Droplet-Based Digital Microfluidics For Sample Collection

By
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A PROJECT

submitted to

Oregon State University

University Honors College

in partial fulfillment of
the requirements for the
degree of

Honors Baccalaureate of Science in Bioengineering
(Honors Scholar)

Presented May 27, 2015
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Abstract approves:

David Hackleman

Technology is constantly co-evolving with science to produce simple and effective tools that address the needs of researchers. Cost and time effective point-of-care devices are valuable for sample collection and analysis, particularly in medicine and environmental health and safety. Digital microfluidic (DMF) technology provides a possible solution. This study explores the application of DMF as a tool for collecting and analyzing Sputum and exhaled breath condensate (EBC) samples and Environmental dry particle samples.

An assay was created that used DMF technology developed at Sandia National Laboratories to collect and analyze sputum, EBC and environmental dry particle samples. Arizona sample dust was sprinkled onto the DMF device and collected using a 3 μL water droplet that moved by electrowetting until the drop was unable to move. In sputum sample analysis, a 0.05% BSA mock sputum was sprayed onto the DMF board using a perfume bottle. EBC was collected on a chilled ITO slide. Both sputum and EBC were collected by a 3 μL droplet of Pluronic® F-127. Dust was visibly picked up, picking up 2/3 of it's own weight in dust. The pluronic droplet increased 4-6 fold in the EBC collection and a concentration of 1.6 mg/mL of BSA was collected.

Key Words: Microfluidics, biological, environmental

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I understand that my project will become part of the permanent collection of Oregon State University, University Honors College. My signature below authorizes release of my project to any reader upon request.

Branden Kusanto, Author

Introduction

Digital microfluidics has been an interest to engineers for its versatility since the late 1990s. One of the main goals of digital microfluidics is creating a wet lab on a handheld chip where one can perform data analysis and sample collection. Researchers address this by using a microliter-sized droplet placed onto the device, which can then be manipulated by various mechanisms such as electrowetting, [5,11,13] dielectrophoresis, [6] thermocapillary transport, [2] and surface acoustic wave transport. [15] Furthermore, another goal for companies is to create a device that is cheap and disposable after performing a certain set of operations.

Historically, microfluidic devices have been designed from the bottom up, combining components that are needed to perform a particular application. Companies and research groups have been creating highly specialized components for their own microfluidic devices. This has hindered the creation of standard commercial parts for microfluidic devices to be used for multiple purposes.

At Sandia National Laboratories, the digital microfluidic device (DMF) that they created was designed to be versatile, cheap, and disposable. It is comprised of multiple parts and Figure 1 shows an exploded assembly view of Sandia's DMF device. Two compression frames on both sides of the device hold the device together with the central manifold frame in between the PCB electrode board and glass slide. The central manifold frame carries the electrical wiring to make electrowetting possible and holes for capillaries to be inserted into the device for introducing and extracting various liquid samples. The central frame can hold seven capillaries on either side of the device and are all coinciding with the long sides of

the patterned electrode substrate. The ports are placed for precise positioning for wires, fiber optics, liquids, or other elements into the DMF device. These capillary tubes can be left in position for extended periods of time with standard CapTite™ capillary ferrules. These ferrules can be installed or removed whenever necessary. The inside and outside of the capillary tubes are coated with Teflon or another super hydrophobic solution to prevent liquids from adhering to the tube surface. Figure 2 shows a cross section of a droplet being introduced into the device. An example of a liquid being introduced into the DMF device and breaking off from the capillary tube after the proper amount of liquid was dispensed and extracted is shown in Figure 3.

