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Digital Microfluidic Chips for Chemical and Biological Applications

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Abstract-**The advent of digital microfluidic lab-on-a-chip (LoC) technology offers an excellent platform for developing diagnostic applications. In diagnostics raw physiological samples must be introduced onto the chip and then further processed by lysing blood cells and extracting DNA. However, types of applications that can be implemented on a digital microfluidic platform are largely determined by detection or sensor technology as well as the compatibility of the liquids with electrowetting. In this paper we will focus on analyte detection technologies and discuss suitable applications, both potential and demonstrated, based on digital microfluidics.**

I. INTRODUCTION

Digital microfluidics involves the manipulation of microliter to picoliter droplets on hydrophobic surfaces under voltage control, whereby many lab-on-a-chip functions can be performed on a common, programmable platform. We and others have demonstrated that a wide diversity in biomedical applications can be parsed into manageable components and assembled into an architecture proving the advantages of being programmable, reconfigurable, and reusable. This capability opens the possibility of handling all of the protocols that a given laboratory or a class of applications would require. And, it provides a path toward realizing the true lab-on-a-chip [1].

Much of the reported work on lab-on-a-chip (LoC) microfluidic devices has focused on miniaturization of analytical methods and protocols for the purpose of improving performance and throughput. The benefits of miniaturization such as smaller sample requirements, reduced reagent consumption, decreased analysis time, and higher levels of throughput and automation have been demonstrated. In addition, most lab-on-a-chip examples have been directed to performing chemical or biological protocols on chip in which pre-prepared samples have been processed off-chip. Thus, to date little work has been reported on integrating the front-end functions, such as sample collection, analyte extraction, pre-concentration, and filtration with the required analytical operations then performed on the chip. Also, there only has been a limited amount of effort aimed at sensor integration.

The types of applications that can be implemented on a digital microfluidic platform are largely determined by detection or sensor technology as well as the compatibility of the liquids with electrowetting. In this paper we will focus on several analyte detection technologies and discuss suitable applications, both potential and demonstrated.

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II. DIGITAL MICROFLUIDICS

The concept of digital microfluidics arose in the late 1990s and involves the manipulation of discrete volumes of liquids on a surface. Manipulation of droplets can occur through various mechanisms, including electrowetting [2-4], dielectrophoresis [5], thermocapillary transport [6], and surface acoustic wave transport [7]. In the digital microfluidic architecture the basic liquid unit volume is fixed by the geometry of the system (fluid quantization), whereas volumetric flow rate is determined by the droplet transport rate and the number of droplets transported. Thus, transport occurs in multiples of the minimum unit volume (fluid packetization). Unlike continuous flow systems, the minimum flow volume in a digital microfluidic system is not determined by the sensitivity of a flow sensor, since there is no flow sensor. Rather, minimum droplet volume is set by detector sensitivity [8].

The use of unit volume droplets allows a microfluidic function to be reduced to a set of basic operations, allowing numerous elemental fluidic operations to be accomplished with a common set of elemental components, i.e. combinations of electrodes on an array [11]. An example of digital microfluidic architecture is shown in Fig. 1. Depicted is a two-dimensional array of electrodes configured for an electrowetting-on-dielectric (EWD) system [9].

Electrowetting-on-dielectric microfluidics is based on the actuation of droplet volumes up to several microliters using the principle of modulating the interfacial tension between a liquid and an electrode coated with a dielectric layer [10]. An electric field established in the dielectric layer creates an imbalance of interfacial tension if the electric field is applied to only one portion of the droplet on an array, which forces the droplet to move [2]. The architecture of Fig. 1 capitalizes on the flexibility of a unit flow grid array. At any given time, the array can be partitioned into "cells" that perform fluidic functions, such as storage, mixing, or transport. If the array is actuated by a clock that can change the voltage at each electrode on the array in one clock cycle, then the architecture has the potential for dynamically reconfiguring the functional cells at least once per clock cycle. Thus, once the fluidic function defined by a cell is completed, the cell electrode voltages can be reconfigured for the next function.

