Anal Bioanal Chem (2009) 393:419–426
DOI 10.1007/s00216-008-2397-x

TRENDS

# Digital bioanalysis

**Elizabeth M. Miller · Aaron R. Wheeler**

Published online: 28 September 2008


**Abstract** Digital microfluidics has recently emerged as a new paradigm in the world of lab-on-a-chip technology. A wide variety of bioanalyses have been successfully implemented in this format. This paper reviews the various techniques that have been adapted to digital microfluidic systems, and the current state of the field.

**Keywords** Lab-on-a-chip · Digital microfluidics · Droplets · Electrowetting · Miniaturization

## Introduction

The name "digital microfluidics" has been used to describe two different technologies—multiphase systems in which droplets are manipulated inside microchannels and open systems in which droplet position is controlled by actuating electrodes arranged in a two-dimensional array. While the former technique has become popular for a wide variety of applications [1–3], we promote the use of the term digital microfluidics (DMF) exclusively for the latter. In DMF, the potential paths and locations of any particular droplet are defined by coordinates in an array—thus, location of each droplet in space is discrete, rather than continuous. As is the case for digital electronics, each "bit" (or droplet) can be addressed individually. In contrast, droplets in microchannels are limited to actuation in series and each droplet lacks spatial digitization, making the system more closely related to (analog) microchannel systems than to the 2-dimensional, array-based geometry used in digital microfluidics. Here, we review the state-of-the-art of digital microfluidic systems applied to biology and chemistry.

Digital microfluidics is characterized by the use of electrical fields to manipulate droplets on an array of electrodes coated with a dielectric material. The dielectric layer is typically coated with Teflon to render it hydrophobic. When a potential is applied to an electrode, charges (and/or aligned dipoles) build up at the interface between the device surface and the surrounding medium. The electrostatic forces generated by these accumulated charges, dipoles, or induced dipoles facilitate movement of the droplet on to each successive actuated electrode. A related phenomenon, electrowetting, is sometimes observed in such systems—droplets with high surface tension experience reduction in the contact angle formed relative to the actuated electrode. In the past [4, 5], capillary forces generated by electrowetting (or electrowetting-on-dielectric, or EWOD) were viewed as the driving force for droplet manipulation, but we posit that this characterization is too limited—it is possible to move liquids that do not actually wet the surface upon actuation [6], and even those without permanent dipoles [7]. Thus, it is probably more accurate to describe the droplet movement phenomenon in terms of a combination of electrostatic and dielectrophoresis forces. In order for droplets to move (or to be merged, split, or dispensed from reservoirs), DC or AC electrical potentials are applied between actuation and ground electrodes. In a one-plate device, the ground electrode is in the same plane as the actuation electrodes (Fig. 1a), while in a two-plate device, the ground electrode is positioned in a top plate, typically formed from glass coated with indium—tin oxide (Fig. 1b).

Digital microfluidic chips are not a substitute for microchannel chips, but rather are a complement to them. Channels are typically used to control sample volumes much smaller than those of DMF devices (although, since

E. M. Miller · A. R. Wheeler
Department of Chemistry, University of Toronto,
80 St George St.,
Toronto, ON M5S 3H6, Canada

E. M. Miller · A. R. Wheeler (✉)
Donnelly Centre for Cellular and Biomolecular Research,
160 College St.,
Toronto, ON M5S 3E1, Canada
e-mail: awheeler@chem.utoronto.ca

