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Transportation and mixing of droplets by surface acoustic wave

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ABSTRACT

Unit operations for complicated biochemical analysis cannot usually be integrated into one substrate. A possible solution to solve this problem is to integrate multi-unit operations into two or more substrates. In this case, transporting droplets from one substrate to another is essential. In this work, a new method to transport droplets from a hydrophobic glass substrate to a piezoelectric substrate is proposed. An interdigitated transducer (IDT) and reflectors were fabricated on an optic grade 128° *YX*-cut lithium niobate ($LiNbO_3$) substrate, and its working surface between the IDT and a reflector was modified to be hydrophobic. Droplets to be transported were first pipetted onto a glass substrate. Adjust the glass substrate so that the droplets could contact the working surface of the piezoelectric substrate, and then was moved down. These droplets could be successfully transported from the glass surface to the piezoelectric substrate because of their "adhesion work" difference. By using this mechanism, water and red dye droplets were successfully transported from glass substrate to piezoelectric substrate. As an application, droplets mixing process was demonstrated in the piezoelectric substrate by using surface acoustic wave after they have been transported from the glass substrate.

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1. Introduction

Microfluidics devices (also called lab-on-chips) have proven to be powerful platforms for bioanalysis due to their advantages such as rapid speed, low reagent consumption, more environmentally appealing, and easy automation and potential portability. Due to these various advantages, microfluidics devices for DNA sequencing, protein analysis, single cell analysis, drug screening, and food safety have been established [1–3].

Compared to more conventional systems, lab-on-chips working with droplets have many advantages such as less analysis time, smaller device size, and lower costs. Therefore, transporting and manipulating micro-fluids in a digital micro-fluid (droplet) have attracted wide attention for chemical and biochemical analysis microfluidics systems [4,5]. Electrowetting on dielectrics (EWOD) [6] and the surface acoustic waves (SAW) [7–10] are the two convenient methods for displacement of liquid droplets in two dimensional planes. The EWOD method requires a surface padded with electrodes. Droplet is displaced by a driving electrical field of some tens of volts applied between two adjacent electrodes. The problem of electrolysis in solution was overcome by coating the electrode surface with a thin dielectric layer (e.g. polymer substrates) several microns to millimeters in thickness. This method is simple and well suited to portable micro-device. However, it cannot be used to condensate and purify target samples. These drawbacks can be overcome by using the SAW method in which no electrode existing in the working area. The driving force for droplets displacement is determined by excitation power. This SAW method has been successfully used to manipulate droplets in microfluidics systems [7–10], to concentrate particles or bio-particles [11–13], and to analyze trace amount mass [14]. In addition, this approach is particularly used to displace droplets in open systems [9], which could help in integration extensive functional units in microfluidics devices.

However, interdigitated transducer (IDT) arrays on piezoelectric substrate are limited. It is difficult to implement all unit operations of a complicated analysis system in one piezoelectric substrate. A good solution is to integrate the operations into two or more substrates. Thus, the efficient transport of droplets from a substrate to another substrate is essential.

In this paper, we proposed a new method to transport droplets between two substrates in space. For generality, two different substrates have been used: one is a piezoelectric substrate and the second one is a glass substrate which can also be a piezoelectric substrate or other substrates. For demonstration, transportation and mixing of two kinds of droplets (see supplementary data, Fig. S1) were studied and mixing efficiency was analyzed. The present method provides a new strategy to integrate unit operations of complicated biochemical analysis system into a microfluidics system with less size and then less operating procedure.

