

# INVESTIGATION OF ELECTROWETTING-BASED MICROFLUIDICS FOR REAL-TIME PCR APPLICATIONS

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## ABSTRACT

An investigation was conducted to determine the suitability of electrowetting-based droplet actuation for microfluidic PCR applications. We experimentally verified that droplets containing standard PCR reagents could be microactuated with the same facility previously demonstrated for droplets of deionized water or salt solution. We further verified that the manipulations and the environment inside the electrowetting chip did not inhibit PCR and we were able to successfully perform real-time PCR assays in 300 nL droplets within the electrowetting chip.

**KEYWORDS:** Droplets, electrowetting, PCR

## INTRODUCTION

The polymerase chain reaction (PCR) is a key technique that finds widespread use in modern molecular biology. The centrality of PCR has led it to become a major focus for miniaturization and automation through emerging microfluidic and lab-on-a-chip technologies. Electrowetting-based approaches which use electrical fields to directly manipulate discrete droplets have been gaining increasing interest in recent years. While the use of electrowetting arrays to rapidly transport, dispense, mix and split salt water droplets has been amply demonstrated by our lab and others [1-3], to date there have been few demonstrations of actual biological assays using these systems. We report here experimental results demonstrating allelic discrimination of a human single nucleotide polymorphism (SNP) using a real-time (TaqMan®) PCR assay performed on an electrowetting microfluidic chip.

## METHODS

Electrowetting chips were fabricated consisting of a bottom-plate with linear arrays of 800  $\mu\text{m}$  pitch square control electrodes and a top-plate machined with four 1.0 mm diameter circular holes aligned to the control electrodes beneath. The top and bottom plates were aligned and glued together with a spacer to provide a fixed 170  $\mu\text{m}$  gap. The substrates were glass and all electrodes and wires were patterned in indium-tin-oxide (ITO) so that the chip was transparent throughout. The entire chip was filled with silicone oil and placed inside a custom-built hot-air thermocycler which was mounted on a fluorescence microscope to permit real-time observation of the droplets contained inside (Fig. 1). The real-time PCR reagents were obtained from a commercially available TaqMan<sup>®</sup> allelic discrimination kit<sup>1</sup> which contained differentially labeled (FAM/VIC) fluorescent probes corresponding to each of the two alleles.

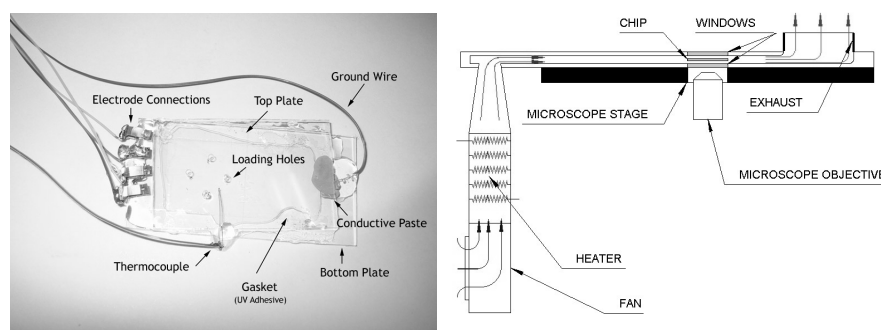


Fig. 1: LEFT: Photograph of the assembled electrowetting chip showing holes for dispensing microdroplets, thermocouple for PID feedback control, and electrode connections. The transparent electrode array lies in the region between the holes. The dimensions of the chip are 25  $\times$  50 mm. RIGHT: Schematic diagram of the custom-built hot-air thermocycler system.

## RESULTS AND DISCUSSION

In order to establish the feasibility of the electrowetting microfluidic platform for PCR-based applications we performed experiments to address three key issues. The first was the effect of PCR reagents on electrowetting-based microfluidic manipulations. Our

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<sup>1</sup> ABI PN:4312559, allelic discrimination kit for C440T mutation of the *CYC2C9\*2* gene.

standard electrowetting microfluidic operations (i.e. transport, mixing, splitting, dispensing) were tested using droplets of PCR mixture and it was found that the addition of PCR reagents had little noticeable effect on our ability to manipulate droplets compared to standard electrolyte droplets (i.e. 0.1 M KCl).