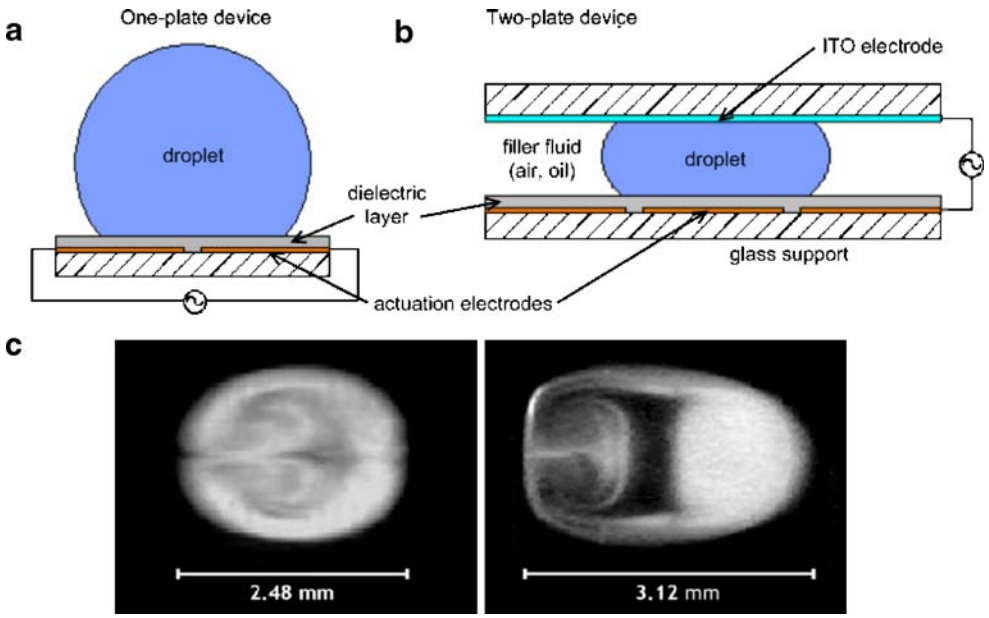


**Fig. 1** Digital microfluidic devices. In the single-plate format (**a**), the ground electrodes are in the same plate as the actuation electrodes (droplet movement is into the plane of the page). In the two-plate format (**b**), the top plate is grounded, allowing reconfigurable, two-dimensional movement of the droplet. Mixing within a coalesced droplet is induced as the droplet is shuttled between electrodes (**c**) [11]. Reproduced by permission of The Royal Society of Chemistry

no sample is diverted to waste when dispensing on DMF devices, the actual amount of sample consumed is comparable). In addition, the geometry of microchannels is much better suited to applications requiring uninterrupted flow, such as chemical separations. In contrast, DMF devices can be used to address each sample or reagent individually, rather than in series. A second advantage is reconfigurability. Each 2-dimensional array of electrodes contains a large number of possible paths—droplets can be re-routed for any number of applications without re-designing the device. Recently, rapid prototyping methods for DMF device fabrication have been developed that make it easier to produce these systems and tailor them to specific applications [8, 9]. While digital devices are not capable of performing separations, they are well suited to performing preparatory reactions, syntheses, and non-separations analyses such as colorimetric assays. Due to the slightly larger dimensions in DMF devices, they are compatible with UV-visible absorbance detection (typically incompatible with microchannels and their very small path lengths).

When applied to the right problems, digital microfluidics is emerging as a useful format for labs-on-a-chip. In this review, we summarize the state-of-the-art of this promising new technology, with an emphasis on practical design considerations and biological applications.

## Experimental design considerations

There are two important factors that must be considered when designing a DMF system for a particular bioapplication. First, thorough and rapid mixing of droplets is essential—in particular, large molecules diffuse slowly, which requires active mixing to achieve a homogeneous merged droplet. Second, one must ensure that samples, reagents, solvents, etc. can be reliably actuated on the device. While this is straightforward for small-molecule applications, it is more complicated when biological molecules are involved. A number of fundamental studies have evaluated these concerns for DMF devices.

### Mixing

One of the limitations in microscale analysis is that diffusion is the only force available for mixing. When working with large molecules (e.g., antibodies or enzymes), the laminar flow conditions that exist in some DMF devices or in microchannels require active mixers to ensure that the reagents will be homogenized for analysis. This is particularly important in time-sensitive experiments such as kinetic studies or cellular assays, where the system may change in the time required to mix its components. Different DMF device designs require different mixing protocols—it is generally more difficult to fully mix droplets in the more confined two-plate format. Paik et al. [10] demonstrated that mixing is most efficient on two-plate devices when a coalesced droplet is moved in a circular or figure-eight configuration, rather than when shuttled back and forth along a linear path (Fig. 1c). The same group then improved upon this technique, with a split-and-merge scheme, able to achieve mixing in less than 2 s [11]. This is adequate for most applications, but the single-plate geometry is better suited for faster mixing. When an AC field is applied in such devices, the deformation of the droplet as it wets and de-wets the surface causes mixing at a much faster rate than in a comparable two-plate device [12]. Mixing times as low as 15 ms have been reported for single-plate devices [13].

### Actuation reliability

Since the actuation mechanism of DMF is dependent upon the hydrophobicity of the device surface, any modification of that surface may be detrimental to droplet actuation. This poses a significant challenge for bioanalytical applications, as molecules such as proteins, DNA, and lipids are known to adsorb onto hydrophobic surfaces, often irreversibly. In the case of a DMF device, this adsorption may alter the properties of the surface, making actuation impossible. Surface fouling can occur as the result of hydrophobic interactions with the surface, via electrostatic interactions between the analytes and the charged electrode surface, or some combination thereof. Careful tuning of voltage bias and adjustments to pH and ionic strength can limit the electrostatic attraction of proteins to device surfaces [14]; however, this method does not prevent hydrophobic interactions, and it is unlikely to be useful for assays requiring reagents and solutions with different pIs or biological pH conditions. Keeping droplets in constant motion also reduces fouling, but this may not be practical for longer analyses.

