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DESIGN AND LOW COST IMPLEMENTATION OF DIGITAL MICROFLUIDIC BIOCHIP

Vishal D. Karade
M.E Student, Dept. of ENTC Engineering,
K.I.T's College of Engineering, Kolhapur,
Shivaji University, Maharashtra, India

Atul R. Nigavekar
Associate Professor, Dept. of ENTC Engineering,
K.I.T's College of Engineering, Kolhapur,
Shivaji University, Maharashtra, India

Abstract— An easy to develop and innovative platform for "Digital Microfluidic Biochip" is presented. In this method devices are formed by considering cost as a main factor. Relays are used as the switching devices, Atmel 89C51 is used as a controlling device and high voltage power supply which provides both PWM AC and DC voltage is built on a general purpose board. This system is able to apply and switch up to 700V both PWM AC and DC voltages. PCB's are designed with an inter-electrode gap of 500 micron. PCB's are designed and etched with an innovative method of making stickers of design, affixing and etching with very low cost. 'Wonder 555' adhesive tape is used as an insulating material to replace expensive material such as 'Parylene-C'. 'Rain-X' is used as a hydrophobic material to replace expensive 'Teflon-AF 1600'. '1cSt Silicone Oil' is used to reduce surface resistance. Tank of plastic box is created to avoid evaporation of silicone oil. Fabrication of device is successfully tested for program controlled droplet manipulation like moving and mixing. We anticipate that this method will bring "Digital Microfluidic Biochip" within the reach of any diagnostic laboratory with minimal modification and facilities.

Keywords: EWOD, DEP, Single and two plate actuation, Electrode patterning, dielectric coating, hydrophobic treatment.

I. INTRODUCTION

In digital microfluidics liquids are manipulated by two techniques namely EWOD and DEP. EWOD is used to manipulate conductive fluids and DEP is used to manipulate insulating fluids [3], [12], [13]. Digital microfluidics is applicable to variety of fields like clinical diagnosis which includes blood serum, tears, saliva, sweat, urine, plasma [5], glucose assay, TNT assay, sulfate assay, and DNA pyrosequencing [6], DNA extraction, repair and amplification, Proteomics and Enzyme Assays, Immunoassays, Cell Assays, electronic hotspot cooling etc [9]. There are two approaches namely 'two plate actuation' and 'single plate actuation' by which liquids can be

manipulated [8], [9], [10]. To have an ideal method to manipulate liquids this would be fast, low cost and not require complicated technologies and clean room access, we have designed and implemented a system which is easily implementable with low cost by all who wish to implement digital microfluidics with minimal facilities. This fabrication technique we have divided into two parts: 'Electronic system design' and 'chip fabrication'. Under electronic system we have designed a 'High voltage power supply' which is able to provide both variable DC voltage up to 500V and variable PWM AC voltage up to 500V. Sugercube relays having both AC and DC switching capability are used as switching devices. AT89C51 is used as a controlling and action taking device. Programming is done in 'Keil'. Chip fabrication we have divided into three parts: Patterning of electrodes, dielectric coating and hydrophobic coating. Generally electrode patterning is done with gold, chrome, Indium Tin Oxide, Aluminum with the help of photolithography [2], [7], [9], [11], [12], [15], [19]. We replaced all these elements by copper. We replaced photolithography and used an innovative method for patterning of electrodes. We made design of electrode pattern in 'Coreldraw' and 'Catia' and made radium stickers of design using cutter/ plotter machine used for radium sticker design cutting, with very low cost. We pasted designed radium sticker on copper clad and etched in ferrous chloride. Chips can be formed by using permanent maker and using blade [1]. Both this methods are useful and low cost methods but needs extensive practice and not having accuracy and uniformity in design. Generally dielectric coating is done with 'Parylene-C' [10], [19], 'Spin-on-glass' [12], 'Al2O3' [14] which are costly and requires special coating machines and expert with clean room access. We replaced these materials by using adhesive 'Wonder 555' tapes and 'Microscopic cover slip'('Bluestar') as dielectric materials which are low cost materials and easily available. 'Cling film' gives best results with low cost but it needs to anneal to chip and its dielectric strength is weak because of which it breaks with higher voltage application. To avoid this we used

'Wonder 555' adhesive tape which is capable to sustain voltages up to 1000 V without breakdown. Generally hydrophobic treatment is done with the help of an expensive chemical 'Teflon-AF 1600', with special coating machines and experts. We used 'Rain-X'(snapdeal) as a hydrophobic material. 'Rain-X' improves hydrophobicity of 'Microscopic cover slip' but doesn't improve significant performance of 'Wonder 555' adhesive tape, so we didn't applied it on tape instead we used '1cSt silicone oil'(MR silicones, Mumbai) on tape to reduce its surface resistance. This work is our best efforts to make possible digital micro fluidics implementation with low cost, fast and to all who wish to use it without access to complicated, costly technologies and clean room.

