Microfluidic Cell Trapping Device Based on Standard PCB Technology

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***Abstract*—Nowadays, the methods used to determine cell concentration either count the cells per volume or measure the cells weight per volume. This paper presents the design, fabrication and measurement of a microfluidic cell trapping device envisaged for cell concentration measurements. The work presented here focuses mainly in the fabrication of the device. Measurements are made to validate the fabricated device. The device separates different size particles by using dielectrophoresis. Horizontal as well as vertical electric fields can be used to trap the particles by controlling the amplitude and frequency of AC voltage signals. The device presented here is fabricated using low-cost and low-temperature technologies.**

Keywords-dielectrophoresis; cell trapping; microfluidic;, vertical electric field; PCB

I. INTRODUCTION

Cell concentration is of high importance to medicine and food industry. In the case of medicine, measuring cell concentration is relevant for medical diagnosis; for food industry, it is useful for monitoring the concentration of yeast cells in food production.

Nowadays, the methods used to determine cell concentration either count the cells per volume or measure the weight of cells per volume. Those methods consume time and resources. The cell trapping device presented here is envisaged for cell concentration determination.

This paper presents the design, fabrication and measurements of a low-cost cell trapping microfluidic device. The design feasibility of the device was tested using COMSOL; however, this is not part of this work. Special attention is given to the fabrication process/method of the device since it uses other technologies than silicon and glass. The fabrication of the device is based on a standard PCB (Printed Circuit Board) and the use of SU-8 photoresist, making the device fabrication a low-temperature process. The PCB offers mechanical stability to the device.

An innovative fabrication method is presented making use of an ITO (Indium Tin oxide) glass slide to close the channel. The ITO glass offers optical transparency and the possibility to apply vertical electric fields in the channel.

Since different size cells respond in a different way when inhomogeneous electric fields are present, the dielectrophoretic technique is used to trap and release different size cells. Measurements are performed to validate the fabricated device and therefore the study of the associated models with the control of the particles' position with the use of an electric field is not part of this work.

Furthermore, the cell trapping device in combination with the packaging technology presented in [1] can be used for fast prototyping of low-cost and low-temperature fabricated μTAS (Micro Total Analysis Systems).

The packaging technology presented in [1] consists of inlaying an either fluidic or electronic chip in a PCB material and to build microfluidic channels on top of them. The electrical connections between the components are inkjet-printed.

In summary, the benefits of the device presented here with respect to commercially available devices are little consumption of sample volume, economic, high speed detection and flexibility of integration. Furthermore, it is small with a size of 70 mm x 70 mm x 1.6 mm.

The paper describes the relevant properties of the materials used for fabrication of the device. The fabrication process is detailed as well as the fabrication challenges and how to overcome them.

The experimental setup is explained and the results are presented. The paper finalizes presenting the conclusion and future work.

This paper does not include how to measure the cell concentration itself. Furthermore, analysis of the kinematic model of the particles as well as a deeper study of the associated control method to achieve very accurate positioning are not within the scope of this work.

II. MATERIALS

The materials used to fabricate the device are ITO glass, SU-8, and PCB material. ITO glass consists of a glass with a thin layer of indium oxide (In_2O_3) and tin oxide (SnO_2) on it. The two properties that make it very attractive for microfluidic devices are its electrical conductivity together with its optical transparency. Furthermore, it is biocompatible. Its main disadvantage is its high electrode impedance; 4 to 5 times higher than similar size gold electrodes [2]. The ITO glass used in this project was ordered from DELTA Technologies LTD., USA and Table I enlists its properties.

TABLE I. ITO GLASS PROPERTIES [3]

ITO glass properties	
Property	***Value***
Sheet resistance R_s	5-15 Ω

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ITO glass properties	
Property	***Value***
Normal transmittance	>85 %
Nominal coating thickness	1200-1600 Å
Substrate thickness	0.5 mm
Substrate dimensions	25 mm x 50 mm x 0.5 mm

SU-8 is an epoxy-based negative photoresist used to pattern high aspect ratio structures [4]. This material is widely used as a structural material for microfluidics due to its physical properties as excellent chemical resistance, great biocompatibility and good adhesion to a wide range of materials. SU-8, when properly hard baked, is difficult to remove; moreover, if it is not completely exposed it tends to outgas. SU-8 2000 series offer low viscosity. SU-8 2002 is used to flatten the PCB surface and SU-8 2025 is used to build the microfluidic channel wall. Table II enlists the properties of SU-8 2002 and SU-8 2025.

