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Mohammad Salman Parvez
The University of Texas Rio Grande Valley

Md Fazlay Rubby
The University of Texas Rio Grande Valley

Shanzida Kabir
The University of Texas Rio Grande Valley

Meah Imtiaz Zulkarnain
The University of Texas Rio Grande Valley

Nazmul Islam
The University of Texas Rio Grande Valley, nazmul.islam@utrgv.edu

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EFFECT OF NON-PLANAR TUNGSTEN V-ELECTRODE PATTERN IN A 3D PRINTED MICROFLUIDIC SYSTEM

Mohammad Salman Parvez, Md Fazlay Rubby, Shanzida Kabir, Meah Imtiaz Zulkarnain, Nazmul Islam
Department of Electrical Engineering, The University of Texas Rio Grande Valley, TX 78539, USA.

ABSTRACT
Manipulation, guiding, and focusing of particles is an important phenomenon in the area of biomedical research. In most cases, particles are suspended in a microfluidic environment. These microfluidic environments can be high or low conductive. Most importantly these flows seeded with the micro-particles are manipulated and guided in microfluidic channels. Microfluidic channels have very low dimensions and considering the flow rate the characteristic of the flow in a microfluidic channel is laminar in nature. There are many micromachining methods available for fabricating microfluidic channels such as soft-lithography, wet etching, electroforming , PDMS molding, laser ablation followed by wet etching but in most of these cases, a microfabrication facility is required which is very costly in nature. Now a days 3D printing process is widely used to design microfluidic channels as a cheap process for conducting laboratory experiments. In this work, a 3D printed microfluidic channel fabrication process was presented along with a CAD drawing with microstructural dimension analysis. Previously V-electrode pattern was used in the static fluid system. In this work, a V-elect rode pattern was inserted in the microfluidic system for the first time to analyze the behavior of the flowing fluid of different conductivity under the application of AC current. The flow characteristics were presented and analyzed with the Reynolds number and the flow region of maximum velocity before and after the implementation of the AC electric field. The direction of the flow was also observed in the V-shaped microfluidics environment.

Keywords: Soft-lithography, Microfluidic Channel,3D Printer, Micromixer.

1. INTRODUCTION
In the sector of biomedical science sorting, guiding, mixing, manipulation and isolation of bioparticles suspended in a microfluidic system are essential for bioanalysis, diagnostic and early detection of diseases like cancer [1], malaria [2], HIV [3]. Inclusion of microfluidic devices paved a new way for the researchers and provided with a cost-effective; point-of-care miniaturized diagnostic and lab-on-a-chip devices. This includes increased processing rates, less sample volume, reduced time and wide range of accessibility due to lower cost [4].
The mechanism of manipulating particles can be classified as active methods and passive methods. Active methods require external forces to stimulate the fluid particles. Not only for manipulating particles but also for micro-mixing active and passive methods are used. Some of the most active techniques are: Magnetophoresis, Acoustophoresis, Di-electrophoresis,Electro-hydro-dynamics, Electrokinetics etc. are used to sort ,trap ,transport, mix etc. [5].
For some of those active techniques like Electrokinetics (AC-electro-osmosis, AC Electro thermal) the pattern of electrode plays a vital role to create a strong flow to manipulate particles such as orthogonal or T-shaped electrode pattern [6,7].

Following that trend a new electrode pattern has been introduced which is called V-shaped electrode pattern that has been used in a glass-slide & PDMS surface to create microfluidic action on a static fluid system[8].After that the pattern has been re-created using sputtered gold on a silicone substrate to create a unidirectional microflow in a static fluid system as well [9]
In this work, this electrode pattern is used in a microfluidic channel in order to observe the effect in a pressure driven flow through system generated by a syringe pump. For this purpose, a straight microfluidic channel has been used with low and high conductive fluids such as PBS solution and NaCl solution respectively seeded with fluorescent particles. The microfluidic channel has been manufactured by using a cheap 3D printed technique which has also been used to manufacture another microsystem called Y-channel Active microfluidic mixing device.

