Magnetic Digital Microfluidics

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Open surface droplet microfluidic platforms manipulate droplets on a substrate with low surface energy. Droplets are driven using various actuation methods including electrowetting, dielectrophoresis, surface acoustic waves and magnetic forces. Magnetic actuation is realized by adding magnetic particles (MPs) to the droplets. Magnetic field is then applied to drive MPs which in turn control the motion of droplets. One can easily move the droplet, merge two droplets and split MPs from the droplet (**Fig. 1**). Magnetic Actuation has additional advantages because MPs can also serve as the solid substrate for molecule adsorption once the MPs surface is functionalized with proper chemistry. As a result, magnetically droplets are often developed into lab-on-chip platforms for various applications. Despite its numerous benefits, magnetic droplet platform lacks of functional components for complex fluidic handling such as droplet metering and aliquoting, or making dilution series. To address the aforementioned issue, we have developed a novel surface energy traps (SETs) assisted platform for open surface droplet manipulation capable of complex liquid operation, which greatly improves the performance and greatly extends the applications of magnetic actuated droplet platforms.

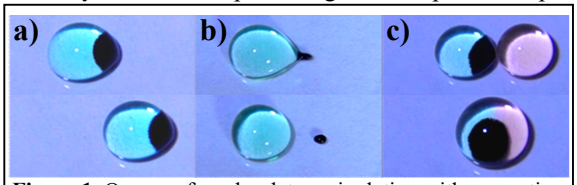

Figure 1. Open surface droplet manipulation with magnetic actuation. **a**) droplet moving, **b**) MPs splitting and **c**) Merging of two droplets.

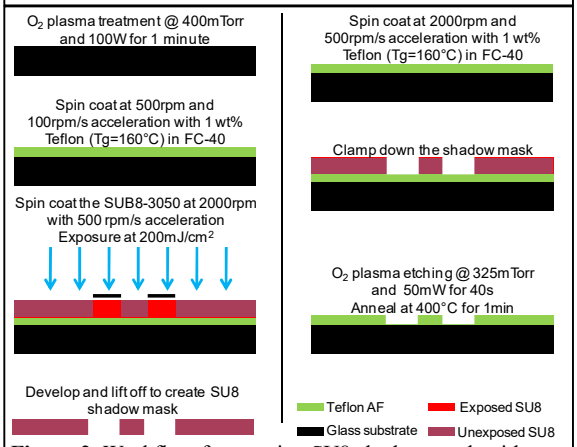

Figure 2. Workflow for creating SU8 shadow mask with Teflon AF as sacrificial layer and patterning SETs with the SU8 shadow mask. SETs patterning completes in 5 min.

SETs are created by selectively modifying the surface wettability of the substrate with oxygen reactive ion etching through a lithographically patterned SU8 photoresist shadow mask (**Fig. 2**). The substrate is initially coated with Teflon AF, leading to low surface energy. Once etched, the underlying substrate is exposed, forming high energy regions that trap the droplet within their boundaries. The platform allows common droplet operation including droplet moving and merging. In **Figure 3a**, the orange droplet is driven towards the blue droplet using magnetic actuation through SSPs. In addition, as the merged droplet moves towards the SET, the liquid droplet is immobilized by the SET whereas the SSPs plug continues travelling and splits from the droplet (**Fig. 3a**). Furthermore, droplets of pre-determined volumes can be easily metered and aliquoted from the parent droplet using SETs (**Fig. 3b-c**). Because the surface tension along the SET contact line is weaker than that around the SSPs plug, SSPs plug does not split from the droplet. Instead, a daughter droplet is metered and aliquoted from the parent droplet by SETs. The SET does not only operate in air (**Fig. 3b**) but also oil (**Fig. 3c**) environment, showing versatility for potentially broader applications. The size of the SET determines the volume of the daughter droplet (**Fig. 4a**). The variation in volume of daughter droplets is only ~3.3% (**Fig. 4b**), as estimated by fluorescence measurement.

