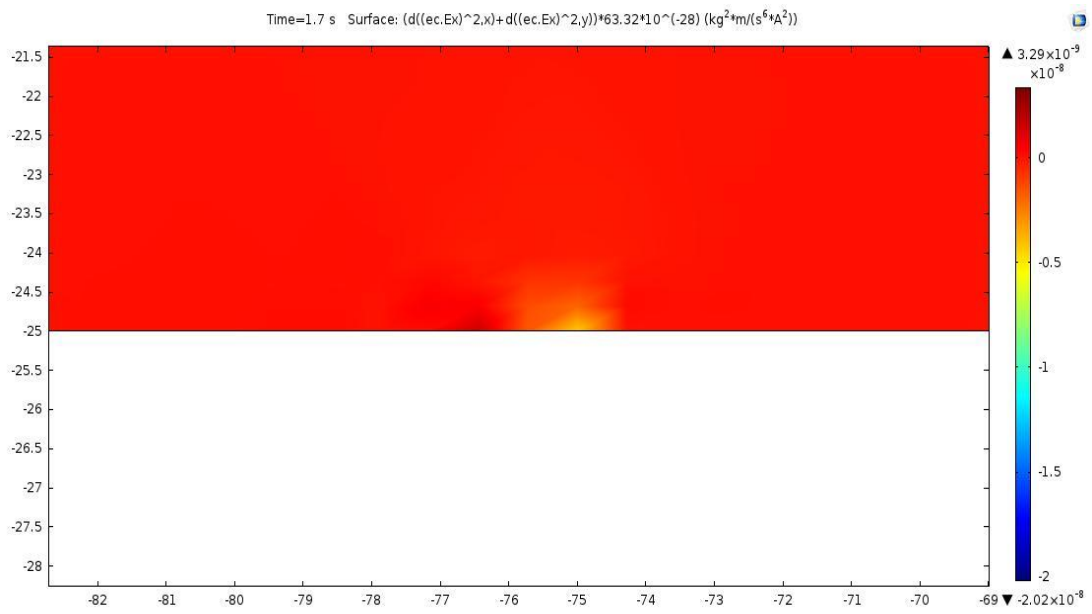


Figure 16(b): F_{DEP} at $t=1.7$ s

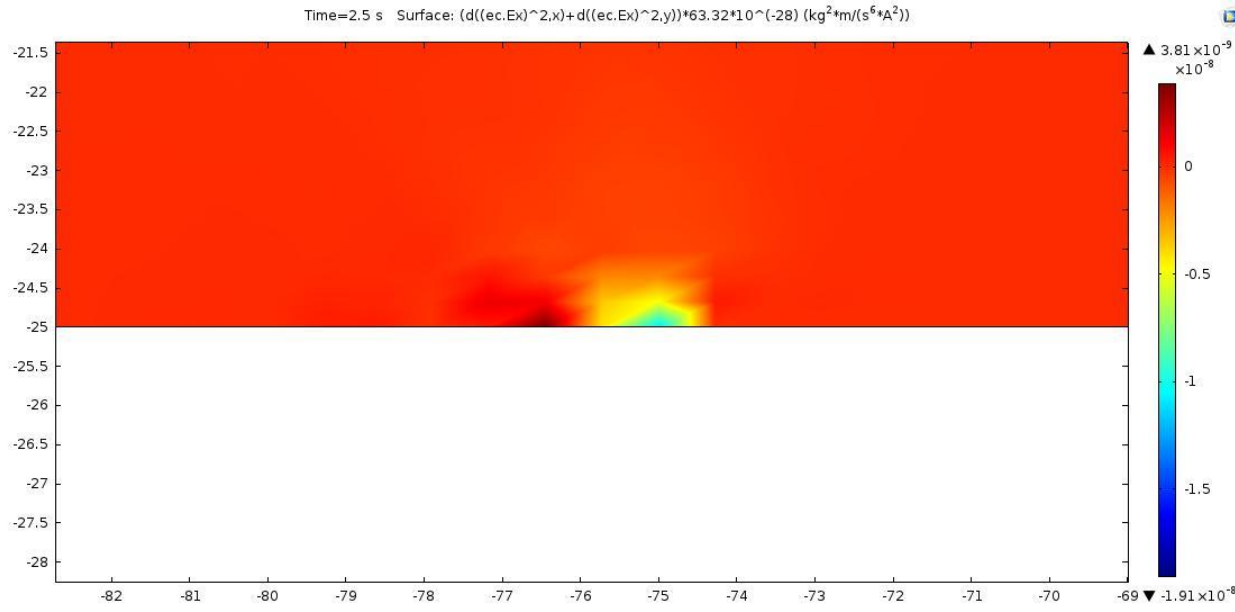


Figure 16(c): F_{DEP} at $t=2.5$ s

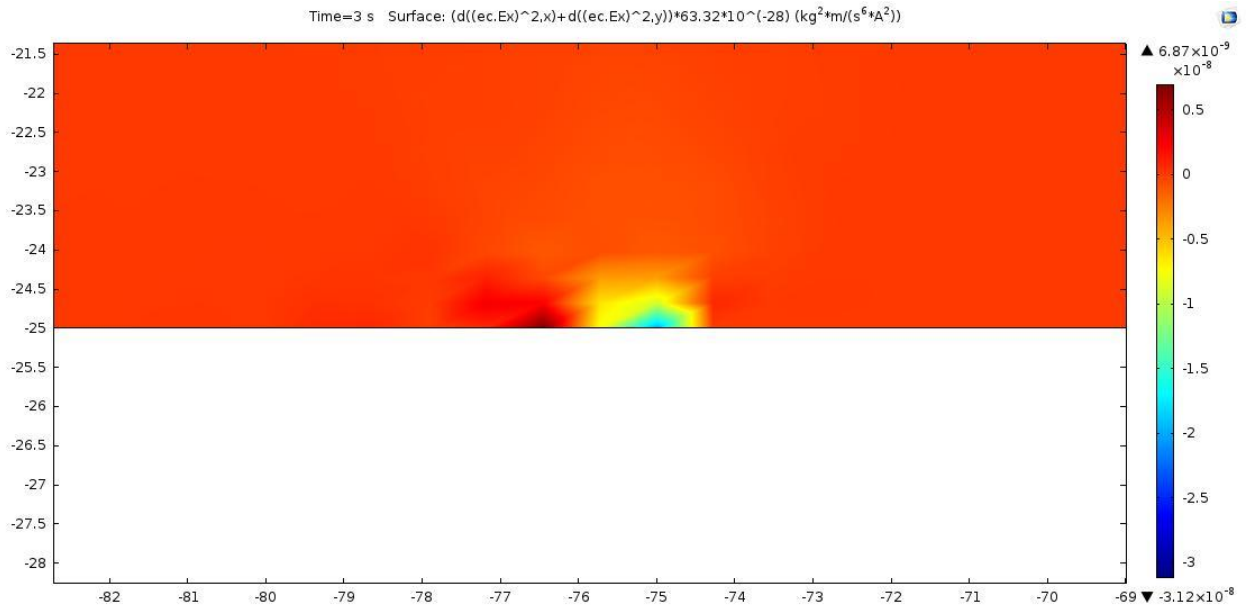


Fig. 16(d): F_{DEP} at $t=3$ s

For 3D model, the potential variation is shown in fig. 17

