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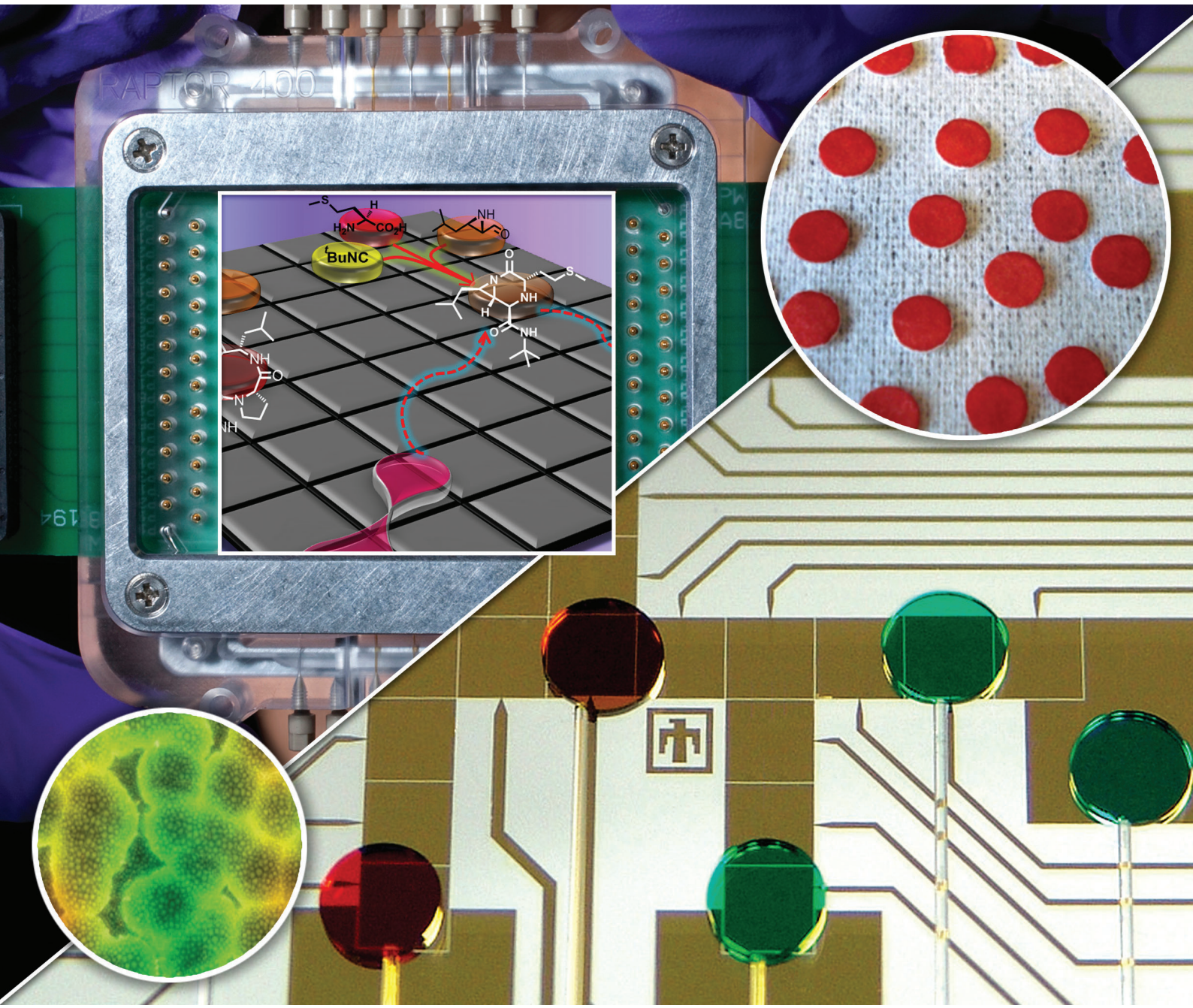

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CRITICAL REVIEW

Kamlesh D. Patel *et al.*

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CRITICAL REVIEW

Digital microfluidics: a versatile tool for applications in chemistry, biology and medicine

Mais J. Jebrail,[a] Michael S. Bartsch[b] and Kamlesh D. Patel*[b]

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Digital microfluidics (DMF) has recently emerged as a popular technology for a wide range of applications. In DMF, nanoliter to microliter droplets containing samples and reagents can be manipulated to carry out a range of discrete fluidic operations simply by applying a series of electrical potentials to an array of patterned electrodes coated with a hydrophobic insulator. DMF is distinct from microchannel-based fluidics as it allows for precise control over multiple reagent phases (liquids and solids) in heterogeneous systems with no need for complex networks of connections, microvalves, or pumps. In this review, we discuss the most recent developments in this technology with particular attention to the potential benefits and outstanding challenges for applications in chemistry, biology, and medicine.

Introduction

The most common format for microfluidics or lab-on-a-chip devices is based on enclosed microchannels in a monolithic substrate. While such systems have been successfully used for applications in chemistry,[1–5] biology[6–9] and medicine,[10–12] in this review, we focus on a related but distinct technology called digital microfluidics (DMF). Like microchannel-based fluidics, DMF is being used to miniaturize a wide range of applications, with the advantages of reduced reagent and solvent consumption, faster reaction rates and the capacity for integration (*i.e.*, the lab-on-a-chip concept). In DMF, discrete droplets of samples and reagents are manipulated (*i.e.*, dispensed from reservoirs, split, merged and mixed) with high-fidelity[13,14] on an open surface by applying a series of electrical potentials to an array of electrodes.[15,16] Although microchannels can also be used to manipulate droplets,[17,18] typically entrained in an immiscible fluid stream, DMF is a distinct paradigm that offers several unique assets to the lab-on-a-chip field.

One unique feature of DMF is the capacity to address each reagent individually with no need for complex networks of tubing or microvalves (Fig. 1a). A second advantage is the ability to control liquids relative to solids with no risk of clogging – thus, as shown in Fig. 1b, DMF is a good match for the accommodation and analysis of solid samples.[19–22] A third asset of DMF is its compatibility with a large range of volumes (see Fig. 1c), making it useful for preparative-scale sample handling.

[a]*Department of Biotechnology and Bioengineering, Sandia National Laboratories, 7011 East Avenue, MS 9292, Livermore, CA 94550*
[b]*Advance System Engineering and Deployment, Sandia National Laboratories, 7011 East Avenue, MS 9292, Livermore, CA 94550*
E-mail: kdpatel@sandia.gov; Fax: +1 925 294 3020; Tel: +1 925 294 3737

Mais J. Jebrail

Mais J. Jebrail is an NSERC Postdoctoral Fellow in the Department of Biotechnology & Bioengineering at Sandia National Laboratories in Livermore, California. Dr Jebrail received his BSc and MSc in Chemistry from York University and his PhD in Interdisciplinary Chemistry from University of Toronto. His primary research focuses on developing microfluidics-based systems for identifying pathogens triggered by bioweapons and infectious diseases.

Michael S. Bartsch

Michael S. Bartsch is a Senior Member of the Technical Staff at Sandia National Laboratories in Livermore, California. He received a B.M.E. degree from the University of Dayton and MS and PhD degrees in mechanical engineering from Stanford University. His current research focuses on scalable microfluidic systems for micro- and nanoparticle synthesis, digital microfluidics for small scale laboratory automation, and novel bioassay implementations for rapid DNA forensic analysis and biosurveillance applications.

