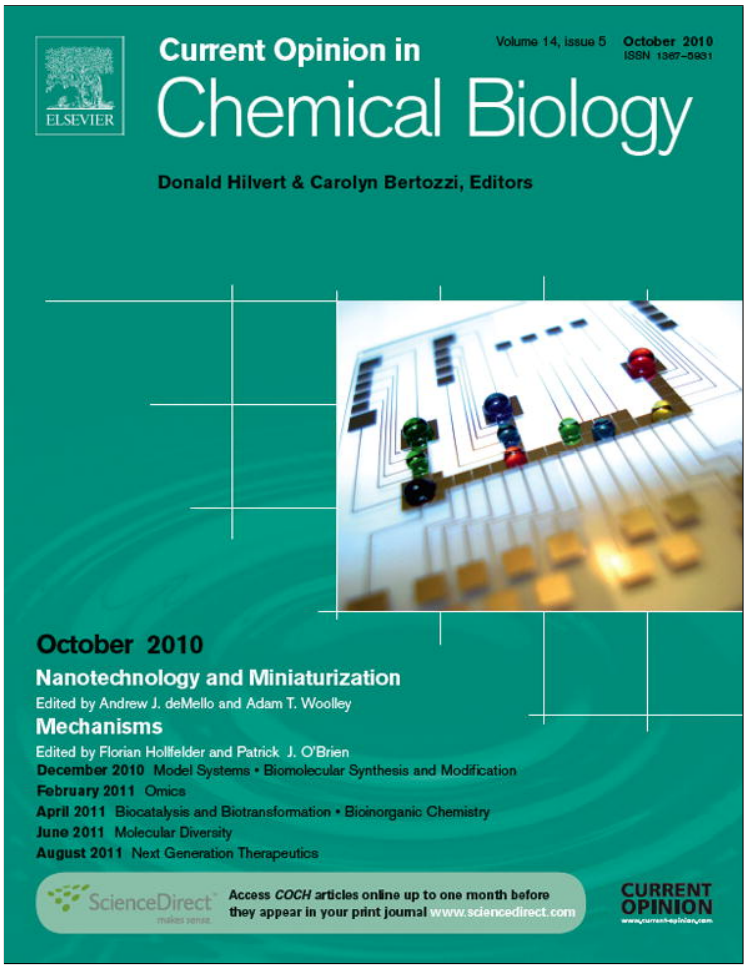

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Current Opinion in Chemical Biology

Let's get digital: digitizing chemical biology with microfluidics

Mais J Jebrail and Aaron R Wheeler

Digital microfluidics (DMF) has recently emerged as a popular technology for a wide range of applications in chemical biology. In DMF, nL–mL droplets containing samples and reagents are controlled (i.e., moved, merged, mixed, and dispensed from reservoirs) by applying a series of electrical potentials to an array of electrodes coated with a hydrophobic insulator. DMF is distinct from microchannel-based fluidics as it allows for precise control over multiple reagent phases (liquid and solid) in heterogeneous systems with no need for complex networks of microvalves. Here, we review the state-of-the-art in DMF as applied to a wide range of applications in chemical biology, including proteomics, enzyme assays and immunoassays, applications involving DNA, cell-based assays, and clinical applications.

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Introduction

The most common format for microfluidics is based on networks of enclosed microchannels. Here, we review a related but distinct technology called digital microfluidics (DMF). In DMF, samples are manipulated as discrete droplets on an open surface by applying a series of electrical potentials to an array of electrodes [1•,2]. As shown in Figure 1a there are two common configurations for DMF — the closed format (also known as the two-plate format) in which droplets are sandwiched between two substrates, and the open format (also known as the one-plate format) in which droplets are positioned on top of a single substrate. In both configurations, the droplets to be manipulated are isolated from the electrodes by a hydrophobic insulator. When an electrical potential is applied, charge accumulates on either side of the insulator, a phenomenon that can be exploited to make droplets move, merge, mix, split, and dispense from reservoirs (Figure 1b) [3].