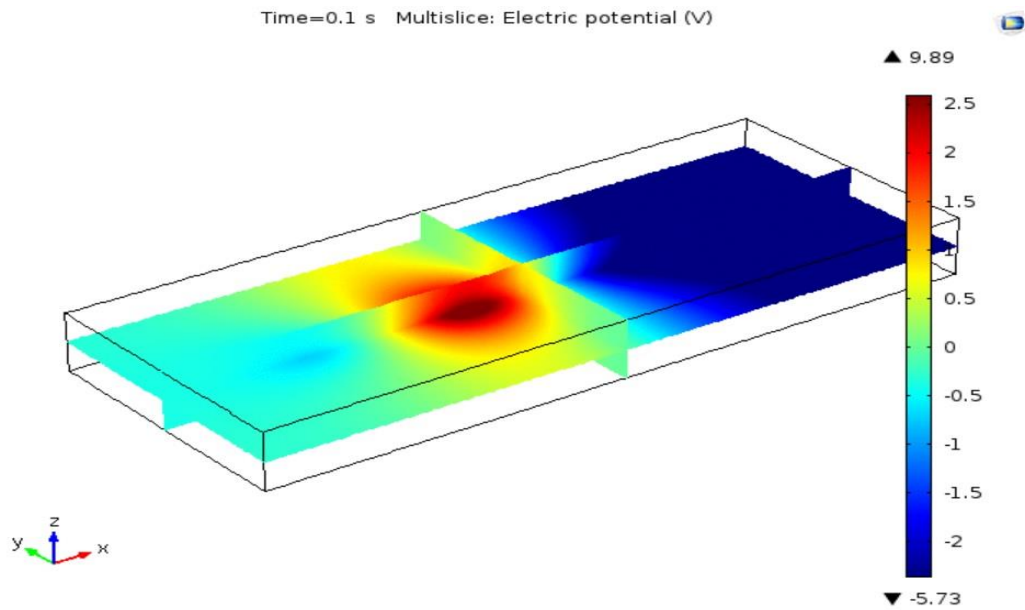


Figure 17(a): Electric potential at $t=0.1$ s

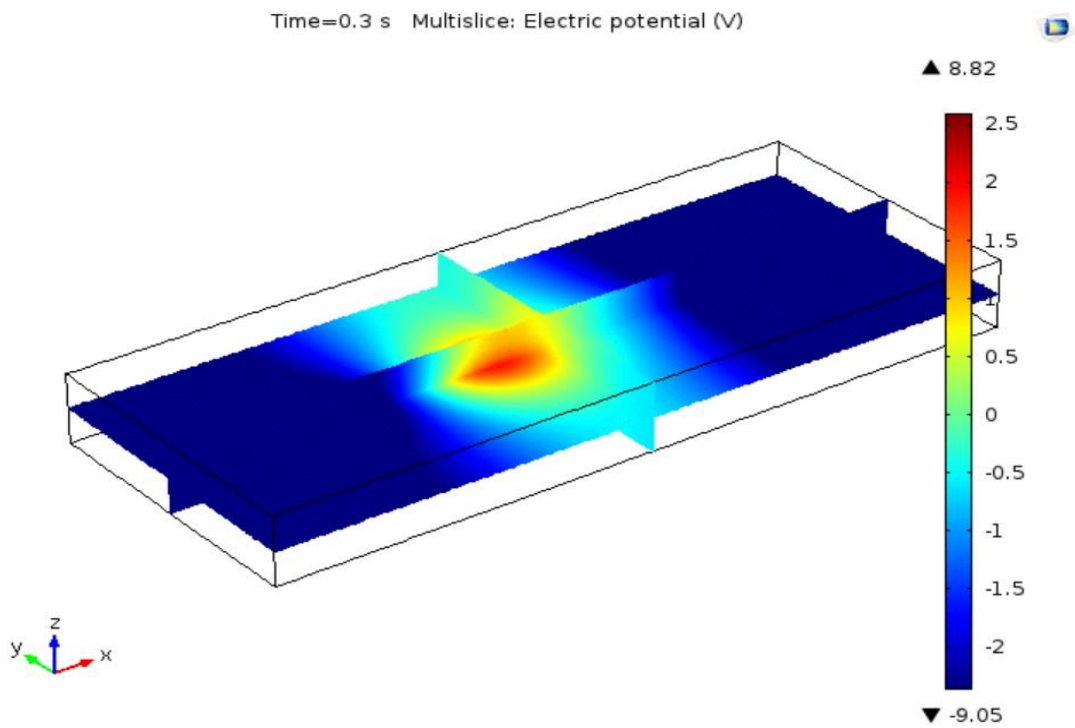


Figure 17(b): Electric potential at $t=0.3$ s

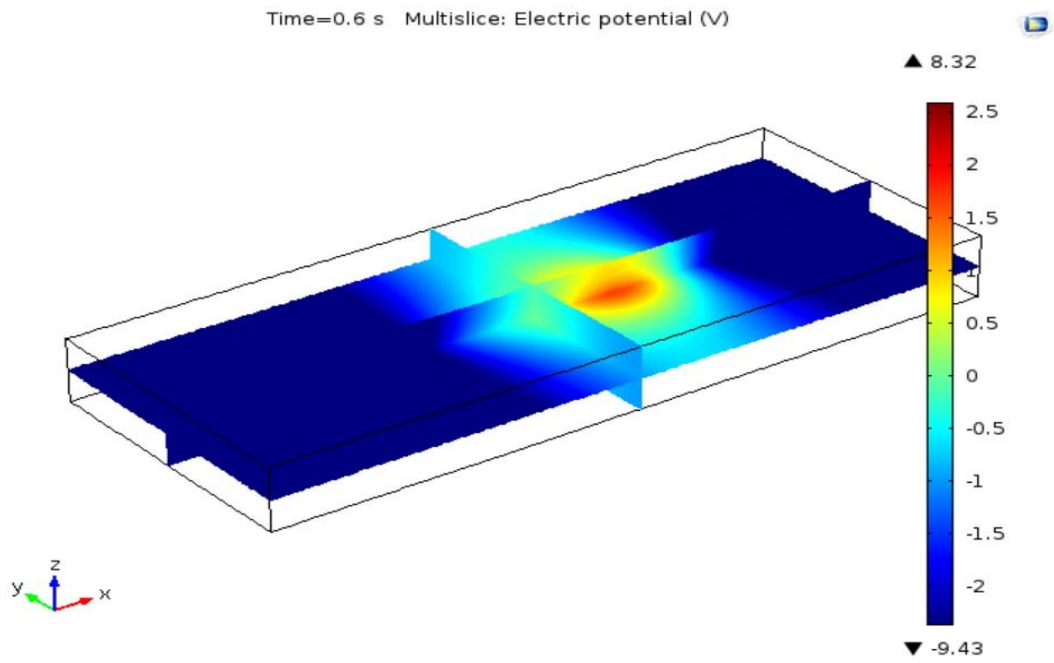


Figure 17(c): Electric potential at $t=0.6$ s