2. DEVICE FABRICATION

The microfluidic channel has been manufactured using a 3D printing machine. The channel designing process is divided into two stages. The manufacturing process of both the straight channel and Y-channel microfluidic mixing device is similar. For simplicity the manufacturing process for the Straight channel has been described below:

1. Drawing & Slicing 2. 3D Printing

2.1. Drawing & Slicing: Determining all the design considerations and measurement parameters the drawing has been created using a CAD tool. The entire drawing is shown below (**Figure**:1)

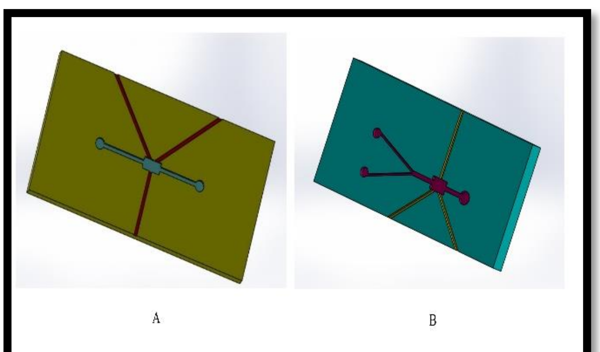

Figure 1: A) Drawing of straight Microfluidic Channel
B) Drawing of Y-channel active Microfluidic Mixing Device

The microfluidic channel is consisted of three parts.

1.**Straight channel:** It consists of the inlet & outlet. the dimension for the channel is shown in Figure 2. The diameter of the inlet and outlet is 1.5mm or 1500µm and the width of the channel is 600 µm & 670 µm for micromixer. The channel has a depth of 700 µm.

2.**Experiment Zone/Mixing Zone:** The channels from the inlet and outlet are merged in a rectangular box which has an arrangement for inserting the V-electrodes. This rectangular zone is an Experiment zone. (Figure 2 A). This zone has a length of 3mm and a width of 1.6mm and for micro mixing device (Figure 2 B) this zone is called Mixing zone which has width of 2mm, and another dimension is similar

3.**Electrode Groove:** There is a grooved arrangement for inserting tungsten electrodes in a V-shape manner inside the Experiment zone/Mixing Zone (Figure :1). The width and depth for the groove have been considered 500 µm since the diameter of the shank of tungsten electrode is 490 µm. and 10 µm has been considered for clearance.

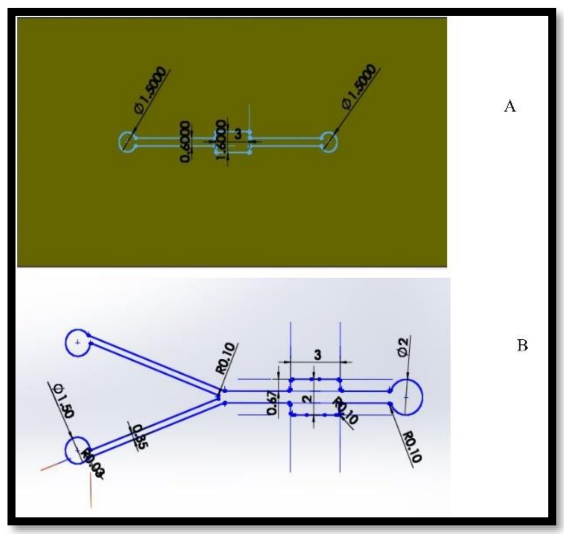

Figure 2: A) Dimensioning of straight Microfluidic Channel, B) Dimensioning of Y-channel Microfluidic Mixing Device

Once the drawing is completed the drawing file has been saved in (.stl) format so that it can be opened in Chitubox software and ready for slicing.
After setting some printing parameters the drawing has been sliced and saved. After slicing the time and the volume of material along with the cost estimation can also be determined by the slicing software. As from Figure: 3 it is shown that the time for printing is 26 min 4 second. The file has been saved in (ctb)

format which is the format required for printing. Finally, the ctb formatted file has been transferred to the dedicated USB for printing.

