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ADVANCED EWOD-BASED DIGITAL MICROFLUIDIC SYSTEM FOR MULTIPLEXED ANALYSIS OF BIOMOLECULAR INTERACTIONS

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ABSTRACT

This paper presents a low-cost technique for the fabrication of complex electrowetting-on-dielectric (EWOD) digital microfluidic devices. Using this original technology, we have developed devices in which 560 electrodes are used to mix and split nl-size liquid droplets and transport them to 100 analysis spots patterned on a disposable plastic top plate. We demonstrate the multiplexing capability of the developed devices by creating on-chip arrays of droplets with various concentration gradients. Finally, automated biomolecular immobilization and hybridization assays are performed in nl-size droplets under numerous conditions simultaneously with only a limited number of stock solutions.

INTRODUCTION

Quantitative analysis of biomolecular interactions is a key element to numerous biological assays in fields such as clinical diagnostic, pathogen detection, as well as for fundamental studies related to biomarkers discovery. Numerous techniques have been developed to study at high-throughput the binding interactions between probe and target biomolecules such as DNA or proteins. However, these traditional methods, including array-based assays, typically require advanced laboratories, specialized equipments, and are based on the repetition of lengthy and labor-intensive procedures. The important expenditure associated with these technologies limits the number of preventive analysis that can be performed and the relatively long response time allows pathogens or contaminants to cause further damage before proactive actions can be undertaken.

Microfluidic systems offer the interesting prospect to automate of the numerous fluidic handling steps of biological assays, reduce reagent consumption, increase throughput, and achieve high-portability [1]. Among the various types of microfluidic devices developed in the past years, digital microfluidics (DMF) has emerged as a promising technology that offers key advantages to perform miniaturized bioassays [2-4]. In DMF devices, nl-size liquid droplets are manipulated at will on a 2D surface using electrowetting-on-dielectric (EWOD) forces applied with an array of computer-controlled electrodes (figure 1). By using this technology, there is no need to integrate complicated mechanical components such as pumps and valves to perform the fluidic operations because the driving EWOD force is applied locally on the droplets. Also, the real-time software-based control of the droplets confers very high flexibility and dynamic reconfigurability to the assays.

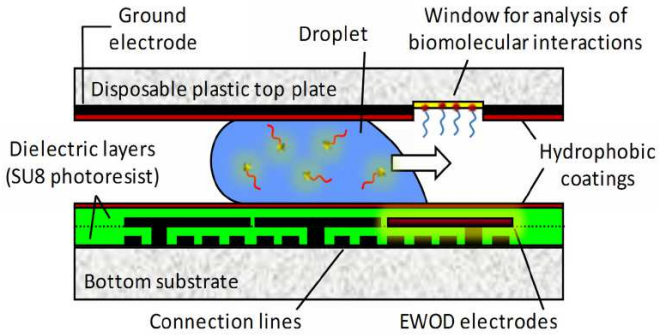

Figure 1: Side view schematics of a DMF device based on multiple layers of dielectrics and connection lines. Windows are patterned in the disposable plastic top plate for the analysis of biomolecular interactions.

On the other hand, most EWOD-based DMF devices demonstrated to date lack the sophistication required to perform advanced multiplexed bioassays in which numerous tests are carried out concurrently. One of the most important fabrication challenge results from the relatively high voltage required to generate the EWOD force driving the droplets (typically > 50V), which imposes the integration of high-quality and relatively thick dielectric layers in the DMF devices [5]. Unfortunately, the high cost involved with traditional technologies to grow high-quality thick dielectrics (e.g., vapor deposition) is often difficult to justify in biological applications where single-use devices are the norm. Also, the polymeric dielectrics typically used with DMF devices (such as Parylene) have relatively low breakdown voltages, low dielectric constant, very low hardness, and are difficult to pattern so as to create devices with multiple levels of metallization interconnected with vias. Because of these limitations, it is still challenging to achieve a high degree of integration in DMF devices and they have thus mostly been limited to the realization of simple proof-of-concept biological assays.

