

INVESTIGATION OF AC ELECTRO-OSMOTIC FLOW BASED MICROFLUIDIC MIXER WITH MICRO RODS

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By
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Investigation of AC electro-osmotic flow based microfluidic mixer with
micro rods

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August 2018

We certify that we have read this thesis and that in our opinion it is fully adequate,
in scope and in quality, as a thesis for the degree of Master of Science.

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ABSTRACT

INVESTIGATION OF AC ELECTRO-OSMOTIC FLOW BASED MICROFLUIDIC MIXER WITH MICRO RODS

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M.S. in Mechanical Engineering

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AC electrokinetics (ACEK) is useful technique for providing species manipulation and liquid transportation. This method is applicable to many fields like cell separation, synthesis, pumping, mixing in microfluidics technologies. Mixing in micro-scale is very problematic issue due to the domination of surface forces over inertial forces. For acquiring complete mixing, extended channels or longer time is needed. To accelerate mixing, active and passive micromixers are presented in the literature. Among active micromixer types, ACEK-based micromixers are preferred techniques due to their advances in preventing electrochemical reactions and electrolysis. In this thesis, micromixer based on AC electroosmosis (ACEO) around micro rods is introduced. In this study, mixer performance is evaluated according to variable rod configurations, two different surface material and flow rates. Both computational modeling and experimentation are handled. Easy and cheap fabrication with commercially available metal wires is adopted. By introducing 3D electrode pairs, 3D vortex profile is presented and the most influential configuration is discovered.

Keywords: Microfluidics, micromixing, AC electrokinetics.

ÖZET

AC ELEKTRO-OZMOTİK AKIŞ TABANLI MİKRO TELLERLE SAĞLANAN MİKRO AKIŞKAN KARIŞTIRICININ İNCELENMESİ

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Parçacık manipülasyonu ve sıvı taşınması gibi olaylarda AC elektrokinetik tekniği çok etkili bir metottur. AC elektrokinetik, hücre ayrılması, sentezleme, pompalama ve karışma gibi mikro akışkan teknolojilerinin bir çok alanında kullanılmaktadır. Mikro akışlarda karışma, yüzey kuvvetlerinin eylemsizlik kuvvetlerini domine etmesi sebebiyle çok zordur. Tamamen karışma elde etmek için, uzun kanallar ya da uzun zaman gereklidir. Literatürde, karışmayı hızlandırmak için aktif ve pasif olmak üzere iki farklı karıştırıcı tiplerinden bahsedilmektedir. Aktif mikro karıştırıcılar arasında AC elektrokinetik metodu, elektrokimyasal reaksiyonları ve elektrolizi önlediği için daha çok tercih edilmektedir. Bu tezde, metal teller etrafında AC elektroozmoz temelli bir mikro karıştırıcı sunulmuştur. Bu çalışmada, mikro karıştırıcı performansı, farklı tel konfigürasyonlarına, iki farklı yüzey metaryaline ve akış hızlarına göre değerlendirilmiştir. Hem sayısal hesaplama hem de deney yapılmıştır. Ticari olarak ulaşılabilen metal tellerle, kolay ve ucuz üretim metodu seçilmiştir. Üç boyutlu elektrot çiftleriyle, üç boyutlu vorteks profile elde edilmiş ve en etkili elektrot konfigürasyonu belirlenmiştir.

Anahtar sözcükler: Mikro akışkanlar, mikro karıştırıcılar, AC elektrokinetik.

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Chapter 1

Introduction

Microfluidics is a technology through which micro-liters amounts of fluids are manipulated inside of micrometers size devices [2]. Lab-on-a-chip (LOC) systems, utilizing microfluidics theory, are composed of miniaturized chemical or biological laboratory platforms in which micrometer sized channels, electrodes, sensors, actuators are introduced. Microfluidics and lab-on-a-chip systems have huge impact on molecular diagnostics, pharmacology, biotechnology, microelectronics by interfering into chemical, biological and single-cell analysis, synthesis, separation, sorting [3]. These systems provide many advantages:

- low amount of samples and reagents usage,
- increased reaction speed comparing with room-sized counterparts,
- reduced manufacturing cost,
- high throughput,
- high sensitivity,
- portable, compact, wearable,
- integrable with other systems.

In addition to these advantages, microfluidic systems have some characteristic features. Firstly, the flow is in laminar regime and Reynolds number ($Re = \rho U D_h / \mu$), non-dimensional parameter that compares inertial forces and viscous forces, is typically much smaller than 1.0 (in some case, it may be reach up to $Re = 10$). Apart from flow at macro scale, in micro scale, viscous or surface forces dominate inertial forces so that $Re \ll 1.0$. This condition enables to neglect the nonlinear term of the Navier-Stokes equation and ease the solution. Furthermore, Peclet number (Pe) is smaller than 1.0. For instance, a T-type channel with two inlets and one outlet, liquids do not mix directly and a clear interface appears between the two liquid streams. After some distance and residence time of the liquid streams, mixture is realized. The distance and time can be defined as $L = U\tau$ and $\tau = W^2/D$. Pe is calculated by getting the proportion of L to channel width, W so, $Pe = UL/D$ where D is diffusivity which is typically around $2 \times 10^{-10} \text{ m}^2/\text{s}$ in microfluidics [4].

Currently, with the developments in fabrication, equipments and imaging tools, application areas of these systems have been extended. For instance, these systems are utilized in protein crystallization by observing the differences between crystal growth and nucleation, drug technology, bioanalyses by manipulation and transportation of DNA, RNA or single cells, monodisperse bubble manipulation and transportation, droplet generation in gas or liquid phase, polymerization by mixing, chemical synthesis of organic or medical chemistry, separation of motile or non-motile cells [2].

1.1 Electrokinetics Theory

When an electrolyte or aqueous solution is in contact with charged solid surface, the charges of the solid get collected on the surface [5]. Due to this attraction, counter ions in the electrolyte come closer to the solid surface and this region of immobile ions constitutes compact layer or stern layer. The region where ions are free and mobile is called diffuse layer. Combination of these two layers is named as Electric Double Layer (EDL). This charge transfer depends on the

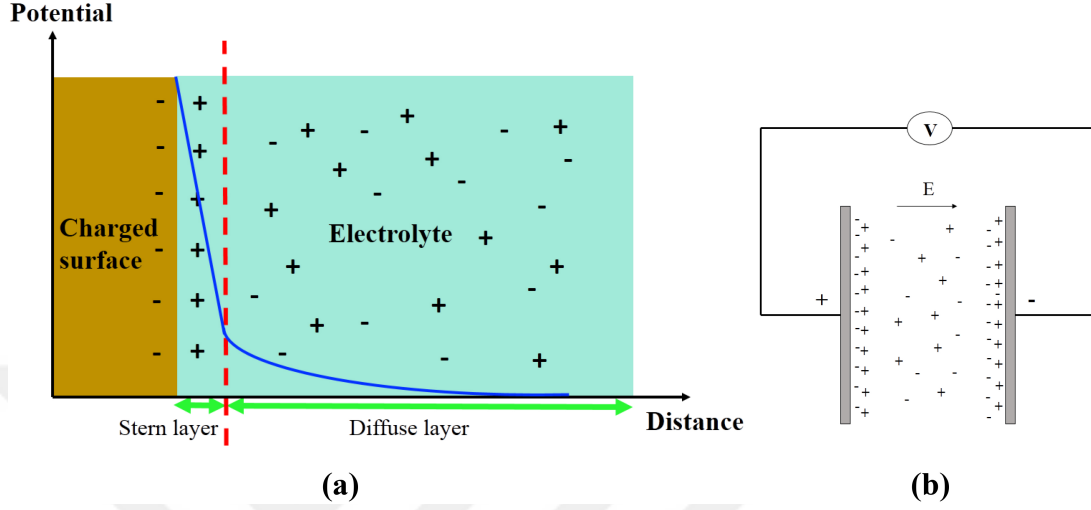


Figure 1.1: Schematic drawing of EDL and electrode polarization: (a) EDL with its components; stern layer, diffuse layer and shear plane, (b) Representation of capacitor

chemical compositions of the surface and electrolyte. In the Figure 1.1-(a), there is a boundary shown with dashed red line representing shear plane and electric potential at this plane is called zeta potential, ζ . From compact layer to diffuse layer, electric potential diminishes gradually (see blue line in Fig.1.1-(a)). Here, under applied electric field, EDL acts like a capacitor with linear potential drop between two plates (see Fig. 1.1-(b)). This potential drop results in dramatic electrical polarization in the material [6]. The length where surface's ζ potential drops to zero is called Debye length, λ_D . Because there is a charge redistribution between aqueous solution and metal surfaces under potential application, the Debye layer behaviour is represented as a capacitor seen in the Fig. 1.2. Bruus [1] modeled Debye layer with two parallel plates under 2ζ voltage difference and electrolyte having width of h .

The charge density inside the electrolyte is gathered by integrating the charge density inside the double layer. The charge density formula defined for single parallel wall

$$\rho_{el} = -\frac{\varepsilon\zeta}{\lambda_D^2} \exp\left[-\frac{z}{\lambda_D}\right] \quad (1.1)$$

and by integrating Eq. 1.1 from 0 to ∞ , charge density per unit area inside the

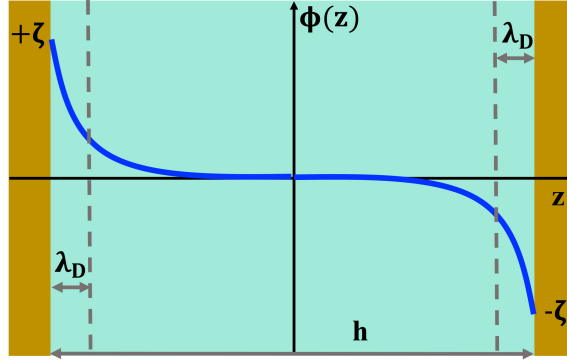


Figure 1.2: Debye layer under voltage difference of 2ζ (adapted from [1])

electrolyte is found [1]:

$$\rho_{liq} = \int_0^\infty \rho_{el}(z) dz = - \int_0^\infty \frac{\varepsilon \zeta}{\lambda_D^2} \exp \left[- \frac{z}{\lambda_D} \right] dz = - \frac{\varepsilon}{\lambda_D} \zeta \quad (1.2)$$

This linear relationship between applied voltage and the charge density supplies capacitance of double layer as $C_D = \varepsilon/\lambda_D$.

In the other technique, Green *et al.* [7] scaled up the real channel by conserving electrode gap and measure the double layer impedance by impedance analyzer. They tried to find out frequency dependence of double layer impedance under applied voltage. Upon gathering impedance, it is used to discover double layer capacitance. According to the observations of the variations in the resistance and capacitance based on frequency, the expression found is constant phase element

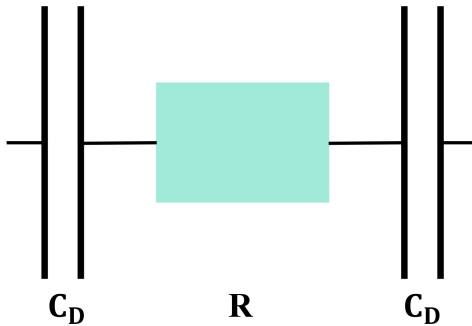


Figure 1.3: Debye layer representation in electrical diagram; two capacitors and one resistor in serial (adapted from [1])

impedance [7]:

$$Z_{DL} = \frac{A}{(i\omega)^\beta} = \frac{A}{(\omega)^\beta} \left[\cos\left(\frac{\pi}{2}\beta\right) - i\sin\left(\frac{\pi}{2}\beta\right) \right] \quad (1.3)$$

where A and β are constants according to variable conductivities and ω is the angular frequency ($\omega = 2\pi f$). It is significant to mention that electric field inside the electrolyte is highly dependent on frequency due to electrode polarization fact [1, 7]. High frequency results in voltage drop throughout the electrolyte and low one causes voltage drop across double layer [7]. Thus, at very high and very low frequency levels, slip velocity around the electrodes would get closer to zero but at intermediate frequency levels, it would reach its maximum value [7]. For finding out transition or characteristic frequency, $\omega_0 = (\sigma/\varepsilon)(\lambda_D/L)$ and for preventing voltage drop across double layer or electrolyte, it should be below charge relaxation frequency, $f_C = \sigma/\varepsilon$ [7]. Green *et al.* [7] defined another way by introducing a parameter, non-dimensional frequency, Ω . They solved this expression separately to take all the conductivity values of electrolyte into account:

$$\Omega = \frac{\omega\varepsilon L}{\sigma\lambda_D} \quad (1.4)$$

Under applied DC electric field to the charged surfaces, it exerts homogeneous electric field and ions start to move in EDL relative to the surface so, motion occurs in the electrolyte. This liquid motion is called EOF. In microfluidics, EDL thickness is on the order of nanometers so, comparing with micrometer-sized channels, it is very small value. In many microfluidic applications, the microchannel size is typically in the order of $100\mu\text{m}$, and the thickness of the EDL region, which is in the order on 10 nm , becomes negligible. With the effect of the viscosity, plug like velocity profile is obtained [5] (see Fig. 1.4). Here, in the figure the region between black dashed lines and channel walls corresponds to EDL and u_{EO} is shown out of these EDL regions so, the velocity profile is shown as plug-like. In the literature, this solution out of the double layer is called as outer solution. In ideal case, velocity magnitude diminishes from u_{EO} to 0 gradually throughout the Debye length. The solution throughout the double layer is mentioned as inner solution. Basically, when assuming very small EDL, inner solution is neglected and just outer solution is taken into account. EOF velocity is defined

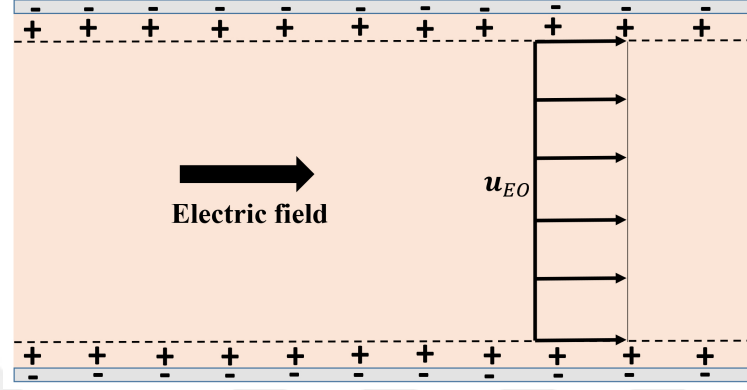


Figure 1.4: Schematic drawing of EOF

by Helmholtz-Smoluchowski equation as:

$$u_{EO} = \frac{\varepsilon \zeta}{\mu} \mathbf{E}_{\parallel} \quad (1.5)$$

and the combination of terms $\mu_{EO} = \varepsilon \zeta / \mu$ corresponds to electroosmotic mobility.

In the case of an AC electric field, molecules inside of a dielectric material form a dipole having equal amount of opposite charges separated by a distance [8]. The electric field, ϕ , in a dielectric medium is defined by Poisson equation:

$$\nabla^2 \phi = -\frac{q}{\varepsilon_0 \varepsilon} \quad (1.6)$$

where ρ , ε_0 and ε are free charge density, permittivity or dielectric constant of the vacuum and permittivity of the medium respectively [8]. When there is no net charge in which q is zero and electric potential equation turns into Laplace equation:

$$\nabla^2 \phi = 0 \quad (1.7)$$

In the case of an sinusoidal electric field, same equation can be used for the phasor of the electric potential. Outside of the EDL, the electric potential on the electrode surface is given in Green *et al.* [7] for 1D case. For 3D applications, electric potential on the electrode surface is defined on surface normal direction, $\mathbf{n}$.

$$\sigma \frac{\partial \phi}{\partial \mathbf{n}} = i\omega q_{DL} \quad (1.8)$$

where σ is the electrical conductivity of electrolyte and q_{DL} is the charge per unit area in the EDL [7]. By the assumption of very small voltage drop across EDL, q_{DL} and voltage show linear relationship and q_{DL} is defined as $q_{DL} = C_{DL}(\phi - V_j)$ in Green *et al.* [7]:

$$\sigma \frac{\partial \phi}{\partial \mathbf{n}} = i\omega C_{DL}(\phi - V_j) \quad (1.9)$$

where C_{DL} is the EDL capacitance per unit area, ω is the applied frequency and V_j is the applied voltage on the electrode j [7]. Based on Debye-Huckel theory, the double layer capacitance (C_{DL}) can be approximated as ε/λ_D [7]. Here, λ_D is the Debye length, which defines the length of the double layer.