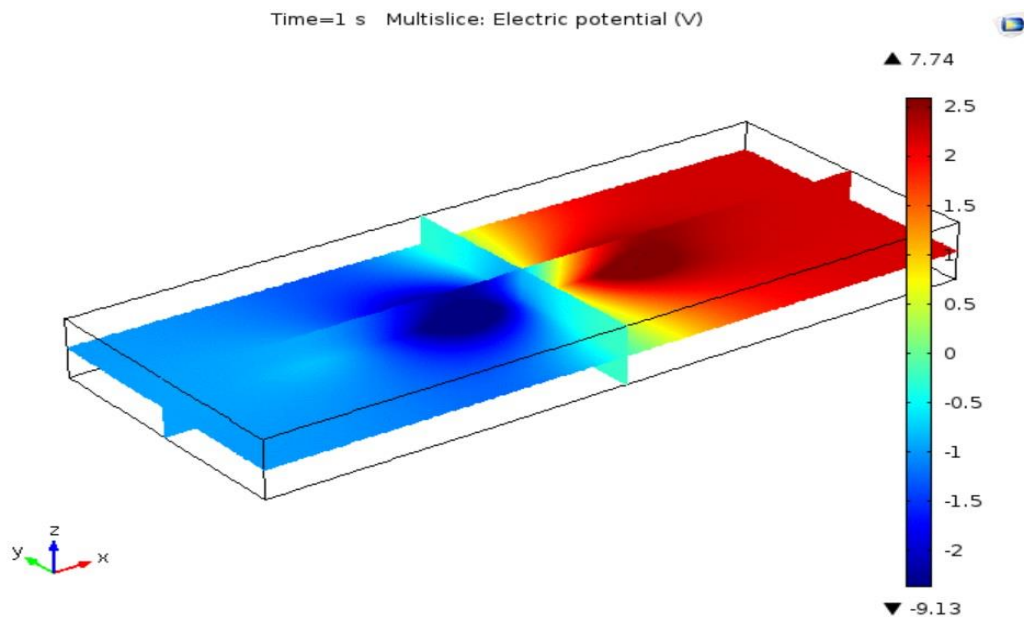


Figure 17(d): Electric potential at $t=1$ s

Similar to 2-D model, the electric potential in 3-D has same variation. From 3-D geometry, a slice is taken to see the variation which resembles to the variation that is seen in 2-D.

