Design and realization of an electrophoretron cycler

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DESIGN AND REALIZATION OF AN ELECTROPHORETRON CYCLER

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science in Mechanical Engineering

in

The Department of Mechanical Engineering

by

Celine Raymonde Ramet
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Abstract

Polymerase Chain Reaction (PCR) is a powerful enzymatic reaction commonly used to amplify specific sequences of Deoxyribo Nucleic Acid (DNA). Since the introduction of the lab on a chip concept, numerous Continuous Flow PCR cyclers were realized with success at the micro scale. As reducing the reactor size and improving thermal management led to reduced sample volumes, results could be achieved much faster with these CF-PCR cyclers than with common commercial cycler. Furthermore, most of these demonstrated CF-PCRs are nowadays evolving towards high-throughput systems. However, most CF-PCR cyclers require complex manipulations and are not flexible (e.g. fixed number of cycles, and/or only usable for PCR …).

The concept of the electrophoretron cycler was introduced and demonstrated at the macro scale in 2001. The present work aims at using this electrokinetic cycler combining electroosmosis and electrophoresis in order to achieve cycling of the DNA species in a micro scale on-chip device, while applying only one potential difference. Even limited by polymers properties, appropriate design of the closed-loop microchannel allows the hydrodynamic effect resulting from mass conservation to drastically improve cycling time and species profile.

This result has been justified by appropriate theoretical analysis combined with numerical simulations, while polymers properties have been carefully characterized using experiments, resulting in the first micro scale electrophoretron prototype which has been tested in PCR like conditions.
Chapter 1 Introduction

1.1. Polymerase Chain Reaction (PCR)

Invented in 1983 by Karry Mullis, the Polymerase Chain Reaction (PCR), which will grant its creator the Nobel Prize ten years later, is an enzymatic reaction used in Deoxyribo Nucleic Acid (DNA) analysis to replicate a specific fragment of the original DNA, called template. Copied fragment is selected by the adequate choice of primers, single stranded DNAs of a few dozen base pairs (bp), whereas complete DNA molecules comport thousands or even million of base pairs. The forward primer indicates the beginning of the copy process and the reverse primer the end. Over the last 25 years, this reaction has become increasingly critical in biology for projects such as the Human Genome Project, which mapped the whole human genetic information, and appearing virus identification, such as at the SRAS apparition in 2003.

The PCR reaction includes 3 different steps: denaturation, annealing (also called renaturation), and extension. During denaturation, the heat separates the two strands of DNA, allowing primers to anneal to their specific locations on the single DNA strands throughout renaturation. Finally the extension consists of the formation of two new double stranded DNA. This last step is permitted by the presence of the enzyme polymerase, which allows the additional nucleotides to link together in the same order as in the DNA template, starting at the primers locations.

Even though its principle is simple, this reaction requires specific conditions to be met in order to take place. First, the solution must be at pH close to 8.3 (or 8.6), which justifies the common use of a Tris based buffer. In addition, because they activate the Taq polymerase enzyme, magnesium ions need to be present in the solution. Potassium, even though it has been shown optional (Chen 2005), also assists the polymerase action. Reactants volumes of PCR working conditions for amplification of 500 bp amplicon of λ-DNA are shown in Table 1.
Table 1 Typical Composition of a PCR Mix

<table>
<thead>
<tr>
<th>Reactants</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris HCl</td>
<td>10 mM</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>1.5 mM</td>
</tr>
<tr>
<td>KCl</td>
<td>50 mM</td>
</tr>
<tr>
<td>Nucleotides (dNTPs)</td>
<td>0.2 mM</td>
</tr>
<tr>
<td>Forward primer</td>
<td>1 µM</td>
</tr>
<tr>
<td>Reverse primer</td>
<td>1 µM</td>
</tr>
<tr>
<td>Template DNA</td>
<td>5 ng/µL</td>
</tr>
<tr>
<td>Polymerase</td>
<td>0.5 U/µL</td>
</tr>
</tbody>
</table>

To perform amplification, the 3 PCR steps (denaturation, annealing and extension) are continuously repeated, for usually about 30 cycles. Unfortunately, the optimum temperature for each of these steps differs drastically. For example, denaturation requires a very high temperature to separate the 2 strands of DNA, whereas at such a high temperature the Polymerase enzyme is also denatured and can not react. Consequently, the temperature must be cyclically switched in each of the three thermal steps, so that highly efficient amplification can be achieved. In most cases, each cycle produces two copies of the template. These new strands can be used in the next cycle as “template”, giving $2^n$ copies after n cycles. This number is valid up to the point where there is no nucleotide (dNTP) or polymerase left. Numerous chemistry works have been dedicated to the optimization of the cycling times, temperatures, time ratios, etc… As a reference, in this work, when amplifying a 500 bp of λ-DNA (a 48 kbp bacterial DNA), unless otherwise specified, the conditions in Table 2 have been used (Courtesy of Dr Witek).
Table 2 Standard Cycling Conditions used for PCR (Courtesy of Dr Witek)

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preliminary</td>
<td>Denaturation</td>
<td>94°C</td>
</tr>
<tr>
<td>Cycling</td>
<td>Denaturation</td>
<td>94°C</td>
</tr>
<tr>
<td>(30 cycles)</td>
<td>Renaturation/Annealing</td>
<td>55°C</td>
</tr>
<tr>
<td></td>
<td>Extension</td>
<td>72°C</td>
</tr>
<tr>
<td>Final</td>
<td>Extension</td>
<td>72°C</td>
</tr>
</tbody>
</table>

Products from the PCR are separated electrophoretically. Very often the method employed is gel electrophoresis (Figure 1), which permits separation of the DNA fragments based on their size. Indeed, DNA molecules are negatively charged, implying DNAs motion towards the positive electrode (anode) when an electrical field is applied. If placed inside an appropriately sized gel matrix, the shorter the DNAs, the faster they will move.

![Figure 1 Example of Gel Electrophoresis of PCR Products](image)

After sufficient time, different fragment sizes are clearly delimited. To allow evaluation of the fragments sizes, a standard is also placed inside the matrix (Column 6 in Figure 1). The gel
contains an intercalating dye (e.g. Ethydium Bryomide) to stain the DNA fragments. Finally, the gel is visualized under UV light. An example of gel electrophoresis of PCR product (amplification of 500 base pairs fragment from a λ-DNA template) may be seen in Table 2.

1.2. PCR Cyclers

The Polymerase Chain Reaction (PCR) is, in most laboratories, realized in programmable benchtop PCR thermal cyclers successively heating up and cooling down the PCR mix. Even though a lot of ameliorations have been brought to the devices since the first generation of commercial PCR thermal cyclers, these are obviously energy and especially time consuming. For example, the PCR cycle described in the previous section (1.1.) can take about an hour and a half in a regular commercial PCR thermal cycler to be completed. Moreover, these commercial cyclers ironically require important volumes of reagents, while the aim of the reaction is to amplify rare templates.

Reducing cyclers’ volume down to the micro scale on a chip has had much impact on reducing cycling time and reagents volume. Most on chip cyclers can be differentiated into two categories: Chamber Type and Continuous Flow Cyclers (CF-PCR). The former, first implemented in 1998 (Daniel 1998), is a reduction to the micro scale of the reactor and has a drastically positive effect in terms of volume reduction, but tends to make manipulation and thermal management more difficult. The later consists in driving the PCR mix inside microchannels heated at different temperatures. CF PCR facilitates manipulation and connection with previous and following steps (Obeid, Christopoulos et al. 2003). However, the thermal management remains one of the most important limitations (Chen 2006) and the reagent volume is usually more difficult to minimize with CF PCR than with Chamber type devices.

Continuous flow PCR cyclers have been an active research area since the first device was reported in Science by Kopp et al. in 1998. One area in which researchers demonstrated the most
creativity is the driving force used to force the flow into the device. Most designs have been pressure driven devices, in which the pressure was controlled by programmable syringes (Kopp, De Mello et al. 1998; Obeid and Christopoulos 2003; Hashimoto, Chen et al. 2004). This well behaved driving force is still used in more recent and insightful works (Crews, Wittwer et al. 2008). There are two main disadvantages to pressure driven continuous flow cycler. First, since the path is an open loop, the loop length on a chip device can not be changed after fabrication, meaning that the number of cycles is predefined before fabrication. The second inconvenience becomes increasingly important while the microchannels length is decreased to implement more cyclers on a chip for high throughput devices. Indeed, the pressure drops required in order to have flow increase exponentially while the microchannel size decreases.

An uncommon but interesting driving force for continuous flow PCR cycler is the buoyancy reported by Ugaz group in 2002. Based on the Rayleigh-Bénard effect, it requires a vertically positioned chip in which the buffer flows due to its buoyancy change due to the temperature variations and subsequent changes in density. Even though it might not be as easily manageable as pressure driven flow, for buoyancy variation is only a consequence of the regulated heat transfer, it eventually simplifies drastically the manipulation of the cycler, as well as the energy consumption since the heaters are the only power expenditures. A last advantage of this technique consists in the fact that the user is capable of fully controlling the number of cycles. However; this cycler would only be suitable for a reaction requiring important temperature differences.

Magnetism is another unusual driving force which also allows control of the number of cycles (Sun, Kwok et al. 2007). Cycling of the PCR reagents throughout the temperature zones is achieved by the movement of an immiscible ferrofluid submitted to magnetic forces. One
interesting evolution of this technique has been the parallelization of this design to create a high throughput continuous flow cycler (Sun, Nguyen et al. 2008).

Use of electrokinetic CF-PCR cyclers have also been demonstrated in numerous applications (Chen 2005; Chen 2006). This kind of PCR cycler allies the convenience of the number of cycles control with low power consumption. However, the use of electrokinetically driven microflows presents two main disadvantages: electrical current circulation generates Joule heating which may compromise the accuracy of the temperatures applied and the electrolysis that occurs at the electrodes when placed in contact with a water-based solution, which may generate enough gases to rapidly clog microchannels, as underlined by Dr Soper’s group (Chen 2005). An original example of electrokinetically driven PCR cycler was provided by Li’s group where the Joule heating was used as the only heat generation (Hu, Xiang et al. 2006). However, all previously cited works require synchronization of the applied voltages and/or currents to make the PCR reagents go through all temperatures. This limitation justifies why an electrokinetic cycler for DNAs that wouldn’t require more control than an on/off function would be an excellent choice for PCR. Furthermore, in most cases, the negatively charged DNAs are solely cycling due to electrophoresis, whereas electroosmosis is only a cause of sample diffusion and not used as driving force. Finally, having the heaters separated from the PCR reagents driving force brings the opportunity to use the cycler for other applications, such as mixing or Ligase Detection Reaction (LDR), a reaction realized on DNA and commonly used to detect gene mutations.

Parallelization of PCR cycling is another tempting goal for researchers, because it allows high throughput treatment (Sun, Nguyen et al. 2008). As the size of the on-chip cyclers decreases, several works have been dedicated to the parallelization. In particular, Park et al aims at integrating their device inside existing chemical plants, by choosing for example the shape of a
titer plate, with 96 or 384 devices. This work is particularly interesting since it could integrate any cycler made of polycarbonate that would fit into the 8x8 mm constraints (Park 2008).

1.3. Electrophoretron Principle

The principle of the electrophoretron was first introduced by Choi’s group in 2001. Their goal was to create an infinitely long channel for electrokinetic separation (Choi 2001). The setup of the prototype realized by this group is represented in Figure 2.

![Figure 2 Scheme of the Electrophoretron Prototype (Choi 2001)](image)

The innovative idea in this cycler was the combination of the electrophoretic and electroosmotic effects. Electrophoresis describes the motion of charged particles in the presence of an electrical field, while electroosmosis corresponds to the motion of the fluid induced in a microchannel when an electrical field is applied. In this prototype, two capillaries with opposite electroosmotic mobility are joined to form a close loop. When a voltage is applied across the loop, the buffer naturally starts cycling. If a sample, with adequate electrophoretic mobility, is introduced, it should also engage cycling once the potential difference is applied. Since the sample will only move in one direction due to electrophoresis, in one capillary, electrophoresis
and electroosmosis are complementary, whereas in the other, electroosmosis has to overcome the electrophoresis to achieve samples cycling. In the case of a micro scale electrophoretron, first introduced by Dr Nikitopoulos group (Elmajdoub 2006), the device is scaled down to a plastic chip where microchannels play the former role of the capillaries. In the schematic presented as Figure 3, assuming the electroosmosis is such as the buffer is cycling in the arrows direction and negatively charged particles, in Channel 1 showed in blue, electrophoresis has to be overcome by the electroosmosis, whereas in Channel 2 pictured in red color, electroosmosis and electrophoresis are complementary. Buffer and species flow are expected to go in the direction given by the arrow in both channels.

![Figure 3 Scheme of an Electrophoretron](image)

This explanation is valid as long as no other force enters in action. Since it is a close loop, pressure may build up inside the device, leading to an acceleration of the fluid in one part and deceleration in the other one.

The principle of this micro scale electrophoretron in polycarbonate (PC) to be used as a PCR cycler was demonstrated using simulations (Elmajdoub 2006).
1.4. Outline of the Thesis

This work is focused on the understanding of the possibilities and limitations of a microscale electrophoretron cycler used under PCR conditions and presents the first prototype to attempt cycling DNAs.

Chapter 2 exposes a thorough theoretical study of electroosmosis and electrophoresis in rectangular microchannels, leading to an extensive understanding of the device. This chapter closes with the search for an optimal design for PCR cycler and with a series of Monte Carlo simulations examining the influence of uncertainty on some parameters. The following chapter displays simulation setups and results, using a commercial solver adapted to electrokinetic at the micro scale. Chapter 4 consists in the study of two polymers, polycarbonate (PC) and Cycle Olefin Copolymers (COC), which were considered as substrate for the realization of the electrophoretron, while Chapter 5 explains the realization of the 1st prototype of the micro scale electrophoretron, from the design choice to the first experimental results. Finally, Chapter 6 concludes this work with conclusions and future objectives of this project.
Chapter 2 Theoretical Study of the Electrophoretron

2.1. Electrostatic Introduction

2.1.1. Dielectric Material (Bansal 2004)

A dielectric material is a material in which the dominant phenomena occurring in presence of an electrical field is polarization. Polarization consists in a reorganization of the electrical dipoles along the direction of the electrical field, creating another electrical field to oppose the first one and reach equilibrium. Because of the polarity of the water molecule, liquid water and all water based solutions are considered as dielectric materials.

2.1.1.1. Equation Derivation

The electrical flux density $\vec{D}$ is given by:

$$\vec{D} = \varepsilon_0 \vec{E} + \vec{P}$$

where $\varepsilon_0$ is the dielectric permittivity of the vacuum, $\vec{E}$ the electric field vector, and $\vec{P}$ the polarization vector.

Assumption: the medium is a linear isotropic dielectric:

$$\vec{P} = \varepsilon_0 \chi_e \vec{E}$$

$\chi_e$ is the electric susceptibility of the material, which characterizes its ability to get polarized (dimensionless).

The electrical flux becomes:

$$\vec{D} = \varepsilon_r \varepsilon_0 \vec{E}$$

with $\varepsilon_r$, dielectric permittivity of the medium ($\varepsilon_r = 1 + \chi_e$).

The Maxwell’s equations are given in Table 3.

<table>
<thead>
<tr>
<th></th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>$\nabla \times \vec{E} = -\frac{\partial \vec{B}}{\partial t}$</td>
</tr>
<tr>
<td>b</td>
<td>$\nabla \times \vec{B} = \vec{j} + \frac{\partial \vec{D}}{\partial t}$</td>
</tr>
<tr>
<td>c</td>
<td>$\nabla \cdot \vec{D} = \rho_e$</td>
</tr>
<tr>
<td>d</td>
<td>$\nabla \cdot \vec{B} = 0$</td>
</tr>
</tbody>
</table>
The different parameters of these equations are: \( \rho_e \) charge density, \( \vec{B} \) magnetic field vector, and \( \vec{J} \) the magnetic flux density vector.

The magnetic field vector can be expressed as:

\[
\vec{B} = \nabla \times \vec{A}
\]

The first Maxwell equation becomes then:

\[
\nabla \times \left( \vec{E} + \frac{\partial \vec{A}}{\partial t} \right) = 0
\]

This last equation means that there is an electrical potential \( \psi \) such that:

\[
\vec{E} + \frac{\partial \vec{A}}{\partial t} = -\nabla \psi
\]

From the third Maxwell’s equation and the expression of the electrical flux density we get:

\[
\nabla \cdot \vec{E} = \frac{\rho_e}{\varepsilon_r \varepsilon_0}
\]

In association with the last equation, we find:

\[
\nabla^2 \psi + \frac{\partial (\nabla \cdot \vec{A})}{\partial t} = -\frac{\rho_e}{\varepsilon_r \varepsilon_0}
\]

Using the Lorenz condition to decouple electric and magnetic field \( (\nabla \cdot \vec{A} = -\mu \varepsilon_r \varepsilon_0 \frac{\partial \psi}{\partial t}) \), the following result is obtained:

\[
\nabla^2 \psi - \mu \varepsilon_r \varepsilon_0 \frac{\partial^2 \psi}{\partial t^2} = -\frac{\rho_e}{\varepsilon_r \varepsilon_0}
\]

Assuming a static field, Poisson Equation (1), called Laplace equation when \( \rho_e = 0 \), is obtained:

\[
\nabla^2 \psi = -\frac{\rho_e}{\varepsilon \varepsilon_0}
\]

2.1.2. Electrical Double Layer (EDL) (Li 2004)

The Electrical Double Layer is responsible for the electroosmosis, which is the main effect on which the electrophoretron is based. First described by Helmholtz in the XIX\textsuperscript{th} century, the current mathematical model is from Chapman and Gouy.
2.1.2.1. Formation of the EDL

There are several origins to the charged surfaces when a solid surface enters in contact with a water based solution:

- Natural affinities of differently charged ions
- Ionization of surface group
- Charged surface in a crystal

When the EDL is forming, the solid surface in contact with the water based solution gets charged. Then counterions are attracted near the surface, whereas coions are rejected in the bulk flow. As represented in Figure 4, two different layers can be identified inside the Electrical Double Layer: the compact layer, which is constituted of immobilized counterions near the solid surface, and the diffuse layer, where ions (mostly counterions) are mobile and the electrical potential decreases up to zero when counterions and coions balance in the bulk fluid.

![Figure 4 EDL Schematization (Assuming the Solid Surface is Negatively Charged)](image)

As shown in Figure 4, these two layers are separated by the shear plane, which is generally used as boundary condition in fluid mechanics (null velocity but electric potential different from 0 and called zeta potential ($\zeta$)). It has to be underlined that this assumption is not the one considered for the simulations presented in Chapter 4.
2.1.2.2. Ion Distribution inside the EDL

Assumptions:

- Charged surface in contact with infinitely large medium
- Electrolyte solution at equilibrium (i.e. electrical and diffusion forces balance each other)

One may object the validity of this last assumption, but even though most liquid flows are not in equilibrium, as long as the Reynolds number $R_e$ is lower than 10, the ion distribution does not change much from the distribution found with this assumption (2).

- Homogeneous EDL field
  \[ \nabla \mu_i = -z_i e \nabla \psi \]

- 1 dimensional surface
  \[ \frac{d\mu_i}{dx} = -z_i e \frac{d\psi}{dx} \]

- Chemical potential per ion: $\mu_i = \mu_i^\infty + k_B T \ln n_i$

where $n_i$ is the number of ions of type $i$ per unit volume.

\[ k_B T \frac{d \ln n_i}{dx} = k_B T \frac{1}{n_i} \frac{dn_i}{dx} = -z_i e \frac{d\psi}{dx} \]

**Boundary Conditions**: as $x \to \infty$, $\psi \to 0$ and $n_i \to n_i^\infty$.

The integration of the last equation over the domain $x>0$ finally gives the ion concentration inside the EDL (2), found to follow a Boltzmann distribution.

\[ n_i = n_i^\infty \exp \left( \frac{-z_i e \psi}{k_B T} \right) \]  \hspace{1cm} (2)

2.1.2.3. Theoretical Model of the EDL

The aim of this part is to find the equation governing the electrical potential inside the EDL starting from (1) and (2).

The charge density inside an electrolyte may be calculated by:
\[ \rho_e = \sum_i z_i e n_i \]

where \( z_i \) is the charge of the ion I, \( e \) the electrical charge of an electron and \( n_i \) the concentration in this ion.

Using (2), the previous equation becomes:

\[ \rho_e = e \sum_i z_i n_i^e e^{-\frac{z_i \psi}{k_B T}} \]

In this equation, \( k_B \) is the Boltzmann constant, \( T \) the absolute temperature in Kelvin and \( \psi \) the electric potential.

Assuming a symmetric solution (i.e. as many cations as anions—for example KCl solution), the charge density can be described by the following equation:

\[ \rho_e = z e (n_+ - n_-) \]

Using (2), the charge density can be expressed as follows:

\[ \rho_e = z e n_+ \sinh \left( -\frac{z \psi}{k_B T} \right) \]

Inserting the last result into Poisson equation (1), Poisson-Boltzmann equation is obtained (3).

\[ \nabla^2 \psi = \frac{2ze n_+}{\epsilon_0} \sinh \left( \frac{ze \psi}{k_B T} \right) \]  

Equation (3) is usually solved in its dimensionless form (4), using the dimensionless electrical potential: \( \Psi = \frac{ze \psi}{k_B T} \) and the Debye Hückel parameter: \( k^2 = \frac{2ze^2 n_+}{\epsilon_0 k_B T} \).

\[ \nabla^2 \Psi = k^2 \sinh (\Psi) \]  

It is a fact commonly agreed on that the thickness of the EDL is proportional to \( 1/k \). Depending on the concentration, the permittivity and the temperature; \( 1/k \) varies from some nm up to some \( \mu \)m. In general, the higher the concentration, the smaller the EDL (this phenomenon is called EDL compression). The characterization of the EDL thickness, called Debye length and
commonly noted \( \lambda_d \) varies with the authors, but all are equivalent. For example, Probstein computes \( \lambda_d \) as follows (Probstein 1994):

\[
\lambda_d = \sqrt{\frac{\varepsilon_0 RT}{2P^2 z^2 n_\infty}} = \sqrt{\frac{\varepsilon_0 k_B N_A T}{2N_A^2 e^2 z^2 n_\infty}} = \sqrt{\frac{\varepsilon_0 k_B T}{2N_A e^2 z^2 n_\infty}} = \frac{1}{\kappa}
\]

In this expression \( n_\infty \) is the concentration in mol.m\(^{-3}\) and \( N_A \) the Avogadro number. The expressions given in the literature frequently seems different because of the relation \( 4\pi = \varepsilon_0 \).