Using the thin EDL approximation, the slip-velocity on the electrode surface can be written as [9]:

$$\mathbf{u} = -\frac{\varepsilon}{4\mu}\Lambda \frac{\partial}{\partial \mathbf{t}}|\phi - V_j|^2 \quad (1.10)$$

where μ is the viscosity and Λ is a ratio of the potential drop in the diffuse layer to the potential drop in the whole EDL ($\Delta\phi_d/\Delta\phi_{DL}$) so, it is smaller than unity. In Green's study [7], Λ value is taken as 1.0 because of the Debye-Huckel theory of very small double layer approximation. However, based on the experimental data, Λ is slightly changed to match experimental data with numerical data because in real cases, the capacitance of the double layer does not depend only on diffuse layer but it also depends on compact layer so that electrode polarization isn't purely capacitive, which means potential drop occurs not only throughout the diffuse layer but also compact layer. [7]:

$$\Lambda = \frac{C_{DL}}{C_d} = \frac{C_s}{C_s + C_d} \quad (1.11)$$

where C_s and C_d are stern layer and diffuse layer capacitances. In general, these capacitance values are measured or in some cases, impedance values are measured via impedance analyzer [10] as mentioned before and from $Z = R/1 + i\omega RC$, capacitance is found and these values are integrated into C_{DL} . By this approach, researchers are able to model electrode polarization and potential drop across EDL and electrolyte as a function of frequency [10].

1.2 Electrokinetics in microfluidic applications

In microfluidics, manipulation of species and liquid transportation can be accomplished by variable ways like pressure, thermal gradients and centrifugal impacts [11]. Furthermore, electrokinetics (EK) is the other technique that is incorporated into microchannels to provide motion [12] and it doesn't require mechanical parts [13]. Electrokinetic transport occurs when polar fluid is in contact with charged solid surface [4] and under applied electric field charge redistribution is realized on both solid surface and polar fluid that induces fluid motion [14]. This electrokinetic motion near the charged solid surface is called electroosmotic flow (EOF). EOF provides uniform, non-pulsating, plug like velocity profile [11, 13] and its response to the applied electric field is fast. In biomedical applications, direct current (DC) is mostly used in manipulation and transportation of cells or molecules by electrophoresis phenomena [15]. Alternating current (AC) is mostly sight in DNA, RNA, protein manipulation or in general cell separation via dielectrophoresis (DEP) [16–18], pumping [9, 10] and mixing [9, 10] via AC electroosmosis (ACEO) or induced-charge electroosmosis (ICEO) [11].

In microfluidics area, there are reactions, for instance, enzyme reactions, nucleic acid and protein synthesis and sequencing that require rapid mixing [19]. Notwithstanding many benefits, in microfluidics, mixing couldn't be achieved readily and long channels are needed and time period to get full mixing extends. The reasons behind the inadequacy of microfluidics in mixing are that surface forces prevail body forces and Reynolds number (Re) is extremely small. In laminar flow regime, flow are fluid streamlines are parallel with each other and with constant boundary conditions streamlines are independent of time [20]. Hence, mixing is realized mainly by molecular diffusion and advection [21].

To overcome micro-mixing problem, variable ways have been introduced by researchers and mainly these methods are separated into two groups; passive and active micro-mixers. In passive type micro-mixers, the geometries of the channels are altered such that fluids' interface area is increased [21]. In active micro-mixers,

external energy is applied and stable flow profile is perturbed [21]. Pressure perturbation [22], acoustic [23], ultrasonic [24], dielectrophoretic [25], magnetic [26], thermal [27] and electrokinetic methods are the fundamental active micromixer methods in the literature. EK facilitates manufacturing and integration with other microfluidic chips and also it decreases the possibility of tremendous pressure drop so, comparing with other techniques EK is more favorable technique in mixing application [28]. One problematic issue about EK is that electrolysis may occur which results in gas bubbles and pH-gradient variation [29]. In this case, AC EK is more advantageous because it may be a solution for undesirable electrochemical reactions such as electrolysis of water and charged particles or proteins [30].

In electrokinetic-based micromixers, applied electric field disturbs stability of the laminar flow inside the micro-channel and causes fluctuations [31]. This disturbance can be ensured by two ways; in one of them, electric field is applied directly to the electrodes or channel walls and bulk fluid motion is observed (EOF) [32], in the other one, electric field is applied to the inlet and outlet of the channel and around conducting objects, charge redistribution occurs and flow is observed (ICEOF) [4]. As an example of ICEOF mixing, Wu *et al.* [33] presented a micromixer by using nonlinear ICEOF which is rooted in non-uniform zeta potential distribution of conducting hurdles. Based on different hurdle geometries and variable electric fields, performance of the mixer is evaluated. Sugioka *et al.* [34] proposed a numerical study of a mixer by following Strouhal number (St) and Péclet number (Pe). In the study, it is mentioned that at extremely large Pe, presented mixer displays better performance with low voltage values [34]. In one the recent studies, Ren *et al.* [35] proposed the same theory and they extended ICEOF with leaky dielectric blocks. In the field of EOF mixing, one of the preliminary studies is presented by Oddy *et al.* [36] in which a mixing chamber made out of platinum electrodes is created inside of a micro channel. By applying AC field to those electrodes, electroosmotic flow is induced and instabilities in the flow profile is appeared [36]. In this study, applied voltage is around 4 kV and as a result of this extreme voltage application, rise in temperature is observed [36]. Glasgow *et al.* [37] presented another version of Oddy

et al. study and instead of creating mixing chamber, pulsating electric field is applied to the wells, located at inlets and outlet of the channel, and mixing by electroosmosis is provided. In this study, it is claimed that once correct pulsation parameters are found, complete mixing can be gathered [37]. Nevertheless, in this application, all the channel is exposed to electric field so, when cells are introduced into the channel, this voltage may cause alterations of the behavior of cells or it may harm the cells. Qian *et al.* [38] presented a mathematical modeling of an electroosmosis based mixer in which ζ potential of the channel walls is altered so that nonuniform ζ potential distribution is ensured. In this study, flow patterns and Poincaré sections are provided but efficiency study isn't addressed [38]. Lastochkin *et al.* [39] presented micromixer and micropump based on different polarization technique (ac faradaic polarization) which is said to be advantageous for preventing gas bubbles and pH gradients. Different channel or electrode geometries are also combined with EOF, to enhance mixing performance. By introducing asymmetric-herringbone-electrode configuration, Wu *et al.* [40] demonstrated a micromixer with periodically out-of-phase AC field application. In the other study, time-pulsating inlet flow rates are inserted by introducing phase shift in the electric fields [41]. In another varying channel or electrode geometry study, Campisi *et al.* [42] combined EOF with pressure-driven flow (PDF) and electrode placement is determined by linked twisted map (LTM) strategy. This study is also performed at high Pe regime and it is mentioned that the study is restricted due to the independence of axial and transverse flows [42]. Sasaki *et al.* [43] used meandering electrode configuration to create EOF and rise the mixing efficiency. In the other study using varying electrode shape, Huang *et al.* [44] demonstrated in-plane vortex patterns inside of Y-shaped channel. For observing the impact of varying ζ potential, which is constant along the surface in general, Lee *et al.* [45] proposed a micromixer by introducing capacitor electrodes and it is mentioned that fluid flow can be controlled easily with this study. In another study, by positioning four symmetric electrodes on the boundary of a circular chamber, mathematical model is published based on inlet mean velocity and applied voltage [46]. Harnett *et al.* [47] fabricated a micromixer having array of metallized posts inside the channel and electrodes are covered through the channel walls. By applying electric potential, with the help of ICEO, mixing

is created. With another novel approach, Ng *et al.* [48] developed a micromixer applying DC-biased AC voltage to the electrodes. By considering the impacts of AC frequency, DC-biased level, polarity of electrodes, flow rate and conductivity, the study is extended. In one of the promising studies, Feng *et al.* [49] provided a numerical study of a mixer based on EOF and ICEOF at the same time in different geometry, eccentric annulus. They enhanced the efficiency of micromixing by integrating both EO and ICEO in a channel and prepared Poincaré sections of Lagrangian chaos in this geometry. The summary of the literature on micromixer comparison is given in Table 1.1.

1.3 Objectives and motivation

Even if low Re provides many benefits, most of the microfluidic reactions suffer from the ineffectiveness of micromixing. To solve this problem, one of the frequently used method, EK is realized and used in mixing application of microfluidics. However, EK literature is mostly confined to deposited electrodes which require cleanroom fabrication [4,9–11,15–19,21,23–26,28–31,33,35–45,47,48,50–54]. In this study, as electrode pairs, commercially available metal rods are used together with micro machined T-shaped metal mold. Apart from common manufacturing techniques, this study doesn't require any cleanroom facilities so, comparing with cleanroom manufacturing, it is more repeatable because by one mold, many channels can be produced and it is faster because PDMS curing process takes shorter period of time, extra time isn't necessary for electrode deposition. Moreover, electrodes could be settled inside of the microchannel which is impossible via cleanroom fabrication. This study implements T-shaped microchannel in which flow is ensured by pressure driven flow is combined with ACEO around 3D electrode pairs. Through this combination, when there is electric field effect, ACEO is introduced inside of the channel and vortices are observed around electrodes. It is assumed that presence of 3D electrode pairs result in 3D vortex profile hence, mixing performance enhances. In molecular diagnostics, applying electric field to the whole channel harms the cells, DNA, RNA or protein structures. Therefore, one of the motivating aspect of this study is that electric field

is just applied to the regions near the electrodes and comparing with the channel length, this region corresponds to very small part of it. EK principle can be integrated into many applications as mentioned in the EK literature but here in this work, mixing aspect of ACEO is examined. The study is further extended by different electrode orientations. Electrode orientation, number of electrodes and spacing between electrodes have huge impact on EOF velocity so that by finding out the suitable configuration, mixing can be realized with lower voltages. As a last step, surface coating impact is observed. EOF is basically charge transfer between charged solid surface and electrolyte and it is highly dependent on chemical composition of surface and electrolyte. Therefore, gold and brass electrodes' performances are discovered. In the literature, there is not much study discussing this fact.

1.4 Outline of the thesis

The complete thesis comprises of the following major sections:

Chapter 1 gives brief introduction to microfluidics and LOC technologies, their characteristics, areas of applications. Electrokinetic theory and how EDL and EOF occurs with chemical point of view are mentioned. Different models of double layer capacitance and impedance are discussed. Furthermore, ACEOF theory with the solution of electric potential and fluid flow equations are given. Transportation techniques in microfluidics are mentioned and electrokinetic transportation with the examples from the literature is compared based on fabrication method, width, electric field, Re , Pe , flow rate, efficiency. The motivation of the present work is addressed.

Table 1.1: Literature micromixer comparison

Reference	Year	Method	Fabrication	Channel Width [μm]	Electric Field [V/cm]	Re	Pe	Flow rate [$\mu\text{L}/\text{min}$]	Eff.
Oddy <i>et al.</i> [36]	2001	EO	Clean room	300	1000	< 1.5	30000	0.5	–
Glasgow <i>et al.</i> [41]	2003	EO	Milling and bonding with optical adhesive	200	–	0.3	3000	0.287	78
Glasgow <i>et al.</i> [37]	2004	EO	Clean room	200	187	0.3	3000	0.5	80
Lee <i>et al.</i> [45]	2004	EO	Clean room	120	–	–	$< 2 \times 10^3$	2	90
Wu <i>et al.</i> [40]	2004	EO	Clean room	200	86	–	–	–	90
Fu <i>et al.</i> [50]	2005	Periodic EK force	Clean room	100	100	–	–	–	95.3
Park <i>et al.</i> [51]	2005	EK instability (EKI)	Clean room	150	240 – 880	–	–	–	65
Shin <i>et al.</i> [55]	2005	EK instability	Clean room	60	–	10	–	–	–
Sasaki <i>et al.</i> [43]	2006	EO	Clean room	200	–	< 5	0.58×10^6	17.4 – 55.8	80 – 90
Huang <i>et al.</i> [44]	2007	EO	Clean room	400	–	–	$< 2 \times 10^3$	2	90
Harnett <i>et al.</i> [47]	2008	ICEO	Clean room	200	–	< 2	10^5	0.1	–
Matos <i>et al.</i> [52]	2008	EO	Clean room	60	–	10	–	–	–
Campisi <i>et al.</i> [42]	2009	EO	Clean room	200	–	< 5	0.58×10^6	17.4 – 55.8	80 – 90
Ng <i>et al.</i> [48]	2009	ACEO & AC electrothermal	Clean room	100	–	–	2071–6213	2	92
Yan <i>et al.</i> [53]	2009	EO	Clean room	300	200	–	–	–	90
Lim <i>et al.</i> [54]	2010	Periodic EOF	Clean room	200	258	0.18	–	–	90
Feng <i>et al.</i> [49]	2016	EO & ICEO	–	$R_r = 0.1$ (radius ratio of the annulus)	100	–	100	–	–

Chapter 2 demonstrates COMSOL Multiphysics modeling with assumptions and micromixer design procedure. Utilized COMSOL Multiphysics module; electric currents, fluid flow and transport of diluted species with equations and boundary conditions are mentioned. Results of the numerical modeling based on variation in voltage, volumetric flow rate are presented. How the mixer design is changed and how it is improved are mentioned. Mixing index findings of each configuration are compared.

Chapter 3 gives experimental setup preparation with channel fabrication, equipments and procedures. Initial experiments with encountered problems are mentioned and the changes in the experimental setup are given. Moreover, post-processing procedure of the acquired experimental data is discussed and experimental results are proposed. How surface coating effect is implemented on the simulations are mentioned and comparisons with experiments are given.

Chapter 4 summarizes the major findings of this study, what could have been done better is mentioned briefly. Future research directions are referred.

Chapter 2

Computational Modeling and Chip Design

Computational analysis are realized by COMSOL Multiphysics software. The simulations first start with the basic geometry and based on observations of the basic case, the orientation of the electrodes and the distances between the electrodes and the walls of the channel are evaluated. What is important for determining the best configuration is that vortices in Fig. 2.1 generated by each electrode should engage in the other electrode's vortices so that mixing will become easier. Three physics; electric field, velocity field and concentration field of COMSOL Multiphysics are applied to the drawn geometry with suitable material parameters and alterations are provided.

The assumptions of this study are as follows:

- (i) Flow is laminar and steady,
- (ii) Fluids are Newtonian,
- (iii) Fluids have the same ρ , σ , μ
- (iv) Electrodes are ideally polarizable,
- (v) Electrodes have homogeneous surface of ζ

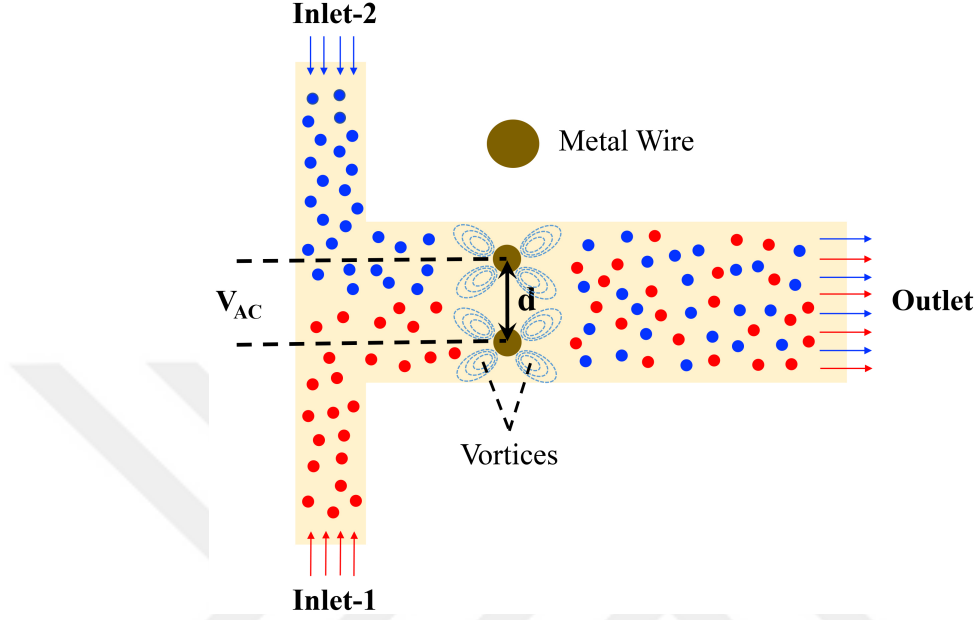


Figure 2.1: Schematic drawing of micromixer: Side channels (Inlet-1 and Inlet-2) and main channel have $500 \mu\text{m}$ and $1000 \mu\text{m}$ wide successively. Metal wire diameter is $200 \mu\text{m}$.