Creeping flow module is solved for steady state and the velocity profile for 2D and 3D is shown in fig. 18 and figure -19 respectively. And the settings done for Creeping flow module is given in table- 3.

Table 3 : Settings in Creeping flow Module

Normal inflow velocity of medium(DI Water) (u)	10 μ m/s
Dynamic viscosity of medium(μ)	1Pa.s(at Room temp.)
Density of medium(ρ)	1000kg/m ³
Wall boundary condition	No Slip
Outlet pressure condition	0 Pa (Suppress Backflow)

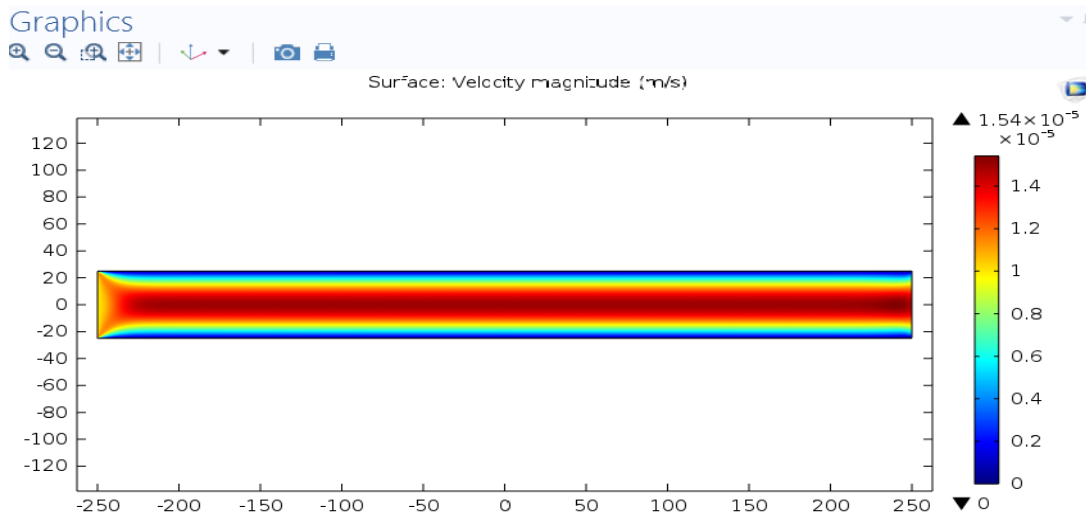


Figure 18: Velocity profile in 2D

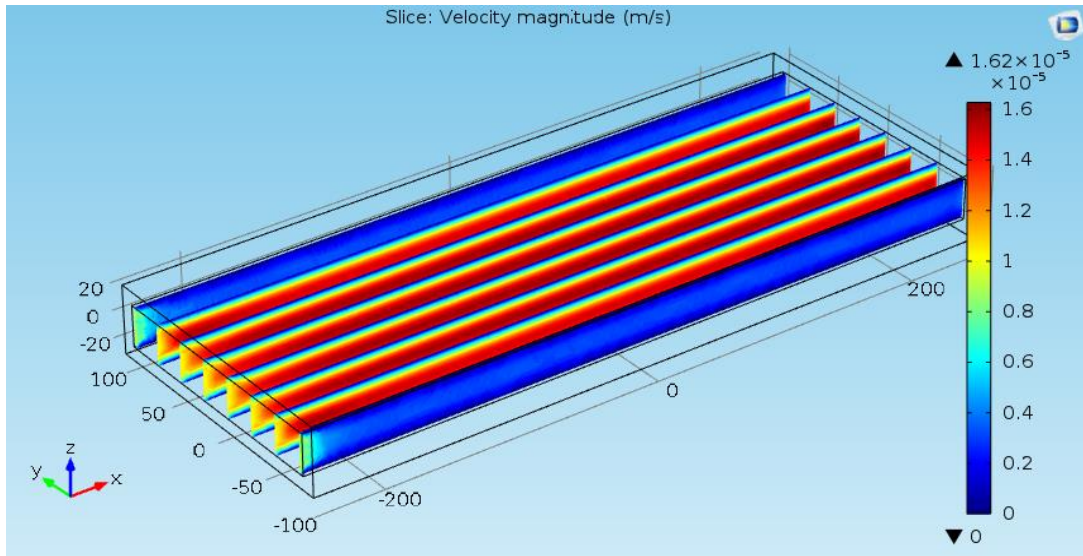


Figure 19: Velocity profile in 3D

The concentration profile of Cryptosporidium obtained by considering steady state as mentioned in chapter - 3 is a dimensionless profile such that each point is a ratio of original value of concentration at that point to the initial concentration at the inlet (C_0) as shown in fig. 20. And same thing follows for length and height of the channel. The properties of Cryptosporidium used are as mentioned in table-2. This is performed using Matlab linked with COMSOL for two different values of DC voltage which is at 2V and 4V. The results are as shown in fig. 21(a,b)