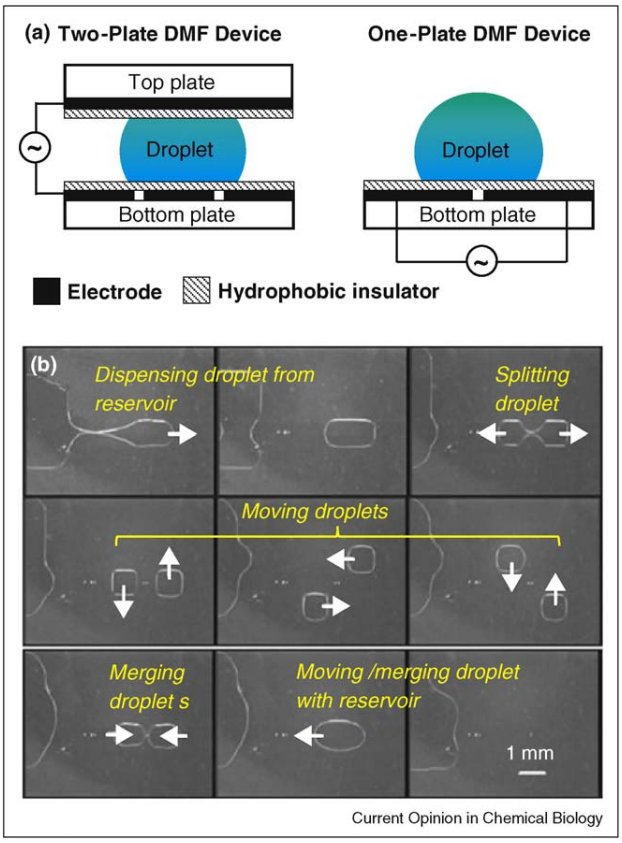
The digital microfluidic format has several unique advantages for lab-on-a-chip applications. For example, as depicted in Figure 2a, DMF is capable of addressing of all of the reagents in an assay individually with no need for complex networks of microvalves. A second advantage, depicted in Figure 2b, is the capacity to control liquids relative to solids with no risk of clogging [4,5••]. Finally, DMF is compatible with a very wide range of volumes (see Figure 2c), making it useful for preparative-scale sample handling. This is contrasted with microchannels, in which volumes are typically much smaller.

DMF is an attractive platform for applications related to chemical biology, which often require the use of expensive or precious reagents. However, a non-trivial challenge is non-specific adsorption of biological molecules to device surfaces (or fouling), which can lead to sample loss, cross-contamination, or droplet sticking (which renders devices useless). Several strategies have been developed to overcome this problem. Srinivasan *et al.* [6] demonstrated that fouling could be minimized by suspending droplets in an immiscible oil, which facilitates manipulation of a variety of fluids containing high concentrations of potential surface fouling molecules (i.e., blood, serum and plasma). Oil is not compatible with all applications, and an alternative strategy is to mix samples and reagents with low concentrations of amphiphilic polymer additives, which facilitates the actuation of serum and other concentrated biochemical reagents with reduced fouling [7]. Finally, a third technique is to use a removable hydrophobic insulator, such that each successive experiment is implemented on a fresh device surface [8•]. These advances and others have made DMF compatible with a wide range of applications in chemical biology, including proteomics, enzyme assays and immunoassays, applications involving DNA, cell-based assays, and clinical applications. These topics are reviewed below.

Proteomics

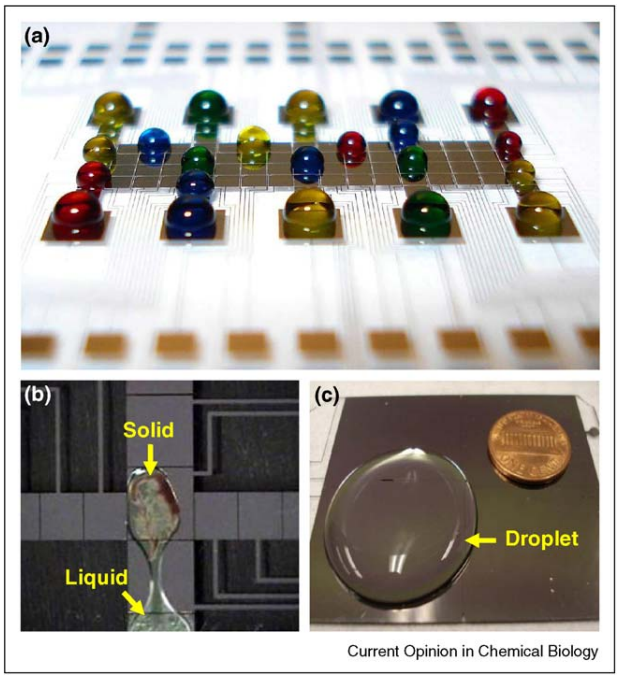
Proteomic experiments typically require tedious, multi-step sample processing before analysis by mass spectrometry or other detectors, and the capacity of DMF for individual addressing of many reagents simultaneously makes it a good fit for such processes. In an early demonstration of proteomics in a DMF format, the Garrell and Kim groups developed DMF-based methods to purify peptides and proteins from heterogeneous mixtures [9,10]. The methods comprised a series of steps, including drying the sample droplet, rinsing the dried spot with DI water droplet to remove impurities, and finally

Figure 1



DMF. **(a)** Side-view schematics of two- and one-plate DMF formats. **(b)** Pictures from a movie depicting the four principle digital microfluidic processes: dispensing, splitting, moving, and merging. The electrodes on this device are not visible because they are formed from transparent indium–tin oxide (ITO). Reproduced with permission from [3] Copyright © 2003 IEEE.