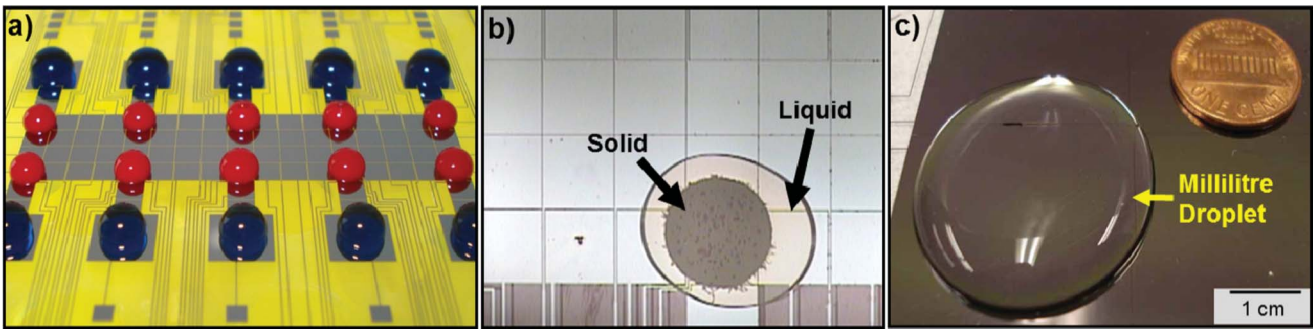

Fig. 1 Unique features of digital microfluidics a) DMF platform controlling twenty reagent droplets with no need for external hardware (*i.e.*, connectors, valves, and pumps). Reproduced with permission from ref. 26. Copyright © 2011 Futurity.org. b) Picture of extraction liquid processing a solid dried blood spot by DMF. Reproduced with permission from ref. 21. Copyright © 2011 The Royal Society of Chemistry. c) Picture of a DMF platform used to manipulate a ~ 3 mL sample droplet. Reproduced with permission from ref. 27. Copyright © 2008 The Royal Society of Chemistry.

In this review, we will explore the key capabilities and challenges for DMF, some of which are summarized in Table 1.

The last digital microfluidic review was published in *Lab on a Chip* in 2009 by Malic *et al.*[23] Since that time, DMF has matured significantly with publications not reflected in the last review and a number of novel applications now being reported. In the following sections, we describe the physics and formats of digital microfluidics, followed by a discussion of the state-of-the-art for a variety of applications in chemistry, biology, medicine, and beyond.

Physics and formats of digital microfluidics

Digital microfluidics was popularized in the early 2000s by the Fair[24] and Kim[25] groups at Duke and UCLA, respectively. In these pioneering works, water droplets were made to move across an array of insulated electrodes upon application of electrical potentials. The technique was explained as being driven by surface tension, and was called "electrowetting" or "electrowetting-on-dielectric" (EWOD). This naming convention arose from the observation that the contact angle between an aqueous droplet and the device surface is dramatically reduced (*i.e.*, wetted) during droplet movement. In the electrowetting paradigm, the two phenomena (droplet wetting and movement) were viewed as being cause-and-effect: droplet movement was understood as being a consequence of a force imbalance arising from non-symmetrical contact angles. However, this understanding does not explain droplet motion for liquids with a low surface tension that are movable yet exhibit no apparent changes in contact angle;[28] nor can it explain related phenomena such as contact angle saturation (*i.e.*, the observed limit on contact angle change above a threshold in applied potential).

A more complete understanding of the physics of droplet actuation can be derived from electromechanical analysis,[29–32] which explains both wetting and droplet movement phenomena in terms of the electrical forces generated on free charges in the droplet meniscus (for conductive liquids) or on dipoles inside the droplet (for dielectric liquids). For the purposes of modeling, these forces can be estimated by integrating the Maxwell–Stress tensor, T_{ij} (eqn (1)) (which can be derived from the Lorenz equation[33]), over any arbitrary surface around the droplet:[30,34]

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

where ε is the dielectric constant of the medium surrounding the droplet, i and j refer to pairs of x, y, and z axes, δ_{ij} is the Kronecker delta and E is the applied electric field. Unlike electrowetting, the electromechanical formulation explains the motion of dielectric liquids and liquids that do not experience a change in contact angle. In addition, it provides a rationale for the phenomenon of contact-angle saturation as an equilibrium between electrical and surface-tension forces.[30,31]

As shown in Fig. 2a, digital microfluidic technology is typically implemented in one of two different configurations: the two-plate or closed format in which droplets are sandwiched between an actuation electrode substrate and a ground plane substrate, and the one-plate or open format in which droplets are placed on top of a single substrate patterned with both actuation and ground electrodes. In both configurations, an insulating layer is deposited on top of the actuation electrodes, and is typically covered by an additional hydrophobic coating to prevent the droplet sticking to the surface.

The two-plate and one-plate digital microfluidic configurations have complementary advantages. Two-plate DMF devices are compatible with the full range of fluidic operations: droplet

Kamlesh D. Patel

Kamlesh D. Patel is a research scientist and manager for the Advance Systems Engineering and Development Group at Sandia National Laboratories in Livermore, California. Dr Patel received his BS in Chemistry from Truman State University and PhD in Analytical Chemistry from UNC-Chapel Hill. With a background in separation science and years of experience in microsystems engineering, Dr Patel has applied microfluidic-based technology to new frontiers. Most recently, Dr Patel has turned his focus towards automating sample preparation techniques for next-generation sequencing, where he hopes to help in developing a strategy to characterize unknown pathogens related to disease outbreak.

Table 1 Capabilities of and challenges for digital microfluidics

Capabilities	Challenges
Easy to manipulate reagent droplets with no need for pumps, tubing and microvalves	Not suitable for chemical separations or continuous-flow synthesis
Can handle wide range of volumes (nL–mL), suitable for preparative applications	Incompatible with high temperatures and pressures
Compatible with aqueous and organic solvents	Difficulty moving concentrated biological samples without additives or oil matrix
Straightforward control over different phases	Incompatible with centrifugation
	Dielectric breakdown with high voltage usage

