AUTOMATED SAMPLE PREPARATION PLATFORM FOR NEXT GENERATION DNA SEQUENCING USING A DIGITAL MICROFLUIDIC HUB

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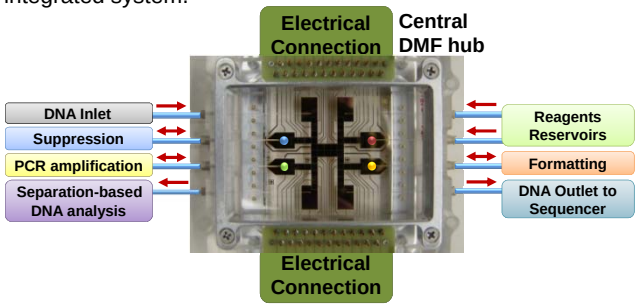
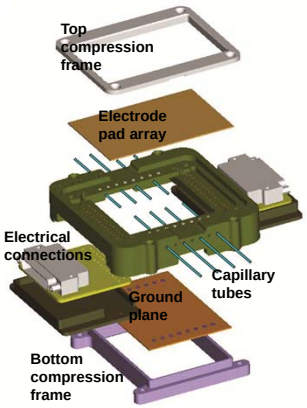
Abstract

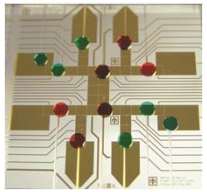
We have developed a digital microfluidic (DMF) platform functioning as a central fluid distribution hub integrated with multiple sample processing subsystem modules to automate library preparation steps for the next-generation sequencing system (NGS). A novel capillary interface enables highly repeatable transfer of liquid between the DMF device and external fluidic modules, allowing both continuous-flow and droplet-based sample manipulations to be performed in one integrated system.

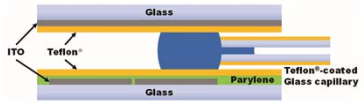


DMF Hub Platform

The platform enables plug-and-play installation of a two-plate DMF device with consistent spacing of 185 µm. This spacing enables the insertion of Teflon-coated glass capillaries with less than 170-µm outer diameter into the interstitial space between the DMF and ground plane substrates for flexible connectivity to transfer samples between modules via the DMF hub.



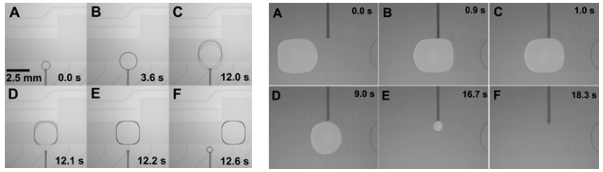




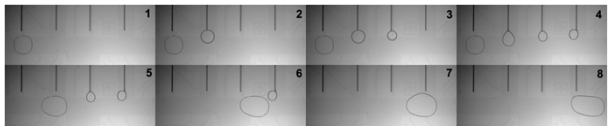
Left: Droplets in contact with capillaries on the gold DMF device.
Right: Cross sectional view of the DMF platform with an in-plane Teflon-coated capillary in contact with a droplet.

Capillary interface

Liquid dispensing and aspiration through the capillary interface have been tested. In case of dispensing, DMF actuation forces can reproducibly separate droplets with high precision: the volume of seven droplets serially dispensed from a capillary was determined to be 2.25 ± 0.02 µL by microscope image analysis.



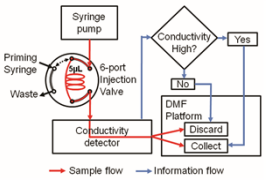
Left: A 2-µL droplet dispensed through a capillary, separated and actuated away.
Right: A 2-µL droplet actuated to make a contact with a capillary and cleanly aspirated.

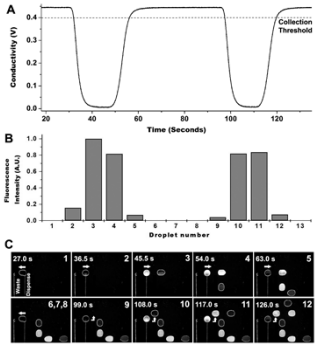


A 2-µL droplet is mixed with three submicroliter reagent droplets dispensed through capillaries by actuating horizontally along the capillary-interfacing pads.

Fraction collection on DMF

Using an in-line contactless conductivity detector in conjunction with the capillary interface, we have demonstrated closed-loop automated fraction collection of target analytes from a continuous flow sample stream into droplets on the DMF device.





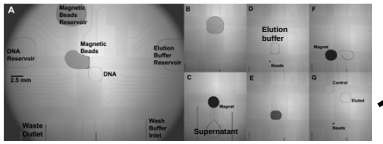
Conductivity trace of the incoming buffer flow through the capillary.

Fluorescence intensity of the dispensed droplets.

Hybrid images of dark field and fluorescence microscopy.

Buffer exchange/ Clean up

Buffer exchange and sample clean-up, the most repeated steps in NGS library preparation, are also demonstrated on the DMF platform using a magnetic bead assay and achieving an average DNA recovery efficiency of 80±4.8%.

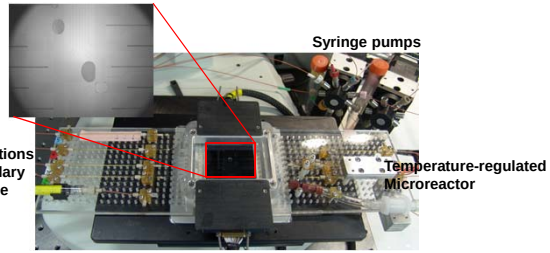


Fluorescence-based DNA quantification.

Droplets of DNA and magnetic bead solution are split from their respective reservoirs (A), merged together, and actively mixed (B). Cylindrical magnets placed on the DMF hold the DNA-bound beads in place so that the supernatant can be removed (C) and the beads are thoroughly washed with ethanol (not shown). A droplet of elution buffer is introduced (D) and mixed with the beads (E) to unbind the DNA from the beads, which are then magnetically separated from the eluted sample (F). The separated beads, the eluted DNA droplet, and a reference control DNA droplet are then imaged for fluorescence analysis (G).

Integration with NGS

We are working towards integrating this platform with next generation sequencers to ultimately detect unknown pathogens by enriching informative nucleic acids sequences (from the pathogen) and suppressing background DNA (from the host) with an upstream suppression module.



Acknowledgements

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