- (vi) Debye-Huckel limit ($eZ \ll k_B T$) is valid (e is the elementary charge, Z is the integer valence number, k_B is the Stefan-Boltzmann constant and T is temperature).

The micromixer composes of a T-shaped microchannel in which flow is introduced by pressure-driven flow (PDF) from side channels and metal electrodes are located in the main channel providing mixing impact (see Fig.2.1). Mixing impact is ensured by applying AC electric field. It is assumed that under applied field, electrodes will generate vortices resulting from AC-EOF around the wire electrodes and mixing will be realized.

The governing equation (Eq. 2.1) and constitutive relations (Eq. 2.1 and Eq. 2.2) of the electric potential are as follows:

$$\nabla \cdot \mathbf{J} = Q_{j,v} \quad (2.1)$$

$$\mathbf{J} = \sigma \mathbf{E} + \mathbf{J}_e \quad (2.2)$$

$$\mathbf{E} = -\nabla \phi \quad (2.3)$$

where $\mathbf{J}$, $Q_{j,v}$, $\mathbf{E}$ and $\mathbf{J}_e$ are current density, current source, electric field and electron current density successively. Boundary conditions of electric field around the metal wires are defined as follows;

$$\phi = \frac{\sigma(1+\delta)}{i\omega C_D} \frac{\partial \phi}{\partial \mathbf{n}} + V_j \quad (2.4)$$

$$\phi = \frac{\sigma(1+\delta)}{i\omega C_D} \frac{\partial \phi}{\partial \mathbf{n}} - V_j \quad (2.5)$$

where $\delta = C_D/C_s$ is the ratio between diffuse layer and stern layer capacitances [56]. Apart from Green's *et al.* study [7], in the equation `refe:neweq`, there is a constant $1/(1+\delta)$ that changes electric potential magnitude based on potential drop across the double layer.

The flow field is governed by continuity (Eq. 2.6) and incompressible Navier-Stokes equation (Eq. 2.7),

$$\nabla \cdot (\rho \mathbf{u}) = 0 \quad (2.6)$$

$$\rho(\mathbf{u} \cdot \nabla) \mathbf{u} = -\nabla p + \mu \nabla^2 \mathbf{u} + \rho \mathbf{g} \quad (2.7)$$

where ρ is the density of the liquid, p is pressure, μ is the viscosity of the liquid and g is the gravity. The above equations are solved with the no-slip boundary condition ($\mathbf{u} = 0$) at the channel walls, specified flow rates ($\mathbf{u} = Q/A$) at the reservoirs and zero pressure ($P = 0$) boundary condition at the exit. For the boundaries around electrodes, slip velocity equation is defined as follows;

$$\mathbf{u} = -\frac{\varepsilon}{4\mu(1+\delta)} \frac{\partial}{\partial \mathbf{t}} |\phi - V_j|^2 \quad (2.8)$$

This equation is similar with the previous slip velocity equation (Eq. 1.10) $\Lambda = 1/(1+\delta)$.

The concentration field is governed by convection-diffusion equation,

$$\mathbf{u} \cdot \nabla c = D \nabla^2 c \quad (2.9)$$

subjected to insulated boundary at the channel walls and the specified concentration at the reservoirs. Herein, c represents the dimensionless mole fraction of

the acetic acid [57]. Therefore, c is assigned as unity at the inlets of acetic acid, and assigned as zero for the inlets of water. At the exit, convective flux boundary condition is assigned.

In COMSOL Multiphysics, three modules; electric currents (ec), creeping flow (spf) and transport of diluted species (tds) are used by defining initial values between three studies, which means in the first study, electric potential is solved then, the electric potential solution is defined as the initial value of the variables in the flow field equation. Upon gathering flow field, the results of the flow field is utilized as initial variable in the concentration solution.

Numbers of degrees of freedom (DOF) solved in three physics are 3 M, 14 M and 3 M respectively. Free triangular mesh is applied to the boundaries of the electrodes and side channel walls with the growth rate of 1.05. Upon increasing the mesh quality more, it is observed that the solution of COMSOL Multiphysics doesn't vary so, increasing the mesh quality more isn't needed. The simulations are performed with HP Z840 Workstation (Intel Xeon E5-2620, 6-CORE, 2.40GHz, 128GB RAM). Variables of computation are given at the Table 2.1.

Table 2.1: Parameters

Parameter	Value
ε_r	80
ε_0 [F/m]	8.85×10^{-12}
σ [mS/cm]	1.0
λ [nm]	4.858
f [kHz]	100
μ [Pa · s]	0.001
ρ [kg/m ³]	1000
Λ	1.0
D [m ² /s]	2×10^{-10}

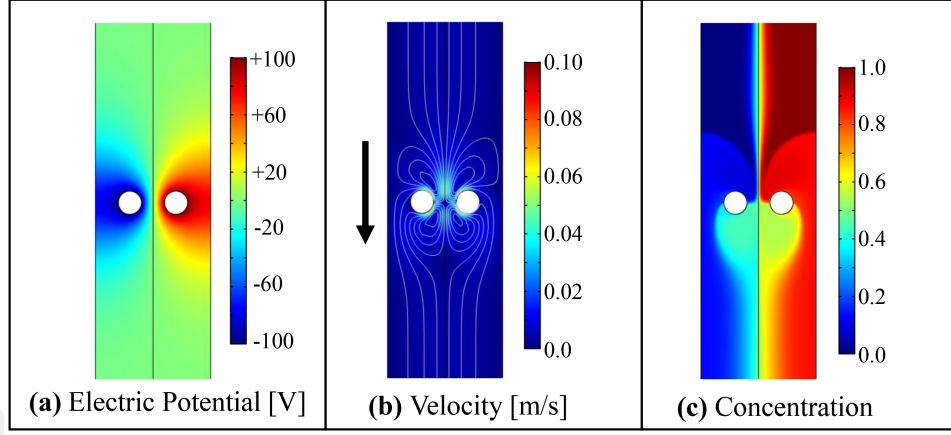


Figure 2.2: COMSOL Multiphysics results of the micromixer ($Q = 1.0$ mL/h) (Black arrow line represents the flow direction.)

2.1 Case A: 2-wire original configuration

Figure 2.2 belongs to the electric potential, velocity and concentration results of the proposed schematic under 200 V voltage difference between two electrodes and 1.0 mL/h volumetric flow rate. As seen in Figure 2.2-(a), electric field doesn't spread over the whole channel but it is restricted to the areas near the electrodes. Therefore, when this mixer is used in cellular or molecular studies, the possibility of harming the cell structure would be diminished. In theory of this study, it is assumed that near the electrodes, vortices will occur that enhances mixing. In Figure 2.2-(b), vortices are present and around the electrodes, EOF dominates PDF, velocity magnitude reaches to 0.1 m/s. When getting away from the electrodes, EOF impact drops off and so, the velocity magnitude as well. Concentration solution of the mixer demonstrates that electric field has impact on the concentration and just behind the electrodes, there is a small mixed portion. However, at approximately $400 \mu\text{m}$ distance away from the electrodes electric field loses its influence immediately so, mixed part disperses.

To have more insight into the concentration field of this micromixer, a line (almost 1 mm away from the wires) just beyond the electrodes throughout the channel length is taken and concentration data on this line is plotted. For comparison, different voltages are experienced and behaviour under variable flow rates

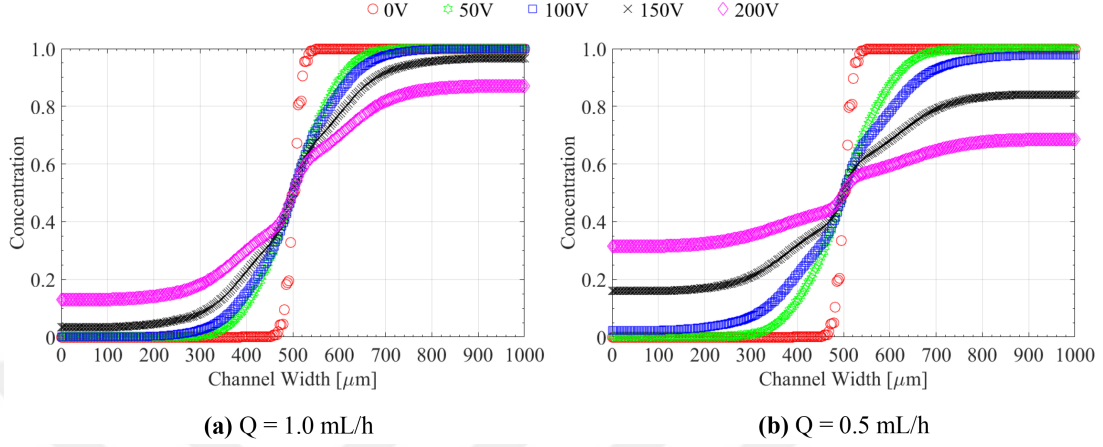


Figure 2.3: COMSOL Multiphysics results of the concentration data of the micromixer under different flow rates

is also examined. Figure 2.3 shows concentration profiles of two different flow rates 1.0 mL/h and 0.5 mL/h. Under higher electric potential, from Eq.1.10, slip velocity escalates. In Fig. 2.3-(a), under 50 V and 100 V electric potential, concentration data changes between 0 and 1, when potential is above 150 V, the concentration data take values between 0.15 and 0.85. These conclusions indicate this orientation isn't enough for mixing but modifications should be done. Furthermore, when dropping volumetric flow rate off, under the same electric field, EOF should dominate the PDF so that there should be some enhancement in the concentration field. In the figure, above 100 V, concentration data seem to be improved because 150 V and 200 V potentials give concentration data between 0.2 – 0.8 and 0.3 – 0.7 respectively. There is improvement but still some modifications should be integrated to have better mixing.

Upon observing concentration result of the proposed mixer, it is concluded that this design isn't appropriate to enhance mixing performance and as a solution, some design modifications are made. As a design improvement, instead of placing the electrodes horizontally, crosswise placement is thought to enhance the mixing because number of vortex cells resulting from EOF would increase due to the presence of asymmetry in the electrode orientation. With this point of view, 2-staggered and 4-staggered rod orientations are assigned. Figure 2.4 shows schematic electrode orientations with suitable distances.

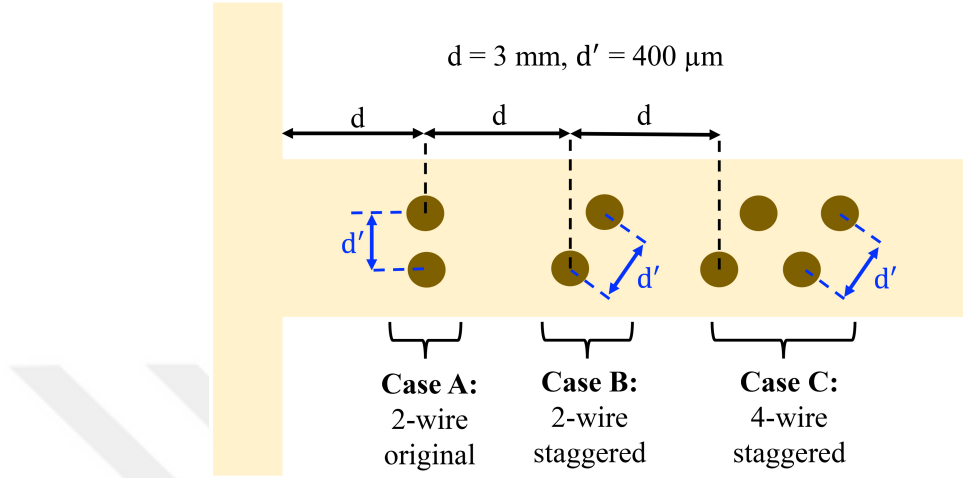


Figure 2.4: Electrode configurations

When deciding the electrode configurations and the distances, some important points are considered;

- If the distance between the electrodes are decreased, the voltage required to have mixing decreases as well so that with lower voltage application, better mixing could be acquired. However, the distance can't be decreased so much due to the manufacturing constraints while opening electrode holes on the mold.
- Angle between two electrodes are chosen as a result of vortex cell observations. It is thought that rise in the number of vortex cells and creating asymmetry between the cells would increase mixing.

2.2 Case B: 2-wire staggered configuration

Under 200 V, electric potential, velocity and concentration results of the 2-wire staggered and 4-wire staggered orientations are supplied and compared with each other. In Figure 2.5-(a), it can be inferred that in contrast with 2-wire original case, electric field seems to expand through the channel even if it isn't directly applied to whole channel. This could create a drawback for cellular applications.

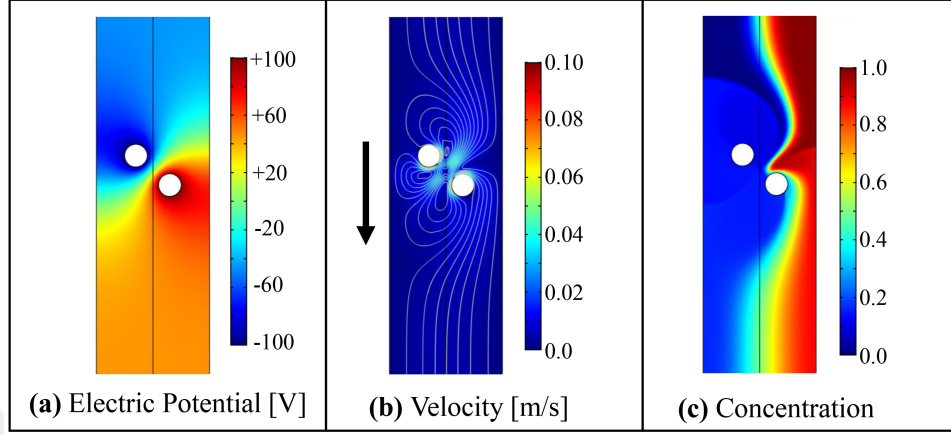


Figure 2.5: COMSOL Multiphysics results of the 2-wire staggered orientation ($Q = 1.0$ mL/h) (Black arrow line represents the flow direction.)

Figure 2.5-(b) reveals that under the same electric potential application with 2-wire case, this time vortex profile seems to be asymmetric and number of vortex cells decreases from four to three. Furthermore, the maximum value of the velocity is almost the same with 2-wire orientation, the reason behind this same velocity magnitude is in both of the cases, the number of electrodes are equal. As a result, even if the number of vortex cells decrease from four to three, the maximum velocity magnitude doesn't diminish so it can not be exactly said that there is a direct relationship between number of cells and velocity magnitude however it can be concluded that 2-wire staggered configuration provides asymmetric vortex profile which is better in mixing performance comparing with symmetric one. The effectiveness may not be seen clearly by only looking at the Fig. 2.5-(c) but by examining the line concentration graphs (Fig. 2.6) or quantifying mixing efficiency (Fig. 2.9), this result may be perceived.

Fig. 2.6 demonstrates concentration profile on a line just 1 mm beneath the wires. In comparison with 2-wire configuration, there is not much progress in 2-wire staggered orientation. Consequently, just introducing asymmetric electrode profile seems not to solve mixing problem but maybe rise in the number of rods may develop the performance considerably. Therefore, as a next step, 4-wire staggered configuration of the Fig. 2.4 is monitored.