Another method of reducing the amount of time a droplet spends in contact with a surface is to ensure that it never actually comes into contact with the surface, such as by surrounding the droplet with an immiscible medium (e.g., silicone [5] or fluorinated [15] oil). DMF devices constructed in this manner are robust and resistant to surface fouling [16, 17]. This approach has an additional advantage when used for longer, multistep protocols—the oil medium prevents evaporation of the sample droplet during the process. Although a wide variety of biological samples can be actuated in this manner [16], it is not compatible with all applications—some commonly used organic solvents, such as ethanol and DMSO are miscible with these oils. Such systems are also prone to other concerns, as analytes may either partition out of the droplet into the oil or be pinned at the outer surface of the droplet, altering enzyme activity or other structure-dependent properties.

An alternative method of reducing surface fouling is the use of a non-ionic surfactant additive. It has been reported that Tween-20, a surfactant commonly found in buffers used for bioanalysis, is compatible with the DMF actuation mechanism [18]. In addition, the PPO:PEO:PPO triblock copolymer Pluronic F-127 has been shown to effect a drastic reduction in the adherence of proteins [19, 20] and cells [21] without adverse effects on protein function or cell viability. Using Pluronic in buffer solutions enables the actuation of higher concentrations of proteins and cells without the need for oil or drastic changes in pH. Pluronic is an excellent tool for reducing protein adsorption; however, analyses of long duration do require a humidified environment to control droplet evaporation.

Without the advances described above, sophisticated bioanalytical methods would not be compatible with digital microfluidics. Taken together, these methods can be used in a variety of bio-applications, such as enzymatic assays, immunoassays, proteomics, DNA analyses, and cell-based assays.

## Applications

### Enzyme assays

The simplest biological application of DMF is the enzymatic assay, and a variety of these have now been implemented. The first enzymatic reaction performed on a DMF chip used luciferase, luciferin, and ATP to demonstrate the actuation of an enzyme and a substrate, and luminescence detection to verify that reactions occurred [22]. The first quantitative DMF-based enzyme application comprised a glucose/glucose oxidase assay with a silicone oil medium and absorbance detection [23]. The detection system exhibited a linear range of approximately two orders of magnitude, and was successful in detecting glucose concentrations spanning the range of clinical interest. Because detection was achieved using an LED and photodiode as source and detector, respectively, this system is small and inexpensive, making it appropriate for point-of-care applications.

Recently, we developed a fluorescent assay (Fig. 2a), using a Pluronic additive instead of silicone oil to prevent adsorption of the enzyme to the device surface [20]. This assay proved to be very reproducible for both small-molecule quantitation and enzyme kinetics experiments—the kinetic constants calculated using the DMF device agreed with those generated using well plates, but used sample volumes that were nearly 150 times smaller. Detection limits were also lowered by two orders of magnitude, and the entire analysis was performed using an existing, commercially available fluorescence plate reader—no dedicated instruments with specialized sources and detectors were required. In another enzyme kinetics study, Nichols and Gardeniers [13] used a single-plate DMF device to investigate pre-steady-state kinetics of PTPase—an extremely time-sensitive measurement—using an electrohydrodynamic mixing scheme and MALDI-TOF mass spectrometry. DMF was used to merge and mix pipetted droplets of enzyme, substrate, quencher, and MALDI matrix at carefully defined time intervals. The formation of a phosphorylated enzyme intermediate was monitored and quantified by mass spectrometry, and mixing times as low as 15 ms resulted in excellent temporal resolution in the measurement (Fig. 2b). These techniques demonstrate the potential of DMF to become a powerful and versatile tool for analysis of enzymes.