Digital microfluidic architecture is under software-driven electronic control, eliminating the need for mechanical tubes, pumps, and valves that are required for continuous-

flow systems. The compatibility of each chemical substance with the electro- wetting platform must be determined initially. Compatibility issues include the following: 1) does the liquid's viscosity and surface tension allow for droplet dispensing and transport by electrowetting? 2) Will the contents of the droplet foul the hydrophobic surfaces of the chip? 3) In systems with a silicone oil medium, will the chemicals in the droplet cross the droplet/oil interface, thus reducing the content in the droplet? 4) What type of detection method is suitable?

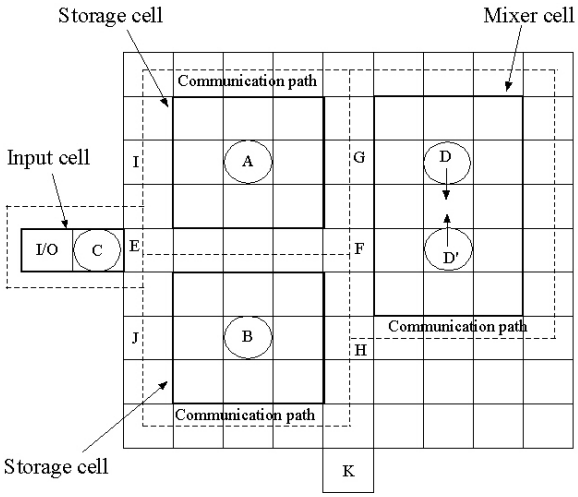

Fig. 1: Two-dimensional electrowetting electrode array used in digital microfluidic architecture. [9]

III. APPLICATIONS

A. Colorimetric Assays

On-chip colorimetric assays for determining the concentrations of target analytes is a natural application for digital microfluidics [12-14]. The specific focus of work in this area has been on multiplexed assays, where multiple analytes can be measured in a single sample. The on-chip process steps include the following: 1) pre-diluted sample and reagent loading into on-chip reservoirs; 2) droplet dispensing of analyte solutions and reagents; 3) droplet transport; 4) mixing of analyte solutions. Srinivasan, *et al*, have demonstrated a colorimetric enzyme-kinetic method based on the Trinder's reaction used for the determination of glucose concentration. (Srinivasan et al. 2004) At the end of the mixing phase, the absorbance is measured for at least 30 seconds, using a 545nm LED-photodiode setup. The mixed droplet is held stationary by electrowetting forces during the absorbance measurement step, depicted in Fig. 2.

The integration of optical sources and detectors based on absorbance is relatively easy to perform on a digital microfluidic platform, especially since the platform is made using plates and see-through indium-tin-oxide electrodes. However, optical absorbtion detection scales poorly with miniaturization, since Beer's law incorporates a pathlength dependence [15]. Regarding the detectors reported by Srinivasan et al. the optical path length typically was 100-300µm [13], which is 30 to 100 times smaller than conventional systems (10mm). This small path length poses serious sensitivity issues, and limits the use of absorbance to assays with very high analyte concentrations.

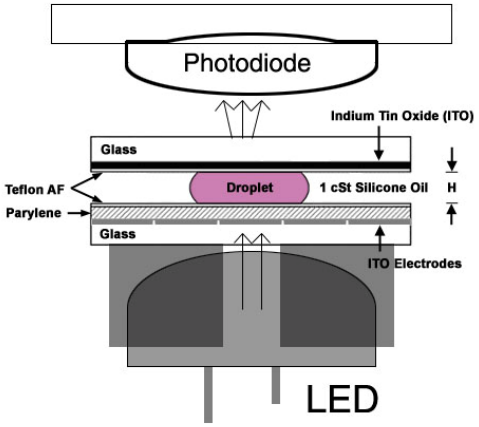

Fig. 2: Optical absorbance measurement instrumentation used to monitor color change due to colorimetric reactions on chip.

B. Chemiluminescent Assays

Chemilumescent detection has been shown to be compatible with the digital microfluidic platform (Luan, et al. 2008) and with diagnostic applications as well as sequencing DNA by synthesis [16]. In general, the on-chip chemistry must result in optical signal generation in the vicinity of a photodetector. Work in this area has been reported by Luan et al. with an integrated optical sensor based upon the heterogeneous integration of an InGaAs-based thin film photodetector with a digital microfluidic system [17].