TABLE II. PROPERTIES OF SU-8 2002 AND 2025 [5], [6]

Properties of SU-8 2002 and 2025		
Property	***Value (SU-8 2002)***	***Value (SU-8 2025)***
Viscosity	7.5 cSt	4500 cSt
Density	1.123 g/ml	1.219 g/ml
Thickness	~2-3 µm	~20-80 µm
Glass transition temperature Tg	210 ˚C	210 ˚C
Thermal conductivity	0.3 W/mK	0.3 W/mK

The PCB is used to mechanically support the electronic components, the microfluidic channel structure and to offer electrical connections. Compared with silicon, the PCB is much cheaper. Table III enlists relevant properties of FR-4 (flame retardant 4).

TABLE III. RELEVANT PROPERTIES OF FR-4 [7]

Relevant properties of FR-4	
Property	***Value***
Glass transition temperature Tg	135 ˚C
Thermal conductivity	0.255-0.290 W/mK
Substrate thickness	1.6 mm
Substrate dimensions	70 mm x 70 mm x 1.6 mm

The fabricated PCB has no solder mask and the electrical connections are gold plated to keep the biocompatibility of the device.

III. DEVICE DIMENSIONS

Figure 1 illustrates the cross-section of the electrodes area in the device, showing the dimensions of the microelectrodes as well as the microfluidic channel.

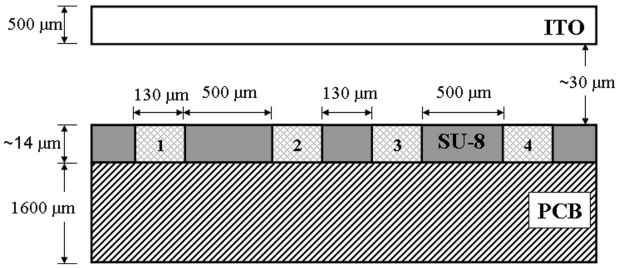

Figure 1. Dimensions of the microfluidic device. Cross-section of the electrodes area.

The PCB used is a 7 cm x 7 cm FR-4 PCB. Four electrodes and two holes for fluidic connections are pre-fabricated in the PCB.

The four electrodes have the same dimensions, 130 µm width and 1000 µm length. The distances between each couple of electrodes are 500 µm between electrodes 1 and 2, 130 µm between electrodes 2 and 3, and 500 µm between electrodes 3 and 4. The radius of the inlet and outlet holes is 400 µm. The microchannel length is 30 mm and the width of the channel is 1 mm. The depth of channel is approximately 30 µm.

IV. FABRICATION

The fabrication of the devices is divided in two stages: the flattening of the PCB surface and the fabrication of the microfluidic channel.

Since the electrodes and other electrical connections are pre-fabricated in the PCB, using standard PCB fabrication procedures, the thickness of the tracks is around 13 µm. The ratio between the channel depth and the thickness of the electrodes is rounded to 2. The flow of the media in the channel would be distorted due to this surface profile thus the surface of the PCB has to be flattened before building the microfluidic channel.

Figure 2 shows the steps followed to flatten the PCB surface. The steps in Figure 2 are performed 6 times since the SU-8 2002 provides layers of 2-3 µm thick, depending on the spinning speed.

First, a plasma treatment is performed to improve the adhesion between the SU-8 and the PCB (a). SU-8 2002 is spun on the surface of the PCB (b), (c). The specimen is subject to a soft baking step at 90 º C (d). The SU-8 is exposed (e) and a post exposure bake step is performed at 90 ºC (f). The photoresist is developed thus the SU-8 covering the electrodes and electrical connections area is removed (g). A hard baking step is performed at 120 °C. The cycle initiates again performing the plasma treatment to the new surface.

The cycle is performed 6 times in order to achieve an SU-8 layer of around 13 µm thick, achieving the same level between the new PCB surface and the electrodes.

Figure 3 shows the steps followed to fabricate the microfluidic channels without going into detail in the surface flattening process.

The PCB is cleaned with ethanol to remove dust, grease and other forms of contamination from the surface. To

remove the humidity adsorbed during storage, it is dehydrated for two hours at 120 ºC (b). Subsequently, an oxygen plasma treatment is performed to improve the wetability of the substrate and the surface of the PCB is flattened using SU-8 2002 (c). The walls of the microchannel are fabricated using SU-8 2025 (d). Silver ink is deposited in order to make the electrical connection from the ITO glass to the PCB (e). The channel is closed using ITO glass. The ITO glass is attached to the SU-8 channel structure using a non-conductive adhesive (NCA) (f) and the adhesive is cured at 80 ºC during 3 hours (g).

