Front. Mech. Eng. 2017, 12(4): 510–525
DOI 10.1007/s11465-017-0460-z

REVIEW ARTICLE

He WANG, Liguo CHEN, Lining SUN

Digital microfluidics: A promising technique for biochemical applications

© Higher Education Press and Springer-Verlag Berlin Heidelberg 2017

Abstract Digital microfluidics (DMF) is a versatile microfluidics technology that has significant application potential in the areas of automation and miniaturization. In DMF, discrete droplets containing samples and reagents are controlled to implement a series of operations via electrowetting-on-dielectric. This process works by applying electrical potentials to an array of electrodes coated with a hydrophobic dielectric layer. Unlike microchannels, DMF facilitates precise control over multiple reaction processes without using complex pump, microvalve, and tubing networks. DMF also presents other distinct features, such as portability, less sample consumption, shorter chemical reaction time, flexibility, and easier combination with other technology types. Due to its unique advantages, DMF has been applied to a broad range of fields (e.g., chemistry, biology, medicine, and environment). This study reviews the basic principles of droplet actuation, configuration design, and fabrication of the DMF device, as well as discusses the latest progress in DMF from the biochemistry perspective.

Keywords digital microfluidics, electrowetting on dielectric, discrete droplet, biochemistry

1 Introduction

The microfluidics system, which generally comprises microfabricated structures for liquid handling, has developed rapidly in the last few decades. Although micromachined systems were introduced in the 1970s [1], microfluidics devices did not receive much attention until the 1990s [2]. Lab-on-a-chip devices and integrated microfludics systems are considered as the bio-chemical analogue of the microelectromechanical system, because the former can automate multiple reaction processes that must be manually implemented in conventional laboratories in the past. For example, such a device can extract and purify certain components from a complicated mixture, isolate the target compositions, and then detect them. Therefore, microfluidics systems have significant potential in the fields of biochemical analysis, clinical diagnosis, environmental monitoring, and food safety analysis.

Digital microfluidics (DMF), a novel microfludics technology, has attracted the interest of many researchers because it is a promising and popular technique with several outstanding advantages [3]. Its most distinct feature is its capacity to manipulate each droplet independently to perform different processes without the need for complicated structural networks of channels, pumps, microvalves, or mechanical mixers, while droplets are limited to actuation in series in the micro-channels. A second unique advantage is DMF’s reconfigurability from one experiment to another.

In DMF, the droplets are independently controlled on an array of electrodes coated with a layer of hydrophobic dielectric material. Once a series of potentials are applied to the electrodes in series, each droplet can be individually manipulated to move, merge, mix, split, and dispense from the reservoirs. This review mainly focuses on the latest progress of DMF in recent years. We first review the DMF formats and theories proposed, after which we summarize the fabrication technique. The last section discusses the recent research developments of DMF devices from the perspective of biochemistry applications in the last five years.

2 Formats and theory of digital microfluidics

DMF devices have two different configurations (Fig. 1(a)):

Received December 29, 2016; accepted April 13, 2017

He WANG, Liguo CHEN (✉), Lining SUN
Robotics and Microsystems Center, Soochow University, Soochow 215006, China
E-mail: chenliguo@suda.edu.cn

He WANG
School of Mechanical Engineering, Henan University of Engineering, Zhengzhou 451191, China