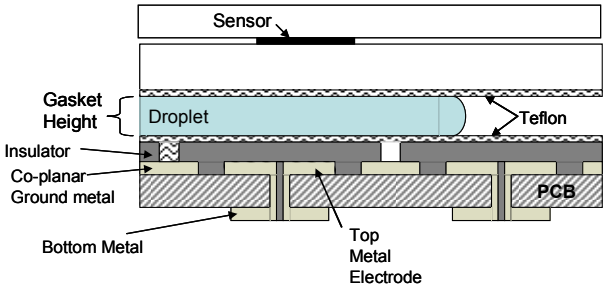

Fig. 3 Side view of a co-planar electrowetting chip made on a printed circuit board. The top plate can be customized to accommodate the optical sensor and its sensing surface.

To integrate a compound semiconductor photodetector with an electrowetting microfluidics system, a coplanar digital microfluidic chip fabricated by Advanced Liquid Logic in printed circuit board (PCB) technology was used. The chip was attached to the controller, and electrodes were switched through a computer GUI connected to the controller. Silicone oil (~2cSt) was dispensed onto the area of the chip used for this experiment. Electrode voltages of 220V were applied to the chip. A top view of the integrated sensor is shown in Fig. 3 [17].

In order to generate a substantial signal in an aqueous medium, the oxidation of pyrogallol (1, 2, 3-trihydroxibenzene) in an alkaline solution was used (the Trautz-Schorigin reaction). When two droplets are mixed it generates a short-lived, bright orange light if the solution is fresh, or a longer lasting, lower intensity light if the solution is not fresh (has been given time to cool). Both solutions are immiscible in silicone oil. Chemicals used in the experiments were dispensed from on-chip reservoirs, and the dispensed droplets were actuated together underneath the sensor. When the droplets mixed underneath the sensor, the chemiluminescent reaction began to generate light.

Another example of chemilumescent detection is DNA sequencing by synthesis. Sequencing-by-synthesis methods involve enzymatic extension by polymerase through the iterative addition of labeled nucleotides, often in an array format. The cascade begins with the addition of a known nucleotide to the DNA (or RNA) strand of interest. This reaction is carried out by DNA Polymerase. Upon nucleotide incorporation, pyrophosphate (PPi) is released. This pyrophosphate is converted to ATP by the enzyme ATP sulfurylase. The ATP then provides energy for the enzyme lucerifase to oxidize luciferin. One of the byproducts of this final oxidation reaction is light at approximately 560 nm. This sequence is shown in Fig. 4.

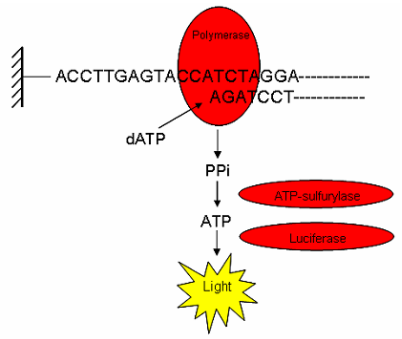

Fig. 4: Illustration of solid-phase pyrosequencing. After incorporation of a nucleotide (in this case dATP), a washing step is used to remove the excess substrate.

The light can be easily detected by a photodiode, photomultiplier tube, or a charge-coupled device (CCD). Since the order in which the nucleotide addition occurs is known, one can determine the sequence of the unknown strand by formation of its complimentary strand. The entire pyrosequencing cascade takes about 3-4 seconds from start to finish per nucleotide added.

Pyrosequencing of DNA has been performed on a digital microfluidic platform [16]. The chip was covered with a transparent top plate and filled with oil to create a microfluidic chamber in which droplets were programmably manipulated (dispensed, transported, merged, split) using electrical fields. Using a 211 bp DNA fragment derived from *C. albicans* genomic DNA, single stranded templates were prepared and attached to 2.8µm magnetic beads.

