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A biocompatible open-surface droplet manipulation platform for detection of multi-nucleotide polymorphism

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We present a novel and simple method to manipulate droplets applicable to an open-surface microfluidic platform. The platform comprised a control module for pneumatic droplets and a superhydrophobic polydimethylsiloxane (PDMS) membrane. With pneumatic suction to cause a deflection of the flexible PDMS-based superhydrophobic membrane, the sample and reagent droplets on the membrane become transported and mixed. A facile one-step laser micromachining technique serves to fabricate a superhydrophobic surface; contact angle 150° and hysteresis 4° were achieved without chemical modification. Relative to previous open-surface microfluidic systems, this platform is capable of simultaneous and precise delivery of droplets in two-dimensional (2D) manipulation. Droplets were manipulated with suction, which avoided interference from an external driving energy (e.g. heat, light, electricity) to affect a bio-sample inside the droplets. Two common bio-samples, namely protein and DNA, verified the performance of the platform. From the experimental results, operations on protein can be implemented without adsorption on the surface of the platform. Another striking result is a visual screening for multi-nucleotide polymorphism with hybridization-mediated growth of gold-nanoparticle (AuNP) probes. The detection results are observable with a naked eye, without the aid of advanced instruments. The entire procedure only takes 5 min from addition of sample and reagent to the results obtained, which is much quicker than the traditional method. The total sample volume consumed in each operation is only 10 μL, which is significantly less than what is required in a large system. According to this approach, the proposed platform is suitable for biological and chemical applications.

Introduction

With advances in micro-electro-mechanical systems (MEMS) and microfluidic techniques since 1993, droplet-based microfluidic systems have been applied to various research fields from fundamental analyses to practical applications as their operation provides many advantages. For instance, samples and reagents are isolated in a well defined reaction environment of each droplet.[1] A large ratio of surface to volume, rapid response and small diffusion length also make possible high-throughput screening, massive biochemical synthesis and highly efficient parallel reactions.[2] Droplet manipulation platforms have hence been used for various investigations, including drug screening, cell cultures, chemical and biological detection, nanoparticle synthesis, PCR reaction, enzyme, cell and organism assay.[3] A droplet manipulation technique is typically implemented in one of two configurations:[4] one is a two-plate or closed format, in which droplets are sandwiched between an actuation electrode substrate and a ground plane substrate; the other is a one-plate or open-surface format, in which droplets are placed on top of a single substrate patterned with actuation and ground electrodes or a textured surface.[5] Open-surface microfluidic devices offer several advantages over conventional closed microfluidic systems, such as a clear optical path, ease of access, simple monolithic construction, direct environmental accessibility, and no cavitation or interfacial obstruction.[5] Established techniques of droplet actuation in open-surface microfluidic systems including optowetting,[6] dielectrophoresis,[7] electrowetting,[8] vibration[9] and thermocapillary actuation[10] have been reported, but these methods also suffer drawbacks. The applied energies, such as optical, electrical and thermal for manipulation of the droplets, are generally accompanied with interference of the biomolecules carried in the droplets. For instance, electric actuation induces a charging attractive force to pose adsorption of the bio-molecules carried on a surface, which might also cause a switching mechanism to fail.[11] Thermal energy would accelerate evaporation of an aqueous droplet and the response is slow (about tens of minutes).[12] Vibrational actuations require expensive equipment and precise control to realize the manipulation of a droplet, which might hinder their practical applications.[9] Alternatively, a surface treated with chemical or biological modifications (e.g. self-assembled monolayer, SAM) can be used to drive droplets without external energy.[13] Nevertheless, these methods involve poor handling. Droplets are efficient only for irreversible transport. In our previous

work, we utilized a stretched elastic surface with nano- or micro-composite structures to control structured densities, thus generating wettability gradients to drive the droplets, but this method also requires precise control to realize the manipulation of droplets. Furthermore, the droplets are transported in only a single direction on the textured surface at the same time, and a stretched elastic surface is difficult to integrate with other devices as their external control system is not compact. To address these issues, we here introduce a pneumatic microfluidic technique and facile one-step laser micromachining to fabricate a new open-surface droplet manipulation platform.[5] Droplets on the superhydrophobic surface can be simultaneously and precisely delivered in a 2D operation using suction without interference from a driving energy. The platform is also easily integrated with another functional microfluidic device for further development and analysis. This platform has hence a great potential for digital fluidic systems in biological and chemical applications.

