A Low Cost Solution for the Fabrication of Dielectrophoretic Microfluidic Devices and Embedded Electrodes

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Abstract— The versatility of a simple method for producing microfluidic devices with embedded electrodes is demonstrated through the fabrication and operation of two dielectrophoretic devices; one employing interdigitated electrode structures on glass and the other employing contactless electrode reservoirs. Device manufacture is based on the precipitation of silver and subsequent photolithography of thin film resists conducted outside of a cleanroom environment. In current experiments, minimum channel widths of 50 microns and electrode widths of 25 microns are achieved when the distance between features is 40 microns or greater. These results illustrate this technique's potential to produce microfluidic devices with embedded electrodes for lab on chip applications while significantly reducing fabrication expense.

I. INTRODUCTION

DIELECTROPHORESIS, the motion of a particle in a non-uniform electric field[1], has become a robust method for analyzing nano-particles, cells, viruses, and DNA[2]. Traditional DEP devices use metal electrodes patterned on glass which create the non-uniform electric field required to manipulate a target particle. Several variations to this technique exist. For example, insulator based dielectrophoresis (iDEP) utilizes insulating structures within the channel to produce electric field non-uniformities[3] and contactless dielectrophoresis (cDEP)[4].

Contactless dielectrophoresis is a new method of cell manipulation developed by the authors. In this technique, insulating barriers separate two fluid electrode channels from a sample channel. When an AC voltage is applied across the fluid electrodes they capacitively coupled to the sample channel inducing a non-uniform electric field. This in turn imparts a dielectrophoretic force on the particles within the sample channel.

Many devices which exploit dielectrophoretic forces require the use of photolithographic processes in a cleanroom environment. Due to their cost, these facilities are not available to many researchers, and the per-unit expense of devices is a limiting factor in their experimental use. Cleanrooms can have an initial build cost of millions of dollars and require a significant investment annually to cover the cost of salaries, maintenance, consumables, chemicals, and infrastructure. The prohibitive cost of these facilities limits their availability to only large universities and government laboratories. Individual investigators are often charged per hour rates to use these facilities for access to the room and additional fees for the use of specialized equipment. In general, the sub-micron resolution these facilities are capable of far exceeds the necessary feature size of dielectrophoretic and microfluidic devices.

Numerous investigators have presented solutions to cleanroom-free fabrication including toner-mediated lithography[5], toner transfer masking[6], photomask replication[7], lab on a print[8], solid object printing[9], and direct photo projection[10]. The resolution of direct printing has been improved by employing novel materials which shrink features when heated[11], numerous techniques have been borrowed from those established for the production of printed circuit boards[12], and significant work has been done in the evaluation of laser and inkjet printed photomasks[13]. In this technical note we present a low cost solution for creating microfluidic devices containing metal electrodes on glass with better than 50 µm resolution. Two functional dielectrophoretic devices were fabricated using this method; one employing interdigitated electrode structures on glass and the other employing contactless electrode reservoirs. Both devices successfully demonstrated DEP on polystyrene microspheres.

II. METHODS

Glass microscope slides were polished with a cerium oxide polishing compound (Angel Gilding Stained Glass Ltd, Oak Park, IL), rinsed with deionized water, and dried using compressed air. The slides were then sensitized using 3 mL of a tinning solution (Angel Gilding Stained Glass Ltd, Oak Park, IL) for 30 seconds. After this time had passed the solution was poured off the slide and it was rinsed with deionized water.

A commercially available mirroring kit was used to deposit pure silver onto the microscope slides. 3mL each of silver reducer, silver activator, and silver solution (Angel Gilding Stained Glass Ltd, Oak Park, IL) were combined and immediately poured onto the sensitized slide. Silver was allowed to precipitate onto the slide for 5 minutes. This process was repeated, without tinning, one additional time resulting in a layer of silver approximately 100nm thick. It should be noted that a similar commercially available kit exists for the deposition of gold on glass.
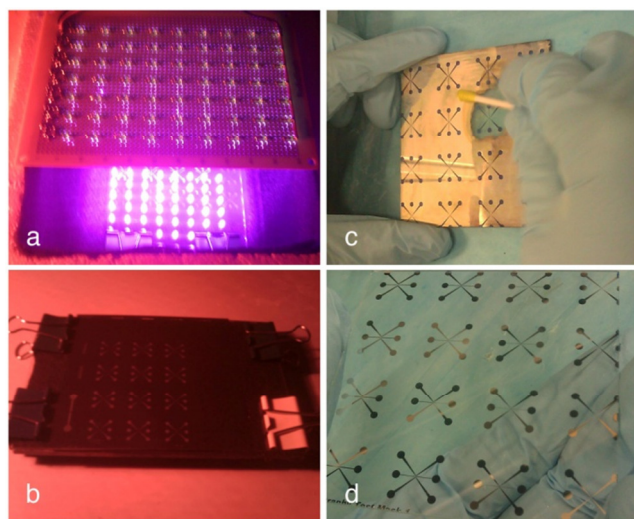

