

DIGITAL MICROFLUIDICS FOR SCREENING ASSAYS

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ABSTRACT

We present the first methods combining digital microfluidics (DMF)-driven enzyme assays and cell-based assays with a multiplexed fluorescence plate reader. These results are an important step towards DMF-driven high-throughput screening.

KEYWORDS: digital microfluidics, enzymes, cells, high-throughput screening

INTRODUCTION

Integrated robotic workstations are revolutionizing the life sciences, enabling high-throughput screening (HTS) on an unprecedented scale. Such technology does not come cheap, however – robotic workstations are expensive, and the consumables costs (multiwell plates, pipette tips, chemicals, etc.) can make the running of even a single assay prohibitive. If these powerful methods are to be available to any but the most wealthy of laboratories, new HTS technologies must be developed.

One such technology is microfluidics – several research groups are developing microchannel-based HTS methods [1,2], which are characterized by reduced reagent consumption, analysis time, and assay cost. While these strategies are promising, they are limited by the difficulty of controlling many different reagents simultaneously in interconnected channels. We are developing new screening assays using an alternative paradigm for fluid transport, called digital microfluidics (DMF). In DMF, fluid is manipulated as discrete droplets on an open platform [3,4], which makes simultaneous control of reagents straightforward and facile. Here, we present the first methods combining DMF-driven enzyme assays and cell-based assays with a multiplexed fluorescence plate reader.

EXPERIMENTAL

Digital microfluidic devices were fabricated using established methods [5-7] in the University of Toronto Emerging Communications Technology Institute (ECTI) cleanroom facility. Briefly, gold and chromium electrodes were patterned by photolithography and wet etching; the electrodes were then coated with 2 μm of Parylene-C and 50 nm of Teflon-AF. In addition to patterned devices, unpatterned indium-tin oxide (ITO) coated glass substrates were coated with 50 nm Teflon-AF. Devices were assembled with an ITO-glass top plate and a patterned bottom plate separated by a spacer formed from one or two pieces of double-sided tape. Droplets were sandwiched between the two plates and actuated by applying driving potentials (80-150 V_{RMS} , 15 kHz) between the top electrode and sequential electrodes on the bottom plate.

The droplets used in all experiments were supplemented with pluronic additives [7] to minimize surface fouling. Quantitation was achieved by positioning DMF devices on plastic multiwell plates and inserting them into a fluorescence microplate reader (Pherastar, BMG Labtech, Durham, NC) equipped with a module for 480 nm excitation and 520 nm emission.

RESULTS AND DISCUSSION

To illustrate compatibility with enzyme assays, we used DMF to implement the model enzyme-substrate reaction of alkaline phosphatase (AP) with fluorescein diphosphate (FDP); a full description of this work can be found in Miller and Wheeler [5]. As depicted in Figure 1, droplets of AP and FDP were dispensed, merged, and mixed on a DMF device, resulting in a 140-nL stopped-flow reaction chamber. Substrate quantitation was achieved with a linear range of 2 orders of magnitude and a detection limit of approximately 7.0×10^{-20} moles. Analyses of the enzyme kinetics were performed and K_m and k_{cat} values of $1.35 \mu\text{M}$ and 120 s^{-1} agreed with those obtained in a conventional 384-well plate under the same conditions ($1.85 \mu\text{M}$ and 155 s^{-1}). As shown in Figure 1e, in on-going efforts, we have developed a multiplexed device capable of performing 10 assays simultaneously.

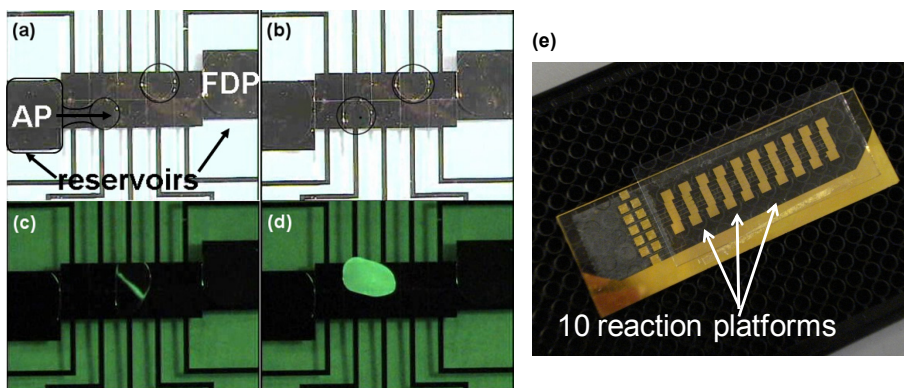


Figure 1. DMF for cell-free assays. **(a-d)** Sequence of frames from a movie depicting a typical assay between alkaline phosphatase (AP) and fluorescein diphosphate (FDP). **(e)** Picture of a device designed for simultaneous control of ten independent DMF-driven assays.

To illustrate compatibility with cell-based assays, we used DMF to implement cytotoxicity screens of Jurkat T-cells with the surfactant, Tween 20; a full description of this work can be found in Barbulovic-Nad et al. [6]. As depicted in Figure 2(a-f), in a typical assay, 150-nL droplets containing cells were dispensed and merged with 150-nL droplets containing Tween 20, and were then actively mixed by moving them on neighboring electrodes in a circle. After 20 min of incubation in a humidified environment, the combined droplets containing cells and Tween 20 were merged and mixed with 150-nL probe droplets containing viability dyes, and then incubated for a second time in a humidified environment (20 min). As shown in Figure 2(g), dose-

response curves were generated using the fluorogenic reporter, calcein-AM, and the sensitivity was ~20-fold higher than comparable assays implemented by pipetting in multiwell plates (not shown). In on-going work, we have demonstrated that there are no adverse effects of actuation by DMF on cell viability, proliferation, and biochemistry.

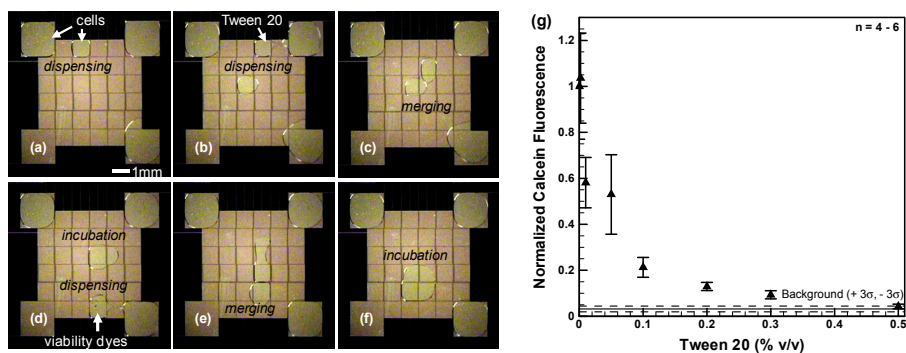


Figure 2. DMF for cell-based assays. **(a-f)** Sequence of frames from a movie depicting a cytotoxicity assay. **(g)** Plot of calcein-AM fluorescence (proportional to the number of living cells) as a function of the concentration of Tween 20.

CONCLUSIONS

In summary, we present the first marriage of digital microfluidics with a multiplexed fluorescence plate reader. The results suggest great potential for DMF in high-throughput screening, perhaps as a part of (or in addition to) the gold standard of robotic workstations.

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