Optimization of Dielectrophoretic Cell Trapping in a Microfluidic Channel

Claire Amadio[1]; Alexandre Kehren[1]; Hadrien Meriah[2]

1. Bioengineering; 2. Mechanical.

Abstract

In the global framework of research against cancer, in vitro-designed cell aggregates have become a commonly used model for cancerous tumors. Microfluidic platforms now allow researchers to build those aggregates with accurate control of size and shape by cell micro-manipulation through the use of dielectrophoretic force. In this work we listed all the parameters necessary to design a microfluidic chip for cell-aggregates construction and modeled the physical problem of dielectrophoretic trapping in a microchannel. We simulated cell trapping with various parameters to find the optimal set of values for this application. Finally, we validated our optimization approach with a more complex static model of a solid cell within the channel.

1 Introduction

Innovation in microfluidic technologies has lead to multiple scientific advancement in the recent years. The extremely fine and precise manipulation of cells – and even single cell – through this approach offers researchers new experiments to further their understanding of cells and their dysfunctions. One example is the formation of "aggregates". Aggregates are a group of cells joined together to form a spherical block, through cell-cell interactions. They are used in the study of different diseases where the collective behavior of cells are important, such as in cancer. Aggregates can be a faithful model of tumors where the cell are packed together and microfluidic platforms have allowed scientists to create such aggregates for the first time.

The formation of aggregates uses consecutive cell trapping in a microfluidic channel to add cells – by stopping them – to the cells already trapped. Cell trapping is achieved by equilibrating two different forces : the drag due to the fluid flow (forward direction) and the dielectrophoretic force due to the non uniform electric field (backward direction). The cell comes in pushed by the flow and is stopped – trapped – when it reaches a gradient of electric field strong enough to compensate the drag and undergoes some deformation.

The goal of this project is to model one key step in aggregate formation : individual cell trapping. Modeling this complex situation could help researcher improve this technique by tackling different unanswered questions. First, since a numerical modelisation of the dielectrophoretic trapping is possible, can it be used to optimize the microfluidic platform? What are the optimized values of all relevant parameters? And second, all the current simulations (as in [1] or [2]) model the cell as a point – surrounding by a shell to compute the drag –, therefore making two hypothesis. One that cell deformation is negligible and has no impact on the aggregate formation. Two that the electrical and fluid field are not disturbed by the cell presence in a significant manner. In order to validate our own simplified model and question those two well accepted hypothesis, a second model allowing the cell deformation is used. The present work aims to find quantitative information about the point cell model simplification: how does introducing cell deformation and fields disturbance change the model? Is a simplified model realistic enough?

2 Methods

2.1 System

The system studied is composed of two elements : a section of the microfluidic channel – including walls, the rightly positioned electrodes and the flowing fluid – and a cell. All the numerical values and range of variations of the relevant parameters were based on values found in the literature and can be found in the COMSOL file. For the purpose of simplification, a 2D geometry is modeled. This assumption that a 2D geometry will be realistic enough to derive use-

ful result is supported by the very high fraction of publications that use the same assumption.

Many different approaches for the electrodes positioning have been investigated by research teams. Based on the work of Jonathan Cottet at the LMIS4 of the EPFL [3], a simple and effective geometry – with two negative electrodes positioned in branching channels and a ground electrode in the outlet channel – is chosen, as presented in figure 2.1.

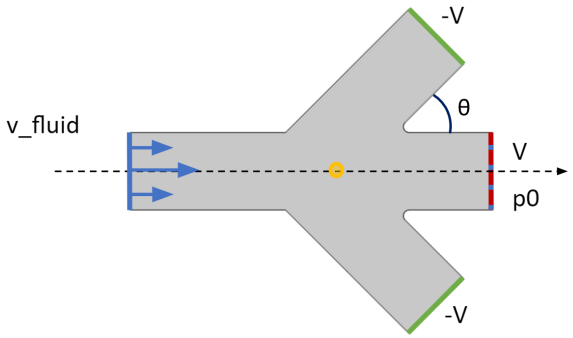

Figure 2.1: Scheme of the geometry and the different boundary conditions.