Fig.1 (a) Ultraviolet LED array exposing a laminated slide through a photo mask which is held in place by a (b) custom exposure frame. (c) Photoresist features cover silver which will be left after processing to create (d) silver electrodes on glass.

A negative thin film photoresist (#146DFR-4, MG Chemicals, Surrey, British Colombia, Canada) was cut into an 80 x 100 mm rectangle and the inner protective film was removed. A silvered slide was sprayed lightly with deionized water and the photoresist was laid on top of the slide such that approximately 20 mm of film extends over one edge. Any existing bubbles were pushed to the edges resulting in a smooth surface. The film extending over one edge was then bent around to the bottom of the slide to form a leading edge for lamination. The slides were then passed through an office laminator (#4, HeatSeal H212, General Binding Corporation, Lincolnshire, IL) twice at low heat, cleaning the laminator between each pass.

A 7x9 array of low cost 400 nm 20 mW light emitting diodes (LEDs) was fabricated to produce the ultraviolet light necessary for exposure. An exposure case was fabricated by lining the top, bottom and sides of a styrofoam container with black felt in order to reduce internal reflections. A 4 by 6 inch piece of sheet glass from a photo frame and a piece of 4 by 6 inch piece of fiberboard covered by black felt formed the front and back of the exposure frame. A laminated side was placed with photoresist up onto the back plate of the exposure frame. A photomask printed at 20k DPI on a transparent film (Output City, Cad / Art Services Inc, Bandon, OR)

was placed ink side down onto the photoresist. The top plate was then placed on top and the entire assembly was held in place using large binder clips.

The exposure frame was placed inside the exposure case and the LED array placed 12 cm above the exposure frame. Slides then were exposed to UV light for 45 seconds. After exposure, the outer protective film was removed from the photoresist. The slides were then placed in a 200 mL bath containing a 10:1 DI water to negative photo developer (#4170-500ML, MG Chemicals, Surrey, British Colombia, Canada) solution for approximately 4 minutes. A foam brush was used to gently brush the surface of the slide in order to expedite the development process. Cotton swabs soaked in developer were used gently wipe areas with small features to ensure complete development. The slides were placed in a beaker containing DI water to halt the development process and gently dried using pressurized air.

Electrode structures on the microscope slides were fabricated by removing all silver not covered by the patterned photoresist. A two part silver remover was included in the mirroring kit used to deposit the silver. 1 mL of each part of the silver remover was combined in a 5 mL beaker. A cotton swab was used to apply the silver remover to the glass slide until only the silver covered by photoresist remained on the slide. The photoresist was then removed by placing the slide in a bath of acetone.
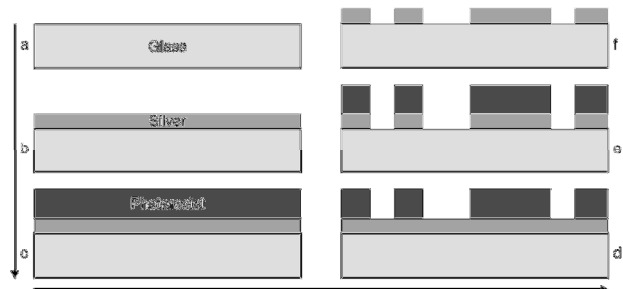

Fig.2 Schematic representation of the fabrication process. (a) A glass slide is cleaned and polished. (b) Silver is deposited onto the glass using a commercial mirroring kit. (c) Thin film photoresist is laminated on top of the silver. (d) The photoresist is exposed and developed. (e) The exposed silver is chemically removed and (f) the photoresist is dissolved.