In this work, we present a method based on spin-coated SU-8 photoresists to enable the fabrication of advanced multi-level DMF devices without the need for costly equipment. The challenges involved in the design and fabrication of such devices are discussed. We then demonstrate the fabrication of fully operational DMF devices based on 560 EWOD electrodes to transport nl-size droplets to a 10x10 array of analysis spots patterned on a disposable plastic top plate. Complex fluidic tests are successfully performed with the fabricated devices and multiplexed analyses of biomolecular binding interactions are demonstrated.

DESIGN AND FABRICATION

For complex DMF devices with hundreds of electrodes, it is highly unpractical to assign an independent electrical input pin to each EWOD electrode. To keep the number of electrical inputs as low as possible, each input pin is rather connected to multiple EWOD electrodes simultaneously. This pin to electrode assignment is then optimized to minimize the possible unwanted interactions between the various fluidic operations that will be performed on-chip. Unfortunately, for complex DFM devices, this optimization is often impossible to realize when the connection lines and EWOD electrodes are patterned simultaneously on the same level of metallization. The patterning of the connection lines and EWOD electrodes on two different metallization layers (as shown in figure 2) is then required despite the increased fabrication complexity. As discussed next, we found that the SU8 photoresist provides an interesting avenue to minimize the cost and number of fabrication steps required to create and pattern the multiple dielectric layers of such devices.

SU8 is an epoxy-based negative photoresist commonly used to achieve high-aspect ratio structures. We found that it also possesses many valuable characteristics as a dielectric for the fabrication of EWOD-based DMF devices. With this approach, only very simple laboratory equipment is indeed required since the dielectric layer of appropriate thickness can be applied in a single step by spin-coating SU8 photoresist. Also, the capacity to pattern directly the SU8 layers by UV exposure reduces considerably the number of steps required to define vias and contact pad regions (Fig. 2). SU8 additionally has a much higher hardness and improved mechanical and chemical stability compared to other photoresists and polymer-based dielectrics. Finally, we found that SU8 has excellent dielectric properties with a measured breakdown voltage on the order of 4 MV/cm, a dielectric constant of about 4, and a low pinhole density. For example, while DMF devices with a 2 µm thick SU8 dielectric require operation voltages of about 80 V, the dielectric breakdown occurs only at voltages close to 800 V.

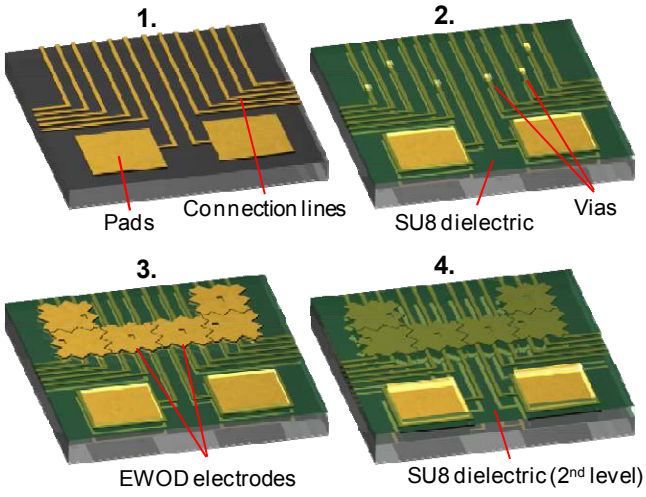

Figure 2: 3D schematics showing the fabrication steps for the DMF devices with multiple levels of metallization.