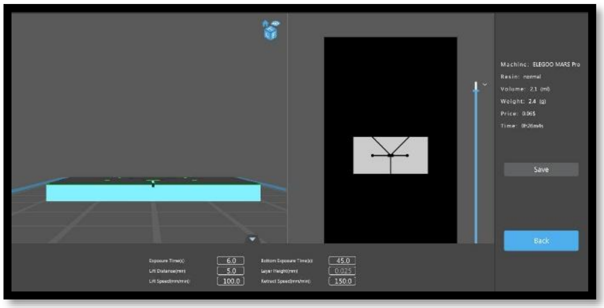

Figure 3: Slicing and Saving

2.2. 3D Printing Process: There are several steps for 3D printing. To mention that, ELEGOO Mars Pro 3D printer has been used to manufacture the microfluidic channel which consists of 1. Rotary Knob, 2. Z-Axis, 3. Build Platform, 4. Resin Tank, 5. Touch Screen 6. Levelling Pad, 7. Cover etc. and Resin is used as a printing material. The steps are:

Step 1: At first the Resin tank and the plate have been properly cleaned with the Acetone and later rinsed with DI water.

Step 2: After cleaning the tray properly; it has been fixed tightly (not too much) on top of the LED screen of the printer.
Later liquid resin to be poured inside the tray. The resin should be shaken properly before pouring and it should be poured through s filter paper. Mask and glove must be worn before pouring the resin as it is harmful to inhale. The resin tank should be filled at least one third of its volume. After filling the tank, the whole system has been covered.

Step 3: In this step, the USB containing the ctb formatted file has been connected to the USB port of the printer the file to be printed has been selected.

Step 4: After the completion of printing process printer has been uncovered and the Aluminum plate has been cleaned properly with Acetone.

Final Step: Finally, the tray has been cleaned with Acetone again because it helps to loosen the attachment of the printing material with the surface of the tray.

After the cleaning with Acetone, the printed channel has been removed carefully with the help of a chisel Care has been taken; because the material might break, so the removal has been done slowly and carefully. (Figure:4)

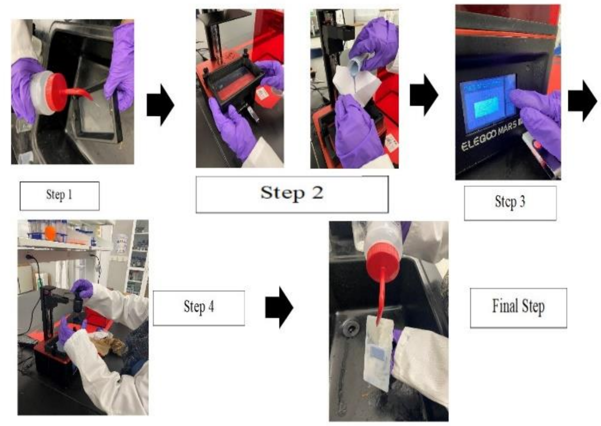

Figure 4: Printing Steps

3. METHODOLOGY

3.1. Setup Configuration

Before preparing the microfluidic channel for carrying on the experiments it has been analyzed under microscope to check for any defects. Figure 5 shows the Microfluidic Channel along with its corresponding microscopic images.

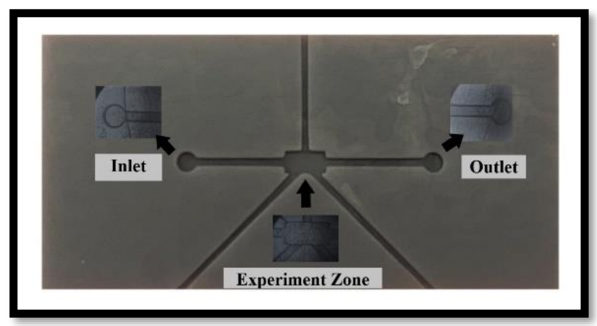

Figure 5: Microscopic view of the straight Microfluidic Channel

The Microfluidic Channel has one inlet and outlet of diameter 1.5mm. It also has an experiment zone where the grooves for the tungsten electrodes coincide in a V-shape manner. This is the zone where the effect of the electric field to the flowing fluid to be observed. For conducting the experiment two types of fluid have been used one is highly conductive NaCl solution and another is less conductive PBS (Phosphate Buffer Saline) solution. Two different setup orientations have also been used for conducting experiments on straight microfluidic channel One is covered with PDMS and another is covered with perfusion chamber. For further verification of the results these two experiments have been conducted in two different orientations. After checking the channel under microscope for any defects the tungsten electrodes have been placed inside the groove using calibration microscope. A distance around 400µm has been kept between the V-electrode with the third electrode. The electrodes have been finally covered by the PDMS. The electrodes and the PDMS/Perfusion Chamber have been fixed by superglue. After that two holes have been made on the surface of the PDMS/Perfusion chamber pointing towards the inlet and outlet. (Figure: 8)