$$X^* = X/l, \quad l \text{ is length of the channel}$$

$$Y^* = Y/h, \quad h \text{ is height of the channel}$$

$$C^* = C/C_0, \quad C_0 \text{ is concentration at inlet}$$

$$C_0 = 1 \text{ (assumed)}$$

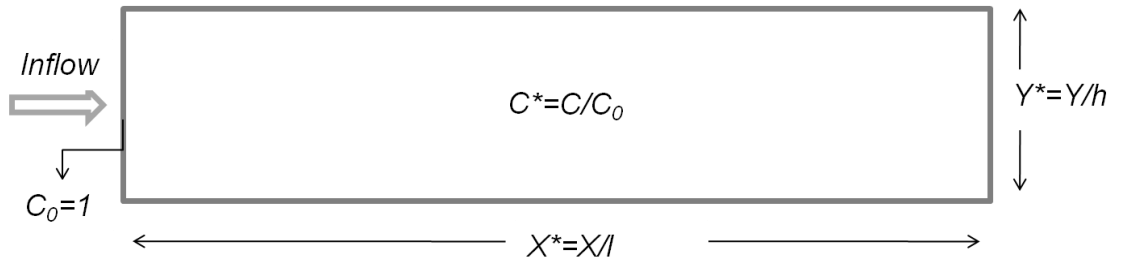


Figure 20: Dimensionless model for a small portion of the channel

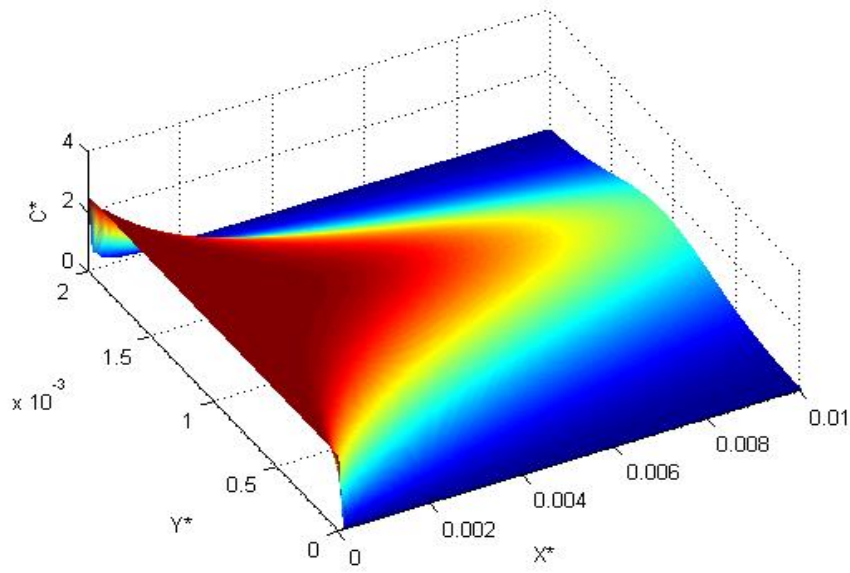


Figure. 21(a): Concentration profile at $V=2V$
 (Concentration variation in a small part of the given channel)

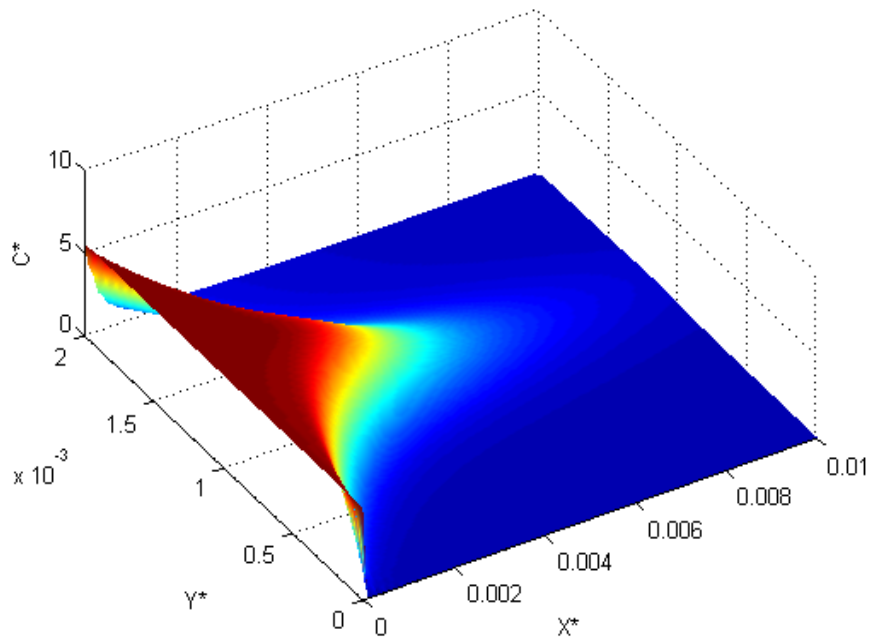


Figure. 21(b): Concentration profile at $V=4V$

(Concentration is increased at a particular height due to increase in voltage value)

As we can see from fig. 21(a) and 21(b) when the applied voltage was 2V the concentration gradient is spread in the given region but at 4V, due to increase in voltage applied, the concentration at a particular height is very intense and spread of gradient is shrunk.