The second issue we investigated was the effect of the electrowetting system and manipulations on the quality of the PCR reaction. Droplets (~300 nL) of complete PCR mix containing each of four allelic controls<sup>2</sup> were dispensed into the chip using electrowetting and subsequently transported back and forth several hundred times under electrowetting control. The droplets were then positioned at opposite ends of the array, the control voltage was removed and the entire chip was thermocycled. Fluorescence time-courses revealed successful amplification of DNA within the droplets and sufficient specificity was obtained in order to permit discrimination of alleles differing in only one base pair (Fig. 2).

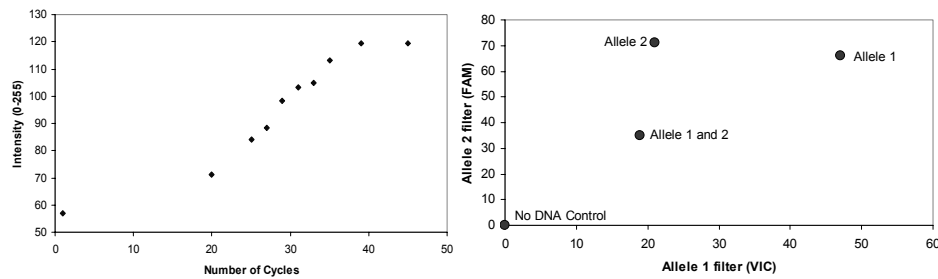


Fig. 2: Real-time (TaqMan®) PCR results in the electrowetting chip. A typical time-course indicating DNA amplification (left) and allelic discrimination of four droplets each containing differentially labeled allelic controls (right).

The third issue we investigated was the potential for cross-contamination between droplets either through the oil phase or by transport over common surfaces. A simple experiment was conducted in which two droplets, one containing control DNA template and one containing no DNA were programmed to flow around the chip in a cross-intersecting flow pattern for 5,000 cycles. Each cycle of flow included 3 transfers on electrodes common to the flow paths of both droplets, providing 15,000 opportunities for transfer of DNA through contamination of the surfaces or the oil. The droplets were then collected, added to PCR mix and amplified on a conventional real-time PCR system. The results shown in Fig. 3 indicate that no substantial cross-contamination could be detected between the droplets. The results confirm the basic compatibility of electrowetting microfluidics for PCR applications.

<sup>2</sup> i.e. allele 1 template only, allele 2 template only, both templates, no template

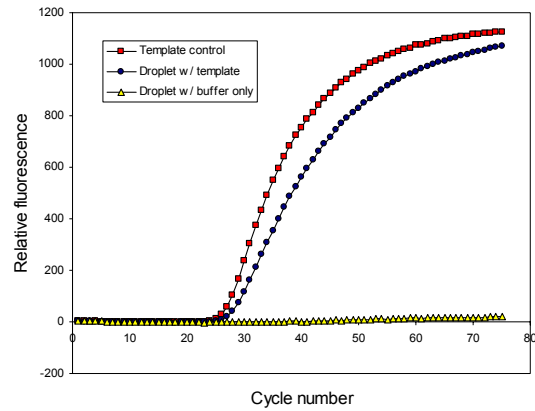


Fig. 3: Cross-contamination results. Droplets both with and without DNA were collected after 15,000 intersecting transfers, added as the template component to a PCR mixture and amplified in a commercial real-time PCR system.

#### REFERENCES

- [1] M.G. Pollack, R.B. Fair and A.D. Shenderov, *Appl. Phys. Lett.*, 77 (11), 1725-1726 (2000).
- [2] M.G. Pollack, A.D. Shenderov and R.B. Fair, *Lab Chip*, 2 (2), 96-101, (2002).
- [3] J. Lee, H. Moon, J. Fowler, T. Schoellhammer and C.J. Kim, *Sens. Actuat. A*, 95 (2-3), 259-268, (2002).