A DIGITAL MICROFLUIDIC PLATFORM FOR AUTOMATED IMMUNOASSAYS OPTIMIZED USING "DESIGN OF EXPERIMENTS" (DOE) METHODS

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ABSTRACT

We introduce an automated digital microfluidic platform capable of performing immunoassays from sample-to-analysis with minimal manual intervention. This platform features a 90 Pogo pin interface, an integrated photomultiplier tube, and an adjustable magnet. The new platform was used to implement a design of experiments optimization for immunoassays, resulting in an optimized protocol that reduced detection limit and sample incubation time by up to 5-fold and 2-fold, respectively, relative to previous work. We propose that this new platform paves the way for a benchtop tool that is useful for implementing immunoassays in near-patient settings around the world.

KEYWORDS: Digital Microfluidics, Immunoassay, Magnetic Separation, Design of Experiments

INTRODUCTION

The *in-vitro* diagnostic industry is dominated by robotic immunoanalyzers—the gold standard for high-throughput protein and small molecule quantitation [1]. These instruments are capable of quantifying disease biomarkers from patient samples at clinically relevant concentrations at a rate of hundreds of tests per hour. Importantly, the throughput of these instruments allows developers to rapidly determine optimal assay parameters via a Design of Experiments (DOE) approach, leading to reduced assay development and optimization timelines. Unfortunately, robotic immunoanalyzers are large, complex instruments found only in well-funded centralized facilities such as hospital reference laboratories, to which patient samples are transported after collection. As health care costs continue to rise, the global in-vitro diagnostic market is gradually shifting from centralized facilities to point of care testing. This market trend is facilitated by technological advances in nanomaterials, integrated sensors, and microfluidics. In particular, microfluidics is proving useful for the miniaturization of liquid handling, leading to the development of various microfluidic immunoassay systems and the commercialization of these platforms.

Digital microfluidics (DMF), a technique in which fluids are manipulated as discrete droplets on devices bearing an array of electrodes buried under an insulating dielectric [2], is a promising format for immunoassays. Recent work has seen DMF applied to implementing immunoassays in a format using an oil carrier fluid [3] and in an oil-free format [4]. Here, we report a significant advance over the state-of-the-art for DMF immunoassays, featuring three new characteristics: complete sample-to-analysis automation, parallel sample processing, and full factorial DOE optimization. The latter is a particularly significant advance; DOE is becoming increasingly important for maximizing information output from minimum experimental effort [5]. To date, however, there have been no reports of DOE optimization of microfluidic immunoassays (of any format), likely because of a lack of automation, parallelization, and control. We propose that this report will be a useful touchstone for future work applying microfluidic DOE to a wide range of applications.

EXPERIMENTAL

Device fabrication and operation

Digital microfluidic devices were formed by standard photolithography and wet etching as described previously [4]. The device design features an array of 80 actuation electrodes (2.2 × 2.2 mm ea.) connected to 8 reservoir electrodes (16.4 × 6.7 mm ea.) with inter-electrode gaps of 30-80 µm. 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 µm). Unit droplet and reservoir droplet volumes on these devices were ~800 nL and ~3.5 µL, respectively.

An automated platform was designed and built to manage droplet operation, magnet and photomultiplier tube (PMT) position, and data collection. Droplet movement was controlled via the open-source Microdrop software and an Arduino-based (Smart Projects, Italy) high-voltage switching instrument described in detail elsewhere [6]. A custom plugin for the Microdrop software was used to control the motors in the platform, read signals from their respective optical limit switches, and trigger PMT reading. To drive droplet movement, an AC sine wave (100-120 V_{RMS}, 10 kHz) was applied between the top-plate (ground) and sequential electrodes on the bottom-plate.