Figure 2



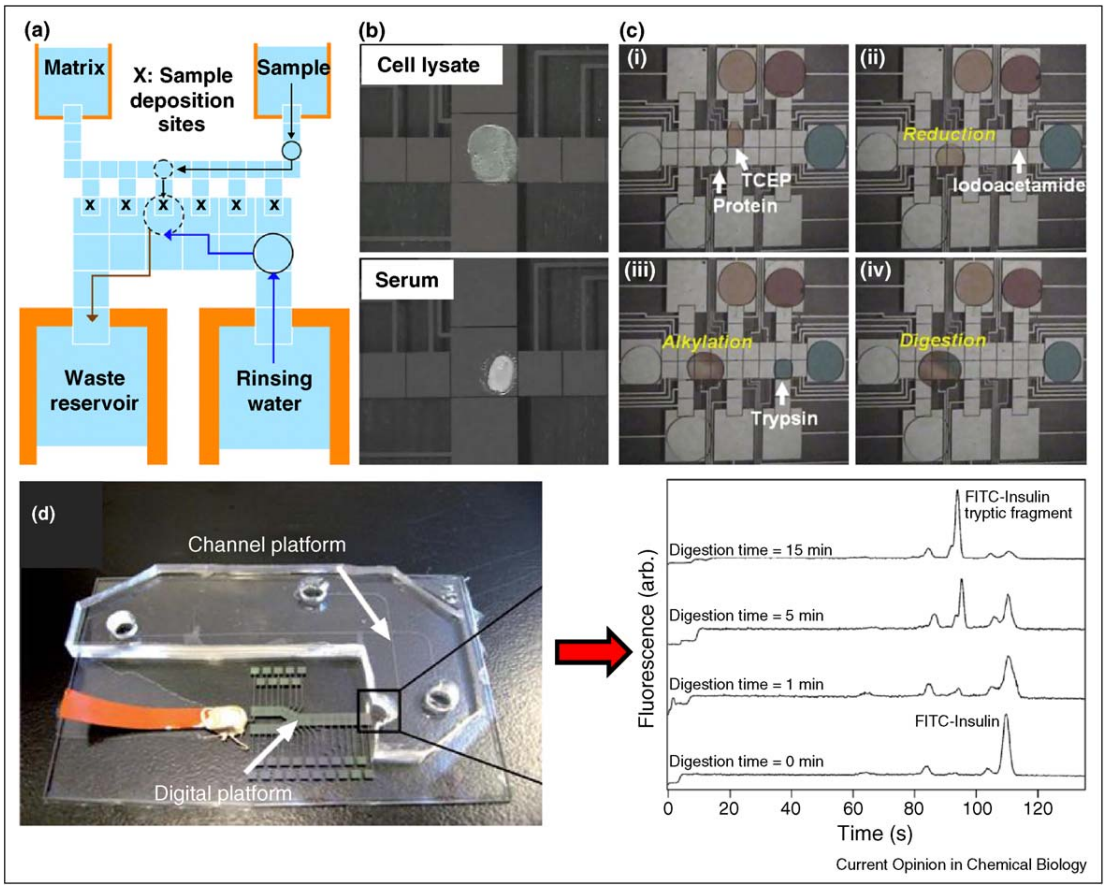
Distinct capabilities of DMF. **(a)** A picture of a DMF platform controlling more than 20 reaction microvessels (i.e., droplets) with no microvalves or other moving parts. **(b)** Picture of supernatant liquid being driven away from a solid precipitate by DMF. **(c)** Picture of a DMF device used to manipulate a 2.8 mL droplet. Reproduced with permission from [23] Copyright © 2008 The Royal Society of Chemistry.