Sandia's DMF device uses electrowetting for liquid transport. Figure 4 is a cross section of the DMF and shows how the droplet moves from electrode to electrode. The droplet is placed in between a hydrophobic PCB board that is patterned with individual, addressable electrodes to define the path of the droplet and a hydrophobic glass slide with an indium tin oxide (ITO) conductive layer on top of the droplet. The patterned array is coated with an insulated material to prevent shortage. The hydrophobic layers are used to increase the contact angle between the droplet and the DMF to allow easier movement when electrodes are actuated. When the droplet needs to move in a certain direction, the nearest electrode is energized through a remote controlled device and causes the droplet to move. When an electric field is induced, the contact angle of the leading edge of the droplet is reduced, creating an imbalance in surface tension of the leading and trailing edge of the droplet to cause the droplet to move.

A better understanding of the physics of droplet actuation is derived from electromechanical analysis. This analysis explains both the wetting and droplet movement phenomena in terms of electrical forces that are formed on free charges in the droplet meniscus. These forces can be estimated by integrating the Maxwell-Stress Tensor, T_{ij}, over any arbitrary surface around the droplet, which is shown in Equation 1. [1, 8,10]

$$T_{ij} = \left(E_i E_j - \frac{1}{2}\delta_{ij}E^2\right) \qquad [1]$$

ε is the dielectric constant of the medium surrounding the droplet, *i* and *j* represent the pairs of x, y, and z axes, d_{ij} is the Kronecker delta, and E is the applied electric field. This formula explains the motion of dielectric liquids and liquids that do not experience a change in contact angle.

Unlike continuous-flow microfluidic devices, digital microfluidic devices are software-driven electronic controlled, which eliminate the need for mechanical tubes, pumps and valves. Furthermore, droplets in digital microfluidics can be merged, split, transported, mixed and incubated by specific electrodes. This is one main advantage of digital microfluidics. There are many other advantages, which are as follows: [6]

- No moving parts: All operations are carried out between the two plates under direct electrical control without any use of pumps or valves
- No channels are required: The gap is simply filled with liquid. Channels only exist in the virtual sense and can be instantly reconfigured through software.
- Many droplets can be independently controlled: Because the electrowetting force is localized at the surface.

- Evaporation is controlled/prevented: Depending on the medium surround the droplets.
- Low ohmic current: Although capacitive currents exist, direct current is blocked, thus sample heating and electrochemical reactions are minimized.
- Works with a wide variety of liquids: Most electrolyte solutions will work.
- Near 100% utilization of sample or reagent is possible: No fluid is wasted for priming channels or filling reservoirs.
- Compatible with microscopy: The use of glass substrates and ITO transparent electrodes makes the chip compatible with observation from a microscope.
- Low energy use: Nanowatts-microwatts of power per transfer
- High speed: Droplet speeds of up to about 25 cm/s achieved.
- Droplet-based protocols are functionally equivalent to bench-scale wet chemistry: Thus established assays and protocols can simply be scaled down, automated and integrated.
- Conditional execution steps can be implemented: Direct computer control of each step permits maximum operational flexibility.

Currently, digital microfluidic devices are being used in the fields of chemistry, biology, and medicine. Chatterjee et al. utilized the DMF device to demonstrate the actuation of organic solvents such as acetone, acetonitrile, ethanol, and many others for microreactors. Millman et al. synthesized micro-particles such as semiconducting microbeads, capsules, and “eyeball” particles. This was done by mixing and merging different types of particles with a suspension of micro/nano

particles, polymer solutions, and polymer precursors. Digital microfluidics can be of use in handling DNA. An early study by Jary et al. handled DNA and repaired oxidized lesions in oligonucleotides. As large volume techniques for cell based assays are rather expensive, digital microfluidics offer a cheaper alternative. Bogojevic et al. created a digital microfluidic device for multiplexed cell-based apoptosis assays using HeLa cells. This method generated a comparable dose-response profile (of caspase-3 activity as a function of Staurosporine concentration) relative to conventional techniques, along with lower detection limits, greater dynamic range for generated data, and a 330-fold reduction in reagent consumption. The Fair group, [16] developed a series of glucose assays in physiological fluids with digital microfluidics, and Sista et al. developed a digital microfluidic device to extract DNA from whole blood samples using magnetic beads.