After making the setup properly the next task was to take the setup for a leak test. Leak test is very crucial and important step for carrying the experiments this system because if the setup passes the leak test successfully that setup can be used for further experiments but if the setup fails the leak test the setup cannot be taken for experiments rather the setup to be reconstructed and the process to be repeated. That is why this step is very crucial because if the fluid does not flow entirely from the inlet to outlet through the channels there might be a chance for overflow and if overflow occurs it will influence the flow inside the channel thus results of the experiments will be distorted . Figure: 6 shows a glimpse of a leak test. It shows that if the fluid is flown from the inlet it will flow through the channel and create a water bubble at the outlet only.

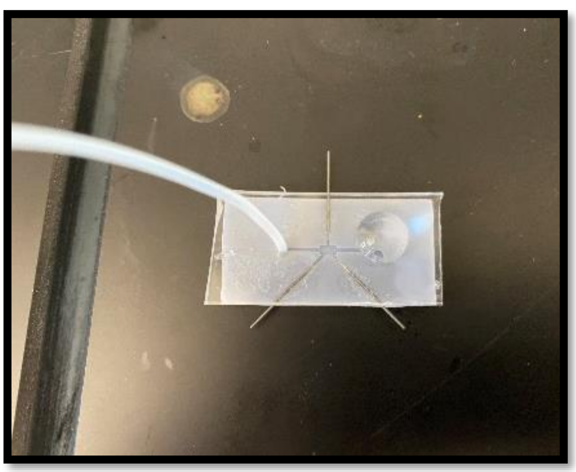

Figure 6: Leak Test

After leak test the setup was taken for further experiments.

3.2 Experimental Parameters

For conducting the experiments on flow through system parameters such as Flow rate, Voltage and Frequency were considered. Table 1 shows the parameters in an increasing order which have been used in different combinations based on several trials.

Flow Rate (µL/h)	Voltage (mV PP)	Frequency (Hz)
1.5	50	100
2.0	200	400
2.5	1000 (1V)	1000
5	4000 (4V)	10 kHz
7.0	5000 (5V)	100 kHz

Table 1: Experimental Parameters

for both types of fluids. In each case the experimental process has been started by adjusting the focus of the experimental setup under microscope and setting up a Mask for analyzing the experiment

4. EXPERIMENTAL PROCEDURE

Figure: 7 (A) & (B) show the experimental setup under microscope respectively. It can be perceived that the orientation for both setups is opposite to each other.

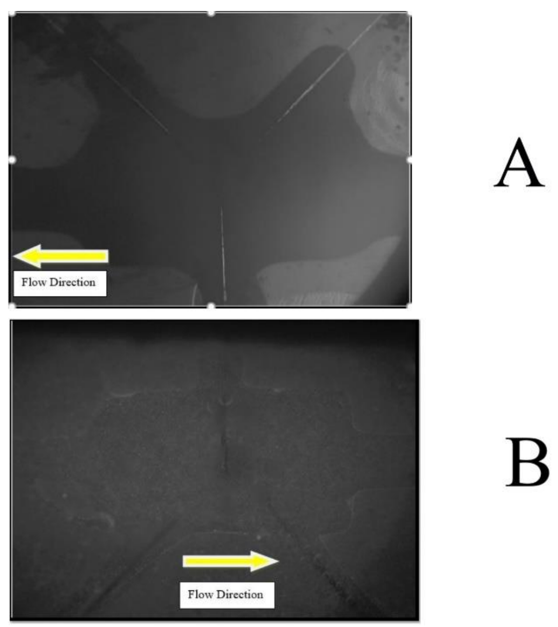

Figure 7: A) Setup configuration under Microscope for NaCl solution
B) Setup configuration under Microscope for PBS solution

