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Microfluidic Cellular and Molecular Detection for Lab-on-a-Chip Applications

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1. ABSTRACT

The power of the "Lab on a Chip" concept lies primarily in its ability to detect and manipulate at the cellular and molecular level with sufficiently high throughputs. With careful design and scaling considerations, molecular and cellular detectors (or sensors) facilitated by controlled microfluidic separation, purification, sorting, and mixing operations are more sensitive and specific. In this presentation, I will depict three levels of detection: DNA, protein, and cell. For DNA detection, a droplet microfluidic platform enables rapid and homogenous mixing in confined picoliter volumes for molecular hybridization fluorescence images. For proteins, I will discuss how an acoustic cavity mixer enabled an order of magnitude increase in speed of detection. Finally, a novel microfluidic device based on dielectrophoresis (DEP) enables the detection and sorting of biological cells based on their dielectric properties.

2. DNA HYBRIDIZATION DETECTION

The monodispersed picoliter microfluidic droplet generation system developed by our group and others [1] can serve as a promising micro-reactor for biological and chemical assays. It employs pressure-driven flow to inject aqueous solutions into aqueous immiscible solutions and form picoliter microdroplets. The biological or chemical reagents are all encapsulated in microdroplets and each droplet is isolated by the immiscible liquid (e.g. mineral oil), thus greatly reducing sample contamination on the microchannel side walls and eliminating reagent dispersion problems. The system is useful not only for DNA sample identification, but also for quantitative analysis. The liquid-liquid reaction rate of DNA hybridization in homogeneous liquid is about 40-fold faster than the hybridization rate in a solid-liquid interface [2]. Hence, it is advantageous to improve the DNA sample/sensing probe hybridization rate using this technique rather than the conventional immobilized-probe approach. Compared to conventional microfluidics with continuous laminar flows, the microdroplet allows rapid mixing among reagents in droplet [3] . The microdroplet formation system that we developed is capable of generating over five hundred monodisperse droplets per second with a size deviation of less than two percent [4]. However, because of the fundamental differences between the microdroplet generation system and other conventional techniques, a liquid-liquid reaction based DNA sensing probe is needed to conduct DNA detection in microdroplets.

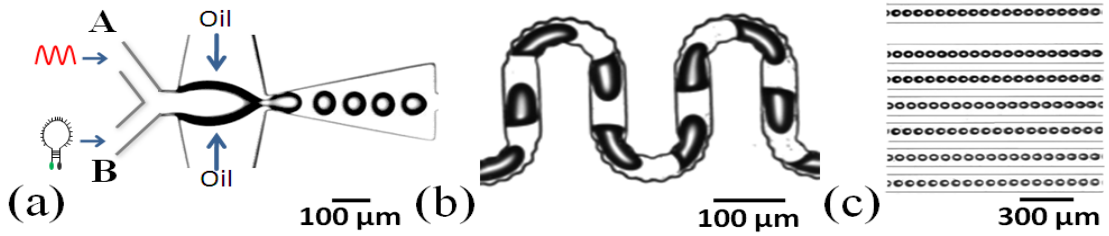

Fig. 1 The merged A and B streams are broken off by side streams and form microdroplets. (a) Consecutive micrographs of droplet generation. The generation time is 2.66 ms/droplet. (b) DNA and MB are rapidly mixed in sawtooth edged U-shaped channel (c) Picoliter droplets moving in hybridization region [5].

In order to achieve rapid DNA detection in homogeneous liquid phase with single nucleotide mismatch sensitivities, we used molecular beacon (MB) as the DNA sensing probe in the microdroplets. The target DNA and MB were all encapsulated in monodispersed picoliter droplet emulsions to reduce sample volume and to enhance the DNA detection efficiency. In our paper [5], we demonstrate a fast DNA sample and mutant detection in a few seconds, and evaluate dynamic MB-

DNA duplex formation using Label-Free DNA Analysis in Microdroplet (LFDAM) system.

The more stable the MB-DNA duplex, the stronger the MB fluorescence restored. The measured fluorescence intensity is directly proportional to sample concentration, droplet volume, droplet generation rate, and fluorescence acquisition time. Figure 1 illustrates (a) the microfluidic droplet generation device, (b) the mixing serpentine channel, and (c) a snapshot of the droplets travelling in the microchannels. Figure 2 illustrates the gradual increase in fluorescent intensity as the droplets travel in the microchannels and more time is lapsed so the MB-DNA duplex has a chance to reach equilibrium.

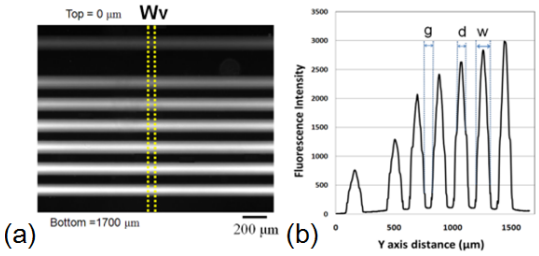

Fig.2 The dynamic binding reaction of target DNA with MB can be observed from the fluorescence change of MB inside droplet. (a) The horizontal average-multiple-line-scan method is used to measure the fluorescence intensity. The yellow dash line represents the fluorescence analysis from the top to the bottom of an image. (b) The measured result shows that the MB fluorescence gradually increases downstream in the LFDAM [5].