Microfluidic channels were created through polymer replication on stamps which had not undergone the final acetone wash, leaving the patterned photoresist intact. Liquid phase polydimethylsiloxane (PDMS) in a 10:1 ratio of monomers to curing agent (Sylgrad 184, Dow Corning, USA) was degassed under vacuum prior to being poured onto the photoresist master and cured for 1 hour at 100°C. After removing the cured PDMS from the stamp, fluidic connections to the channels were punched in the devices using 1.5 mm core borers (Harris Uni-Core, Ted Pella Inc., Redding, CA). Glass microscope slides (75 mm x 75 mm x 1.2 mm, Alexis Scientific) were cleaned with soap and water, rinsed with distilled water, ethanol, isopropyl alcohol, and then dried with

compressed air. The PDMS replica was bonded to the glass slides after treating with air plasma for 2 minutes in a PDC-001 plasma cleaner (Harrick Plasma, Ithaca, New York).

Electrical connections to the embedded electrodes were formed by securing high voltage electrical wires to contact pads using high purity silver paint (Structure Probe Inc., West Chester, PA). This was allowed to dry for one hour creating a solid connection. A drop of 5 minute epoxy (Devcon Inc., Danvers, MA), used to secure the electrical connections, was placed on top of each electrode pad and allowed to cure for 24 hours.

Polystyrene microspheres were used to prove the functionality of these devices through the demonstration of dielectrophoresis. 1 µL of 1 µm and 4 µL of 4 µm beads (FluoSpheres sulfate, Invitrogen, Eugene, Oregon) were suspended in 5 mL of DI water with a final conductivity of 6.2 µS/cm. 40 uL of this sample solution was pipetted into the devices. A syringe pump was used to drive samples at a rate of 0.02 mL/hour (PHD Ultra, Harvard Apparatus, Holliston, Massachusetts).

An AC electric field was created by amplifying (AL-50HF-A, Amp-Line Corp., Oakland Gardens, NY*)* the output signal of a function generator (GFG-3015, GW Instek, Taipei, Taiwan). A step up transformer was used when voltages greater than 30 V_{RMS} were required. Voltage and frequency were measured using an oscilloscope (TDS-1002B, Tektronics Inc. Beaverton, OR) connected to the output stage of the amplifier.
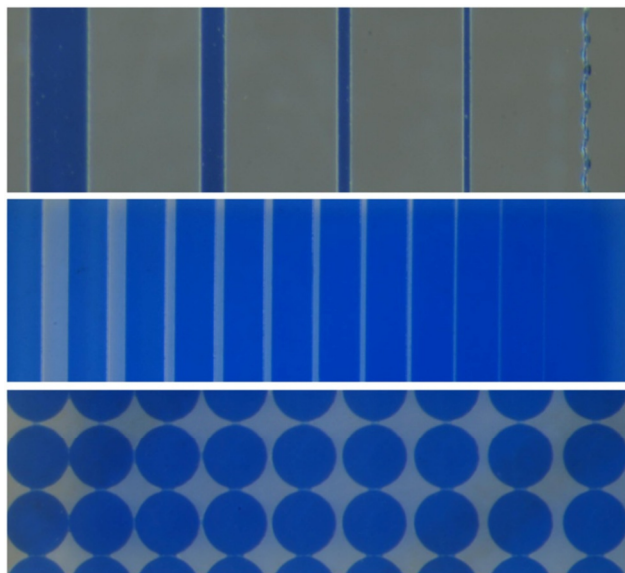

Fig 3: [Top] 500, 250, 100, 50, and 25µm (left to right) thick structures. A 10µm test structure existed on the mask, but did not develop. [Center] 500µm structures separated by 300, 200, 100, 90, 80, 70, 60, 50, 40, 30, 20, and 10µm left to right. [Bottom] 250µm diameter pillars separated by 10, 20, 30, 40, 50, 60, 70, and 80µm from left to right.

III. RESULTS

In the absence of the silver substrate, test structures 50

µm wide and greater could be reliably fabricated using this process. Structures 25 µm thick formed successfully after exposure, however, they did not have enough surface area to adhere completely onto plain glass slides during the development process. The resulting photoresist structures did not form perfectly straight lines as seen in Fig. 3[Top]. 10 µm test structures on the mask did not develop. 500 µm wide test structures consistently developed when separated by 40 µm or more as seen in Fig. 3[Center].

Some photoresist could not be removed between features separated by distances of 20 and 30 µm resulting in poor PDMS replication. A 10 µm gap could not be developed between structures. Similarly, 250 µm pillars were easily developed and replicated when separated by 40 µm or more as seen in Fig. 3[Bottom].

A single photoresist layer produced channels with a minimum width of 50 µm and a nominal depth of 50 µm. 100 µm deep channels were produced by removing the outer protective sheet after lamination, laminating another sheet on top of the previous layer, and exposing for 105 seconds.