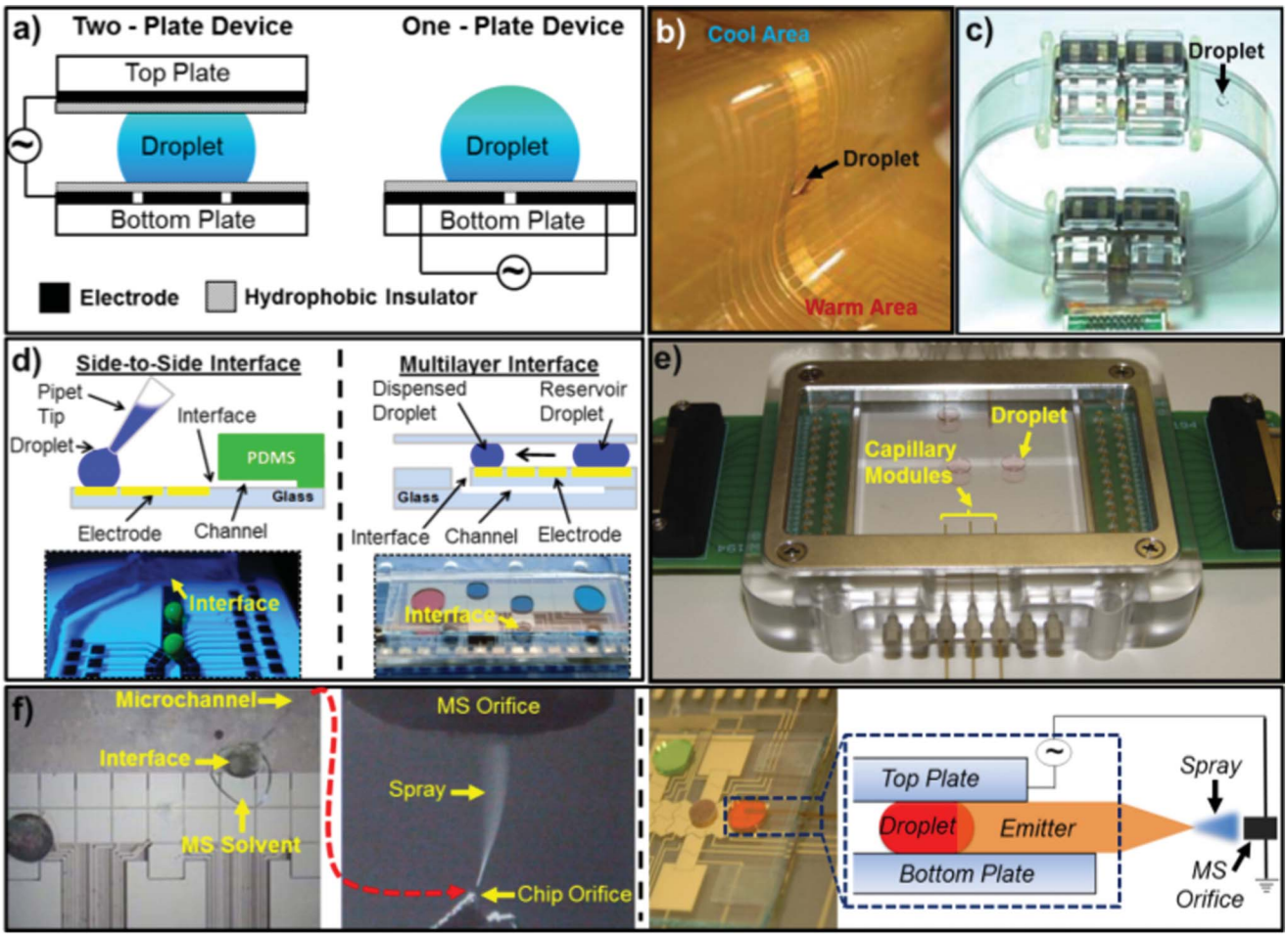

Fig. 2 Digital microfluidic formats. a) Side-view schematics of two- (left) and one-plate (right) DMF formats. Reproduced with permission from ref. 43. Copyright © 2010 Elsevier B.V. b) Picture of a flexible "All-Terrain Droplet Actuation" device moving a droplet from a warm to a cool area. Reproduced with permission from ref. 27. Copyright © 2008 The Royal Society of Chemistry. c) Picture of a wearable "droplet-on-a-wristband" device. Reproduced with permission from ref. 40. Copyright © 2011 The Royal Society of Chemistry. d) Schematics (top) and pictures (bottom) of two formats of "hybrid microfluidics," which integrates DMF for sample processing with microchannels for separations. The side-to-side configuration (left) comprises a one-plate DMF device mated to a PDMS microchannel on a common substrate and the multilayer design (right) comprises a DMF array patterned on a top substrate mated to a network of microchannels in a glass substrate below. Reproduced with permission from ref. 42. Copyright © 2010 The American Chemical Society, and ref. 41. Copyright © 2008 The Royal Society of Chemistry. e) Image of a DMF device routing droplets to-and-away-from capillary modules that are fixed between lower and upper substrates of DMF device. The electrodes on this device are not visible because they are formed from transparent Indium-Tin Oxide (ITO). Reproduced with permission from ref. 44. Copyright © 2011 Society for Laboratory Automation and Screening. f) Pictures (left) of mass spectrometry (MS) solvent introduced into spray microchannel (*via* interface hole) and sprayed off-tip, and top and side views (right) of a capillary emitter sandwitched between the top and bottom plates of an assembled DMF device. Reproduced with permission from ref. 21. Copyright © 2011 The Royal Society of Chemistry, and ref. 45. Copyright © 2012 The American Chemical Society.

dispensing, moving, splitting, and merging are all feasible.[35] In contrast, one-plate DMF devices are typically not capable of splitting or dispensing functionalities, but facilitate rapid sample and reagent mixing,[36] offer the capacity to manipulate large droplets[27] and provide better access to samples for external detectors or pipette-based liquid handling.[23]

A second key distinction in format for digital microfluidic systems is the nature of the matrix surrounding droplets on the device. For many applications, this matrix is simply ambient air. This format is the most straightforward, but is susceptible to evaporation and may require humidified chambers to overcome this issue.[37] Another common format uses a matrix of oil,[38] which limits evaporation and reduces the surface energy, and thus requires lower electrical potentials for droplet actuation. Oil-immersed systems have drawbacks, however, including the requirement of gaskets or other structures to contain the oil, the potential for the unwanted liquid–liquid extraction of analytes into the surrounding oil,[27] incompatibility with oil-miscible liquids (*e.g.*, organic solvents), and incompatibility with applications requiring drying droplets onto the device surface.[39]

A third distinction for digital microfluidic device format is device geometry. DMF is typically implemented in planar formats (Fig. 1a), but the use of flexible platforms is growing in popularity. For example, Abdelgawad *et al.*[27] described the format of "All Terrain Droplet Actuation" (ATDA) using devices fabricated on flexible substrates, which were capable of droplet actuation on inclined, declined, and inverted surfaces. This format allows for straightforward integration of multiple physicochemical environments on the same device for applications requiring temperature cycling (Fig. 2b). Similarly, Fan *et al.*[40] developed a wearable "droplet-on-a-wristband" device formed from flexible substrates that can fit around patients' wrists for the potential application of point-of-care testing (Fig. 2c).

A very recent trend for digital microfluidic device format is integration with microchannel-based elements such as separation columns. For example, "hybrid microfluidics"[41,42] combines the strength of DMF for sample processing with the speed and sensitivity of microchannel-based separations. Two formats for hybrid microfluidics have been reported. A side-by-side configuration[41] comprises a one-plate DMF device mated to a PDMS microchannel (Fig. 2d, left). Devices formed in this manner were demonstrated to be useful for applications such as in-line sample labelling with fluorogenic reagents followed by separations. A multilayer configuration[42] comprises a two-plate DMF device on top layers mated to a network of channels on a bottom layer (Fig. 2d, right). This format facilitates the implementation of complex processing regimens (*e.g.*, multi-enzyme digestion of a proteomic sample) followed by electrophoretic separations. A strategy similar to hybrid microfluidics was reported by Gorbatsova *et al.*,[46] who mated samples controlled by DMF to the inlet of an external capillary for separations. In other work, our group[44,47] recently developed a novel in-plane capillary-digital microfluidic interface for robustly interconverting liquid samples between continuous-flow and discretized droplet formats (Fig. 2e). The architecture uses the DMF primarily as a central hub for scheduling, routing, and coordinating the transport of multiple reagents between external sample processing modules – in this particular case to prepare nucleic acid samples for next generation sequencing. This strategy has the unique benefit of enabling seamless sample manipulation at the microliter scale while maintaining the flexibility of modular integration.