3. PROTEIN BINDING DETECTION

The proposed microfluidic system is an acoustic bubble-array micromixer that can be categorized as an active mixer based on a phenomenon termed cavitation microstreaming. Cavitation microstreaming has been

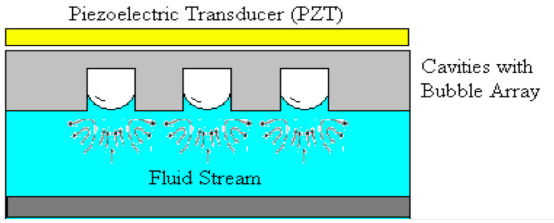

Fig.3 Schematic of Acoustic Bubble-Array Micromixer

previously demonstrated as capable of achieving high degree lateral fluid transport and mixing [6]. The theory employs bubbles as the functional element. Cavities arrayed within a hydrophobic substrate in essence become the functional bubble array once fluid is passed over the substrate trapping air bubbles within the cavities (Fig.3). The interfacial membrane between the air and fluid created on the bubble surface can be resonated or vibrated when an acoustic sound field is applied through a piezoelectric transducer (PZT).

Figure 4 shows the results comparing microfluidic enhanced protein hybridization detection versus conventional protein binding relying on conventional pipetting into the chip[7].

4. DIELECTROPHORETIC DETECTION AND SORTING OF CELLS

When interdigitated electrodes are set at the side walls of the channel, lateral DEP force can be generated which can attract or repel the particles to the walls with the electrodes. If another series of electrodes is configured to face the first set of electrodes, the secondary DEP force can balance the DEP force generated from the first electrode array. Figure 5 illustrates the force balance of particles between the two sets of side wall electrode arrays [8].

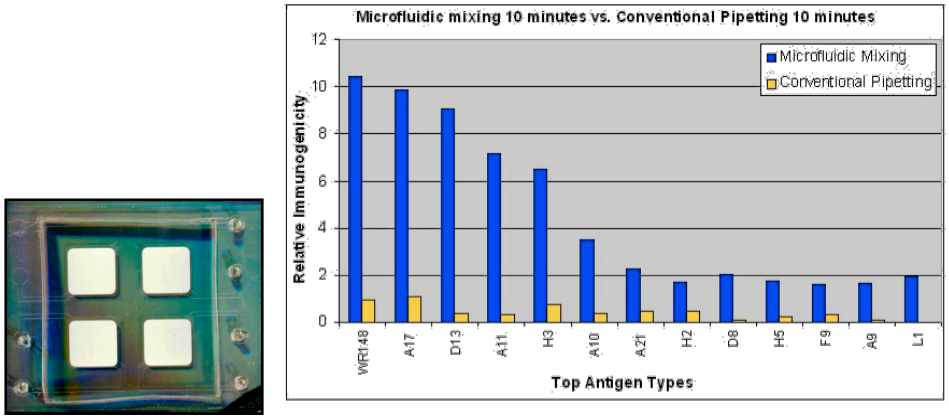

Fig. 4 Left: acoustic bubble array mixer fabricated on top of four protein array nitrocellulose pads. Right: The fluorescence intensity signal comparing acoustic microfluidic mixing results with pipette conventional results.

Negative DEP forces from both sets of interdigitated electrodes can also be used to position objects at different equilibrium points. This can prevent the objects from contacting the sidewalls of the channel which may cause adhesion to the channel, and also prevent the concentration of objects (such as cells) at high electric field regions, which could potentially cause cell damage. Experimental results successfully demonstrate the separation of polystyrene beads and modified HEK293 cells. For the cells/beads separation, both the cells and beads experience negative DEP forces from the electrodes and are continuously directed by fluid flow out of the channel to the outlets. Compared to the cells, the beads are relatively homogeneous and could therefore be more uniformly directed to a single well.

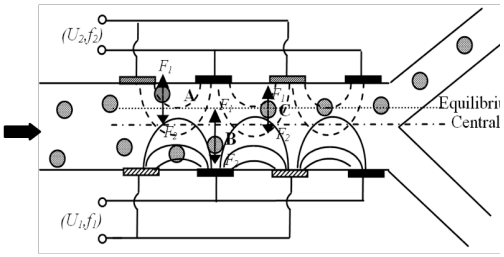

Fig. 5 Principle of the DEP sorting design. Particles are flowing through the channel by the free flow, in the DEP sorting zone force balance plots are shown for the particles with negative DEP properties in the switching region. *U:* magnitude of the voltage, f : the frequency [8].

5. REFERENCES

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