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Immunoassays and DOE Analysis

Using DMF magnetic separation for reagent exchange and particle washing as described previously [4], on-chip immunoassays for DOE analysis were implemented in seven steps: (1) A droplet containing paramagnetic particles was dispensed from a reservoir and separated from the diluent. (2) 1, 3 or 5 droplets (representing 0.8, 2.4, or 4 µL after merging) of TSH standards (0, 0.4, or 4 µIU/mL) were dispensed, delivered to the immobilized particles, and mixed for 3, 6, or 9 minutes. (3) The particles were washed four times in wash buffer and separated from the supernatant. (4) One droplet of HRP conjugate solution was dispensed, delivered to the immobilized particles, and mixed for 2 minutes. (5) The particles were washed five times in wash buffer; after the fifth wash, the particles were kept suspended in wash buffer and queued for analysis. (6) The particles were separated from the wash buffer, resuspended in one droplet of H_2O_2, and this droplet was merged and mixed (for 40 seconds) with one droplet of luminol-enhancer solution. (7) The pooled droplet was incubated for 100 seconds, and the chemiluminescent signal was recorded using the integrated PMT. In these assays, two experiments were run simultaneously and four replicates were evaluated for each condition. The ratio of the chemiluminescent signal from each sample relative to the average signal generated from the blank (0 µIU/mL TSH) was collected for each sample volume and incubation time. These measured signal-to-background ratio (SBR) responses were fitted via centered polynomial regression using a modified second-order model. Statistical analysis, model construction, and data interpretation were performed using the JMP 10 statistical analysis package (S.A.S. Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

We report here a new integrated platform (approximately the size of a shoebox) capable of performing complete immunoassays (i.e., sample in, analysis out) with minimal manual intervention. This instrument comprises three core components: a Pogo pin interface for digital microfluidic control, an integrated photo multiplier tube for chemiluminescent detection, and an adjustable magnet for particle separation; each component is controlled by a motor and two optical limit switches. Using this adjustable magnet, eight samples can be processed simultaneously (Figure 1), with the potential to extend to higher levels of multiplexing in the future.

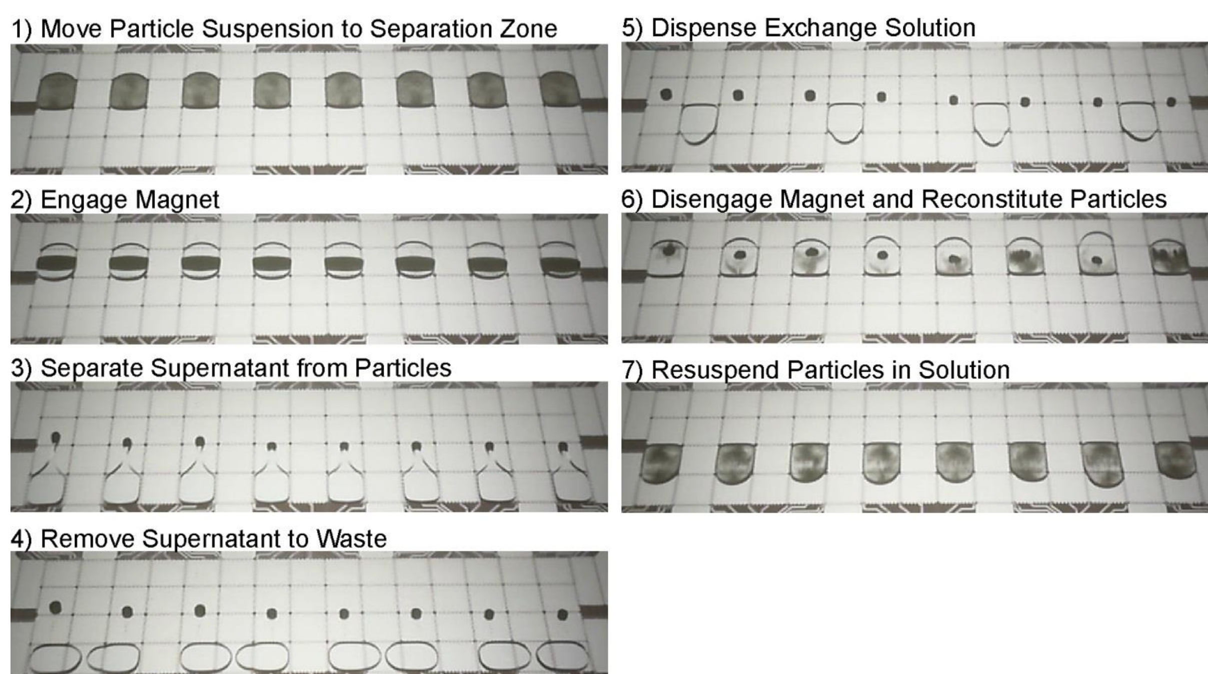

Figure 1: Frames from a movie depicting eight parallel particle separation on-chip position from magnet surface.