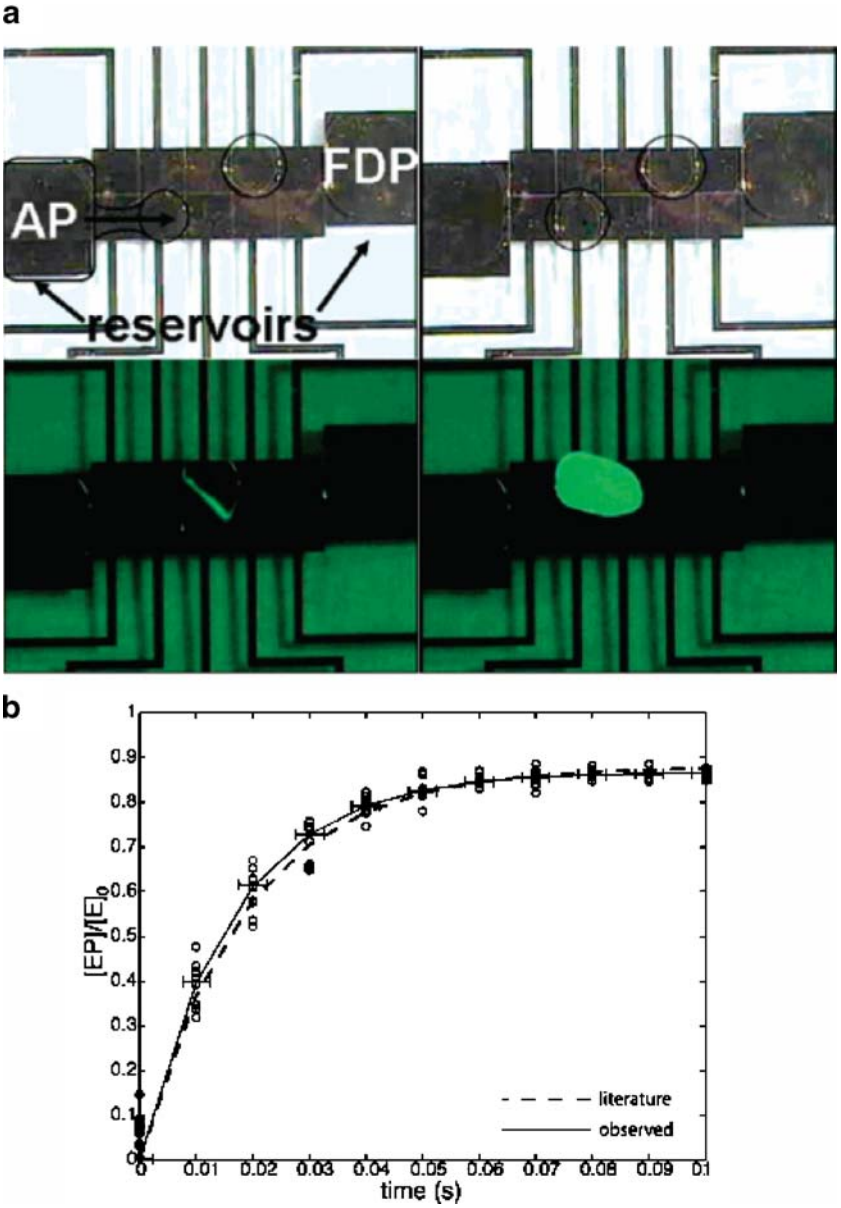


**Fig. 2** Enzymatic assays on a DMF device. (**a**) Droplets of fluorescein diphosphate (*FDP*) and alkaline phosphatase (*AP*) are dispensed, merged, and mixed on the device, turning over a fluorescent product. Reproduced with permission from Ref. [20]. Copyright 2008 American Chemical Society. (**b**) Formation of a phosphorylated PTPase intermediate (quantified via MALDI-MS), monitored within a DMF device via quenching of the reaction at specific time intervals spanning a 100-ms period in a pre-steady state kinetic analysis. Reproduced with permission from Ref. [13]. Copyright 2007 American Chemical Society

## Immunoassays

Another application to which digital microfluidics is well-suited is heterogeneous immunoassays. Because chemical separations are not required for such assays, channels are not necessary—the droplet format of DMF mimics the macroscale equivalent of pipetting reagents into wells. DMF has a major advantage over well plates, not just in terms of decreased sample size but also because the assay time is not limited by the kinetics of diffusion. In wells, antigen and detection antibody molecules must diffuse to the surface in order to be detected, but on a DMF device droplet movement and mixing decreases the amount of time required for the target molecules to come into contact with the capture antibody.

So far, two immunoassay approaches on DMF devices have been reported, both of which rely upon particles suspended in droplets. In the first approach, Rastogi and Velev [24] developed assays for IgG and ricin based on the agglutination of latex and gold particles—a droplet sus-

pended in fluorinated oil on a single plate device was captured by dielectrophoresis and served as a container with a controlled evaporation rate. The pattern assumed by the antibody-coated latex and gold particles as the droplet evaporated was indicative of the quantity of antigen present in the sample (Fig. 3a). This method, compared with a commercially available ricin assay, had a limit of detection 10× lower and consumed 100× less sample volume. In the second approach, magnetic beads were used as a support surface for a chemiluminescent ELISA [17]. The use of beads ensured high surface area for contact between the antibody-coated surface and the sample and took advantage of the faster mass transport conditions in the microscale (relative to well plates), while an external magnet was used to separate the beads from the supernatant for the requisite wash steps. We speculate that a wide variety of other immunoassay applications for DMF are on the horizon.

## Proteomics

Digital microfluidics also has great potential for proteomic sample preparation, because these applications tend to require a sequence of reactions and purification steps. Because MALDI-TOF-MS is an inherently array-based technique, it is a natural match for DMF. Wheeler et al. [25] demonstrated that DMF actuation is compatible with a variety of MALDI matrices, including DHB, FA, and SA.