As previously mentioned, the current approach to develop digital microfluidic devices is to create highly specific parts to perform a certain function, limiting the function of the device. Creating a cheap and disposable universal digital microfluidic device that can be used in multiple fields would be beneficial for quick collection and analysis and remote controlled experiments.

This study aimed to use the DMF device that was created at Sandia National Laboratories and find multiple ways in which this device could be used for collection and analysis. By looking at a specific field of interest, individual assays were developed and data was collected to see if viable. After discussion, the group believed that the DMF device could prove beneficial for environmental and biological analysis. For environmental collection and analysis, dust would be

collected on the DMF board and concentrated using a 3 μL droplet. This would be used for scenarios to remotely test the dust in an area where there is known radioactivity levels or hard to reach areas. The group wanted to look into the field of medicine and try to use the DMF device to help diagnose patients through their breath and sputum. It is thought that if the DMF device could help diagnose lung diseases through their breath and cough.

Materials and Methods

Materials

All materials and supplies were provided by Ken Patel and Sandia National Laboratories.

Preparing PCB Board and Insulated Glass Slide

Preparation of all PCB boards and ITO glass slides were performed in a clean room before use. Both the boards and sliders were cleaned with ethanol and dried using an air gun to remove and dust particles that would cause an uneven hydrophobic coating. After cleaning, the boards and slides were coated with Teflon dissolved in FC40, spun at 1500 RPM for 30 seconds, and placed in an oven at 100 °C for 4 hours.

Environmental Dry Particle Assay

Sample test dust shipped from Arizona was dispersed randomly throughout the DMF board. This was done by placing dust on a sheet of a paper that was charged through static charge. The sheet of paper was then tapped lightly to release dust particles randomly on the board, one electrode was left untouched by dust for the introduction of a 3 μL droplet. The droplet was dispensed through a pipette after

an adequate amount of dust was put on the board, which is shown in Figure 5a. The DMF device was then assembled and the droplet moved across the DMF board picking up dust until it could no longer move through electrowetting (Figure 5b). The droplet was then extracted using a pipette and put on a pre weighed weigh boat, and placed on a hotplate to let the water evaporate. After evaporation, the sample was weighed to determine the amount of dust collected from the droplet.

Exhaled Breath Condensate Assay

ITO slides were placed in a refrigerator and chilled for two hours. When ready, the ITO slide would be taken out and breathed on. tThe DMF device would be assembled with a 3 μL droplet of 0.05% Pluronic® F-127, which is shown in Figure 6a. The droplet went across the DMF board until it could no longer move like in Figure 6c. The size of the droplet was measured before and after EBC collection.

Mock Sputum Assay

0.2 mL of mock sputum made of 0.05% BSA was sprayed onto a PCB board and a 3 μL of 0.05% Pluronic® F-127 droplet was placed on the board. The device was then assembled and the droplet collected the mock sputum until it could no longer move due to saturation shown in Figure 7. The droplet was then extracted through a pipette and protein concentration was quantified using a Nano-Drop 1000.

Results and Discussion

Environmental Dry Particle Assay

Arizona test dust (1-5 microns) was spread over the PCB board and a 3 μL droplet was used to pick up dust particles until it could no longer move. After eight

trials, it could be visibly seen that the droplet picked up dust particles as it moved across the board. On average, 88% of the droplet's weight was composed of dust particles after the experiment was completed. The weight of the droplet was determined by multiplying the volume of water dispensed onto the board before the experiment started by the density of water. The droplet was placed on the hot plate for an extended period of time to ensure that all water has evaporated and record the weight of dust that was collected.

Exhaled Breath Condensate Assay

An ITO glass slide was chilled in a refrigerator and breathed on before assembling the DMF board with 3 μL of 0.05% Pluronic® F-127. The pluronic droplet was then used to pick up as much condensate within 30 seconds. The volume of the droplet was measured at the end of the experiment and determined that the droplet increased 4-6 fold. The droplet was extracted and placed in a microfuge tube to be used for analysis. After collecting a sufficient amount of samples, the droplet would be analyzed with a mass spectrophotometer. However, time restraints restricted the opportunity.