The experimental procedure has been started with a no voltage condition in order to mark the uninfluenced flow as well as calibrate the PIV system

After that several combinations of voltage, flow rate and frequency have been applied to analyze the behavior of the fluid flow using Micro particle Image Velocimetry (PIV) system which consists of Nikon Eclipse LV150N microscope, fitted with a charged couple device (CCD) camera. It also has a laser system (TSI YAG70-15-QTL). The experimental setups have been placed under the microscope and the electrodes were actuated with a function generator, which has been connected to a voltage amplifier. The flow has been generated using a syringe pump 45 seconds before energizing the electrodes. The movement of the fluorescent particles in the fluid has been traced by a synchronized laser emission and image capturing process. Insight 4G, has been used to analyze each experimental run of 50 captured frames. These 50 frames are processed after checking spatial calibration and masking the boundaries. Insight 4G generates an excel sheet consisting of vector positions of fluorescent particles. The software detects the particle flow and creates vector files, which are then used to plot the graph by Tecplot Focus 2013R1 software. [7]

5. RESULTS & DISCUSSIONS

Figure 8 shows the numerical simulation result of the fluid flow under the application of electric field. Since the simulation has been done for 2-dimensional case and the experimental setup is 3-dimensional so the simulation result and the experimental result might vary slightly.

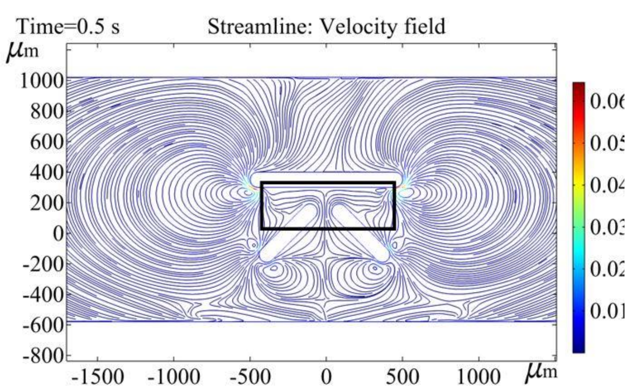

Figure 8: Fluid flow under the application of electric field

In this Figure: 8, the region of interest has been enclosed by a black box. Also, from this Figure we can observe that due to electric field the flow stream is bent in the gap of V-electrodes. Since the simulation is 2-dimensional so we can observe a separation or discontinuity of flow in the V-electrode zone but for a 3-dimensional case there is no chance for separation of flow as the depth of the channel works as a barrier and maintain the continuity of the flow which we are going to observe in the next segment.

5.1 NaCl Solution

The Tecplot data for Sodium Chloride solution is given. Figure 10 shows the flow vector before the application of the electric field at a flow rate of 2.5µL/h & Reynolds number 5.73x10^{-4} which clearly is a laminar flow.