Preventive medicine of genetic diseases attracts considerable interest and shows their great potential for the understanding of how genetic variation leads to disease. People who have defective genes might be detected and pretreated in early stages before they cause significant morbidity. Developing a simple and rapid tool to detect a genetic mutation and genetic diseases is hence essential. Ample tests have revealed that diseases such as cancer, hypertension and diabetes are associated closely with mutations in gene sequences.[14-16] Among these studies, single-nucleotide polymorphisms (SNP) comprise a genetic variation of a most common type. SNP have a DNA sequence diversity occurring when a single nucleotide — A, T, C or G — in the genome (or other shared sequence) differs between members of a biological species or paired chromosomes, which serve as an important biomarker both for diagnosis of the risk of diseases, and for determining optimum specific medication.[15, 16] Although SNP are certainly an important source of human genome variation, they are not the only form of divergence between genomes. Recent investigations indicate that multi-nucleotide polymorphisms, such as triple-nucleotide polymorphisms (TNP), have a greater propensity to be involved in disease- causing mutation. For example, a SNP can result in the change of only one amino-acid sequence at most, whereas a TNP can alter the residue at two adjacent positions and cause a more appreciable change.[17] Furthermore, SNP have a great predilection for synonymous substitution whereby they alter the nucleotide sequence, but do not alter the amino-acid sequence. These synonymous changes are typically silent mutations and do not affect the phenotype. In contrast, a TNP would affect multiple positions in a codon;[18] as a result, the detection of TNP should be relatively straightforward. Most existing techniques to profile genetic variation with a specific disease or trait are based on either a primer extension or direct hybridization. The primer-extension methods include DNA sequencing,[19] allele-specific primer extension[20] and polymerase chain reaction,[21] which use the ability of a polymerase to incorporate specific deoxyribonucleosides complementary to a template DNA for analysis of genetic variation. The direct hybridization approach, such as single-strand conformation polymorphism,[22] heteroduplex analysis,[23] and denaturing gradient-gel electrophoresis[24], allows the unknown target sequence to be identified based on its ability to suffer sequence-specific binding with a designed probe. Those methods generally require, however, a long analytic process, expensive equipment, well trained technicians, and consumption of sample and reagents in appreciable amounts.

Since 1993, MEMS and microfluidic techniques have enabled the miniaturization of a system for biomedical analysis. Several microfluidic systems for detection of a DNA sequence have been developed.[25] This detection can be performed even in a microchannel, thus requiring fewer pieces of large-scale equipment and simplifying the experimental protocol.[26] Those systems have been proved to improve the sensitivity, detection limit and specificity of DNA biosensor.[27] The microchannel-based systems can be applied, however, only to hybridization; detection of the results still requires other advanced instruments such as a PCR machine or fluorescence readout to identify the heterogeneous SNP. These methods also require precise control to achieve a satisfactory melting temperature of DNA: the cost of a bulk integrated system is still necessary. To our knowledge, these systems are designed to identify SNP, but they are ineffective in detecting variations involving more than one consecutive nucleotide. Elghanian et al. demonstrated a colorimetric method of polynucleotide detection based on the distance-dependent optical properties of AuNP.[28] But the precise control of experimental temperature and delicate manual handling are still needed to be improved. Development of a new technical platform, with little human intervention, free from thermal control and with direct readout of detection results to preform multi-nucleotide polymorphism detection would hence be highly desirable.

We therefore utilized a new open-surface droplet-manipulation microfluidic platform, which integrates a module for pneumatic droplet control and a superhydrophobic surface to detect multi-nucleotide polymorphism. We adopted a novel colorimetric approach based on a concept of DNA hybridization-mediated growth of AuNP probes to detect a DNA sample.[29] With pneumatic suction as a driving energy, droplets on a superhydrophobic membrane can be delivered simultaneously and precisely with 2D manipulation without interference to a DNA sample inside the droplets. The multi-nucleotide polymorphism can be detected with a naked eye, without the aid of advanced instruments. The proposed platform is suitable for biological and chemical applications.