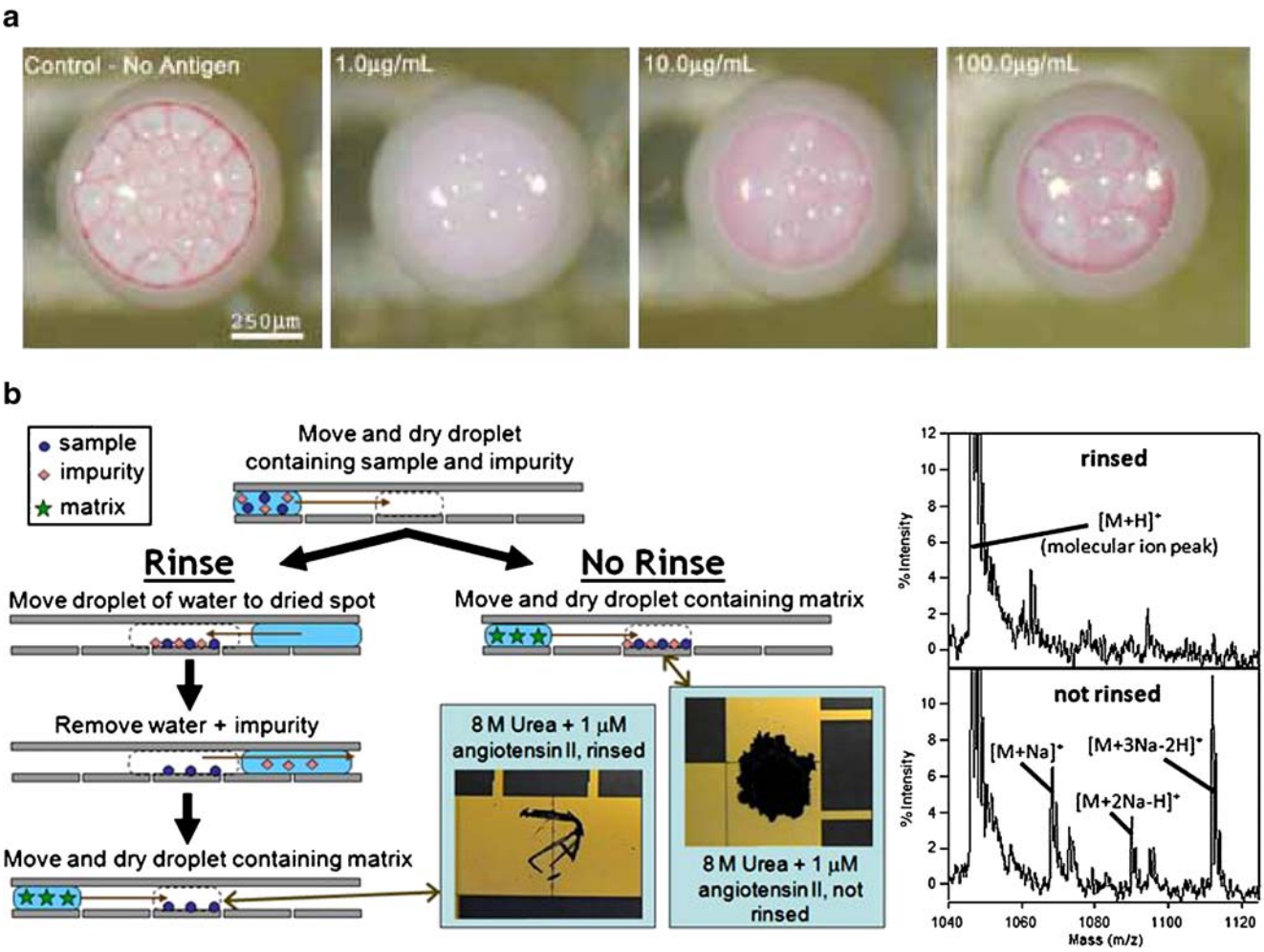


**Fig. 3** (**a**) A DMF immunoassay. Latex (*white*) and gold (*red*) particles are conjugated to an anti-IgG antibody. As the droplet slowly evaporates, the particles agglutinate to form a pattern—if a small amount of IgG is present, the smaller gold particles are bound to the latex and cannot rise above them; if a larger amount of IgG is present, the antibodies on both sets of particles become saturated, and the gold particles can interpolate themselves around the latex and rise to the top. Reprinted with permission from Ref. [24]. Copyright 2007, American Institute of Physics. (**b**) Proteomic sample preparation for MALDI-MS on a DMF device. DMF is used to control droplets of sample (angiotensin), matrix, and rinse buffer. When analyzed by mass spectrometry, a number of sodium adduct peaks are visible in the spectrum for the unrinsed sample spot, but only the molecular ion is present in the spectrum when a rinse step is included prior to matrix crystallization. Reproduced with permission from Ref. [26]. Copyright 2005 American Chemical Society