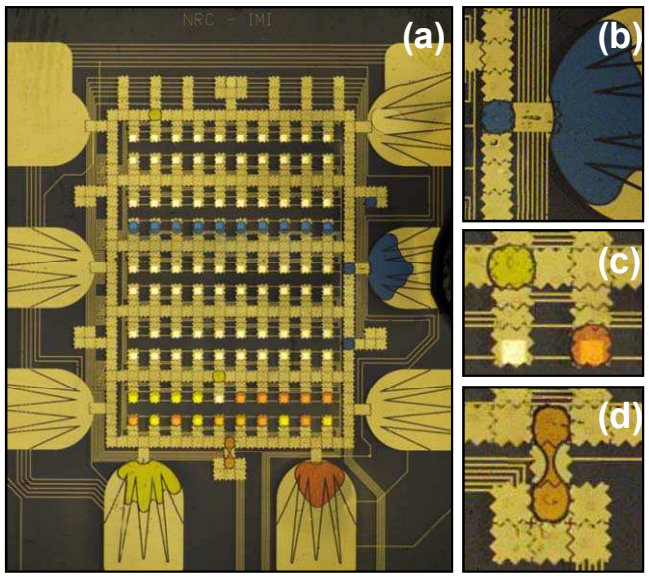

Figure 3: (a) Top view of an advanced EWOD-based digital microfluidic device. The 560 EWOD electrodes of the device control (b) 8 reservoirs, (c) 100 analysis spots, and (d) multiple regions for mixing, storing and splitting droplets.

Such a high difference between the breakdown voltage of the dielectric layer and the operation voltage improves markedly the reliability of the DMF devices.

We have developed a process for the fabrication of multi-level EWOD-based DMF devices where the direct patterning capability of SU8 has been used to easily open vias and contact pads regions (figure 2). The electrodes and contact lines were patterned in a 100 nm thick gold thin film using standard wet etching. It is noteworthy that the first SU8 dielectric layer is not affected by the chemicals used during the subsequent patterning of the top metal layer (developer, etching solutions, solvents, etc.), thus enabling the fabrication of devices with multiple metallization layers. The top plate of the DMF devices consists of a thermoplastic substrate, onto which a semitransparent 10 nm thick gold layer and a hydrophobic coating were patterned by lift-off. The high-voltage required to control the droplets on the DMF devices was obtained by amplifying the 5V DC output of a USB port to a 0-150V AC square wave (at 1 kHz) using a homemade circuitry. The USB connection was simultaneously used to transfer the instructions from the control software to high-voltage switches controlling the state of the input pins in contact with the DMF device. Finally, it is noteworthy that, in order to facilitate the operations of the DMF devices, all the assays shown in this paper were performed with droplets encapsulated in a thin layer of silicone oil [6].

Figure 3 shows an example of advanced DMF devices that were fabricated based on this process. The layout of the devices was designed so as to create a universal platform for the study of the interaction between biomolecules. In this design, 560 EWOD electrodes were used to independently address 100 analysis spots (bright regions seen in Fig. 3c) patterned on a disposable plastic top plate, allowing the

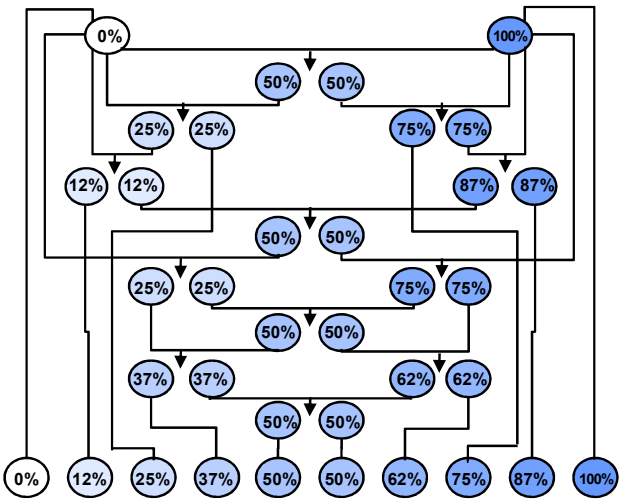

Figure 4: Schematic representation of the program used to generate an array of droplets having a linear concentration gradient from two reservoirs (denoted as 0% and 100%).

monitoring of interactions between immobilized probe and target biomolecules. The 30×40 mm devices comprised 7 input reservoirs from which elementary droplets can be dispensed (Fig. 3b) and one waste reservoir. Extra electrodes have also been positioned around the chip to provide regions for temporary storage, mixing, and splitting of the droplets (Fig. 3d).