II. PROPOSED SYSTEM ARCHITECTURE

A. Elecronic system design.

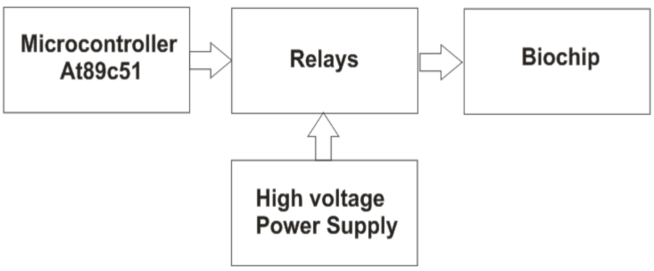

Fig.1 Block diagram of proposed system.

Fig .1 shows block diagram of our system. In this a high voltage is switched through relays and applied to biochip according to program done in microcontroller.

i. High voltage power supply:
This circuit is built with the help of push-pull topology. Round bobbin ferrite core transformer is used for stepping up operation with primary turns 26-0-26 and secondary turns 543-0-543. It is able to give variable output of up to 500 V both PWM AC and DC.

ii. AT89c51:
Atmel AT89c51 is used as a controlling and action taking device. It takes action on how to route droplets on chip, with varying delays. Programming is done in 'Keil'.

iii. Relays:
12V sugercube relays, having both AC and DC voltage switching capability are used for high voltage switching purpose.

iv. Biochip:
It is the electronically etched pattern of electrodes on which droplet routing takes place in order to perform clinical diagnosis, according to program.

B. Chip design

i. Patterning of electrodes

Generally for electrode patterning gold, chrome, Indium-tin-oxide, aluminum is used with the help of photolithography, but this technique becomes costly and need clean room access with variety of electronic machines and expert person who is able to coat precisely. Fig. 2 shows the steps in chip patterning. Here at first the design of electrode pattern with required size of electrodes and inter-electrode spacing is done in 'CorelDraw' as shown in a). This design is then imported in 'FlexiSignPro' or 'SignWizard', the software's compatible to cutter/plotter machine, used to make radium stickers of design. Imported design is then cut with cutter/ plotter machine and radium sticker is prepared. This radium is then pasted on copper clad as shown in b).

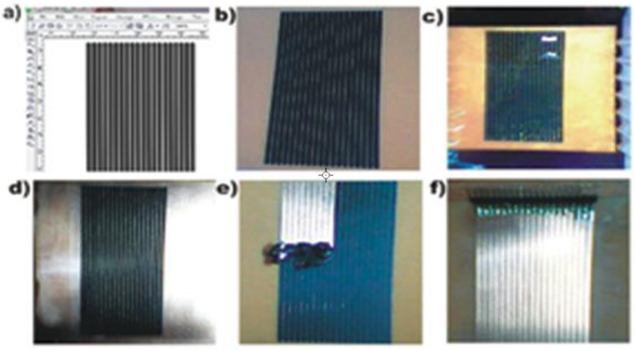

Fig. 2: Chip Patterning

Design pasted copper clad is then etched in ferrous chloride as shown in c). The surface which is in contact with ferrous chloride got removed and remaining part which was covered beneath the radium remained as it was as shown in d). Design sticker is then removed as shown in e). Connector pins are then soldered as shown in f). This technique provides accuracy and sharpness in chip. The technique of drawing the design with permanent markers and etching also beneficial but it doesn't provide accuracy while drawing pattern on chip to be etched [1]. The technique of cutting with the blade is also beneficial but still this requires extensive practice and doesn't provide accuracy [1]. Electrode patterning can be done with laser printing [18]. This technique is fast but requires laser printer and flexible copper sheet. Also its quality depends on quality of lesser printer and toner. By our technique it is possible to design any kind of shapes of electrodes like triangular, square, circular with greater accuracy than that of marker and blade method. Also it is possible to make zigzag pattern electrode to make the droplet easily movable. With this technique it is easy to change distance between electrodes and variation in angle of zigzag pattern. Some of the times we observed problem of sparking with the chips formed with photolithography and screen printing when inter-electrode gap is below 200 micron. With our technique we also faced problem of sparking but it is removable by cutting shorts in between electrodes with the application of high voltage. We predict that our method is very cost effective, easily implementable at home and serves the purpose for digital micro fluidics.