2.2 Physical Laws

One characteristic of this project is the multitude of physics involved. For the purpose of understanding by the reader, some theoretical background of the most 'exotic' physic are detailed in the following section. For the other physics – solid mechanics and fluid mechanics –, only the main physical laws will be briefly recapitulated.

2.2.1 Physics of Dielectrophoresis

Dielectrophoresis (often abbreviated DEP) is an electrostatic phenomenon which describes the motion of suspended particles in a fluid resulting from polarization forces produced by an inhomogeneous electric field. In fact, if an object consists of positive and negative charges, but the average positions of those charges do not lie in the same place, the object is a dipole. These particles are then polarized when these average positions of positive and negative charge are moved apart by an electric field. The dielectrophoretic force can be written as

$$\mathbf{F}_{DEP} = 2\pi r^3 \epsilon_0 \epsilon_f Re[K(\omega)] \nabla |E_{rms}|^2 \qquad (2.1)$$

where r is the radius of the particle, ϵ_0 is the absolute permittivity of the vacuum, ϵ_f is the permittivity of the fluid, E_{rms} is the root mean square electric field strength and ω is the frequency of the AC electric field. $K(\omega)$ is the Clausius-Mossotti factor - or polarization ratio - and will be introduced afterwards.

If the electric field is equal everywhere, then the forces on the dipole are equal and opposite, and this particle will not move. However, if the electric field is non-uniform - in other words, a gradient of electric field is present - then the force on the charge at the higher electric field will be greater than the force on the dipole in the lower electric field. The particle will then move in the direction governed by the charge in the highest field, along the direction of the field gradient. The force can be in the direction of increasing or decreasing electric field - these are termed "positive dielectrophoresis" and "negative dielectrophoresis" respectively. In order to know what case is present, one needs to pay attention to the ratio K which is expressed as

$$K(\omega) = \frac{\bar{\epsilon_p} - \bar{\epsilon_f}}{\bar{\epsilon_p} + 2\bar{\epsilon_f}} \qquad (2.2)$$

where $\bar{\epsilon_k} = \epsilon_0 \epsilon_k - i\frac{\delta_k}{\omega}$ is the complex permittivity where δ_k is the corresponding conductivity. If this polarization ratio is positive, the DEP force will be oriented toward the domain with a higher electric field and the inverse when this ratio is negative.

2.2.2 Transient Analysis : A Moving Cell

In the first model, we want to simulate the dynamic trapping of the cells within the channel. To do so, we will use the *particle tracing* physics of COMSOL to model the behavior of cells in a flow, under dielectrophoresis. Cells will be depicted as solid spherical particles flowing in the channel, featuring the following characteristics: diameter: 10 μm, membrane thickness: 9 nm, conductivity: σ_{cell} = 5 mS/m, relative permittivity: ϵ_{rcell} = 5. For the solution, we will have to consider a medium that is viable for living cells: phosphate buffered saline (PBS), with the following parameters: conductivity: $\sigma_{solution}$ = 1.6 S/m, relative permittivity: $\epsilon_{rsolution}$ = 80, density: $\rho_{solution}$ = 1000 kg/m^3, dynamic viscosity: $\mu_{solution}$ = 1 mPa.s.