Methods have also been developed to couple digital microfluidics to nanoelectrospray ionization emitters for direct analysis by mass spectrometry. Jebrail and Yang *et al.*[21] initially reported a method relying on multilayer hybrid microfluidics[42] for in-line analysis, in which samples were transferred from a DMF module (on the top of the device) to a microchannel (on the bottom of the device) with an integrated nanoelectrospray ionization (nESI) emitter for mass spectrometry (Fig. 2f, left). One drawback of this method is the complexity of device fabrication and alignment and the need to thermally bond the two substrates together to form the device. Recently, Shih and Yang *et al.* reported a new DMF–nESI interface that requires significantly simpler fabrication.[45] The interface is assembled by inserting a conventional pulled-glass capillary nESI emitter between the top and bottom substrates of an assembled digital microfluidic device (Fig. 2f, right). This interface is similar to work reported simultaneously by Baker and Roper,[48] but with the key difference being that the nESI interface requires external hardware (*i.e.*, pressure source and N_2 gas) for sampling into the mass spectrometer. These approaches show significant potential for future lab-on-a-chip systems.

Digital microfluidic applications in chemistry

The format of digital microfluidics, in which droplets can be used as individually addressable microreactors, seems well suited for chemical synthesis. This idea was reinforced when Chatterjee *et al.*[28] demonstrated the capacity of DMF to actuate organic solvents including acetone, acetonitrile, ethanol, dichloromethane and others. In an early demonstration of chemical applications in DMF format, Millman *et al.*[49] synthesized a wide variety of micro-particles, including capsules, semiconducting microbeads, and inhomogeneous striped and "eyeball" particles (Fig. 3a). Droplets containing suspensions of micro/nano particles, polymer solutions, and polymer precursors were merged and mixed to yield the different types of particles. In another example of synthesis applications on digital microfluidc devices, Dubois *et al.*[50] implemented Grieco's reaction using ionic liquid droplets as microreactors to synthesize tetrahydroquinolines. In this reaction, an onium salt is reacted with benzaldehyde derivatives and excess indene in an ionic liquid matrix to obtain tetrahydroquinolines. Ionic liquids are advantageous for this application because of their low vapor pressure – reactions can be implemented in small droplets (< 1 μL) on single-plate devices with no evaporation. In addition, ionic liquids have other advantages for use with DMF, such as intrinsic conductivity and thermal stability.

While the studies described above established the compatibility of digital microfluidics with chemical synthesis, they used simple one-plate devices that were capable of carrying out only a single, serial reaction with no dispensing, splitting, active mixing, or flexibility in droplet volumes. The Wheeler group introduced the first two-plate DMF platform for chemical synthesis that is suitable for the control of multi-step reactions in parallel.[51] This platform was used to carry out the synchronized synthesis of five peptide macrocycles from three different components (amino

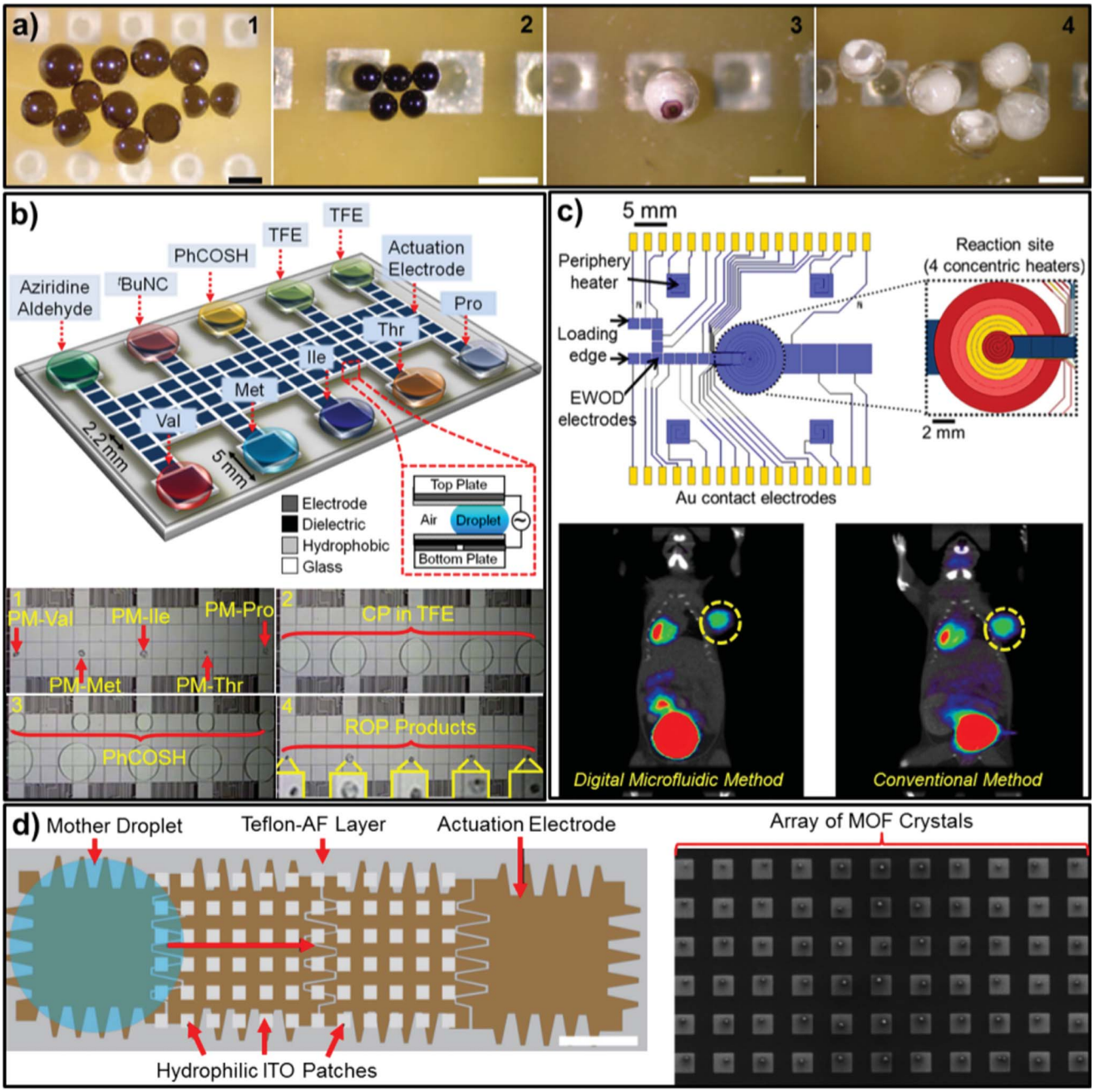

Fig. 3 Digital microfluidic applications in chemistry. a) Pictures depicting micro-particles synthesized using DMF. The products include conductive gold/SU-8 particles (1), semiconducting polypyrrole particles (2), ''eyeball'' microbeads (3), and cup-shaped particles formed by drying water droplets that were originally encapsulated in latex (4). Scale bars are 1 mm; reprinted with permission from ref. 49. Copyright © 2005 Nature Publishing Group. b) Schematic (top) of a DMF device used for synchronized synthesis, and sequence of frames from a movie (bottom) illustrating the steps in DMF synthesis of aziridine ring-opened peptide (ROP) products. Peptide macrocycles (PM) are solubilized in trifluoroethanol (TFE) (frames 1,2), then merged (frames 3,4) with droplets containing thiobenzoic acid (PhCOSH), followed by isolation of the ROP products. The insets in frame 4 are magnified images of the dried products. Reproduced with permission from ref. 51. Copyright © 2010 John Wiley & Sons, Inc. c) Schematic (top) of a DMF device with four concentric heaters for synthesis of radiotracer [^{18}F]FDG, and positron emission tomography images of a mouse bearing a lymphoma xenograft tumor in the right shoulder (dashed circle) after administration of [^{18}F]FDG prepared by DMF (left) and conventional method (right). Reproduced with permission from ref. 52. Copyright © 2012 National Academy of Sciences. d) Top-view schematic (left) of a mother droplet moving across an array of indium-tin oxide (ITO) micropatches formed in a Teflon-AF background matrix and a scanning electron microscope image of single metal–organic framework (MOF) crystal arrays (right) synthesized by DMF. Reproduced with permission from ref. 53. Copyright © 2012 John Wiley & Sons, Inc.