Finally, Karniadakis (Karniadakis 2002) defines the Debye length for a non symmetrical electrolyte as follows:

\[
\lambda_d = \frac{1}{\kappa} = \sqrt{\frac{\varepsilon_0 k_B T}{N_A e^2 \sum_i n_{i,\infty} z_i^2}}
\]

This result is proportional to the one proposed by Probstein, showing no contradiction.

Assumption: the electrical potential is small compared to thermal energy: i.e. \( |ze\psi| \ll |k_B T| \) (Debye-Hückel approximation). This simplifies equations (3) and (4) to respectively give (5) and (6).

\[
\nabla^2 \psi = \frac{2zen_\infty ze\psi}{\varepsilon_0 k_B T}
\]

Equation (6) is sometimes called a Helmholtz equation and can be solved with a Partial Differential Equation solver, such as PDETool, a toolbox available in Matlab (Mathworks, Arlington, VA).

For an asymmetric electrolyte solution (for example LaCl3), the expression for the charge density becomes more complicated (7).

\[
\rho_e = e \sum_i z_i n_{i,\infty} e^{\frac{z_i e\psi}{k_B T}} = -3en_\infty \left( \exp\left(\frac{e\psi}{k_B T}\right) - \exp\left(-\frac{3e\psi}{k_B T}\right) \right)
\]
Though all other equations are still valid, no analytical solution exists for such a case, since the Debye-Hückel approximation can not simplify (7). However, these problems could be treated using simulations.

2.1.3. Electrical Potential inside Rectangular Microchannels (Li 2004)

This section focus on determining an analytical expression for the electrical potential inside a rectangular microchannel, using the parameters defined in Figure 5 and the Poisson-Boltzmann equation (6) restated below.

\[ \nabla^2 \psi = k^2 \psi \]

In Figure 5, W is the width of the channel, whereas the H is the depth.

![Figure 5 Rectangular Channel Parameters](image)

Using the following scaling: \( \bar{x} = \frac{x}{w} \), \( \bar{y} = \frac{y}{w} \), \( \bar{k} = kW \), and assuming that the length in the z-direction is much longer than the width and the depth of the channel, the Poisson Boltzmann equation can be simplified as follows.

\[ \frac{\partial^2 \psi}{\partial \bar{x}^2} + \frac{\partial^2 \psi}{\partial \bar{y}^2} = \bar{k}^2 \psi \]

In Figure 6, AR is the aspect ratio, defined as the ratio of the depth over the width \( AR = \frac{H}{w} \).
Boundary Conditions:

- Symmetry in the center of the channel: At $\bar{x} = 0$, $\frac{\partial \psi}{\partial \bar{x}} = 0$; at $\bar{y} = 0$, $\frac{\partial \psi}{\partial \bar{y}} = 0$.
- Electrical potential at the wall equal to zeta potential: at $\bar{x} = \frac{1}{2}$, $\Psi = \zeta$; at $\bar{y} = \frac{AR}{2}$, $\Psi = \zeta$.

After separation of variables and using the superposition theorem, the dimensionless electrical potential inside a rectangular microchannel (8) is found:

$$
\Psi = 4\bar{\zeta} \sum_{m=0}^{\infty} \frac{(-1)^m}{(2m+1)\pi} \cos\left((\pi + 2m\pi)\bar{x}\right) \frac{\cosh\left(\sqrt{\left(\frac{\pi + 2m\pi}{\pi + 4\pi}\right)^2 + k^2\bar{y}}\right)}{\cosh\left(\sqrt{\left(\frac{\pi + 2m\pi}{\pi + 4\pi}\right)^2 + k^2\bar{y}}\right)}
$$

$$
+ 4\bar{\zeta} \sum_{n=0}^{\infty} \frac{(-1)^n}{(2n+1)\pi} \cos\left(\frac{\pi + 2n\pi}{AR}\bar{y}\right) \frac{\cosh\left(\sqrt{\left(\frac{\pi + 2n\pi}{\pi + 4\pi}\right)^2 + k^2\bar{x}}\right)}{\cosh\left(\sqrt{\left(\frac{\pi + 2n\pi}{\pi + 4\pi}\right)^2 + k^2\bar{x}}\right)}
$$

Because both infinite series converge quickly (10 terms are sufficient for a $10^{-4}$ precision), this result can be easily plotted (Figure 7). This semi-analytical solution can be compared with the Partial Differential solver built in Matlab (PDE Toolbox), shown in Figure 8.
There is excellent agreement between Figure 7 and Figure 8, which validates our theoretical analysis. The major differences which may be observed are either due to a lack of term in the plot of the analytical solution or due to a mesh without enough refining using Matlab PDETool.
2.2. Fluidic Solution

2.2.1. Electroosmosis

Electroosmosis is the flow of a fluid due to the unbalanced ions repartition inside the EDL, when this fluid is placed in an electrical field. The flow moves due to the electrokinetic attraction of the excessive counterions concentration along the walls. In other words, as in the case depicted in Figure 9, an overabundance of positive ions (cations) generates a flow towards the negative electrode (cathode). Such case would be called a positive electroosmotic flow (EOF). Glass and most polymers without surface treatments show positive EOF (Shadpour 2005).

\[ \mu_{eo} = -\frac{\varepsilon \varepsilon_0 \zeta}{\mu} \] (9)

The strength of the EOF is usually characterized by the electroosmotic mobility, defined as in equation (9) (Probstein 1994), where \( \mu \) is the fluid viscosity.
2.2.2. Formulation (Li 2004)

As usual in Fluid Mechanics, the flow can be solved using the Navier Stokes flow equation (10), where \( \vec{F}_e \) is the electrical force, \( p \), the pressure, and \( \vec{v} \), the fluid velocity.

\[
\vec{F}_e + \mu \nabla^2 \vec{v} - \nabla p = 0
\]  

Projecting (10) along the z-axis gives:

\[
\frac{d^2w}{dx^2} + \frac{d^2w}{dy^2} = \frac{1}{\mu} \frac{dp}{dz} - \frac{\rho_e E_z}{\mu}
\]

where \( w \) the velocity and \( E_z \) the electrical field in the z-direction. Because of this projection, in this entire chapter, the velocities calculated and plotted are all stream wise. Cross stream velocities will be studied by the only means of simulations (c.f. Chapter 3) but are negligible in the straight parts of the device.

Using the scaling: \( \vec{w} = \frac{w}{w_0} \), \( \vec{p} = \frac{p-p_0}{\rho_l w_0^2} \) (scaling by the dynamic pressure), \( \vec{E}_z = \frac{E_z W_R e_0}{\varepsilon_0} \), with \( R_{e_0} = \frac{\rho_l W w_0}{\mu} \) (Reynolds Number) and \( F_1 = \frac{2 \varepsilon_0 \varepsilon_n}{\rho_l w_0^2} \), this equality becomes:

\[
\frac{d^2\vec{w}}{dx^2} + \frac{d^2\vec{w}}{dy^2} = R_{e_0} \frac{d\vec{p}}{dz} + F_1 \vec{E}_z \sinh \Psi
\]

Boundary Conditions:

- Symmetry relatively to the channel centerline : at \( \vec{x} = 0 \), \( \frac{d\vec{w}}{d\vec{x}} = 0 \), \( \vec{y} = 0 \), \( \frac{d\vec{w}}{d\vec{y}} = 0 \)
  
- No slip at the walls; at \( \vec{x} = \frac{H}{2h} \), \( \vec{w} = 0 \), \( \vec{y} = \frac{w}{2h} \), \( \vec{w} = 0 \)

2.2.3. Any Point Solution

Using Green’s function (Duffy 2001), the exposed problem can be solved. The detailed calculations are in Appendix B. The velocity in a microchannel under the effect of a longitudinal electrical field can be expressed by the following expression:
\[ \bar{w}(x,y) = -\frac{16}{\pi^2} \frac{d}{dz} \int_0^\infty \int_0^\infty \cos((2m-1)\pi \bar{x}) \cos\left(\frac{(2n-1)\pi \bar{y}}{AR}\right) \frac{4}{\pi^2 AR} F_1 F_2 \]

\times \left[ \frac{\cos((2m-1)\pi \bar{x}) \cos\left(\frac{(2n-1)\pi \bar{y}}{AR}\right)}{(2m-1)^2 + \left(\frac{2n-1}{AR}\right)^2} \right] \int_{x=-\frac{1}{2}}^{x=\frac{1}{2}} \int_{y=-\frac{AR}{2}}^{y=\frac{AR}{2}} \cos((2m-1)\pi \bar{x}) \cos\left(\frac{(2n-1)\pi \bar{y}}{AR}\right) \sinh \Psi \, d\bar{x} \, d\bar{y} \]

This solution is still dependent on an integral that will be called \( I_{m,n} \), which can be calculated as follows:

1. Using the Debye approximation: \( \sinh \Psi \sim \Psi \)

\[ I_{m,n} = \int_{x=-\frac{1}{2}}^{x=\frac{1}{2}} \int_{y=-\frac{AR}{2}}^{y=\frac{AR}{2}} \cos((2m-1)\pi \bar{x}) \cos\left(\frac{(2n-1)\pi \bar{y}}{AR}\right) \psi(\bar{x}, \bar{y}, W, AR) \, d\bar{x} \, d\bar{y} \]

2. The electrical potential inside a rectangular microchannel when a voltage difference is applied was computed in the previous section and can also be expressed as:

\[ \Psi = 4\xi \sum_{j=1}^\infty \frac{(-1)^{j+1}}{(2j-1)\pi} \cos((-1 + 2j)\pi \bar{x}) \frac{\cosh\left(\sqrt{(-\pi + 2j\pi)^2 + k^2 \bar{y}}\right)}{\cosh\left(\sqrt{(-\pi + 2j\pi)^2 + k^2 \frac{AR}{2}}\right)} \]

\[ + 4\xi \sum_{l=1}^\infty \frac{(-1)^{l+1}}{(2l-1)\pi} \cos\left(-1 + 2l\pi \frac{\bar{x}}{AR}\right) \frac{\cosh\left(\sqrt{\left(-\pi + 2l\pi\right)^2 + k^2 \bar{x}}\right)}{\cosh\left(\sqrt{\left(-\pi + 2l\pi\right)^2 + k^2 \frac{1}{2}}\right)} \]

3. After calculation (see Appendix C), the result is:

\[ I_{m,n}(AR) = \frac{4 \, AR \, \xi (-1)^{m+n}}{(2m-1)^2 \pi^2 + k^2 + \left(\frac{2n-1}{AR}\right)^2} \times \frac{\left(\frac{2n-1}{AR}\right)^2 + (2m-1)^2}{(2m-1)(2n-1)} \]

4. Finally, the stream wise flow velocity inside a rectangular microchannel is explicitly found (11).
2.2.4. Average Velocity

Equation (11) can easily be averaged over the cross section.

$$\bar{w}(\bar{x}, \bar{y}) = \int_{\text{Crosssection}} \bar{w}(\bar{x}, \bar{y}) \, d\bar{x}d\bar{y}$$

This integration finally gives the dimensionless flow velocity at any cross section inside a rectangular microchannel (12), which is also the expression of the dimensionless flowrate.

$$\bar{w}_{av}(W, AR) = \int_{\text{Crosssection}} \bar{w}(\bar{x}, \bar{y}) \, d\bar{x}d\bar{y}$$

2.3. Fluid Flow in the Electrophoretron

To find the velocity at any point in our device, the same process used by Elmajdoub (Elmajdoub 2006) is followed, with the more accurate assumption of a rectangular channel rather than a cylindrical one. However, this last choice was proven to be a reasonable way to treat the basic functioning of our device, as supported by the simulations (Elmajdoub 2006).
2.3.1. General Results

2.3.1.1. Electroosmotic Velocity in a Rectangular Microchannel

Using that $\bar{w} = \frac{w}{w_0}$, $k^2 = \frac{2z^2e^2n_e}{\varepsilon_0k_BT}$, $\bar{p} = \frac{p - p_0}{\rho_0w_0^2}$, $\bar{E_z} = \frac{E_zWRe_0}{z_0}$, with $Re_0 = \frac{\rho_0w_0}{\mu}$, $\zeta = \frac{ze^2}{k_BT}$ and $F_1 = \frac{2z\varepsilon_0e^2}{\rho_0w_0^2}$, dimensional results can be found. Furthermore, the definition of the electroosmotic mobility (9) was used to find the electroosmotic velocity at any point inside a rectangular microchannel (13) and its averaged value over the channel cross section (14) while a potential difference $\Delta \phi$ is applied.

\[
\begin{align*}
\bar{w}(x, y) & = \sum_{m=1}^{\infty} \sum_{n=1}^{\infty} \frac{(-1)^{m+n} \cos \left( \frac{2m-1}{W_i} \pi x \right) \cos \left( \frac{2n-1}{H_i} \pi y \right)}{(2m-1)(2n-1) \left[ \left( \frac{2m-1}{W_i} \right)^2 + \left( \frac{2n-1}{H_i} \right)^2 \right]^2} + \frac{16 \Delta \phi}{\pi^2 L_i k^2 \mu_{eo,i}} \\
& \quad \times \sum_{m=1}^{\infty} \sum_{n=1}^{\infty} \frac{(-1)^{m+n} \cos \left( \frac{2m-1}{W_i} \pi x \right) \cos \left( \frac{2n-1}{H_i} \pi y \right)}{(2m-1)^2 + (2n-1)^2 + k^2} \\
\end{align*}
\]  

(13)

\[
\begin{align*}
\bar{w}_{av}(W, H) & = \sum_{m=1}^{\infty} \sum_{n=1}^{\infty} \frac{1}{(2m-1)^2(2n-1)^2 \left[ \left( \frac{2m-1}{W} \right)^2 + \left( \frac{2n-1}{H^2} \right)^2 \right]} + \frac{64 \Delta \phi}{\pi^4 L_i k^2 \mu_{eo}} \\
& \quad \times \sum_{m=1}^{\infty} \sum_{n=1}^{\infty} \frac{1}{(2m-1)^2(2n-1)^2 \left[ \left( \frac{2m-1}{W} \right)^2 + (2n-1)^2 \right]^2} \\
\end{align*}
\]  

(14)

The flowrate (15) can be found by multiplying (14) by the cross section of the channel $HW$.

\[
\begin{align*}
Q(W, H) & = \frac{1}{\mu L} \sum_{m=1}^{\infty} \sum_{n=1}^{\infty} \frac{1}{(2m-1)^2(2n-1)^2 \left[ \left( \frac{2m-1}{W} \right)^2 + (2n-1)^2 \right]} \\
& \quad + \frac{64 \Delta \phi}{\pi^4 L_i k^2 \mu_{eo}} \sum_{m=1}^{\infty} \sum_{n=1}^{\infty} \frac{1}{(2m-1)^2(2n-1)^2 \left[ \left( \frac{2m-1}{W} \right)^2 + (2n-1)^2 \right]^2} \\
\end{align*}
\]  

(15)
2.3.1.2. Pressure Drop inside an Electrophoretron

As specified in Chapter 1 and can be seen in Figure 10, an electrophoretron is a close loop microchannel where part of the loop has reversed electroosmotic mobility. In all the following, we will consider that width, depth, length, and electroosmotic mobility may differ between the two parts. As a reference, the channel with natural positive electroosmotic mobility will be called Channel 1, whereas the part with reverse electroosmotic mobility will be referred as Channel 2.

As a convention, a positive velocity will have the direction given by the blue arrow in Figure 10. In other words, in normal functioning of our device, Channel 1 velocity will be positive whereas Channel 2 velocity will be negative. Moreover, since the electrophoretron is a closed loop, what flows in one channel is going to be transferred in the other. Assuming no leakage, the mass is conserved from one channel to the other, this translates into:

\[ Q_1(W_1, H_1) = -Q_2(W_2, H_2) \]

Using (15) in this last equality, the pressure drop inside an electrophoretron while a potential difference \( \Delta \phi \) is applied can be determined (16).
\[ \Delta p = \pi^2 \mu \Delta \varphi \ k^2 \times \frac{\sum_{m=1}^{\infty} \sum_{n=1}^{\infty} \frac{1}{(2m-1)^2(2n-1)^2}}{\sum_{m=1}^{\infty} \sum_{n=1}^{\infty} (2m-1)^2(2n-1)^2} \left( \frac{\mu_{eo1} H_1 W_1}{L_1 a_{m,n,1}} + \frac{\mu_{eo2} H_2 W_2}{L_2 a_{m,n,2}} \right) \]  

with \(a_{m,n,i} = \left(\frac{(2m-1)\pi}{W_i}\right)^2 + k^2 + \left(\frac{(2n-1)\pi}{H_i}\right)^2\) and \(b_{m,n,i} = \left(\frac{2m-1}{W_i}\right)^2 + \left(\frac{2n-1}{H_i}\right)^2\) \((i=1, \text{ or } 2)\).

Note: \([a_{m,n,i}] = [b_{m,n,i}] = \text{Length}^{-2}\)

### 2.3.1.3. Velocity inside the Electrophoretron

Replacing the expression of the pressure drop (16) into (13), we get the velocity inside Channel \(i\) of the electrophoretron (17), from which the centerline and the average velocities can easily be found.

\[
w_i(x,y) = \frac{16 \Delta \varphi}{\pi^2 L_i} k^2 \left( \frac{\mu_{eo,i} \sum_{m=1}^{\infty} \sum_{n=1}^{\infty} (-1)^{m+n} \cos \left( \frac{2m-1}{W_i} \pi x \right) \cos \left( \frac{2n-1}{H_i} \pi y \right)}{(2m-1)(2n-1)a_{m,n,i}} \right) \]

\[
= \frac{\sum_{m=1}^{\infty} \sum_{n=1}^{\infty} \frac{1}{(2m-1)^2(2n-1)^2}}{\sum_{m=1}^{\infty} \sum_{n=1}^{\infty} (2m-1)^2(2n-1)^2} \left( \frac{\mu_{eo1} H_1 W_1}{L_1 a_{m,n,1}} + \frac{\mu_{eo2} H_2 W_2}{L_2 a_{m,n,2}} \right) \times \frac{\sum_{m=1}^{\infty} \sum_{n=1}^{\infty} (-1)^{m+n} \cos \left( \frac{2m-1}{W_i} \pi x \right) \cos \left( \frac{2n-1}{H_i} \pi y \right)}{(2m-1)(2n-1)b_{m,n,i}} \]

### 2.3.2. Simplified Results

#### 2.3.2.1. Assumption

Assuming that the Debye layer is much thinner than the microchannel cross section implies:

\[ k \gg \frac{1}{W_i}, \frac{1}{H_i} \]

Then, \(a_{m,n,i}\) can be simplified as follows:
2.3.2.2. Validity of the Assumption

The critical length is given by the smallest depth in the main channel, varying from 25\(\mu\)m to 50 \(\mu\)m (c.f. Chapter 5). Using the definition from Karnidakis for the Debye length in asymmetric solution, the subsequent shows numerical values for 2 different buffers:

\[
\lambda_d = \frac{1}{\kappa} = \sqrt{\frac{\epsilon\epsilon_0 k_B T}{N_A e^2 \sum_i n_{i,\infty} z_i^2}}
\]

- **Buffer composition 1**: 1.5 mM MgCl\(_2\), 50 mM KCl, 10 mM Tris-HCl, and 1% Polymerase Buffer (100 mM KCl and 10 mM Tris-HCl)

\[
\sum_i n_{i,\infty} z_i^2 = 0.030002 \text{ M. L}^{-1}
\]

\[
\begin{array}{c|c|c}
T=25^\circ\text{C} & T=95^\circ\text{C} \\
\lambda_d & 79.4 \text{ nm} & 88.2 \text{ nm}
\end{array}
\]

\(\lambda_d\) was computed at room temperature (25\(^\circ\)C) because principle demonstration experiments are conducted in this configuration (c.f. Chapter 5). However it was also considered at 95\(^\circ\)C since this temperature represents a worst case scenario; indeed, 95\(^\circ\)C is the highest temperature at which the electrophoretron will ultimately be used.

Saying that \(W_i \gg \lambda_d\), would commonly be mathematically translated into \(W_i \leq 10^{-3}\lambda_d\). This condition is not met with this buffer, particularly at high temperature, nevertheless the order of magnitude is satisfying. Consequently, the velocity profile will defer from the one expected in the region next to the walls. Therefore, the centerline velocity predicted by our model in Channel 1 should always be valid. This will be particularly important for the optimized configuration search that will be conducted using the centerline velocity in Channel 1.
Buffer composition 2: 1.5 mM MgCl₂, 10 mM Tris-HCl, and 1% Polymerase Buffer (100 mM KCl and 10 mM Tris-HCl)

This buffer had been used previously in order to reduce the current induced in the buffer when applying a difference of potential, resulting in a decrease of gas production due to electrolysis (Chen 2005). As calculated below, this buffer has an alternate consequence: it increases the ionic strength, leading to an increase of the Debye length.

\[
\sum \frac{n_{i,z} z_i^2}{T=25^\circ C \quad T=95^\circ C} = 6.104 \times 10^{-3} \text{ M} \cdot \text{L}^{-1}
\]

\[
\lambda_d \quad 176 \text{ nm} \quad 196 \text{ nm}
\]

Using this buffer, our assumption will be twice less valid, thus the wall zone in which the velocity profiles are no trustworthy should be twice as large.