delivering a droplet of matrix-assisted laser desorption/ionization (MALDI) matrix to the purified proteins for analysis on-chip by mass spectrometry. The same team then improved upon this process by implementing simultaneous purification of 6 samples (Figure 3a) [11]. Recently, we implemented a DMF-based protocol for extracting and purifying proteins from complex biological mixtures by precipitation, rinsing, and resolubilization (Figure 3b) [4]. The method had comparable protein recovery efficiencies (≥80%) relative to conventional techniques, combined with the advantages of no centrifugation, and 2× faster extraction and purification. In a separate study, we applied DMF to key proteomic processing steps that commonly follow protein extraction, including protein reduction, alkylation, and digestion [12]. Peptide mixtures processed in this manner were analyzed off-chip by MALDI-MS, and identified by Mascot database search engine yielding correct sample identifications with confidence levels >95%. In related work, Garrell's group also demonstrated on-chip protein biochemical processing combined with *in situ* analysis by MALDI-MS [13]. For a complete proteomic sample workup, we developed an automated DMF-based platform integrating all common processes, including protein precipitation, rinsing, resolubilization, reduction, alkylation, and digestion [14•] (Figure 3c). Finally, in ongoing work, we have integrated DMF with microchannels for in-line sample processing and separations [15••]. This 'hybrid microfluidics' format comprises a one-plate DMF platform mated to the front end of a network of microchannels used to implement electrophoretic separations (Figure 3d). In this work, droplets containing proteomic analytes were digested by droplets of trypsin on the DMF platform, and then driven by DMF to the microchannels for separation and analysis.

Immunoassays and enzyme assays

Immunoassays and enzyme assays are routinely used to detect analytes in biological samples with high selectivity, and the former (immunoassays) has recently emerged as a good match for DMF. Sista *et al.* [16••] reported a droplet-based magnetic bead immunoassay using DMF to detect insulin and interleukin-6. In this work, a droplet