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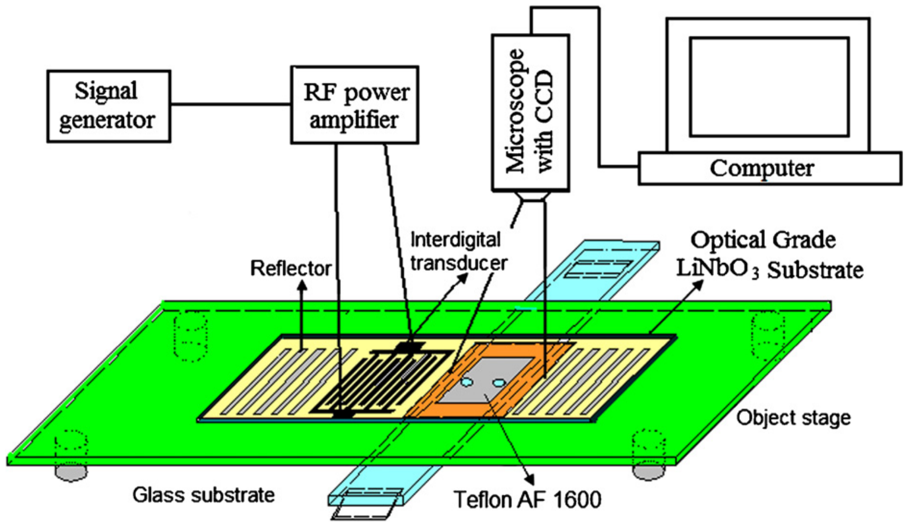

Fig. 1. Experimental set-up for transporting droplets between a glass and a piezoelectric substrate.

2. Experimental methods

Fig. 1 illustrates the experimental set-up for transporting droplets between a piezoelectric and a glass substrate. An aluminum interdigital transducer (IDT) and two aluminum reflectors were fabricated on an optic grade 128° (128 degree) rotated *Y*-cut *X*-propagating $LiNbO_3$ substrate (Deqing Huaying Electronics Co., Ltd., China. Purity: >99.99%). The substrate was adhered to a flat object stage. Working surface of the piezoelectric substrate was designed to face down for easily transporting droplets from the glass substrate. Sizes of the IDT are listed in Table 1.

The working surface of the piezoelectric substrate between the IDT and reflector was coated by a thin film of Teflon AF 1600 (DuPont China Holding Co., Ltd.) to make the surface hydrophobic, which decreased the resistance for aqueous droplets movement. The thin film of Teflon AF 1600 was coated by moving a card with a little fluoropolymer solution (Teflon AF 1600) on the piezoelectric substrate and baked for an hour at 150 °C. The surface of glass substrate was also coated by Teflon AF1600 thin film using the same method. This glass substrate was placed on a movable shelf, which can be adjustable in horizontal and vertical directions. A highly sensitive CCD color video camera (DCE-2, Gentaur, Europe) was used to monitor the droplets movement and mixing process. A MDVNT software (Novel) was used for camera control and image processing.

3. Principles of transporting droplets between two substrates

The interfacial tension of a droplet to a solid surface is sketched in Fig. 2.

According to the Young's equation, the relationships of the solid–gas (*S*–*V*) interfacial energy (γ_1), the liquid–gas (*L*–*V*) interfacial energy (γ_2), the solid–liquid (*S*–*L*) interfacial energy (γ_3) and the contact angle (θ) in Fig. 2 satisfy the following equation:

$$\cos\theta = \frac{\gamma_1 - \gamma_3}{\gamma_2} \quad (1)$$

Delivering a droplet from one solid surface to another substrate is mainly dependent on the difference of their "adhesion work", which is defined as the work to separate two-phase matters contacting each other. It represents adhesion strength of the solid to a droplet. The larger is the "adhesion work", the securer is the droplet combined with the solid. The value of "adhesion work" (W) satisfies the following equation [15]:

$$W = \gamma_1 + \gamma_2 - \gamma_3 \quad (2)$$

Substituting Eq. (1) into Eq. (2), we can obtain the following equation:

$$W = \gamma_2(1 + \cos\theta) \quad (3)$$

When considering the gravitation of a droplet, to deliver a droplet from the glass substrate to the $LiNbO_3$ substrate must satisfy the following conditions:

$$\int_L W_{top-droplet}\,dl > \int_{L'} W_{down-droplet}\,dl + G;$$
$$\int_L W_{co}\,dl > \int_{L'} W_{down-droplet}\,dl + G \quad (4)$$

where $W_{top\text{-}droplet}$ represents the "adhesion work" to separate a droplet from the working surface of $LiNbO_3$ substrate, $W_{down\text{-}droplet}$ represents the "adhesion work" to separate a droplet from the glass substrate, W_{co} is "cohesive work" of the droplet, and G is the gravitation of the droplet.