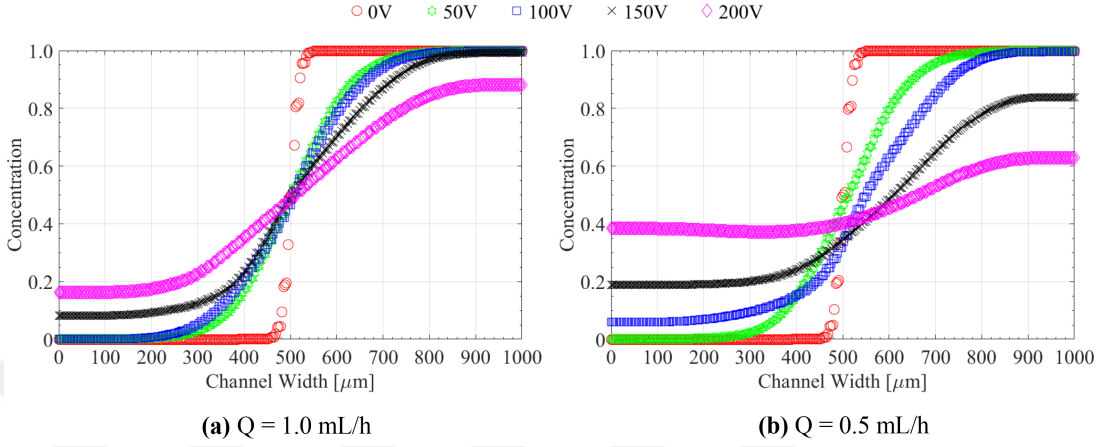


Figure 2.6: COMSOL Multiphysics results of the line concentration of the 2-wire staggered orientation under different flow rates

2.3 Case C: 4-wire staggered configuration

In Figure 2.7, electric field seems to expand through the channel as in the 2-wire staggered case. However, streamlines of the velocity field show that number of vortex cells escalates and the maximum velocity magnitude around the electrodes increases slightly. Here with this arrangement, there is rise in both the number of vortex cells (in concurrence with asymmetric streamline profile) and velocity magnitude. By just looking at the streamline profile, there should be boost in the mixing performance exactly. As expected, concentration field of the 4-wire staggered configuration reveals that just beyond the wires, concentration takes values between 0.4 and 0.6, which means mixing is almost acquired.

To decide whether this arrangement is suitable or not, evaluating line concentration will provide better insight. Therefore, as the other cases, line concentrations under variable voltages and volumetric flow rates are also taken into consideration. At $Q = 1.0 \text{ mL/h}$ - 200 V and $Q = 0.5 \text{ mL/h}$ - 150 V conditions, concentration values are between 0.35 and 0.65 and mixing is seen to some extent. 4-wire staggered case gets closer to 0.5 concentration value via $Q = 0.5 \text{ mL/h}$ flow rate and 200 V electric potential application, it reaches almost perfect mixing. What is effective on this case is 4-wire configuration has more vortex cells (see Figure 2.7-(b)) and because of the existence of two more electrodes, EOF

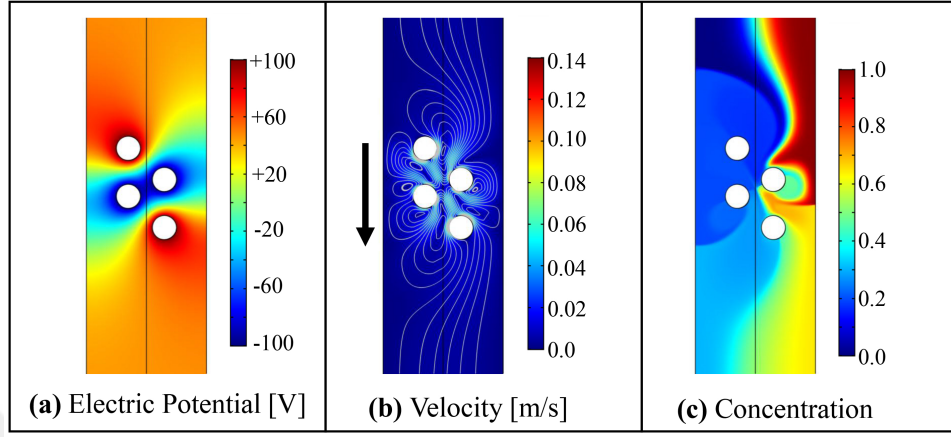


Figure 2.7: COMSOL Multiphysics results of the 4-wire staggered orientation ($Q = 1.0$ mL/h) (Black arrow line represents the flow direction.)

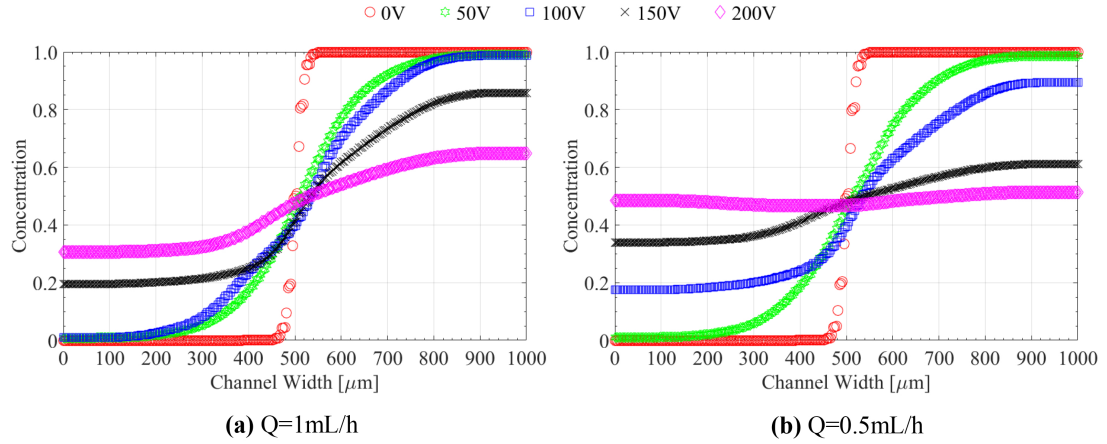


Figure 2.8: COMSOL Multiphysics results of the line concentration of the 4-wire staggered orientation under different flow rates

slip velocity enhances and mixing index as well. What could have been done is that by changing positive and negative poles of the applied electric field, different cases may proceed but it is neglected in this study.

Significant points deduced from the computational studies:

- rise in number of vortex cells,
- asymmetric vortex profile,
- increase in number of rods,

- higher voltage,
- lower flow rate

boost mixing performance.

In order to quantify the mixing performance, the mixing index at the exit of the mixing section is determined. Looking at the line concentration gives idea related to performance but quantification of mixing index is significant to compare this study with the literature. Mixing index can be determined via [58],

$$\sigma = 1 - \frac{\int_A |c - c_\infty| dA}{\int_A |c_o - c_\infty| dA} \quad (2.10)$$

where c is the sample concentration, and c_o and c_∞ are the sample concentrations in the completely unmixed and completely mixed conditions, respectively and A is cross sectional area. Therefore, $\sigma = 0$ indicates a completely unmixed state, and $\sigma = 1.0$ indicates complete mixing.

Based on acquired line concentration fields in Figures 2.3, 2.6 and 2.8, mixing index, σ is calculated by Eq. (2.10). According to the previous observations from surface and line concentration plots, in each configuration, lower flow rate should reflect better mixing performance. Therefore, it isn't surprising that $Q = 0.5 \text{ mL/h}$ cases are better at mixing performance than $Q = 1.0 \text{ mL/h}$ in each electrode configurations (compare red and black, green and magenta, blue and cyan data of Figure 2.9). At the same flow rate, the efficiency magnitude should follow 4-staggered, 2-staggered and 2-original. As expected blue line is at the top comparing with red and green lines and cyan line has highest values comparing with black and magenta lines. The maximum index belongs to 4-staggered configuration under $Q = 0.5 \text{ mL/h}$ and 200 V , the index is around 100%.

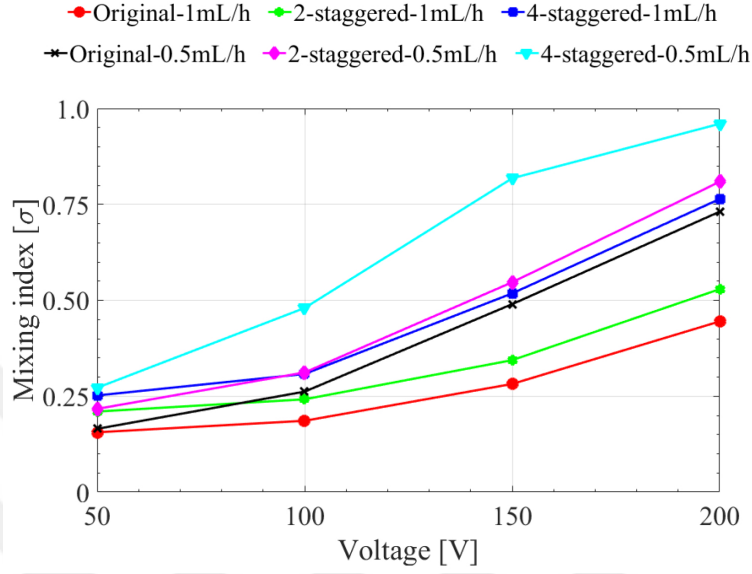


Figure 2.9: Mixing index vs. voltage graph of the simulation results based on different flow rates and electrode orientations ($\Lambda = 1$)

Table 2.2: Computational results

Parameter	Value
U_{av} [mm/s]	2.771 ($Q = 1.0$ mL/h) 1.454 ($Q = 0.5$ mL/h)
Re	0.90 ($Q = 1.0$ mL/h) 0.48 ($Q = 0.5$ mL/h)
Pe	100
$\mathbf{E}$ [V/m] (Case A) 100 V	$-11091 - 500.32i$ (x-dir.) $-6.9153 \times 10^{-5} + 1.4675 \times 10^{-5}i$ (y-dir.)
$\mathbf{E}$ [V/m] (Case B) 100 V	$-8172.1 - 366.70i$ (x-dir.) $6924.6 + 305.80i$ (y-dir.)
$\mathbf{E}$ [V/m] (Case C) 100 V	$0.0011225 + 7.1860 \times 10^{-5}i$ (x-dir.) $-6.4576 \times 10^{-4} - 6.0400 \times 10^{-5}i$ (y-dir.)
ω_0 [Hz]	23
f_C [MHz]	150

Table 2.2 presents average velocity (U_{av}), Re , Pe , ω_0 , f_C and electric field ($\mathbf{E}$) results of each electrode configuration (under 100 V). It can be stated that Re is below unity for both of the volumetric flow rates, and the flow is in the laminar regime. Element Pe is approximately 100 and it decreases to 40 at the middle of the channel, where maximum velocity magnitude is gathered. Pe is acquired from simulations by using the expression;

$$Pe = \frac{\|\beta\|h}{2c} \quad (2.11)$$

with β , h and c are convective velocity vector, mesh element size and diffusion coefficient respectively.

Electric field values of three configurations belong to the 100 V cases as in the figures 2.2, 2.5 and 2.7. The fields are supplied both in x - and y -directions and because of the AC field, they have both imaginary and real parts. It is mentioned before that charge relaxation frequency should be higher than characteristic frequency and as it is seen ω_0 is several orders smaller than f_C .

Chapter 3

Experimentation

3.1 Chip fabrication

For producing the device, T-shaped mold made out of brass is manufactured with micro CNC machining technique. The mold composes of two side channels and one main channel to produce T-shape and the widths of the side channels and main channel are 0.5 mm and 1 mm respectively and their height is 200 μm . To place the wires, holes with 200 μm diameter are drilled on the mold according to the aimed configuration (see Fig. 3.1).

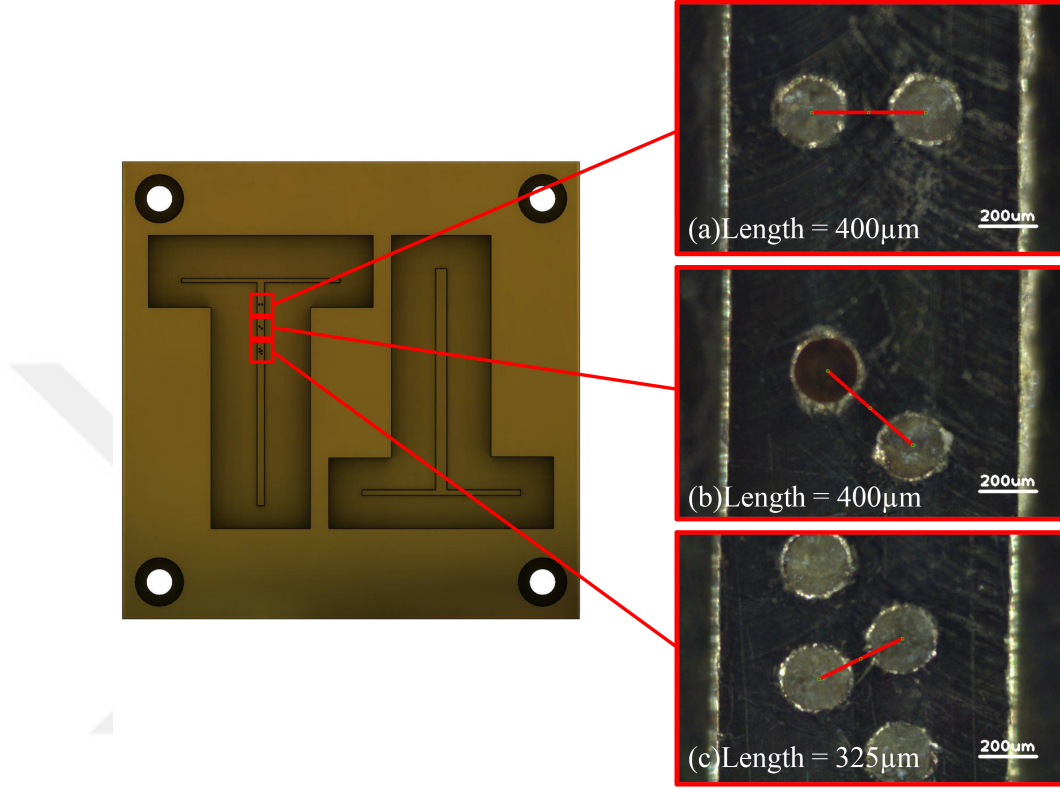


Figure 3.1: Microscope images of the channel (The images are taken via AIV μ MIT Microscope Research Station)

Upon manufacturing, the mold is cleaned with the ultrasonic cleaner (Kudos SK1200H) and the mold is steeped in acetone for deeper cleaning. Because the height of the holes are very thin, it is almost impossible to hold the wires into the holes. Therefore, a supporting plexiglass part is prepared (see Fig. 3.2-(b)) and the wires are settled into the holes with the help of supporting part, PDMS (Sylgard 184 silicone elastomer base and curing agent) is poured and the mold is placed into incubator (Nüve EN 025) for almost 2 hours for curing. Upon curing, PDMS is taken out of the mold (Fig. 3.2-(e)), inlets and outlet are opened with punch (Fig. 3.2-(f)). By plasma cleaner (Harrick plasma PDC-32G), PDMS is bonded to the silica glass (Marienfeld $76 \times 52 \times 1$ mm microscope slides) (Fig. 3.2-(g)) and tubings are sealed to the inlets and outlet of the channel (Fig. 3.2-(h)).