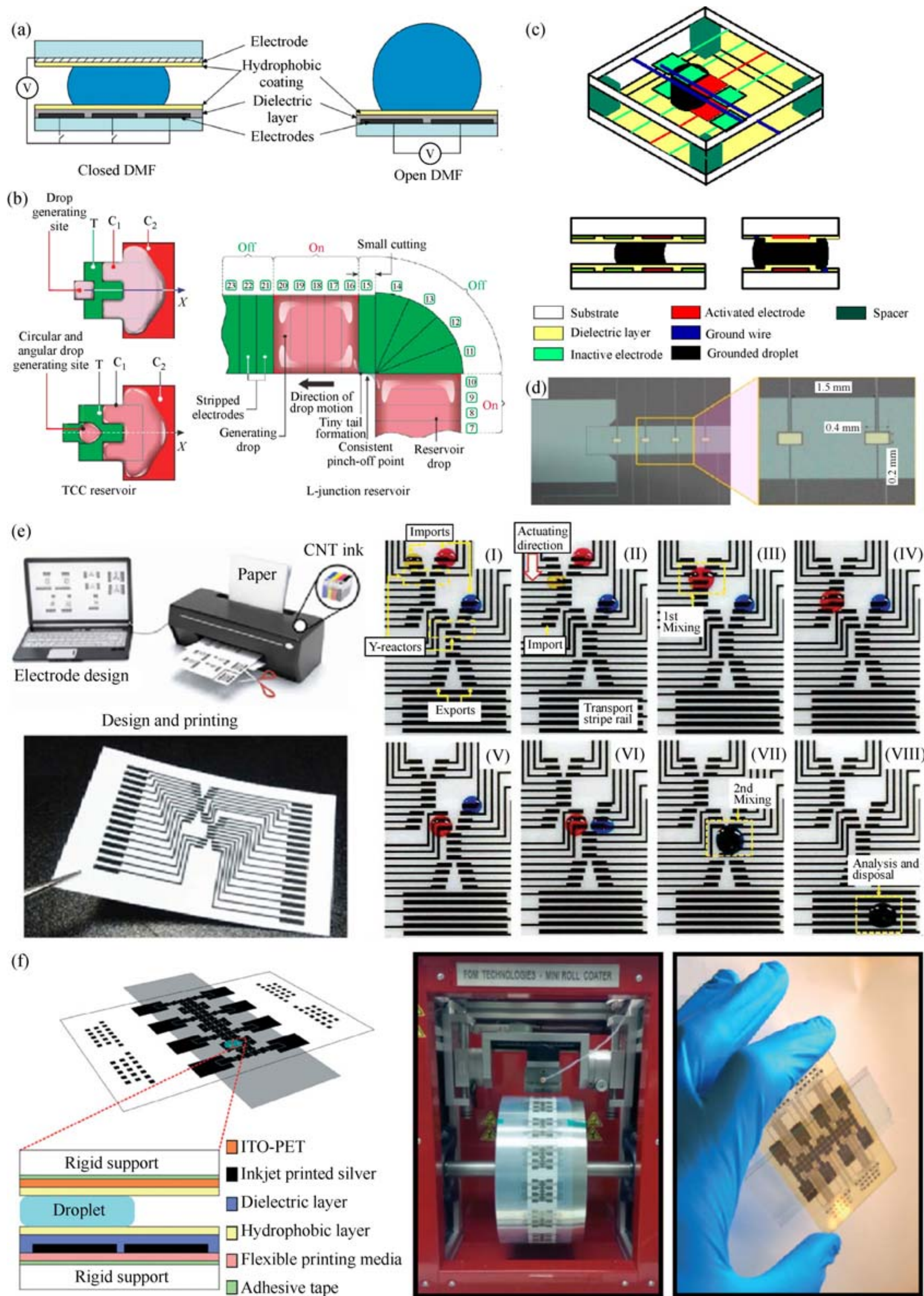

Fig. 1 DMF: (a) Side-view schematics of the parallel-plate (left) and single-plate (right) DMF devices; (b) photos of the TCC and L-junction reservoirs; (c) schematics of the twin-plate electrowetting configuration; (d) photo (upper left) of the fabricated single-plate electrowetting-on-dielectric device with island-ground electrode (IG-SEWOD) device, snapshots (lower left) of the droplet transport, and comparison of the droplet dynamic properties in the *X*-direction (upper right) and *Y*-direction (lower right) transport between the IG-SEWOD and floating EWOD device; (e) photo (left) of the inkjet-printed paper chip, and a series of frames (right) from a movie (I to VIII) that depicts the mixing process of the three droplets; (f) isometric-view schematic (left) and photo (right) of the inkjet printed, roll-coated paper DMF chip, as well as photo (middle) of a roll of inkjet-printed bottom plate placed on a mini roll coater to coat a layer of Cyanoresin CR-S cyanoethyl pullulan as the dielectric layer