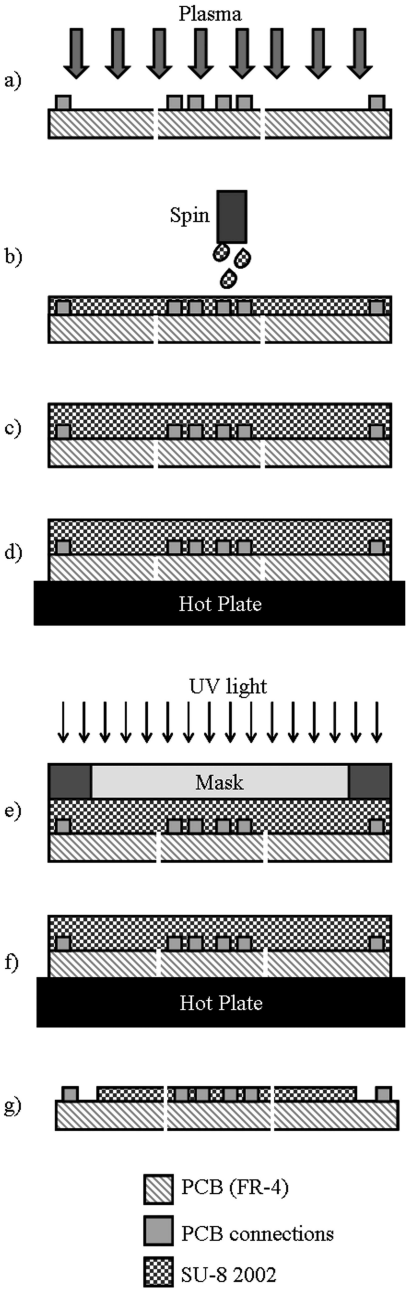

Figure 2. Process to flatten the PCB surface.

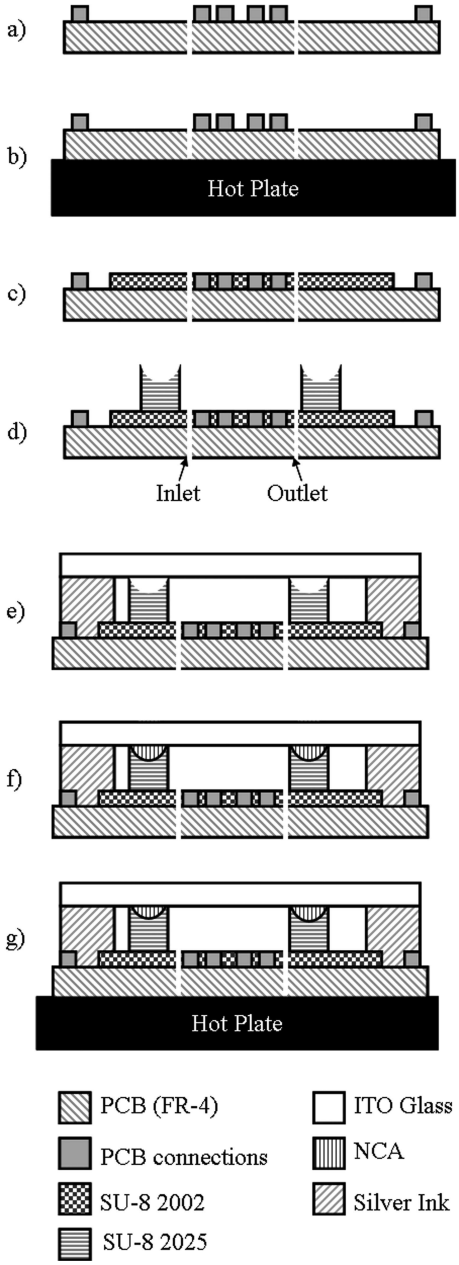

Figure 3. Flowchart to fabricate the microfluidic channels.

The fluidic connections to access the channel are made with a through vias during the fabrication of the PCB. The tubes to access the channel are press fit in the through vias on the back side of the device and glued. The device is ready to be used.

Figure 4 shows a fabricated device. The device is built on a prefabricated PCB including the electrodes.