Mock Sputum Assay

A mock sputum was created with a 0.05% BSA solution, which was verified by a Nano-Drop 1000, and placed in a spray bottle which was then used on the PCB board. The DMF device was assembled with a 3 μL of 0.05% Pluronic® F-127 droplet. After six trials, it was determined that an average of 1.6 mg/mL was collected with a standard deviation of 1.4 mg/mL. There was a high variability in the data as it was hard to determine when the droplet stopped moving due to

biofouling. Pressure was applied on the ITO glass slide to aid in droplet movement to determine the amount of BSA the droplet could physically pick up without biofouling. It was observed that the chances of biofouling increased if the mock sputum was left on the PCB board. This probably occurs because the mock sputum has time to degrade the hydrophobic Teflon layer. Another possibility that could have occurred is that the BSA stuck to the hydrophobic layer, creating a new surface and making the droplet immobile.

Cost

Costs of the DMF device is shown in table 1. In 2012, the cost of the Sandia Digital Microfluidic Hub would cost less than $3,000 per unit. The reusable PCB boards and ITO slides approximately cost $50-$75 each. However, with higher production and production at scale outside of Sandia, it is projected to cost $1,000, and less than $5 for the hub and reusable boards respectfully. If mass-produced outside of Sandia National Laboratories, this product will prove beneficial in terms of finding a cheap, disposable and universal DMF device for collection and analysis.

Conclusions

Three experiments were conducted to see if a digital microfluidic device designed by Sandia National Laboratories would prove beneficial in multiple fields. These experiments were an exhaled breath condensate (EBC) assay, a mock sputum assay, and an environmental dry particle assay. Both the EBC and the environmental dry particle assay proved that the droplet used in the device was capable of increasing its size four to six fold and 88% of the droplets weight respectfully. The DMF device was able to collect mock sputum, but biofouling significantly affected

droplet movement. Further investigation on a suitable hydrophobic material would need to be conducted to prevent biofouling. These preliminary results can now be used towards multiple fields of science. In the field of medicine, the EBC and sputum collection and analysis can be used for respiratory and cardiovascular disease and detection. Along with this, the DMF device can be used for biosurveillance of aerosolized pathogens by concentrating the pathogens in a small microliter sized droplet. The dry particle dust collection and analysis can be used for nuclear radiation detection in fall out areas. A DMF device can remotely pick up radioactive dust and concentrate it into a droplet for analysis. Furthermore, the DMF device can be used in space exploration by collecting and analyzing air and soil samples. Finally, the device can help monitor air quality in homes and the environment.

Sandia's DMF device can help start the creation of a DMF device for use in multiple fields. Furthermore, this device will only require a small investment for the DMF hub, and the PCB boards and ITO slides can be made incredibly cheap through mass production. This universal, cheap, disposable device can be used to help save lives and analyze unknown places.

A) B)

