DROPLET ACTUATION ON A SURFACE WITH ANISOTROPIC WETTABILITY USING IN-PLANE SYMMETRIC CYCLIC VIBRATION

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ABSTRACT

This paper reports a simple droplet manipulation approach that can displace aqueous droplets over a long distance within the working plane. Repeated gradient wettability patterns were produced on the working surface. The aqueous droplet can be moved along the gradient direction under in-plane symmetric cyclic vibration. Typical droplet manipulations, such as unidirectional movement, droplet mixing and merging, were successfully demonstrated. A bichinchoninic acid (BCA) assay was implemented to illustrate the use of this approach for biochemical detection.

INTRODUCTION

Microfluidic systems that perform biochemical reactions with less sample and reagent consumptions have been widely employed in various fields in the past two decades [1, 2]. Droplet-based microfluidic systems that exhibit reduced biomolecule adsorption with a low risk of cross-contamination [3] comparing to conventional closed-channel continuous-flow microfluidic systems have received extraordinary attentions. In droplet-based microfluidics (also known as digital microfluidics), liquid droplets containing various chemical species can be separated from each other or mixed with other droplets on demand. Each droplet with the volume of a few μL or less may serve as an individual microreactor. High-throughput experimentations with a low sample volume and a small overall device footprint are thus possible [4]. Among numerous droplet-based microfluidic designs, open-channel microfluidics owns several distinct advantages. It is free from the reliance on excessive driving fluids and complicated pumping units. The entire working surface can be utilized through reconfiguring the trajectories of droplet motions. In addition, the leaking issue associated with closed-channel continuous-flow microfluidic systems is no longer a concern.

In open-channel microfluidics, liquid droplets are manipulated on the working surface by various physical means. Electrowetting-on-dielectric (EWOD) is a prevalent approach for droplet manipulation [5], where the droplet motion is induced by creating a wettability difference between the front and the rear edges of the droplet by the electrowetting effect. Typical droplet motions including linear translocation, droplet merging, and mixing were successfully demonstrated. Nonetheless, droplet manipulation by EWOD is limited by possible electrical leakage/breakdown [6]. In particular, a droplet with a high ionic concentration can significantly reduce the breakdown voltage and fail the EWOD chip [5]. In view of this, other non-electrical digital microfluidic stimulation approaches have been developed for droplet manipulation [7-10]. The stimulation can be applied to either the droplets [11] or to the working substrates [7]. Among these methods, mechanical stimulations exhibit a great potential for droplet manipulation due to the relatively simple setup and high process flexibility [11]. In particular, droplets can be moved on a non-wettable surface under asymmetric mechanical vibration [7, 12], or along the gradient of surface wettability under out-of-plane mechanical agitation [13]. Gravitational force and mechanical agitation can also be combined to induce droplet motion [14]. Unfortunately, current mechanical droplet

manipulation approaches have several drawbacks. The moving direction of the droplets under asymmetric mechanical vibration highly depends on the droplet size. Simultaneous manipulation of multiple droplets with varied volumes is challenging.

In this study, an alternative mechanical stimulation approach was developed for open-channel digital microfluidics. Repeated patterns with gradient surface wettability were arranged head-to-tail on a superhydrophobic working substrate (**Figure 1**). The wettability gradient in each pattern aligns with the longitudinal axis of the patterns. An aqueous droplet sitting within the pattern thus sees different magnitudes of wettability at its two edges. Upon an in-plane symmetric mechanical vibration along the longitudinal axis of the patterns, the droplet has the tendency to move along the longitudinal axis of the pattern, and start moving once the vibration magnitude exceeds a certain threshold. The inertial motion allows the droplet to reach the next pattern. Continuous vibration can cause a net displacement of the droplet along one direction. Basic droplet motions, including linear translocation, mixing, and merging were successfully demonstrated. In addition, droplet-based on-chip protein concentration measurement was performed. Different from previous mechanical stimulation methods that require complicated motorized stages, the mechanical stimulation was produced by a modified vertex mixer that is widely available in a biochemical laboratory. Besides, the working surface with repeated gradient wettability was generated using a commercial laser engraver. The low-cost fabrication and simple operation of the digital microfluidic system show the potential for various droplet-based on-chip analyses.

Figure 1: Schematic of the droplet actuation on a surface with repeated patterns of gradient wettability using in-plane cyclic vibration.

EXPERIMENTAL SECTION

Device Preparation

Before the laser irradiation, a superhydrophobic substrate was prepared. A PDMS (Sylgard 184, Dow Corning, MI) substrate was fabricated by mixing a base pre-polymer and a curing agent at a

weight ratio of 10:1. The well-mixed pre-polymer solution was degassed in the vacuum for 30 min. The gas-less solution was then dispensed and spread on a petri dish to achieve a 2 mm thick PDMS layer. The solution was cured on a hotplate at 65°C for 2 hours. A super-hydrophobic surface layer (Rust-Oleum NeverWet™, IL, USA) was spun on the PDMS substrate at 6000 RPM. The substrate was placed in a ventilation cabinet for 3 hours for solvent evaporation, and then cut into rectangular pieces (70 mm × 30 mm) before use.