Table 1
Designing parameters of the IDT and reflectors.

Piezoelectric substrate	128°-*Y* cut *X* $LiNbO_3$
Aperture (μm)	4300
Wave length (μm)	144
The number of IDT pairs	35
Synchronous frequency (MHz)	27.7
The number of reflectors	110
Electrode thickness (nm)	700
Metallization ratio	0.5

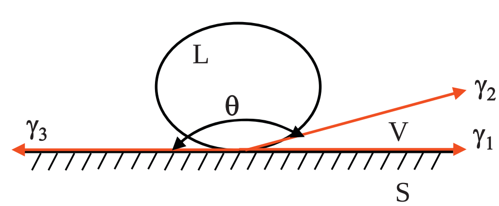

Fig. 2. Schematic representation for the interfacial tensions between a droplet and a solid substrate. *S* denotes the solid substrate, *L* the liquid phase and *V* the gas phase.

According to Eq. (3), the “adhesion work” can be calculated from the contact angle, since γ_2 can be obtained from literature. The contact angles of water–Teflon modified glass substrate and water–Teflon modified piezoelectric substrate were measured by a contact angle measuring apparatus (DIGIDROP, GBX corp., France), which were 119.5° and 87.5°, respectively. The contact angles of glycerine–Teflon modified glass substrate and glycerine–Teflon modified piezoelectric substrate were also measured. They were 102.9° and 84.8°, respectively.

After the droplets have been transported from the glass substrate to the $LiNbO_3$ substrate, they thus can be further implemented unit operations such as mixing, split, reaction with other droplets on the same substrate.

When surface acoustic wave passing through a droplet, part of the Rayleigh wave will transfer into leaky wave and radiate energy into the droplet along an angle θ_R.

$$\theta_R = \arc\sin\left(\frac{V_W}{V_R}\right) \quad (5)$$

where V_W represents the propagation velocity of wave in the droplet and V_R represents the propagation velocity in the piezoelectric substrate. Thus, the unit body force acting on the droplet generated by SAW can be represented as [16]:

$$F = -\rho(1+\alpha_l^2)^{3/2}A^2\omega^2K_i \exp 2(K_i x + \alpha_l K_i z) \quad (6)$$

where ω is the angular frequency, ρ is the viscosity of droplet, A is the SAW amplitude, K_L is the wave number of leaky wave, and K_i is the imaginary part of K_L, and $\alpha_l = j\alpha$ with $\alpha = \sqrt{1-(V_R/V_W)^2}$. When the unit body force is large enough, the droplet will then be driven in the working surface of the piezoelectric substrate.

4. Results

In order to develop biological lab-on-chips, aqueous solution droplets are usually transported and handled. Therefore, in our experiment, droplets were transported from the glass substrate to the piezoelectric substrate. By adjusting the glass substrate to a place so that a droplet to be transported can be easily pipetted onto its hydrophobic surface. After the droplet was pipetted onto its surface, the glass substrate was adjusted in horizontal direction and vertical direction. When the droplet was contacted with the hydrophobic surface of the $LiNbO_3$ substrate, the glass substrate was move down to detach the droplet. In this case, the droplet would attach to the hydrophobic surface of the $LiNbO_3$ substrate because of the difference of their “adhesion work”.