Mass spectra were obtained for several standard proteins representing a range of molecular weights (insulin, cytochrome c, and myoglobin), using DMF to dispense a droplet of protein which was allowed to dry on the surface. A droplet of matrix solution was then dispensed and dried in the same manner, before analysis via MALDI-TOF-MS. In later work, an on-chip purification step was incorporated to remove urea and other water-soluble impurities from the dried matrix spot (Fig. 3b) [26, 27]. This step dramatically increased signal-to-noise ratios and actually outperformed the standard ZipTip purification process used in the macroscale.

In on-going work, we recently demonstrated that DMF is compatible with other common proteomic processing steps, including enzymatic digestion [19]. The use of Pluronic additives is particularly important for these kinds of processes; its successful implementation suggests that a wide range of proteomic applications will soon be implemented using DMF technology.

### DNA-based applications

DNA analyses tend to be performed on small, precious samples (e.g., cell extracts or de novo synthesized oligonucleotides) and/or in a highly multiplexed format, making them a natural fit for microfluidics. Thus, there is a great deal of interest in using DMF to develop fluidic microprocessors and small-volume microreactors for these sorts of applications. A DMF processor was developed for analysis of the kinetics of DNA repair enzymes [28]. This device was used to characterize the repair of DNA lesions with fapy glycosylase—in addition to an analysis of enzyme activity and kinetics, it was determined that there was less than 3% variation in the size of droplets dispensed, and *E. coli* extracts were analyzed with no noticeable device fouling. DMF devices have also been used as microfluidic reactors for DNA ligation [29]. While a comparable macroscale method consumes 15 μL of reagent (of which only 2 μL is required to transform cells), the total reaction volume on a two-plate device amounts to 2.1 μL, greatly reducing reagent waste. However, certain steps of the ligation process required higher temperatures, and thus were performed off-chip.

Other DNA-based applications present the same challenge of requiring multiple environments (i.e., temperatures) on the chip for different stages of the analysis. As a result, a system for PCR has been developed that controls the temperature environment of droplets actuated by DMF (Fig. 4a) [30]. In a silicone oil-filled device, droplets of primers and cDNA were merged, mixed, and transported to a PCR reaction chamber containing a heater and a microsensor to monitor the chamber temperature. The results of the PCR amplification were verified offline, via slab gel electrophoresis. As an alternative strategy, we developed a three-dimensional DMF geometry, called all-terrain droplet actuation (ATDA), to provide greater flexibility when different environments are required. By fabricating actuation electrodes in flexible substrates, droplets can be exposed to (a) different temperatures simply by placing one end of a device on an external heater, or (b) anaerobic environments by submerging one portion of the device in oil [31].

The latter system was used to extract histones from a sample of DNA—after submerging a sample droplet in phenol-containing oil, enough histones were removed to allow detection of a DNA signal by mass spectrometry. This technique shows promise for sample preparation for DNA analysis, and for the analysis itself.

### Cell-based applications

Applications such as cell-sorting and cell-based assays provide an increased challenge in DMF devices, as cells must be transported in high electrical fields without altering their function or viability. In addition, cells are likely to adsorb onto hydrophobic surfaces, or to contain proteins that will adsorb. There are two reports of applications involving cells on DMF devices. Huang et al [32] demonstrated concentration of cells within a droplet (portions of which were then manipulated by DMF), and we recently implemented the first cell-based assay by digital microfluidics [21]. In the latter, a Pluronic additive enabled the actuation of cell suspensions with densities as high as $6\times 10^7$ cells $mL^{-1}$, and 150-nL droplets containing a few hundred cells were subjected to vitality, proliferation, and toxicology-screening assays (Fig. 4b). By modeling the electric field and experimental data, it was determined that the actuation mechanism has no significant effect on the function of the cells. In addition, the on-chip assay—using DMF for all dispensing, merging, and mixing operations—had a much lower background signal, and 20× greater sensitivity relative to conventional methods. Because of the lower LOD, the DMF method was able to accurately predict the lethal dose of a toxic compound, which was an order of magnitude higher than the value obtained using the well-plate assay.