In Particle Trajectory module by using the properties in Table -2 for DEP force , particle movements are captured at different times as shown in Fig. 22(a,b,c,d)

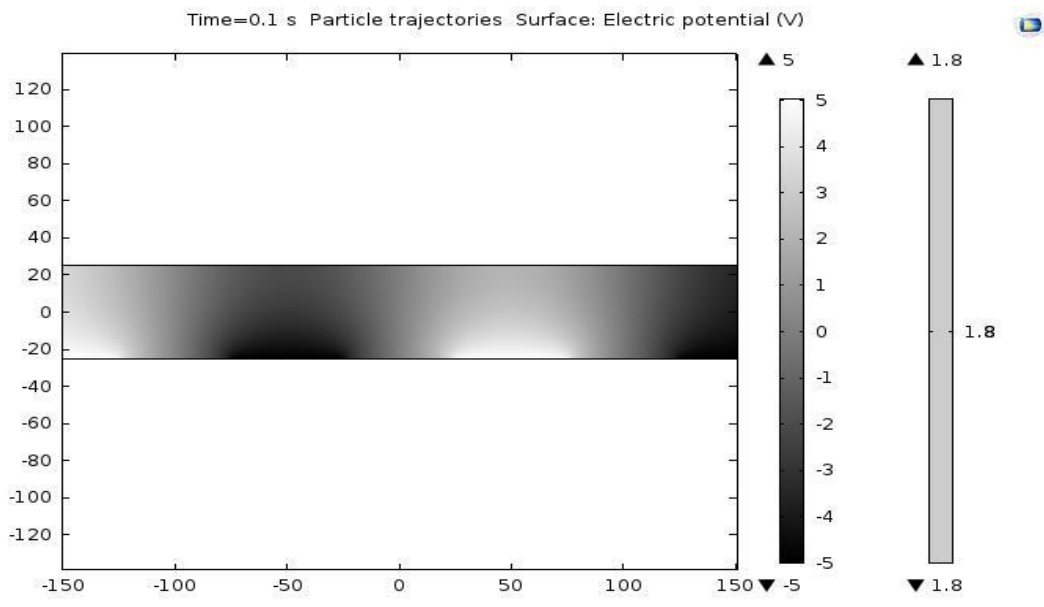


Figure. 22(a): Particle trajectory at $t=0.1$ s

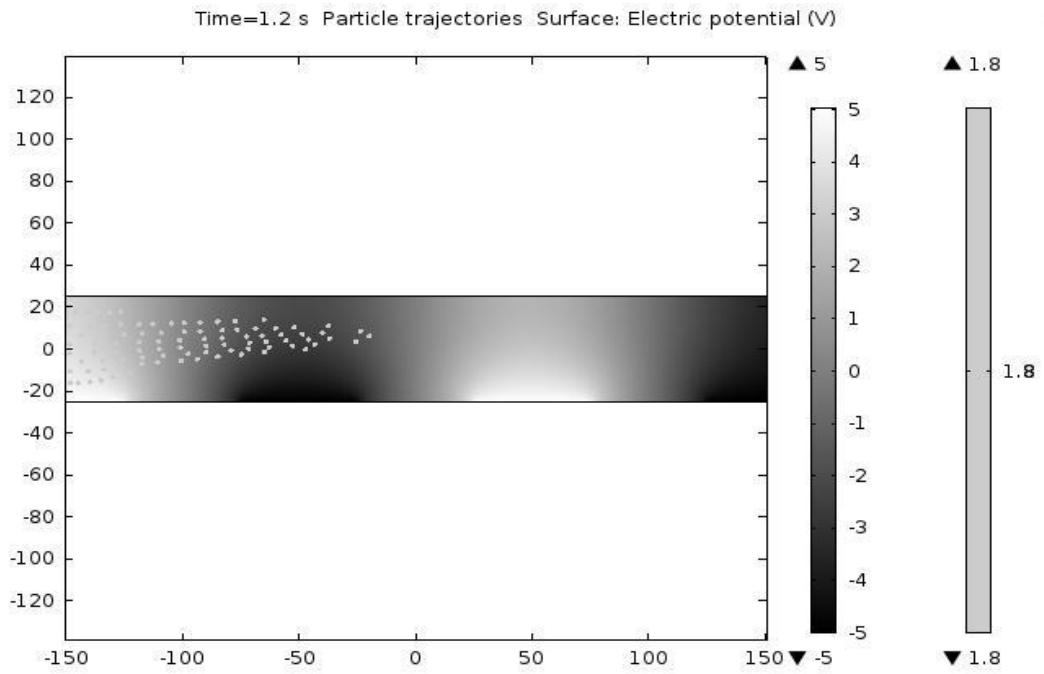


Figure. 22(b): Particle trajectory at $t=1.2$ s

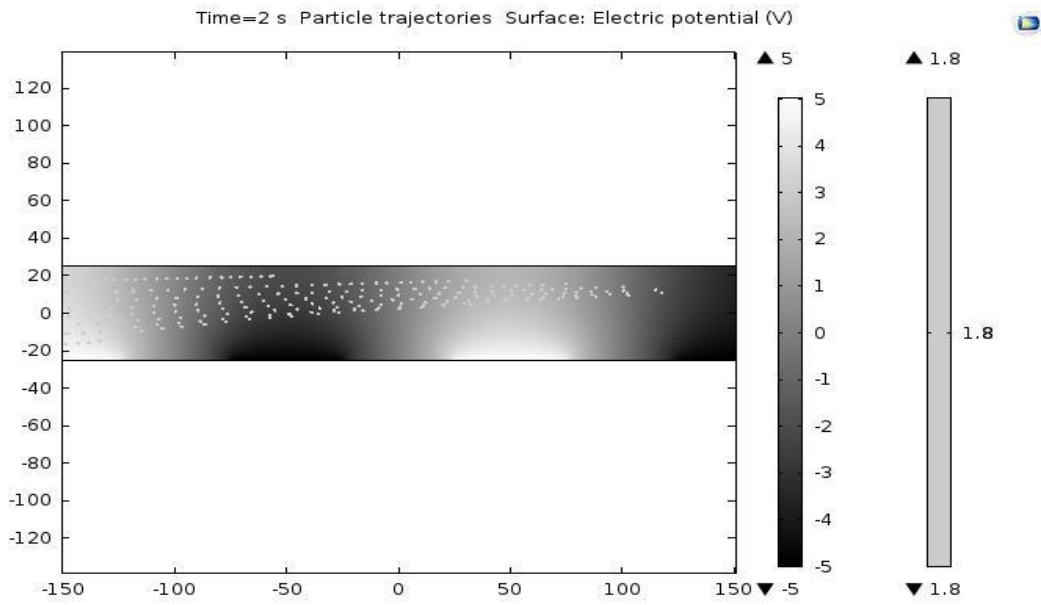


Figure. 22(c): Particle trajectory at $t=2$ s

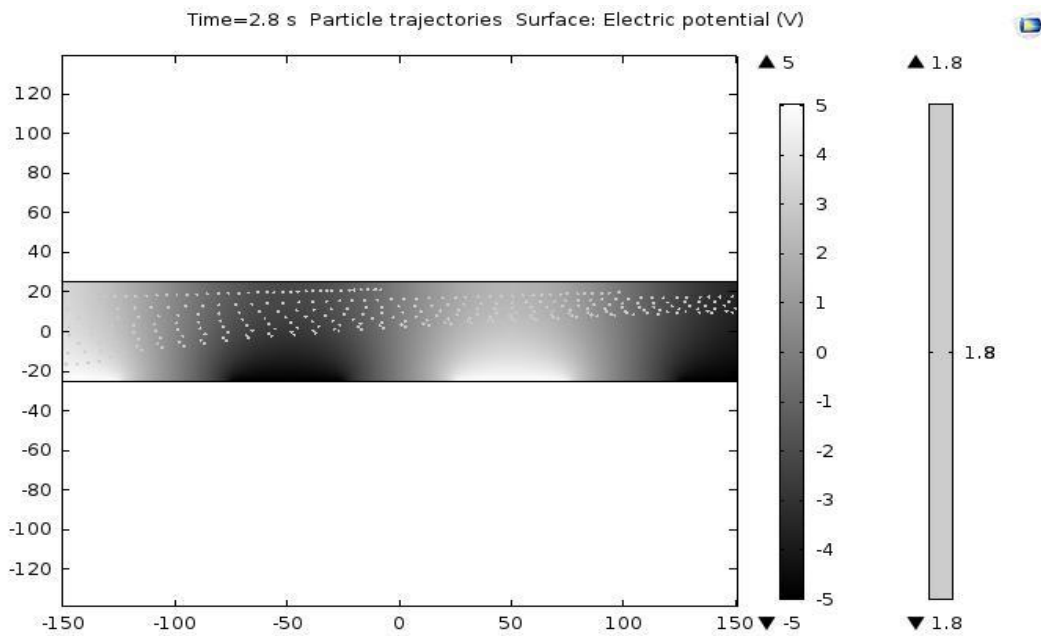


Figure. 22(d): Particle trajectory at $t=2.8$ s

From particle trajectory images (Fig. 22(a,b,c,d)) we can see at $t=0$ the particles are released from inlet in parallel. Which then due to the effect of electric field and hence due to DEP force get deflected and ultimately reaches outlet. Color variation apart from particles is the variable electric potential.

CHAPTER 5

Summary and future work

Hence we can see that how using one of the electrokinetic method called Dielectrophoresis and Travelling wave dielectrophoresis is useful in manipulating *Cryptosporidium*. We observed how change in frequency and/or applied voltage can change the concentration profile. The direction of the DEP force exerted on the cells depend on the contrast between dielectric constant of the medium and that of the cell. From results we can also see how change in the medium can change DEP force behavior. The cross-over frequency, where DEP force changes its sign is different for different medium as well as for different type of cells. This tells us what range of frequencies should be used for operating. The plots also showed that the intensity of DEP force is stronger near the electrode regions.

The future aim is to simulate 3-D electrodes structures like pyramidal and spiral and see its effect on different types of cells of different shapes and also finding concentration in the entire channel for time dependent module for both 2-D and 3-D structures. Aim would also be at designing 2-D and 3-D electrodes and channels by MEMS techniques.