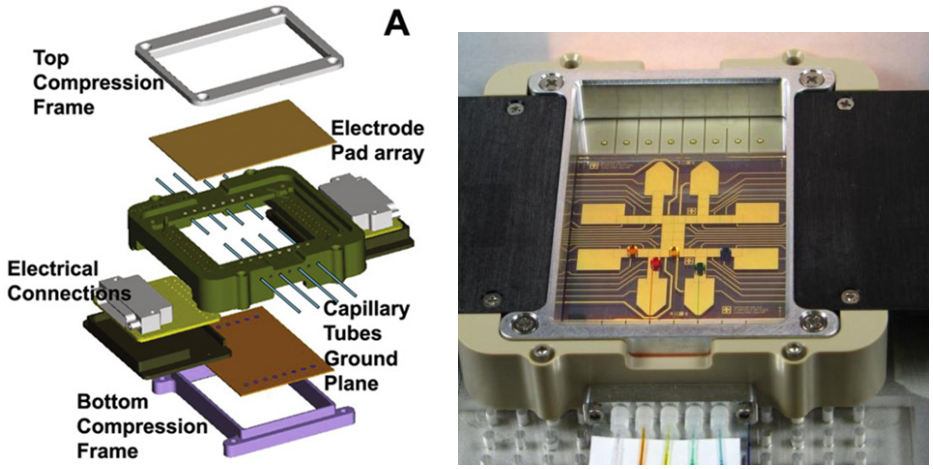


Figure 1: a) Exploded view of the Sandia Digital Microfluidic Hub with capillary interface. B) The Digtial Microfluidic Hub when assembled. PC: Hanyoup Kim

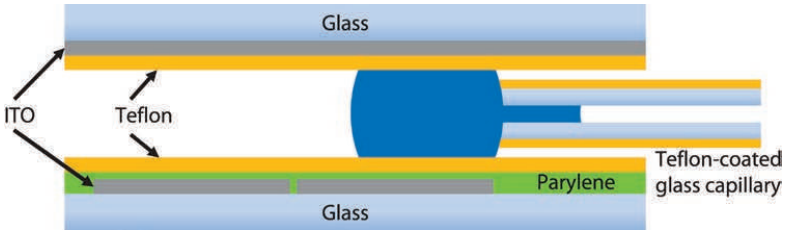


Figure 2: Cross section of Sandia's DMF device showing how a droplet can be inserted into the device from a micro capillary. PC:Hanyoup Kim

A) B)

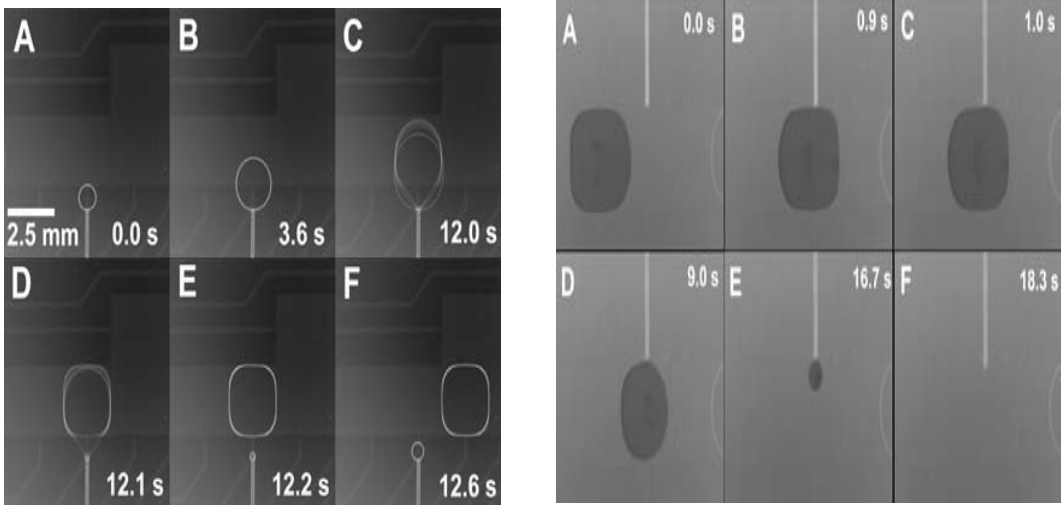


Figure 3: A) The introduction of a droplet of liquid entering the device and detaching from the capillary. The capillary tip is coated with a super hydrophobic coating to prevent liquid from adhering to the tip. B) Extracting a droplet from a DMF device. The droplet would move towards the electrode where the capillary is located and then extracted using a syringe. PC: Hanyoup Kim