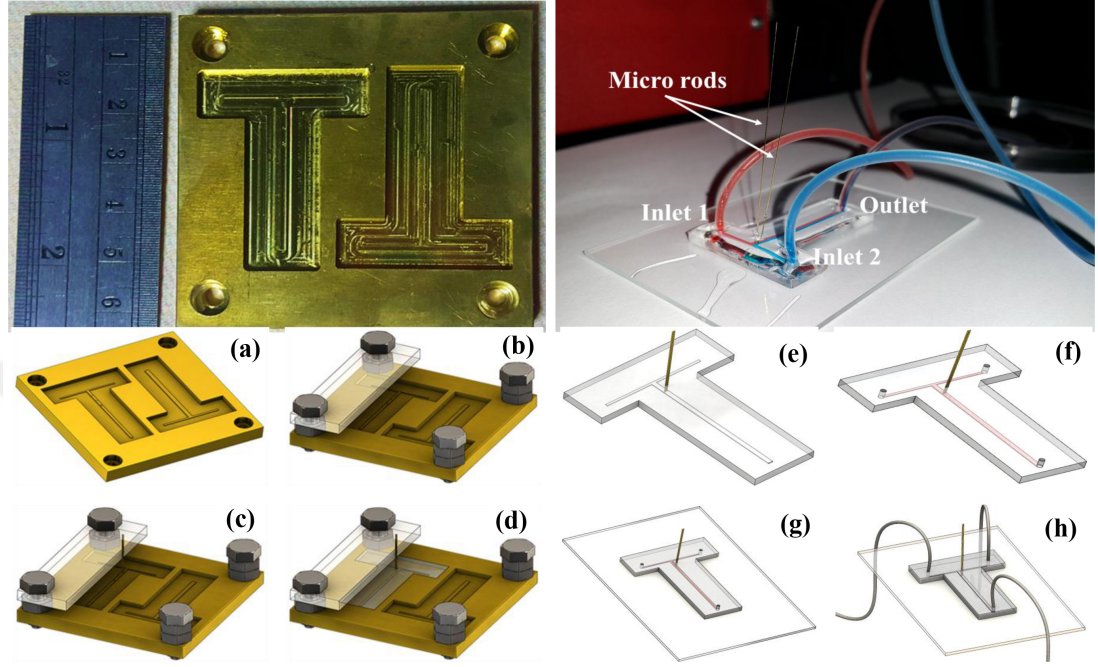


Figure 3.2: Manufacturing process: (a) Manufactured mold by milling, (b) Assembly of the supporting part, (c) Metal wires' placement, (d) Pouring of PDMS into the mold, (e) Peeling PDMS off the mold, (e) Inlets and outlet punching, (f) PDMS bonding with silica glass, (h) Attachment of tubings

It is known that when electric field is applied, potential drop occurs across EDL and surface ζ potential, which depends on chemical properties of surface material and the solution inside the channel, changes [32]. Hence, it is supposed that EOF velocity varies based on different surface material. One of the significant material property for material selection is surface conductivity and it is thought that if surface material has larger conductivity, then, EOF velocity around metal wires rises so that the system reflects better performance. When ranking metals based on electrical conductivity, the highest metal seems to be silver but because silver undergoes electrochemical reaction in electrolyte solution, the best choice of metal seems to be gold. For experimental works, commercially available brass wire having $200\ \mu\text{m}$ diameter is used and for seeing material impact, the brass wire is covered with pure gold by electroplating technique. In this method, brass wire and pure gold metal is put into an ionic solution and they are attached to

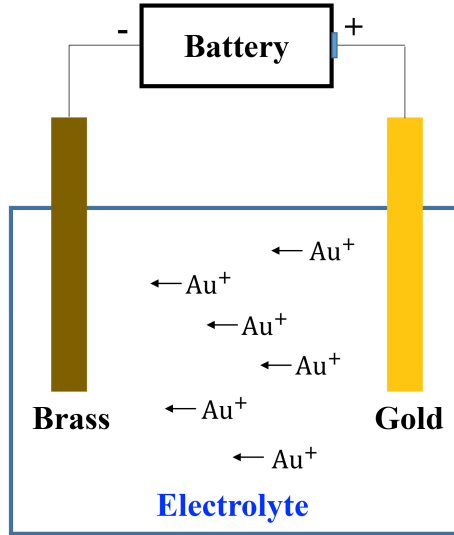


Figure 3.3: Electroplating schematic: Au^+ ions start to move from gold metal to brass metal and cover its surface.

the DC power supply (Gwinstek GPS-3303). Because of the reason that electrons are wanted to move from gold to brass, gold is attached to the positive side of the supply and brass is chosen to be ground. Under low voltage (3 – 4 V) and low current (0.02 – 0.03 A), coating is realized. This technique is preferred because it is cheaper and faster but in fact, it is not possible to control the thickness of the coating.

3.2 Preliminary experiments

Initial experiments are conducted with the channel having 0.75 mm width at the side channels and 1.5 mm width at the main channel, its height is 200 μm as mentioned in Section 3.1. Furthermore, in this initial fabrication, just two holes (original configuration) are drilled and the center to center distance between these two holes was 700 μm . It was manufactured wider because getting familiar with the manufacturing stages is aimed.

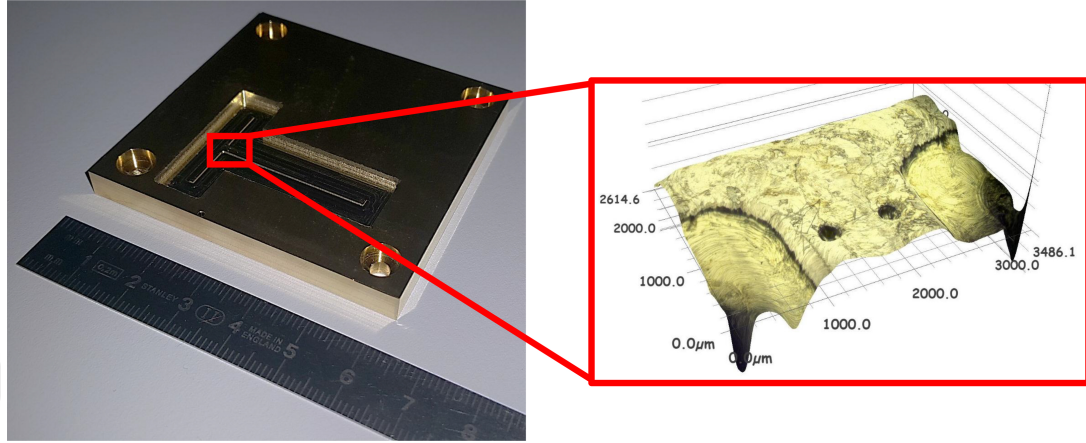


Figure 3.4: First fabricated microchannel mold: The center to center distance between two holes is $700 \mu\text{m}$. 3D image of the channel is taken with Keyence Laser Microscope

Prepared microfluidic chip is positioned on the inverted microscope (AIV $\mu\text{MIT-inv}$). Inlet tubings of the channel are combined with double syringe pump (New Era Pump Systems, NE-300). For seeing the mixing visually, red and blue food dyes (KRK Gda) are used. For electric field application, waveform generator (Agilent 33250A) with high voltage amplifier (Falco Systems WMA-300) are attached to the wire electrodes. Figure 3.5 shows preliminary experimental setup with the equipments.

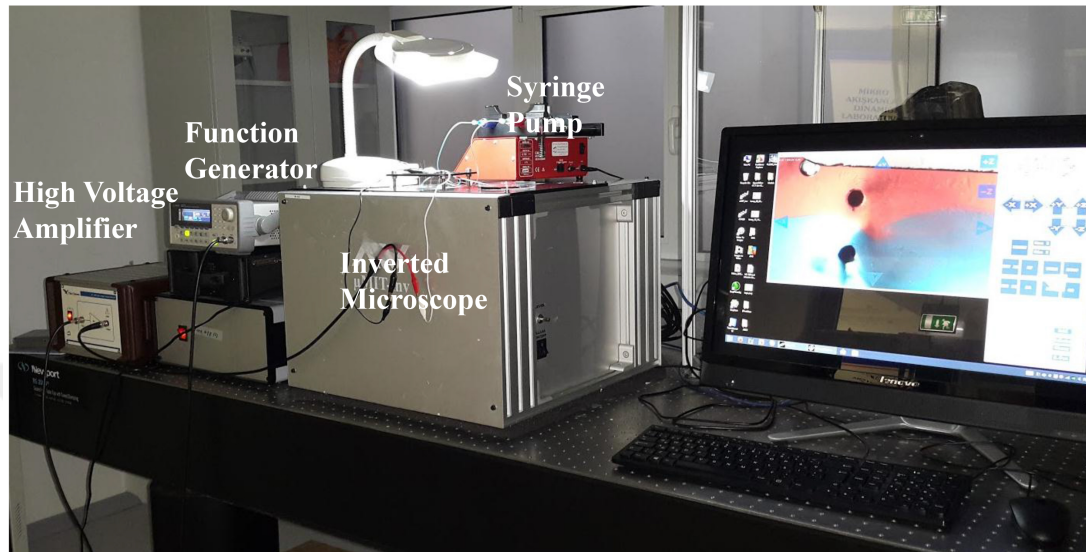


Figure 3.5: Initial experimental setup: In this setup, for imaging inverted microscope is utilized.

Getting images via inverted microscope created problems during image processing stage. Due to microscope illumination, there are shining or dark regions, shadows and colors of images become whitish, greenish or pinky (see Figure 3.6). Furthermore, colors couldn't be observed in a clear way. Due to these discrepancies, intensity values of each data reflect variations and unexpected peaks or slumps occur, lower intensity values are gathered. These illumination issues couldn't be prevented so, imaging technique is changed.

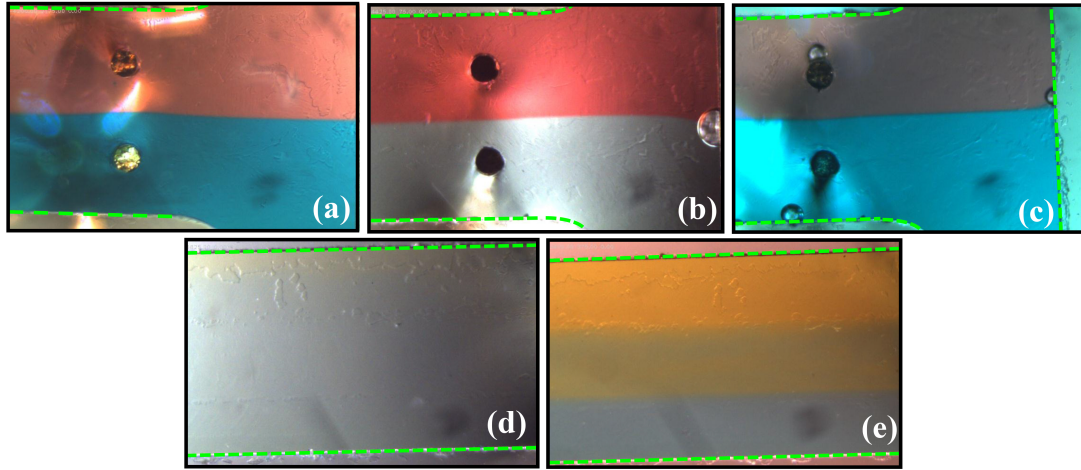


Figure 3.6: Inverted microscope imaging results: (a) There are flash points or shining regions. (b) There are still shining parts and even if the blue color dye is the same with (a), blue color seems to be whitish. (c) The picture color becomes greenish. (d) It belongs to mixed case of the blue and yellow colors but mixture color, green, couldn't be seen clearly and green intensity of this data closes to white color. (e) Picture color becomes pinky (see the region out of the channel wall) and blue color doesn't have good quality so that blue intensity stay around 100.

Mixing is also observed via asid-base indicator, phenolphthalein and NaOH [24]. However, there is a fact that when NaOH is solved in water, it leaves its ions inside the water so that under applied electric field, electrolysis is an inevitable problem. Furthermore, again with inverted microscope, it is hard to observe pink color of phenolphthalein and NaOH mixing.

3.3 New imaging platform

Upon facing with difficulties in imaging procedures, in stead of using inverted microscope, fluorescent imaging platform is preferred. In this approach, it is assumed that the inlet with fluorescent dye will be reflected through the microscope

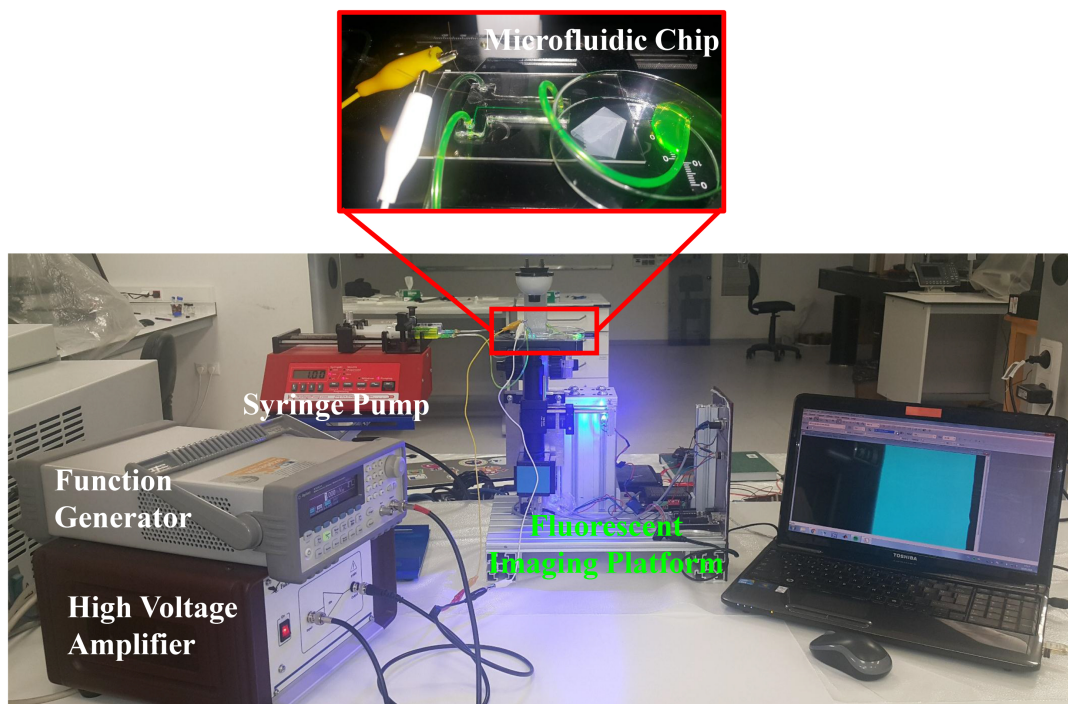


Figure 3.7: Experimental Setup

and the other inlet will be seen as black region. Therefore, flash point in the initial experiments will be handled. The Figure 3.7 shows new experimental setup with fluorescent imaging platform.

From syringe pump inlets, yellow phosphorous dye (appropriate for blue laser light absorption and emission wavelength) and DI water are supplied. This platform is generally used for fluorescent imaging but while searching for fluorescent dyes, it is seen that these dyes are mostly implemented in cell colouring and also, for preparation, these dyes need other chemicals. There are also other dyes but they again require some toxic chemicals like calzein, auramine so that under electric field application, there may be some reactions with these chemicals. Furthermore, both of these dyes are very expensive. Therefore, in stead of these, phosphorous dye taken from highlighter is utilized. Only drawback of using phosphorous dye is that in phosphorous dyes, emission occurs via phosphorescence not fluorescence so, emission continues several seconds more after cutting off the laser light. However, this condition doesn't create any problem for this study.