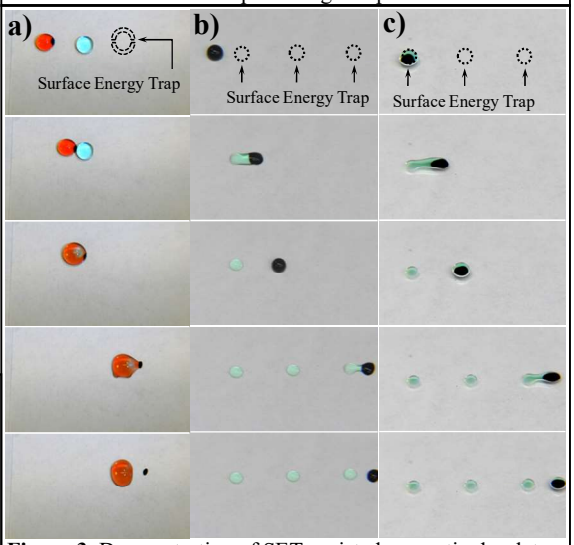

Figure 3. Demonstration of SET-assisted magnetic droplet manipulation. **a**) SET-assisted droplet immobilization and SSP splitting. Orange droplet moves to merge with blue droplet and the merged droplet is immobilized by the SET, which facilitates the splitting of the SSP plug from the droplet. **b**) and **c**) Droplet metering and aliquoting **b**) in air and **c**) in oil environment. Small SETs are not able to immobilize the entire droplet. Instead, an aliquot is metered and held back by the SET.

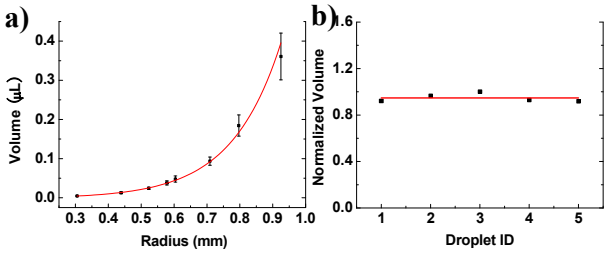

Figure 4. ***a)*** *Volumes of the aliquoted daughter droplets are dependent on the size of SETs.* ***b)*** Five identical daughter drops are metered with SETs from the same parent droplet. The variation in volume is ~3.3%.

To make a dilution series with SETs, the solution buffer droplet is first pulled over an array of SETs by magnets. The SETs array consists of SETs of difference sizes calculated to generate daughter droplet aliquots of desired sizes (**Fig. 5a**). Then the dilution buffer droplets are dragged to merge with the daughter droplet aliquoted from the solution buffer (**Fig. 5b**). In the end, the MPs used to drive solution buffer droplets are removed from the dilution series (**Fig. 5c**). The amount of MPs for solution buffer droplets actuation is small so that SETs can hold the final droplets in position and facilitate MPs splitting. Two dilution series of fluorescein with respective dilution factors of 2 and $10^{1/3}$ are created using water as dilution buffer. The expected concentrations are plotted against the measured concentration and the linear fitting yields slopes of 0.95 (**Fig. 6a**) and 0.9 (**Fig. 6b**), both of which are close to 1 in which case the measured concentrations exactly with expectations.

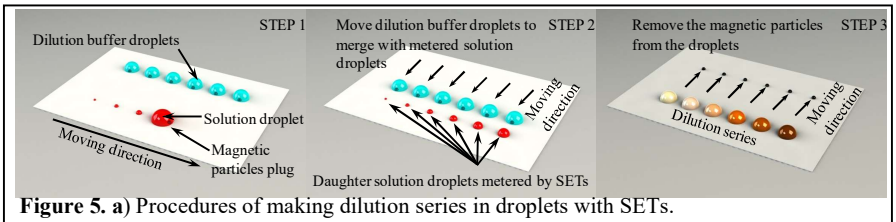

Figure 5. a) Procedures of making dilution series in droplets with SETs.

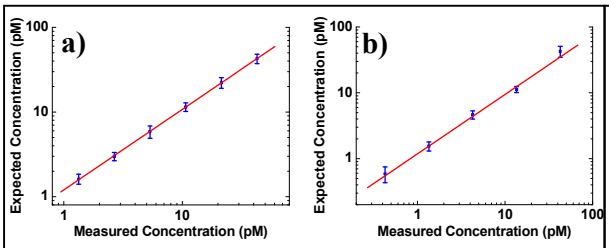

Figure 6. a) Dilution series made by SETs with a dilution factor of 2. The expected concentration is plotted against the measured concentration. The best linear fitting yields a slope of 0.95. **b**) Dilution series with a dilution factor of $10^{1/3}$. The best linear fit has a slope of 0.9.