Figure 3

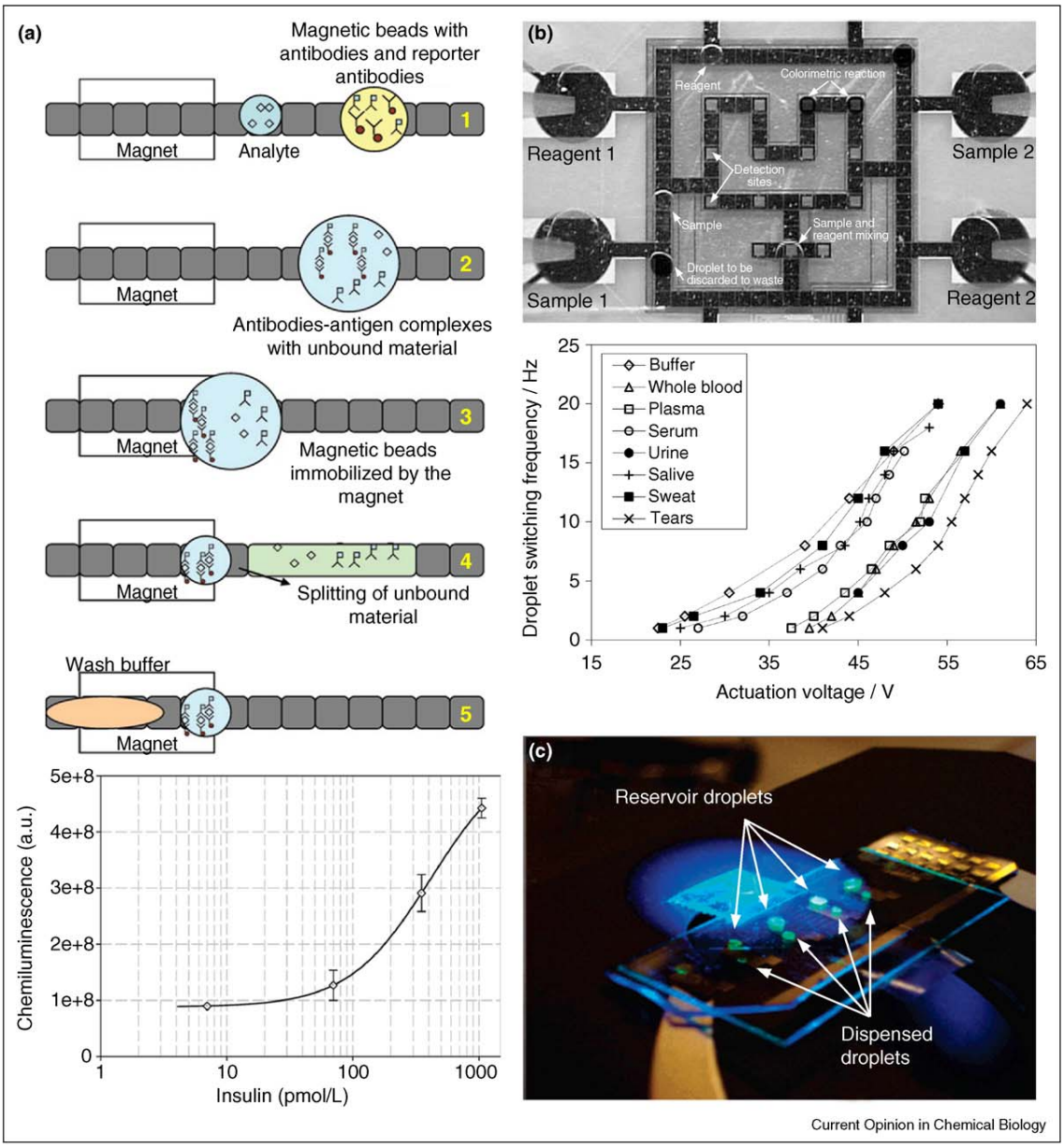


Digital microfluidic systems for proteomics. **(a)** Schematic of a DMF device used to perform multiplexed proteomic sample de-salting. Reproduced with permission from [11] Copyright © 2006 The Royal Society of Chemistry. **(b)** Pictures of protein precipitates isolated from droplets of cell lysate (70 nL) and fetal bovine serum (140 nL) using DMF. Reproduced with permission from [4] Copyright © 2009 The American Chemical Society. **(c)** Frames from a movie illustrating sequential reduction, alkylation, and digestion of a droplet of resolubilized protein. Reproduced with permission from [14•] Copyright © 2009 JoVE. **(d)** Picture (left) of hybrid device which comprises an electrode array for sample preparation by DMF and a network of microchannels for chemical separations. Electropherograms (right) illustrating on-chip digestion of singly labeled FITC-Insulin by trypsin over a time period of 15 minutes. Reproduced with permission from [15] Copyright © 2009 The Royal Society of Chemistry.

of analyte and a second droplet containing magnetic beads (modified with primary capture antibodies), blocking proteins, and reporter antibodies were merged to form capture antibody–antigen–reporter antibody complexes. A magnetic field was then used to immobilize the beads such that the supernatant could be driven away by DMF. Close to 100% bead recovery was realized after 7776-fold dilution-based washing of the supernatant. The beads were then resuspended in a new buffer droplet and the analytes were detected by chemiluminescence (Figure 4a). The assay had low detection limits: less than 10 pmol L^{-1} and 5 pg mL^{-1} for insulin and interleukin-6. In a separate study, the same group implemented a similar method to perform immunoassays for cardiac troponin I in whole blood in less than 8 minutes [17].

Enzyme assays have long been a popular target for DMF. In one of the first reports, Taniguchi *et al.* [18] demonstrated a bioluminescence assay for ATP using the enzyme, luciferase. In another early report, the Fair group developed an automated glucose assay compatible with a range of physiological fluids (serum, saliva, plasma, and urine) on a DMF device (Figure 4b) [19]. Droplets of glucose oxidase were merged with sample droplets spiked with glucose, then mixed, and the glucose concentration was measured using an integrated LED/photodiode detector. Nichols and Gardeniers [20•] carried time-sensitive measurement-using DMF to mix reagents and MALDI time-of-flight mass spectrometry to investigate pre-steady-state kinetics of the enzyme, tyrosine phosphatase. We applied DMF to the study of enzyme kinetics by mixing and merging droplets of alkaline phosphatase with fluor-