3.4 Postprocessing procedures

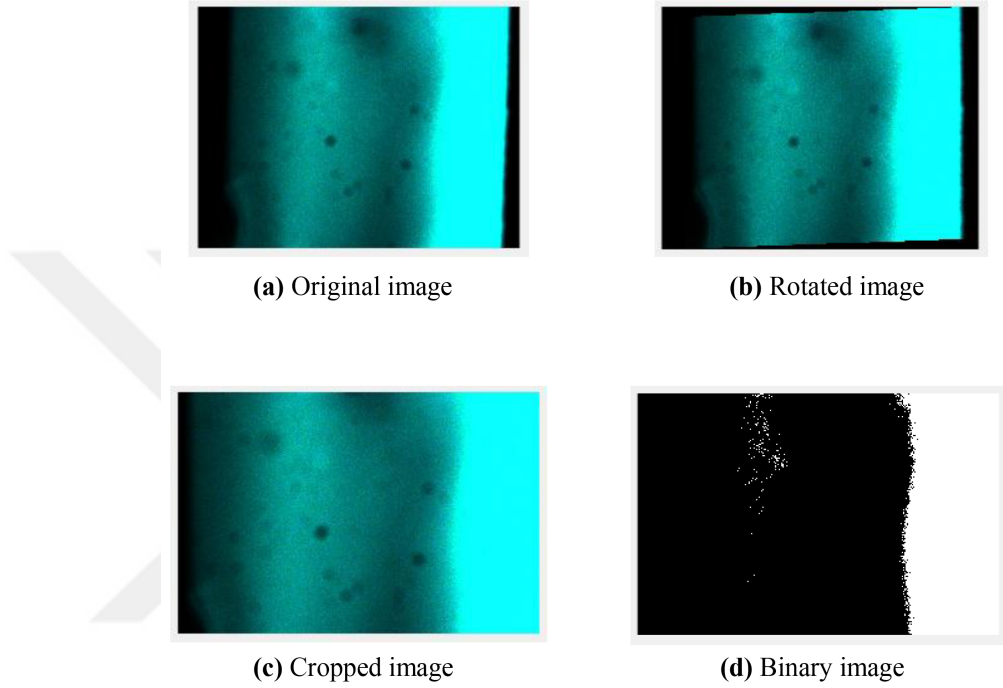


Figure 3.8: Example of the postprocessing stages

During experiments, channel is observed and data are taken via IC Capture 2.4 program and data are processed with MATLAB. The experiments are repeated 5 times for each electrode and material arrangements and the average of 5 images are taken. The procedures of the post-processing are as follows:

- The imaging platform axis is arranged to visualize the correct place.
- If the walls of the channel is not perpendicular, then, the image is rotated.
- To get rid of the regions out of channel, the image is cropped.
- The image is turned into binarial form.

Figure 3.8 shows an example of the gathered data via microscope (a) and how it is handled (b-c-d).

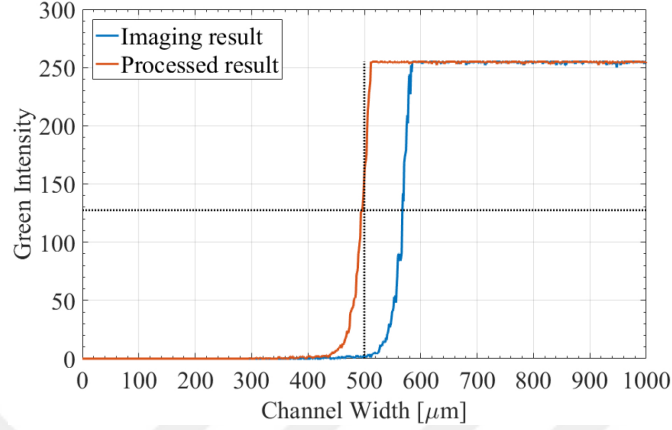


Figure 3.9: Example of the postprocessed intensity result

The Figure 3.9 reflects the example of how post-processing affects the intensity results. In the figure, blue line represents the image taken via microscope (unprocessed image) and it can be seen that centerline slipped to the right, which has huge impact on intensity results. Nevertheless, upon performing the stages b and c in the Fig. 3.8, the line comes the centre (red line in Fig.3.9). Figures 3.8 and 3.9 contain example data belonging to 2-wire brass staggered configuration.

To create binary images in MATLAB, threshold value is needed to be found. For this study, a correct threshold corresponds to a value at which mixing occurs. However, by looking at the image it is not easy to find out threshold level. If it is not specified, MATLAB itself impose its own threshold level for images. The Figure 3.10 compares the threshold levels, one is given by MATLAB and the other is defined by the user.

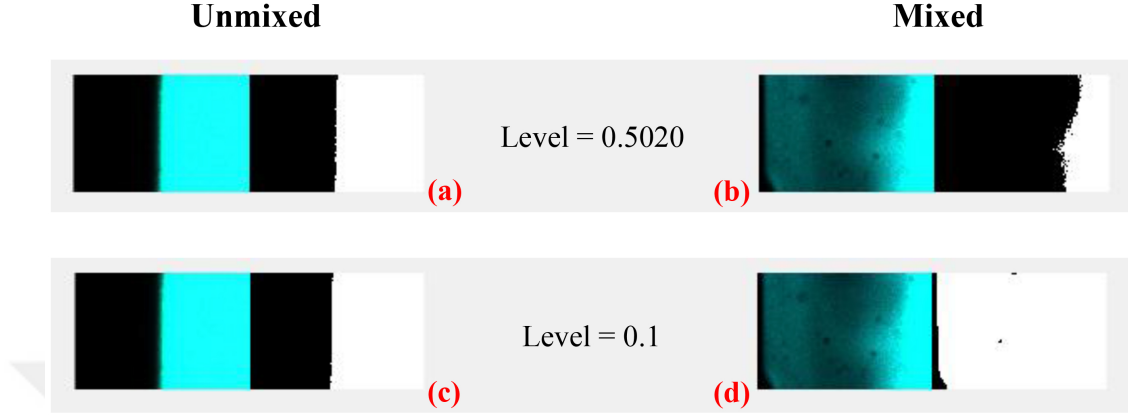


Figure 3.10: Threshold determination

In the figure, images are merged with their binaral ones based on two different threshold levels. MATLAB gives 0.502 as a threshold value but as seen in the Figure 3.10-(a) and (b), while 0.502 is a suitable value for (a), it is not suitable for (b). Because there are mixed (enlightened by fluorescent dye) regions but this value behaves as if there is no mixing (giving black pixels in the mixed regions). When 0.100 is defined by the user, there is almost no variation in Fig. 3.10-(c) but (d) alters so much. In this case, when looking at the mixed case, there are unmixed fluorescent parts at the right side of the channel but this threshold value delivers these sides as mixed regions as well (see Fig. 3.10-(d)). In Fig. 3.10-(b), unmixed fluorescent part (white region) can be determined and in Fig. 3.10-(d), unmixed DI water part (black region) can be observed. That is to say, via this method, the interfaces between mixed and unmixed liquids can be seen clearly at those threshold levels. If number of white and black pixels in each image are counted and difference between black pixels is taken, then, total change in fluorescence intensity or in other words, mixing index (σ), can be acquired (see Eq. 3.1). There are different forms of this equation, for instance, while Munson et al. [59] mentions it as the extend of mixing, Wu et al. [60] calls it as mixing index or mixing degree. In other cases, it is referred to as mixing efficiency [33].

$$\sigma = \frac{P_{1,b.p.} - P_{2,b.p.}}{P_{total}} \quad (3.1)$$

where $P_{1,b.p.}$, $P_{2,b.p.}$ and P_{total} are number of black pixels with threshold level 0.5020, number of black pixels with threshold level 0.1 and total number of black and white pixels respectively.

3.5 Experimental results

Experimental results are also evaluated by observing RGB intensities before turning the images into the binarial ones. Upon getting rid of channel walls, a horizontal line is taken at the middle of each picture and concentration values on this line are plotted. The Figure 3.11 reflects the concentration values on the magenta dashed line of unmixed and mixed cases. While unmixed case gives a step function like graph, the mixed case differs and at the middle of the channel the green intensity values are between 100 and 170. In the mixed phase Fig. 3.11-(b), there are black dots resulting from the spots on the lenses. Even if it is cleaned, these spots couldn't be prevented but they don't have much impact on the experimental results.

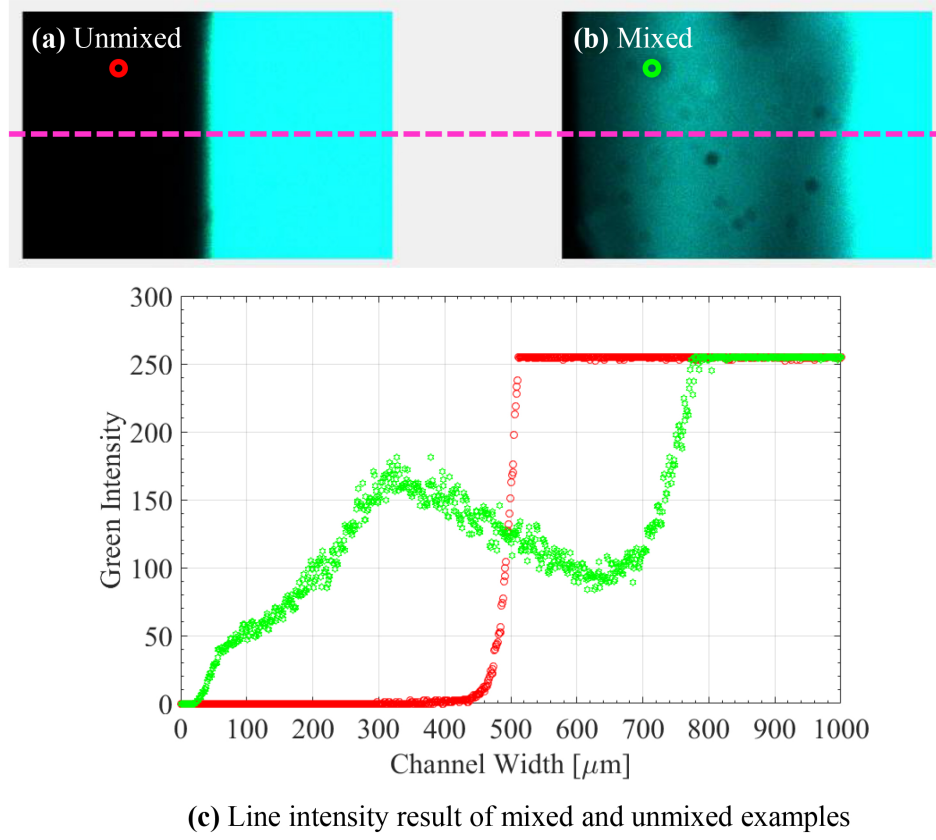


Figure 3.11: Line concentration implementation

For each configuration, both gold and brass surface materials under variable voltages are observed. Figure 3.12 expresses the first electrode orientation results. At $Q = 1.0$ mL/h flow rate, 50 V, 100 V don't give good performance but more than 100 V some mixed regions at the middle of the channel are seen via looking at the figure. When comparing brass cases of two different flow rates, it can be clearly seen that at lower flow rate, there is mixed region, having green intensity around 130, under even 100 V electric potential application. Therefore, it can be concluded that 0.5 mL/h flow rate has better performance. Here, what better performance means that under low voltage application, there are more regions having moderate intensity values, which is the average of maximum (256) and minimum (0) intensity values. If Figure 3.12 is compared with its computational result, Figure 2.2, rise in performance with increasing voltage and negative correlation between flow rate and performance are common results of

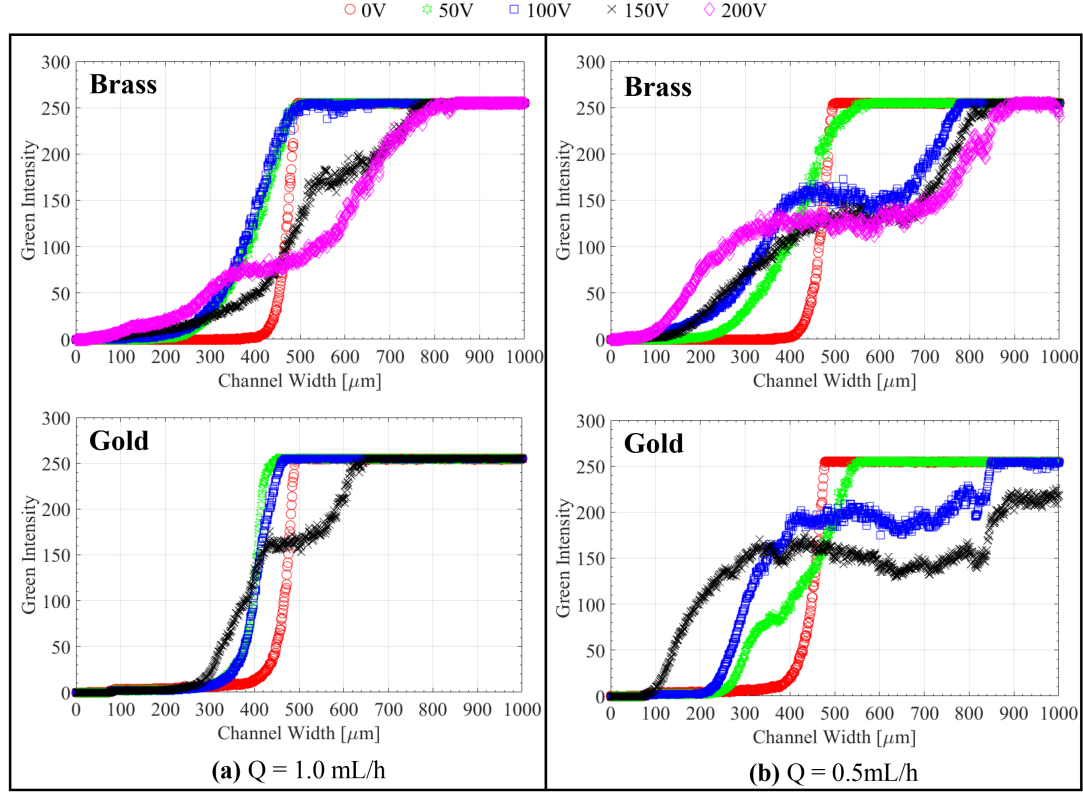


Figure 3.12: 2-wire configuration experimental results (surface coating is brass.)

two studies. However, there is a discrepancy between curve forms. For instance, if magenta data of two studies are compared, in the experimental one, there is almost $100 \mu\text{m}$ unmixed black region and at the right hand of the graph above $800 \mu\text{m}$, there is also unmixed fluorescent region having green intensity of 256. However, magenta data of the simulations (see Fig. 2.2-(a)) don't show such regions, in stead, it gives 0.1 and 0.9 concentration values on these regions, which means these regions have little mixing. Some improvements like decreasing mesh size and trying different diffusion techniques are made to reach experimental data curves but exact profile couldn't be acquired.

As the next step, gold-coated channel is processed. In theory, with gold arrangement, at lower voltage, line concentration should give intensity values around 128. For instance, in Fig. 3.12-(b)-gold case, under 100 V and 150 V, concentration lines come to moderate values differing between 150 and 200 and these values are seen between $300 \mu\text{m}$ and $900 \mu\text{m}$ distances. However, in brass

($Q = 0.5 \text{ mL/h}$), these regions are narrower (between $400 \text{ }\mu\text{m}$ and $700 \text{ }\mu\text{m}$). It seems that gold case demonstrates better intensity consequences in contrast to brass case. Despite of little improvements via gold statement, in fact, there is not much performance rise comparing four figures of original arrangement. Therefore, evaluating the other arrangements would be better approach.

For 2-wire staggered configuration, line concentration plots are also observed for both brass and gold surface materials. With this arrangement, according to computational consequences, under the same voltage, there should be more area having moderate green intensity values comparing with case A. In contrast to Fig. 3.12-(a)-brass case, step function like 100 V data, in Fig. 3.13-(a)-brass, there is mixed region at the middle of the channel between $300 \text{ }\mu\text{m}$ and $600 \text{ }\mu\text{m}$ channel width. This data reflects that mixing performance rises with case B. The other way of defining better mixing performance is that under the same electric potential, the region having moderate intensity should increase. In Fig. 3.13-(b)-brass, magenta data reflects medium data from $50 \text{ }\mu\text{m}$ to $800 \text{ }\mu\text{m}$ but Fig. 3.12-(b) has data from $300 \text{ }\mu\text{m}$ to $750 \text{ }\mu\text{m}$. Therefore, it can be concluded that case B is better than case A. In these figures, there is surprising peaks (black curve in both flow rates). The same peak can be observed in Fig. 3.11 mixed data. If Fig. 3.11-(b) is examined in detail, at the left there is unmixed black region from $0 \text{ }\mu\text{m}$ to $20 \text{ }\mu\text{m}$ and some mixed region from $100 \text{ }\mu\text{m}$ to $250 \text{ }\mu\text{m}$ and a peak around $300 \text{ }\mu\text{m}$. This condition is probably resulted from a little phosphorous dye dominated region at this point.