Whether a droplet can be successfully transported from one substrate to another substrate, the “adhesion work” difference plays a determining role. When the droplet volume is enough, its gravitation can be ignored. However, when the volume of a droplet is larger enough, its gravitation will be an important element and should be considered. First, transport experiment of a 12 μl water droplet was done and analyzed. The adhesion forces of the droplet to the Teflon modified glass substrate is 0.291 mN, while the adhesion forces of the droplet to the Teflon modified piezoelectric substrate is 0.598 mN. The gravitation of the 12 μl water droplet is 0.118 mN. Thus the adhesion forces of the droplet to the Teflon modified piezoelectric substrate is greater than the sum of the adhesion forces of the droplet to the Teflon modified glass substrate and the droplet gravitation. In the meantime, the cohesive force of the 12 μl water droplet is about 1.150 mN, which is also greater than the sum of the adhesion forces of the droplet to the Teflon modified glass substrate and the droplet gravitation. Therefore, the two conditions of Eq. (4) are satisfied and the 12 μl water droplet can be transported from the glass substrate to piezoelectric substrate, which has been verified by the experiment (Fig. S2).

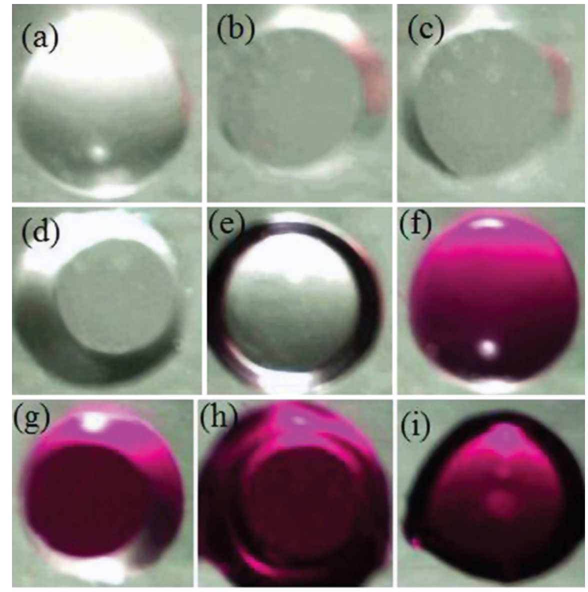

Fig. 3. A 3 μl water droplet (a–e) and a 3 μl red dye droplet (f–i) have been transported from the glass substrate to the piezoelectric substrate. The scale bar is 1 mm.

Fig. 3 shows the successful transportation of a 3 μl water droplet (a–e) and a 3 μl red dye (red dye used in our experiment is red ink, which will not substantially affect the liquid drop) droplet (f–i) from the glass substrate to the piezoelectric substrate. Fig. 3a and f shows the water droplet and red dye droplet on hydrophobic glass surface. As the droplets moved up to contact the hydrophobic surface of the $LiNbO_3$ substrate (Fig. 3b and g), the droplets were squeezed to change their morphologies. As soon as the contact area of the droplets is larger enough (e.g., Fig. 3c), the glass substrate was moved down again (Fig. 3d and h). In this case, since the adhesion work of the droplet to the hydrophobic surface of the $LiNbO_3$ substrate is larger than to the sum of adhesion work of the droplet to glass and gravitation of the droplet, the droplets were successful transported to the hydrophobic surface of the $LiNbO_3$ substrate (Fig. 3e and i).

As an application, a 2 μl red dye droplet and a 2 μl water droplet were transported from a glass substrate to a piezoelectric substrate, and mixed on the piezoelectric substrate by SAW. As shown in Fig. 4, after the two droplets were successfully transported to the working surface of $LiNbO_3$ substrate (Fig. 4a), a 27.5 MHz RF sinusoidal signal amplified by power amplifier was applied to the IDT. When amplitude of the generated SAW was appropriate, the water droplet could be moved along the propagation direction of the acoustic wave (Fig. 4b), while the 2 μl red dye droplet was stable because of SAW attenuation and reflection in propagation. This requirement could be met when the RF sinusoidal signal power was 28.5 dBm. (The dBm is used to define the absolute value in power. Its value can be calculated using the equation: $1\,\text{dBm} = 10\lg(E/1\,\text{mW})$, E is the value of power applying on the interdigital transducer.) As soon as the moving water droplet contacted to the red dye droplet, fast mixing starts (Fig. 4c). Mixing continuously processed as the water droplet moved to the place where the still red dye droplet was. This experiment clearly shows that two droplets can be mixed very fast with the help of SAW.