The bead suspension and pyrosequencing reagents were loaded in wells formed in the top-plate. Unit-sized 400 nL droplets were dispensed from the wells and manipulated within the chip according to the user program. At each cycle the sample droplet containing the beads was combined with one droplet containing nucleotides and one droplet containing the three-enzyme mixture. The combined droplet was mixed and transported to a detector where a luminescent signal proportional to the number of bases incorporated was detected by a photomultiplier coupled to the transparent top plate. The combined droplet was then transported to a wash station with a permanent magnet located underneath the chip. Washing was performed by repeated addition and removal of fresh buffer droplets to the sample droplet while the magnetic beads were immobilized on the chip surface. The entire cycle was then repeated with a fresh enzyme droplet and a fresh nucleotide droplet selected from one of the four nucleotide wells. Up to 20 bases in three different regions of the 211 bp template were successfully sequenced using this technique. Results of a 20bp read are shown in Fig. 5.

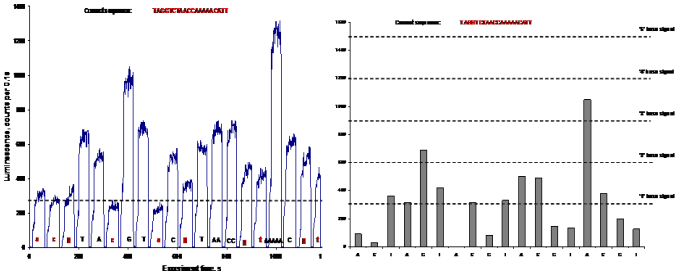

Fig. 5: On-chip pyrosequencing results showing 17-bp sequencing of a 211-bp long *C.albicans* DNA template.

C. High Sensitivity Integrated Sensors

To expand the applications of digital microfluidics to new areas requires integration of sensing systems that are sensitive enough to detect latent and subclinical infections. The input to the digital microfluidic platform would be a drop of blood. The blood is then processed by the microfluidic system to separate the red blood cells containing the parasite, the cells are lysed, and the DNA is extracted from the contents of the cell (including the parasite). The microfluidic system contains stabilized reagents that are then used to isolate the targeted DNA strands, and to "unzip" the DNA, resulting in single strand DNA that is the target DNA. The DNA is then amplified using PCR or LAMP, and then is presented to the integrated optical sensor. The amplification step time will be minimized through the use of a high sensitivity optical sensor and amplification at the sensing surface. The optical sensors are surface customized with the single strand DNA (the "probe") that is complementary to the target [18].

D.Plate Reader Assays

Assays performed on digital microfluidic chips based on biological events would include cytotoxicity assays. In most cases, a high-intensity lamp passes light to the chip surface and the light emitted by the reaction happening on chip is quantified by a detector. Common detection modes for micro reader assays are absorbance, fluorescence intensity, luminescence, time-resolved fluorescence, and fluorescence polarization.

A digital microfluidic biochip has been proposed to perform cytotoxicity testing [19]. The proposed chip would dilute the compound to be tested over a range of concentrations (user defined) so that the cytotoxic behavior of the compound could be characterized in more detail than a single concentration measurement would allow. The chip would then mix the compound dilutions with a cell suspension and then incubate the mixture for a user specified period of time. Following incubation, the chip would add the cytotoxicity assay reagent(s) to the mixture and then optically detect the amount of cell death. Although the chip design was directed for use in cytotoxicity assays, the chip could be easily interfaced with other digital microfluidic biochip capabilities as well.

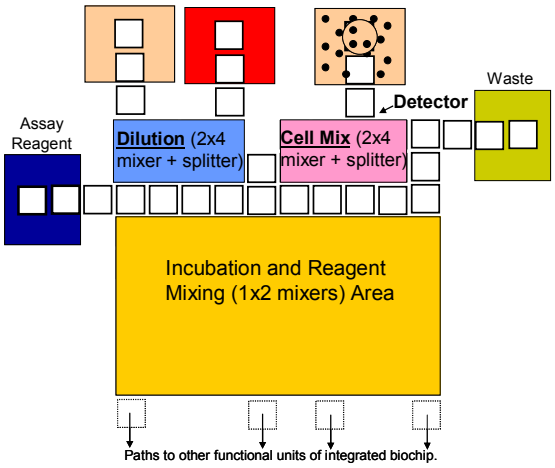

Fig. 6: Floor plan of a digital microfluidic chip to perform cytotoxicity assays.