References

- [1] D. Beebe, M. Wheeler, H. Zeringue, E. Walters, and S. Raty, “Microfluidic technology for assisted reproduction,” *Theriogenology*, vol. 57, no. 1, pp. 125–135, 2002.
- [2] J. Yang, Y. Huang, X.-B. Wang, F. F. Becker, and P. R. C. Gascoyne, “Cell separation on microfabricated electrodes using dielectrophoretic/gravitational field-flow fractionation,” *Anal. Chem.*, vol. 71, no. 5, pp. 911–918, 1999.
- [3] P. Belgrader, M. Okuzumi, F. Pourahmadi, D. A. Borkholder, and M. A. Northrup, “A microfluidic cartridge to prepare spores for PCR analysis,” *Biosens. Bioelectron.*, vol. 14, no. 10, pp. 849–852, 2000.
- [4] E. T. Lagally, I. Medintz, and R. A. Mathies, “Single-molecule DNA amplification and analysis in an integrated microfluidic device,” *Anal. Chem.*, vol. 73, no. 3, pp. 565–570, 2001.
- [5] D. Li, *Electrokinetics in microfluidics*, vol. 2. Academic Press, 2004.
- [6] E. P. Technology, E. Dynamics, and E. Science, “Book Review Electromechanics of Particles,” vol. 89, pp. 177–178, 1996.
- [7] M. Lungu, R. Giugiulan, M. Bunoiu, N. Strambeanu, and a. Neculae, “Submicron particle trapping using traveling wave dielectrophoresis,” vol. 111, pp. 111–116, 2013.
- [8] U. Lei, C. W. Huang, J. Chen, C. Y. Yang, Y. J. Lo, A. Wo, C. F. Chen, and T. W. Fung, “A travelling wave dielectrophoretic pump for blood delivery.,” *Lab Chip*, vol. 9, no. 10, pp. 1349–1356, 2009.
- [9] A. Neculae, R. Giugiulan, M. Bunoiu, and M. Lungu, “Effects of fluid flow velocity effectss of fluid flow velocity upon nano particle distribution in microfluidic devices under DeP” *Rom. Reports Phys.*, vol. 66, no. 3, pp. 754–764, 2014.
- [10] N. Green, “Numerical solution of the dielectrophoretic and travelling wave forces for interdigitated electrode arrays using the finite element method,” *J. Electrostat.*, vol. 56, no. 2, pp. 235–254, 2002.
- [11] C. Ab, “COMSOL Multiphysics User’s Guid,” 2012.
- [12] <https://www.COMSOL.co.in/>.

- [13] <https://en.wikipedia.org/wiki/Multiphysics>. .
- [14] www.freeFEM.org.
- [15] C. Ilescu, G. Tresset, L. Yu, and G. Xu, “3D dielectrophoretic chips: Trapping and separation of cell populations,” *Rom. J. Inf. Sci. Technol.*, vol. 13, no. 1, pp. 49–64, 2010.
- [16] C. Ilescu, L. Yu, G. Xu, and F. E. H. Tay, “A dielectrophoretic chip with a 3-D electric field gradient,” *Microelectromechanical Syst. J.*, vol. 15, no. 6, pp. 1506–1513, 2006.
- [17] D. Liu and S. V. Garimella, “Microfluidic Pumping Based on Traveling-Wave Dielectrophoresis,” *Nanoscale Microscale Thermophys. Eng.*, vol. 13, no. 2, pp. 109–133, 2009.
- [18] J. A. Eastman, S. R. Phillpot, S. U. S. Choi, and P. Keblinski, “Thermal transport in nanofluids 1,” *Annu. Rev. Mater. Res.*, vol. 34, pp. 219–246, 2004.
- [19] H. Morgan, N. G. Green, M. P. Hughes, W. Monaghan, and T. C. Tan, “Large-area travelling-wave dielectrophoresis particle separator,” *J. Micromechanics Microengineering*, vol. 7, no. 2, pp. 65–70, 1999.
- [20] M. Camara, O. Camara, H. Ilboudo, H. Sakande, J. Kaboré, L. N’Dri, V. Jamonneau, and B. Bucheton, “Sleeping sickness diagnosis: use of buffy coats improves the sensitivity of the mini anion exchange centrifugation test,” *Trop. Med. Int. Heal.*, vol. 15, no. 7, pp. 796–799, 2010.
- [21] a. Menachery, C. Kremer, P. E. Wong, a. Carlsson, S. L. Neale, M. P. Barrett, and J. M. Cooper, “Counterflow dielectrophoresis for trypanosome enrichment and detection in blood,” pp. 1–5, 2012.
- [22] H. L. DuPont, C. L. Chappell, C. R. Sterling, P. C. Okhuysen, J. B. Rose, and W. Jakubowski, “The infectivity of *Cryptosporidium parvum* in healthy volunteers,” *N. Engl. J. Med.*, vol. 332, no. 13, pp. 855–859, 1995.
- [23] P. C. Okhuysen, C. L. Chappell, J. H. Crabb, C. R. Sterling, and H. L. DuPont, “Virulence of three distinct *Cryptosporidium parvum* isolates for healthy adults,” *J. Infect. Dis.*, vol. 180, no. 4, pp. 1275–1281, 1999.
- [24] M. Elitas, R. Martinez-Duarte, N. Dhar, J. D. McKinney, and P. Renaud, “Dielectrophoresis-based purification of antibiotic-treated bacterial subpopulations,” *Lab Chip*, vol. 14, no. 11, pp. 1850–1857, 2014.

- [25] H. Narayanan Unni, D. Hartono, L. Yue Lanry Yung, M. Mah-Lee Ng, H. Pueh Lee, B. Cheong Khoo, and K. M. Lim, “Characterization and separation of Cryptosporidium and Giardia cells using on-chip dielectrophoresis,” *Biomicrofluidics*, vol. 6, no. 1, 2012.
- [26] P. Pham, I. Texier, and F. Perraut, “From Numerical to Experimental Study of Microsystems for Dielectrophoresis on Bioparticles,” *COMSOL Conf. Paris*, pp. 1–8, 2006.
- [27] https://en.wikipedia.org/wiki/Laplace's_equation.
- [28] https://en.wikipedia.org/wiki/Navier%E2%80%93Stokes_equations .
- [29] “Thomas B. Jones-Electromechanics of particles-CUP (1995).pdf.” .