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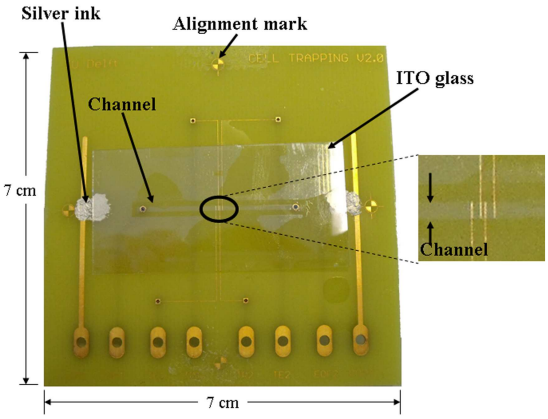


Figure 4. Fabricated device.

The electrical connection from the PCB to the ITO glass is made with silver ink. The fluidic connections for accessing the channel are made in the back side of the PCB.

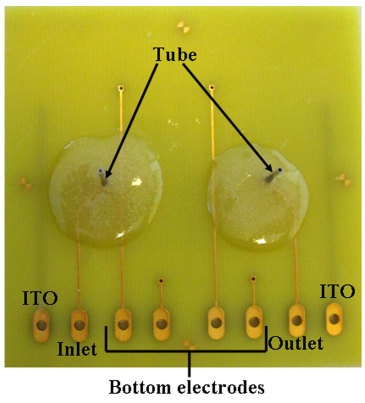


Figure 5. Fluidic connections for accesing the channel.

Figure 5 shows the back side of the PCB. The metal tubes for the inlet and the outlet holes are press fit in the holes and secured on the back side of the device with an epoxy adhesive.

V. FABRICATION CHALLENGES

During the fabrication of the device, there are two challenges to overcome.

The first one is to flatten the PCB surface. Figure 6 shows the PCB surface profile in the area of the electrodes measured with a Dektak profiler; the electrodes have a height of around 13 µm above the PCB surface.

The electrodes and electrical connections are fabricated with copper gold plated on the PCB. The surface is flattened with SU-8 in order to avoid contact of the media with copper and to avoid the flow of the media to be disturbed by the electrodes profile. This process is described in the previous section.

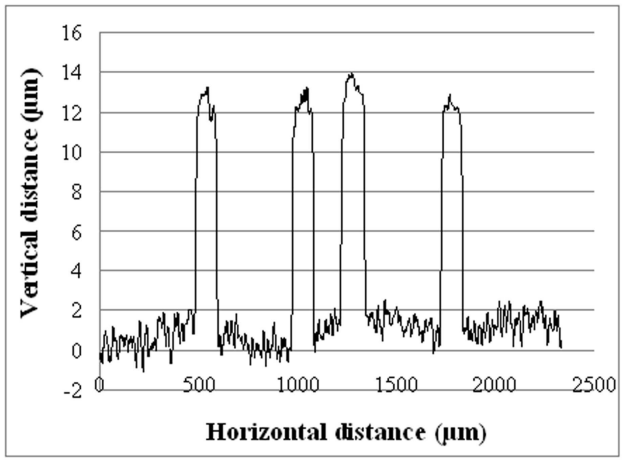
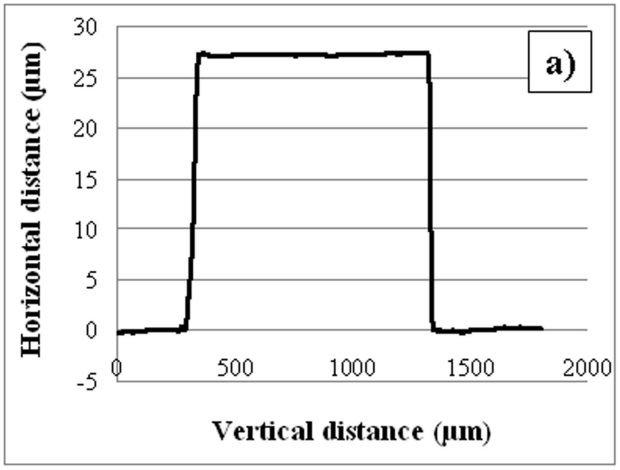


Figure 6. PCB surface profile in the area of the electrodes.

