AUTOMATED SAMPLE PREPARATION PLATFORM FOR NEXT GENERATION DNA SEQUENCING USING A DIGITAL MICROFLUIDIC HUB
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ABSTRACT
We have demonstrated a novel capillary interface enabling highly repeatable transfer of liquid between a digital microfluidic (DMF) platform, which functions as a central fluid distribution hub, and multiple sample processing subsystem modules. This unique capillary interface allows both continuous-flow and droplet-based sample manipulations to be performed in one integrated system for automating library preparation steps for next-generation sequencers. Here we describe the use of this capillary-to-DMF interface in conjunction with an in-line contactless conductivity detector to enable and automate the closed-loop fraction collection and sorting of analyte droplets delivered to the DMF device.

KEYWORDS: Digital microfluidic hub, capillary interface, next generation sequencing, library preparation, fraction collection, automated droplet sorting

INTRODUCTION
While the throughput and speed of next generation sequencing (NGS) technology is advancing at an unprecedented rate, the library preparation technology still relies primarily on manual bench-top processes, which can often be slow, labor-intensive, inefficient, and inconsistent. Automation of sample preparation using microfluidic techniques is well-suited to address many of these limitations. However, fabricating a monolithic microfluidic device that replicates all the relevant bench-top processes can be prohibitively complicated or lack the flexibility to execute the diverse protocols for processing different sample types and volumes. We are developing a nucleic acid sample preparation platform to generate DNA libraries for interfacing with NGS (Figure 1). Our goal is to detect unknown pathogens by enriching informative nucleic acid sequences (those derived from the pathogen) and suppressing background DNA (those from the host) to maximize the sensitivity of state-of-the-art NGS. The system leverages the capabilities of a central DMF platform with a novel the capillary interface to provide flexibility in integrating different sample processing subsystem modules. Moreover, the DMF platform allows the precise temporal and spatial manipulation of microliter-scale droplets containing nanograms of DNA to accomplish the library sample processing steps.

EXPERIMENTAL METHODS
DMF Platform and capillary interface
The platform consists of an engineered polymer frame with opposed recesses that automatically register the DMF substrates relative to each other, yielding a closed-format DMF device with a fixed substrate-to-substrate spacing of 185 µm. Capillary tubes with a 150 µm outer diameter are positioned in the interstitial space between the DMF electrode and ground plane substrates and are fixed in place using CapTite™ capillary fittings (Sandia National Labs, Livermore, CA). The hy-
drophobically-coated fused-silica capillaries enable the transfer of sample droplets between the central DMF device and processing modules. DMF devices are fabricated by patterning an electrode array onto a 50 x 75 mm ITO-coated glass slide (Delta Technologies, Stillwater, MN), coating the electrodes with dielectric material (parylene C), and spinning Teflon AF onto the coated surface to render it hydrophobic. A Teflon-coated ITO glass slide without any pattern is used as the ground plane. Droplets are actuated by applying an optimized AC voltage pulse (typically ~50-100 Vrms at 15 kHz) to the DMF electrodes. A computer-controlled electronic interface allows the activation of individual electrode pad(s) either by manual keystrokes or predetermined scripted sequences. Droplet actuation is monitored with an MVX10 (Olympus, Center Valley, PA) microscope with a high speed digital camera (QIClick, Qimaging, Surrey, Canada). Further details of the DMF platform and its operation are described elsewhere [1].

**Automated fraction collection**

For the automated fraction collection experiment, a syringe pump is used to deliver a continuous flow of “background” buffer solution (10 mM Na$_3$PO$_4$ with 0.005% w/v SDS) at 15 µL/min through a Teflon-coated capillary onto the DMF platform. A 5 µL “sample” bolus of high ionic strength (160 mM Na$_3$PO$_4$ buffer with 0.005% w/v SDS) containing fluorescein dye is introduced upstream of the DMF interface with an HPLC-style 6-port injection/switching valve (Valco EHMA, Houston, TX). The conductivity of the liquid delivered through the capillary to the DMF device is monitored by a contactless, flow-through conductivity detector (Edaq C4D, Denistone, Australia). A second capillary also interfaced to the DMF platform is connected to a vacuum line to dispose of “waste” droplets as needed. Custom control software implemented using Labview 10 (National Instruments, Austin, TX) collects digitized conductivity measurements and triggers automated, synchronous droplet actuation on the DMF platform to accomplish fraction collection and sorting. Droplets identified as containing high conductivity sample are automatically routed and parked at predetermined collection locations while those lacking sample content are actuated into contact with the waste capillary for aspiration by the vacuum.

**RESULTS AND DISCUSSION**

**Droplet dispensing through a capillary interface**

Figure 2 illustrates the process of dispensing a droplet from a continuous flow stream of 10 mM Na$_3$PO$_4$ buffer through a Teflon-coated capillary and onto the DMF platform. The dispensed liquid forms a droplet at the tip of the capillary tube, overlapping the receiving electrode and growing to a size at which it can be moved away by applying voltage to that electrode. The hydrophobic coating of the capillary and substrates prevents the dispensed liquid from wicking backwards along the capillary during droplet formation. Once the droplet reaches a threshold volume of approximately 2 µL, 300 ms voltage pulses are applied to the receiving electrode and the neighboring pad in sequence to overcome the capillary-face surface tension and separate the droplet cleanly from the capillary as shown in Figure 2. DMF actuation forces can reproducibly separate droplets with high precision: the volume of seven droplets serially dispensed from a capillary with a 15 µL/min flow rate was determined to be 2.25 ± 0.02 µL by microscope image analysis.

![Figure 2](image)

*Figure 2: Captured images of the sequence of a 2 µL droplet dispensed through the capillary interface and separated from the capillary by DMF actuation.*

**Automated fraction collection**

The automated fraction collection experiment is illustrated in Figure 3, which shows the conductivity trace of a continuous buffer flow with two sample bolus injections and video frames of the automated processing of sample and waste droplets on the DMF device. Based on their measured conductivity, individual collected sample droplets were automatically routed in succession to predefined “parking spaces” on the DMF platform. Droplets that did not contain the eluted sample fraction were automatically actuated to the waste pad and pulled off the platform using the vacuum as shown in Figure 3. As expected, dispensed droplets containing only the background buffer showed no significant fluorescence.

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Real-time conductivity monitoring can detect the distinct step-changes in buffer concentration corresponding to the elution conditions of a general chromatography separation method. For example, it can be applied to selective collection and sorting of single stranded DNA and double stranded DNA fractions separated by a hydroxyapatite separation column. The approach presented here can be readily adapted for use with other in-line flow-through sensors as a generalized method for automating the subsampling of heterogeneous continuous or segmented flows onto droplet-based DMF devices.

CONCLUSION

In this work, we have demonstrated the high degree of reproducibility with which fluids may be transferred to and from a DMF device through an in-plane capillary interface, enabling the collection of target analyte fractions from a continuous flow stream. The automated fraction collection experiments have further demonstrated the benefits of the DMF platform and capillary interface for automatically selecting and archiving fractions from a continuous sample stream applicable to automating the DNA sample preparation workflows for NGS. Future work will use the capillary interface to couple additional library preparation processing modules to the DMF platform, including microreactor chambers for enzymatic reactions, low-volume PCR for DNA amplification, and electrophoretic gel separations for quantitative analysis.

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REFERENCES


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