Materials and Methods

Oligonucleotides and chemicals

The 20-mer oligonucleotides consisted of a probe DNA (5'-thiol-GAGCTGGTGGCGTAGGCAAG-3') and three target DNA, namely perfect match DNA (5'-CTTGCCTACGCCACCAGCTC-3'), three base-pair mismatched DNA (5'-CTTGCCTAC**TTT**ACCAGCTC-3') and six base-pair mismatched DNA (5'-CTTGCCT**TTTTTT**CCAGCTC-3'). The probe DNA was labelled with thiol at the 5'-end for immobilization onto the 13-nm AuNP surface (17 nM, TAN Bead Inc., Taiwan). The surface-immobilized probe DNA stabilized the AuNP dispersion. Detail information on the preparation of probe DNA-modified AuNP is available in previous literature.[30] In this work there was no need to modify the target DNA. All oligonucleotides were purchased from MDBio (Taiwan).

13-nm AuNP solution (17 nM, TAN Bead Inc., Taiwan), hydroxylamine hydrochloride (NH2OH•HCl, 99.9999 %, Sigma-Aldrich) and hydrogen tetrachloroaurate(III) hydrate (HAuCl4•3H2O, 99.999 %, Sigma-Aldrich) were purchased from the indicated suppliers. DI (Deionized) water was used to dissolve NH_2OH, $HAuCl_4$, target DNA samples and the probe DNA.

Experiments

Figure 1 is a schematic illustration of the colormetric detection for rapid identification of multi-nucleotide polymorphisms based on the hybridization-mediated growth AuNP probe. The target DNA sample droplet (10 μL, 0.5 μM) was first placed on the superhydrophobic area of the chip. Probe DNA-modified AuNP (1 μL, 25 nM) was then dripped into the target DNA sample (Fig. 1(a)), followed by utilizing suction to cause the deflection of the PDMS membrane to roll the droplet forward and backward. The target DNA and probe DNA-modified AuNP become well mixed and hybridized within about 3 min (Fig. 1(b)). Subsequently, NH_2OH (2 μL, 400 mM) and $HAuCl_4$ (2 μL, 25.4 mM) were dripped into the droplet for the growth of the AuNP probe (Fig. 1(c)). After rigorous blending performed on the proposed chip for 1 min, the target-probe DNA duplex of the AuNP shift the absorption peak and alter the color of the solution (Fig.1 (d)). The experimental results were quickly detected with the naked eye based on the shape-dependent optical properties of AuNP. All experimental process described above was undertaken at room temperature (25 °C).

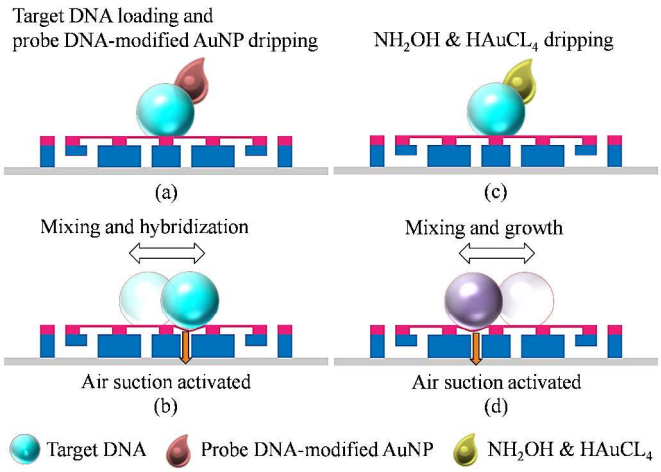

Fig. 1 Operating principle of the proposed platform. (a) a droplet containing target DNA and probe-modified AuNP are loaded on the chip; (b) mixing and hybridization is performed; (c) NH_2OH and $HAuCl_4$ are added into the droplet; (d) target-probe DNA duplex of AuNP shift the absorption peak and alter the color after the mixing and growth.

Chip design

The major objective of the current work is to present a highly biocompatible open-surface droplet-manipulation platform for biochemical application. To realize the experiment described above, we developed a new droplet manipulation fashion and method of fabrication of a PDMS-based superhydrophobic membrane. A droplet can be activated using pneumatic suction on the superhydrophobic surface without interference from the driving energy. Figure 2(a) is an exploded view of the microfluidic chip, which consists of two PDMS structures including a thin-film PDMS membrane with a superhydrophobic surface and a thick-film air channel layer, and one glass plate. A PDMS membrane with a superhydrophobic surface is located on the air channels, which are used to connect the air chamber and an external vacuum pump. The layout of the microfluidic platform is schematically shown in Fig. 2(b). The dimensions of the microfluidic chip are 2.9 cm x 2.7 cm.