The silver substrate improved photoresist adheasion. As a result, photoresist features with widths down to 25 µm could be fabricated. The photoresist effectivly protected features from silver the removal process resulting in the successful formation of electrodes with line widths down to 25 µm.
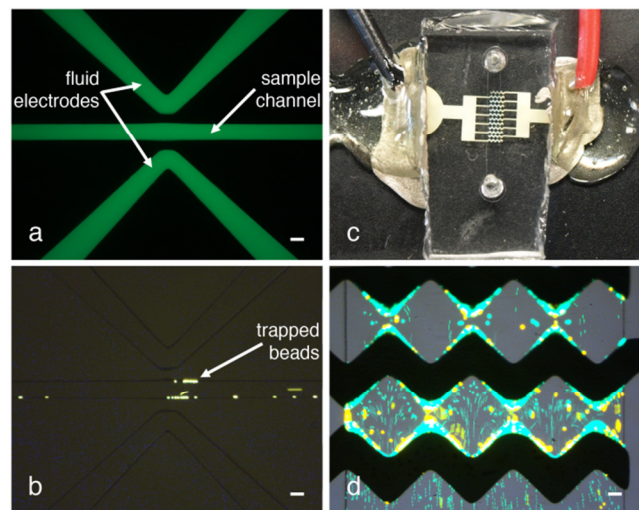

Fig. 4: (a) Examples of cDEP devices with 50 µm minimum feature sizes which can be produced using this process. (b) 4 µm beads driven by pressure are trapped in the region between the two electrodes when a 150 V_{RMS} 600 kHz signal is applied. (c) Silver electrodes deposited on glass encapsulated in a 1mm wide microfluidic channel. Conductive silver paint is used to ensure an electrical connection between the wires and the deposited silver. Epoxy holds the wires permanently in place. (d) 1 and 4 µm beads driven by pressure are entrapped by dielectrophoretic forces when a 7.3 V_{RMS} 60 Hz signal is applied to the electrodes. The scale bar is 50 µm.

The fluid electrode channels in the cDEP device (Fig. 4a)

are filled with a highly conductive fluid, typically phosphate buffered saline. The 50 µm insulating membrane which isolates the fluid electrode channels from the sample channel acts as a large resistor in parallel with a capacitor. When a high frequency signal is applied across the fluid electrode channels, the impedance of the barriers is over come and a voltage drop occurs across the sample channel. The electric field generated within the sample channel is non-uniform due to the shape of the insulating barriers. When a 600 kHz signal is across the sample channel, 4 µm beads suspended in deionized water feel a positive dielectrophoretic force which acts to push them into regions of highest electric field non-uniformity. When the applied voltage is increased to 150 V_{RMS}, the dielectrophoretic force overcomes the fluid drag force and the beads are trapped along the channel wall, as shown in Fig. 4b. This action is reversible and when the voltage is turned off, the particles are released downstream.

Traditional DEP devices employ metal electrodes patterned on glass. The device in Fig. 4c has an array of interdigitated saw tooth electrodes, separated by 50 and 350 µm at their minimum and maximum respectively. This device was encapsulated by a 1 mm wide, 50 µm deep channel which allowed pressure driven flow to drive particles over the electrodes. The geometry of the metal electrodes creates a non-uniform electric field when an AC signal is applied. At 60 Hz, the 1 and 4 µm beads experience a negative DEP force that acts away from the electrodes and opposes the fluid drag force. When the applied voltage is increased to 7.3 V_{RMS} the DEP force and drag force become balanced and the particles are trapped, as shown in Fig. 4d.

IV. CONCLUSIONS

This fabrication technique has successfully demonstrated working dielectrophoretic devices of two different forms. This is the first demonstration of a cDEP device with insulating barriers greater than 20 µm and this process has been used to create functional cDEP devices which include insulating features in the sample channel (results not shown). Additionally, embedded electrodes are currently being evaluated to determine the Clausius-Mossotti crossover frequency for mammalian cells to aid in dielectrophoretic separation.

While this process contains numerous manual steps, the low cost of fabrication, simplicity, availability of supplies, and exclusion of toxic chemicals make this technique ideal for researchers that do not have access to a cleanroom. Furthermore, this process displays great potential as a rapid, low cost solution for the fabrication of complex multi-phased devices capable of sorting, isolating, and enriching samples as well as performing downstream analysis, such as impedance detection. Future work will focus on evaluating the reproducibility of this technique between batches.

V. REFERENCES

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