2.3.2.3. Series Convergence

Normal convergence of both series appearing in (17) gives:

\[
\sum_{m=1}^{\infty} \sum_{n=1}^{\infty} \frac{(-1)^{m+n} \cos \left(\frac{2m-1}{W_i} \pi x\right) \cos \left(\frac{2n-1}{H_i} \pi y\right)}{(2m-1)(2n-1)}
\]

\[
= \sum_{m=1}^{\infty} \frac{(-1)^m \cos \left(\frac{2m-1}{W_i} \pi x\right)}{2m-1} \times \sum_{n=1}^{\infty} \frac{(-1)^n \cos \left(\frac{2n-1}{H_i} \pi y\right)}{2n-1}
\]

\[
= \sum_{m=1}^{\infty} \sum_{n=1}^{\infty} \frac{1}{(2m-1)^2(2n-1)^2} = \sum_{m=1}^{\infty} \frac{1}{(2m-1)^2} \times \sum_{n=1}^{\infty} \frac{1}{(2n-1)^2}
\]

Furthermore, the development in Fourier series of \( \pi \) provides:

\[
\forall x \in \mathbb{R}, \quad \pi = 4 \sum_{n=1}^{\infty} \frac{(-1)^n \cos((2n-1)x)}{(2n-1)}
\]
Thus,
\[
\sum_{m=1}^{\infty} \sum_{n=1}^{\infty} \frac{(-1)^{m+n} \cos \left( \frac{2m - 1}{W_i} \pi x \right) \cos \left( \frac{2n - 1}{H_i} \pi y \right)}{(2m - 1)(2n - 1)} = \frac{\pi^2}{16} \tag{18}
\]

Moreover, \( \pi \) may also be expressed by:
\[
\pi^2 = 8 \sum_{m=0}^{\infty} \frac{1}{(2m + 1)^2} = 8 \sum_{m=1}^{\infty} \frac{1}{(2m - 1)^2}
\]

Thus,
\[
\sum_{m=1}^{\infty} \sum_{n=1}^{\infty} \frac{1}{(2m - 1)^2(2n - 1)^2} = \frac{\pi^4}{64} \tag{19}
\]

### 2.3.2.4. New Parameters

To simplify (17), new parameters are defined:
\[
g_i = \sum_{m=1}^{\infty} \sum_{n=1}^{\infty} \frac{1}{(2m - 1)^2(2n - 1)^2} b_{m,n,i}
\]
\[
F_i(x, y) = \sum_{m=1}^{\infty} \sum_{n=1}^{\infty} \frac{(-1)^{m+n} \cos \left( \frac{2m - 1}{W_i} \pi x \right) \cos \left( \frac{2n - 1}{H_i} \pi y \right)}{(2m - 1)(2n - 1) b_{m,n,i}}
\]
\[
f_i = F_i(0,0) = \sum_{m=1}^{\infty} \sum_{n=1}^{\infty} \frac{(-1)^{m+n}}{(2m - 1)(2n - 1) b_{m,n,i}}
\]

with \( b_{m,n,i}(W_i, H_i) = \left( \frac{2m - 1}{W_i} \right)^2 + \left( \frac{2n - 1}{H_i} \right)^2 \).

### 2.3.2.5. Pressure Drop

These simplifications ((17), (18) and (19)) and parameters can be used to shorten the expression of the pressure drop created by the flow circulation in an electrophoretron while a potential difference \( \Delta \phi \) is applied (20):
\[
\Delta p = \frac{\pi^6}{64} \frac{\mu_0 H_1 W_1}{L_1} \frac{\mu_0 H_2 W_2}{L_2} \left( \frac{1}{g_1} + \frac{H_1 W_1}{L_1} \frac{1}{g_2} + \frac{H_2 W_2}{L_2} \frac{1}{g_2} \right) \tag{20}
\]
2.3.2.6. Velocity

2.3.2.6.1. Velocity Derivation

With the same simplifications, the velocity at any point in Channel $i$ inside an electrophoretron can be found (21).

$$w_i(x,y) = \frac{\Delta \varphi}{L_i} \left( \mu_{eo,i} - \frac{\pi^2}{4} F_i(x,y) \frac{\mu_{eo1} H_i W_i}{L_1} + \frac{\mu_{eo2} H_i W_i}{L_2} \right)$$  \hspace{1cm} (21)

2.3.2.6.2. Velocity Plot

From (21), the flow velocity profile inside the electrophoretron can be plotted in both channels, as can be seen in Figure 11. These plots were obtained using Matlab in order to evaluate $F_i$, and exported in Tecplot 360 (Bellevue, WA) to ease graphic manipulation. It has to be emphasized that the apparent infinite series $F_i$ actually converges extremely quickly (10 terms are sufficient to obtain a $10^{-4}$ precision). The code written to obtain the plot is presented in Appendix D.1. One flow velocity profile for each Channel (i.e. $i=1$ or 2) is provided.

a ) Channel 2

b ) Channel 1

Figure 11 Flow Stream Wise Velocity Profiles
(Length= 8 mm, \(\frac{L_1}{L_2} =1.97\), \(\frac{W_2}{W_1} = \frac{H_2}{H_1} =1\), and \(\frac{H_i}{W_i}=3\))
Using the same configuration allowed the comparison with previously reported simulations results in Figure 12.

a) Analytical Solution

b) Simulations Results

Figure 12 Flow Stream Wise Velocity Profiles in Channel 1
(Length = 8 mm, \( \frac{L_1}{L_2} = 1.97 \), \( \frac{W_2}{W_1} = \frac{H_2}{H_1} = 1 \), and \( \frac{H_1}{W_1} = 3 \))

One can notice how similar both profiles in Figure 12 are. The shape of the velocity profile is the same in both theory results and simulations, and even longitudinal velocity values are extremely close. The differences may be explained by the bends effects that can only be taken into account in the simulations. Indeed, the velocities found in the simulations show a reduction in magnitude when compared to the theory results. Because the velocity close to the wall is mainly determined by the EOF, it is logical that the decrease is essentially noticed in the center part of the channel.

It has to be emphasized though that the validity of the thin EDL assumption cannot be evaluated by these simulations; the same assumption is actually used by the solver (c.f. Chapter 3).

Centerline (22) and average (23) velocity in Channel \( i \) can also be computed.

\[
 w_{\text{center},i}(W_i, H_i) = \frac{\Delta \varphi}{L_i} \left( \mu_{eo,i} - \frac{\pi^2}{4} f_i \left( \frac{\mu_{eo1} H_1 W_i}{L_1} + \frac{\mu_{eo2} H_2 W_2}{L_2} \right) \right) 
\]  

(22)
2.3.2.6.3. Centerline Velocity Comparison between Rectangular and Cylindrical Channels

Assuming square channels of side $a_i$: $H_i = W_i = a_i$, the centerline velocity is then modified as follows:

$$w_{\text{center},i}(a_i) = \frac{\Delta \varphi}{L_i} \left( \mu_{e_{0,i}} - \frac{\pi^2}{4} f_i \left( \frac{\mu_{e_{01}}}{L_1} \frac{a_1^2}{g_1} + \frac{\mu_{e_{02}}}{L_2} \frac{a_2^2}{g_2} \right) \right)$$

The different parameters are modified as follows:

$$b_{m,n,i}(a_i) = \frac{(2m - 1)^2 + (2n - 1)^2}{a_i^2}$$

$$g_i = a_i^2 \sum_{m=1}^{\infty} \sum_{n=1}^{\infty} \frac{1}{(2m - 1)^2 (2n - 1)^2 [(2m - 1)^2 + (2n - 1)^2]}$$

$$f_i = a_i^2 \sum_{m=1}^{\infty} \sum_{n=1}^{\infty} \frac{(-1)^{m+n}}{(2m - 1)(2n - 1) [(2m - 1)^2 + (2n - 1)^2]}$$

Given that (computed with Matlab)

$$\frac{\sum_{m=1}^{\infty} \sum_{n=1}^{\infty} (-1)^{m+n}}{(2m - 1)(2n - 1) [(2m - 1)^2 + (2n - 1)^2]} \approx 0.8496$$

The centerline velocity in Channel $i$ for an electrophoretron with square cross section (side $a_i$) is given by the following expression:

$$w_{\text{center},\text{square},i}(a_i) = \frac{\Delta \varphi}{L_i} \left( \mu_{e_{0,i}} - 2.0963 \ a_i^2 \times \frac{\mu_{e_{01}}}{L_1} \frac{a_1^4}{a_1^4 + a_2^4} + \frac{\mu_{e_{02}}}{L_2} \frac{a_2^4}{a_1^4 + a_2^4} \right)$$

This result should be compared to the centerline velocity with a cylindrical cross section (radius $a_i$):
The good adequacy (same form, similar numerical values) between these two results validates our solution. It can also be noticed that considering a cylindrical channel instead of square channel tend to increase (in absolute value) the centerline velocity in Channel i.

### 2.3.3. Dimensionless Results

In order to be able to compare velocity results from both channels to run an optimization routine, a common dimensionless velocity must be selected. The chosen parameter is the electroosmotic velocity generated by a straight channel of the total length of our loop when applying the potential difference above it, assuming the whole loop has the electroosmotic mobility of Channel 1, which can be translated numerically (24).

\[
W_{0,1} = \frac{\mu_{eo,1}}{L_1 + L_2} \frac{\Delta \varphi}{L_1 + L_2} \tag{24}
\]

Defining the dimensionless ratios \( \alpha = \frac{L_1}{L_2}, \gamma = \frac{H_2}{H_1}, \) and \( \beta = \frac{W_2}{W_1}, \) the dimensionless flow velocities at any point inside an electrophoretron become (25: Channel 1) and (26: Channel 2):

\[
\bar{w}_1(x,y) = \left(1 + \frac{1}{\alpha}\right) \left(1 - \frac{\pi^2}{4} F_1(x,y) \frac{1 + \frac{\mu_{eo2}}{\mu_{eo1}} \gamma \alpha \beta}{g_1 + \gamma \alpha \beta g_2}\right) \tag{25}
\]

\[
\bar{w}_2(x,y) = (1 + \alpha) \left(\frac{\mu_{eo2}}{\mu_{eo1}} \frac{\pi^2}{4} F_2(x,y) \frac{1 + \frac{\mu_{eo2}}{\mu_{eo1}} \gamma \alpha \beta}{g_1 + \gamma \alpha \beta g_2}\right) \tag{26}
\]

Neither \( g_i, f_i \) nor \( F_i \) are dimensionless, so in order to get the velocity expressions depending only on dimensionless parameters, they are redefined as follows:

\[ f_i = W_i^2 f_i'; \quad g_i = W_i^2 g_i'; \quad F_i = W_i^2 F_i' \]
One may notice that \( g_{1}', f_{1}' \) and \( F_{1}' \) do not depend on any dimensional parameter but only on the aspect ratio \( (AR_1 = \frac{H_1}{W_1}) \). (25) and (26) become then (27) and (28) respectively.

\[
\bar{w}_1 = \left( 1 + \frac{1}{\alpha} \right) \left( 1 - \frac{\pi^2}{4} F_1'(x,y) \frac{1 + \frac{\mu_{e02}}{\mu_{e01}} \frac{AR_2}{AR_1} \alpha \beta^2}{g_{1}' + \frac{AR_2}{AR_1} \beta^4 g_{2}'} \right) \tag{27}
\]

\[
\bar{w}_2 = (1 + \alpha) \left( \frac{\mu_{e02}}{\mu_{e01}} - \frac{\pi^2}{4} F_2'(x,y) \beta^2 \frac{1 + \frac{\mu_{e02}}{\mu_{e01}} \frac{AR_2}{AR_1} \alpha \beta^2}{g_{1}' + \frac{AR_2}{AR_1} \beta^4 g_{2}'} \right) \tag{28}
\]

2.4. Species Flow inside an Electrophoretron

2.4.1. Electrophoresis

Electrophoresis describes the motion of charged particles inside an electrical field, as shown in Figure 13.

![Figure 13 Motion of DNA (Negatively Charged) under an Electrical Field](image)

It had been shown (Probstein 1994) that electrophoresis occurring in a microchannel filled with a fluid have negligible influence on the fluid flow field. In other words, electroosmosis and electrophoresis are decoupled. Physically, the electrophoresis velocity can be interpreted as the relative velocity of the species in the moving frame of the fluid.
As well as electroosmosis, a molecule electrophoresis is characterized by its electrophoretic mobility (29), where $\zeta$ here is the zeta potential of the species.

$$\mu_{\text{eph}} = \frac{e \varepsilon_0 \zeta}{\mu} \quad (29)$$

In addition, the electrophoretic velocity can be related to its mobility and the electrical field by the following equation:

$$\vec{u}_{\text{eph}} = \mu_{\text{eph}} \vec{E}$$

### 2.4.2. Species Velocity at any Point inside an Electrophoretron

Since electroosmosis and electrophoresis are decoupled, the following equation is valid to obtain the velocity of the species inside the electrophoretron:

$$\vec{u}_{\text{species}} = \vec{u}_{\text{eph}} + \vec{u}_{\text{eo}}$$

Projecting this equation along the channel axis allows getting the velocity of the species at any point in Channel $i$ of the electrophoretron cycler (30).

$$w_{\text{species},i}(x,y) = \frac{\Delta \varphi}{L_i} \left( \mu_{\text{eo},i} + \mu_{\text{eph}} - \frac{\pi^2}{4} F_1(x,y) \right) \left( \frac{\mu_{\text{eo}1} H_{11} W_1}{L_1} + \frac{\mu_{\text{eo}2} H_{22} W_2}{L_2} \right)$$

(30)

One may object that because electrophoresis is the only dispersion effect taken into consideration in this analytical study and diffusion is neglected, the analytical expression of the velocity of the species (30) is not as realistic as the flow velocity (21) can be. This is a valid argument; however, the diffusion, which is coupled with the flow velocity, will be taken into account with the simulations presented in Chapter 3.

After using the scaling defined in section 2.3.3, the dimensionless expressions of the species velocities in both channels can be found (31: Channel 1) and (32: Channel 2).

$$w_{\text{species},1} = \left( 1 + \frac{1}{\alpha} \right) \left( 1 + \frac{\mu_{\text{eph}}}{\mu_{\text{eo}1}} \cdot \frac{\pi^2}{4} F_1'(x,y) \frac{1 + \frac{\mu_{\text{eo}2}}{\mu_{\text{eo}1}} \frac{A R_2}{A R_1} \alpha^2}{g_1' + \frac{A R_2}{A R_1} \beta^4 g_2'} \right)$$

(31)
2.5. Geometry Optimization

2.5.1. Motivation and Principle

Previous electroosmotic measurements (Elmajdoub 2006) showed low electroosmotic values in polycarbonate (PC), the polymer aimed to be used for the electrophoretron prototype. In order to reduce DNA cycling time, an optimal situation would be one maximizing the velocity in the unmodified channel, namely Channel 1. Since its EOF mobility cannot be increased, the only possible modification is building up a pressure drop using geometry variation. Because the wall velocity is mainly dictated by the EOF mobility, increasing the mean velocity in Channel 1 means increasing the center velocity (that will further be designated as the maximum velocity in Channel 1). It has to be emphasized that in this case the maximum velocity (in absolute value) in Channel 2 can be found at the walls, as illustrated in Figure 14.

\[
\overline{w_{\text{species},z}} = (1 + \alpha) \left( \frac{\mu_{\text{eo}2}}{\mu_{\text{eo}1}} + \frac{\mu_{\text{eph}}}{\mu_{\text{eo}1}} - \frac{\pi^2}{4} F_2'(x,y) \beta^2 \left( 1 + \frac{\mu_{\text{eo}2} AR_2}{\mu_{\text{eo}1} AR_1} \alpha \beta^2 \right) \frac{g_1'}{g_1'} + \frac{AR_2}{AR_1} \alpha \beta^4 g_2' \right)
\]

(32)

Figure 14 Flow Velocity Profiles
(Length = 8 mm, \( \alpha=2.77 \), \( \beta=\gamma=1 \), and ARi=3)
In other words, increasing the center velocity in Channel 1 will decrease the center velocity in Channel 2. Practically, if the built up pressure gets too important, it is possible to observe reverse flow in the center of Channel 2. This case is illustrated in Figure 15, where the whole middle section of Channel 2 shows reverse flow. However, since in this channel, DNAs are electrophoretically attracted in the same direction as the wall velocity, it is not critical towards DNA cycling, which is the electrophoretron purpose.

Another important motivation of creating this type of velocity profiles is to prevent the DNA species to go into the wall region, in order to limit wall absorption. Indeed, the theory of particles inside a pipe stipulates that:
1. If the particles lag behind the flow, they will be going towards higher velocity gradient.

2. If the particles lead the flow, they will be going down the gradients.

Assumption: DNAs’ behavior inside a microchannel is comparable to particles behavior in macro size channels

In Channel 1, due to electrophoresis, DNAs are willing to go in the direction opposite of the electroosmotic flow. DNAs will then be attracted towards higher velocity gradients, i.e. towards the center part. On the other hand, DNAs are ahead of the flow in Channel 2: descending gradients, they will also tend to go to the center part.

2.5.2. Cylindrical Channel

From the analytical study realized in a previous work (Elmajdoub 2006), and using the same notations as before, the velocity at any point in an electrophoretron considering a cylindrical channel geometry is:

\[
w_{cyl,1}(r, a_1) = \frac{\Delta \varphi}{L_1} \left( \frac{\mu_{eo,1} a_1^2}{L_1} + \frac{\mu_{eo,2} a_2^2}{L_2} \right) \left( 1 - \left( \frac{r}{a_1} \right)^2 \right) \left( \frac{L_1}{a_1^4} + \frac{L_2}{a_2^4} \right)
\]

As explained earlier, our goal is to obtain a velocity profile which would maximize the velocity in the center of Channel 1.

Explicitly, we are looking for \((a_i)_{i=1,2}\) such as maximizing:

\[
w_{center,cyl,1} = \frac{\Delta \varphi}{L_1} \left( \frac{\mu_{eo,1} a_1^2}{L_1} + \frac{\mu_{eo,2} a_2^2}{L_2} \right) \left( 1 - \left( \frac{r}{a_1} \right)^2 \right) \left( \frac{L_1}{a_1^4} + \frac{L_2}{a_2^4} \right)
\]

Using the \(\alpha\) and \(\beta\) parameters defined earlier, the expression to maximize can be rewritten as:

\[
w_{center,cyl,1} = \frac{\Delta \varphi}{L_1} \left( \mu_{eo,1} - 2 \frac{\mu_{eo,1} a_1^2}{L_1} + \frac{\mu_{eo,2} a_2^2}{L_2} \right) \left( 1 - \left( \frac{r}{a_1} \right)^2 \right) \left( \frac{L_1}{a_1^4} + \frac{L_2}{a_2^4} \right)
\]
Deriving relatively to $\beta$ (assuming $\alpha$ constant) and equalizing this expression to 0, the $\beta$ maximizing $w_{\text{center,cyl,1}}$ is solution of:

$$\beta^4 + 2 \frac{\mu_{e01}}{\mu_{e02} \alpha} \beta^2 - \frac{1}{\alpha} = 0$$

Considering only the positive root, the optimum $\beta$ would have the following expression:

$$\beta_{\text{opt}} = \sqrt[4]{-\frac{\mu_{e01}}{\mu_{e02} \alpha} + \sqrt{\left(\frac{\mu_{e01}}{\mu_{e02} \alpha}\right)^2 + 1}}$$

It has to be noticed that this result depends on $\alpha$, that should be chosen arbitrarily or optimized by a comparable method (while assuming $\beta$ constant).

This result can be used to find an optimum radii set, that can then be translated into width and depth values using the hydraulic diameter. The main inconvenient using this method is that we do not take into account the more precise results that we derived previously with the rectangular channel assumption, resulting in a loss of precision. Moreover, the aspect ratios are not optimized and have to be subjectively selected. Finally, this analytical derivation simply proved that optimum solutions might be derived but that another method should be used in order to provide precise results.

### 2.5.3. Rectangular Channel

For a rectangular channel, the center velocity in Channel 1 ((32) at $x = y = 0$) depends on 5 parameters ($\alpha$, $\beta$, $\gamma$, AR$_1$, and AR$_2$), linked by one relation (33), making it a 4th degree problem. In other words, a simple derivation would not be sufficient to find an optimal solution.

$$\frac{\beta}{\gamma} = \frac{\text{AR}_1}{\text{AR}_2} \quad (33)$$

To solve this problem, a Matlab code was written (Appendix D.2), using the Optimization Toolbox. The process consists of initializing each parameter and constraining them (values given
in Table 4) before using the function “fmincon” of Matlab that locates the maximum (or precisely the minimum of the opposite) of the given function.

<table>
<thead>
<tr>
<th></th>
<th>Minimum</th>
<th>Initial</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>AR$_1$</td>
<td>1</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>AR$_2$</td>
<td>0.1</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>1/3</td>
<td>1.33</td>
<td>3</td>
</tr>
<tr>
<td>$\beta$</td>
<td>0.1</td>
<td>2.6</td>
<td>10</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>0.5</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

The results generated by the program are the maximum value of the centerline velocity Channel 1 found, as well as values of the parameters to reach it. Moreover, the overall cycling time may be computed from the mean velocities. However, the result found using this code is affected by the limitations imposed on the parameters, but also by their initial values. This initialization must then be done carefully since it is likely that the program will stop when finding a local maximum close to these values. After noticing that some parameters always seemed limited by the same boundary, some constraints were relaxed to observe how the “maximized” solution evolved. For instance, the maximum values of $\alpha$ and $\beta$ were increased, resulting in a set of optimized solutions. The final decision among these was taken by weighing the parameters that we were more willing to relax, and/or to what extent we were willing to do so, using graphs as Figure 16.

As can be seen in Figure 16, there are two types of optimization results: considering $\gamma$ as a variable or considering $\gamma=1$. The reason for that is simple: having $\gamma$ different from 1 means having a depth variation. Because single side hot embossing was the only fabrication method
considered, a change of depth implies having asymmetric geometry in the z-direction. In other words, having a change of depth is a possibility, but its utility had to be studied with caution.

In Figure 16, the interest of the change of depth is not very strong. From this figure, one can see that although the cylindrical optimization was drastically improving the design, the optimization with Matlab improves it even more.

To consider the worst case scenario, the optimization search was conducted with the most unfavorable measurements of EOF mobility (c.f. Chapter 4). The results, plotted in Figure 17, will be used to determine the designs to be selected for the prototypes.