acids, aziridine aldehyde and *tert*-butyl isocyanide) followed by late-stage modification with thiobenzoic acid to generate aziridine ring-opened products. As shown in Fig. 3b, the DMF device featured ten reagent reservoirs and eighty-eight actuation electrodes dedicated to dispensing, merging, and mixing droplets of reagents and products. In other work, Keng *et al.*[52] developed an integrated two-plate DMF device with four concentric heaters for synthesizing 2-[^{18}F]fluoro-2-deoxy-D-glucose ([^{18}F]FDG), a widely used radiotracer for imaging living subjects with positron emission tomography, with high and reliable radio-fluorination efficiency (> 85%) (Fig. 3c, top). DMF synthesized [^{18}F]FDG was biodistributed (*in vivo*) in mice bearing lymphoma xenograft tumors and exhibited comparable uptake in tumors to [^{18}F]FDG prepared using conventional methods (Fig. 3c, bottom). Finally, Witters *et al.* used DMF to synthesize single metal–organic framework (MOF) crystals in a high-throughput fashion.[53] As shown in Fig. 3d, library HKUST-1 [$Cu_3(BTC)_2$] crystals were printed by transporting a mother droplet of HKUST-1 precursor solution over an array of indium-tin oxide micropatches (in a Teflon-AF background matrix) on the top plate of a DMF device. Among other benefits, the authors anticipate that this technology can pave the way for straightforward post-synthesis modification of printed MOF crystals with different functionalities.

In comparison with other microfluidic technologies (*e.g.*, enclosed microchannels), digital microfluidics is particularly well suited to synthesis, as DMF allows for precise control over multiple reagent phases. For example, a critical step in the synthesis of aziridine ring-opened products (as described above) is the removal of the solvent and re-dissolution of the crystalline peptide macrocycles for further processing (Fig. 3b, frames 1–2). This highlights the flexibility of DMF – the solvent volumes used to re-dissolve a particular solid can be readily varied. This stands in contrast to microchannel-based systems, in which working volumes are defined by the channel dimensions and cannot be changed.

The most useful features of digital microfluidics for synthesis include the individual addressing of all reagents with no need for complex networks of microvalves,[54,55] chemically inert Teflon-based device surfaces that facilitate the use of organic solvents and easy access to reasonably large amounts of products for off-chip analysis. On the other hand, DMF is not appropriate for all synthetic applications; for example, reactions performed at high temperatures and pressures or those that require in-line purification are better suited for closed microchannel systems[56] and there are several unique advantages associated with modular continuous flow reactors formed in microchannels (also known as mesofluidics[1,57]). Nevertheless, the potential benefits of DMF as a synthetic platform have yet to be fully explored, suggesting considerable room for innovation in the future.

Digital microfluidic applications in biology

Digital microfluidics is an attractive platform for biological applications,[43] which often require the use of expensive or precious reagents. A challenge for such applications is the non-specific adsorption of biological molecules to device surfaces (biofouling), which can lead to sample loss or cross-contamination. In the context of DMF, biomolecules adsorbed to device surfaces can result in droplet sticking, which can render fouled devices useless. Strategies have been developed to overcome this problem. For example, DMF devices making use of an oil matrix reduce the opportunity for biomolecules to come into contact with surfaces, which limits the extent of fouling.[58] For applications that are not compatible with oil, an alternative strategy is to mix samples and reagents with low concentrations of additives such as Pluronic©[59,60] or graphene oxide[61], which facilitates the actuation of serum and other concentrated biochemical reagents while reducing fouling. Lastly, one can use a removable hydrophobic insulator, such that each successive experiment is implemented on a fresh device surface.[62] These adaptations have made DMF compatible with a wide range of applications in biology, as described below.

Handling and characterizing samples of DNA have become critical steps for a wide range of applications, especially molecular biology. Accordingly, a number of examples of digital microfluidic DNA manipulation have been published. Early studies included using DMF to study the repair of oxidized lesions in oligonucleotides by Jary *et al.*[63] 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. Liu *et al.*[64] 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 other studies, Malic *et al.*[65,66] carried out the on-chip immobilization of thiolated DNA probes followed by hybridization with droplets containing complementary oligonucelotide target sequences. Measurements were carried out by surface plasmon resonance imaging and revealed a two-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.*,[67] who implemented the polymerase chain reaction (PCR). In this work, a DMF device with an embedded micro-heater was developed to facilitate thermal cycling. 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. In other work, Sista *et al.*[68] performed a 40-cycle real-time PCR in 12 min by shuttling a droplet through different temperature zones. In addition, Pollack and coworkers from Advanced Liquid Logic (ALL; www.liquid-logic.com) improved on previous methods by developing a multifunctional DMF cartridge capable of performing real-time PCR, immunoassays and sample preparation assays.[69] The cartridge was operated using a custom-built benchtop instrument equipped with all of the required control and detection capabilities for performing multiplexed real-time PCR. More recently, the same group utilized their digital microfluidic-based instrument to sequence DNA using a pyrosequencing method (Fig. 4a).[70] For proof-of-concept, a portion of a 229 bp *Candida parapsilosis* template was sequenced and over 60 bp of sequence was generated with 100% accuracy. Lastly, our group developed a DMF distribution hub for integrating multiple subsystem modules for automated library template construction for next generation sequencing (NGS). This DMF hub platform not only executed the sample preparation protocol,[44] but also integrated a