In addition, a 2 μl red dye droplet and a 2 μl glycerol droplet could also be transported from the glass substrate and then fast mixed on the piezoelectric substrate with the help of SAW. The whole process was shown in Fig. 5. In this case, a 27.5 MHz RF sinusoidal signal amplified by power amplifier was applied to the IDT and the RF signal power which fed on the IDT was 29.0 dBm.

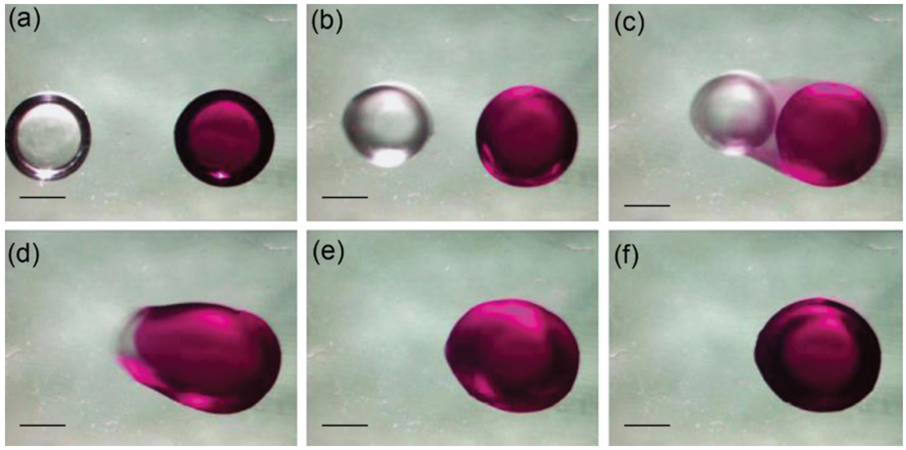

Fig. 4. A 2 μl water droplet and a 2 μl red dye droplet mixture at (a) $t = 0$ s, (b) $t = 4.066$ s, (c) $t = 4.4$ s, (d) $t = 4.466$ s, (e) $t = 5.000$ s, and (f) $t = 6.133$ s. The scale bars are 1 mm.

5. Discussion

As a droplet can easily be transported in the two-dimensional plane by the IDT arrays [9], it is easy to make two droplets in one direction for mixing. Thus, the original position of the two droplets in the glass substrate is not important. For simplicity, the two droplets to be mixed are placed in the same direction as shown in Figs. 4 and 5.

The mixing efficiency can be assessed quantitatively by a mixing index σ [17]:

$$\sigma = \sqrt{\langle (C - \langle C \rangle)^2 \rangle} \tag{7}$$

where C is the gray value of every pixel of the droplet, <> is the average gray value of the droplet. From Eq. (7), full mixing of two droplets results in $\sigma = 0$, and complete segregation results in $\sigma = 0.5$.

In order to calculate the mixing index, the distribution region of the mixed droplets must be defined at first. As shape of most droplets is ellipsoidal, we used ellipsoid to fit the edge of a droplet for simplicity. The contour of a droplet is determined by the Matlab program, then several coordinates of pixel points in contour (x_i,y_i) ($i = 1, 2, 3, \ldots, N$) can be obtained, which approximately equal interval distribution in the contour. According to these coordinates (x_i,y_i), we can approximately determine the range of center coordinate (x_0,y_0) and that of long axis (a) and short axis (b) of fitting elliptic equation. Searching all values of x_0, y_0, a, and b to find the fittest elliptic equation so that the distance sum of all points to the elliptical is minimum. The flow process chart of the program can be found in Supporting Information (Fig. S3).