Figure 4



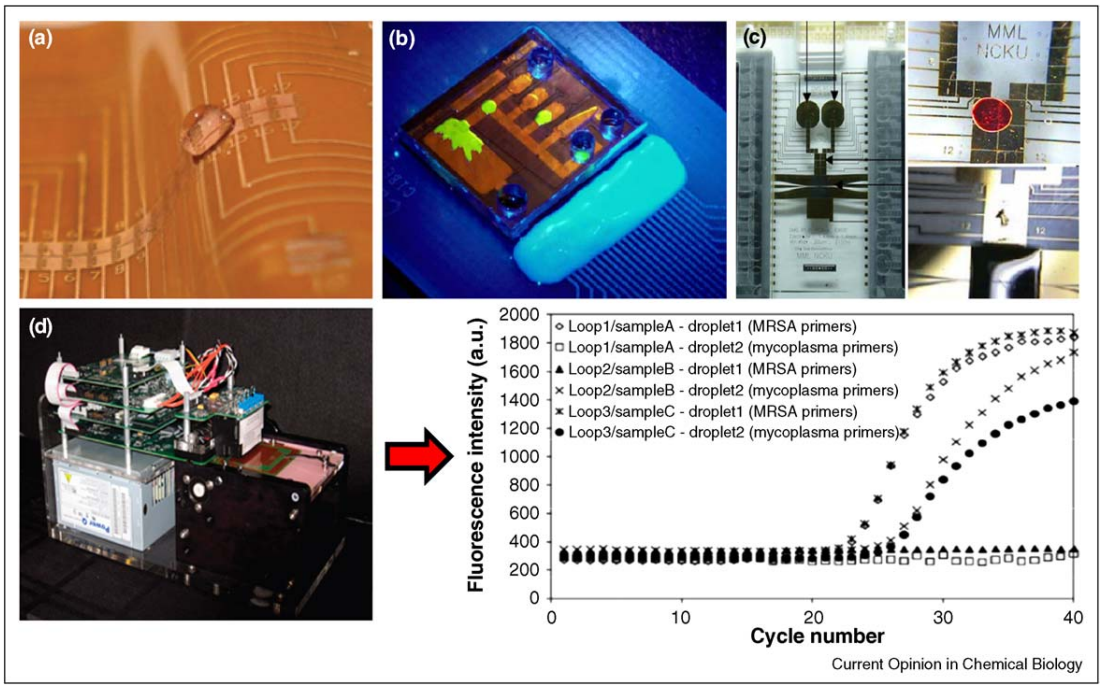
DMF for immunoassays and enzyme assays. **(a)** Schematic (top) of droplet-based magnetic bead immunoassay protocol using DMF: (1) dispensing reagent droplets, (2) incubation, (3) immobilization of magnetic beads by magnetic field, (4) washing away unbound material, and (5) adding fresh wash buffer for analysis. A standard curve (bottom) for insulin generated on DMF platform. Reproduced with permission from [16••] Copyright © 2008 The Royal Society of Chemistry. **(b)** Picture (top) of DMF device used to perform glucose assays, and plots (bottom) of actuation voltage of various physiological fluids as a function of switching frequency. Reproduced with permission from [19] Copyright © 2004 The Royal Society of Chemistry. **(c)** Picture of a multiplexed DMF device used to study enzyme kinetics. Reproduced with permission from [21] Copyright © 2008 The American Chemical Society.

escein diphosphate on a multiplexed DMF device (Figure 4c) [21]. Enzyme reaction coefficients, K_m and k_{cat}, generated by DMF agreed with literature values, and the assays used much smaller volumes and had higher sensitivity than conventional methods. Finally, Martin *et al.* [22] described initial steps for constructing artificial Golgi organelle through the use of DMF, recombinant enzymes, and magnetic nanoparticles.

Applications involving DNA

Handling, purifying, detecting, and characterizing samples of DNA have become critical steps for a wide

Figure 5



DMF for applications involving DNA. **(a)** Picture of an ATDA device, capable of manipulating droplets on flexible substrates. In this work, ATDA was used to implement liquid-phase extraction for purification of DNA from heterogeneous solutions. Reproduced with permission from [23] Copyright © 2008 The Royal Society of Chemistry. **(b)** Picture of DMF device used to implement DNA repair reactions. Reproduced with permission from [24] Copyright © 2006 Nano Science and Technology Institute. **(c)** Picture of DMF device used to perform PCR; the pictures on the right are frames from a video depicting a droplet (red) generated after mixing target DNA and droplets containing primers and other droplets. Reproduced with permission from [27•] Copyright © 2006 Springer Netherlands. **(d)** Picture (left) of self-contained DMF system for multiplexed real-time PCR, and plots (right) of real-time PCR of three different DNA samples in parallel. Reproduced with permission from [28•] Copyright © 2010 The American Chemical Society.

range of basic and applied fields of science. Thus, it is not surprising that such processes have been an attractive match for DMF. For example, we demonstrated the first of these processes, DNA handling and purification, using DMF to implement liquid–liquid extraction of a heterogenous mixture of DNA and proteins [23]. In this work, all-terrain droplet actuation (ATDA) (Figure 5a) was used to drive aqueous droplets containing a mixture of DNA and proteins into and out of a pool of phenolic oil, which had the effect of removing proteins from the droplet and purifying the nucleic acid. A second application, repair of oxidized lesions in oligonucleotides, was implemented in DMF format by Jary *et al.* [24]. In this work, droplets containing a DNA repair enzyme and damaged DNA were merged by DMF, incubated, and then the repaired DNA was detected by fluorescence microscopy (Figure 5b). Liu *et al.* [25] demonstrated a similar application, in which a DMF device was developed to facilitate DNA ligation by merging droplets containing vector DNA and the enzyme, DNA ligase. In a different application, Malic *et al.* [26•] carried out on-chip immobilization of thiolated DNA probes followed by hybridization with droplets containing complementary oligonucelotide target sequences. Surface plasmon resonance imaging measurements revealed a 2-fold increase in the efficiency of DNA immobilization under an applied potential in comparison to passive immobilization.