The parallel-plate format [4] (also known as closed) and the single-plate format [5] (also known as open). In the parallel-plate format, droplets are sandwiched between two parallel plates wherein electrodes are patterned. The lower plate consists of an array of actuation electrodes, whereas the upper plate serves as the ground electrode consisting of a transparent conductive material (e.g., indium tin oxide (ITO)). In the single-plate format, each droplet sits on top of a single substrate that bears both the actuation and the ground electrodes. Regardless of the DMF format, all the actuation electrodes are covered with a layer of dielectric material to restrict the electric current and prevent electrolysis. A hydrophobic coating is also deposited on top of the insulating layer, which is mainly to increase the contact angle and reduce contact angle hysteresis. The droplets in many DMF devices are manipulated in the air, although other filler media (e.g., silicone oil) can lower the evaporation rate and decrease the voltage required to actuate the droplets [5].

The two DMF configurations have their distinct advantages even though they have similar actuating principles. Parallel-plate DMF devices are fit for all droplet operations; transporting, splitting, merging, mixing, and dispensing from the reservoirs are all feasible [6,7]. Such devices also provide reliable and reproducible droplet volume control. By contrast, the single-plate format cannot easily split or dispense droplets from reservoirs, and the aforementioned functions can only be attained under special conditions [8]; however, it is more easily integrated with other liquid handling and operation platforms or surface analytical facilities. Moving and mixing are faster and easier in the single-plate format as the resistance to droplet movement decreases. A third advantage for the single-plate structure is that spherical droplets can more easily focus on the fluorescence, significantly improving the detection sensitivity [9].

When applying a series of electrical potentials to an array of actuation electrodes in DMF devices [10], each droplet is controlled independently to perform corresponding operations. This has been previously explained as a phenomenon called "electrowetting" (EW) or "electrowetting-on-dielectric" (EWOD). In the EWOD paradigm, the droplet wettability on a solid surface is electrically changed to produce a motion, and this is caused by the electrical force concentrating on the triple-phase contact line. The concentrated electrical force results in a change in the contact angle, which in turn, leads to a capillary pressure that causes the droplet movement. These theoretical analyses are based on a thermodynamic perspective through the Young-Lippman equation [4,7] given by

$$\cos\theta = \cos\theta_0 + \frac{\varepsilon_0 \varepsilon_r V^2}{2\gamma d}, \tag{1}$$

where θ_0 is the initial contact angles with zero applied voltage, θ is the static contact angles under the applied voltage of V, ε_0 is the vacuum permittivity, ε_r is the relative permittivity of the dielectric, V is the externally applied voltage, γ is the surface tension coefficient between the droplet and surrounding fluid media, and d is the thickness of the dielectric layer.

However, the EWOD understanding can neither explain why dielectric liquids or other liquids with low surface tension can be actuated with almost no significant changes in the contact angle [11], nor explain other related phenomena. For example, the contact angle does not increase with the applied voltage when it exceeds the threshold voltage, which is referred to as the contact angle saturation (CAS).

A more direct and complete understanding of the phenomenon of droplet actuation is derived from the electromechanical perspective, which posits that the electrical forces exerted on the liquid along the triple-phase contact line lead to droplet motion [12–14]. When an electric potential is applied, a large amount of the free charges and polarized dipoles gather near the triple-phase contact line by the external electric field, resulting in the Maxwell-stress on the liquid-fluid media interface. Consequently, the curvature of the liquid-fluid media interface must be decreased to balance this stress, which also lowers the Laplace pressure. The decrease in the contact angle can then be realized according to the magnitude of the applied voltage [15]. From the modeling perspective, these forces that act on the droplet surface are calculated by integrating the Maxwell Stress tensor $\boldsymbol{T}$ over an arbitrary surface surrounding the droplet [16], using the equation

$$\begin{cases} F = \int T_{ij} \boldsymbol{n} \mathrm{d}S \\ T_{ij} = \varepsilon \left(-\frac{1}{2} \boldsymbol{E}^2 I_{ij} + E_i E_j \right) \end{cases}, \tag{2}$$

where $\boldsymbol{n}$ is the unit normal vector, i and j denote respective pairs of the x, y, and z axes, ε is the dielectric constant of the fluid media surrounding the droplet, $\boldsymbol{E}$ is the electric field around the droplet, and I_{ij} is the Kronecker delta.