Three physics have to be coupled here to model this situation: laminar flow (static study) to obtain the velocity field, electric current (frequency domain) for the electric field that induce the dielectrophoretic force and particle tracing to compute cell behavior under the conditions induces by the two other physics. Concerning boundary conditions, we set electrical insulation on all edges but the inlet and outlet of the channel and the location of the electrode, the flow inlet is set with the normal flow inlet velocity of v_{fluid}. The electrodes will be defined

as electrical terminals according to the geometry presented in figure 2.1, with a voltage +/-V. The particle tracing module will then use the solution of the two previous physics to compute both drag force and dielectrophoretic force to derive the trajectory of a cell, modeled as solid particles, throughout the channel. The purpose of this first model is first to check if dielectrophoresis is able to compensentate the drag force to trap cells. The second objective is to vary different parameters to find the optimal set that enables cell trapping. The values of V and v_{fluid}, as well as the lateral channel angle θ (see figure 3.3) are parameters that we will optimize for an optimal cell trapping.

2.2.3 Static Analysis : A Deformable Cell

In this second model, the main difference is the presence of a deformable cell, following the physical laws of solid mechanics and disturbing the electrical and flow field around it. The cell is modeled as an elastic material, with a Young Modulus of 5 kPa, with the same relative permittivity as before – values found in the literature. The density and poisson ratio are taken equal to the ones of water. The circulating fluid is taken with the same constitutive laws as in the previous model. All boundary conditions such as inlet, outlet and electrical potential are taken as before, see figure 2.1 for a reminder.

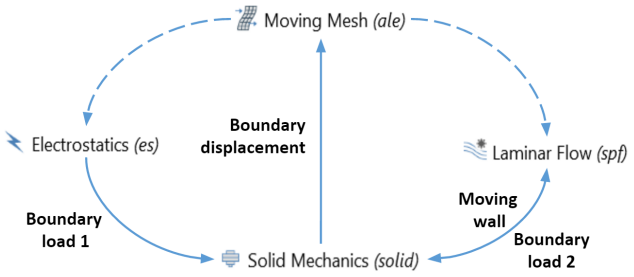

Figure 2.2: Scheme of the physics coupling through boundary conditions.

The complex part of this model resides in the multiplicity of physical laws : Solid Mechanics for the cell deformation, Fluids Mechanics for the flow and an Electrical Field for the dielectrophoretic force. COMSOL does not contain a preexisting multiphysics module that allows the coupling of those three physics. Therefore a manual coupling was realized through boundary conditions on the surface of the cell, as presented in figure 2.2. A Moving Mesh module is also necessary for the coupling to move the fluid area according to the cell deformation and movement. First, the fluid and the electric field impose a stress on the cell (Boundary load). Then the deformation of the cells imposes the deformation of the moving mesh (Boundary displacement). Finally the speed of the cell imposes the speed of the fluid at the cell surface (Moving Wall).

2.2.4 Heat analysis

The main objective of the transient analysis is to optimize the microfluidic chip parameters for particle trapping. However the particles we want to consider in the end are isolated living cells in physiological conditions (adapted medium, body temperature). So for the chip optimization to be complete, we need to see if those physiological conditions are respected, and in particular we need to make sure that the electrodes used for dielectrophoresis are not overheating the medium.

To do so, we built a model based on the same channel geometry than the first model. Based on the work of Shizhi Qian and Ye Ai [4], we used the following three coupled physics: *electric current*, *laminar flow* and *heat transfer in fluids*, in a stationary study. The boundary heat sources of the model were located at the electrode with a general source given by the solution of the electric current study. The initial values as well as the open boundaries conditions were taken at room temperature. Global parameters were the same than for the transient analysis, and in addition we defined the heat-related parameters as: fluid thermal conductivity: $k_f = 0.6$ W/(K.m); fluid thermal capacity $C_p = 4181$ J/(kg.K); ratio of specific heats: $\gamma = 1.33$. Those are values for water in our conditions of pressure and temperature, these values are close from those of PBS solution that is actually used for our application. However, what is significantly different between water and PBS is the electrical conductivity: for 1X standard solution of PBS: $\sigma_{PBS} = 1.6$ S/m, whereas for deionized water: $\sigma_{DIW} = 5{,}5{*}10^{-6}$ S/m, This parameter affects a lot the heating in channel since it involved in Joule heating effect. For this reason we will study here how changing the conductivity of the solution can change heating in the channel and what might be the optimal solution for cell dielectrophoretic trapping.