Obviously, all 3 designs that showed a positive mean DNA velocity in Channel 1 were selected to figure on the final mold insert. A design with no depth change, Design 10, was also added in case the change of depth would create problems.
2.6. Monte Carlo Simulations-Effect of Mobilities Uncertainties and Geometry Variations

To consider the uncertainties on the physical characteristics of the electrophoretron, such as the electroosmotic mobility, we conducted some Monte Carlo simulations.

The principle of a Monte Carlo simulation is very simple: considering statistical distributions of the entry parameters, such as a Gaussian or a Uniform distribution, arbitrary values are chosen among these distributions and results are computed for this set of values. Repeating the process for a number of entry sets N gives the statistical distribution of the results.

2.6.1. Simulations Conditions

Since they usually provide a realistic representation of experimental data, Gaussian distributions (34) were selected as entry distributions.

\[ f(x) = \frac{1}{\sigma \sqrt{2\pi}} \exp \left[ -\frac{1}{2} \left( \frac{x - \bar{x}}{\sigma} \right)^2 \right] \] (34)
The equation (34) gives the density of a Gaussian distribution. This density is function of \( \sigma \), the standard deviation, and \( \bar{x} \) the mean value of the parameter \( x \) (34). In other words, characterizing a Gaussian distribution requires the standard deviation and the mean value.

For the electroosmotic mobility, the means and standard deviations from actual experimental data were used; whereas for geometrical variations, the following assumptions were considered:

- Channels width and depth variations: 3 \( \mu \)m
- Channels length variations 1 mm

From these assumptions, standard deviation was found using the logarithmic derivative. The detailed calculations are given for \( \beta \) and results are provided for \( \gamma \), \( AR_i \) and \( \alpha \). \( \beta \) was defined as:

\[
\beta = \frac{W_2}{W_1}
\]

Using the logarithmic derivative on the precedent equation gives:

\[
\frac{d\beta}{\beta} = \frac{dW_2}{W_2} - \frac{dW_1}{W_1}
\]

This result leads to the following relative variation of \( \beta \):

\[
\frac{\Delta \beta}{\beta} = \frac{\Delta W_2}{W_2} + \frac{\Delta W_1}{W_1}
\]

The relative variation then directly gives the absolute variation:

\[
\Delta \beta = \frac{W_2}{W_1} \left( \frac{\Delta W_2}{W_2} + \frac{\Delta W_1}{W_1} \right)
\]

Using \( \Delta W_2 = \Delta W_1 = \Delta W \), the \( \beta \) absolute variation can be simplified (35).

\[
\Delta \beta = \frac{\Delta W}{W_1} (1 + \beta) \quad (35)
\]

Doing the same computation with \( \gamma \), \( AR_i \) (36) and \( \alpha \) (37), their absolute variation can be found.

\[
\Delta \gamma = \frac{\Delta W}{H_1} (1 + \gamma) \quad \Delta AR_i = \frac{\Delta W}{W_i} (1 + AR_i) \quad (36)
\]
All results were simulated $N=10^6$ times and the simulations were implemented using Matlab. The part of the code corresponding directly to the Monte Carlo simulation was based on an open source code (Wittwer 2004), and the results were computed with the analytical expressions computed in earlier. The full code is given in Appendix D.3.

The different designs considered in these simulations are the four designs selected in section 2.5 as to figure on the mold insert used to realize the first prototypes of the electrophoretron.

2.6.2. Results

The statistical distributions were plotted in order to compare the efficiency of the different selected optimized designs. Statistical distributions are usually presented using vertical bars; however, for visibility while plotting values for the four selected design on the same graph, a line representation was chosen. Firstly, the species velocities (in the center of the channel, at the wall, or averaged over the cross section) in the different channels of the electrophoretron were plotted. These three velocities of interest in Channel 1 are presented in Figure 18. The same plots for Channel 2 are provided in Appendix E.

One may notice in Figure 18-c that the different designs have no effect on the minimum velocity. This lack of efficiency may be explained by the fact that the minimum velocity here is the wall velocity, i.e. the pressure drop created by the geometry optimization does not affect it. The main conclusion from Figure 18-a & b is that the optimized designs actually increase mean and maximum velocities in Channel 1, compared to Figure 18-c, which would be the overall velocity without additional pressure drop. 

\[
\Delta \alpha = \frac{\Delta L}{L_T} (1 + \alpha)^2 \tag{37}
\]
In other words, the optimization of the design is valuable, even considering the variations in the mobilities and the geometry. All designs have a positive impact, but this impact becomes less important from Design 1 to 10. The statistical distribution of the overall cycling time was determined by means of the averaged velocities in Channels 1 and 2. The Channel 1 dimensionless cycling time statistical distribution is plotted in Figure 19.
In Figure 19, it is again clear that designs 1, 2, 3 and 10 procure less and less optimization in this order. The effect of depth change (Designs 1, 2 & 3) appears really strong, because these designs feature most probable cycling time much smaller than Design 10. Moreover, this most probable value has much more probability to be attained in the case of the designs with change of depths.

Additional information, such as the percentage of working cases, i.e. percentage of cases in which the mean velocity in Channel 1 is positive (Figure 20), or the comparison between the most probable values and the theory predicted values (Figure 21), were also computed and plotted. The biggest interest of Figure 20 is probably that it permits to summarize the overall statistical distributions in one plot simple to compare and analyze.
Figure 20 Percentage of Working Cases (Channel 1 Velocity Positive Condition)
The green bars show the percentage of working cases calculated from the minimum velocity in
Channel 1, while the red ones illustrate the same percentage based on the mean velocity in
Channel 1.

The relative difference between the red and green bars in Figure 20 illustrates once more
the positive effect of all optimized designs. Design 1 appears again as the most desirable
configuration, while designs 1, 2 and 3 present all a probability higher than 50% to work.

Figure 21 presents the comparison between the velocities that can be calculated from the
mean values of all parameters and the most probable velocities given by the Monte Carlo
simulations while taking into account the uncertainties on these parameters. The immediate
conclusion that can be drawn from Figure 21 is that the most probable velocity in Channel 1 are
higher when taking into account the uncertainty on the mobilities and the geometry than with
straight forward calculation. Although it might seem surprising that taking into account the
uncertainties provides a higher working probability, this can be explained by the facts that mean
mobilities in PC are extremely unfavorable for the device to work (c.f. Chapter 4) and that the uncertainties on mobilities have much more impact than the uncertainties on the geometrical parameters. Indeed, it is to be expected that the same plot only taking into account the geometrical uncertainties would show a decrease in velocities.

![Figure 21 Comparison of the Mean Dimensionless Velocity in Channel 1 between the Theory Prediction and the Most Expected Value from the MC Simulations](image)

However, the effect of the different designs is overall the same between the results found via Monte Carlo simulations and the analytical theory. The only examples that do not verify that are Designs 2 and 3; indeed, design 3 shows a higher most expected value than 2 from the simulations, whereas the analysis would let think the opposite. However, this result can not be seen in Figure 18-b and thus is probably due to a mistake in the program evaluating the most probable values. Therefore, we can conclude that the analysis presented earlier is sufficient to evaluate the efficiency of the different designs found in the optimized configuration search.
2.6.3. Conclusions

In conclusion, Monte Carlo simulations allowed a better understanding on the designs requirements for the electrophoretron to work. Indeed, all optimized designs showed good probability to serve their purpose while considering the important uncertainties on mobilities and geometry. Furthermore, we also concluded that these simulations usually provide the same trend as the simple analysis, resulting in no need to conduct these CPU consuming simulations to choose the most desirable designs but only to illustrate the uncertainties effect.
Chapter 3 Simulations

3.1. Purpose

Concerning the principle demonstration, most of the computational part of this project had already been completed previously (Elmajdoub 2006). Moreover, since the analytical study of the present work has been more exhaustive and has proved providing comparable velocity profiles as simulations (c.f. Chapter 2), the latest simulations presented in this chapter serve more precise purposes:

- Confirming working conditions using new electroosmotic measurements
- Taking into account realistic micro channel features such as reservoirs, electrodes, geometrical variations, and exact length.

3.2. Simulations with Coventorware 2006®

Coventorware 2006 (Cary, NC) was used to simulate the functioning of the electrophoretron. The solver selected (only one of the available solvers in Coventorware) was NetFlow, which is dedicated to electrokinetic flows. More precisely, NetFlow plays the role of an interface between the user and Fluent (Ansys Inc, Canonsburg, PA), which is the actual solver.

3.2.1. Equations Solved by the NetFlow Solver

The equations solved by the NetFlow solver are the same as the one solved analytically in Chapter 2, with comparable assumptions. These equations are summarized in Table 5.

<table>
<thead>
<tr>
<th>Equations</th>
<th>Table 5 Equations Solved with NetFlow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrical Potential</td>
<td>( \nabla^2 \Psi = k^2 \Psi ) (6)</td>
</tr>
<tr>
<td>Flow</td>
<td>( \rho \vec{E} + \mu \nabla^2 \vec{v}_{eo} - \nabla p = 0 ) (10)</td>
</tr>
<tr>
<td>Species</td>
<td>( \frac{\partial c}{\partial t} + (\vec{v}<em>{eo} + \vec{v}</em>{eph}) \nabla c = D \nabla^2 c ) (38)</td>
</tr>
</tbody>
</table>
The only additional equation (38) is used to resolve species motion. In this equation, D is the diffusion coefficient for DNA species and c their concentration. The diffusion can then be taken into account in the simulations whereas it wasn’t in the theoretical analysis. As exhaustively described by Chen, there are different types of diffusion (molecular, thermal, hydrodynamic, etc…) (Chen 2006), which all interfere with a nice plug flow of the species.

3.2.2. Simulations Set Up

Considering the similarity in the models, some previous findings (Elmajdoub 2006) were used directly without further demonstration. For instance, a new grid independency study was not conducted and in all cases, an Extruded Bricks Pave-Q-Morph mesh was used. Moreover, when preparing the simulations, the finite volume element option was chosen as well. The simulation results could also be validated using the analytical solution developed.

All geometry designs were first realized on AutoCAD 2008 (Autodesk, San Rafael, CA), using as many layers as required to take into account the different boundary conditions. Unfortunately, because of the specificities of the design, no simplification could be done using symmetry. Once these designs had been converted in native geometry files in Coventor, the solid model was created using the processor file. The mesh was then generated based on this solid model. Since a full-size geometry was considered and in order to limit the number of cells, the x-y element size was set quite important in the overall geometry (as big as 75 µm in some cases). However, it was refined in the main channels to get at least 8 to 10 cells by width, while the z elements were simply limited to 8 to 10 cells in the whole geometry. Once the mesh is generated, the solver selected and all parameters defined, the simulation can be started. After convergence, the results are directly transmitted to the Coventorware interface. Most results are directly plotted with Tecplot and can be saved independently at that point.
The value of the diffusion coefficient considered in all simulations is 120 µm²/s. If compared with results from Chen, the value considered for D represents the worst case scenario for the PCR cycler application. Indeed, this result is valid for very small DNAs (smaller 200 bp) and for high temperature (close to 100°C).

When time dependent simulations are run, the choice of time step can have a drastic impact on the simulations (Table 6). Depending on the duration of the phenomena studied, once the number of output timesteps is chosen (usually larger than 10), computing timestep can be selected. This timestep should be at least 10 times smaller than the output timestep. However, a smaller timestep might be required to completely resolve the species. In case of unexpected loss of species for example, reducing the computing timestep to 1/100th or 1/1000th of the output timestep, especially at the beginning of the simulation, might give better results (Rani 2008).

<table>
<thead>
<tr>
<th>Table 6 Example of Timestep Selection</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Time (cycling time)</strong></td>
</tr>
<tr>
<td><strong>Number of Timesteps</strong></td>
</tr>
<tr>
<td><strong>Output Timestep</strong></td>
</tr>
<tr>
<td><strong>Computing Timestep</strong></td>
</tr>
</tbody>
</table>

In conclusion, Coventorware 2006 is extremely user-friendly software, which has the main drawback of leaving little action for the user. For example, the data files are inaccessible until the simulation completely converged, making it impossible to visualize only partially converged data sets. This tends to make the debugging extremely difficult, especially when the simulations would not converge.

3.3. Cases

3.3.1. Cycling Comparison between PC and COC

These first simulations were realized previously to new EOF measurements and only aimed to validate cycling in the conclusions given in the previous work (Elmajdoub 2006).
Indeed, the length of the two channels was chosen in this case order to minimize the pressure drop, which is totally opposed to what was exposed at the end of Chapter 2. The values of EOF mobilities used for PC and COC in these simulations are the one previously reported in the literature (Elmajdoub 2006; Pu 2007).

Table 7 EOF Mobility Values in cm²/(V.s) Used in these Simulations (c.f. 3.4.1)

<table>
<thead>
<tr>
<th></th>
<th>Pristine Polymer</th>
<th>Aminated Polymer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Channel 1</td>
<td>Channel 2</td>
</tr>
<tr>
<td>PC (Elmajdoub 2006)</td>
<td>3.934</td>
<td>-1.366</td>
</tr>
<tr>
<td>COC (Pu 2007)</td>
<td>5.3</td>
<td>-1.9</td>
</tr>
</tbody>
</table>

3.3.2. Geometrical Variations

This set of simulations was only conducted in order to study the effect of the width change. The theoretical analysis presented in Chapter 2 was demonstrated to be sufficient to predict stream wise velocity profiles with confidence far from the change of width. However, simulations should be used in order to obtain predictions at the transition zones. Depth changes were not simulated because they would require a different mesh, involving a new grid independence study in order to be validated. The design used for this simulation is Design 10, previously selected to be one of the first prototypes of the electrophoretron. A 3D representation of Design in shown in Figure 22.

These simulations, realized later in the project, were done with more realistic values for PC with PCR buffer (c.f. Table 8).

Table 8 EOF Mobility Values in cm²/(V.s) Used in these Simulations (c.f.3.4.2)

<table>
<thead>
<tr>
<th></th>
<th>Pristine Polymer</th>
<th>Aminated Polymer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Channel 1</td>
<td>Channel 2</td>
</tr>
<tr>
<td>PC</td>
<td>2.3</td>
<td>-2.31</td>
</tr>
</tbody>
</table>
3.4. Results

3.4.1. Cycling Comparison between PC and COC

DNAs’ success in cycling in the COC electrophoretron is illustrated in Figure 23. As can be noticed, more than a quarter of the path is covered in 16 s (i.e. ~18% of the overall cycling time). This change in velocity is due to the change of material: Channel 2, with reverse EOF mobility, is the first channel in which the DNAs pass through. This effect is even more prominent in the same evolution in PC depicted in Figure 24, where only the first timestep shows an important DNAs position change.
The diffusion effect of opposed electrophoresis is also more visible in Figure 24, which shows similar evolution in a PC-based device, since the mobility ratio $\mu_{\text{eph}}/\mu_{\text{eo,1}}$ is larger in this case than for COC.
Figure 24 Evolution with Time of DNAs inside a Microscale Electrophoretron in PC

The interest of preferring COC over PC, if COC actually features EOF mobility as high as reported, is evident from the comparison between these two simulations. Indeed, the time required to complete a cycle in the first channel made of COC is more than twice as fast as the one required in the channel made of PC. However, this is only based on the electroosmotic mobility values and does not take into account other properties such as the wettability.
3.4.2. Geometrical Variations

The main result of this set of simulations is constituted by the flow structures at the changes of width. This zone follows and precedes the material change and the electrodes position, whose effects were thoroughly studied previously without width variation (Elmajdoub, Nikitopoulos et al. 2006). In all detailed pictures shown below the electroosmotic flow and the species are going from right to left.

Figure 25 shows the logical effect of the diverging channel: strong gradients of the cross stream secondary velocity. The width variation has also an impact on the electrode zone since the streamlines starts diverging in this region. This can be seen in Figure 26-a, where there is inversion between the direction of high magnitude cross-stream velocity, when moving away from the negative electrode zone.

![Figure 25 Cross-Stream Secondary Velocity Profile and Streamlines in the Diverging Channel Velocities are given µm/s. The blue area represents the location of the – electrode (Anode) on the top side.](image)

Figure 26 allows a complete understanding of the 3D flow motion at the electrodes. This motion is briefly reminded below but was already observed and thoroughly explained in comparable manner in simulations without width change.
A three dimensional flow occurs in the electrodes zone due to the shape of the electrical field as shown Figure 26-a, the change in electroosmotic mobility between the two channels, and mass conservation. Explicitly, because the electrical field is vertical in the electrode region, the electroosmosis effect creates a close to the wall flow motion towards the negative electrode and away from the positive one. Mass conservation implies then an opposed flow motion in the center region. This vertical cross stream velocities variations are visible in Figure 26-b in the case of the cathode and represented at both electrodes in Figure 27. This cross stream circulation also explains the horizontal cross stream opposed velocities in Figure 26-a and the curvature of the central plane streamlines visible in Figure 26-b.

Figure 26 Negative Electrode (Cathode) Region (a) Horizontal Cross-Stream Velocity with Electrical Field Lines- (b) Center Plane Streamlines with Vertical Cross-Stream Velocity

Figure 27 Cross Stream Flow Motion in the Electrodes Regions
(a) Negative Electrode (b) Positive Electrode
Furthermore, the flow motion is complicated by the separation vortex occurring because of the zeta potential change at the extremity of the electrode area. This vortex is clearly visible in Figure 28.

Figure 28 Streamlines-Visualization of the Separation Vortex at the Negative Electrode (Cathode)
The rake is issued in the bottom 10 µm of the channel.

All these flow features will also appear on the flow of DNA species, which also undergo electrophoretic attraction or repulsion from the positive or negative electrode respectively. In other words, DNAs motion at the electrodes is reinforced by the flow, at the exception of the separation vortex zone.

3.5. Conclusions

The simulations were extremely useful in the process of understanding the whole complexity of the problem. However, because the length scale of all process studied here
(electroosmosis, electrophoresis) is extremely small (order of magnitude of the dozens of nm), even the simulation of a device that can fit into a 8 mm x 8 mm square was extremely time and CPU consuming, especially when dealing with time dependent simulations. These limitations explained the moderate use of this powerful tool.
Chapter 4 Material Study

In micro-devices history, used materials have traditionally been glass, PolyMethyl Methacrylate (PMMA), Polydimethylsiloxane (PDMS) or Polycarbonate (PC) (Henry 2000; Witek 2004; Shadpour 2005; Chen 2006). However, even though manufacturers propose these polymers with variety of additives, these materials have limited possibilities. For an electrophoretron used as PCR reaction cycler, the main properties of interest are the electroosmotic mobility at pH 8.3 (with PCR buffer) and the ability to chemically reverse this mobility in part of the microchannel. To the best of our knowledge, such ability has been demonstrated on three different polymers commonly used for MEMs fabrication: PMMA (Henry 2000), PC (Elmajdoub 2006), and Cycle Olefin Copolymer (COC) (Pu 2007). Moreover, higher mobility of the pristine polymer enables our application. Ideally, this electroosmotic mobility should even be higher in absolute value than the electrophoretic mobility of DNA ($-3.75 \times 10^{-4}$ cm$^2$/Vs) (Stellwagen 1997).

On one hand, both PMMA and PC have been widely used for multiple on chip application, including PCR cyclers. Therefore, their properties and fabrication processes have been extensively studied and documented. However, for the micro scale electrophoretron; PMMA was rapidly abandoned for this application because of its low glass transition temperature and the fact that it showed the lowest electroosmotic mobility (Elmajdoub 2006).

On the other hand, some Topas® COC have been reported to have very high electroosmotic mobility in basic buffer (Pu 2007): up to $5 \times 10^{-4}$ cm$^2$/Vs, i.e 66% higher than for PC. In addition, COCs is a particularly attractive type of material because as a copolymer, almost any additives combination is possible, leading to infinite properties possibilities. For our application, a high glass transition temperature is necessary to ensure no deformation while operating the device at temperature up to 95°C. This property is determined by the amount of
cycle-olefin comonomer: the higher it is, the higher the heat resistance is. In the present study, PC and some Topas COCs (5013, 6013, 6015, 8007) have then been studied for their different properties in order to choose adequate material for principle demonstration and best working conditions. Regarding to the heat resistance, the more suitable grade of COC for PCR application would be 6015, whereas 5013 and 6013 might work and 8007 can not be envisaged.

4.1. Experimental Section

4.1.1. Modifications

On both PC and COC, the modification process used to reverse the electroosmotic mobility was demonstrated earlier (Pu 2007) and is based on the amination of carboxylic groups. The main difference consists in the method used to create these carboxylic groups on the polymer surface (Figure 29).

a) PC

b) COC (Pu 2007)

Figure 29 Process Used to Reverse EOF Mobility on (a) PC and (b) COC
Details in Table 9 and Table 10
As can be seen in Figure 29, on COC, the creation of carboxylic groups relies on the photografting of a Methacrylic Acid film on the COC surface. In other words, a MAA layer is polymerized on COC, using UV light as an activator in this process. The use of UV exposure (specifications of this UV exposure figure in Table 9 and Table 10) in both carboxylic groups’ creation processes is a deliberate choice allowing the manipulator to control precisely where the surface modification occurs.

<table>
<thead>
<tr>
<th>Table 9 Polycarbonate Modification Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Step</strong></td>
</tr>
<tr>
<td>UV exposure</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Amination solution (1mL)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Amination exposure</td>
</tr>
<tr>
<td>Cleaning</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

It has to be emphasized that when the surface modification has to be realized inside microchannels, the processes presented in Figure 29 have to be slightly modified in order to incorporate thermal bonding of the coverplate on top of the embossed polymer sheet. On both PC and COC, this additional step would be located between the creation of the carboxylic groups and the amination solution exposure. The effect of this thermal bonding on the surface properties will be evaluated with water contact angle (c.f. 4.1.3.).
Table 10 COC Modification Conditions (Modified from Pu et al.)