## Outlook

Because the technology is still very new, the future of digital microfluidics is wide open. It remains to be seen which applications will secure a foothold in this format, and which have not even been considered yet. We believe that a wide variety of enzymatic assays will be performed on DMF devices—the use of detection methods such as absorbance ensure that nearly any assay could be adapted

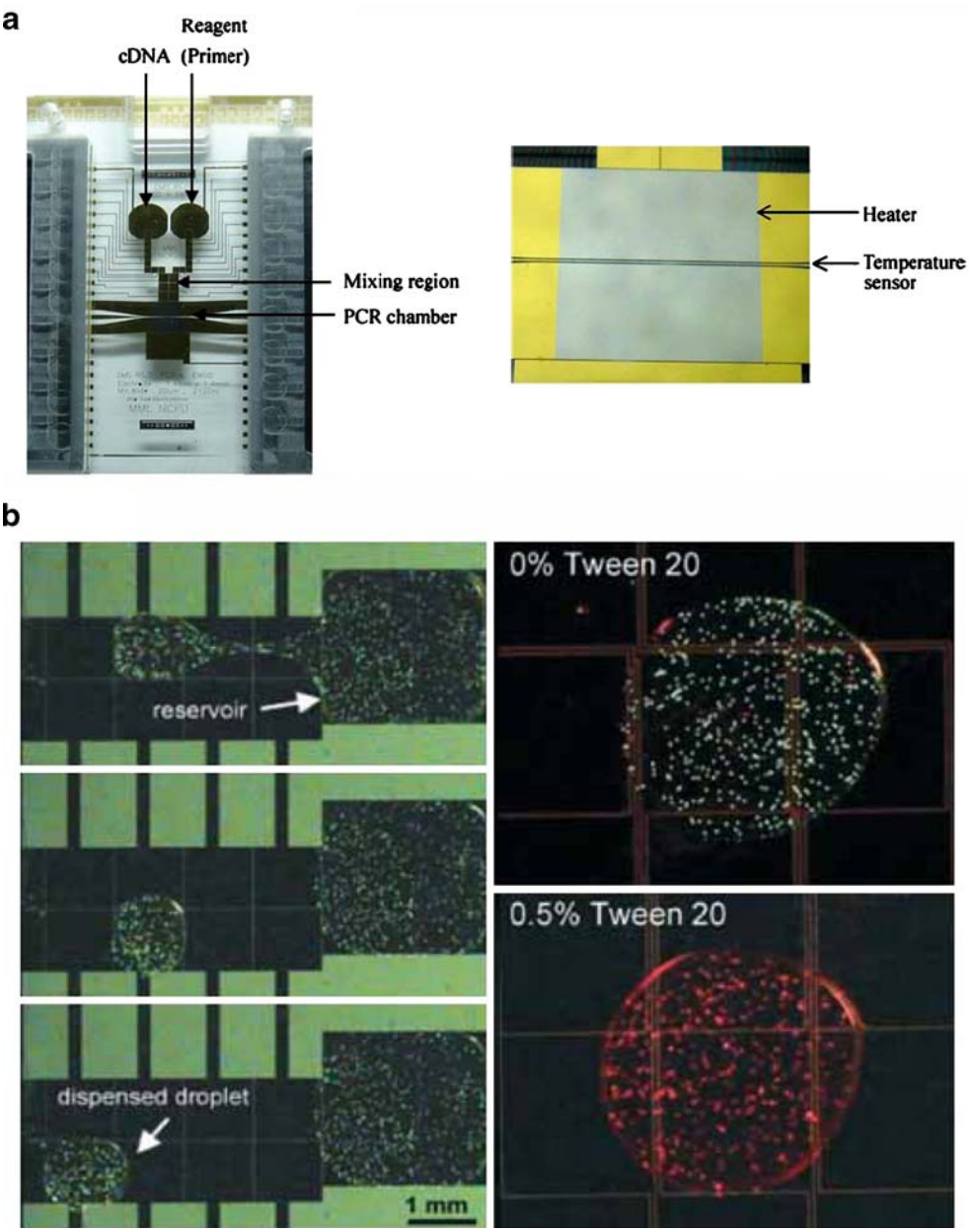


**Fig. 4** (**a**) A DMF device for PCR. The device contains reservoirs for a cDNA sample and a primer, a 2×3 matrix of electrodes for mixing coalesced droplets, and a PCR chamber containing an integrated heater and temperature sensor. Reprinted from Ref. [30] with kind permission from Springer Science + Business Media. Copyright Springer Science + Business Media, LLC 2006. (**b**) A DMF cell-based assay. A droplet containing ~260 cells is dispensed from a reservoir, then merged with various concentrations of Tween-20 (a surfactant) to determine the lethal dose. The cells are stained with viability dyes in order to quantify live (*green*) and dead (*red*) cells after each dose [21]. Reproduced by permission of The Royal Society of Chemistry