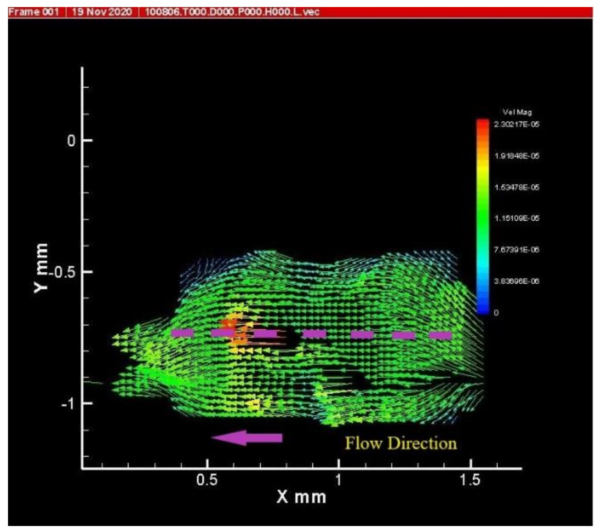

Figure 9: NaCl solution TECPLOT before the application of electric field

If we compare the Figure 9 with the Figure 7 A), we can observe that the flow direction is from right to left and the position of the V-electrode is at the top. The purple dotted lines in Figure: 9 indicate a certain zone in the flow stream where the velocity of the stream is maximum which is at the center and it can also be verified from the velocity magnitude at the top right corner of Figure 9. It can also be observed that the flow is undisturbed and uniform. Now from the Figure 10 we can observe that the at a flow rate of 2 µL/h and under the application of 200mVpp and 100kHz the dotted lines of maximum velocity is well deflected and creates a prominent cleavage at the zone where the V-electrodes are placed which is quite similar to the simulation but unlike the simulation the simulation the flow has maintained its continuity at the region of V-electrode and has formed the cleavage The maximum velocity can also be verified form the velocity bar provided in the Figure :10 where the purple dotted lines follow the maximum velocity vector lines .

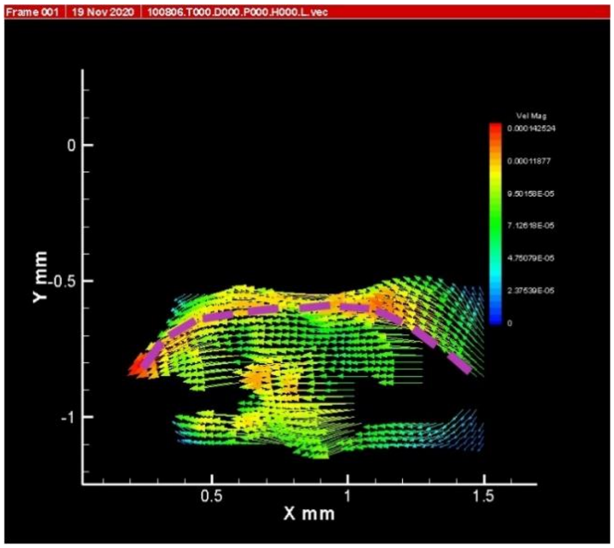

Figure 10: The velocity profile at 2µL/h, 200mVpp & 100kHz of NaCl solution

5.2 PBS Solution

Similar type of experiments has been carried out in the case of PBS solution but in opposite orientation. In that case the orientation and the flow direction have been opposite to the experiments of NaCl solution. Figure: 11 shows the direction of flow for PBS solution before the application of electric field at a flow rate of 2.5 µL/h, where the Reynolds number is $6x10^{-4}$ when no voltage was applied and again the purple dotted lines indicate a certain zone in the flow stream where the velocity of the stream is maximum

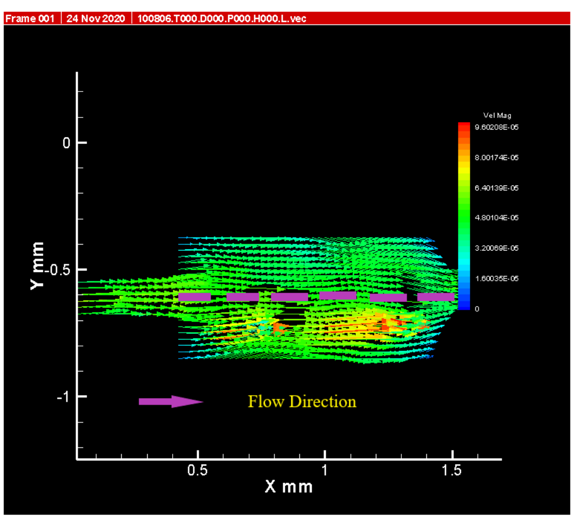

Figure 11: PBS solution TECPLOT before the application of electric field

In that case, the V-electrode is placed at the bottom of the figure and the flow direction is from left to right (Figure:12).