<table>
<thead>
<tr>
<th>Step</th>
<th>Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV exposure</td>
<td>Wavelength: 254 nm</td>
</tr>
<tr>
<td></td>
<td>Power: 18 mW/cm²</td>
</tr>
<tr>
<td></td>
<td>Time: 20 min</td>
</tr>
<tr>
<td>MAA solution</td>
<td>0.1% Benzophenone (diluted in Acetone)</td>
</tr>
<tr>
<td></td>
<td>10% v/v 99.9% MAA</td>
</tr>
<tr>
<td></td>
<td>Steering: &gt; 2 hours</td>
</tr>
<tr>
<td>Filtering through</td>
<td>0.2µm nylon filters</td>
</tr>
<tr>
<td>MAA exposure</td>
<td>Synchronized with the UV exposure</td>
</tr>
<tr>
<td>Cleaning</td>
<td>(DI Water Rinse + 15 min sonication) × 3</td>
</tr>
<tr>
<td></td>
<td>Dried with pure air jet</td>
</tr>
<tr>
<td>Amination solution</td>
<td>48 mg of EDC</td>
</tr>
<tr>
<td>(1mL)</td>
<td>250 mL of 0.1 M Ethylenediamine solution</td>
</tr>
<tr>
<td>Amination exposure</td>
<td>Between 2 and 12 hours</td>
</tr>
</tbody>
</table>

The difference between both processes can be explained by the fact that unlike PC, COC does not contain Oxygen in its composition, and simple UV exposure would not generate carboxylic groups on its surface. However, MAA contains a full carboxylic group as circled in its chemical structure, presented as Figure 30.

![Methacrylic Acid Chemical Structure](image)

Figure 30 Methacrylic Acid Chemical Structure  
(Carboxylic Group is circled)
Consequently, polymerizing MAA on the COC surface covers it with extremely high density carboxylic groups, as reported by Pu.

However, this treatment on COC had previously only been reported on one type of COC: Topas® 8007. Because of its low glass transition temperature ($T_g$) this specific type wouldn’t suit our application. Reproducing the treatment on types of Topas® COC with higher $T_g$ (5013, 6013 and 6015) was attempted. To demonstrate carboxylic sites presence on the surface of COC after MAA polymerization, the process previously used after UV exposure of PMMA (Wei 2005) was chosen (c.f. 4.1.2.).

4.1.2. Fluorescence Characterization of Carboxylic Groups

To characterize carboxylic groups presence, the last step of the modification process (Figure 29-b) is replaced by a Fluorescein Glycine Amide treatment (Molecular Probes, Invitrogen, Carlsbad, California) (Figure 31). Details of this last stage figure in Table 11. Moreover, as can be seen in Figure 31, the UV exposure is this time realized through a Nickel grid in order to provide a control. The grid also offers a way to assess the achieved precision on the location of the surface chemistry modification. As a control, the fluorescence process was employed on UV PMMA, following the procedure described in Wei’s article.

Table 11 Details on Fluorescence Process (Modified from Wei 2005)

<table>
<thead>
<tr>
<th>Step</th>
<th>Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fluorescence solution</strong></td>
<td>0.5 mM of EDC</td>
</tr>
<tr>
<td></td>
<td>0.5 mM of Fluorescein Glycine Amide</td>
</tr>
<tr>
<td></td>
<td>in pH 7.0 100 mM Phosphate Buffer</td>
</tr>
<tr>
<td><strong>Fluorescence exposure</strong></td>
<td>&gt;12 hours protected from UV light</td>
</tr>
<tr>
<td><strong>Cleaning</strong></td>
<td>Rinse with 100 mM Phosphate Buffer</td>
</tr>
<tr>
<td></td>
<td>Dried with pure air jet</td>
</tr>
</tbody>
</table>
After treatment, pictures are taken immediately by means of a Nikon Photoshot FxA fluorescence microscope. The excitation and emission filters were centered at 488 nm and 520, respectively.

Since auto-fluorescence has been reported to be important for these types of COC at the wavelength used (Mair 2006), all experiments were done on 0.5 mm thick polymer sheets. To reach this thickness, the 5013, 6013 and 6015 COC samples were micro-milled from the original 1/8” thick inject molded sheet, whereas the 8007 COC was actually directly injected into a mold of this thickness by Topas®.

![Fluorescence Process](image)

**Figure 31 Characterization of Carboxylic Groups via Fluorescence Process**

### 4.1.3. Topology Measurements

Since MAA is photografted on COC surface, theoretically, a new polymer layer is added on top of the COC. The thickness of this layer has to be precisely evaluated so that it can be taken into account while designing microchannels. Indeed, thicknesses as high as several microns had been reported on COC 8007 (Pu 2007), which would not be not negligible in a 50
µm wide microchannel. Ellipsometry was initially considered to conduct these measurements, but was abandoned since COC was not a reflective surface. Indeed, to use ellipsometry, COC would have had to be coated on a gold surface for example.

4.1.3.1. Contact Profilometer

A contact profilometer (Tencor, Milpitas, CA) was used as a first attempt to determine the thickness of the MAA film. However, precise measurements were found difficult to realize. This could be explained by the very small size of the grid used (the Ni grid used was about 25µm wide), probably too small compared to the resolution of the profilometer. Finally, an Atomic Force Microscope was employed to obtain 3D measurements.

4.1.3.2. Atomic Force Microscope (AFM)

The Atomic Force Microscopes used were Agilent 550 and 5500. The following describes the method used while doing topology measurements.

4.1.3.2.1. Limitations and Possibilities

The maximum height that can be measured with this AFM is 6 µm, but it is particularly appropriate to measure a height with an order of magnitude of 1 µm, with a resolution of 0.1 Å. The maximum range is 100 µm.

The two main modes used are Contact Mode and AC or Tapping Mode. In the former, the tip is screening the surface by staying in constant contact with it, whereas in the latter, the tip is vibrating and, depending on the material hardness, the amplitude (hard material) or the phase (soft material) decreases when getting in contact of the surface. To avoid damaging the surface, it is preferable to have reduced amplitude vibrations when getting closer to it.

In AC mode, the parameters deflection and friction that can be measured allow the characterization of two different physical properties of the material: deflection measures topological variations whereas the friction characterizes the surface chemistry.
4.1.3.2.2. AFM Use

After starting computer, light source, controllers and Picoview1.4 software, the AFM tip, at the end of which is the cantilever, should be mounted on the scanner and this one on the holder. Then, once the scanner is connected to the AFM, the laser light is adjusted so that it illuminates the top of the cantilever. Finally, the photodiode detector is aligned in order to receive the laser reflection on the cantilever. The position of the detector can be evaluated using the sum, deflection and friction parameters (visible on Picoview Software and on the controller display). The last step before performing the measurements is to approach the scanner close to the sample (fixed on the magnetic holder via double sided tape). For it is the most delicate manipulation, software help should be used to approach the scanner from the surface. To avoid damaging the scanner, the final approach should always be done with the Picoview software.

For topology measurements, scanning was realized on relatively wide areas (between 50 and 100 um side square) in AC mode using silicone cantilever. Some measurements were also done using the Contact Mode in an attempt to characterize the COC surface chemistry change after the fluorescence characterization of carboxylic groups. Silicon cantilevers were still used in this case, even though silicone nitrate ones, more flexible, could be less prone to generate surface damage.

After scanning, data was processed using the freeware Gwyddion. This software allows getting the 3D representations (Figure 37 & Figure 38), as well as profile (Figure 39), from which it is easier to evaluate the MAA layer thickness.

4.1.4. Contact Angle Measurements

The first purpose of the contact angle study was to evaluate the wettability to understand the effect of the different steps on the modification process (c.f. 4.1.1). Studying the consequence of the coverplate bonding on PC was done in an attempt to determine a bonding process for chips
UV irradiated on only half their surface. This will also allow realistic evaluation of the pressure drop due to the capillary forces in both parts of the device.

### 4.1.4.1. Background

Water contact angle can be used to characterize the wettability of a material. This may be important in term of characterization of the surface tension but also to evaluate the capillary forces when a microchannel is first wetted.

The contact angle of a liquid on a solid surface in a gaseous atmosphere is measured between the tangent to the droplet and the interface liquid/solid, as shown in broad outline on Figure 32. Of course, two water contact angles can be measured from a 2D picture, but they should normally be identical if the material is isotropic.

![Figure 32 Water Contact Angle (a) Hydrophilic Surface (b) Hydrophobic Surface](image-url)
Two different types of material may be differentiated with respect to their water contact angle. With a water contact angle over 90° (Figure 32-b), a material is called hydrophobic (e.g. Teflon), while if below 90°(Figure 32-a) it is hydrophilic (e.g. glass).

These measurements are extremely sensitive to humidity/temperature conditions, water properties, as well as to the algorithms used to compute the contact angle. That’s why several measurements need to be done and averaged to have trustable values. Furthermore, the contact angle may vary with time, meaning that, for consistent results, measurements should be realized after the same amount of time after the water droplet deposition.

The additional pressure gradient due to capillary forces while first wetting a channel can be evaluated using the Young Laplace equation (39).

\[ \Delta p = \frac{4\gamma \cos (\theta)}{D_h} \]  

(39)

where \( \gamma \) is the surface tension at the water/air interface, \( \Theta \) is the contact angle and \( D_h \) the hydraulic diameter.

One might object that the contact angle between the actual buffer and the solid surface may slightly vary from the contact angle between distilled water and the surface, but the trend and the order of magnitude should be the same. Indeed, the PCR buffer is a water-based solution.

4.1.4.2. Experimental Conditions

Contact angle measurements were carried using the sessile drop method (Shadpour 2005). All measurements were realized at room temperature (~70°F), with approximately 2 \( \mu \)L water droplets manually deposed on the surface with a pipette. The water used was Nanopure water (18 MΩ). All polymer samples were preliminary cleaned following the same process (Alconox solution, DI Water rinse, IPA rinse, DI Water rinse, Dried with pure air jet). The different cases that were studied are summarized in Table 12.
Table 12 Contact Angle Measurements Realized
“M” stands for “Measured”

<table>
<thead>
<tr>
<th>No Further Treatment</th>
<th>NH₂ Exposure to 150°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC</td>
<td>M</td>
</tr>
<tr>
<td>UV PC</td>
<td>M</td>
</tr>
<tr>
<td>COC</td>
<td>M</td>
</tr>
<tr>
<td>Modified COC</td>
<td>M</td>
</tr>
</tbody>
</table>

The results reported here were all computed using VCA 2000 software (VCA, Billerica, MA). To compute the contact angle, points were manually selected on the digital pictures: the liquid/solid interface extremities, the top of the droplet, and 2 other points at the air/liquid interface. These points were adjusted so that both contact angles have the same value. 95% confidence intervals were directly computed from the measurements (40).

$$\Delta \theta = \frac{\sigma t_{95}}{\sqrt{n}} \quad (40)$$

where $\sigma$ is the standard deviation, $t_{95}$ is the 95th percentile of the Student distribution for the (n-1) degree of freedom and n is the number of measurements.

Using this method to evaluate the uncertainty rather than the common standard deviation, allows taking into account the number of measurements realized. Indeed, for a small number, $\Delta t_{95\%}$ would be bigger than the standard deviation $\sigma$, whereas for a large number of experiments (>10), $\Delta t_{95\%}$ will be much smaller than $\sigma$.

4.1.5. Electroosmotic Mobility Measurements

Because of the strong dependence of our device on electroosmotic flow, the strength of the electroosmosis that may be expected if in the device was evaluated as precisely as possible. The electroosmotic mobility allows characterizing electroosmosis in one specific micro-channel,
independently of the electrical field. It has to be emphasized that the electroosmosis depends on the channel (its material) and the buffer (pH, concentration, conductivity ...). This justifies the use of buffers and channels as close as possible to the ones used in the aimed application.

4.1.5.1. Current Monitoring Method

One of the simplest way to measure the electroosmotic mobility is the current monitoring method, developed by Zare group (Huang 1988) to measure the mobility inside capillaries. This method has been widely used by variety of chemistry groups when characterizing polymers and polymer modifications (Henry 2000), (Li 2000), (Witek 2004) and (Pu 2007). With the current monitoring method, the electroosmotic mobility is evaluated by measuring the time for the conductivity of a buffer to settle after adding some different conductivity buffer at one extremity of the channel/capillary, while applying a constant electrical field. Nevertheless, this method is applied differently depending on the group using it: some put the 2\textsuperscript{nd} buffer immediately (Huang 1988) whereas other let previously time for the conductivity to settle with the initial buffer (Shadpour 2005). Furthermore, the application of this method is not straightforward when used with small micro-channels and reservoirs rather than capillaries. Indeed, in this case, avoiding hydrodynamic flow influence or manipulation disturbance has been found difficult. Another reported challenge is to determine with precision the conductivity settling time. Indeed, as can be seen on the typical current measurement shown as Figure 33, in most cases, the current doesn’t reach a perfect plateau, but rather increases much slower. This leads to some difficulties in determining the exact settling time. Moreover, as shown in 4.1.4.3., a small uncertainty on this may have a drastic impact on the uncertainty on the mobility. To solve this problem, Li’s group reported the use of the slope of the current evolution to determine directly the mobility (Li 2002). However, to give precise results, using the slope requires other parameters such as the
conductivity of the buffer, parameters not necessarily readily available for an uncommon buffer, such as the one used (PCR buffer).

![Figure 33 Example of Current Evolution with Time during Current Monitoring Method](image)

In addition, the question of which points should be used to find the slope is still important; even though it is far less dramatic than in traditional current monitoring method. This inconvenient justifies that the method proposed by Li was only used as an evaluation of the measurements realized more conventionally. In other words, the slope was compared with the mobility value found for each measurement, in order to check that their proportionality. This facilitated the elimination of some doubtful results, caused by badly sealed chips or poor manipulation.

The last difficulty to overcome in order to obtain reliable measurements in a precise configuration (i.e. with specific buffer, pH and channels material) is to use a buffer solution as close as possible of the actual buffer of interest, even though the current monitoring method requires a buffer change. A way to comply with this limitation is to use two identical buffers with a small difference in concentration (and so conductivity) (Kirby 2004). Indeed, the current-monitoring method has been showed to work with concentrations as close as 5% (Huang 1988).
It has to be mentioned though that increasing the difference between the two buffers conductivity facilitates the measurements because the difference in current rises.

4.1.5.2. Measurements

The measurements realized in this study used a high voltage power supply Spellman CZE 1000R (Plainview, NY). The delivered current was directly monitored using a Fluke 79 multimeter, whose acquisition was achieved using Fluke View Forms software. Measurements were performed with PC microchannels, 50 µm wide and 150 µm deep. Three types of chemical treatments were used on these microchannels:

1. No treatment
2. UV treatment (same characteristics as UV preceding the amination process-Table 9)
3. UV and amination treatment (same characteristics as presented in Table 9).

It has to be noted that in order to create microchannels, the hot embossed chip had to be bounded with a coverplate (as explained precisely for the electrophoretron in Chapter 5). This means a heat exposition at 150°C for 20 min and 30 min for the untreated chips and the previously UV treated ones respectively.

In all measurements, we waited for stabilization before adding the second buffer (as explained in section 4.1.4.1).

4.1.5.3. Precision Evaluation

Using the current monitoring method, the electroosmotic mobility can be related to the time measured for current stabilization (t) as follows;

$$\mu_{eo} = \frac{L}{E \cdot t} = \frac{L^2}{V \cdot t'}$$

where L is the distance between the reservoirs (i.e. the length of the channel), E the electrical field, and V the applied voltage.
The logarithmic derivative gives:

$$\frac{d\mu_{\text{eo}}}{\mu_{\text{eo}}} = 2 \frac{dL}{L} - \frac{dV}{V} - \frac{dt}{t}$$

This provides the relative uncertainty on the EOF mobility:

$$\frac{\Delta \mu_{\text{eo}}}{\mu_{\text{eo}}} = 2 \frac{\Delta L}{L} + \frac{\Delta V}{V} + \frac{\Delta t}{t}$$

Finally, the total uncertainty on the EOF mobility can then be computed (41):

$$\Delta \mu_{\text{eo}} = \mu_{\text{eo}} \left(2 \frac{\Delta L}{L} + \frac{\Delta V}{V} + \frac{\Delta t}{t}\right) = \frac{L^2}{Vt} \left(2 \frac{\Delta L}{L} + \frac{\Delta V}{V} + \frac{\Delta t}{t}\right)$$ (41)

**Assumption 1: Ideal Measurement (i.e. good manipulation)**

- $\Delta L = 0$
- $\Delta V = 0$ This means that the power supply delivers exactly the measured voltage
- $\frac{\Delta t}{t} = 10\%$ Uncertainty to evaluate the time for current stabilization
- $\mu = 3 \text{cm}^2/(\text{Vs})$

**Assumption 2: Realistic Measurement**

- $\Delta L = 1 \text{mm}$. For a 3 cm long channel, this gives: $\frac{\Delta L}{L} \approx 3.33\%$
- $\frac{\Delta V}{V} = 10\%; \quad \frac{\Delta t}{t} = 10\%; \quad \mu = 3 \text{cm}^2/(\text{Vs})$

<table>
<thead>
<tr>
<th>Uncertainty</th>
<th>Ideal Assumption</th>
<th>Realistic Assumption</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\frac{\Delta \mu}{\mu}$</td>
<td>$\frac{\Delta t}{t} = 10%$</td>
<td>$2 \frac{\Delta L}{L} + \frac{\Delta V}{V} + \frac{\Delta t}{t} = 26.66%$</td>
</tr>
<tr>
<td>$\Delta \mu$</td>
<td>$0.3 \text{cm}^2/(\text{Vs})$</td>
<td>$0.7998 \text{cm}^2/(\text{Vs})$</td>
</tr>
</tbody>
</table>

The previous calculations compute the uncertainties for one measurement. A large number of experiments (at least 10 for each buffer/treatment) was carried in order to obtain statistical accuracy and consider a statistic uncertainty using the Student Distribution.
To compute the results, the mean time measured was first calculated, and then the mean electroosmotic mobility was computed (42).

$$\mu_{ee,\text{mean}} = \frac{l^2}{Vt_{\text{mean}}} \quad (42)$$

To compute the uncertainty, the standard deviation on the measurement of $t$ calculation was first carried out and then a 95% confidence interval was evaluated using (43).

$$\Delta t = \frac{\sigma t_{95}}{\sqrt{n}} \quad (43)$$

where $\sigma$ is the standard deviation, $t_{95}$ the 95th percentile of the student distribution for the degree of freedom (n-1), and $n$ is the number of measurements.

From this confidence interval on the mean time, the 95% confidence interval for the electroosmotic mobility was computed (41) assuming no error in channel length and voltage.

4.2. Results

4.2.1. Fluorescence Characterization of Carboxylic Groups

As can be seen in Figure 35, carboxylic groups creation was achieved on all types of Topas® Cycle Olefin Copolymers 5013, 6013, 6015 and 8007. Figure 34 is the control on UV irradiated PMMA through the same grid.

![Figure 34 Fluorescence Characterization of Carboxylic Groups on UV-Modified PMMA](image)
The small size of the grid (squares are about 30 µm wide), clearly visible on all pictures, is an assessment of the precision achieved, due to UV exposure. In addition, one can notice, especially on Figure 36, that the fluorescence intensity and contrast is much lower on COC 6013 and 6015 than in the other types of COC. Furthermore, in comparison with the fluorescence on PMMA pictured in Figure 34, the fluorescence on 6013 and 6015 seems also to show less intensity. This might be explained by a lower concentration of carboxylic groups on the surface. However, fluorescence pictures have only visualization and not quantification purpose. Therefore, no conclusion can be drawn from this difference in intensity.

Figure 35 Fluorescence Characterization of Carboxylic Groups on Modified Topas COCs
The grid is identical in all cases

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In addition, it seems unlikely that the observed fluorescence would only come from autofluorescence of the COC after UV exposure. Indeed, previously to these results, we had several failures that were due to a bad preparation of the MAA solution. These samples were UV exposed through a grid and did not show any fluorescence while observed under the fluorescence microscope. Therefore, we can conclude to successful photografting on all types of COCs.

4.2.2. Topology Measurements on Photografted COC

Topology measurements with the AFM could only be achieved on the 8007 samples. On any other samples than 8007, the modified zone, clearly visible under UV fluorescence as shown in Figure 35, couldn’t be detected under the camera or the tip with bright field. This may be due
to the older age of the COC samples 5013, 6013 and 6015 compared to the 8007. Indeed, these samples were injected 3 years earlier and the modification treatment was completed a week earlier.

From these measurements (in particular Figure 39), the MAA layer may be estimated to be about 0.6 µm thick, which is much thinner than previous reported results (Pu 2007). However,
the uncertainty on this result is extremely high because of the lack of additional experiments. Indeed, these measurements couldn’t be reproduced after a few days due to a too important number of particles on the surface that made the tip lose contact.

4.2.2.1. Conclusions

Even though precise quantification of the MAA layer thickness was not achieved, its photografting on all types of Topas COC used was a success. Our inability to detect a thickness on all samples and the increasing number of particles on the MAA layer might be explained if the photographed layer is not stable. It had been proposed to add a preliminary cleaning step to the modification with a short Oxygen Reactive Ion Etching (O₂-RIE) treatment. Indeed, such a cleaning treatment had been shown having a positive effect on photografting (Goddard 2007). However, because of time issue, this was not tested.

4.2.3. Contact Angle Measurements

Reported values for untreated Lexan® polycarbonate show that this material is almost hydrophobic (Shadpour 2005), but no mention was made to the variation of contact angle throughout the modification process used to reverse the PC electroosmotic mobility.
4.2.3.1. Effect of the Bonding (i.e. 150°C for 20/25/30 min) on PC and UV-Treated PC

To compare the effect of the bonding process on both pristine PC and UV modified PC, the contact angles of the samples previously submitted at 150°C for different periods of time were plotted on the same graph (Figure 40).

As visible on Figure 40, on both untreated and UV-treated PC, the “baking” tends to increase the contact angle and longer exposure to high temperature increases the contact angle. This effect is more visible after UV treatment. Indeed, the UV treatment generates a surface much more hydrophilic due to the creation of the hydrophilic carboxylic groups. The increase in contact angle following the heat exposure may be explained by the fact that the material, damaged by the UV, is cured by the heat exposure close to its $T_g$. Indeed, while the heat exposure corresponding to the binding process, the polymer molecules reorganize, leading to this curing effect.