The super-hydrophobic coating was modified by laser irradiation (25W, VSL2.30, Universal Laser Systems, AZ) (Figure 2a). The operating parameters of the laser have been described in a previous report [15]. Briefly, the laser irradiation was controlled by varying the Gray Scale (GS, 10~18%). Other operating parameters, including the Raster Intensity (RI, -40%), the number of the engraving cycles ($n=8$), the laser spot size ($\sim 127\ \mu\text{m}$), the lateral traveling speed of the laser head (0.2629m/s), the laser repetition rate (5000Hz), and the spatial resolution (500 PPI (point per inch)) were kept constant throughout the experiments. After the treatment, the PDMS substrate was air blown and visually examined (Figure 2b).

Wettability Measurements of Laser Irradiated Patterns

The wettability of the substrate was examined by measuring the static contact angle of a 10 μL water droplet dispensed on the substrate using a contact angle goniometer (Figure 2c). The contact angle hysteresis was examined by titling the substrate and examining the minimal angle that can move a 20 μL droplet on the surface (Figure 2d&e). The titling angle increased from 0° with the increment of 1°. The stage was held still after each increment.

Testing Rig

After laser irradiation, the substrate was mounted on a customized stage, and connected to a commercial vortex mixer. The stage translated the two-dimensional vibration generated by the vortex mixer into a linear sinusoidal wave with the amplitude of $\sim 4\text{mm}$. The frequency can be adjusted from 1~50 Hz. After a droplet was dispensed on the substrate, the motion of the droplet was recorded using a high-speed camera (Exilim Pro EX-F1, CASIO, Tokyo, Japan).

RESULT AND DISCUSSION

Gradient Wettability

An area of 4 mm×2.5 mm were irradiated by the laser. By changing the GS from 10% at one edge of the area to 18% at the other edge (Figure 1c), the degree of decomposition of the super-hydrophobic coating varied spatially, and so did the surface wettability. Such areas were repeatedly patterned on the surface, where the edge subject to the laser irradiation with the GS of 18% in one pattern met the edge subject to the laser irradiation with the GS of 10% of the neighboring pattern. The contact angle measurements showed that a high GS led to a more wetted surface. Within each pattern, the contact angle varied from $\sim 126^\circ$ (measured at the edge with 18% GS) to $\sim 153^\circ$ (measured at the edge with 10% GS) (Figure 2c). The spatially varied wettability also caused different minimal sliding angles towards opposing directions (Figure 2f). The 20 μL droplet started to move towards the direction of increasing surface wettability at the minimal sliding angle of $\sim 37^\circ$, whereas it started to move towards the direction of decreasing surface wettability at the minimal sliding angle of $\sim 53^\circ$ (Figure 2d-f). This indicated that the minimal inertial forces that can displace the droplets along opposing directions were different.

Figure 2: The gradient wettability induced by the laser irradiation. (a) Laser engraver used for irradiation. (b) A substrate after laser irradiation. (c) The contact angle of a 10 μL water droplet on the substrates treated with varied laser doses. (d-f) The minimal sliding angles towards the opposing directions.

Linear Droplet Translocation

A 20 μL droplet was dispensed on the pattern with gradient wettability. The droplet spontaneously migrated to the most wetted edge of the pattern. This is because the gradient of the surface wettability is large enough to overcome the contact angle hysteresis of the droplet. When an in-plane cyclic vibration of 10 Hz was applied, the droplets moved along the longitudinal axis of the pattern towards the less wetted area and came across the edge to the neighboring pattern (Figure 3a). In particular, the droplet moved across an edge of the neighboring within each vibration cycle, and travelled over a distance of 40 mm within 1s (Figure 3b). Continuous vibration can move the droplet over a long distance.

Figure 3: Droplet manipulation by cyclic in-plane symmetric vibration. (a) The top and cross-sectional views of a droplet manipulation in one pattern. (b) Long distance droplet movement ($\sim 16\text{ mm}$). 5 time-lapsed photographs were overlapped. The faded droplets represent the positions of a droplet at the different time.

Droplet Mixing and Merging

Droplet mixing is an essential functions of droplet based microfluidic and is used extensively for biochemical analysis. The mixing of two droplets by the symmetric in-plane vibration was

demonstrated by arranging two groups of patterns towards the opposite directions. As shown in the *Figure 4*, both pattern groups were connected to a square area which had been irradiated by laser with the GS of 25%. The square area was thus more hydrophilic than the hydrophilic edge of the patterns. Two 20 μ L droplets stained with different colors were dispensed on the distal ends of the two opposite patterns. Once the in-plane vibration of 10Hz was applied, both droplets moved toward the square area at the center due to the gradient wettability. The two droplets met with each other within $\sim$ 300 ms. The in-plane vibration also helped to quickly mix the two droplets, which was evidenced by the color of the merged droplet. The color of the merged droplet became homogenous within 2.5 s (25 cycles of vibration). When the two droplets of the same volumes were mixed without vibration, it took more than 25 s before the color of the merged droplet became homogenous.