ii. Dielectric coating

Generally dielectric coating is done by using Parylen-C, SiO2, Al2O3 etc. by using variety of coating techniques like spin coating, dip coating, sputtering, vacuumed deposition etc. These chemicals are costly and require access to clean room with special coating machines and expert. To avoid this we tried to get results on paints, adhesives but there were problems of non-uniform coating, hydrophilic behavior, sticky nature and pilling out of coating with respect to time, no sustainability of higher voltages etc. Also once coated the same chip can't be reused under non-performing action of chip because of reasons like dielectric breakdown, pilling off, non-uniformity in coating, failure of hydrophobic treatment etc. 'Polymethyl methacrylate' is coated on copper substrate with spin coating with 1000-5000 rpm, but this failed to perform EWOD operation and got removed with respect to time. Although few of the paints with spray coating showed EWOD operation with the application of DC voltage in the range of 100V to 150V. To avoid all these problems and to make cost effective coating we tried "Cling film" with thickness of ~10 micron, and generally used for fruits packaging which is same as saran wrap [1]. This gives best results but it is unable to sustain higher voltages. It is observed that with increase in voltage it breaks down. There are two methods by which we can form coating of 'Cling film'. In first method we wrapped 'Cling film' around the chip and performed experiment. In second method at, first we spread few drops of silicon oil on chip, 'Cling film' is then wrapped around the chip, and paper is placed on this and pressed with hot iron until the film makes tight contact with the chip. Care must be taken that the film should not melt and form wrinkles on its surface. Pressing with hot iron improves the results by removing air particles beneath the wrap but its durability over time is weak. 'Microscopic cover slips also give best results. Its thickness is ~130 micron. This glass is able to sustain up to 500V. We observed breaking of glass after 500V. This glass is by nature hydrophilic. To make it hydrophobic we made use of 'Rain-X'. It is observed that spreading few drops of '1cSt silicone' oil beneath the glass improved speed of movement of droplet. Also use of '1cSt silicone oil' on glass improved speed and reduced required potential to move the droplet. Same experiment is performed with 'Wonder 555' adhesive tape. Best results are observed with this tape. In this we spread few drops '1cSt silicone oil' on the chip and 'Wonder 555' tape is pasted on this. We created a tank of plastic box to hold '1cSt silicone oi'l as shown in fig. 3.

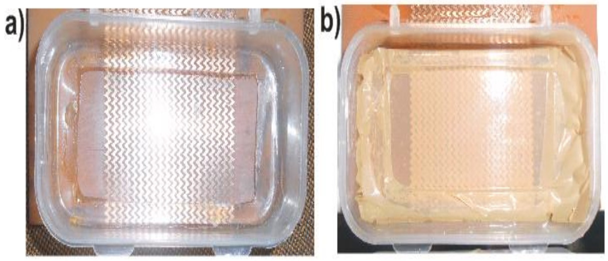

Fig. 3: Tank arrangement and dielectric coating.

In fig 3-a), plastic box is cut from bottom and its edges are pasted with adhesive material, 'Bondtight'. In fig-3 b), 'Wonder 555' tape is pasted on chip by spreading few drops of '1cSt silicone oi'l on PCB. Tank is having a transparent lock at the upper side which is used to reduce evaporation rate of fluids inside the tank and to visualize inside of the tank. It is observed that droplet moved from one electrode to other with the application of 100V DC. This tape is checked with application of 1000V both DC and PWM AC and it is observed that it doesn't break. Its advantages are higher voltage sustainability without breaking, no need of annealing as it is adhesive, fast applicability, low cost, easy availability, and uniformity. Once used with 'Wonder 555' tape we can reuse this chip for other dielectric coating applications like wrap, glass or any other coatings as it is easily removable. Lamination sheets, texo tape, screen guard, Teflon tape used in plumbing, mica sheets, acrylcoat spray also tried as adielectric material. Some of these have shown results but it is found that "wonder 555' tape gives best results of all and with this the chip becomes reusable.