![Figure 40 Bonding Effect on Contact Angle of PC and UV PC](image)

The measurements were realized on samples under the same treatments, as shown by the color code, except that only the ones showed on the right hand side were UV exposed for 30 min (254 nm, 18 mW/cm²) previously to the heat treatment.
Concerning the search for an adequate process to bond PC chips that have been UV treated on only part of their surface, Figure 40 shows that the surface tension is likely to be extremely different on untreated PC and UV PC. Therefore, bonding under the same conditions will not give the same results on both parts. However a longer heat exposure time might have a positive effect on the successful bonding of the UV treated part.

### 4.2.3.2. Effect of the Amination Solution on PC and UV PC

Figure 41 aims at comparing the wettability between pristine and previously UV treated PC after 2 hours under the amination solution (see Table 9).

![Graph: Figure 41 Contact Angle on PC and UV PC after Exposure to the Amination Solution](image)

Even though the amination solution should only have an effect on the UV treated chips, a change on the contact angle on untreated PC is clearly visible on Figure 41. However, this may be only due to sticking of NH₂ molecules, rather than a real covalent bonding as occurs on UV treated PC. This contact angle change on untreated PC should be overcome with sufficient
cleaning of the chip after exposure to the amination solution. It is essential to do so; otherwise this may have a detrimental effect on the electroosmotic mobility of pristine PC, primordial for the electrophoretron application.

After adequate cleaning, the contact angle on PC is expected to return to the same level as before the amination solution exposure. This will lead to an important contact angle difference (>20°) between the aminated part (Channel 2) and the untreated part (Channel 1), resulting in additional pressure due to the capillary forces (39).

4.2.3.3. Effect of the MAA Photografting on COC

The contact angle on 3 different types of Topas® COCs: 5013, 6013 and 6015 was compared to the same COCs right after the MAA polymerization process (without the use of the grid in order to have a homogenous surface). These results are plotted on Figure 42.

The change of color characterizes the surface treatments endured by the different types of COC. The different types are specified on the x-axis. The “MAA Sol+UV” legend refers to the modification process described in 4.1.1.
All studied types of studied COCs appear slightly hydrophobic, which is consistent with was reported earlier in the literature (SHIN 2005). COCs thus have less ability to be wet by water than PC, which would have to be taken into account in case of a device realized in COC. Indeed, capillary forces are going to be opposed to the filling of the chip (42). In addition, a clear reduction of the COC contact angle after polymerization of the MAA film is visible. In comparison with the measurements realized on PC (Figure 40), it is noticeable that even though they both figure carboxylic groups on their surface, UV PC has lower contact angles than modified COCs. This could be explained by the fact that these measurements were done after the first modification experiments on COC; so the modification process might not have been mastered at that time. However, the decrease of the water contact angle was an encouraging sign. Because the amination increased the contact angle of UV PC, it is to be expected that the same behavior could be observed on COC.

4.2.3.4. Conclusions

On both polymers, the surface modification effects can be measured using water contact angle. Even though these measurements didn’t allow us to determine an ideal bonding process for half UV treated chips, these results show that the surface chemistry change between the 2 channels of the electrophoretron leads to a more hydrophilic surface in Channel 2 than in Channel 1 (unmodified). In addition, the hydrophobicity of pristine COC will probably present a disadvantage when first filling a device made in COC.

4.2.4. Electroosmotic Mobility Measurements

Combining all EOF measurements realized in polycarbonate microchannels in PCR-like conditions in one plot gives Figure 43. Overall, the first remark that can be made on Figure 43 is that Polycarbonate has a positive electroosmotic mobility, which can be reversed using the process described earlier. A deeper look at Figure 43 shows that values of positive EOF in PC
microchannels are of the order of 3 cm²/(Vs), and that all measurements are below the critical value of 3.75 cm²/(Vs)-shown with the dashed-dotted line in Figure 43. This value is the magnitude of the electrophoretic mobility of DNA (Stellwagen 1997) in TBE buffer. If the electroosmotic mobility of PC was higher than this critical value, our device could work without having to induce a pressure gradient (cf Chapter 2).

Figure 43 EOF Measurements in PC Microchannels (pH 8.3)

Table: EOF Measurements in PC Microchannels (pH 8.3)

<table>
<thead>
<tr>
<th>Buffer</th>
<th>Electroosmotic Mobility (cm²/(Vs))</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBE 1M/5M</td>
<td></td>
</tr>
<tr>
<td>10mM Tris-HCl, 1.5mM MgCl₂</td>
<td></td>
</tr>
<tr>
<td>0.5X/1X</td>
<td></td>
</tr>
<tr>
<td>10mM Tris-HCl, Commercial PCR Buffer</td>
<td></td>
</tr>
<tr>
<td>0.8X/1X</td>
<td></td>
</tr>
<tr>
<td>10mM Tris-HCl, Polymerase</td>
<td></td>
</tr>
<tr>
<td>0.8X/1X</td>
<td></td>
</tr>
</tbody>
</table>

“Commercial PCR buffer”: 10mM TrisHCl, 1.5 mM MgCl₂, 50 mM KCl + Polymerase

The first column shows measurements previously reported (Elmajdoub 2006) but re-computed using the method explained before. Excel spreadsheets are visible in Appendix E.

The results shown in the first two columns may be criticized because of the high difference in buffer concentrations used, especially the first one realized in the early part of the study. The overall trend may be explained by the difference in ionic strength between the
different buffers. Indeed, it had already been showed that the higher the ionic strength of the buffer, the lower the electroosmotic mobility in absolute value (Kirby 2004), which is exactly what can be seen when comparing column 3 to the others. From the comparison of column 2 and 4 of Figure 43, it is also possible to see the effect of the presence of Polymerase inside the buffer. As a protein, Polymerase is expected to tend to stick to the PC walls and reduce the electroosmotic mobility of pristine PC. Such reduction had already been quantified with the BSA protein on a variety of polymers (Shadpour 2005). Indeed, Taq Polymerase (New England BioLabs, MA) has an isoelectric point (pI) of 6.42. In other words, at a basic pH as used for PCR, polymerase is negatively charged, leading to a diminution of the zeta potential when the polymerase goes into the wall region. On the other hand, inside aminated channels, the polymerase increases the zeta potential when it goes into the wall region. This would explain the increase in absolute value of the electroosmotic mobility that may be noticed between the measurements without polymerase (1st two columns of Figure 43) and those with polymerase (last two columns of Figure 43). Moreover, it may be noticed that the UV exposure successfully increased the electroosmotic mobility of PC, as reported earlier (Witek 2004). This increase is due to the fact that UV exposure creates carboxylic groups (negatively charged) on the surface of PC. Therefore it the overall negative charge on the PC surface rises, resulting in an increase in absolute value of zeta potential which results in an increase of the electroosmotic mobility.

4.3. Polymer Choice

Both PC and COC show basic requirements to be used for our application: high Tg, demonstrated possibility to chemically reverse the EOF in the areas of interest,… However, both of them have important drawbacks. On one hand, pristine PC shows low EOF mobility, which requires the use of an optimization process in order to get cycling only in the center part of the channel (cf Chapter 2). On the other hand, COCs, not so common commercially yet and existing
in variety of different types, are not so well documented in term of available process. Not only our work on the modification process to reverse the EOF was not sufficient to fully characterize it, but its optimized embossing conditions are not fully known. This explains why, whereas we anticipate future development of the electrophoretron in COC, for the demonstration purpose of our work, we choose to use PC.
Chapter 5 Electrophoretron Prototype Realization and First Experiments

Parameters for an optimal design have been determined in Chapter 2, using a theoretical analysis validated by means of simulations presented in Chapter 3. Chapter 4 showed the material study realized which ultimately led to the choice of polycarbonate for the prototype of the microscale electrophoretron. Chapter 5 intends to present the process used to obtain the final design of the mold insert used for the fabrication of the microchip.

5.1. Final Design

The results found at the end of Chapter 2 led to four different designs, whose characteristics were given in function of dimensionless parameters. These parameters have to be translated into physical dimensions while taking into account the size limitation induced by the 8x8 mm constraints associated with the titer plate design. These constraints are summarized in Table 14.

<table>
<thead>
<tr>
<th>Table 14 Design Constraints</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Minimum</strong></td>
</tr>
<tr>
<td>( W_1, W_2, H_1, H_2 )</td>
</tr>
<tr>
<td>( L_1, L_2 )</td>
</tr>
<tr>
<td>( L_T )</td>
</tr>
</tbody>
</table>

From these constraints and the designs selected in Chapter 2, the characteristics of the four designs to be realized were computed and presented in Table 15. As may be seen in this table, the volume of the cycler is much smaller (~10\(^{-3}\) times) than the usual 100 µL used in commercial PCR cyclers. In comparison with other CF-PCR cyclers (Table 16), the volume reduction is also rather consequent. This is due to the electrokinetic propulsion, which allows having a closed loop configuration and also to the small footprint selected.
The surface to volume ratio, also visible in Table 15, is another way to compare the different PCR cyclers. Experimentalists are interested in having high surface-to-volume ratio since it eases the heat transfer. CF PCR cyclers usually provide an important improvement of this factor, as can be seen in Table 16.

Comparison between Table 15 and Table 16 reveals that although all chosen electrophoretic designs have lower surface-to-ratio than other CF PCR cyclers, it is still several order of magnitudes higher than a commercial PCR. This is partly due to the fact that the design optimization, which led to the very small aspect ratio in Channel 2, also increased the surface-to-volume ratio. The next step consists of the actual fabrication of the design.
5.2. Fabrication

5.2.1. Chip Fabrication

From Table 15, the designs were drawn using AutoCAD 2008. One example may be found in Figure 44, while all others designs are illustrated in Appendix G.

![Figure 44 AutoCAD Layout of Electrophoretron Design 3](image)

The 4 selected designs were regrouped on a single AutoCAD drawing in order to get a single nickel mold insert realized. The difference of depths could not be realized gradually, so the limit between the two depths was set right at the extremities of the thin channel. Moreover, as mentioned earlier, the change of depth is asymmetrical. However, since the depth ratio never exceeds 2, neither the step nor the asymmetry should have long term effect on the velocity profile.
Once the mold is micro-milled in Nickel (Figure 45) with a Kern micro-milling machine, it has to be hot embossed into polycarbonate (Lexan®) sheets. Because of the very thin channels (25 µm) connecting the main channel to the reservoirs, the hot embossing, whose conditions are provided in Table 17, was realized at the Center of the Advanced Microstructures & Devices at LSU.

a) General View
b) Detail on one Design

Figure 45 Nickel Mold Insert

<table>
<thead>
<tr>
<th>Table 17 Hot Embossing Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molding Temperature</td>
</tr>
<tr>
<td>Maximum Force</td>
</tr>
<tr>
<td>Demolding Temperature</td>
</tr>
</tbody>
</table>

After hot embossing the chips are cut individually and the 1 mm holes are drilled to form the reservoirs. Following the cleaning process (Alconox solution rinsed with DI water, sonication, and IPA rinse), chips are dried and inspected under the microscope. The parts which are not supposed to be treated with UV are then hidden under aluminum foil on both the chip and the 0.5 mm PC coverplate. After the UV exposure (18 mW/cm² at 254 nm for 30 min), chip and coverplate are clamped between two glass sheets. Care is taken to make sure that the UV
irradiated parts are in contact with one another and that the 1 mm holes previously drilled (for the electrodes) on the coverplate are over the microchannels. Then the coverplate is bonded to the chip at 149°C for 20 min. The temperature is slightly lower than for pristine PC in order to avoid wall collapsing in the UV treated region. Indeed it has been shown that the UV irradiation lowers the glass transition temperature of the polymer (Witek 2004). After bonding and examination under the microscope, the amination treatment is completed by filling the whole channel with the amination solution (c.f. Chapter 4). The channel part which was not affected by the UV (Channel 1) is then not modified by the amination solution, whereas Channel 2 fixes the amine groups. The amination solution is vacuum pumped, and abundant rinsing with DI water is eventually used in order to make sure non-covalently bonded amine groups are pulled away from the channels (c.f. 4.2.).

5.2.2. Electrodes

5.2.2.1. Background

As mentioned earlier, the hydrolysis of water is a concern when dealing with such small volume (c.f. Table 16). The hydrolysis (44-a & b) is an oxidation-reduction reaction that occurs when a current goes through a water-based solution.

\[
2 \text{H}_2\text{O} + 2 \text{e}^- \rightarrow 2 \text{OH}^- + \text{H}_2 \quad (44-a)
\]
\[
2 \text{H}_2\text{O} \rightarrow 4 \text{H}^+ + \text{O}_2 + 4 \text{e}^- \quad (44-b)
\]

The first reaction (44-a) occurs at the negative electrode (i.e. the cathode), whereas the second (44-b) takes place at the positive electrode (i.e. the anode). The problem of gas production for an electrokinetic cycler has already been studied and minimizing the current by reducing the buffer conductivity has been shown effective (Chen 2005). However, in the case of a very small volume electrode area, this may still not be sufficient. Indeed, at 24°C, with a current of only 50 mA, the production rate of hydrogen is 400 nL/min (and 200 nL/min for oxygen) (Bard). This production
rate would unfortunately increase with the temperature. Different solutions were studied: palladium electrodes, open electrodes, and home-made agarose gel electrodes.

Palladium has naturally the ability to absorb up to 500 times its own volume of hydrogen. However, the rate of absorption is not known. Moreover, palladium is relatively expensive and would only be a solution for part of the problem (the hydrogen). In addition, by using electroplating, the volume of palladium that would be obtained would be extremely small and would not allow hydrogen absorption for more than a few minutes.

Open electrodes are simply regular electrodes (metal wire, such as platinum wire) immersed in the solution without a coverplate on top. This would have for main advantage to give the opportunity for the produced gases to exit the channel. However, it is to be expected that the produced gas bubbles would stick to the electrodes because of surface tension, and even moving these electrodes doesn’t guaranty that the gas would leave the buffer fluid. Moreover, the gas bubbles could perturb the flow and/or be entrained in it. To avoid this kind of perturbation, an open electrode capable of preventing the gas from penetrating the flow while allowing the current to go through was designed, fabricated and tested.

### 5.2.2.2. Electrodes Fabrication

A pipette tip filled with buffer with a layer of Agarose gel at the bottom of tip was used as electrode support. In other words, the platinum wire was inserted into this tip to create an electrode that shouldn’t let gas bubbles enter the channel. The agarose gel is usually used for gel electrophoresis (c.f. Chapter 1) and has the advantage of preventing the buffer to leak from the tip. The complete electrode setup is shown in Figure 46.
The microchannel was first filled with the buffer. Then, a 2% Agarose Gel previously prepared from the buffer (10 mM TrisHCl+ 1.5 mM MgCl$_2$) and is manually inserted inside a micro pipette tip. The tip was then placed over the previously drilled holes and attached to the coverplate using tape. At that point the tip can be filled with buffer and once the platinum wire is introduced inside of it, the electrode is complete. As a validity test of this setup, two electrodes were fixed over a 3cm long channel, separated by about 2 cm, as visible in Figure 47. In the following, in order to use these electrodes while visualizing the channels using the fluorescence microscope, adhesive putty was used in order to fix the electrodes on the coverplate.
The current delivered was monitored using the same setup as for the EOF measurements (c.f. Chapter 4). Relatively stable current could be observed for two hours as long as the gas bubbles don’t clog the pipette tip, as can be seen in Figure 48. The clogging can however be easily avoided if the wire is fixed high enough inside the pipette tip. The y-coordinate in Figure 48 is a voltage proportional to the current delivered by the HV source. Variations in currents are not important here: a non-zero current means that there is electrical contact through the electrodes. These variations can nevertheless be explained by Joule effect, and variations in the surface area of the immersed platinum wire, when a bubble forms or disconnects itself from the wire or when the wire slides inside the tip.

![Figure 48 Variations of the Current Delivered by the HV Source with Time](image)

It has to be noted that preliminarily imbibing the gel with buffer gives better results in terms of conductivity. The main drawback of this electrode is that it can only be used for demonstration purpose. Indeed, an agarose electrode could not be used in a real PCR experiment,
since agarose boils at ~60°C. In other words, the matrix gel would be destroyed at PCR
temperatures and another solution would have to be implemented.

5.3. Preliminary Experiments

5.3.1. Experimental Process

Both preliminary experiments were realized using an inverted Nikon EFD-3 fluorescence
microscope; while movies were recorded instantaneously.

5.3.1.1. Straight Channels Experiments

The main purpose of these experiments was to evaluate the apparent mobility in PCR
buffer of DNA inside PC and aminated PC microchannels. After filling the channel with PCR
buffer, these experiments simply consisted in applying a voltage over a 3 cm long PC
microchannel (same as used for EOF experiments-c.f. Chapter 4) and observe fluorescently
tagged DNAs, previously hydrodynamically introduced inside the channel, moving through the
channel under the influence of the electrical field. Using a home-made power supply, the applied
voltage ranged from 200 V to 800 V, resulting in electrical fields from 65 to 265 V/cm.

The intercalating dye used to stain the DNA was Yopro-1 (Molecular Probes, Invitrogen,
Carlsbad, California), which has for maximum excitation and emission wavelengths 491 nm and
509 nm respectively. The solution, prepared as described in Table 18, was incubated for one hour
at 55°C in a water bath protected from the dark, to help the dye fixation on the DNA molecules.

<table>
<thead>
<tr>
<th>Table 18 DNA Solution Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Concentration</strong></td>
</tr>
<tr>
<td>λ-DNA 5 µg/L</td>
</tr>
<tr>
<td>Yopro 1 10 µg/L</td>
</tr>
<tr>
<td>PCR Mix 1X</td>
</tr>
</tbody>
</table>
The PCR mix presented in Table 18 is based on the commercial PCR mix sold with the Taq Polymerase (New England Biolabs, Beverly, MA). The complete composition of this buffer is given in Table 19. This is a real PCR mix as it could be used in thermal cycler without the primers.

Table 19 PCR Mix Constitution (Straight Channels Experiments)

<table>
<thead>
<tr>
<th>Reactants</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris HCl</td>
<td>10 mM</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>1.5 mM</td>
</tr>
<tr>
<td>KCl</td>
<td>50 mM</td>
</tr>
<tr>
<td>Nucleotides (dNTPs)</td>
<td>0.2 mM</td>
</tr>
<tr>
<td>Polymerase</td>
<td>0.5 U/µL</td>
</tr>
</tbody>
</table>

5.3.1.2. Fluorescence Experiment-Leakage Test

As previously realized (Park 2008), a 6 µM water soluble Fluorescein (Aldrich, Saint Louis, MO) solution in a 1X TBE buffer was used to realize the leakage testing. The 4 different designs, bounded with a plain cover without previous treatment for 25 min at 149°C, were filled by means of a syringe and then studied under the fluorescence microscope in order to detect leakage (due to incomplete bonding) or limited filling. Attention was paid to fill the chips by the reservoir located on the smallest channel side (Channel 1). Otherwise, the pressure gradient due to the cross-section reduction would prevent complete filling of the chip.

5.3.2. Results

5.3.2.1. Straight Channels Experiments

Unfortunately, the applied voltage could not be precisely controlled during these experiments, due to current limitation of the available power source. Therefore, the velocity
measurements that could be realized by counting the number of frames in which individual DNA molecules were in the field could not be related with apparent mobility values. These experiments only confirmed that, without additional pressure gradient, DNAs go towards the positive electrode, in both untreated and aminated channels.

![Figure 49 Dyed DNA in PC Microchannels (a) Aminated PC -20X (b) Pristine PC-10X](image)

5.3.2.2. Leakage Testing

In all designs, no leakage due to insufficient bonding was noticed, even though some incomplete filling could be seen, mainly due to small air bubbles stuck inside the channels (Figure 50-d). In other words, design and bonding process were proved suitable to the fabrication of the electrophoretron. However, Design 1 presents signs of collapsing of the cover due to bonding. Indeed, in Figure 50-a, the fluorescence solution only appears on the side of the large channel. In order to avoid this problem in demonstration use of the design, the bonding time was then reduced to 20 min for all designs. This was particularly important because the bonding is realized after UV exposure, which tends to reduce the glass transition temperature and therefore increases the collapsing hazards.

The difference in depth between Channel 1 and Channel 2 can clearly be seen in designs 2 and 3 (Figure 50- c and d respectively) with the variation of fluorescence intensity. Indeed, the deeper channel has more intensity.
Design 10 exhibited leaking probably due to a defect introduced while micro-milling the mold: two rectangular structures designed for support of the channel, are linked to one reservoir. This leaking may explain that the fluorescence intensity is lower in the center part of the large channel in Design 10. Indeed, the fluorescent solution tends to leak into the support structures. The design defectt is clearly visible in bright field in Figure 51.

Figure 50 Fluorescence Images of the Various Designs of the Electrophoretron

Figure 51 Defects in Design 10 (5X Objective)
5.4. Prototype Testing

5.4.1. Introduction

Testing the prototype requires demonstrating cycling of the DNA while applying a potential difference. A first step would have been the demonstration of buffer cycling inside the device. To serve this purpose, the use of fluorescent particles was studied but not selected because all particles are intrinsically charged. In addition, these charges are barely documented and the values reported are of the same order of the DNA (Yan 2006). In other words, using fluorescent particles would not characterize the buffer flow inside the device and would add an unknown parameter: the electrophoretic mobility of the particles, which might even be inconsistent. Since stained DNAs use was already demonstrated, we decided to directly use DNAs in PCR-like proportions.

5.4.2. Experimental Process

Once the chips are prepared as explained in section 5.2.1, the experimental process is sensibly the same as presented earlier for the straight channels. In other words, the chips are first filled with the PCR buffer, whose composition is given in Table 20.