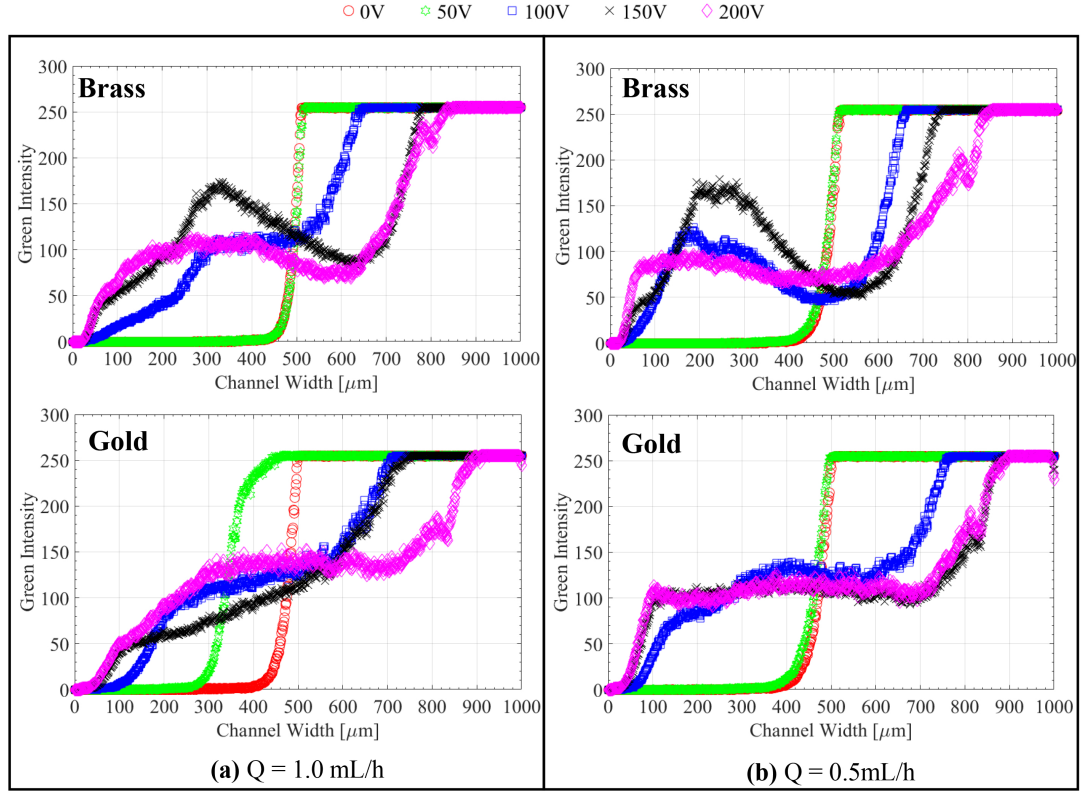


Figure 3.13: 2-wire staggered configuration experimental results (surface coating is brass.)

Gold-coated channel of case B demonstrates wider medium green intensity regions comparing with previous figures. From computational studies, asymmetric vortex profile of case B arrangement enhances mixing performance is also realized with this experimental study. Furthermore, as mentioned at the beginning of the Chapter 3, the metal having higher conductivity is thought to give better performance, here in (b)-gold of the Fig. 3.13, under 100 V, 150 V and 200 V, blue, black and magenta curves locate around 110 green intensity. Case B seems to ensure better mixing performance by both computational and experimental consequences. However, the same problem continues between experimental and computational curves. In experiments, there is unmixed areas close to the channel walls. Nevertheless, these regions couldn't be observed in computations with this arrangement as well.

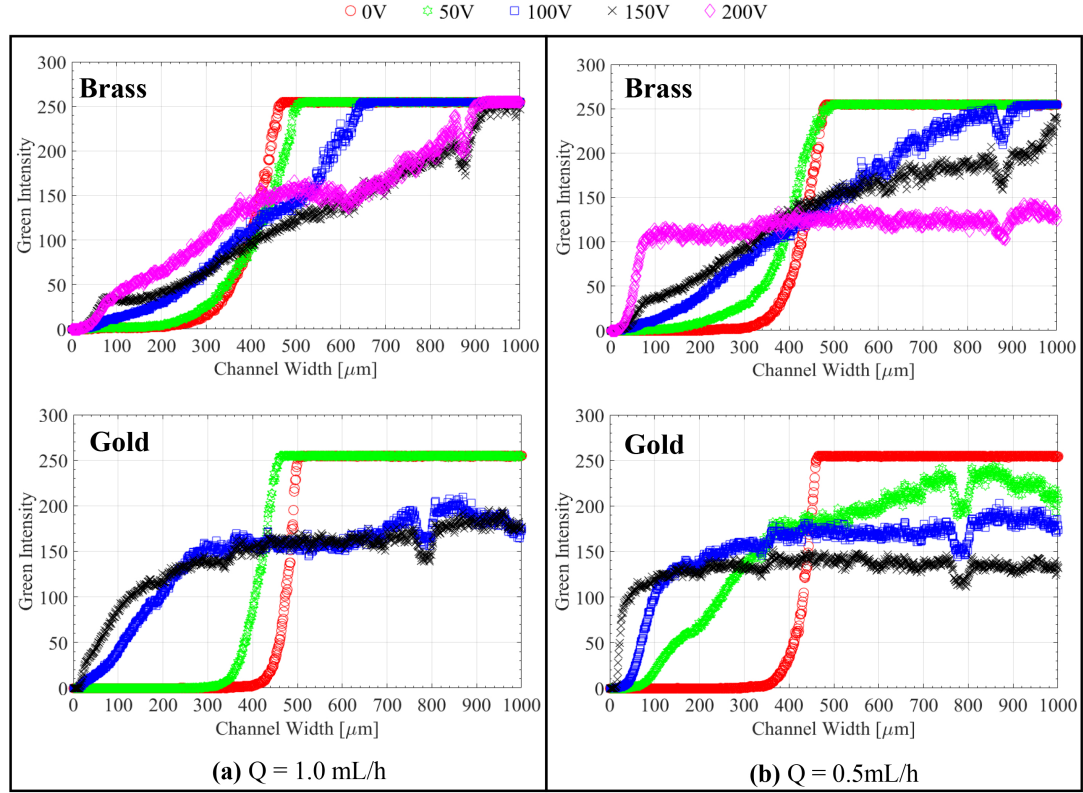


Figure 3.14: 4-wire staggered configuration experimental results (surface coating is brass.)

From computational point of view, case C provides asymmetric streamline profile and more vortex cells due to increase in electrode quantity so, it is the best configuration. When comparing Fig. 3.13-(a)-brass with Case C, it is hard to guess the performance of the Case C due to the curve shape. It isn't obvious that there is a mixed region at the middle of the channel. However, Fig. 3.14-(b)-brass gives better investigation of having intensity value of 100 starting from 50 μm to the end of the channel. At 200 V, magenta curve locates almost at the middle of the channel indicating approximately perfect mixing. When looking at its computational arrangement, the magenta data also gives this profile. The other curves, for instance, 100 V (blue) and 150 V (black) don't reveal much knowledge about the performance but calculating efficiency or index would provide better understanding.

Based on better performance with higher conductivity metal approximation,

gold-coated 4-wire arrangement should medium concentration data at lower voltages comparing with the brass one. In Figure 3.14-(b)-gold, it is clearly seen that at even 50 V, some mixing portions are experienced. Even $Q = 1.0$ mL/h flow rate reflects mixing under 100 V and 150 V. With this case, there is no need to increase voltage more because mixing is already gathered and also, because in very small region, two rods with very high electric potential could result in extreme pressure drop and electrolysis may occur. To get rid of these problems, more electric potential application isn't aimed.

Among all those line concentration graphs, 4-wire staggered gold case denotes the best case, which means at lower voltages, the concentration line starts to place at the middle of the intensity graph. However, just looking at the line concentrations isn't enough to understand mixing performance. To have true insight into the efficiency, σ values are calculated for each case by Eq. 3.1. In each image having 600 pixels of height and 900 pixels of width, whole the area is scanned starting from 0 to 600 with 5 pixels distances. For each image, 121 horizontal lines are drawn (corresponding to 900 pixels for each line) and average mixing indices of 121 lines, standard deviation and uncertainty values are calculated (see Appendix). It is seen that standard deviations are so small therefore, attachment of the error bar is thought to be unnecessary. The same analysis is performed for a smaller region corresponding to 50 pixels at the middle of each image. This time, starting from 275 to 325, 26 lines are drawn with 2 pixels of spaces and mixing indices via Eq. 3.1 are calculated then, the average index of 26 lines are found. The purpose of performing these analysis is that mixing indices would differ from the top of the image (closer to the inlet) to the bottom of the image (closer to the outlet) so, by taking a small region error would be decreased. Fig. reff:50pixels reflects the area at which mixing indices are calculated. This space (50 pixels) corresponds to approximately $56 \mu\text{m}$. Mixing index results of this region are given in Figures 3.16 and 3.17. On this small region, standard deviation values are so small that error bar isn't needed.

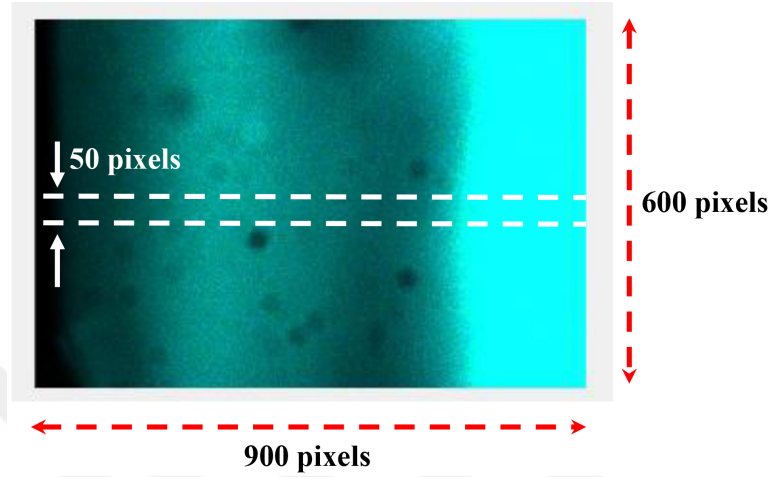


Figure 3.15: Mixing index calculation: Mixing index (σ) is calculated on the area between white-dashed lines corresponding to almost $56 \mu\text{m}$.

Mixing indices based on both surface coating and flow rate are compared. Figure 3.16 demonstrates indices based on material selection. First observation is that voltages between 0 V and 50 V, it is almost impossible to identify mixing because almost all the lines are stacked up on top of each other from 0 V to 50 V and higher voltage application is needed. When comparing case A arrangement, $Q = 1.0 \text{ mL/h}$ flow rate (red line), of gold and brass materials, mixing indices of this arrangement is very low and it seems that this arrangement even with gold material doesn't contribute to mixing. Furthermore, material impact couldn't be perceived. However, with 0.5 mL/h flow rate (black line), efficiency seems to increase above 50 % for brass case (200 V), 70 % for gold case (150 V). Case B orientation (green and magenta) seems to give better mixing index than case A. Even if much investigation couldn't be done for 0 – 50 V, above 100 V, mixing indices of Case B dominate Case A. However, when ranking material impacts, both in brass and gold cases, there is slight difference between indices, for instance, under 200 V and $Q = 0.5 \text{ mL/h}$, brass and gold gives 72 % and 76 % mixing indices. Under $Q = 1.0 \text{ mL/h}$, these values are 69 % and 74 % successively. Therefore, material impact doesn't dominate so much with this arrangement as well. In terms of flow rates, gold material shows more variations comparing with brass one but at 200 V, both coatings of Fig. 3.16 get close to

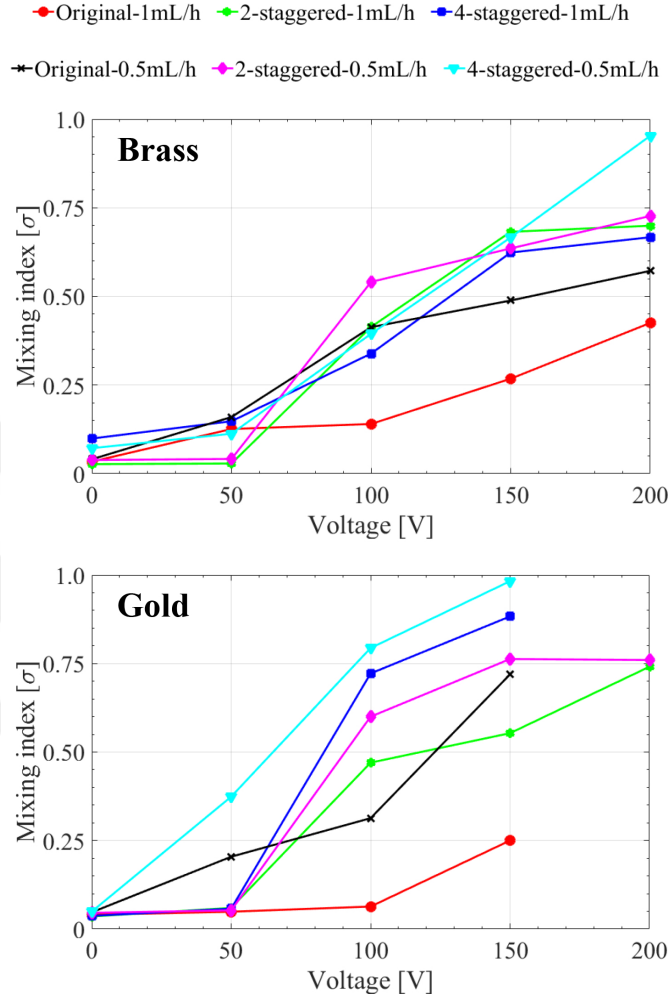


Figure 3.16: Mixing index according to surface material

each other. Fortunately, case C (blue and cyan) distinguishes itself in terms of both flow rate and surface coating. Both in brass and gold materials, lower flow rate dominates higher flow rate and this time domination is clearly observable. Even if Case C reflects its performance at 200 V for brass material, in gold one, it always dominates the graph for $Q = 0.5$ mL/h. Moreover, gold case under 150 V provides almost perfect mixing with 98 % mixing index.

Figure 3.17 shows mixing index vs. voltage curves for two flow rates. First striking discrepancy of these figures is that in $Q = 1.0$ mL/h, gold wire seems to stay under the brass one but in fact, they have almost similar efficiency results of 25 % and 27 %. In $Q = 0.5$ mL/h experiment of Case A, at 100 V, gold stays under

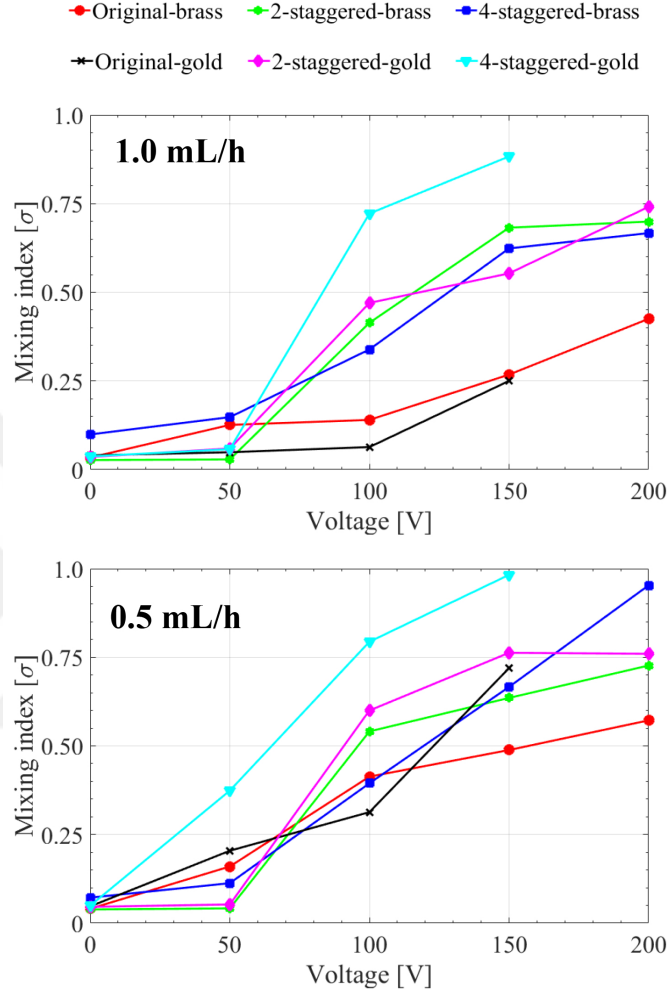


Figure 3.17: Mixing index according to flow rate

the brass one but for the other voltages, there is no such a problem. Therefore, for finding out the material impact, just looking at the efficiency results of Case A would be impractical. In 2-staggered case, it seems that gold material dominates brass one in almost every voltage. 2-wire staggered (case B) study reflects better performance results comparing with case A but even 2-wire staggered case doesn't supply sufficient knowledge in terms of material impact. Two curves of Case C (blue and cyan) demonstrate that increasing the number of electrodes rises the performance and introducing gold-coated wires enhances the performance more. Therefore, The best separable result, which dominates all of the cases, belongs to case C (4-staggered) with almost perfect mixing at 150 V.