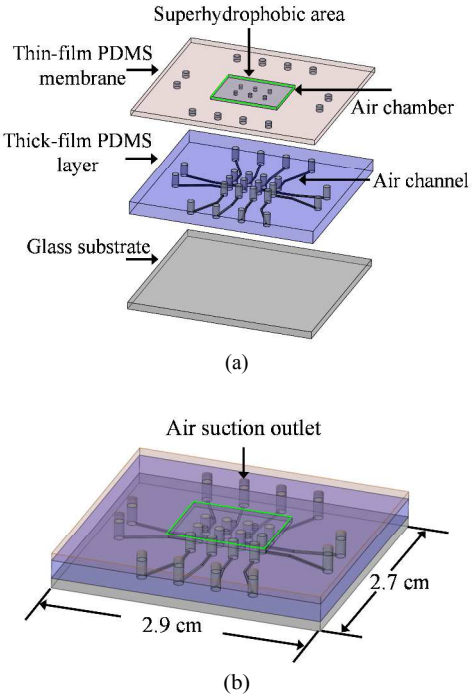

Fig. 2 (a) Exploded view of the microfluidic chip. The chip consists of two PDMS layers and one glass substrate. (b) Layout of the droplet manipulation chip.

Fabrication process

We designed the microfluidic platform to automate the droplet control. The PDMS-based droplet-manipulation platform comprised a thin-film PDMS membrane (100 μm) with a superhydrophobic surface and a thick-film air channel layer (5 mm). The microfabrication including computer-numerical-control (CNC) micromachining, PDMS casting and replication for rapid prototyping of the microfluidic components, and laser micromachining served to fabricate a superhydrophobic surface. Briefly, master molds with microstructures on PMMA plates are first formed using a CNC machine equipped with 0.5-mm drill bit. The feed speed of the drill bit was 7 mm s^{-1} and the rate of rotation was 26,000 rpm. The PDMS was then poured into the PMMA templates to obtain the inverse microstructures of the air chambers and air channels. The CO_2 laser machine directly engraved PDMS to create a superhydrophobic area on the thin-film PDMS membrane (12 W/106.68 mm/s). After the PDMS demolding was completed, the thin-film PDMS membrane, the thick-film PDMS layer and the glass substrate are bonded together utilizing an oxygen-plasma treatment to form the complete microfluidic chip. A photograph of the assembled chip appears in Fig. 3(a). A scanning-electron-microscope (SEM) image of the superhydrophobic surface is shown in Fig. 3(b).

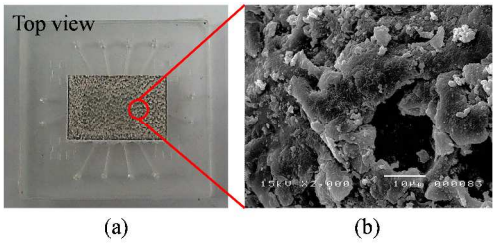

Fig. 3 (a) Photograph of the proposed platform. (b) SEM image of the superhydrophobic surface.

Results and Discussion

Measurement of contact angle and hysteresis angle

The angles of droplet contact and hysteresis were measured with a contact-angle meter (OCA 20, Dataphysics Instruments Inc., Germany). The volume of deionized (DI) water used for these measurements is 10 μL. The contact angle (θ_c) of the droplet was measured to be 150° on the superhydrophobic surface, as shown in Fig. 4(a).We define the hysteresis angle as the difference between the advancing contact angle (θ_A) and receding contact angle (θ_R). The angle subtended at the front of the droplet is the advancing contact angle, whereas at the rear for the receding contact angle. The strategy to measure the hysteresis angle was to place the microfluidic chip on the tilting plate, and then to tilt the surface until the droplet on the chip began to slide. The experimental results showed the advancing contact angle to be 152° and the receding contact angle 148°. The hysteresis angle is hence 4°, shown in Fig. 4(b).

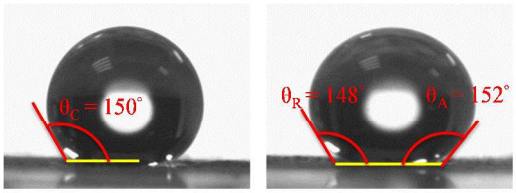

Fig.4 (a) Contact angle of the droplet (θ_C = 150.0°), (b) Hysteresis angle of the droplet is 4° (θ_A = 152°, θ_R = 148°).