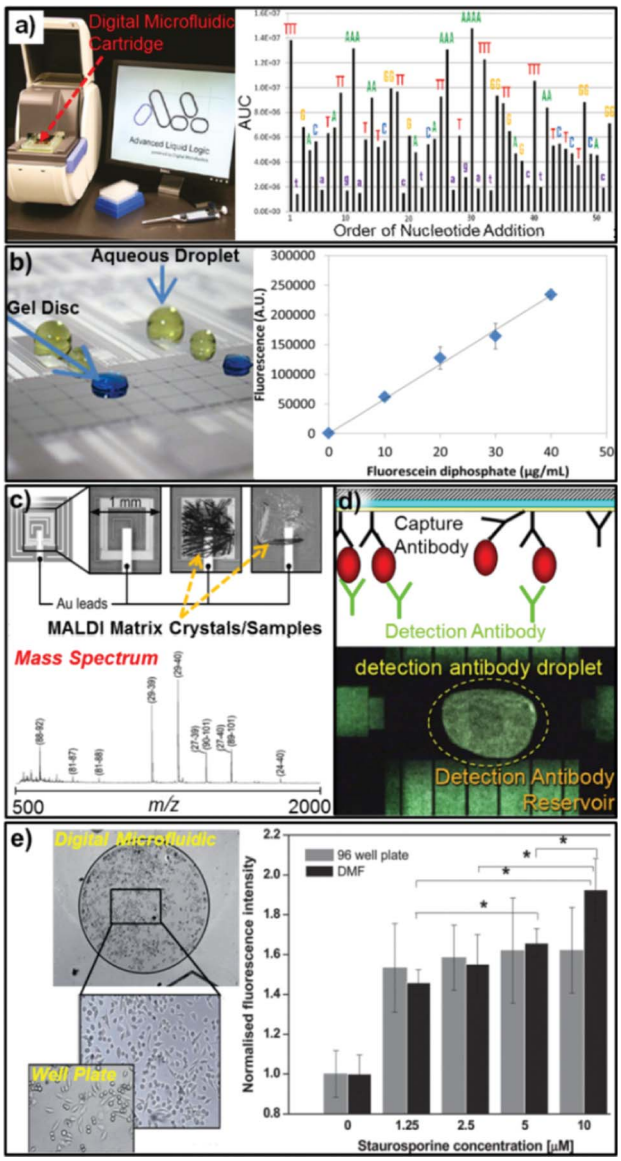

Fig. 4 Digital microfluidic applications in biology. a) Picture (left) of a sequencing instrument from Advanced Liquid Logic, and a pyrogram (right) of the *Candida parapsilosis* template. Reproduced with permission from ref. 70. Copyright © 2011 The American Chemical Society. b) Image (left) of gel discs on a DMF device with no top plate and the fluorescence response curve (right) as a function of fluorescein diphosphate concentration. Reproduced with permission from ref. 22. Copyright © 2012 American Institute of Physics. c) Pictures (top) of a MALDI matrix and sample crystals grown after the evaporation of the solvent by resistive heating and a MALDI-MS spectrum (bottom) of cytochrome *c* digested at 40 °C. Reproduced with permission from ref. 78. Copyright © 2010 The American Chemical Society. d) Schematic (top) depicting an IgG sandwich immunoassay, and picture (bottom) of a droplet containing detection antibody (FITC-labeled *anti*-IgG). Reproduced with permission from ref. 82. Copyright © 2011 Springer. e) Pictures (left) of HeLa cells on a hydrophilic site on a DMF device and (inset) cells grown on a well-plate, and the dose-response curve (right) of caspase-3 activity as a function of staurosporine concentration from cells assayed on a 96 well plate and DMF device. Reproduced with permission from ref. 83. Copyright © 2012 The Royal Society of Chemistry.

quantitative capillary electrophoresis module for the size-based quality control of the library prior to sequencing.[47]

Digital microfluidics has also been applied to conduct enzyme assays. In one of the first reports, Taniguchi *et al.*[71] demonstrated a bioluminescence assay for ATP using the luciferase enzyme. Later, Nichols and Gardeniers[72] carried out time-sensitive measurements by using DMF to mix the reagents and MALDI time-of-flight mass spectrometry to investigate the pre-steady-state kinetics of the enzyme, tyrosine phosphatase. Subsequently, Miller[73] *et al.* applied DMF to the study of enzyme kinetics by mixing and merging droplets of alkaline phosphatase with fluorescein diphosphate on a multiplexed DMF device. 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. Recently, Fiddes and Luk *et al.*[22] also demonstrated the action of alkaline phosphatase on fluorescein diphosphate using cylindrical hydrogel discs incorporated in DMF devices (Fig. 4b). In this work, agarose gel discs were modified with alkaline phosphatase enzyme molecules, and droplets containing fluorescein diphosphate were dispensed and merged onto the gels for the cleavage of phosphate groups and the generation of fluorescein.

The capacity to address many reagents and phases simultaneously makes digital microfluidics a good fit for applications in proteomics as well. Early work in this area focused on the combination of DMF sample handling with detection by matrix assisted laser desorption ionization mass spectrometry (MALDI-MS).[39,74,75] More recently, Jebrail *et al.* implemented a DMF-based protocol for extracting and purifying proteins from complex biological mixtures (*e.g.*, serum and cell lysate) by precipitation, rinsing, and resolubilization.[19] The method has protein recovery efficiencies comparable to conventional techniques ($\geqslant 80\%$) and has the benefit of not requiring centrifugation. Processes relying on centrifugation are challenging to implement in most systems based on lab-on-a-chip devices, including DMF, and are therefore avoided whenever possible. In other work, Luk *et al.*[76] and Chatterjee *et al.*[77] applied DMF to key proteomic processing steps that commonly follow protein extraction, including reduction, alkylation, and digestion. Nelson *et al.*[78] improved upon these techniques by integrating resistive heating and temperature sensing elements for straightforward integration with MALDI-MS (Fig. 4c). Jebrail *et al.*[79] integrated many of these methods into an automated digital platform including protein precipitation, rinsing, resolubilization, reduction, alkylation, and digestion. Finally, Luk and Fiddes *et al.*[80] integrated agarose discs ($\sim$2 mm diameter) bearing immobilized enzymes (*e.g.*, trypsin or pepsin) into DMF systems for digesting proteins.

Digital microfluidics has also proven to be a useful platform for carrying out immunoassays. Sista *et al.*[81] reported a droplet-based magnetic bead immunoassay using DMF to detect insulin and interleukin-6. In this work, a droplet of analyte and a second droplet containing magnetic beads (modified with capture antibodies), blocking proteins, and reporter antibodies were merged to form capture antibody–antigen–reporter antibody complexes. A magnet was then used to immobilize the beads such that the supernatant could be driven away. The assay had low detection limits: less than 10 pmol L^{-1} and 5 pg mL^{-1} for insulin and interleukin-6, respectively. In a separate study, Sista

et al. implemented a similar method for detecting cardiac troponin I in whole blood.[68] Recently, Miller *et al.*[82] developed a DMF platform for similar immunoassay applications implemented without beads or magnets. As shown in Fig. 3d, the method relied on device surfaces modified with spots of capture antibody (Fc-specific *anti*-human IgG), which binds antigen to the droplet sample and is in turn recognized by detection antibodies.

Cell-based assays have also been a popular target for digital microfluidics, as the reagents and other materials are often prohibitively expensive for large-volume techniques. In their initial work, Barbulovic-Nad *et al.* 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 to generate dose-response curves.[84] The DMF assay was more sensitive than conventional methods and had no significant effects on cell vitality. This agrees with a study conducted by Zhou *et al.*, in which no increase in the number of dead osteoblasts was observed after droplet actuation.[85] Fan *et al.*[86] used dielectrophoresis to separate neuroblastoma cells to different regions of droplets that were manipulated by DMF. The original droplets were then split into daughter droplets containing different cell densities. Shah *et al.*[87] improved upon these techniques by integrating DMF with optoelectronic tweezers for precise cell handling.

The Wheeler group developed a DMF-driven method of solution replacement for complete cell culture (*i.e.*, cell seeding, growth, detachment, and re-seeding on a fresh surface, *etc.*) and analysis, which they have termed "passive dispensing."[37,88,89] In this approach, an aqueous droplet is driven across a hydrophilic site formed on the surface of the bottom[37] or top plate[88,89] of a DMF device, which results in the spontaneous formation of a sub-droplet dubbed a "virtual microwell". Utilizing the above mechanism, Bogojevic *et al.*[83] recently developed the first DMF device implementing a multiplexed cell-based apoptosis assay. In this work, HeLa cells were seeded and grown on hydrophilic sites patterned on the top plate of DMF device, and a 6-plex caspase-3 activity assay was conducted using staurosporine as a model agonist (Fig. 4e). The method generated dose-response profiles of caspase-3 activity as a function of staurosporine concentration comparable to conventional techniques (*i.e.*, pipetting, aspiration, and 96-well plates.), but it yielded lower detection limits, greater dynamic range, and a 33-fold reduction in reagent consumption. Note that the above cell based assays required low Pluronic© concentrations (0.02–0.2% w/v) to facilitate cell droplet movement.