C. Hydrophobic treatment

Hydrophobic treatment is generally done with the help of 'Teflon AF 1600' [6], [14], [19], which are costly chemical and again requires special coating machines, experts and clean room access. To replace this we have used 'Rain-X' as a hydrophobic material [1]. 'Rain-X' can improve hydrophobicity of glass but its durability is weak. With respect to time it loses its hydrophobicity. Also to improve results it needs the glass to be heated in oven. But cover slip doesn't sustain higher temperatures and breaks. It is observed that dip coating of glass with 'Rain-X' doesn't improve results. Also it takes time to make the glass hydrophobic by spreading the drop of 'Rain-X' on glass and tilting the chip to wet all over surface of glass. 'Rain-X' didn't improve significant performance with application on 'Wonder 555' Tape. So we neglected hydrophobic treatment with 'Rain-X' on 'Wonder 555' tape. Instead we reduced surface resistance by using '1cst Silicone oil' on tape. '1cst silicone oil' is immiscible with other fluids and dielectric in nature. But it is having a problem of evaporation with respect to time. To avoid its evaporation we created a tank of plastic box on chip. Tank arrangement improved the performance of droplet motion and reduced evaporation rate of '1cSt silicone oil'. The fabricated device is tested for moving operation of droplet with single plate actuation. Droplets of water, NaCl, FeCl3, KMnO4, Hg, Serum, HCl, and Phenolphthalein are moved successfully with both PWM AC and DC voltage application.

III. METHODOLOGY

In digital micro fluidics instead of manipulating flow of liquid, the liquids are divided into discrete droplets which are independently controllable with system clock. Mainly there are two kinds of electrical methods available; Dielectrophoresis (DEP) and electro-wetting-on-dielectric (EWOD) are used for droplet actuation. Both the techniques provide required droplet speeds to carry out tests in the device.

DEP: DEP uses high frequency AC voltages and can be used to move insulating fluids. Liquid DEP actuation is defined as the attraction of polarizable liquid masses into the regions of higher electric-field intensity. There may be problem of excessive Joule heating in DEP actuation which can be reduced by using higher thermal conductivity materials or by reducing structure size [15].

EWOD: EWOD uses DC voltages to carry out droplet actuation which is conductive in nature. In comparison to DEP, there is almost negligible Joule heating occurs in EWOD because the dielectric layer covering the electrodes blocks dc electric current [15]. Electro wetting is a method for modifying the wetting properties of a surface. An electrostatic field is created when we apply voltage. Due to the creation of electrostatic field the interfacial tension between liquid-gas and liquid-substrate is changed. Because of the change in the interfacial tension due to application of electric voltage, the droplet shape is deformed. Deformation of droplets can be used for the fluidic operations like creating, cutting, moving, mixing etc. [16]. The relation among the contact angle change by EWOD, the channel gap and the droplet size through theoretical analysis is carried out [].The voltage dependence of the interfacial energy reduction is described by,

$$\gamma SL(V) = \gamma SL(0) - \frac{1}{2}\frac{\varepsilon}{d}V^2 \quad (1)$$

Where, ε is the permittivity of the insulator, d is the thickness of the insulator; V is the applied potential and γSL is the interfacial energy. In EWOD Force density is confined to the surface of the droplet while in DEP it is spread throughout the bulk [3]. Generally there are two configurations by which we can implement digital micro fluidics, 'single plate actuation' and 'two plate actuation'.

Single plate actuation:

Figure 4 shows single plate actuation configuration. In this configuration electrodes are patterned on the bottom plate. On this a dielectric layer is coated followed by a hydrophobic layer. With this configuration 'moving' and 'mixing' operations are possible but 'creating' and 'cutting' operations are not possible [4].

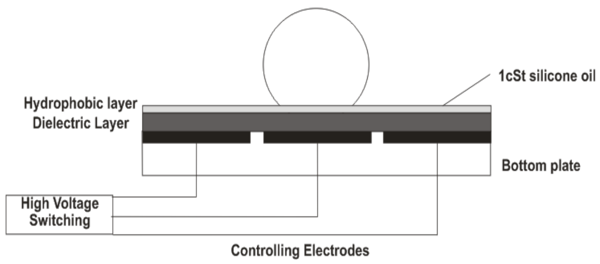

Fig. 4 : Single Plate Actuation

In this configuration evaporation rates are more as it is open to air. This configuration is also called as open plate configuration. In this a control electrodes are used to switch voltages, dielectric layer is used to form electrostatic field and hydrophobic layer is used to reduce the driving force for droplet motion to occur.