Mixing index

In this work, the proposed microfluidic platform has been demonstrated to manipulate droplets to perform mixing. Any enhancement of mixing would decrease the duration of the sample reaction. The bio-sample and reagent were first pipetted onto the superhydrophobic surface. When a suction was applied, the thin-film PDMS membrane with a superhydrophobic surface was then bent by the smaller air pressure in the air chamber that was generated by the suction from the outer vacuum pump. The sample and reagent accordingly rolled to the lower region and coalesced because of the altered contact angle. The relationship between the working pressure and deformation depth of a PDMS membrane can be referenced in the ESI‡ (Fig. S1). On driving the coalescent droplet forward and backward, it became sufficiently mixed within a short period. The mixing efficiency was observed using a charge-coupled-device (CCD) camera. The mixing index was calculated from the measured concentration profile.[31] Figure 5 shows the relation between the mixing index and the duration of mixing for varied driving frequencies. The experimental results reveal that the period for complete mixing can be decreased on increasing the driving frequency. Complete mixing can hence be achieved in 2 s at driving frequency 7 Hz. The mixing index increased from 47.6% to 96.7%. If the driving frequency is 0 Hz, the mixing index is approximately 88.5% for 180 seconds. Video in the ESI‡ show the mixing process of the droplets coalescence on the proposed platform. The driving frequency is 3 Hz, the working pressure is -80 kPa (gauge pressure) and the transport velocity of droplet is around 13.8 mm/s. Note that the platform will not work if the droplet size is under 5 μL, since the wetting state of a droplet on the superhydrophobic surface may change from Cassie state to a Wenzel state.

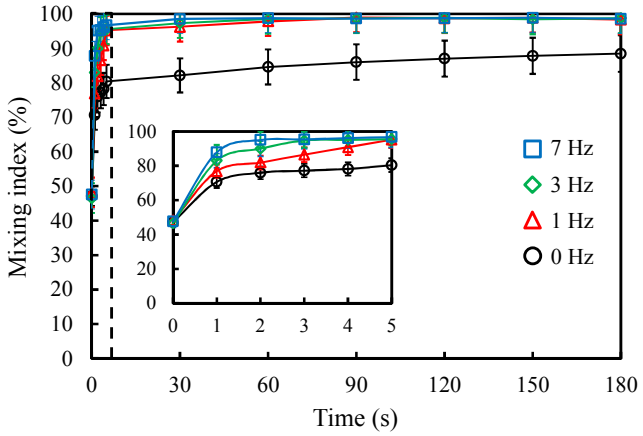

Fig. 5 Relation between mixing index and mixing duration for varied driving frequencies.

Biocompatible test

To estimate whether the driving energy interferes with the bio-molecules in the droplet, we employed a protein solution of goat anti-rabbit IgG, fluorescently labeled (ATTO 590), to observe the adsorption of bio-molecules on superhydrophobic surfaces. The protein solution was dropped onto the microfluidic platform, followed by operation of the droplet utilizing the proposed platform for 10 min. The platform was then purged with DI water to remove the droplet. The residual proteins on the platform surface were visualized with a fluorescence scanner to evaluate the interference of the activation energy on the bio-molecules in a droplet. Figure 6(a) shows fluorescence images of the droplet before actuation; the fluorescent intensity is 1248 au. Figure 6(b) shows the fluorescent image of the droplet after mixing for 10 min; the fluorescent intensity is 1295 au, almost the same. Figure 6(c) shows the fluorescent image of the superhydrophobic surface after washing away the protein solution; the fluorescent intensity is only 114 au. According to the image, no significant non-specific absorption of proteins remained on the superhydrophobic surface (background value 98 au). The avoidance of an exerted energy interference on the bio-sample in a droplet is verified in this device.

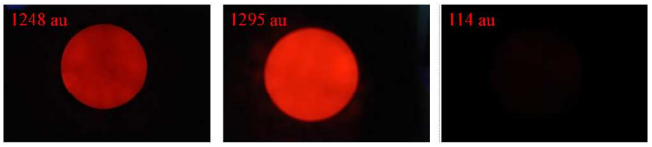

Fig. 6 (a) Fluorescent intensity of the droplet before actuation, 1248 au. (b) Fluorescent intensity of the droplet after actuation for 10 min, 1295 au. (c) Fluorescent intensity of the superhydrophobic surface after washing away the protein solution, 114 au (background value 98 au).