The device layout has been optimized to provide the highest possible flexibility in the control of the droplets despite using only 24 independent high-voltage electrical inputs. For example, the assignment of an input pin to each of the 560 EWOD electrodes was optimized to guarantee that the elementary droplets can be transported, mixed and split without disturbing the droplets already immobilized on the analysis spots. This optimization also ensured that each reservoir of the device can dispense elementary droplets independently. We also demonstrate a split electrode design (Fig. 3c) that improves the independence of the fluidic operations performed close to the analysis spots while minimizing the required real estate on the device. The developed layout thus offers enough flexibility to perform automated biomolecular assays under up to 100 different conditions simultaneously despite using only 24 independent electrical inputs.

OPERATION OF THE DMF DEVICES

The fabricated DMF devices aim at studying biomolecular interactions under multiple different conditions simultaneously. The liquid placed in the seven reservoirs of the devices must therefore be mixed on-chip in various proportions to generate droplets having intermediate concentrations. For this purpose, elementary droplets dispensed from the reservoirs are transported to the regions located around the devices where they are merged and rapidly mixed (Fig. 3d). After splitting, the mixed droplets are then either used as is or brought to temporarily storage regions for further dilution steps. Therefore, by combining multiple mixing, splitting, and storage steps, arbitrarily mixtures and complex dilution patterns can be rapidly generated on-chip from only a limited number of stock solutions.

As an example, figure 4 shows a schematic representation of a program that can be used to generate an array of 10 droplets with a linear variation in concentration. Elementary droplets are first dispensed from the two reservoirs (denoted as 0% and 100% respectively) and mixed to create two droplets with 50% composition. To generate other compositions, multiple dilution steps are required with temporary storage of some droplets. The process shown in figure 4 requires the dispensing of 10 droplets from the reservoirs, 12 mixing steps, and the temporary storage of 8 droplets (up to 3 simultaneously).

As shown in figure 5, the fabricated DMF devices were used to generate such a linear concentration gradient. For this assay, the reservoir on the right hand side has been filled with a blue dye, while the reservoir on the left contains water. Fig. 5a shows a selected frame from a movie with examples of the fluidic operations required to generate the linear concentration gradient. The numbers placed next to the droplets show the targeted concentration of blue dye. The finalized linear concentration gradient is shown in Fig. 5b, where smooth and regular color changes from

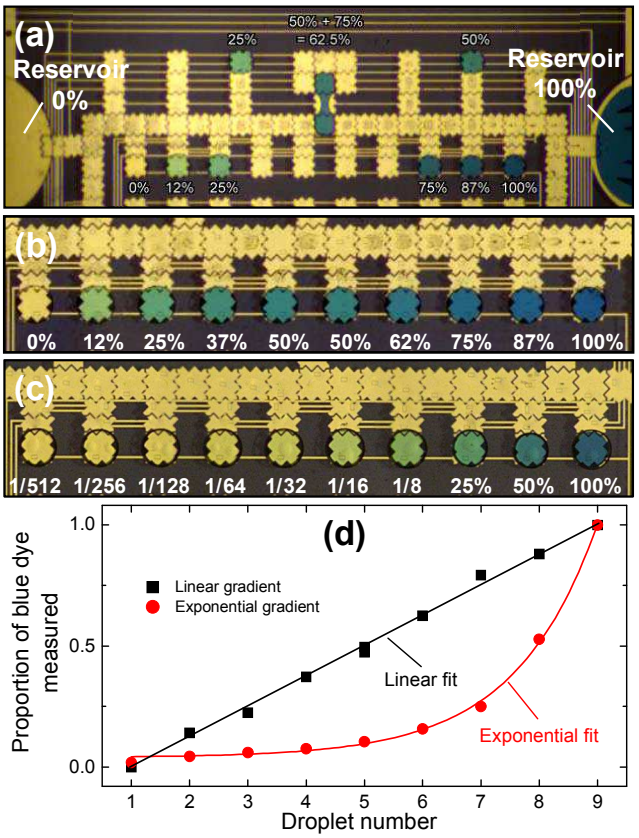

Figure 5: On-chip generation of linear and exponential concentration gradients from 2 reservoirs containing water and a blue dye. (a) Selected frame of a movie showing an example of the fluidic operations required to generate the (b) linear and (c) exponential gradient of concentration. (d) Proportion of dye measured in the droplets.