Two plate actuation:

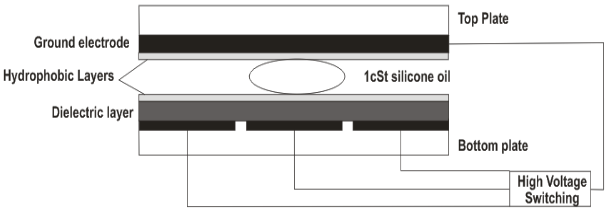

Fig. 5: Two palte configuration

Fig. 5 shows two plate actuation. In this configuration droplet is sandwitched in between top and bottom plates. On bottom plate electrodes are patternerd, on which dielectric layer followed by hydrophobic layer is coated. Top plate is used as a ground electrode. Potential is applied in between top and bottom plate controlling electrodes. ‘1cSt silicone oil’ is used as a filler medium in between two plates. Drop of fluid to be manipulated moves through ‘1cSt silicone oil’. With this configuration all four basic fluidic operations of creating, cutting, moving, and mixing are possible[16]. Also evaporation rate of ‘1cst silicone oil’ is less than that of single plate configuration as it is closed to air. This is also called as closed plate configuration.

IV. RESULTS AND DISCUSSION

Droplet motion took place on Cling film, Microscopic cover slip and on ‘Wonder 555’ tape with both AC and DC potential. Moving and mixing operations are done successfully according to program. Droplet creation and cutting is tried with variety of programs and with varying shapes of electrodes, with varying size of electrodes, with varying inter-electrode gap, with varying volume of droplet, with varying viscosity of droplet, with varying conductivity of droplet, with varying fluid itself (water, NaCl, FeCl3, KMnO4, Hg, serum, HCl, Phenolphthalein), with varying voltages, with varying type of potential (PWM AC and DC) and with varying dielectric material (‘Wonder 555’, glass, Cling film) but no satisfactory results found. Droplet tried to form neck to break but suddenly lost contact from one side and moved as a whole towards other side of electrode. However automatic breaking is observed on chip. Fig. 6 shows video frames of mixing operation of HCl and Phenolphthalein. After mixing color change is observed.

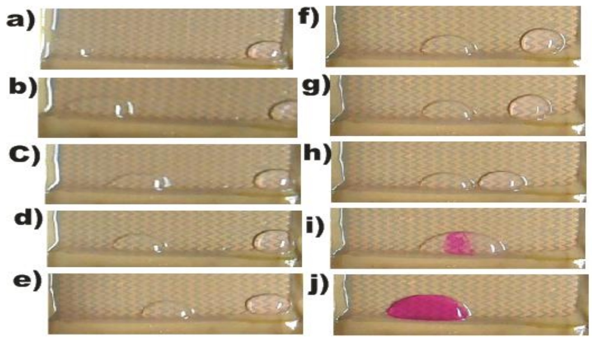

Fig. 6: Mixing of HCl and Phenolphthalein

It is observed that with DC potential the droplet becomes more volatile than that of PWM AC. With DC sparking problem is more as compared to PWM AC. The following fig. 7 shows moving operation of 70 µl, 0.1 M KMnO4. It is observed that with PWM AC the droplet moves without much more changing its shape as shown in fig. 7, a) to f) below. With DC droplet moves with sudden change in its shape and becomes volatile as shown in figure 7, g) to l). It is observed that Droplet moved with a speed of 4.5 cm/s.

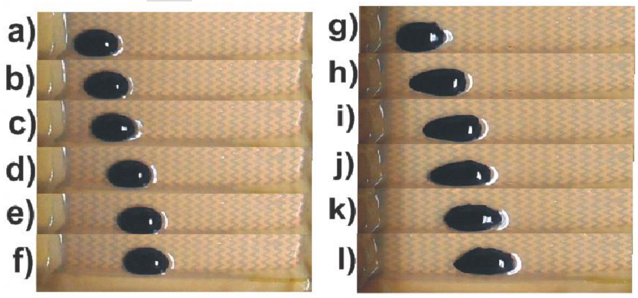

Fig. 7: Moving operation with DC and AC potentials with 0.1 M KMnO4

V. CONCLUSION

Here we have presented an innovative, low cost and easy to implement approach for implementation of “Digital microfluidic biochip” without accessing clean room and complicated technologies. An innovative method for chip patterning is presented. Applicability with variety of dielectric materials is discussed. Dielectric and hydrophobic treatments are discussed with considering cost as a main factor. Fabricated device is tested for successful ‘moving’ and ‘mixing’ operations of variety of fluids. We expect that our efforts will be beneficial to all who wish to implement “Digital microfluidic biochip" with minimal facilities to enhance the scope of "Digital microfluidic biochip".

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