E. DNA Hybridization Testing

There is significant interest in developing microfluidic systems that can function as portable, chip-scale DNA diagnostic sensors. The testing procedure involves isolating parasitic DNA from blood, cleaning the DNA, and detecting. This protocol is illustrated below in Fig. 7 [18]. The main fluidic functions required to achieve the detection of DNA are given in the flowchart. Using malaria detection as an example, the procedure starts having as input a 1µl volume of blood. The first step is the sample preparation. This consists of detaching the infected cells, breaking their cellular membrane and extracting the DNA. As the amount of DNA is too scarce for successful detection to be considered, DNA must be replicated by using an amplification technique. Finally, a detection step is integrated in order to determine if an infection with one of the malaria parasites is present in the organism [18].

Magnetic bead separation implemented on a digital microfluidic platform is the first step in separating infected cells from other cells in whole blood [18, 20]. Magnetic beads can be made to selectively tag infected cells using antibody-antigen bonding. By locating the droplet containing beads and other cells over a magnet, separation can be achieved by washing a 2x droplet through the bead droplet. After 5-10 wash droplets pass, the bead droplet is relatively clean, only containing tagged cells on beads. This method does not result in bead loss [16].

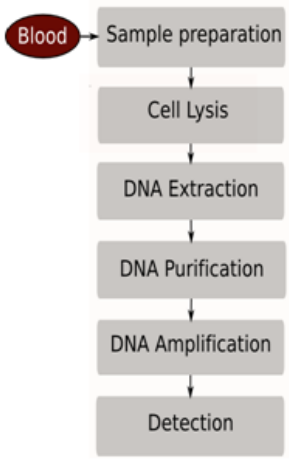

Figure 3: malaria chip flowchart.

Fig. 7: Malaria chip flowchart.

Chemical cell lysis of infected cells is carried out to extract parasitic DNA. Droplets containing lysing agents are dispensed from their reservoirs, and are mixed with the bead droplets. After cell lysis, DNA strands need to be extracted from a mixture of the cell contents suspended in a droplet. To facilitate DNA extraction, the droplet is first heated to 95°C to convert the double stranded DNA to single stranded DNA. The droplet is then mixed with a droplet containing complementary DNA strands attached to magnetic beads. Magnetic bead separation is then repeated as described previously. After DNA extraction from the lysed cells, the droplet is heated to unbind the DNA from the magnetic beads. This droplet undergoes further magnetic bead separation, to separate the magnetic beads from the DNA strands. The resulting droplet, concentrated with DNA strands then undergoes PCR [21].

Dhar et al [18] have pointed out that several detection schemes are possible for the malaria chip. A popular technique is flow cytometry, where sample processing is performed on-chip, and the chip is then used in conjunction with a commercial cytrometry device. An advantage of this technique is that it is well-established, but a major drawback is that the detector is rather large and not on-chip. An on-chip detection option is an integrated thin-film semiconductor light source, waveguide and detector capable of measuring changes in transmission. Or, instead of integrating a light source, such a scheme could measure

changes in the chemiluminescence of the sample, as shown by L. Luan et al [17].

Surface plasmon resonance (SPR) may also be a viable detection scheme. However, no one has shown that SPR can be integrated on a digital microfluidic chip.

IV. CONCLUSIONS

Investigators working in the field of digital microfluidics have conducted extensive research on the basic principles and operations underlying the implementation of electrowetting-based microfluidic systems. The result is a substantial "microfluidic toolkit" of automated droplet operations, a sizable catalog of compatible reagents, and demonstrations of important chemical and biological assays. However, the lack of good integrated on-chip sensing methods and on-chip sample preparation currently are the biggest impediments to broad commercial acceptability of microfluidic technologies, including digital microfluidics. Other issues include system integration and interfacing to other laboratory formats and devices, packaging, reagent storage, and maintaining temperature control of the chip during field operation.

The number and variety of analyses being performed on chip has increased along with the need to perform multiple-sample manipulations. It is often desirable to isolate components that produce a signal of interest, so that they can be detected. Currently, mass separation methods, such as capillary electrophoresis, are not an established part of the digital microfluidic toolkit, and integration of separation methods presents a significant challenge. Nevertheless, we will see the first introduction of commercial digital microfluidic chips into certain laboratory applications in the near future.

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