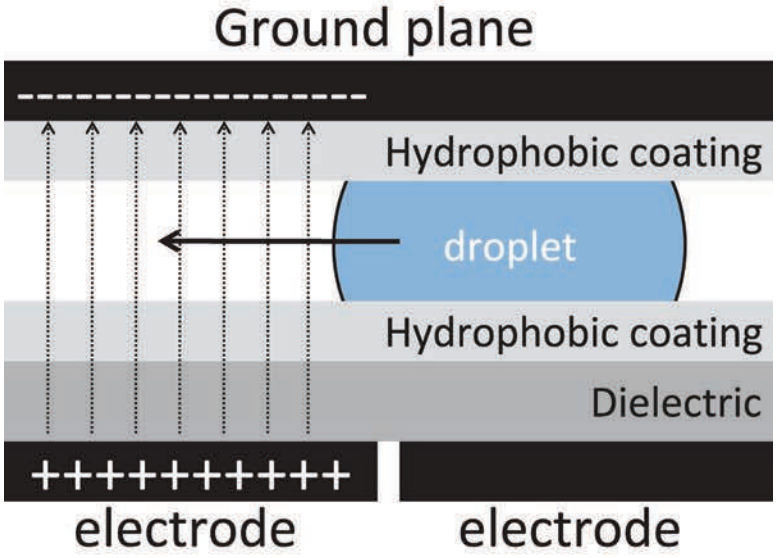
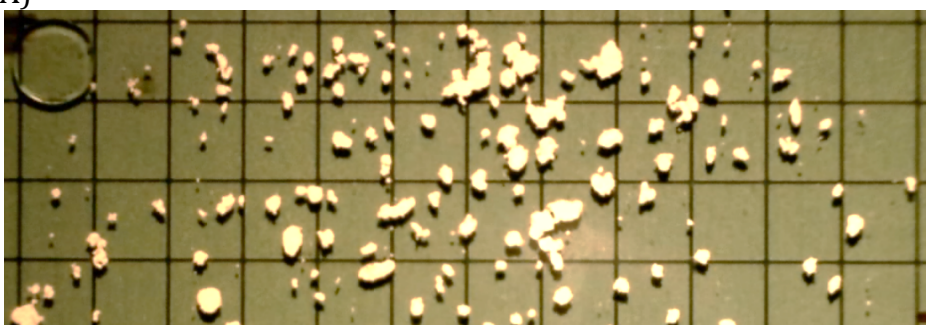


Figure 4: Droplet actuation in a closed-format digital microfluidic device. PC: Hanyoup Kim

A)



B)

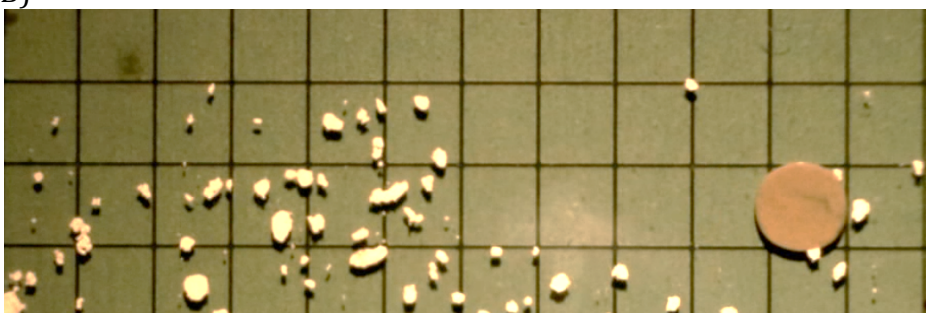


Figure 5: Environmental dry particle assay. A) A 3 µL droplet is placed on the board with Arizona Test dust sprinkled randomly over the board. The droplet would move across the board, picking up dust. B) The droplet saturated with dust particles and can no longer move.