2.3 Numerical Model

For all models we used the software COMSOL Multiphysics® version 5.2a, Academic Class Kit License. For the main model used for transient and heat analysis, we used a mesh calibrated for fluid dynamics, with a free triangular pattern. The maximum

element size of the mesh was 2.17μm nad the minimal element size was 0.0251μm, with a maximum element growth rate of 1.08 and a curvature factor of 0.25. We used two types of solver: one stationary to calculate the electric field and the fluid velocity field and another time-dependent to solve the particle tracing problem. Both were set with a relative tolerance of 0.01.

3 Results

3.1 Cell Trapping and Optimization

The main objective of the transient analysis is to find the optimal set of parameters (θ, v_{fluid}, V) that allow successful cell trapping in the channel. To quantify the impact of those parameter on trapping, we defined trapping "successful", when the cell is stopped within the trapping zone (see figure 3.3).

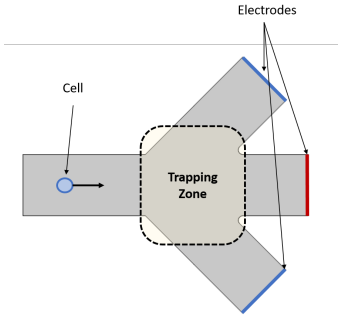

Figure 3.3: Scheme of the trapping zone.

Parameters optimization is done under several requirements: the lower the fluid velocity, the easier it is to trap cell but flow speed must not be too low to avoid risks of cell sedimentation in the channel, so we will always have $v_{fluid} > 50\mu$l/s, Voltage on the other hand must be high enough to induce a high electric field and therefore a high dielectrophoretic force but not too high to avoid damaging the cells. Finally, the angle θ will change the electric field lines map by modifying the channel geometry (see figure 3.4) and thus, impact the effect of the dielectrophoresis. The stake of the optimization is to find a right trade off between all those parameters.

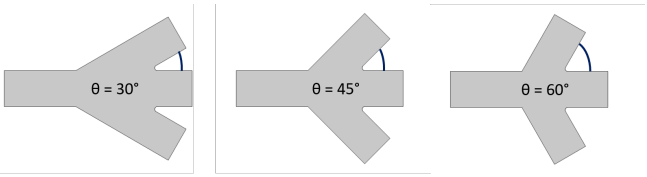

Figure 3.4: Influence of lateral channel angle θ on the overall design

We first simulated dielectrophoretic trapping for different values of θ, and V at a fixed fluid velocity v_{fluid} = 500 μm/s to determine which are trapping cells and which are not. Figure 3.5 shows the final position of the cells in the channel (i.e. at t=∞), as a fraction of the total channel length. A final position of 100% means that the particle is not trapped. However we will consider that the cell is successfully trapped, only if it lands in the trapping zone, which corresponds to less than 85% of the total channel length. All the final positions that are below the dashed line on figure 3.4 are successful trapping. We observed that for high values of θ necessitated lower values of voltage for trapping, which means that increasing the lateral channel angle increases the action of the dielectrophoretic force, and thus facilitates the trapping.

We gathered this information in supplementary figure 5.11, to represent the trapping domain of the parameter set (θ, V). All the values that are located in the white region of the plane will give a successful trapping.

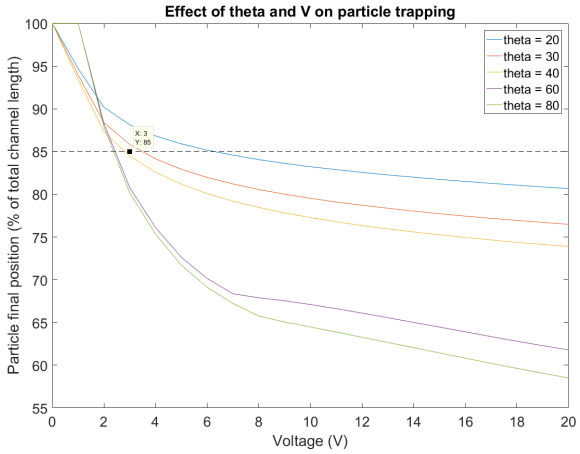

Figure 3.5: Final position of the cell within the channel for different values of θ and V at v_{fluid} = 500 μm/s.