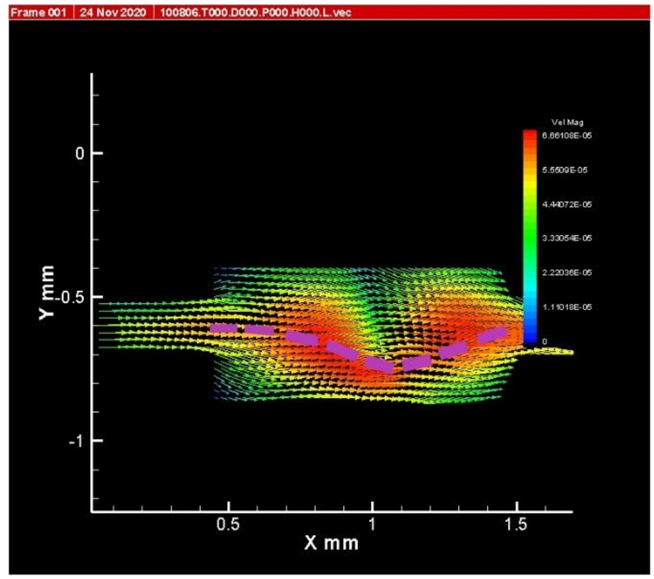

Figure 12: The velocity profile at 2.5µL/h, 200mVpp &400Hz of PBS solution

Maintaining the same velocity, a voltage of 200mVpp has been applied at 400Hz for PBS solution as shown in Figure 12 and the same thing occurred at the zone where the V-electrodes are placed and if it is observed the same bending occurs at the V-electrode zone similar to the simulation and the experiment for the case of NaCl solution.

As both from the Figure:10 & 12 we can see the bending of the flow stream of maximum velocity occurs towards the V-electrode zone and looks perfect which helps to justify the simulation result as well.

5.3 Y-Channel Active microfluidic mixing device

Another set of experiments have been carried out with the similar type of microfluidic channel (Figure:13) which is called Y-Channel Active microfluidic mixing device that has two inlets which can intake two different type of fluids and one outlet with a mixing zone similar to experiment zone with V-electrode inserted.

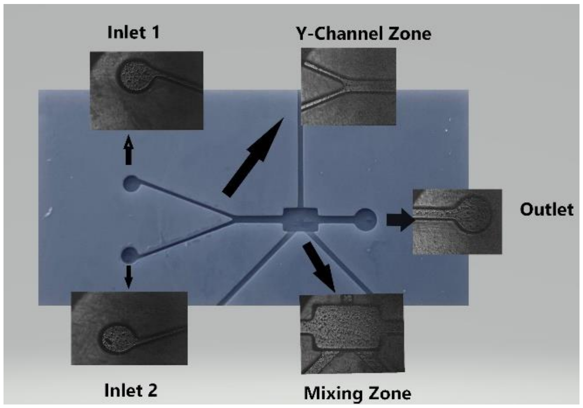

Figure 13: Microscopic view of the active Y-Mixing Microfluidic Channel

For experimental analysis, the average inlet velocity was 212 μm/s. The calculated Reynolds number is 0.178. This small Reynolds number created a laminar profile in the channel. In figure 14, it is observed that the fluid flow pattern is completely laminar. In this condition, the fluorescent particles in the fluid does not have the ability to go to the adjacent layer of the fluid.

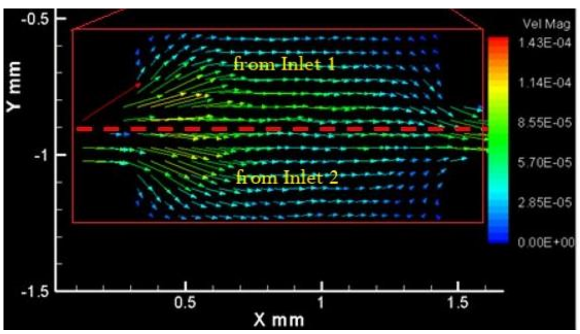

Figure 14: Fluid flow direction from both inlets inside Micromixer without electric field

An imaginary red line has been drawn in the middle of the channel to differentiate between the two fluid streams from two inlets. It is observed that after going out from the mixer zone of the micromixer the upper part of the fluid stream remains in the upper part of the microchannel and the lower fluid stream remains in the lower part. After that the AC electric field has been applied to the electrodes. It has been started from 2 V p-p and continued until 5 V p-p. It has been found that no notable deflection has been found in the flow pattern until 2 V. In figure 15(a), it is observed that the flow field is changing directions at 2 $V_{P\text{-}P}$. The positions of the electrodes in figure 15 are similar to the grooves of electrode shown in figure: 13. Near single electrode, the electric field force the velocity streamlines towards the middle of the channel. It forces the fluorescent particles in the fluid to go to the adjacent layer and mix with that layer. Similar flow disturbance has also been observed near the V-electrode. These flow disturbances are responsible for changing the velocity vector patterns in the mixing zone. Some flow reversal has been observed in that region. It created a mixing environment for the nearby particles in the fluid. So, the direction change in the velocity profile means that the particles in the fluid are mixing with the adjacent layer of the fluid. It is not possible without applying electric potential in the fluid. The effect of frequency in the mixing has been observed experimentally the frequency is increased from 100 Hz to 1000 Hz, the disturbances created by the electric field on the fluorescence particles found different from one another. The AC electric field created a wave like flow pattern inside the micromixer. This wave like flow is responsible for mixing to have occurred at the outlet of the micromixer. The upper fluid stream goes to the lower part of the micromixer and again comes back to the upper part of the microchannel.