The second challenge to overcome is the way of sealing the channels. A low viscosity NCA is used for that purpose. Nevertheless, if the channel walls are fabricated with the SU-8 processing parameters recommended by the manufacturer, capillary forces make the NCA flow into the channel, blocking it. To overcome this, a channel on top of the channel walls is fabricated. Figure 7 shows (a) the cross-section of the wall of the channel when the recommended processing parameters are used and (b) the cross-section of the channel´s wall when different parameters are used for processing.

"The recommended processing parameters" refers to the way the temperature is taken from room temperature to the specified temperature, and vice versa, during the baking steps.



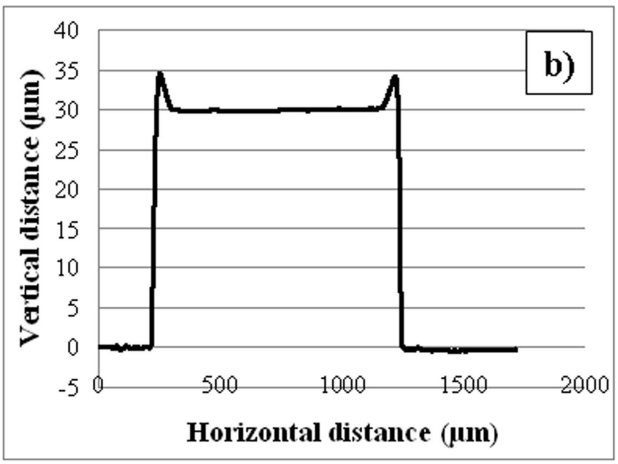


Figure 7. Cross-section of the wall of the channel when slow ramping of the temperatures during the baking steps is used (a) and when no ramping is used (b).

If the step from room temperature to baking temperature, and from baking temperature to room temperature is made in a fast way, stress in the resist will form such that two small walls are created in the top of the channel´s wall, creating a small channel on top of the walls of the channel; this small channel is used to flow the NCA avoiding it from coming into the fluidic channel. The NCA is cured and the channel is sealed. By sealing the channel in this way, no bonding equipment and heavy weights are necessary.

VI. EXPERIMENTS

The DEP (dielectrophoresis) force depends on the flow velocity, frequency and amplitude of the AC voltage signal, and particle size. Frequency and amplitude are isolated in this work to study their effects on trapping particles with a different size.

A syringe pump KdScientific model 200 is connected to the inlet tube of the microfluidic device to drive the flow of demi water with polystyrene particles by applying pressure difference between the inlet and the outlet.

An AC generator KROHN-HITE model 4300 is connected to the electrodes via the pads on the PCB to generate electric fields in the channel.

In order to test horizontal electric fields, a voltage difference is applied between adjacent electrodes, that is to say, between electrodes 1 and 2, 2 and 3, and 3 and 4.

To test vertical electric fields, a voltage difference is applied between the ITO glass and electrodes 2 and 4.

An oscilloscope HP54601B is used to check the applied AC signals.

The device is placed on the table of a microscope IX71 Olympus which has a low noise CCD camera ColorView Olympus to acquire images while the particles are being trapped. The software used to acquire the images is analySIS docu Olympus.

The trapped particles are plain microspheres of polystyrene from Phosphorex, Inc. with a mean diameter of 2, 6 and 10 µm.

VII. RESULTS

Table IV shows the results for trapping different size particles by regulating the frequency of the AC signal. The flow velocity used in this case is 0.1 µl/h.

TABLE IV. FREQUENCY REQUIRED FOR TRAPPING DIFFERENT SIZE PARTICLES WITH A HORIZONTAL ELECTRIC FIELD

Frequency required for trapping different size particles (horizontal electric field)		
Particle radius (µm)	***Voltage Frequency (KHz)***	***Voltage difference between electrodes (V)***
10	>140	26
6	>160	26
2	>1600	26

TABLE V. VOLTAGE AMPLITUDE REQUIRED FOR TRAPPING DIFFERENT SIZE PARTICLES WITH A HORIZONTAL ELECTRIC FIELD

Voltage amplitude required for trapping different size particles (horizontal electric field)		
Particle radius (µm)	***Voltage Frequency (MHz)***	***Voltage difference between electrodes (V)***
10	6	6.4
6	6	10.4
2	6	23.3

Table V shows the results for trapping different size particles by regulating the amplitude of the AC signal. The flow velocity in this case is 2 µl/h.