<table>
<thead>
<tr>
<th>Reactants</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris HCl</td>
<td>10 mM</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>1.5 mM</td>
</tr>
<tr>
<td>Polymerase</td>
<td>0.5 U/µL</td>
</tr>
</tbody>
</table>

Then, the DNA solution (Table 18) is pushed inside the device, and the presence of stained DNA is visually checked under the inverted fluorescence microscope through the coverplate of the chip. This one can then be flipped upside down and the electrodes presented in 5.2.2 are setup
with adhesive putty. The chip is then placed under the microscope for visualization through the thick side of the device, which limits the objective magnification that can be used to 10X. Indeed, the higher the magnification, the lower the depth of field, which leads to the impossibility of getting a focused image on the channel with a magnification higher than 20X (while visualizing through the thick side of the chip).

The focus is made on the channel before applying a 500 V potential difference using the Spellmann HV power supply described previously. The current, in permanent display on the power supply, is regularly checked, in order to monitor that there is still electrical conduction and that the current is not limited.

5.4.3. Results

Although the experimental process was working, as proves the DNA clearly visible in Channel 1 of a design 10 chip in Figure 52, no DNA cycling could be observed during the experiments realized. Two main reasons may be proposed.

First, an important number of air bubbles, as visible in Figure 53, most probably introduced by the open electrodes which are not hermetically sealed to the device, or the reservoirs, were noticed.
Bubbles apparently do not totally prevent the electrical current circulation but absolutely modify the hydrodynamics inside the channel, reducing to zero the effect of the optimization process. The number of bubbles tends to increase while using the device, but bubbles are visible from the very beginning on all devices. In order to reduce the probability of air bubbles, the PCR buffer was sonicated for 10 minutes previously to the experiments.

![Figure 53 Bubbles Visible in a Design 10 Chip after Successive Fillings and Applied Voltages](image)

Then, the filling method of the chips is probably not adequate. Because of the very thin linking channels and of the open electrodes, the pressure to apply in order to fill the whole channel is extremely high, and it is likely that some bubbles are already formed while filling due to insufficient applied pressure.

Finally, as mentioned in section 5.3.2.2, the bonding process is apparently not totally adequate. Indeed, even though it is quite difficult to assess without destructive testing of the prototypes, it is likely that the channels profiles differ from what expected because of cover collapsing, reducing again the effect of the design optimization realized in Chapter 2, therefore preventing the device to be in potential working conditions.
Chapter 6 Conclusions and Future Work

After the introduction of the Polymerase Chain Reaction and the conditions required for its efficiency, we studied the present state of the art of the Continuous Flow PCR Cyclers. The electrophoretron concept, first designed for macro scale electrophoresis, was finally exposed. Chapter 2 consisted in an exhaustive analytical study of the cycler, leading to four optimal designs which greatly increase the probability of the device to work as demonstrated with Monte Carlo simulations. Chapter 3 presented simulations of the working devices realized with Coventorware 2006, a software allowing simulation of electrokinetic microflows. In Chapter 4, a study of polycarbonate (PC) and cycle olefin copolymer (COC) was conducted, concluding that even though COC presents much wider possibilities than PC, the later was eventually chosen to be used to realize the first prototypes. The manufacturing of these prototypes is described in Chapter 5, from the preliminary experiments to the attempts of demonstration of DNAs cycling.

Now that the problems to be solved in order to demonstrate cycling of the DNA species had been precisely identified, DNA cycling should be achievable; the huge potential of this device would then open multiple leads for future work. At short term, quantification of the possibilities of the electrophoretron prototypes is the first goal to pursue. The next aim should be the implementation of the electrophoretron as a PCR cycler, which still involves numerous problem solving situations, as some solutions found in this work would not be viable under high temperature. Once PCR is achieved using the electrophoretron, this one should be integrated into a high throughput platform. Finally, the microscale electrophoretron should be modified in order to comply with other applications requirements, such as the Ligase Detection Reaction (LDR).
Bibliography


Duffy (2001). Green's Functions with Applications, Chapman & Hall.


Kirby (2004). "Zeta potential of microfluidic substrates:

Kirby (2004). "Zeta potential of microfluidic substrates:


Appendix A: Table of Parameters

\( \vec{A} \): arbitrary vector

AR: aspect ratio

\[ a_{m,n,i} = \left( \frac{(2m - 1)\pi}{W_i} \right)^2 + k^2 + \left( \frac{(2n - 1)\pi}{H_i} \right)^2 \]

\( \alpha \): length ratio

\( \vec{B} \): magnetic field vector

\[ b_{m,n,i}(W_i, H_i) = \left( \frac{2m - 1}{W_i} \right)^2 + \left( \frac{2n - 1}{H_i} \right)^2 \]

\( \beta \): width ratio

c: species concentration

\( \gamma \): depth ratio (Chapter 2); surface tension (Chapter 4)

\( \vec{D} \): electrical flux density vector

\( D_h \): hydraulic diameter

\( \vec{E} \): electrical field vector

e: electrical charge of an electron

\( \varepsilon_0 \): electric permittivity of the vacuum

\( \varepsilon_r \): electric permittivity of the medium

\( \vec{F}_e \): electrical force vector

\[ F_i(x,y) = \sum_{m=1}^{\infty} \sum_{n=1}^{\infty} \frac{(-1)^{m+n} \cos \left( \frac{2m - 1}{W_i} \pi x \right) \cos \left( \frac{2n - 1}{H_i} \pi y \right)}{(2m - 1)(2n - 1)b_{m,n,i}} \]

\[ F_i' = \frac{F_i}{W_i^2} \]
\[ f_i = F_i(0,0) = \sum_{m=1}^{\infty} \sum_{n=1}^{\infty} \frac{(-1)^{m+n}}{(2m-1)(2n-1)b_{m,n,i}} \]

\[ f'_i = \frac{f_i}{W_i^2} \]

\[ g_i = \sum_{m=1}^{\infty} \sum_{n=1}^{\infty} \frac{1}{(2m-1)^2(2n-1)^2b_{m,n,i}} \]

\[ g'_i = \frac{g_i}{W_i^2} \]

H: rectangular channel height

\( \vec{J} \): magnetic flux density vector

k: Debye Hückel parameter

\( k_B \): Boltzmann constant

L: length of the channel

\( \lambda_d \): Debye length

\( N_A \): Avogadro number

n: number of measurements

\( n_i \): number of ions i per unite volume

\( n_\infty \): number of ions at the infinity

\( \vec{P} \): polarization vector

p: pressure

\( \psi \): electrical potential

\( \Psi \): dimensionless electrical potential

Q: flowrate

\( R_e \): Reynolds number
\( \rho \): fluid density
\( \rho_e \): charge density
\( T \): absolute temperature
\( t_{95} \): 95th percentile Student distribution
\( \Theta \): contact angle
\( \sigma \): standard deviation
\( \mu \): fluid velocity
\( \mu_{eo} \): electroosmotic mobility
\( \mu_{eph} \): electrophoretic mobility
\( \vec{V} \): fluid velocity vector
\( V \): applied voltage
\( w \): fluid velocity along the z-axis
\( W \): rectangular channel width
\( z_i \): charge of ion i
\( \chi_e \): electric susceptibility
\( \Delta \varphi \): applied difference of potential
Appendix B: Green’s Function Solution of the Equation : (Duffy 2001)

\[ \frac{\partial^2 u}{\partial x^2} + \frac{\partial^2 u}{\partial y^2} = -f(x,y) \]

on

\[ \begin{array}{c}
  \text{y} \\
  \text{a} \\
  \text{b}
\end{array} \]

- Derivation of the solution

This gives an equation for the Green’s function:

\[ \frac{\partial^2 g}{\partial x^2} + \frac{\partial^2 g}{\partial y^2} = -\delta(x - \xi)\delta(y - \eta) \]

For \(0 < x, \xi < a\) and \(0 < y, \eta < b\)

BCs: \(g=0\) at the boundary.

The eigenfunctions of \(g\):

\[ \frac{\partial^2 \varphi}{\partial x^2} + \frac{\partial^2 \varphi}{\partial y^2} = -\lambda \varphi \]

Using the separation of variables, we find:

\[ \varphi_{mn} = \sin \left( \frac{n \pi}{a} x \right) \sin \left( \frac{m \pi}{b} y \right) \]

With the following eigenvalues

\[ \lambda_{mn} = \left( \frac{n \pi}{a} \right)^2 + \left( \frac{m \pi}{b} \right)^2 \]

This implies:

\[ g(x,y|\xi, \eta) = \sum_{n=1}^{\infty} \sum_{m=1}^{\infty} A_{mn} \sin \left( \frac{n \pi}{a} x \right) \sin \left( \frac{m \pi}{b} y \right) \]

A known identity is:
\[ \delta(x - \xi)\delta(y - \eta) = \frac{4}{ab} \sum_{n=1}^{\infty} \sum_{m=1}^{\infty} \sin\left(\frac{n\pi}{a} x\right) \sin\left(\frac{m\pi}{b} y\right) \sin\left(\frac{n\pi}{a} \xi\right) \sin\left(\frac{m\pi}{b} \eta\right) \]

Using the 2 last results in the differential equation (1) gives:

\[ \left(\left(\frac{n\pi}{a}\right)^2 + \left(\frac{m\pi}{b}\right)^2\right) A_{mn} = \frac{4}{ab} \sin\left(\frac{n\pi}{a} \xi\right) \sin\left(\frac{m\pi}{b} \eta\right) \]

We finally get:

\[ g(x, y|\xi, \eta) = \frac{4}{ab} \sum_{n=1}^{\infty} \sum_{m=1}^{\infty} \frac{\sin\left(\frac{n\pi}{a} \xi\right) \sin\left(\frac{m\pi}{b} \eta\right) \sin\left(\frac{n\pi}{a} x\right) \sin\left(\frac{m\pi}{b} y\right)}{\left(\left(\frac{n\pi}{a}\right)^2 + \left(\frac{m\pi}{b}\right)^2\right)} \]

Since using green’s function the solution is:

\[ u(x, y) = \iint f(x, y) g(x, y|\xi, \eta) \, d\xi d\eta \]

We get:

\[ u(x, y) = \frac{4}{ab} \sum_{n=1}^{\infty} \sum_{m=1}^{\infty} \frac{\sin\left(\frac{n\pi}{a} x\right) \sin\left(\frac{m\pi}{b} y\right) \iint f(x, y) \sin\left(\frac{n\pi}{a} \xi\right) \sin\left(\frac{m\pi}{b} \eta\right) \, d\xi d\eta}{\left(\left(\frac{n\pi}{a}\right)^2 + \left(\frac{m\pi}{b}\right)^2\right)} \]

Let \( f(x, y) = A = \text{cte}, \) then:

\[ u(x, y) = -\frac{4A}{ab} \sum_{n=1}^{\infty} \sum_{m=1}^{\infty} \frac{\sin\left(\frac{n\pi}{a} x\right) \sin\left(\frac{m\pi}{b} y\right) \int_0^a \sin\left(\frac{n\pi}{a} \xi\right) d\xi \int_0^b \sin\left(\frac{m\pi}{b} \eta\right) d\eta}{\left(\left(\frac{n\pi}{a}\right)^2 + \left(\frac{m\pi}{b}\right)^2\right)} \]

i.e.:

\[ u(x, y) = -\frac{4A}{ab} \sum_{n=1}^{\infty} \sum_{m=1}^{\infty} \frac{\sin\left(\frac{n\pi}{a} x\right) \sin\left(\frac{m\pi}{b} y\right) \int_0^a \sin\left(\frac{n\pi}{a} \xi\right) d\xi \int_0^b \sin\left(\frac{m\pi}{b} \eta\right) d\eta}{\left(\left(\frac{n\pi}{a}\right)^2 + \left(\frac{m\pi}{b}\right)^2\right)} \]

\[ u(x, y) = -4A \sum_{n=1}^{\infty} \sum_{m=1}^{\infty} \frac{\sin\left(\frac{n\pi}{a} x\right) \sin\left(\frac{m\pi}{b} y\right) \int_0^a \sin\left(\frac{n\pi}{a} \xi\right) d\xi \int_0^b \sin\left(\frac{m\pi}{b} \eta\right) d\eta}{n^2 + mn} \left(\frac{a}{mn}\right)\left(\frac{b}{mn}\right) (1 + (-1)^n)(1 + (-1)^m) \]
We may notice that if either $m$ or $n$ is even, then $u=0$. We may thus reformulate:

$$u(x, y) = -4A \sum_{n=1}^{\infty} \sum_{m=1}^{\infty} \frac{\sin \left( \frac{n \pi}{a} x \right) \sin \left( \frac{m \pi}{b} y \right)}{\left( \frac{n \pi}{a} \right)^2 + \left( \frac{m \pi}{b} \right)^2} \frac{(1 + (-1)^n)(1 + (-1)^m)}{(n \pi)(m \pi)}$$

Finally,

$$u(x, y) = -16A \pi^2 \sum_{n=1}^{\infty} \sum_{m=1}^{\infty} \frac{\sin \left( \frac{(2n-1) \pi}{a} x \right) \sin \left( \frac{(2m-1) \pi}{b} y \right)}{(2n-1)(2m-1)} \frac{4}{\left( \frac{(2n-1) \pi}{a} \right)^2 + \left( \frac{(2m-1) \pi}{b} \right)^2}$$

If we change the coordinate system to be centered on the axis of the channel $x' = x + \frac{a}{2}$ and $y' = y + \frac{b}{2}$

$$u'(x', y')$$

$$= -16A \pi^2 \sum_{n=1}^{\infty} \sum_{m=1}^{\infty} \frac{\sin \left( \frac{(2n-1) \pi}{a} x' - \frac{(2n-1) \pi}{2} \right) \sin \left( \frac{(2m-1) \pi}{b} y' - \frac{(2m-1) \pi}{2} \right)}{(2n-1)(2m-1)} \frac{4}{\left( \frac{(2n-1) \pi}{a} \right)^2 + \left( \frac{(2m-1) \pi}{b} \right)^2}$$

Since $\sin \left( \alpha + \frac{\pi}{2} \right) = \cos (\alpha)$

$$u'(x', y') = -16A \pi^2 \sum_{n=1}^{\infty} \sum_{m=1}^{\infty} \cos \left( \frac{(2n-1) \pi}{a} x' - n \pi \right) \cos \left( \frac{(2m-1) \pi}{b} y' - m \pi \right) \frac{4}{(2n-1)(2m-1)} \frac{1}{\left( \frac{(2n-1) \pi}{a} \right)^2 + \left( \frac{(2m-1) \pi}{b} \right)^2}$$

Using $\cos(a + b) = \cos a \cos b - \sin a \sin b$, we get:

$$u'(x', y') = -16A \pi^2 \sum_{n=1}^{\infty} \sum_{m=1}^{\infty} (-1)^{n+m} \cos \left( \frac{(2n-1) \pi}{a} x' \right) \cos \left( \frac{(2m-1) \pi}{b} y' \right) \frac{4}{(2n-1)(2m-1)} \frac{1}{\left( \frac{(2n-1) \pi}{a} \right)^2 + \left( \frac{(2m-1) \pi}{b} \right)^2}$$

and $a=2W$, $b=2H$, we finally obtain:
\[\begin{align*}
& u'(x', y') = -\frac{64 A}{\pi^2} \sum_{n=1}^{\infty} \sum_{m=1}^{\infty} \frac{(-1)^{n+m} \cos \left(\frac{(2n-1) \pi}{2H} x'\right) \cos \left(\frac{(2m-1) \pi}{2W} y'\right)}{(2n-1)(2m-1) \left(\frac{(2n-1)\pi}{H}\right)^2 + \left(\frac{(2m-1)\pi}{W}\right)^2} \\
& \end{align*}\]

With the adequate adimensionalization,

\[\begin{align*}
\bar{u}(\bar{y}, \bar{z}) &= -\frac{64}{\pi^4 D_h^2 \bar{d} \bar{x}} \sum_{m=1}^{\infty} \sum_{n=1}^{\infty} \frac{(-1)^{m+n} \cos \left(\frac{(2m-1) \pi D_h}{2H} \bar{y}\right) \cos \left(\frac{(2n-1) \pi D_h}{2W} \bar{z}\right)}{(2m-1)(2n-1) \left[\frac{(2m-1)^2}{H^2} + \frac{(2n-1)^2}{W^2}\right]} \\
& \end{align*}\]

- Verification of the solution:

\[\begin{align*}
\frac{\partial \bar{u}}{\partial \bar{y}} &= \frac{32}{\pi^3 D_h \bar{d} \bar{x}} \sum_{m=1}^{\infty} \sum_{n=1}^{\infty} \frac{(-1)^{m+n} \sin \left(\frac{(2m-1) \pi D_h}{2H} \bar{y}\right) \cos \left(\frac{(2n-1) \pi D_h}{2W} \bar{z}\right)}{H(2n-1) \left[\frac{(2m-1)^2}{H^2} + \frac{(2n-1)^2}{W^2}\right]} \\
\frac{\partial^2 \bar{u}}{\partial \bar{y}^2} &= \frac{16}{\pi^2 H^2 \bar{d} \bar{x}} \sum_{m=1}^{\infty} \sum_{n=1}^{\infty} \frac{(-1)^{m+n} (2m-1) \cos \left(\frac{(2m-1) \pi D_h}{2H} \bar{y}\right) \cos \left(\frac{(2n-1) \pi D_h}{2W} \bar{z}\right)}{(2m-1) \left[\frac{(2m-1)^2}{H^2} + \frac{(2n-1)^2}{W^2}\right]} \\
& \end{align*}\]

And:

\[\begin{align*}
\frac{\partial^2 \bar{u}}{\partial \bar{z}^2} &= \frac{16}{\pi^2 W^2 \bar{d} \bar{x}} \sum_{m=1}^{\infty} \sum_{n=1}^{\infty} \frac{(-1)^{m+n} (2n-1) \cos \left(\frac{(2m-1) \pi D_h}{2H} \bar{y}\right) \cos \left(\frac{(2n-1) \pi D_h}{2W} \bar{z}\right)}{(2m-1) \left[\frac{(2m-1)^2}{H^2} + \frac{(2n-1)^2}{W^2}\right]} \\
& \end{align*}\]

Then,

\[\begin{align*}
\frac{\partial^2 \bar{u}}{\partial \bar{y}^2} + \frac{\partial^2 \bar{u}}{\partial \bar{z}^2} &= \frac{16 \bar{d} \bar{x}}{\pi^2} \sum_{m=1}^{\infty} \sum_{n=1}^{\infty} \frac{(-1)^{m+n} \cos \left(\frac{(2m-1) \pi D_h}{2H} \bar{y}\right) \cos \left(\frac{(2n-1) \pi D_h}{2W} \bar{z}\right)}{\left[\frac{(2m-1)^2}{H^2} + \frac{(2n-1)^2}{W^2}\right]} \\
& \times \left(\frac{2m-1}{2n-1} \frac{1}{W^2} + \frac{2n-1}{2m-1} \frac{1}{H^2}\right) \\
& \end{align*}\]

\[\begin{align*}
\frac{\partial^2 \bar{u}}{\partial \bar{y}^2} + \frac{\partial^2 \bar{u}}{\partial \bar{z}^2} &= \frac{16 \bar{d} \bar{x}}{\pi^2} \sum_{m=1}^{\infty} \sum_{n=1}^{\infty} \frac{(-1)^{m+n} \cos \left(\frac{(2m-1) \pi D_h}{2H} \bar{y}\right) \cos \left(\frac{(2n-1) \pi D_h}{2W} \bar{z}\right)}{\left[\frac{(2m-1)^2}{H^2} + \frac{(2n-1)^2}{W^2}\right]} \\
& \times \left(\frac{2m-1}{2n-1} \frac{1}{W^2} + \frac{2n-1}{2m-1} \frac{1}{H^2}\right) \\
& \end{align*}\]
Since \(\frac{2m-1}{2n-1} \frac{1}{W^2} + \frac{2n-1}{2m-1} \frac{1}{H^2} = \frac{1}{(2m-1)(2n-1)} \left[ \frac{(2m-1)^2}{H^2} + \frac{(2n-1)^2}{W^2} \right] \).

\[
\frac{\partial^2 \bar{u}}{\partial \bar{y}^2} + \frac{\partial^2 \bar{u}}{\partial \bar{z}^2} = \frac{16}{\pi^2} \frac{d\bar{p}}{d\bar{x}} \sum_{m=1}^{\infty} \sum_{n=1}^{\infty} \frac{(-1)^{m+n} \cos \left( \frac{(2m-1)\pi D_h \bar{y}}{2H} \right) \cos \left( \frac{(2n-1)\pi D_h \bar{z}}{2W} \right)}{(2m-1)(2n-1)}
\]

\[
\frac{\partial^2 \bar{u}}{\partial \bar{y}^2} + \frac{\partial^2 \bar{u}}{\partial \bar{z}^2} = \frac{16}{\pi^2} \frac{d\bar{p}}{d\bar{x}} \sum_{m=1}^{\infty} \frac{(-1)^m \cos \left( \frac{(2m-1)\pi D_h \bar{y}}{2H} \right)}{(2m-1)} \sum_{n=1}^{\infty} \frac{(-1)^n \cos \left( \frac{(2n-1)\pi D_h \bar{z}}{2W} \right)}{(2n-1)}
\]

Development in Fourier series of \(\pi\):

\[\pi = 4 \sum_{n=1}^{\infty} \frac{(-1)^n \cos((2n-1)x)}{2n-1}\]

(Source: http://functions.wolfram.com, constant function \(\pi\))

Which gives actually \(\frac{\partial^2 \bar{u}}{\partial \bar{y}^2} + \frac{\partial^2 \bar{u}}{\partial \bar{z}^2} = \frac{d\bar{p}}{d\bar{x}}\)
Appendix C: Some Detailed Calculations

\[
\int_{y=0}^{y=H/D_h} \int_{z=0}^{z=W/D_h} \cosh \left( y_j k \bar{y} \right) \cos \left( \Delta_j \bar{z} \right) \cos(\Delta_m' \bar{y}) \cos(\Delta_n \bar{z}) \, d\bar{y} d\bar{z}
\]

\[
= \int_0^{H/D_h} \cosh \left( y_j k \bar{y} \right) \cos(\Delta_m' \bar{y}) \, d\bar{y} \times \int_0^{W/D_h} \cos(\Delta_n \bar{z}) \cos(\Delta_j \bar{z}) \, d\bar{z}
\]