droplet to droplet are observed. To demonstrate the very high flexibility of the developed DMF system, we have also created an array of 10 droplets with an exponential concentration gradient (Fig. 5c) by simply changing the programmed sequence of fluidic operations executed by the control software. For both types of concentration gradients (i.e. linear and exponential), the measured proportion of blue dye was found to closely match the targeted concentration (Fig. 5d). It is noteworthy that, despite necessitating more than 1000 unitary fluidic operations, these two assays could be completed in less than 90s on the DMF device, thus demonstrating both the speed and reliability of the devices.

ON-CHIP BIOMOLECULAR ASSAYS

An example of a biomolecular interaction assay performed with the fabricated DMF devices is shown in figure 6. In this assay, two types of amino-modified DNA probes were first immobilized on the disposable plastic top plate of the DMF devices by dispensing and routing 20 probe droplets (DNA concentration of 40 μM) on two rows of 10 analysis spots. The droplets placed on the top row (as shown in Fig. 6a) contain negative control DNA, while the DNA immobilized on the bottom row is complementary to the target DNA. After incubation for 30 min and rinsing, droplets containing fluorescently-labeled target DNA (20 μM) were dispensed and diluted on-chip in order to create an exponential concentration gradient on both rows of analysis spots. The measured fluorescence intensity (Fig. 6b and 6c) confirmed the expected dependence of probe-to-target interaction with respect to both (i) the type of DNA probe and (ii) the target concentration. This assay demonstrates the possibility to use the developed DMF devices to perform multiplex biomolecular assays where the interaction of probe and target biomolecules is analyzed under numerous conditions simultaneously.

CONCLUSION

A low-cost process has been developed for the fabrication of advanced DMF devices that can be used as a universal platform for the study of the interaction between biomolecules. We believe that the developed technology proposed herein provides a highly flexible tool for the rapid analysis of biomolecular interactions under numerous different conditions. Work is underway to integrate these devices with label-free detection systems [2] and various biological species (DNA, RNA, proteins, cells, etc.).

ACKNOWLEDGEMENT

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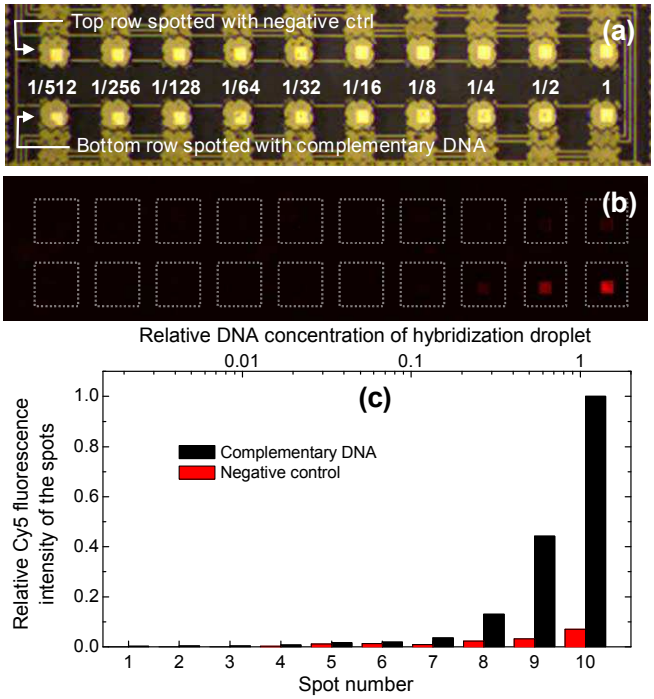

Figure 6: (a) Top view of the DMF device showing the hybridization step of a biomolecular interaction assay with DNA. Top and bottom row of spots were first spotted with negative control and complementary DNA respectively. The numbers denote the relative DNA concentration in the hybridization droplets. Measured fluorescence intensity of the Cy5-labeled target DNA vs. concentration and type of probe is shown in (b) and (c).

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