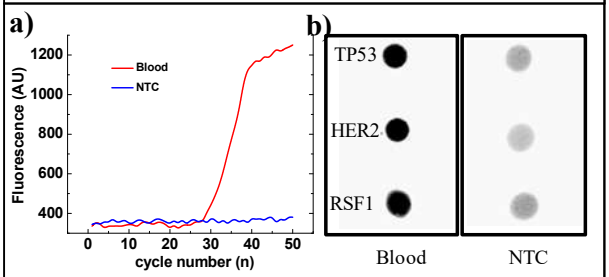

Figure 8. a) Real time amplification curve of RSF1 gene from human whole blood sample processed on the SETs assisted droplet platform. **b**) Multiplex cancer biomarker detection on the SETs assisted droplet platform from single sample. NTC-Non-template control.

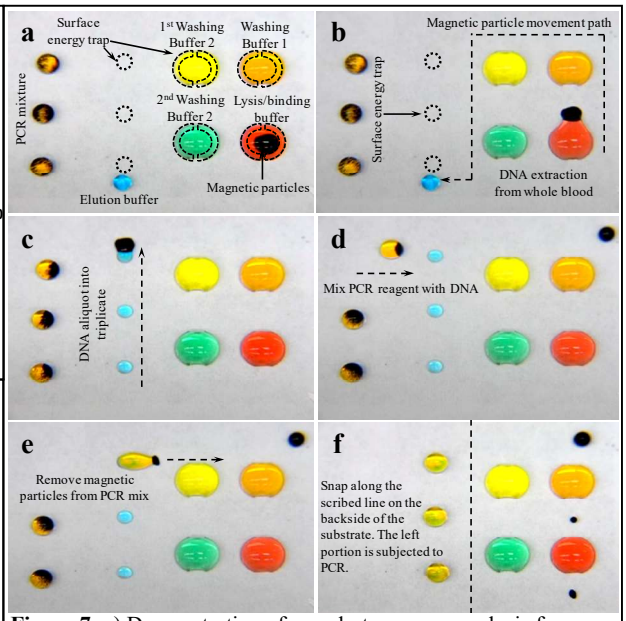

Figure 7. a) Demonstration of sample-to-answer analysis from crude whole blood on SETs-assisted droplet manipulaation platform with food color. a) Blood is incubated with lysis/binding buffer and SSPs, where the DNA molecules bind to the SSP surface. b) SETs hold the buffer droplet in position while the SSPs plug splits from the lysis/binding buffer and merges with the droplet containing washing buffer 1. The SSPs plug moves through the subsequent washing buffer droplets in the same manner, during which the unwanted cellular components are removed. c) The DNA is eluted from the SSPs plug. As the eluent droplet moves through 3 small SETS, aliquots are metered from the parent droplet. d) and e) PCR mixture droplet with different primers are merged with eluent aliquots. f) The reaction droplets are subjected to thermal cycling.

With SETs, magnetic droplet platform is able to replace bench-top equipment and process, performing truly point-of-care molecular diagnostics. By designing various types of SETs, we have developed a droplet based POC platform capable of multiplex cancer biomarker detection from whole blood (**Fig. 7a**). Whole blood is first incubated with the SSPs and lysis/binding buffer where the DNA molecules bind to the SSPs surface. Then the SSPs plug splits from buffer droplet while the SET hold the buffer droplet in position. The SSPs plug moves through the washing buffer droplets in the same manner with the assistance of SETs (**Fig. 7b**). The SSPs plug is then incubated with elution buffer. After that, the SSPs plug drags the eluent through 3 small SETs and split 3 aliquots (**Fig. 7c**). 3 PCR buffer droplets with primers that target TP53, HER2 and RSF1 genes respectively are merged with eluent aliquots (**Fig. 7d** and **7e**). In the end, the 3 droplets are subjected to thermal cycling (**Fig. 7f**). Being able to make aliquots from the eluent allows multiplex detection of different cancer biomarkers from a single sample preparation. A miniaturized fluorescence detection system with a lock-in configuration is included to monitor real time fluorescent signals. The system is able to different 500±50Hz true signals from ambient light noise and has sub-nanomolar sensitivity. Real time amplification curve confirms successful detection of RSF1 gene from human whole blood (**Fig. 8a**). Positive detection of 3 cancer markers is verified by fluorescent scan using Typhoon scanner (**Fig. 8b**).