

# A DIGITAL MICROFLUIDIC APPROACH TO OIL-FREE MAGNETIC PARTICLE-BASED IMMUNOASSAYS

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## ABSTRACT

We report the first particle-based immunoassay on digital microfluidics without the aid of oil carrier fluid to enable droplet movement. This new format allowed the realization of an on-chip particle separation method capable of removing greater than 90% of unbound reagents in one step. Compared to conventional methods, this immunoassay approach reduced reagent volumes and analysis time by 100-fold and 10-fold, respectively, while retaining a level of analytical performance required for clinical screening. We propose that this technique has great potential for use in an inexpensive instrument for the quantitative analysis of proteins and small molecules in low sample volumes.

## KEYWORDS

Digital microfluidics, immunoassay, EWOD, electrowetting-on-dielectric, ELISA, magnetic separation

## INTRODUCTION

There is great interest in miniaturizing immunoassays in microfluidics, as this can potentially speed up analysis, lower reagent consumption, and reduce the cost and size of test instruments.[1] While most fluid handling schemes in microfluidic immunoassays rely on continuous fluid flow or droplets within networks of enclosed micron-dimension channels (i.e., microchannels), an alternative droplet-based fluid handling scheme called digital microfluidics (DMF) is growing in popularity.[2] In DMF, fluids are electrostatically controlled as discrete droplets (pL to  $\mu$ L) on an array of insulated electrodes. By applying appropriate sequences of potentials to these electrodes, multiple droplets can be manipulated simultaneously and various droplet operations can be achieved using the same device design. This flexibility makes DMF well-suited for applications that require complex, multistep protocols such as immunoassays.[3]

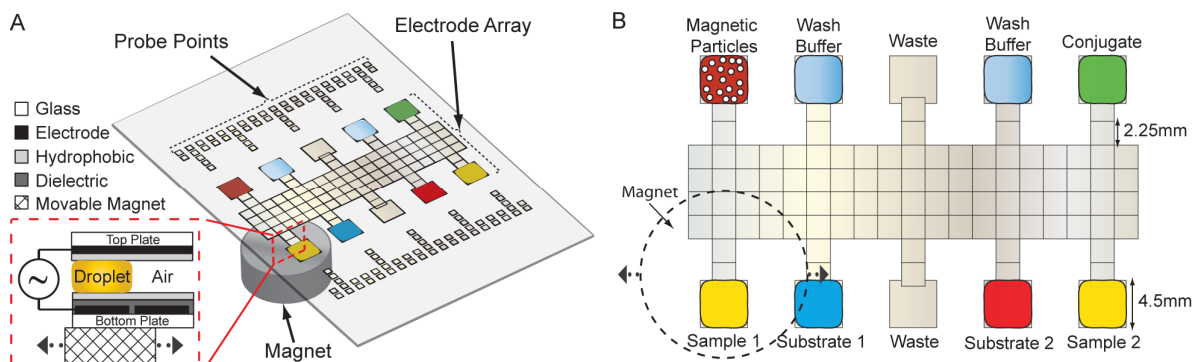


Figure 1: Digital microfluidic design. (A) 3D Schematic and side view of a DMF device, which features a movable magnet underneath the device for particle immobilization. (B) Top view of DMF device, showing the position of the magnet, 10 reservoirs to accommodate reagents, and 96 actuation electrodes to carry out the immunoassay protocol.

To date, two DMF methods for magnetic particle-based immunoassays have been reported.[4-5] In this previous work, an immiscible oil carrier fluid was required to prevent non-specific adsorption and enable droplet manipulation. This is not ideal, as proteins in reagent droplets may adsorb to the water-oil interface, which can undermine assay performance.[6] In addition, oil is problematic for integration with other on-chip functions that involve solid phases, organic solvents, and cell/organism culture. Here, we report the first magnetic particle-based immunoassay on DMF without using oil carrier fluid (i.e., droplets are surrounded by air). This change in format allowed the realization of a novel on-chip particle separation method, representing a significant improvement to the particle separation methods used previously. Using this technique, we developed methods for non-competitive and competitive immunoassays, using thyroid-stimulating hormone (TSH) and  $17\beta$ -estradiol (E2) as model analytes, respectively. We show that, compared to conventional methods, the new DMF approach reported here reduced reagent volumes and analysis time by 100-fold and 10-fold, respectively, while retaining a level of analytical performance required for clinical screening.