We ran a second simulation, this time considering the couple (v_{fluid}, V). Results are depicted in figure 3.6. Here again we consider a threshold of 85% of the total channel length, and all the points that are below the dashed line are trapping combinations. We simulated for values of flow velocity in [50 μm/s, 1000 μm/s], which is a typical range used in microfluidics cell detection and trapping and for V varying in [1V, 11V]. As expected, high values of voltage allow easier trapping and high values of flow velocity does the opposite. We noticed that for V > 6V, the trapping is successful for all of fluid velocities in our range. We summed up those results in a 2D representation of the trapping combination (v_{fluid}, V) in supplementary figure 5.12.

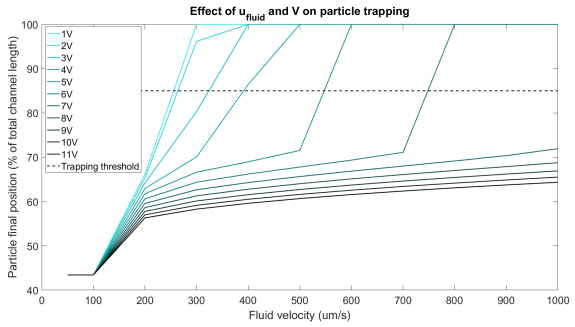

Figure 3.6: Final position of the cell within the channel for different values of v_{fluid} and V, for $\theta = 45°$.

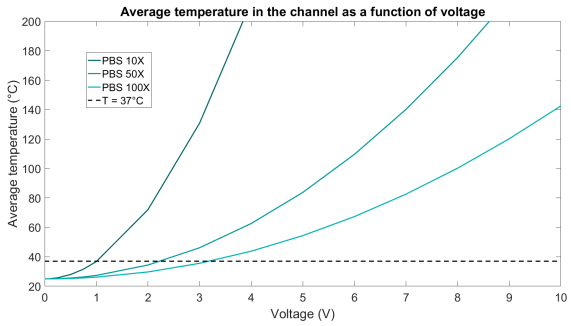

Figure 3.7: Evolution of the average temperature in the channel with different levels of PBS dilution (10X, 50X, 100X). The black dashed line represents the physiological temperature (37 °C). (v_{fluid} = 100 μm/s and $\theta = 45°$).

This information on stability enable parameter selection for designing the dielectrophoretic-trapping microfluidic chips.

3.2 Heat analysis

Transient analysis has provided sets of parameter that allow DEP trapping. Yet, we still have to make sure that the medium remains viable for the cells when those parameters are applied, especially concerning thermal conditions. Indeed, PBS is a highly conductive solution because of its ionic concentration, so it can heat up the medium by Joule effect. To prevent this, one solution consists in diluting PBS to reduce ionic concentration. We ran a heat simulation for different values of the solution electrical conductivity $\sigma_{solution}$, representing different dilution levels of a PBS solution. The electrical conductivity of a PBS solution varies linearly with the dilution (Chaparro et al., *Journal of Physics*, 2016). Simulation were performed with $\theta = 45°$ and $v_{fluid} = 100$ μm/s (relevant values for trapping according to transient analysis). The expected outcome is to find the maximal voltage that can be applied without overheating the medium and thus, avoid damaging the cells. Figure 3.7 shows the evolution of the average temperature in the channel with increasing voltage applied at the electrodes, for different dilution f PBS solution: 10X, 50X and 100X. Suitable voltages are the ones that induce an average temperature in the channel below the physiological temperature T = 37°C (dashed line on figure 3.7).