Screening of multi-mismatched DNA

Colorimetric detection of multi-nucleotide polymorphism has been demonstrated on the proposed open-surface droplet-control system, as shown in Fig. 7. We chose NH_2OH as the reducing agent to facilitate the reduction of $HAuCl_4$ catalysed by the gold surface. While probe-modified AuNP were

hybridized with perfectly matched DNA, three base-pair mismatched DNA and six base-pair mismatched DNA, respectively (for 3 min). The varied target DNA affinity to probe-modified AuNP would induce formation of variously shaped AuNP.[32] The formation of the AuNP can result in selective deposition of the reduced gold metal on the AuNP template (for 1 min).[30] Note that the operation conditions of driving frequency and working pressure were 5 Hz and -80 kPa (gauge pressure). As a result, target-probe duplex AuNP of varied size and shape were formed. The size and shape of these aggregated AuNP dominate their optical properties.[33] In the original state, probe-modified AuNP exhibit a red color (Fig. 7(a)), because such AuNP have a maximum optical absorption about 520 nm attributed to a surface-plasmon resonance. After hybridization with target DNA, the target-probe duplex of AuNP shifts the absorption toward larger wavelength and alters the color of the solution. The target-probe DNA duplex of AuNP in the presence of the perfectly matched DNA was transparent (Fig. 7(b)). The AuNP hybridized with the three base-pair mismatched DNA and six base-pair mismatched DNA were blue and purple, as shown in Fig. 7(c) and 7(d), respectively.

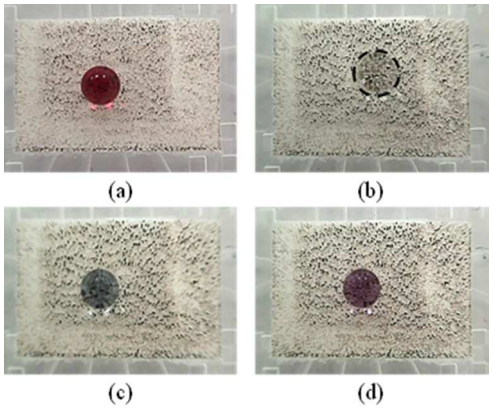

Fig. 7 Images of corresponding target-probe DNA duplex of AuNP on the proposed chip. (a) Initial state of the probe-modified AuNP. (b) Image of perfectly matched DNA-hybridized droplet. (c) Image of three base-pair mismatched DNA-hybridized droplet, and (d) six base-pair mismatched DNA-hybridized droplet.

Conclusions

We have demonstrated a new method of droplet-based manipulation on an open-surface microfluidic platform to detect multi-nucleotide polymorphism. With pneumatic suction to cause a deflection of the PDMS membrane with a superhydrophobic surface, the droplets on the membrane can be delivered simultaneously and precisely with 2D manipulation without interference to the bio-sample in the droplets. A facile one-step laser micromachining technique is used to fabricate a flexible PDMS-based surperhydrophobic surface. A contact angle 150° and hysteresis 4° are achieved on a surperhydrophobic surface without chemical modification. The proposed microfluidic platform provides a gentle actuation to prevent physical damage to the biological materials during the incubation. As a result, an efficient and gentle mixing effect can be generated. Experimental results show no significant interference of the droplets after the incubation and no non-specific absorption remains on the superhydrophobic surface. Another striking result is a simple, visual detection of multi-nucleotide polymorphism that was performed on the proposed chip with hybridization-mediated growth of gold nanoparticle (AuNP) probes. With this approach, whole experimental processes can be carried out at room temperature. The detection is readily observed with a naked eye, without the aid of advanced instruments. The entire procedure takes only 5 min from addition of sample and reagent to the results obtained, which is much quicker than the traditional method. The total sample volume consumed in each operation is only 10 μL, which is significantly less than what is required in a large system. With this approach, the proposed platform is suitable for many applications, especially for the manipulation of biological and chemical reagents.

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Notes and references

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‡ Electronic supplementary information (ESI) available: Supplementary figure and video of mixing process on the proposed platform.

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