The electromechanical perspective may also explain the movement of dielectric liquids and liquids with no change in the contact angle. Although several researchers believe that the electromechanical perspective may be related to the CAS [17], no uniform conclusion has been derived concerning the mechanism that explains CAS completely.

3 Device design, fabrication, and implementation

Various DMF device design schemes, fabrication techniques, and analytical technologies have been developed to

satisfy the needs of different applications. This section mainly focuses on the pros and cons of recent advancements in this field.

3.1 Device design

Except for EWOD, droplets in DMF are also manipulated by many other actuation mechanisms, such as thermocapillary [18,19], surface acoustics [20–23], dielectrophoresis [24–27], magnetic forces [28–31], and optoelectrowetting [32–34]. Regardless of the selected actuation mechanism, the performance improvement has always been one of the key research topics for DMF. In DMF devices, the ability to precisely control elementary droplet manipulations (e.g., dispensing and splitting) is important, because it largely affects the analysis performance once droplets with varying sizes are dispensed from the reservoir or unevenly split into two different sizes of daughter droplets. However, reproducing these manipulation processes of droplet splitting and dispensing is extremely difficult. Vergauwe et al. [35] studied the cumulative effects of different droplet operations on their volume variability by Monte Carlo simulations. They found that reproducible droplet generation, splitting, and transport could be accurately controlled with minimal droplet volume variability by optimizing actuation parameters (e.g., activation time, relaxation time, actuation voltage, and electrode size). The most common method for generating droplets in DMF devices is by using T-junction geometries. Yaddessalage [36] designed different electrode geometries of reservoirs (e.g., TCC-shaped and L-junction reservoirs) to control the droplet pinch-off point, attain high accuracy and consistency of droplet generation, and enhance the speed of the droplet motion (Fig. 1(b)). These researchers attained the minimum average volume error of 0.083% for 50 droplets of repeatable droplet generation, demonstrating a high rate of reproducibility. Nevertheless, the electrode geometries of reservoirs are slightly complicated. If this class of electrode geometry of reservoirs is formed by the printing technique, then the fabrication for DMF devices can be made simpler. Decreasing the spacing height between the two parallel plates can also improve the accuracy and consistency in the volume of droplets dispensed from the reservoir. For example, Elvira et al. [37] studied the long-term reproducibility and reliability of daughter droplet generations. They found that the reliability of the droplet generation is relatively higher when lowering the spacing height, and that the uniformity of the dispensed droplet volume peaks as the volume of the mother droplet in the reservoir and that of the reservoir electrode become equal. Yafia and Najjaran [38] developed a variable gap size actuation mechanism in a parallel-plate DMF device to accomplish this goal. By varying the position of the top plate continuously and precisely according to different droplet manipulations, this technique is able not only to control the uniformity in the dispensed droplets volume, but also to control the droplet transport more reliably, split and merge more effectively, mix faster, and position more accurately. However, the structure is still extremely complicated even though the system performance is excellent.