In this study, because surface coating effect is also experienced, in simulations, this impact is also aimed to be observed. Therefore, parameter alterations according to material change are needed to be defined. Nevertheless, in Eq. 1.9, variables σ , ω and also from $C_{DL} = \varepsilon/\lambda_D$, ε and λ_D don't contain material impact. Moreover, because there is no impedance analyser in hand, capacitance is tried to be measured but because it is very low value, signal couldn't be generated. In the literature, researchers play with the electrode scales by preserving the gap between electrodes and measure the impedance. However, because they are using planar electrodes, it is appropriate to present measurement but in this study, because of the electrode arrangement, which are located through the channel width not the channel length, this scaling method isn't suitable. Therefore, measurement of electrode potential drop is a fail for this study. Other techniques are searched to solve this problem. What is needed to introduce material impact is modelling double layer capacitance or potential drop across the double layer correctly. Therefore, apart from measurement methodology, the articles concerning parameter based studies are revised.

Green *et al.* [7] mentions that Λ is around 0.25 and by using $\Lambda = 0.24$ and $\Lambda = 0.25$ values of Eq. 2.8, numerical data are multiplied by these numbers to find out correct potential drop across the double layer. Du *et al.* [56] and Canpolat *et al.* [4] use different values of δ to model capacitance ratio across the double layer. Green *et al.* [7] uses different parameter (Λ) because they measure impedance across the double layer so that they don't need δ parameter. However, all of these studies contain Debye-Huckel approximation which is applicable when applied voltages are very small. By considering Debye-Huckel approximation, in fact, non-ideal effects such as electrochemical reactions of electrodes, steric effects and polarization of the concentration are neglected [56]. However, in this study because applied voltages are high so, neglecting these non-ideal effects is an inappropriate way. To have more insight into the charging dynamics under high voltages and different surface materials cases, steric effects theory should be taken into consideration [61]. Therefore, at this point making comparison between experimental and computational results wouldn't be suitable. During computational studies, these facts are realized but to have qualitative results

and observing the impacts of electrode configurations, the number of electrodes, neglecting steric effects by introducing Debye-Huckel theory would be enough. Furthermore, even if the same results couldn't be acquired qualitatively, the behaviour under increasing voltages and the number of electrodes shows similarities.

The other factor resulting in variation of computational and experimental results is that during simulations, it is assumed that electrode surfaces have homogeneous ζ . However, this measurable quantity may differ for the electrodes and it may change upon surface coating with gold. Moreover, because the thickness of the coating couldn't be controlled by electroplating technique, the coating thickness may vary from electrode to electrode. Therefore, modeling the electrodes as if they have homogeneous ζ may prevent correct computational results.

3.6 Heating observations

In this study, high electric field reaching to 200 V is applied to very small area which can cause heating up of the liquids inside the channel. Heating is important factor because it can result in vital consequences;

- Rise in temperature alters viscosity of liquids. For instance, when viscosity of water at the room temperature is $1 \text{ mPa} \cdot \text{s}$, it is 0.79 at 30°C and 0.65 at 40°C . This much viscosity alteration causes change in Re .
- Diffusion coefficient is also affected by temperature variation because this parameter is dependent on temperature, pressure and also size of the molecules. Temperature and diffusion coefficient are positively correlated so that rise in temperature will increase diffusion coefficient, enhance mixing.

Therefore, to observe heating, experiments are repeated via thermal camera (FLIR A-300). The highest voltages are given under both of the flow rates and

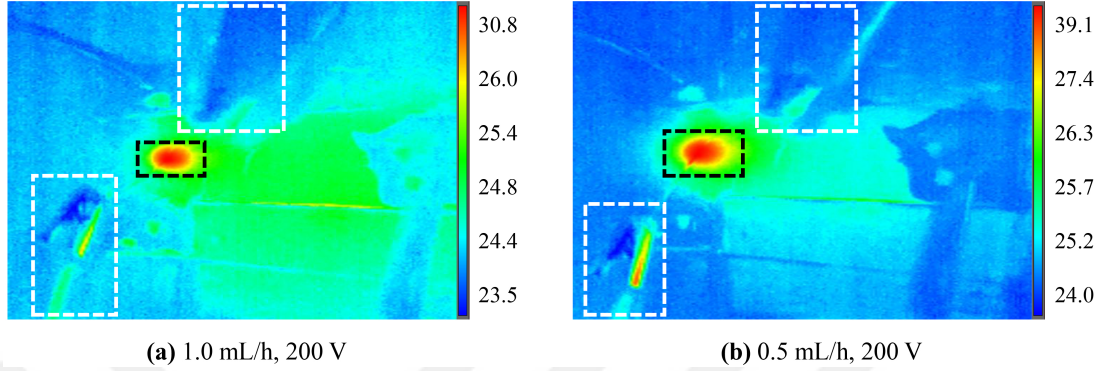


Figure 3.18: Thermal camera images under different flow rates: Black and white boxes denote the area between electrodes and crocodile wires transmitting electric field successively.

temperature rise is evaluated. Figure 3.18 shows thermal camera images of two flow rates under the highest applied voltage.

Upon electric field application, heating occurs immediately on the crocodile wires and the highest temperature rise belongs to these crocodiles. When temperature is around 30 °C on the crocodiles, it is approximately 26 °C between the electrodes (see Fig. 3.18-a). When decreasing flow rate, temperature on the crocodiles boost to 39 °C and between the electrodes, it goes up to 30 °C. Temperature variation is between 2 °C and 6 °C for higher and lower flow rates inside the channel. Therefore, temperature variation is neglected for the simulations via taking ρ and μ constant. Another important notice is that in case of electrolysis, temperature rises dramatically, it breaks the working principle of this mixer due to the bubbles preventing the flow of liquids and creating huge pressure around the electrodes. Fortunately, with this ACEO based microfluidic mixer, electrolysis is prevented.

Chapter 4

Summary and Future Research Directions

Microfluidics & LOC systems are portable platforms that can realize biological, chemical and single-cell analysis with low amount of samples and reagents, cheap fabrications, fast reactions, high throughput and sensitivity.

Species manipulation and transportation in micro regime are provided by variable forces like pressure, temperature, centrifugal or EK. EK transport dominates the other techniques by ensuring rapidly responding uniform, non-pulsating flow. EK is applicable to many fields such as cell separation, synthesis, manipulation, pumping and mixing.

In microfluidic applications, mixing can not be realized easily because Re and Pe are so small that just molecular diffusion is effective on mixing and this condition is not enough for getting fast mixtures. Also, reaction time and channel length expand. There are variable methods; passive and active techniques for creating fast mixing and ACEK-based micromixers are one of the favourable ones due to minimizing the possibility of pressure drop.

The focus of this thesis is to introduce a micromixer based on ACEOF inside of T-shaped microchannel. The flow inside the channel is driven by pressure and

on the metal rod electrodes inside the channel, ACEOF is realized and mixing occurs. This study is extended via running into variable configurations, flow rates and surface coatings observations. The novelty of this study is that most of the EK literature is abounded with clean room fabrication but, in this study commercially available metal rods are used as electrode pairs so, easy and cheap manufacturing technique is aimed. Furthermore, the electrodes provide 3D mixing scheme which enhances mixing index. The other important aspect of this study is electric field is only applied to the electrodes not the whole channel so that this study would decrease the possibility of harming cells, species. According to both experimental and computational implications, it is seen that 4-wire staggered gold electrode configuration gives the best mixing index, σ with around 100%. Furthermore, as expected under lower flow rate, mixing index rises because in this case, velocity magnitude of EOF near the rods prevails the velocity magnitude of pressure-driven flow. Another vital indication of this study is surface coating strength. In theory, the metal with higher electrical conductivity will rise EOF magnitude. In this study, two surface materials; gold and brass are observed and as expected, in the experiments, gold material demonstrates better mixing performance under the same electric field and flow rate. Even if this fact couldn't be observed clearly via Case A and Case B electrode configurations, it could be seen by dominating case; 4-wire staggered configuration. This result is also aimed to be observed via computation however, EOF equations in the literature don't implement this fact because EDL is neglected (Debye-Huckel approximation), in stead different measurements techniques like impedance or capacitance measurement techniques are managed. However, in these measurements, planar electrodes are utilized and by scaling up these electrodes by preserving the gap between the electrodes are aimed. What makes this measurement impossible for this study is that electrodes are placed throughout the channel width so that scaling up would be inefficient. Moreover, Debye-Huckel approximation is valid under low voltage levels however, in this study, applied voltage is high so that while considering Debye-Huckel's $C_{DL} = \epsilon/\lambda$, non-ideal effects like steric effects are neglected. This condition results in unmatched computational and experimental results quantitatively even if the trends match. Therefore, comparing experimental and numerical results would be inefficient at this point. The other

assumptions like homogeneous surface ζ , liquid having the same ρ , μ and neglecting temperature change under applied voltages may be other factors causing discrepancies between computation and experimentation.

As a future research directions, the most prominent step would be modeling steric effects via taking all the non-ideal cases into consideration so that closer computational results with the experiments are acquired. Moreover, this study would be enhanced by inserting this into a specific bio-sensor applications. Under specific claims of the application, it is very easy to change the design, fabricate and integrate or combine with other microfluidic devices. The application doesn't necessarily need to be from mixing but this theory can be applied to pumping as well. Furthermore, preparing Poincaré map of this study would give better knowledge about the unmixed regions inside channel and it can increase the quality of this study. Another important point is that during measurements, mass spectroscopy method would be more suitable because with this method, concentration inside the liquid is gathered directly by comparing it with the blank liquid. In stead of just looking at the intensity level, mass spectroscopy would be better approach.

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Appendix A

Mixing index results of the
experimental data

Table A.1: Mixing index results of 121 horizontal lines (Case A)

Material	Flow rate [mL/h]	Voltage [V]	Average Index	Standard Deviation	Uncertainty
Brass	1.0	0	0.03	0	0
		1.0	0.12	0	0
		2.0	0.15	0.01	0.02
		3.0	0.25	0	0.01
		4.0	0.42	0.01	0.03
Brass	0.5	0	0.04	0	0
		1.0	0.17	0.01	0.02
		2.0	0.40	0.01	0.03
		3.0	0.43	0.05	0.10
		4.0	0.49	0.08	0.17
Gold	1.0	0	0.04	0	0
		1.0	0.05	0	0
		2.0	0.06	0	0
		3.0	0.26	0.01	0.02
Gold	0.5	0	0.04	0	0
		1.0	0.21	0	0
		2.0	0.32	0.05	0.10
		3.0	0.72	0	0.01

Table A.2: Mixing index results of 121 horizontal lines (Case B)

Material	Flow rate [mL/h]	Voltage [V]	Average Index	Standard Deviation	Uncertainty
Brass	1.0	0	0.02	0	0
		1.0	0.03	0	0
		2.0	0.43	0.01	0.02
		3.0	0.68	0	0
		4.0	0.70	0	0
Brass	0.5	0	0.04	0	0
		1.0	0.04	0	0
		2.0	0.54	0	0.01
		3.0	0.63	0	0.01
		4.0	0.72	0	0
Gold	1.0	0	0.03	0	0
		1.0	0.07	0	0
		2.0	0.49	0	0
		3.0	0.57	0	0
		4.0	0.73	0	0.02
Gold	0.5	0	0.04	0	0
		1.0	0.05	0	0
		2.0	0.59	0	0
		3.0	0.76	0	0
		4.0	0.75	0	0

Table A.3: Mixing index results of 121 horizontal lines (Case C)

Material	Flow rate [mL/h]	Voltage [V]	Average Index	Standard Deviation	Uncertainty
Brass	1.0	0	0.1	0	0
		1.0	0.16	0	0.10
		2.0	0.37	0.01	0.03
		3.0	0.60	0	0
		4.0	0.68	0.01	0.02
Brass	0.5	0	0.07	0	0
		1.0	0.12	0	0.01
		2.0	0.43	0.02	0.04
		3.0	0.69	0.01	0.03
		4.0	0.95	0	0
Gold	1.0	0	0.03	0	0
		1.0	0.05	0	0
		2.0	0.74	0.01	0.03
		3.0	0.91	0.01	0.02
Gold	0.5	0	0.04	0	0
		1.0	0.45	0.03	0.06
		2.0	0.84	0.01	0.03
		3.0	0.98	0	0

Table A.4: Mixing index results of 50 pixels region of Fig. 3.15 (Case A)

Material	Flow rate [mL/h]	Voltage [V]	Average Index	Standard Deviation	Uncertainty
Brass	1.0	0	0.03	0	0
		1.0	0.12	0	0
		2.0	0.12	0	0
		3.0	0.25	0	0.01
		4.0	0.41	0	0
Brass	0.5	0	0.04	0	0
		1.0	0.14	0	0
		2.0	0.42	0	0
		3.0	0.51	0	0
		4.0	0.62	0	0
Gold	1.0	0	0.04	0	0
		1.0	0.05	0	0
		2.0	0.06	0	0
		3.0	0.24	0	0
Gold	0.5	0	0.05	0	0
		1.0	0.20	0	0
		2.0	0.26	0	0
		3.0	0.72	0	0

Table A.5: Mixing index results of 50 pixels region of Fig. 3.15 (Case B)

Material	Flow rate [mL/h]	Voltage [V]	Average Index	Standard Deviation	Uncertainty
Brass	1.0	0	0.02	0	0
		1.0	0.03	0	0
		2.0	0.41	0	0
		3.0	0.68	0	0
		4.0	0.70	0	0
Brass	0.5	0	0.04	0	0
		1.0	0.04	0	0
		2.0	0.54	0	0
		3.0	0.63	0	0
		4.0	0.72	0	0
Gold	1.0	0	0.03	0	0
		1.0	0.06	0	0
		2.0	0.47	0	0
		3.0	0.55	0	0
		4.0	0.74	0	0
Gold	0.5	0	0.04	0	0
		1.0	0.05	0	0
		2.0	0.60	0	0.01
		3.0	0.76	0	0
		4.0	0.76	0	0

Table A.6: Mixing index results of 50 pixels region of Fig. 3.15 (Case C)

Material	Flow rate [mL/h]	Voltage [V]	Average Index	Standard Deviation	Uncertainty
Brass	1.0	0	0.09	0	0
		1.0	0.15	0	0
		2.0	0.34	0	0
		3.0	0.62	0	0
		4.0	0.67	0	0.01
Brass	0.5	0	0.07	0	0
		1.0	0.11	0	0
		2.0	0.39	0	0
		3.0	0.66	0	0
		4.0	0.95	0	0
Gold	1.0	0	0.03	0	0
		1.0	0.05	0	0
		2.0	0.72	0	0.01
		3.0	0.88	0	0
Gold	0.5	0	0.05	0	0
		1.0	0.37	0	0
		2.0	0.79	0	0
		3.0	0.98	0	0