Digital microfluidic applications in medicine

The precise control over different reagents, phases (*i.e.*, liquids relative to solids[19,20,51,80,90] and immiscible solvents[20,91]) and volumes afforded by digital microfluidics makes it well suited to applications in medicine. In an important first step toward clinical applications, the Fair group[38] developed a series of glucose assays in physiological fluids (serum, saliva, plasma, and urine) with actuation by DMF (see Fig. 4a). More recently, Sista *et al.*[68] developed a DMF technique to extract DNA from whole blood samples using magnetic beads with integrated analyses by immunoassays and PCR.

Noha and Jebrail *et al.* developed a digital microfluidic method for processing 1 mg samples of breast tissue homogenate and 1 μL samples of blood and serum for the quantitation of steroid hormones.[20] In a typical assay, a sample was chemically lysed, the estradiol extracted into a polar solvent, the unwanted constituents were extracted into a nonpolar solvent by liquid–liquid extraction and the extract was delivered to a collection reservoir for off-chip analysis (Fig. 4b). The DMF method uses a sample size that is 1000–4000 times smaller than conventional methods for the extraction and quantification of steroids and was 20–30 times faster.

Digital microfluidic techniques provide similar benefits for analyzing newborn dried blood spot (DBS, *i.e.*, a sample of filter paper bearing dried blood) samples used to screen for metabolic disorders. Recently, a cover story in *Chemical & Engineering News*[93] described the increasing prevalence of DBS samples for disease detection and drug development, driven by advantages such as small sample consumption, easy shipment and storage and reduced use of animal testing. However, DBS samples present several challenges, including manual processing (*e.g.*, solvent extraction) and lengthy analysis times[93], drawbacks that can potentially be alleviated when using DMF.

The Wheeler group[21] reported the first microfluidic method for in-line extraction and analysis of analytes in DBS samples by mass spectrometry. In this work, biomarkers for amino acid metabolism disorders (*e.g.*, methionine, phenylalanine and tyrosine) were extracted from the DBS of newborn patients and derivatized using digital microfluidics, then quantified by tandem mass spectrometry (Fig. 5c). The prototype microfluidic system was able to correctly identify newborn patients suffering from metabolic disorders (*e.g.*, phenylketonuria) while significantly reducing the required sample volume (20 μL *vs.* 170–450 μL) and analysis time ($\sim$1 h *vs.* $>$ 3.5 h) relative to conventional methods. In a separate study, the same group improved on their previous method by including a new, straightforward interface between DMF and mass spectrometry for in-line analysis.[45] This method was used to perform on-chip extraction and quantification of succinylacetone, a specific marker of tyrosinemia type 1, in DBS samples. Finally, Advanced Liquid Logic validated a disposable DMF cartridge for rapid, multiplexed analysis of newborn DBS extracts for lysosomal storage diseases (Fig. 5d).[92] Eleven DBS extracts were simultaneously processed on a single cartridge and analyzed for Pompe and Fabry disorders (caused by acid α-glucosidase and α-galactosidase deficiencies) using fluorometric enzyme assays. These new DMF methods have the potential to contribute to a new generation of analytical techniques for quantifying analytes in DBS samples in a wide range of applications.

Cross-cutting applications

The unique characteristics of digital microfluidics have also made this technology attractive for a diverse set of applications that do not fit neatly into a single category as described above. For example, Zhao *et al.*[94] manipulated air bubbles instead of droplets on DMF devices and used these bubbles to effect a chemical reaction between gaseous reagents. In other work,

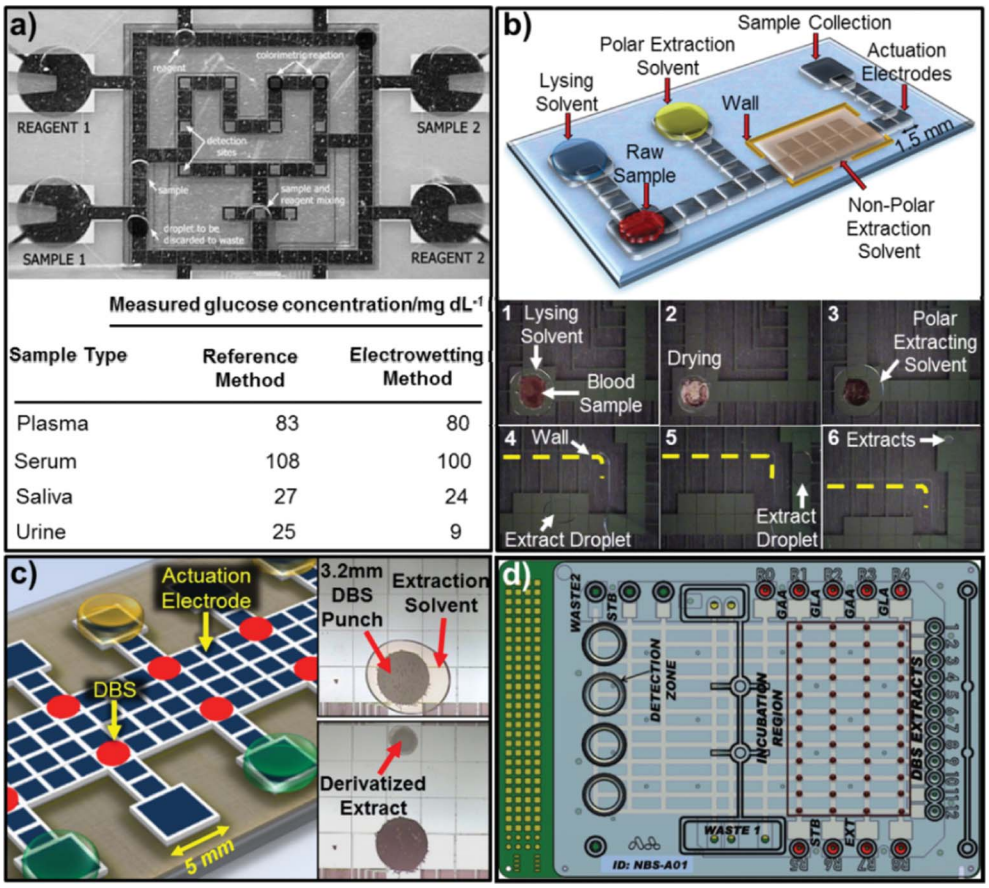

Fig. 5 Digital microfluidic applications in medicine. a) Picture of DMF (top) used to perform glucose assays, and table (bottom) comparing glucose concentrations in different samples obtained by DMF and a reference method. Reproduced with permission from ref. 38. Copyright © 2004 The Royal Society of Chemistry. b) Schematic (top) of a DMF device for extracting and purifying estrogen from tissue, blood, or serum, and a series of frames (bottom) from a movie (1 to 6) illustrating the key steps in the DMF-based extraction of estrogen from a 1 μl droplet of human blood. Reproduced with permission from ref. 20. Copyright © 2009 The American Association for the Advancement of Science. c) Schematic of dried blood spots (DBS) on a DMF device (left) and pictures (right) depicting sample processing of a single 3.2 mm DBS by DMF. Reproduced with permission from ref. 21. Copyright © 2011 The Royal Society of Chemistry. d) Schematic of disposable DMF cartridge developed by Advanced Liquid Logic Inc. for multiplexed enzyme analyses of DBS extracts. Reproduced with permission from ref. 92. Copyright © 2011 The American Association for Clinical Chemistry.