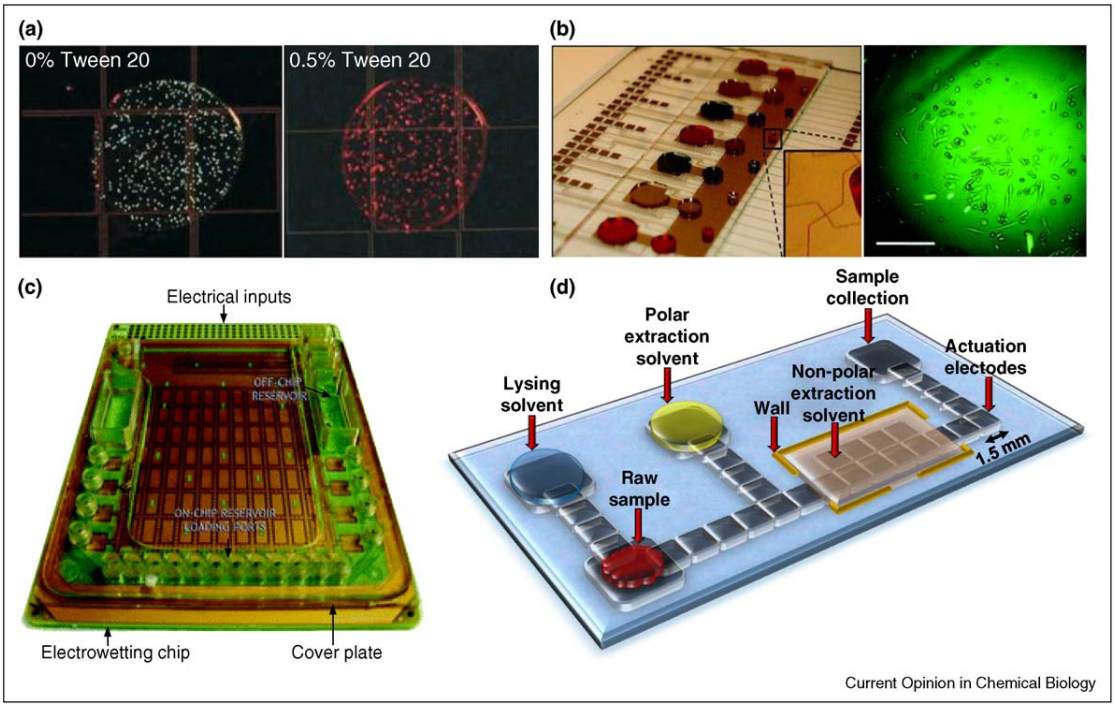
The most complete DNA application using DMF was initially reported by Chang *et al.* [27•], who implemented the polymerase chain reaction (PCR). In this work, a digital microfluidic device with an embedded micro-heater was developed to facilitate thermal cycling. As shown in Figure 5c, droplets containing an oligonucleotide to be amplified and PCR reagents were merged, mixed, and then delivered to the integrated heater. The fluorescent signals from DNA amplified on-chip were comparable to those generated using a bench-scale PCR machine with 50% and 70% reductions in total time and sample consumption, respectively. Sista *et al.* [17] improved on this technique, performing a 40-cycle real-time PCR in 12 minutes by shuttling droplet through two different temperature zones on a DMF cartridge capable of accommodating other assays (immunoassays and sample preparation). More recently, Pollack and co-workers expanded on this technique to develop an automated

and self-contained multichannel DMF platform for multiplexed real-time PCR assays [28•]. The entire system is the size of a shoebox and comprises all of the required control and detection capabilities, and a disposable microfluidic cartridge in which sample processing and PCR takes place (Figure 5d). The system has an amplification efficiency of 94.7% and is capable of detecting the equivalent of a single genome of test samples (methicillin-resistant *Staphylococcus aureus*). As a proof-of-concept for high-throughput multiplexed PCR applications, the authors demonstrated that multiple DNA samples could be amplified and detected simultaneously.