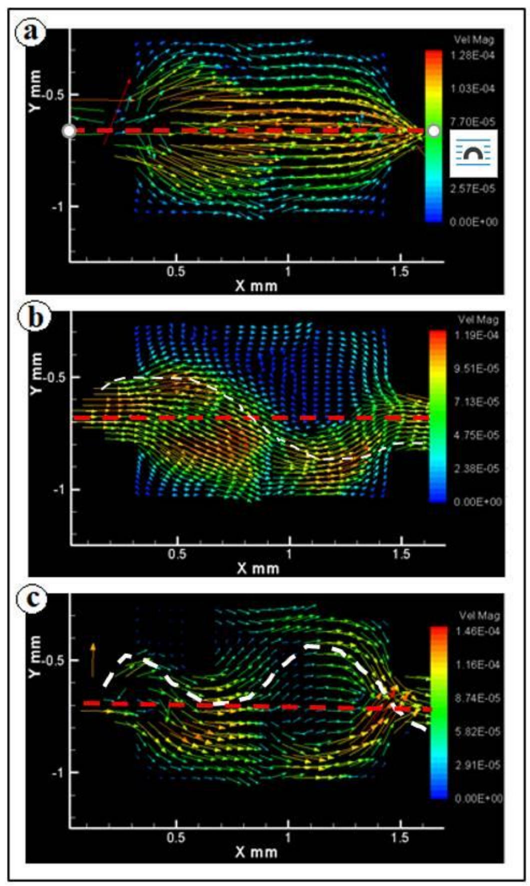

Figure 15: A) Flow field inside the channel at AC-potential V= 2 v & frequency, f= 100 Hz. B) Flow

field inside the channel at AC-potential V= 3 v & frequency, f= 500 Hz Hz. C) Flow field inside the channel at AC-potential V= 5 v & frequency, f= 1000 Hz

It creates a sine wave flow pattern in the mixing zone. This sine wave flow pattern becomes more intensified in figure 15(C). So, increasing the voltage and frequency intensified the mixing process in the micromixer. The sine wave flow pattern is highlighted by a white dotted line in figure 15 (B) and 15 (C).

6. CONCLUSION

From the straight microfluidic channel's experiments, the primary focus was to figure out the effect of V-electrode pattern in both highly conductive and low conductive fluid in a flow-through system. Since in the flow through system the flow was pressure driven and from the comparison of simulation and results from tecplot data it can be analyzed that, in two different configurations the bending of the flow towards the direction of ‘V’ electrodes have been occurred that can be useful for manipulating or guiding particles seeded in the fluid flow in a certain direction **.** Again, as from the Reynolds number in the microfluidic channels the flow that occurs is laminar in fashion so for mixing purpose laminar flow is not a good option for mixing two different types of fluid in a mixing channel rather turbulence is efficient option for mixing purposes [10] but in the case of micro-fluidic devices due to downsizing the dimensions of the channel inertial flow becomes less significant and viscous flow becomes dominant and the flow becomes laminar so from the experimental Reynolds number it can be perceived easily, so in this case turbulence is hard to achieve. As from the experiments of the Y-Channel Active microfluidic mixing device a turbulence is required to create the mixing process faster in the mixing zone otherwise the flow stream will flow separately due to pressure as shown in the Figure:16 so the presence of an electric field can stir by disturbing the fluid streams at the mixing zone which can serve the purpose of turbulence in the mixing zone somewhat.

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