## EXPERIMENTAL

### Device fabrication and operation

Digital microfluidic devices were formed by standard photolithography and wet etching. The device design featured an array of 116 actuation electrodes ( $2.25 \times 2.25$  mm ea.) connected to 10 reservoir electrodes ( $4.5 \times 4.5$  mm ea.) with inter-electrode gaps of 30–80  $\mu\text{m}$ . Devices were assembled with an unpatterned ITO–glass top-plate and a patterned bottom-plate separated by a spacer formed from two pieces of double-sided tape (total spacer thickness 180  $\mu\text{m}$ ) (Figure 1A). Unit droplet and reservoir droplet volumes on these devices were  $\sim 800$  nL and  $\sim 3.5$   $\mu\text{L}$ , respectively. To drive droplet movement, an AC sine wave ( $\sim 150$  V<sub>RMS</sub>, 10 KHz) was applied between the top-plate (ground) and sequential electrodes on the bottom-plate via the exposed contact pads. The DMF system was coupled with a movable neodymium magnet, and its position is controlled manually using a metal clip.

### Immunoassay reagents

Key immunoassay reagents used on-chip included analyte standards (TSH or E2), antibody coated paramagnetic microparticles (Anti- $\beta$  TSH or Anti-E2), Horse-radish peroxidase (HRP) conjugated reporters (anti-TSH-HRP or E2-HRP), and chemiluminescent substrate (luminol/enhancer and H<sub>2</sub>O<sub>2</sub>). The DMF system accommodates all required assay reagents, allowing for the entire assay to be performed on-chip (Figure 1B). To enable droplet movement without oil carrier fluid, we added low concentrations of Pluronic L64 to all reagent droplets.

### Magnetic particle washing protocol

A wash-test assay was developed to evaluate the washing efficiency of the serial dilution and supernatant separation methods (Figure 2A, B). A wash-test suspension was prepared by resuspending anti- $\beta$  TSH particles in TSH conjugate. For serial dilution washing, DMF was used to merge and mix a droplet of wash-test suspension with a droplet of wash buffer. Next, the magnet was positioned such that the particles were immobilized to one side of the pooled droplet, the droplet was split into two daughter droplets, and the droplet not containing particles was moved to waste. After the magnet was removed, the droplet containing the particles was mixed to resuspend the particles in solution. For supernatant separation washing, particles in a droplet of wash-test suspension were first immobilized by the magnet and DMF was used to actuate the supernatant droplet away from the magnet to waste, leaving the particles immobilized on the device surface. Next, the magnet was removed, the particles were reconstituted in a droplet of wash buffer, and the droplet was mixed to resuspend the particles in solution. For both techniques, the washing procedure was repeated four times; after each wash step, the supernatant waste droplet was interrogated for peroxidase activity (Figure 2C).

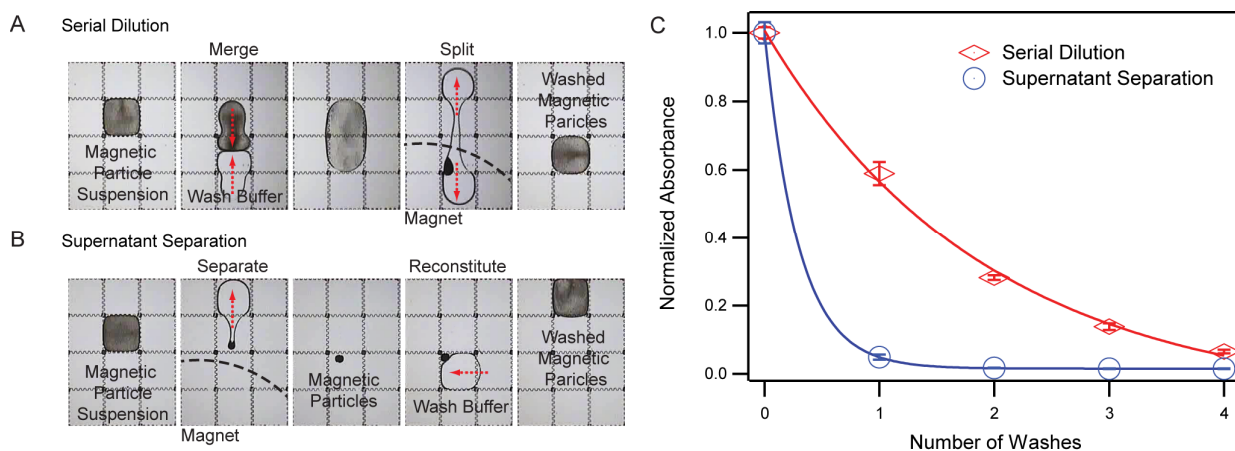


Figure 2: Comparison of magnetic particle washing protocols. (A) Video sequence of conventional serial dilution washing protocol. (B) Video sequence of supernatant separation washing protocol. (C) Comparison of the efficiency of serial dilution (red diamonds) and supernatant separation (blue circles) methods by measuring enzyme leftover in the supernatant after up to 4 wash steps. Error bars are  $\pm 1$  S.D. from three replicates.