For a 10X PBS solution, only voltages below 1V avoid overheating, for a 50X dilution, the maximal voltage is 2.2V and 3V for a 100X dilution, By keeping voltage in this range we prevent cells from being damaged by the Joule effect, but we also have to make sure that the configurations with these maximal voltage values are still trapping. We ran a new transient analysis using the maximal voltages obtained and the parameters used for the heat analysis. Results are summarized in table 3.1. We observed that with a 10X configuration, trapping is not possible if we want to preserved the cells at the same time, whereas 50X and 100X PBS solution allow doing both. The last two configuration of table 3.1 can be used for dielectrophoretic trapping of living cells.

PBS dilution	$\sigma_{solution}$	v_{fluid}	θ	V_{max}	Trapping
10X	1.6 S/m	100 μm/s	45°	1V	NO
50X	0.32 S/m	100 μm/s	45°	2.2V	YES
100X	0.16 S/m	100 μm/s	45°	3V	YES

Table 3.1: Evaluation of the configurations for different levels of PBS dilution.

3.3 Cell Deformation and Validation

As detailed in the methods section, a second model is used to validate our first model and the commonly accepted hypothesis that field disturbance by the cell and cell deformation are negligible. The figure 3.9 compares the electrical field norm (rainbow map) and the fluid velocity field (white lines) for the two models. From the computation, the value of the drag and the DEP are obtained by integrating the surface forces on the cell boundary. Those two forces are the key elements in the cell trapping since they must be equal at the stopping point: the cell stops exactly when the DEP (increasing as the cell travel the electric field) compensates the drag (roughly constant since the flow perturbation is reasonable).

The result of the first model – the point model – are validated by inputing the found parameters into this model. The final cell position is given as the initial cell position, flow speed and theta angle are adjusted. The corresponding real drag and DEP (with disturbed flow) are computed to verify that they are of equal norm. Every combination found with the particle tracing model was successfully validated in the realistic model of a whole cell.

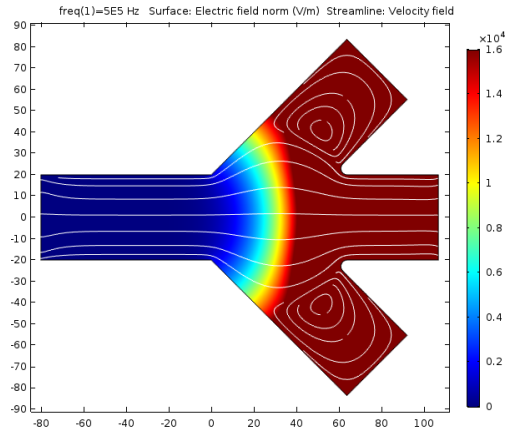

Figure 3.8: Visualization of the electric field norm (rainbow map) and the flow field velocity (white streamlines), without cell in the channel from the first model (transient analysis)

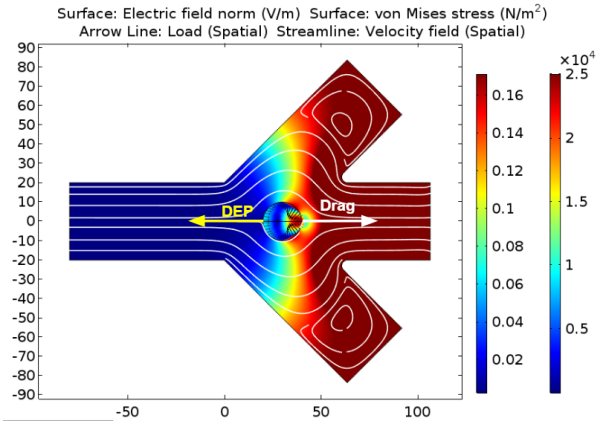

Figure 3.9: Visualization of the electric field norm (rainbow map) and the flow field velocity (white lines) in the microchannel around the cell. The DEP (yellow) and the drag (white) are here to illustrate the concept of cell trapping through force compensation.