To test this platform, we implemented a three-level full factorial DOE optimization for thyroid-stimulating hormone (TSH) immunoassays, varying three factors: 1) analyte concentration, 2) incubation time, and 3) sample volume. Three different models were evaluated by fitting the experimental results obtained by the pilot on-chip immunoassays via regression analysis. The correlation between predicted and actual signal-to-background ratios with the two-factor interactions model is shown in Figure 2A. These results led to a prediction of optimal sample volume and incubation time for on-chip TSH immunoassay (Figure 2B). Relative to previous work [4], this new protocol improved the detection limit of TSH to 0.11 µIU/mL (the clinically relevant range is 0.4 - 2.5 µIU/mL [7]), while further reducing the analysis time (Table 1) mainly due to the parallel droplet actuation from the automation format.

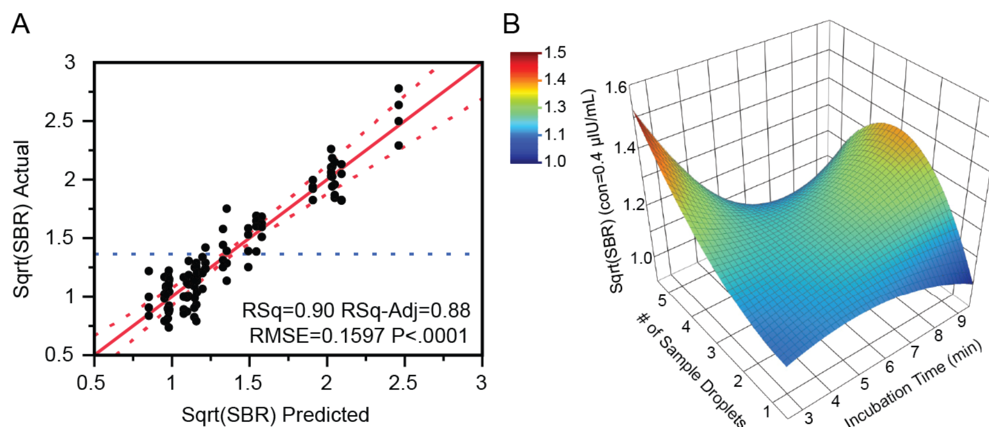

*Figure 2: Design of experiments (DOE) analysis for TSH magnetic particle immunoassays. (**A**) Actual versus predicted plot of signal to background ration (SBR) superimposed with confidence curves (0.05). The plot correlates model predictions to actual data; the P-value, R^2, adjusted R^2, and Root Mean Square Error for the correlation are indicated below the plot. (**B**) A three-dimensional SBR response surface at 0.4 µIU/mL TSH concentration showing the interaction between incubation time and number of sample droplets (heat map with red = high response, blue = low response).*

Table 1. Comparison of TSH immunoassay performance

Immnoassay system	Prototype Setup Described previously [4]	Integrated Platform
Coefficient of Variability	9-21%	6-10%
Limit of detection (absolute)	2.0 nIU	0.6 nIU
Limit of detection (concentration)	0.83 µIU/mL	0.15 µIU/mL
Incubation Time	6 minutes	3 minutes
# of parallel assays	1	8

CONCLUSION

We developed an automated digital microfluidic platform for magnetic particle-based immunoassays. This platform features a 90 Pogo pin interface for digital microfluidic control, an integrated detector for chemiluminescent detection, and a new magnet assembly that facilitates up to 8 simultaneous digital microfluidic magnetic separations. Most importantly, the new platform allows for the implementation of DOE optimization of immunoassay performance. As a test case, a three-level full factorial DOE analysis enabled the development of an optimized protocol that reduced detection limit and sample incubation time by up to 5-fold and 2-fold, respectively. We propose that this new platform has great potential for the quantitative analysis of disease biomarkers at various near-patient settings world-wide.

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