Figure 4: Droplet mixing by moving two droplets toward each other.

In above mixing experiment, the two droplets moved towards each other and met at a center place. In droplet based operation, there were also needs to dislocate only one droplet, while keeping the other stationary. This was demonstrated by patterning the areas with gradient wettability with different widths. The areas with three different widths, i.e., 2.5 mm, 3 mm and 3.3 mm, were patterned. The length of the area were the same (4mm). The areas with different widths were connected along the longitudinal direction (*Figure 5*). Three 20 μ L droplets were dispensed at the locations shown in the figure. The in-plane vibration of 10Hz was applied. At the beginning, only the droplet on the area with the width of 2.5mm was dislocated, while the other two droplets stay stationary. Once the moving droplet met the droplet on the area with the width of 3mm, the merged droplet gained the volume of 40 μ L and started to move under the same vibration. Similarly, once the merged droplet with the volume of 40 μ L met the droplet on the area with the width of 3.3 mm, the merged droplet gained the volume of 60 μ L and started to move further along the longitudinal direction. This study showed that the motion of the droplet not only depends on the wettability of the pattern and the mechanical stimulation, but also on the width of the wettability pattern and the droplet volume.

Figure 5: Sequential merging by selectively manipulating the droplets by placing them on patterns with different widths.

Measurement of Albumin Concentration

The efficacy of this droplet manipulation approach for biochemical analysis was demonstrated through the measurement of the albumin concentration of a sample solution (*Figure 6a*). Multiple groups of repeated patterns with the gradient wettability were first irradiated using the laser with the GS from 10% to 18%. All the groups were terminated with a hydrophilic square that was treated using the laser with the GS of 25%. The 1000 μ g/mL albumin solution was diluted by DI water to obtain the albumin solutions with the concentration from 0.3 to 1000 μ g/mL. These solutions were used to build a standard curve for albumin concentration quantification. A 10 μ L droplet of albumin solution at each concentration was dispensed within a hydrophilic square and dried. The bicinchoninic acid (BCA) working reagent was prepared by mixing the reagent A and the reagent B of Pierce BCA protein assay kit (Thermo Fisher Scientific, MA, USA) with the volume ratio of 50:1.

A 2 μ L droplet with an unknown albumin concentration was dispensed on the hydrophilic square of an unoccupied pattern group. A 20 μ L BCA working reagent was dispensed to the other end of the pattern group. Similarly, a 20 μ L BCA working reagent was dispensed to the distal end of each pattern group that has the immobilized albumin. The in-plane vibration of 10Hz was applied along the longitudinal direction of the pattern. Upon the vibration, all the BCA droplets were transported to the hydrophilic squares within 2 s (*II, Figure 6a*). The substrate was continuously vibrated to allow thorough mixing of the BCA reagents with albumin. The temperature of the substrate was elevated to 37 $^{\circ}$ C to assist the reaction. The colors of the droplets changed due to the reaction between albumin and BCA (*III, Figure 6a*). The concentration of the unknown albumin droplet was quantified by measuring the absorption of light at the wavelength of 562 nm and compare the absorption of the standard samples with known concentrations. In particular, a linear standard curve with a high regression coefficient > 0.98 was obtained (*Figure 6b*). The concentration of the droplet to be measured was calculated as $\sim$ 239 μ g/mL. This value was close to its actual concentration of $\sim$ 230 μ g/mL. The reaction time of the droplet based protein analysis was compared to the BCA assay performed using the standard protocol. The light absorption of the droplet based analysis reached 95% of its maximal within 10 min (*Figure 6c*), which was three times faster than that of the standard BCA assays. The shorter reaction time is

due to the in-plane vibration that continuously accelerated the mixing of reagents and the smaller sample volume.

Figure 6: Droplet-based protein measurement. (a) The schematic of the device. (b) The light absorption at 562 nm of the droplet with unknown albumin concentration and the standard curve. The red arrows indicates the albumin concentration in the unknown sample. (c) The comparison of the droplet based analysis with the measurement using a standard BCA assay protocol.

CONCLUSION

In this paper, we report an approach of open channel droplet manipulation by arranging repeated patterns of gradient wettability on the working substrate and applying symmetric in-plane vibration. The gradient wettability created by decomposing the super-hydrophobic coating by the laser irradiation with different doses causes different minimal inertial forces that can move the droplet along the opposing directions. As such, the droplets can be dislocated and generate a net displacement along the longitudinal direction of the patterns upon in-plane cyclic vibration. Continuous vibration is able to have the droplet travel over a long distance. Typical droplet manipulations including unidirectional movement, and droplet merging and mixing were implemented. The measurement of protein concentration using this approach was demonstrated, which showed a faster reaction rate than the measurements using the standard BCA assay protocol.

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