Finally, the amplitude of the cell deformation was briefly studied. It was found to be on the order of nm. With a cell size of tens of um, this deformation is negligible. No cell damage is expected at this order of magnitude. The figure 3.10 illustrates the shape of the deformed cell for a direct current.

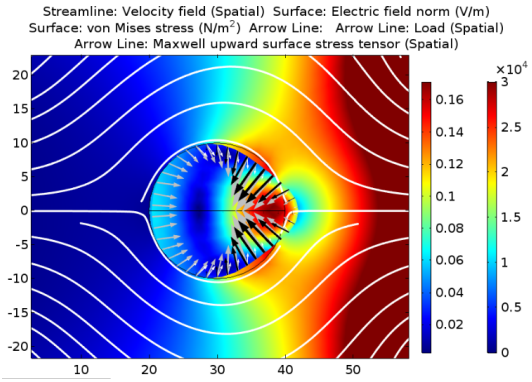

Figure 3.10: Visualization of the cell deformation under a DC electrical field. The arrows represent the different surface loads : fluid (white), DEP from the electrical field (black) and total (grey). The white lines represent flow field, and the rainbow coloring the electrical field.

A study of the modes of deformation of the cell under an alternative current was then realized, but for space reasons, it will not be detailed in this report. It was nonetheless found that the cell resonates for certain frequencies. This phenomenon is observed after a threshold frequency around 7 kHz, see supplementary figure 5.13. The cell are expected to undergo vibrations at those frequencies.

4 Discussion

4.1 Cell trapping and optimization

Transient analysis

This model was efficient because it allowed rapid extraction of design parameters. Still, it is necessary to question the accuracy of the results by keeping in mind that we had to make some strong assumptions to perform our simulations:

2D simplification: we reduced the problem to a planar situation, supposing homogeneity of electric field along vertical direction, which is not exact if all three electrodes are placed at the bottom of the channel. Therefore it could be interesting to run similar simulations with a three-dimensional model to look at the vertical effects of DEP.

Particle tracing: in this physical module, cells are modeled as non-deformable spheric shells. Drag and dielectrophoretic forces are directly computed by the software as acting on this shell. In order to validate this approximation, we had to run models taking solid properties of the cell into account, in a static model. To go even further, we could also run a transient analysis using this more accurate approach.

Heat analysis

The temperature study in the channel imposed a restriction in the range of possibles voltage values. Taking physiological requirements into account induced a refinement in the optimization. A major condition to keep the medium thermally viable for the cells while applying DEP, is to reduce electrical conductivity by diluting the PBS. This will lead to a lower ionic concentration, which is good to avoid overheating, but also reduces the tonicity of the medium and threatens the osmotic equilibrium of the cell. One simple way to circumvent this problem would be to add glucose in the solution, which will not significantly increase electrical conductivity, but will ensure osmotic equilibrium of the cell.

4.2 Cell deformation

The use of a validating model is one of the strength of this project, as it is quite unique. The literature has a gap in terms of validation of the main hypothesis used in all current model : is cell deformation negligible and how are the electrical and flow field affected by a solid cell instead of a point one. Our study has first shown that neglecting cell deformation is a very reasonable assumptions. Second, although the two fields are quite deformed by the presence of a cell, the main results derived from the simple model are still true. Optimizing cell trapping with strong assumptions on cell impact was proven to be sufficient for channel design applications.

A supplementary study was started to tackle one of the limitation of the current model : the absence of time dependent study in the complex model. The initial results were that it is possible to implement one more layer of complexity in COMSOL Multiphysics without much change to the current model and that again, the initially found trapping parameters are still trapping. One limitation is that when the cell passes the trapping point, the DEP becomes higher than the drag and the model cannot converge anymore. The unfinished model was therefore not included in this report, since this is a major hurdle that has yet to be overcome.