In DMF systems, one common approach to decreasing the applied actuation voltage and increasing the driving force is by utilizing a thin film or high-k dielectric. However, the thinner the dielectric layer, the more likely it is that dielectric breakdown can occur. Hence, many attempts have been made to develop other solutions. For example, Chang and Pak [39] proposed a novel twin-plate, EWOD-based DMF structure, in which the two plates are identical, and each plate is equivalent to a ground-type, single-plate format. The two plates are combined with a spacer by adjusting their actuation electrode arrays facing each other (Fig. 1(c)). Given that the chord length of the effective triple-phase contact line is doubled in this new design, the EW force is now twice larger than that in the standard parallel-plate DMF device. Hence, the velocity of the droplet motion becomes significantly faster, and the applied voltage is theoretically decreased to range from 70% and 80% when the same velocity is attained. Cui et al. [40] presented a single-plate EWOD (SEWOD) device with island-ground (IG) electrode which was surrounded by the driving electrodes and resembled an island (Fig. 1 (d)). Although the structure of the IG-SEWOD device is slightly more complex, the driving voltage of the SEWOD device with the IG electrode is only half that of a conventional floating SEWOD device. The dynamic performance is significantly enhanced due to the "double locking" effect and rapid residual charge elimination provided by the IG electrode. IG-SEWOD could ensure that the distance of each step during droplet movement is one electrode length, whereas either excessive or insufficient droplet moving distances occur all the time in the floating SEWOD. Moreover, the transversal displacement of the droplet in the IG-SEWOD is much less than that in the floating SEWOD.

3.2 Device fabrication

The fabrication process of DMF devices is mainly determined by the substrates. Most academic laboratories select glass and silicon as the substrates; thus, photolithography or etching in clean-room facilities can be considered the most common fabrication methods of DMF chips. Given the high material cost, time consumption, lack of rapid prototyping, and stringent requirements on clean-room facilities, the traditional fabrication method of DMF chips hinders their widespread applications in laboratories, which weakens its advantage relative to channel-based microfluidic devices, and are particularly unfit for point-of-care testing (POCT) in low-resource settings, such as remote areas and developing nations. Such disadvantages have led to the rapid development of

different alternatives. Among these, applying printing techniques into the fabrication of DMF chips has received increasing interest with the emergence of paper as the substrate.

Inkjet printing conductive ink on paper is a simpler way to form an electrode array compared with the conventional process. Ko et al. [41] utilized the inkjet printing technique to fabricate patterned electrodes on commercially available photo papers. Using an electrode pattern designed and drawn by a desktop computer, the researchers printed on the photo paper by employing conductive carbon nanotube (CNT) ink. The printable CNT electrodes are coated with a layer of parylene-C through vapor deposition, followed by a Teflon-AF 1600 through spin coating (Fig. 1(e)). Tuning the surface tension with silicone oil and alternating current (AC) voltage at a low frequency is crucial in overcoming the technical barriers caused by lower printing resolution. Fobel et al. [42] also utilized inkjet printing with an in-lab high tech industrial-grade inkjet printer, which is relatively expensive but provides better resolution for fabricating a paper-based DMF. The authors employed silver nanopartical-based ink to print the electrodes. Moreover, the paper DMF devices have been utilized with an integrated paper spray ionization emitter for sample ionization for the first time [43,44]. The fabrication method of the paper-based DMF is simpler than the conventional fabrication techniques of the DMF devices, and can broaden the application of DMF chips. Furthermore, this fabrication approach can likely extend to the roll-to-roll process to attain the mass production of low-cost DMF chips. Although the performances of the paper-based DMF devices in the aforementioned reports are outstanding, the dielectric and hydrophobic layers are still formed by vapor deposition and spin-coating, which typically require a clean-room facility. In response to the disadvantages, Dixon et al. [45] designed a new technique to form paper-based parallel-plate DMF devices. In this new technique, they fabricated electrodes by utilizing a cheap consumer-grade inkjet printer, and a roll-coating process was then applied to the roll-to-roll fabrication to form the dielectric and hydrophobic layers, which could easily be scaled to mass production (Fig. 1(f)). The droplets movement is smooth and robust, and the velocities match those that have been formed using conventional cleanroom techniques. Moreover, this device is considered useful in conducting multistep procedures to implement complex rubella virus (RV) diagnostic assays. Except for inkjet printing, screen printing is also employed to pattern the electrodes on a paper substrate. Yafia et al. [46] also introduced screen printing for fabricating DMF electrodes to print conductive inks based on silver and carbon on the paper substrate. Compared with other rapid prototyping fabrication techniques, the said method exhibits superiority in many aspects in terms of the manufacturing steps, materials, time, cost, mass production capability, and equipment availability. However, this printing process is not only manually operated, but the applied voltage during the droplet transport process is also excessively high, reaching an AC signal of up to 300 Vrms (rms: Root mean square).