Cell-based assays

Cell-based assays have been a popular target for miniaturization, as the reagents and other materials are often prohibitively expensive. Despite this obvious match, cell-based assays have only recently been embraced by the DMF community: in the past two years, five papers have been published describing DMF applications involving cells [29–31,32••,33]. In the first [29], we implemented a toxicity assay in which droplets carrying Jurkat-T cells were merged with droplets containing different concentrations of the surfactant Tween 20 (lethal to cells) and were then merged again with droplets carrying viability dyes (Figure 6a). The DMF assay was more sensitive than an identical one performed in a 384-well plate, such that the DMF-generated results gave a better approximation of the empirical value of the 100% lethal concentration, and also had a 30× reduction in reagent consumption. Additionally, actuation by DMF was found to have no significant effects on cell vitality. This agrees with the second DMF/cell study, in which Zhou *et al.* [30] reported no increase in number of dead osteoblasts after droplet actuation. In the third study, Fan *et al.* used dielectrophoresis to separate neuroblastoma cells to different regions of droplets that were manipulated by DMF [31]. The original droplets were then split into daughter droplets containing different cell densities. In the fourth study, Shah *et al.* developed an integrated DMF-lateral field optoelectronic tweezer microfluidic device for cell handling [33]. Finally, we recently developed the first

Figure 6



DMF for cell-based and clinical applications. **(a)** Pictures of DMF device used for cell-based toxicology assays. The two frames depict droplets containing Jurkat-T cells challenged with 0% (left) and 0.5% (right) Tween-20. Cells exposed to no Tween-20 are alive and fluoresce green when labeled with calcein; in contrast, when exposed to high concentrations of Tween-20 they die and fluoresce red when labeled with ethidium homodimer-1. Reproduced with permission from [29] Copyright © 2008 The Royal Society of Chemistry. **(b)** Pictures DMF platform developed for complete cell culture (left) and transfection of cells in droplets (right). Transfected cells appear green as a result of green fluorescent protein emission. Reproduced with permission from [32••] Copyright © 2010 The Royal Society of Chemistry. **(c)** DMF cartridge capable of performing magnetic bead-based immunoassays, PCR, and sample preparation. Reproduced with permission from [17] Copyright © 2008 The Royal Society of Chemistry. **(d)** Schematic of DMF device for extracting and purifying estrogen from 1 μL human breast tissue homogenate, whole blood and serum. The device includes sample and solvent reservoirs, and a liquid–liquid extraction zone (bounded by a photoresist wall). Reproduced with permission from [5••] Copyright © 2009 The American Association for the Advancement of Science.

microfluidic platform capable of implementing all of the steps required for mammalian cell culture — cell seeding, growth, detachment, and re-seeding on a fresh surface [32••]. The new DMF technique demonstrated cell growth characteristics comparable to those found in conventional tissue culture and were used for on-chip transfection of cells (Figure 6b).

Clinical applications

The precise control over different reagents, phases, and volumes afforded by DMF makes it a good match for clinical applications. In an important step towards establishing this link, Srinivasan *et al.* [19] demonstrated basic compatibility of physiological fluids with DMF. Recently, Sista *et al.* [17] demonstrated the extraction of DNA from whole blood samples using magnetic beads on a DMF cartridge also capable of performing immunoassays and PCR as described above (Figure 6c). Finally, we recently reported a DMF-driven method for sample clean-up and extraction of estradiol (the most biologically active form of estrogen) in 1 μL samples of human breast tissue homogenate, as well as from whole blood and serum [5••]. In a typical assay, a sample was chemically lysed, the estradiol extracted into a polar solvent, unwanted constituents extracted into a nonpolar solvent, and the extract was delivered to a collection reservoir for off-chip analysis (Figure 6d). Extracted estradiol from 1 μL samples (e.g., breast tissue homogenate from a postmenopausal breast cancer patient) was detected with high signal-to-noise ratio using liquid chromatography–MS/MS with selected reaction monitoring (SRM), and quantified using enzyme-linked immunosorbent assay. The DMF method used a sample size that is 1000–4000× smaller than the conventional methods for extraction and quantification of steroids, and was 20–30× faster.

Conclusion

In summary, the technology of DMF is making unique contributions in applications of chemical biology ranging from proteomics to the medical clinic. While the technology is still in its infancy, we propose that it is poised to become an important tool in laboratory science, as well as in unexplored areas such as forensic science and environmental testing.

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