One interesting point and possible prolongation of this study could be a more thorough study of cellular deformation. Especially when applied to the deformation of an aggregate – multiple cell glued together – instead of one single cell. The bigger size of an aggregate would possibly lead to internal deformation and corresponding shear that could tend to disrupt the aggregate integrity.

5 Conclusion

The approach we followed throughout this project was to use numerical simulation to extract design guidelines on how to build a microfluidic chip for dielectrophoretic cell aggregation. Moreover, the complete static approach validates the simple particle tracing model, which we can trust for parameter optimization. We now know what parameters to use to design our device and we could now, for instance, go to a micro-fabrication clean-room to produce those chips for testing. This method spares time in lab testings and preliminary experimentations. It is however necessary, for validation, to test the design in experimental conditions because our model is limited by several approximations.

References

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Supplementary Materials

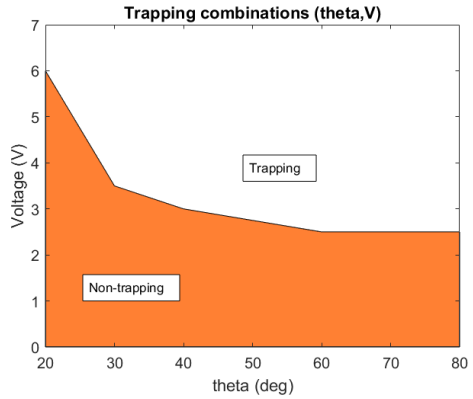

Figure 5.11: 2D representation of stable values of (θ, V). The area in white represents the stable values and the orange, the unstable. Fluid velocity: v_{fluid} = 500 μm/s.

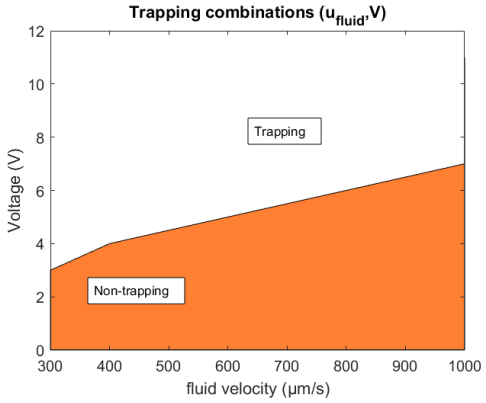

Figure 5.12: 2D representation of stable values of (v_{fluid}, V). The area in white represents the stable values and the orange, the unstable. Lateral channel angle: $\theta = 45°$.

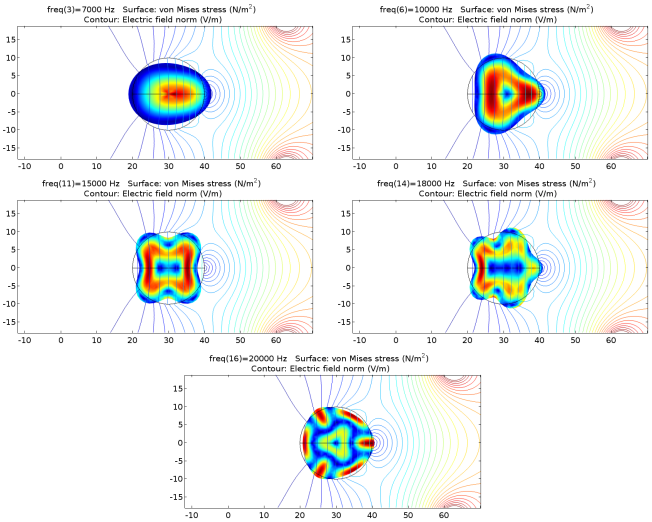

Figure 5.13: Modes of deformation of the cell for different frequencies of electrical potentials. In the presented order, the following modes of resonnance are observable : 2, 3, 4, 5 and 6.