According to the program, we can define the contour of a droplet. Fig. 6 shows the contour of a mixed 2 μl glycerol–2 μl red dye droplet at $t = 15.666$ s and its fitted ellipse. From the simulated results, it is clear that the fitted ellipse almost overlaps with the contour of the droplet.

Then, we can calculate the mixing index of the mixed droplets using Eq. (7). With the help of SAW, the mixing index of the 2 μl water–2 μl red dye droplet is 0.0815 after 1.133 s mixing time, and 0.0768 for the 2 μl red dye–2 μl glycerol droplet after 0.666 s mixing time. These values indicate that the droplets are almost mixed sufficiently. However, without the help of SAW, the mixing indexes of them are 0.1845 and 0.2168 after 30 s and 52.333 s mixing time respectively via free diffusion. These results demonstrate that the

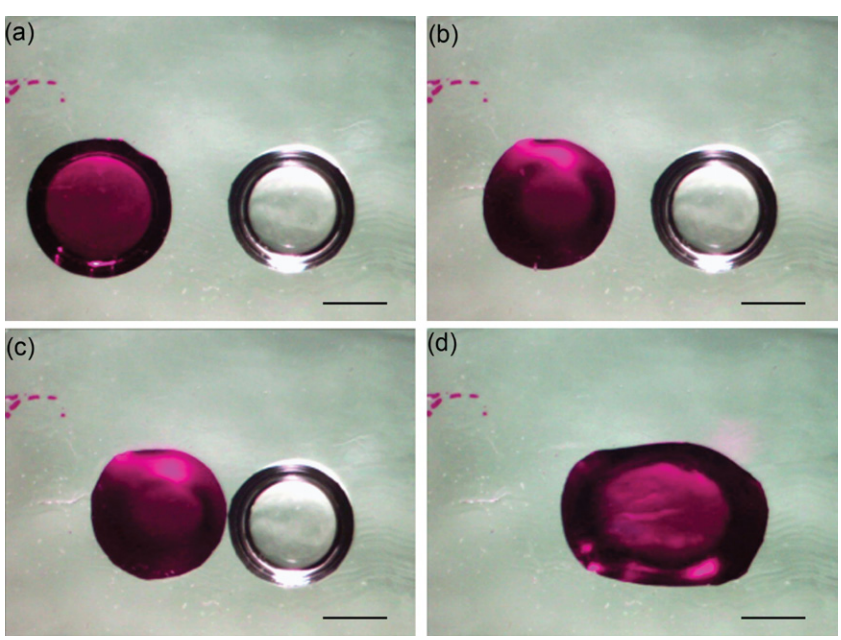

Fig. 5. A 2 μl red dye droplet and a 2 μl glycerol droplet mixture at (a) $t = 0$ s, (b) $t = 11.266$ s, (c) $t = 15$ s, and (d) $t = 15.666$ s. The scale bars are 1 mm.

Fig. 6. The contour of mixed droplet of a 2 μl glycerol droplet–2 μl red dye droplet at t = 15.666 s (white line) and its fitted ellipse (red line). (For interpretation of the references to color in this figure caption, the reader is referred to the web version of the article.)

mixing of droplets can be accelerated significantly with the help of SAW. The main reason is the presence of strong internal bulk convection at high acoustic power [18], which accelerates droplets mixing.

6. Conclusion

In summary, we have reported that droplets can be transported from one substrate to another based on the different of their interfacial tension, which demonstrates that manipulation of droplets in two substrates can be achieved. As an application, mixing operation is implemented after droplets have been transported. The mixing velocity of two droplets can be significantly accelerated with the help of SAW compared to the mixing via free diffusion. The present approach can extends unit operations of droplets from one substrate to two substrates and can provide a novel strategy for integrating more unit operations in lab-on-chips for bioassays.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2011.01.017.

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