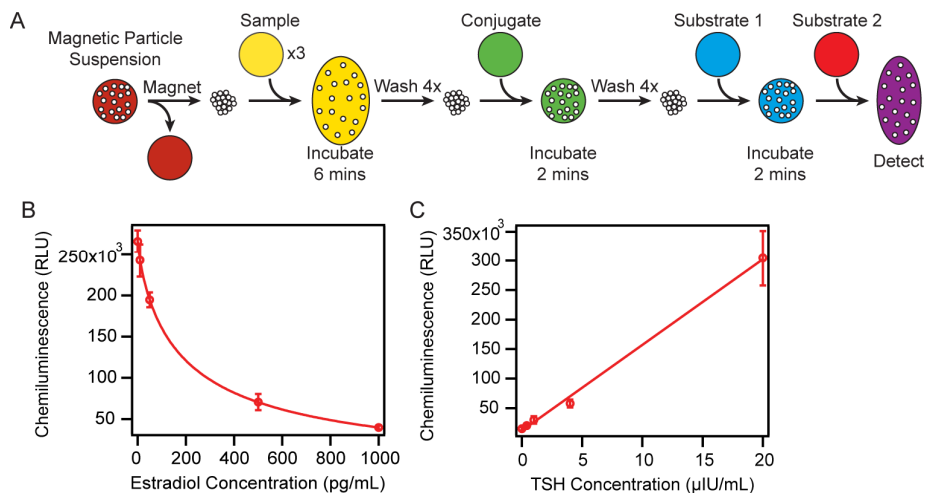
## RESULTS AND DISCUSSION

A key requirement in particle-based immunoassays is the ability to remove the unbound reagents from the surface of the solid support. All previous magnetic particle-based immunoassays implemented in DMF have used a "serial dilution" method [4] to wash unbound reagents from particles (Figure 2A). In this work, we used an alternative approach, termed "supernatant separation" (Figure 2B), which involves the separation of the supernatant from the particles. This washing protocol is more efficient, capable of removing greater than 90% of unbound reagents in one step (Figure 2C).

We optimized an on-chip particle-based immunoassay protocol using luminol-based chemiluminescent substrates, peroxidase-conjugated reagents, and a plate reader for detection (Figure 3A). This protocol was implemented for a non-competitive immunoassay and, for the first time in DMF, a competitive immunoassay, using thyroid-stimulating hormone

(TSH) and 17 $\beta$ -estradiol (E2) as model analytes, respectively (Figure 3B, C). The limit of detection of TSH and E2 are 0.83  $\mu$ IU/mL and 21 pg/mL, respectively, making these assays useful to screen for diseases such as hypothyroidism (TSH > 3.0  $\mu$ IU/mL) or ovarian tumor (E2 > 20pg/mL, depending on patient age).

There are two salient advantages of DMF for immunoassays: reduced consumption of reagent volumes and faster analysis time. As context for the former advantage (reagent consumption), a typical well-plate ELISA assay requires 50  $\mu$ L sample, 100  $\mu$ L conjugate, 900  $\mu$ L wash buffer, and 150  $\mu$ L substrate. In contrast, DMF immunoassay requires 2.4  $\mu$ L sample, 0.80  $\mu$ L conjugate, 6.4  $\mu$ L wash buffer, and 1.6  $\mu$ L substrate. This represents a 100-fold reduction in reagent consumption in the DMF method, from 1.2 mL to 11.2  $\mu$ L. For the latter advantage (analysis time), sample incubation in well-plate ELISA kits requires 60 minutes, while the DMF immunoassays only require 6 minutes of sample incubation time. This advantage is directly related to the reduction of reagent volumes—the same number of particles is packed into a smaller volume, increasing surface area-to-volume ratio and reaction kinetics.



*Figure 3: On-chip immunoassay protocol and calibration curves. (A) Scheme of droplet-based on-chip immunoassay. (B) 17 $\beta$ -estradiol (E2) competitive immunoassay. (C) Thyroid-stimulating hormones (TSH) sandwich immunoassay. Error bars are  $\pm 1$  S.D from four replicates.*

## CONCLUSION

We have developed the first particle-based immunoassay on DMF without the aid of oil carrier fluid. Compared to conventional techniques, this method reduced reagent volumes and analysis time by 100-fold and 10-fold, respectively, while retaining a level of analytical performance required for clinical screening. We propose that the new technique has great potential for use in a fast, low-waste, and inexpensive instrument for the quantitative analysis of proteins and small molecules in low sample volumes. This innovation will not only simplify device design, but also lead to integrated DMF devices in which complex samples can be pretreated and analyzed on a single device.

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