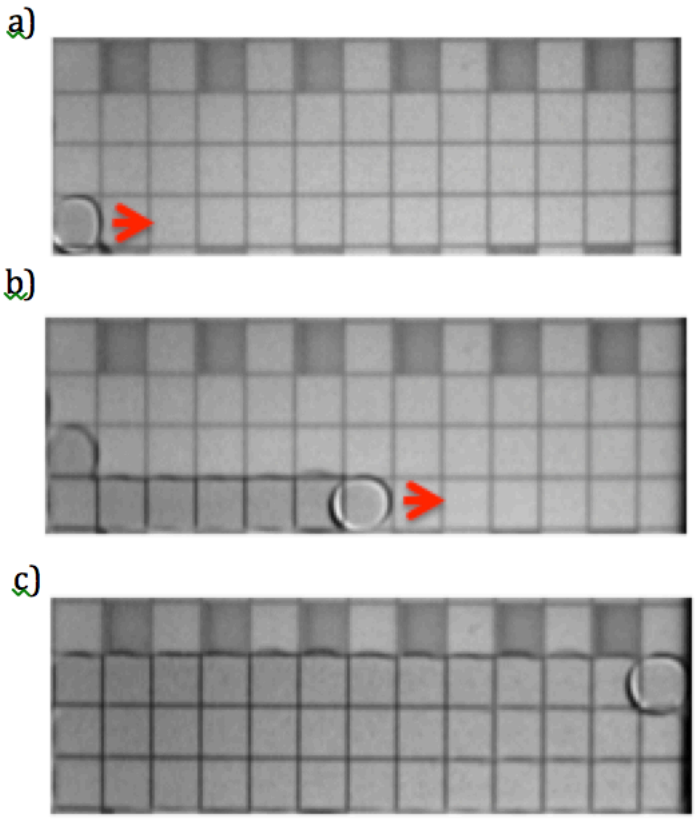


Figure 6: Exhaled Breath Condensate Assay. A) A glass ITO slide is chilled and then breathed on creating the foggy glass shown above. A 3 µL droplet of 0.05% Pluronic® F-127 is placed. B) The droplet moving across the ITO slide picking up the condensation. C) The droplet moved across the board and picked up as much as it could within 30 seconds.

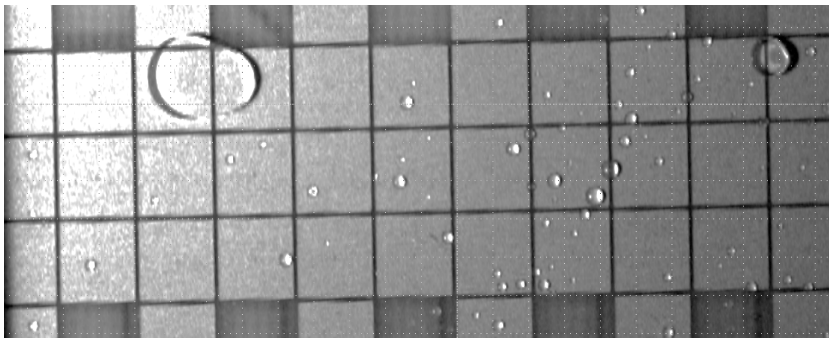


Figure 7: Mock Sputum Assay. A mock sputum made of 0.05 % BSA is sprayed onto the board with a 3 µL droplet of 0.05% Pluronic® F-127. The droplet moves across the DMF device and pick up the mock sputum until it could no longer move.

Table 1: 2012 Cost of Sandia Digital Microfluidic Hub along with parts needed. If this product reaches mass production, all costs for this device will significantly decrease and allow the opportunity for a cheap device. (Hanyoup Kim)

Item	Quantity & Unit Price	Subtotal
Sandia Digital Microfluidic Hub (including electronics and software)	1 × $3,000	$3,000
Digital microfluidic substrates	2 × $50	$100
OEM syringe pumps with multiport valves	2 × $1,200	$2,400
COTS thermal cycling system	1 × $600	$600
Custom enzyme microreactor	1 × $250	$250
Custom magnetic bead capture module	1 × $100	$100
COTS fittings, capillary tubing, etc.	Various	$50
Total		**$6,500**

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