Let’s call the 1\textsuperscript{st} integral J and integrate it by part twice:

\[
J = \frac{1}{y_j k} \left[ \sinh \left( y_j k \bar{y} \right) \cos(\Delta_m' \bar{y}) \right] + \frac{\Delta_m'}{y_j k} \int \sinh \left( y_j k \bar{y} \right) \sin(\Delta_m' \bar{y}) \, d\bar{y}
\]

\[
J = \frac{1}{y_j k} \left[ \sinh \left( y_j k \bar{y} \right) \cos(\Delta_m' \bar{y}) \right] + \frac{\Delta_m'}{(y_j k)^2} \left[ \cosh \left( y_j k \bar{y} \right) \sin(\Delta_m' \bar{y}) \right] - \left( \frac{\Delta_m'}{y_j k} \right)^2 J
\]

This gives:

\[
J = \frac{(y_j k)^2}{(y_j k)^2 + \Delta_m'^2} \left( \frac{1}{y_j k} \left[ \sinh \left( y_j k \bar{y} \right) \cos(\Delta_m' \bar{y}) \right] + \frac{\Delta_m'}{(y_j k)^2} \left[ \cosh \left( y_j k \bar{y} \right) \sin(\Delta_m' \bar{y}) \right] \right)
\]

Finally,

\[
J = \frac{(y_j k)^2}{(y_j k)^2 + \Delta_m'^2} \left( \frac{1}{y_j k} \sinh \left( y_j k \frac{H}{D_h} \right) \cos \left( \Delta_m' \frac{H}{D_h} \right) + \frac{\Delta_m'}{(y_j k)^2} \cosh \left( y_j k \frac{H}{D_h} \right) \sin \left( \Delta_m' \frac{H}{D_h} \right) \right)
\]

\[
\cos \left( \Delta_m' \frac{H}{D_h} \right) = \cos \left( \pi m - \frac{\pi}{2} \right) = \sin(\pi m) = 0
\]

\[
\sin \left( \Delta_m' \frac{H}{D_h} \right) = \sin \left( \pi m - \frac{\pi}{2} \right) = -\cos(\pi m) = (-1)^{m+1}
\]

Then

\[
J = \frac{(-1)^{m+1} \Delta_m' \cosh \left( y_j k \frac{H}{D_h} \right)}{(y_j k)^2 + \Delta_m'^2}
\]

Let’s call the 2\textsuperscript{nd} integral K and use a trigonometric identity to evaluate it:
\[
K = \int_0^{\frac{w}{D_h}} \cos(\Delta_n \bar{z}) \cos(\Delta_j \bar{z}) \, d\bar{z}
\]

\[
K = \frac{1}{2} \left\{ \left[ \frac{\sin \left( \frac{(\Delta_n + \Delta_j) \bar{z}}{\Delta_n + \Delta_j} \right)}{\Delta_n + \Delta_j} \right] + \left[ \frac{\sin \left( \frac{(\Delta_n - \Delta_j) \bar{z}}{\Delta_n - \Delta_j} \right)}{\Delta_n - \Delta_j} \right] \right\}
\]

\[
K = \frac{1}{2} \left\{ \frac{\sin \left( \frac{(\Delta_n + \Delta_j) W}{\Delta_n + \Delta_j} \right)}{\Delta_n + \Delta_j} + \frac{\sin \left( \frac{(\Delta_n - \Delta_j) W}{\Delta_n - \Delta_j} \right)}{\Delta_n - \Delta_j} \right\} = 0, \text{ if } \Delta_n \neq \Delta_j, \text{ i.e. if } n \neq j
\]

If \( n = j \),

\[
K = \int_0^{\frac{w}{D_h}} (\cos(\Delta_n \bar{z}))^2 \, d\bar{z}
\]

i.e.

\[
K = \frac{W}{2D_h}
\]

Another

\[
\frac{\Delta_m'}{(Y_m \bar{k})^2 + \Delta_m'^2} = \frac{(2m - 1)\pi D_h}{2H} \left( \frac{(2n - 1)\pi D_h}{2W} \right)^2 + \left( \frac{(2m - 1)\pi D_h}{2H} \right)^2
\]

\[
\frac{\Delta_m'}{(Y_m \bar{k})^2 + \Delta_m'^2} = \frac{(2m - 1)\pi D_h}{2H} \left( \frac{2n - 1}{W} + \frac{2m - 1}{H} \right)^2 \left( \frac{\pi D_h}{2} \right)^2
\]

\[
\Delta_n \left( Y_m \bar{k} \right)^2 + \Delta_n^2 = \frac{(2n - 1)\pi D_h}{2W} \left( \frac{2n - 1}{W} + \frac{2m - 1}{H} \right)^2 \left( \frac{\pi D_h}{2} \right)^2
\]
D.1: Plot of Velocity Profiles inside an Electrophoretrotron

function plot_velocity_ce_3D_real_1=
plot_velocity(L_1,L_2,mu_eo1,mu_eo2,w_2,h_2,w_1,h_1,m_max)

%L_1: length of the unmodified channel in microns
%L_2: length of the modified channel in microns
%mu_eo1: EO mobility of the unmodified channel
%mu_eo2: EO mobility of the modified channel
%w_2: width of the modified channel in microns
%h_2: depth of the modified channel in microns
%w_1: width of the unmodified channel in microns
%h_1: depth of the unmodified channel in microns
%m_max: number of terms added on both directions

close all

%Plot of the velocity, function of x and y
delta_phi=250;%given in volts
mu_eph=-37500;
n_max=m_max;

x_1=-w_1/2:1:w_1/2;
y_1=-h_1/2:1:h_1/2;
[X1,Y1]=meshgrid(x_1,y_1);
x_2=-w_2/2:1:w_2/2;
y_2=-h_2/2:1:h_2/2;
[X2,Y2]=meshgrid(x_2,y_2);

%Initialization
term_a=zeros(length(y_2),length(x_2));
g_2=zeros(length(w_2),length(h_2));
g_1=zeros(length(w_2),length(h_2));
f_2=zeros(length(w_2),length(h_2));
f_1=zeros(length(w_2),length(h_2));
term_b=zeros(length(w_1),length(h_1));

%Calculations of every term
for m=1:m_max;
    for n=1:n_max;
        term_a=term_a+((-1).^(m+n).*cos((2.*m-1).*pi.*X2./w_2).*cos((2.*n-1).*pi.*Y2./h_2))./((2.*m-1).^2.*(2.*n-1).^2.*bmn(m,n,w_2,h_2));
        term_b=term_b+((-1).^(m+n).*cos((2.*m-1).*pi.*X1./w_1).*cos((2.*n-1).*pi.*Y1./h_1))./((2.*m-1).^2.*(2.*n-1).^2.*bmn(m,n,w_1,h_1));
        g_2=g_2+1./((2.*m-1).^2.*(2.*n-1).^2.*bmn(m,n,w_2,h_2));
    end
end

%Close all figures
close all
\[ g_1 = g_1 + 1/(2. \cdot m-1)^2 \cdot (2. \cdot n-1)^2 \cdot bmn(m,n,w_1,h_1); \]
\[ f_2 = f_2 + (-1)^{(m+n)}/((2. \cdot m-1) \cdot (2. \cdot n-1) \cdot bmn(m,n,w_2,h_2)); \]
\[ f_1 = f_1 + (-1)^{(m+n)}/((2. \cdot m-1) \cdot (2. \cdot n-1) \cdot bmn(m,n,w_1,h_1)); \]
\[
% channel 2
\[
term_sum2 = -term_a \cdot ((\mu_{eo2} \cdot h_2 \cdot w_2 / L_2 + \mu_{eo1} \cdot h_1 \cdot w_1 / L_1) \cdot (h_1 \cdot w_1 / L_1) \cdot g_1 + (h_2 \cdot w_2 / L_2) \cdot g_2) ;
\]
\[
velocity2 = \mu_{eo2} + (\pi^2 \cdot term_sum2) / 4 ;
\]
\[
velocity_species_2 = (\delta \phi / L_2) \cdot (\mu_{eo1} + \mu_{eph}) + (\pi^2 \cdot term_sum2) / 4 ;
\]
\[
v_{mean2} = (\delta \phi / L_2) \cdot (\mu_{eo2} - g_2 \cdot (\mu_{eo2} \cdot h_2 \cdot w_2 / L_2 + \mu_{eo1} \cdot h_1 \cdot w_1 / L_1) / ((h_1 \cdot w_1 / L_1) \cdot g_1 + (h_2 \cdot w_2 / L_2) \cdot g_2)) ;
\]
\[
% channel 1
\[
term_sum1 = -term_b \cdot ((\mu_{eo2} \cdot h_2 \cdot w_2 / L_2 + \mu_{eo1} \cdot h_1 \cdot w_1 / L_1) \cdot (h_1 \cdot w_1 / L_1) \cdot g_1 + (h_2 \cdot w_2 / L_2) \cdot g_2) ;
\]
\[
velocity1 = \mu_{eo1} + (\pi^2 \cdot term_sum1) / 4 ;
\]
\[
velocity_species_1 = (\delta \phi / L_1) \cdot (\mu_{eo1} + \mu_{eph}) + (\pi^2 \cdot term_sum1) / 4 ;
\]
\[
v_{mean1} = (\delta \phi / L_1) \cdot (\mu_{eo1} + \mu_{eph} - g_1 \cdot (\mu_{eo2} \cdot h_2 \cdot w_2 / L_2 + \mu_{eo1} \cdot h_1 \cdot w_1 / L_1) / (h_1 \cdot w_1 / L_1) \cdot g_1 + (h_2 \cdot w_2 / L_2) \cdot g_2) ;
\]
\[
dp = (\pi^6 / 64) \cdot (\delta \phi) \cdot (8.9 \cdot 10^{-4}) \cdot (\mu_{eo2} \cdot h_2 \cdot w_2 / L_2 + \mu_{eo1} \cdot h_1 \cdot w_1 / L_1) / ((h_1 \cdot w_1 / L_1) \cdot g_1 + (h_2 \cdot w_2 / L_2) \cdot g_2) ;
\]

\[
% subplot
\[
% channel 2
\[
surface(X2,Y2,velocity2);
axis tight;
set(gca,'DataAspectRatio',[1,1,5]);
colorbar
xlabel('x');
ylabel('y');
title('EO Velocity in channel 2');
\]
\[
% subplot
\[
surface(X1,Y1,velocity1);
\]
axis tight;
set(gca,'DataAspectRatio',[1,1,5]);
colorbar
xlabel('x');
ylabel('y');
title('EO Velocity in channel 1');

subplot(2,2,3)
surface(X2,Y2,velocity_species_2);
axis tight;
set(gca,'DataAspectRatio',[1,1,5]);
colorbar
xlabel('x');
ylabel('y');
title('Velocity species in channel 2');

shading flat %to not see the grid

% Writing of the .dat file necessary to plot in 3D with Tecplot
s=size(velocity1);
M=[];

for i=1:s(1) % row reading
    for j=1:s(2) % columns reading
        M=[M;[x_1(j),y_1(i),velocity1(i,j)]];
    end
end

input='Velocity_plot.dat';
fid=fopen(input,'w');
fprintf(fid,strcat('VARIABLES = "x", "y", "EO velocity (µm/s)"\n'));
fprintf(fid,strcat('ZONE T="w1", I=',num2str(s(2)),', J=',num2str(s(1)),', DATAPACKING=POINT,\n'));
fprintf(fid,'%02.5f %02.5f %02.5f\n',[M(:,1);M(:,2);M(:,3)]);
fclose(fid)
clear fid

s=size(velocity2);
M=[];
for i=1:s(1)%row reading
    for j=1:s(2)%columns reading
        M=[M;x_2(j),y_2(i),velocity2(i,j)];
    end
end

input='Velocity_plot_2.dat';
fid=fopen(input,'w');
fprintf(fid,strcat('VARIABLES = "x", "y", "EO velocity 2 (µm/s)"','
'));
fprintf(fid,strcat('ZONE T="w1", I=',num2str(s(2)),', J=',num2str(s(1))',',
    DATAPACKING=POINT','
'));
fprintf(fid,'%02.5f %02.5f %02.5f
',M(:,1);M(:,2);M(:,3)');
fclose(fid)
clear fid

D.2. Optimization Program

%optimum search for the maximum of w1max
close all
clear all
x0=[1.36 2.6 1 1];%Alpha, Beta, AR1, AR2, respectively
A=[1 0 0 0;0 1 0 0; 0 0 1 0;0 0 0 1;-1 0 0 0;0 -1 0 0; 0 0 -1 0;0 0 0 -1];
b=[0.8 10 4 4 -0.3 -0.1 -1 -0.1]';
Alpha_max_variation=[0.5:0.05:3];
%Fval=0;

%Let Alpha max vary
for i=1:length(Alpha_max_variation)
    b(1)=Alpha_max_variation(i);
    [x,fval]=fmincon('w1max',x0,A,b,[],[],[],[],'constraints');
    Alpha(i)=x(1);
    Beta(i)=x(2);
    AR1(i)=x(3);
    AR2(i)=x(4);
    Gamma(i)=x(4).*x(2)./(x(3));
    Fval(i)=fval;
    w_2(i)=w2_av(x(1),x(2),x(3),x(4));
    w_1(i)=w1_av(x(1),x(2),x(3),x(4));
    L1_ad(i)=x(1)./(1+x(1));% non dimensionalized by Lt
    L2_ad(i)=1./(1+x(1));% non dimensionalized by Lt
end
dt1=delta_t_ad(L1_ad,w_1);
dt2=-delta_t_ad(L2_ad,w_2);%because the velocity is negative in channel2
dt=dt1+dt2;
subplot(1,2,1)
plot(Alpha_max_variation,Alpha,Alpha_max_variation,Beta,Alpha_max_variation,AR1,Alpha_max_variation,AR2,Alpha_max_variation,-Fval,Alpha_max_variation,Fval,Alpha_max_variation,dt1,
Alpha_max_variation,dt2,Alpha_max_variation,dt)
legend('Alpha','Beta','AR_1','AR2','Max DNA Velocity','dt1','dt2','dt')
title('Optimized values relatively to change in max values of Alpha for Gamma<2')

%Writing of the .dat file necessary to plot in 3D with Tecplot
s=length(Alpha);
M=[];
M2=[];
M3=[];
M4=[];
M5=[];
M6=[];
M7=[];
M8=[];
for i=1:s(1)%row reading
    M=[M;[Alpha_max_variation(i),Alpha(i)]];
    M2=[M2;[Alpha_max_variation(i),Beta(i)]];
    M3=[M3;[Alpha_max_variation(i),AR1(i)]];
    M4=[M4;[Alpha_max_variation(i),AR2(i)]];
    M5=[M5;[Alpha_max_variation(i),-Fval(i)]];
    M6=[M6;[Alpha_max_variation(i),dt1(i)]];
    M7=[M7;[Alpha_max_variation(i),dt2(i)]];
    M8=[M8;[Alpha_max_variation(i),Gamma(i)]];
end
input='PC_Alpha.dat';
fid=fopen(input,'w');
fprintf(fid,strcat('VARIABLES = "Alpha_max", "Whatever"
'));
fprintf(fid,strcat('ZONE T="Alpha", I=',num2str(s(1)),'
'));
fprintf(fid,'%02.5f %02.5f
',[M(:,1)';M(:,2)']);
fprintf(fid,strcat('ZONE T="Beta", I=',num2str(s(1)),'
'));
fprintf(fid,'%02.5f %02.5f
',[M2(:,1)';M2(:,2)']);
fprintf(fid,strcat('ZONE T="AR1", I=',num2str(s(1)),'
'));
fprintf(fid,'%02.5f %02.5f
',[M3(:,1)';M3(:,2)']);
fprintf(fid,strcat('ZONE T="AR2", I=',num2str(s(1)),'
'));
fprintf(fid,'%02.5f %02.5f
',[M4(:,1)';M4(:,2)']);
fprintf(fid,strcat('ZONE T="Velocity", I=',num2str(s(1)),'
'));
fprintf(fid,'%02.5f %02.5f
',[M5(:,1)';M5(:,2)']);
fprintf(fid,strcat('ZONE T="dt1", I=',num2str(s(1)),'
'));
fprintf(fid,'%02.5f %02.5f
',[M6(:,1)';M6(:,2)']);
fprintf(fid,strcat('ZONE T="dt2", I=',num2str(s(1)),'
'));
fprintf(fid,'%02.5f %02.5f
',[M7(:,1)';M7(:,2)']);
fprintf(fid,strcat('ZONE T="dt", I=',num2str(s(1)),'
'));
fprintf(fid,'%02.5f %02.5f
',[M8(:,1)';M8(:,2)']);
fclose(fid)
clear fid
%Let Beta max vary
Beta_max_variation=[5:0.1:10];

for i=1:length(Beta_max_variation)
    b(2)=Beta_max_variation(i);
    [x,fval]=fmincon('w1max',x0,A,b,[],[],[],[],'constraints');
    Alpha(i)=x(1);
    Beta(i)=x(2);
    AR1(i)=x(3);
    AR2(i)=x(4);
    Fval(i)=fval;
    w_2(i)=w2_av(x(1),x(2),x(3),x(4));
    w_1(i)=w1_av(x(1),x(2),x(3),x(4));
    L1_ad(i)=x(1)./(1+x(1)); % non dimensionalized by Lt
    L2_ad(i)=1./(1+x(1)); % non dimensionalized by Lt
end

dt1=delta_t_ad(L1_ad,w_1);
dt2=-delta_t_ad(L2_ad,w_2);
dt=dt1+dt2;

subplot(1,2,2)
plot(Beta_max_variation,Alpha,Beta_max_variation,Beta,Beta_max_variation,AR1,Beta_max_variation,AR2,Beta_max_variation,-Fval,Beta_max_variation,dt1,Beta_max_variation,dt2,Beta_max_variation,dt)
legend('Alpha','Beta','AR_1','AR2','Max DNA velocity','dt1','dt2','dt')
title('Optimized values relatively to change in max values of Beta for Gamma=<2')

%Writing of the .dat file necessary to plot in 3D with Tecplot
(…) Same thing as for Alpha

C.3. Monte Carlo Simulations

function [w1,w2,w1min,w2min,t,tmin,tmax,w1max,w2max] =
Monte_Carlo_normal(Alpha,Beta,AR1,AR2)

%%% Parameters: Alpha, Beta, AR1, AR2
%%% Results:w1,w2,w1min,w2min,t,tmin,tmax,w1max,w2max
%%%!!!!! The mobility values for all Monte Carlo simulations are specified inside this program!!!!!!

% Example Monte Carlo Simulation in Matlab
(http://www.vertex42.com/ExcelArticles/mc/MatlabMCExample.html)

% Generate n samples from a normal distribution
% r = ( randn(n,1) * sd ) + mu
% mu : mean
% sd : standard deviation
n = 1000000; % The number of function evaluations

W1=50;
H1=50;
Lt=25200;
DL=1000;% I decided!
D_W=3% Dr Nik

%Uncertainty evaluation
D_Alpha=(DL./Lt).*(Alpha+1).^2;
D_Beta=D_W./W1+Beta.*D_W./W1;
D_AR1=D_W./W1+AR1.*D_W./W1;
D_AR2=D_W./(Beta.*W1)+AR2.*D_W./(Beta.*W1);

% --- Generate vectors of random inputs
% x1 ~ Normal distribution N
mu_eo1=(randn(n,1)*11539)+22995;%PC
mu_eo2=(randn(n,1)*14638)-23079;%Aminated PC
%mu_eo1=(randn(n,1)*17234)+39342;%PC-Nada
%mu_eo2=(randn(n,1)*5136)-13663;%Aminated PC-Nada
mu_eph=(randn(n,1)*400)-37500;%DNA electrophoretic mobility (Stellwagen, 1997)

Alpha=(randn(n,1)*D_Alpha)+Alpha;
Beta=(randn(n,1)*D_Beta)+Beta;
AR1=(randn(n,1)*D_AR1)+AR1;
AR2=(randn(n,1)*D_AR2)+AR2;

% --- Run the simulation
w1=w1_mean(Alpha,Beta,AR1,AR2,mu_eo1,mu_eo2,mu_eph,n);
w1min=w1_min_wall(Alpha,Beta,AR1,AR2,mu_eo1,mu_eo2,mu_eph,n);
w1max=w1_max(Alpha,Beta,AR1,AR2,mu_eo1,mu_eo2,mu_eph,n);
w2=w2_mean(Alpha,Beta,AR1,AR2,mu_eo1,mu_eo2,mu_eph,n);
w2min=w2_min_center(Alpha,Beta,AR1,AR2,mu_eo1,mu_eo2,mu_eph,n);
w2max=w2_max_wall(Alpha,Beta,AR1,AR2,mu_eo1,mu_eo2,mu_eph,n);
t=Alpha./((1+Alpha).*(1+Alpha).*w1-1./((1+Alpha).*w2);
tmin=Alpha./((1+Alpha).*w1min-1./((1+Alpha).*w2min);
tmax=Alpha./((1+Alpha).*w1max-1./((1+Alpha).*w2max);
Appendix E: Monte Carlo Simulations Results for Channel 2

a) Maximum Channel 2 Velocity

b) Average Channel 2 Velocity

c) Minimum Channel 2 Velocity

Figure 54 Statistical Distribution of Dimensionless Channel 2 Species Velocities from MC Simulations (N=10^6)
### Appendix F: EOF Measurements Excel Spreadsheets

#### F.1. PC

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**F.3 UV PC**

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Appendix G: AutoCAD Designs Chosen for Mold Insert Realization

Figure 55 Design 1
Figure 56 Design 2
Figure 57 Design 10
Vita

Céline Ramet was born in June 1984, in Paris suburbs in France, from Michel and Michèle Ramet. After high school, she prepared national competitive exams for engineering school in Lycée Fénelon, Paris. In 2004, she was admitted to ENSICA, an engineering school specialized in aeronautics based in Toulouse, France. She left for the United States to enter the graduate program of the Mechanical Engineering Department at Louisiana State University (Baton Rouge) in 2006. She graduated from ENSICA in October 2008 with a French Engineering Degree, equivalent of Master of Science in Engineering, and expects to receive the degree of Master of Science in Mechanical Engineering from LSU in December 2008.