to DMF with minimal modification, something that simply cannot be said for microchannel-based systems. We foresee greater sophistication and integration in digital microfluidic systems. While DMF devices have already been fabricated with built-in light sources and detectors and partially integrated with multiwell plate readers, we expect the technique will be similarly integrated with other micro and macro-scale technologies, such as separation channels, microfabricated interfaces with mass spectrometry, microdialysis probes, sample-pipetting robots, etc. At the same time, the development of rapid prototyping methods suggests that disposable DMF devices will become widely available in the near future, which will improve their potential for clinical applications. We also believe that work on DMF-based immunoassays has only just begun—with its two-dimensional array geometry, DMF has potential to become an irreplaceable technology for this application. Integration of DNA or antibody microarrays seems a logical step in developing clinical applications for these devices, and DMF microreactors may even be used some day for multistep reactions involving biomolecules, such as custom DNA or peptide synthesis. Finally, we expect advances in cellular assays, such as on-chip pharmaceutical screening and automated cell culture. In short, digital microfluidics is still in its infancy, but it is poised to become a valuable tool for the lab-on-a-chip community.

## References


1. Song H, Chen DL, Ismagilov RF (2006) Angew Chem Int Ed 45:7336–7356
2. Teh S-Y, Lin R, Hung L-H, Lee AP (2008) Lab on a Chip 8:198–220
3. Huebner A, Sharma S, Srisa-Art M, Hollfelder F, Edel JB, deMello AJ (2008) Lab on a Chip 8:1244–1254
4. Lee J, Moon H, Fowler J, Schoellhammer T, Kim C-J (2002) Sens Actuators A 95:259–268
5. Pollack MG, Fair RB, Shenderov AD (2000) Appl Phys Lett 77:1725–1726
6. Chatterjee D, Hetayothin B, Wheeler AR, King D, Garrell RL (2006) Lab on a Chip 6:199–206
7. Hsieh T-H, Fan S-K (2008) In: MEMS 2008, pp. 641–644, IEEE, Tucson, AZ, USA
8. Watson M, Abdelgawad M, Ye G, Yonson N, Trottier J, Wheeler AR (2006) Anal Chem 78:7877–7885
9. Abdelgawad M, Wheeler AR (2007) Adv Mater 19:133–137
10. Paik P, Pamula VK, Pollack MG, Fair RB (2003) Lab on a Chip 3:28–33
11. Paik P, Pamula VK, Fair RB (2003) Lab on a Chip 3:253–259
12. Cooney CG, Chen C-Y, Emerling MR, Nadim A, Sterling JD (2006) Microfluidics Nanofluidics 2:435–446
13. Nichols KP, Gardeniers HJGE (2007) Anal Chem 79:8699–8704
14. Yoon JY, Garrell RL (2003) Anal Chem 75:5097–5102
15. Velev OD, Prevo BG, Bhatt KH (2003) Nature 426:515–516
16. Srinivasan V, Pamula VK, Fair RB (2004) Lab on a Chip 4:310–315
17. Sista R (2007) In: development of a digital microfluidic lab-on-a-chip for automated immunoassay with magnetically responsive beads, PhD dissertation)
18. Raccurt O, Berthier J, Clementz P, Borella M, Plissonnier M (2007) J Micromech Microeng 17:2217–2223
19. Luk V, Mo GC, Wheeler AR (2008) Langmuir 24:6382–6389
20. Miller EM, Wheeler AR (2008) Anal Chem 80:1614–1619
21. Barbulovic-Nad I, Yang H, Park PS, Wheeler AR (2008) Lab on a Chip 8:519–526
22. Taniguchi T, Torii T, Higuchi T (2002) Lab on a Chip 2:19–23
23. Srinivasan V, Pamula VK, Fair RB (2004) Anal Chim Acta 507:145–150
24. Rastogi V, Velev OD (2007) Biomicrofluidics 1:014107
25. Wheeler AR, Moon H, Kim C-J, Loo JA, Garrell RL (2004) Anal Chem 76:4833–4838
26. Wheeler AR, Moon H, Bird CA, Loo RRO, Kim C-J, Loo JA, Garrell RL (2005) Anal Chem 77:534–540
27. Moon H, Wheeler AR, Garrell RL, Loo JA, Kim C-J (2006) Lab on a Chip 6:1213–1219
28. Jary D, Chollat-Namy A, Fouillet Y, Boutet J, Chabrol C, Castellan G, Gasparutto D, Peponnet C (2006) In: NSTI Nanotech Tech Proc, 2:554–557
29. Liu Y-J, Yao D-J, Lin H-C, Chang W-Y, Chang H-Y (2008) J Micromech Microeng 18:1–7
30. Chang YH, Lee GB, Huang FC, Chen YY, Lin JL (2006) Biomed Microdevices 8:215–225
31. Abdelgawad M, Wheeler AR (2008) Lab on a Chip 8:672–677
32. Fan S-K, Huang P-W, Wang T-T, Peng Y-H (2008) Lab on a Chip 8:1325