droplets controlled by DMF were used to collect particles from the surfaces of perforated microfilter membranes, which may be useful for sampling bioaerosols in environmental monitoring applications.[95] Gao *et al.* used DMF for rapid colorimetric detection of mercury(II) by mixing a detector droplet composed of a conjugated polymer and a label-free mercury-specific oligonucleotide probe with a mercury ion-containing droplet.[96] Recently, Kuehne *et al.*[97] reported the miniaturization of dye-lasers by using DMF. As shown in Fig. 6b, the emission wavelength of the DMF dye laser can be altered by moving droplets of different dye solutions in and out of an excitation beam. This technique has the potential to be beneficial for a wide range of applications requiring on-chip optical sensing.

In another example, Polous *et al.*[98] developed an integrated DMF device bearing thin-film electrodes for the formation and analysis of lipid bilayer membranes (Fig. 6c). In this work, aqueous droplets surrounded by a lipid-containing organic oil were moved close to each other and lipid bilayer formation at the interface was probed using electrochemical techniques. Son *et al.*[99] used DMF to transport and process relatively large living organisms, as shown in Fig. 6d. For example, the dechorionation (*i.e.*, removing the chorion of an embryo to facilitate micro-injection and accelerate growth) of a zebrafish embryo was carried out by merging droplets containing digestive reagents and an embryo. This work represents an initial step towards using DMF as an alternative to microwell plates for applications involving multicellular organisms.

Au and Shih *et al.*[100] reported a microbioreactor powered by DMF for the automated culture and on-chip analysis of microorganisms (*e.g.*, bacteria, algae and yeast). Lapierre *et al.*[101] coupled DMF to a surface-assisted laser desorption–ionization (SALDI) silicon nanowire-based interface for mass spectrometry (MS) analysis. The integrated system allows for a rapid, straightforward and highly sensitive MS analysis of small biomolecules. Finally, Choi *et al.*[102] equipped a DMF device with field effect transistors (FET) to electrically detect biomolecules. As shown in Fig. 6e, FET-based biosensors were embedded in the center of droplet-actuation electrodes for tracing an influenza antibody in real-time without labeling.

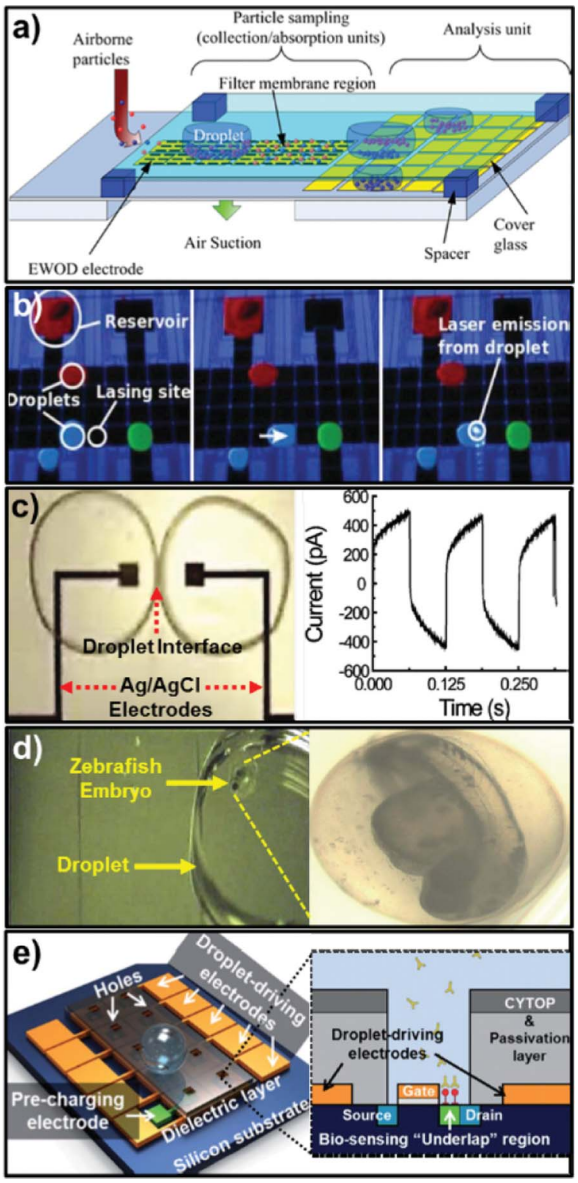

Fig. 6 Cross-cutting applications of digital microfluidics. a) A schematic of a DMF-powered monitoring system for airborne particle sampling and analysis. Reproduced with permission from ref. 95. Copyright © 2009 IOP Publishing. b) Images from left to right of a blue dye droplet actuated to the lasing site by DMF for emission. Reproduced with permission from ref. 97. Copyright © 2011 The Royal Society of Chemistry. c) Picture (left) of a DMF device integrated with Ag/AgCl electrodes controlling two aqueous droplets, each surrounded by a lipid-containing organic phase, and electrical measurement (right) of bilayer formation at the interface of the two droplets. Reproduced with permission from ref. 98. Copyright © 2009 American Institute of Physics. d) Picture of a digital microfluidic device controlling a droplet containing a zebrafish embryo for processing (left) and picture (right) of a magnified embryo on the device. Reproduced with permission from ref. 99. Copyright © 2009 The Royal Society of Chemistry. e) Top-view schematic of a DMF device embedded with field effect transistors (FET) biosensors (left) and an exploded cross-sectional view of the biosensor (right). Reproduced with permission from ref. 102. Copyright © 2012 The Royal Society of Chemistry.

Summary and outlook for the future

Since its debut in the early 2000s as a basic method for moving water droplets on a surface, digital microfluidics (DMF) has matured from something of a curiosity into a technology that is making unique contributions to laboratory practice in chemistry, biology, medicine, and other fields. The most active application area for DMF so far has been biology, but we expect this to change in the future as the unique features of DMF become more widely recognized. For example, we believe that the potential benefits of DMF for chemical synthesis and multi-phase sample manipulation have yet to be fully investigated and exploited. Moreover, we anticipate that DMF will increasingly find applications in portable and deployable applications like forensic science, biosurveillance, and environmental sampling.

Several of the advantages and disadvantages of DMF described here are listed in Table 1. While challenges remain, DMF technology has matured rapidly in a fairly short time and shows great promise for growth beyond academic and research labs. At least one company, Advanced Liquid Logic (ALL), has been established to translate the capabilities of DMF to end users, and there are likely to be others. Moreover, ancillary players such as Luminex Corporation (www.luminexcorp.com) and NuGen Technologies (www.nugeninc.com) are partnering with ALL and others to develop small-footprint, cost-efficient systems that reduce labor costs and produce fast and accurate analytical results. In the next decade, we speculate that an ever-expanding community of researchers spanning academia, industry, and government will continue to push DMF technology to address an ever-growing list of challenging problems in chemistry, biology, medicine, and beyond.

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