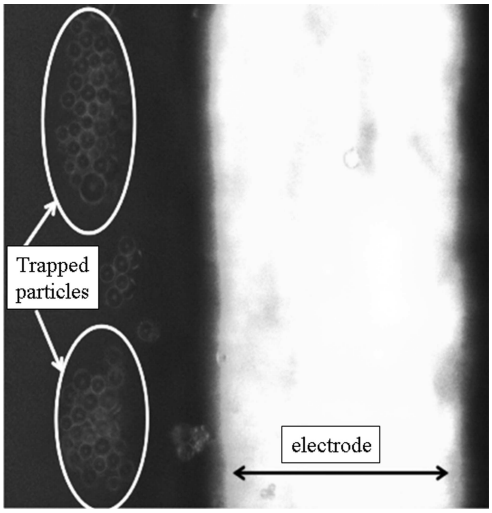


Figure 8. 10 µm and 6 µm particles trapped with a horizontal electric field at 400 KHz and a voltage difference between electrodes of 25 V.

Figure 8 shows trapped particles with a diameter of 10 µm and 6 µm. The frequency of the AC signal is 400 KHz and the voltage amplitude difference between the electrodes is 25 V. The flow velocity used is 0.1 µl/h. A horizontal electric field is used.

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Figure 9 shows trapped particles with a diameter of 10 µm. The frequency of the AC signal is 160 KHz and the voltage amplitude difference between the electrodes is 7.5 V. The flow velocity used is 0.1 µl/h. A horizontal electric field is used.

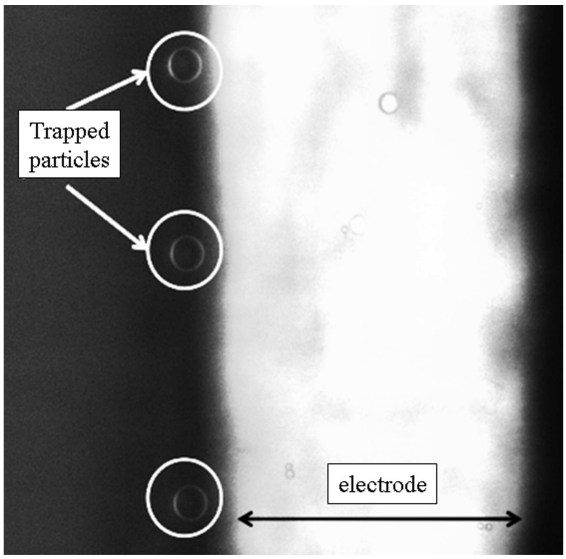

Figure 9. 10 µm particles trapped with a horizontal electric field at 160 KHz and a voltage difference between electrodes of 7.5 V.

Table VI shows the results for trapping different size particles using a vertical electric field. The frequency and amplitude of the AC signal are regulated. The flow velocity in this case is 1 µl/h.

TABLE VI. FREQUENCY AND VOLTAGE REQUIRED TO TRAP DIFFERENT SIZE PARTICLES WITH A VERTICAL ELECTRIC FIELD

Frequency and voltage amplitude required to trap different size particles (vertical electric field)		
Particle radius (µm)	***Voltage Frequency (KHz)***	***Voltage difference between ITO and electrodes 2, 4 (V)***
10	160	8.0
6	160	23.2
2	1200	23.2

The tables and figures show that the device is capable of trapping different size particles by controlling the frequency and the amplitude of the AC voltage signal using horizontal as well as vertical electric fields.

VIII. CONCLUSION AND FUTURE WORK

This work shows the fabrication of a dielectrophoretic microfluidic device for particle trapping and separation. Particles with different size can be separated by controlling the amplitude and frequency of AC voltage signals.

Furthermore, the device can trap particles using horizontal electric fields as well as vertical electric fields.

The device shows better performance in the vertical electric field than in the horizontal electric field; the frequencies required to trap particles of the same size are lower in the vertical direction than in the horizontal direction.

Moreover, the device is fabricated using low-cost and low-temperature technologies allowing fast prototyping and the integration of the device with the technology proposed in [1] to form µTAS.

As a part of future work, the cell concentration determination method is going to be developed enabling the measurements of cell concentration in blood for medical diagnosis and the monitoring of yeast cells' concentration in food production.

Furthermore, the analysis of the kinematic model of the particles and the deeper study of the associated control method to achieve very accurate positioning is also part of future work.

Moreover, future work can enlarge the field of application to the analysis of biological fluids by trapping living cells as different types of microorganisms or nuclei.

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