4 Biochemical application of digital microfluidics

Given that DMF devices are promising fluid-handling platforms with many advantages, they have been employed in different applications. Such systems can largely simplify the conventional and cumbersome laboratory procedures by automatically controlling micro-droplets. This section discusses the latest development of DMF applications in the bio-chemistry field from five aspects. Several applications span these categories and are thus mentioned several times.

4.1 Chemical and enzymatic assays

DMF devices can offer fast chemical reaction time as well as lower reagent and sample consumption. Moreover, multiple functions can be integrated on a single DMF device, so they are well suited for chemical and enzymatic assays. The applications commonly involve the precise measurement of reagents by daughter droplet generation and subsequent splitting, merging, and mixing to produce individual microreactors. Many chemical reactions can be performed simultaneously on a single DMF device by manipulating each droplet independently on an electrode array.

The implementation of chemical reactions on DMF mainly includes the evaluation of certain substances, the analysis of the reaction kinetics performance, and synthesis of new mixtures. Based on the early studies of Taniguchi et al. [47] and Ito et al. [48], many researchers have successfully developed chemical assays for different applications, such as those in the fields of biology and medicine. Given that most biochemical reactions in the body are enzymatic reactions, implementing enzyme assays on DMF have long been one of the most popular application targets. For example, Sista et al. [49] utilized the DMF platform to perform rapid, multiplexed fluorometric enzymatic assays, in which deidentified and affected newborn dried blood spots samples were collected and analyzed to screen for Pompe and Fabry diseases. Their results are highly consistent with those obtained by conventional bench-based fluorometry. Boles et al. [50] demonstrated the feasibility of implementing a three-enzyme pyrosequencing protocol within droplets utilizing DMF. The DMF device sequenced a portion of a 229 base pair Candida parapsilosis template, which initially confirmed the feasibility, through de novo and resequencing protocols.

Although many enzyme applications of DMF devices

have been reported in the literature, knowledge of how to improve the performance of an analytical assay on DMF is still lacking. Vergauwe et al. [35] applied Monte Carlo simulations to study the cumulative effects of the variability caused by different droplet manipulations. Reproducible droplet generation, splitting and transport could be accurately controlled with minimal droplet size variability by optimizing the actuation parameters (i.e., actuation voltage, activation time, relaxation time, and electrode size). They obtained a complete calibration curve for an enzymatic assay with a mean CV-value of 2% through parameter optimization. The "sample-to-analysis" problem is often encountered in many chemical analysis processes. In particular, analytes in many practical samples exist in large volumes of diluted concentration, whereas analysis techniques are often only restricted to small volumes. Solid-phase microextraction (SPME) can effectively solve this problem by rapidly and quantitatively extracting analytes from a large volume into a small volume, which are compatible with many analytical methods. Given that analytes with limited volatility are largely suitable for high-performance liquid chromatography (HPLC), SPME is often integrated with HPLC to perform the analysis, which requires complicated and specialized interfaces to automatically couple SPME with HPLC without human interference. However, these interfaces have some disadvantages. In response to this interface problem, Choi et al. [51] utilized DMF as the interface to integrate SPME with HPLC (Fig. 2(a)). In their proposed technique, analytes were first loaded onto the SPME fiber coating in the system, after which the SPME fibers were inserted into the open area between the two plates on the DMF device. Droplets were actuated onto the fibers and the analytes were desorbed to remove them from the fiber coating. Finally, the analytes were manually or automatically loaded onto the HPLC for subsequent analysis. The SPME-DMF-HPLC system was utilized for the quantitative analysis of anabolic-androgenic steroids and hormones in urine samples by mass spectrometry (MS). This method has proven to be beneficial in quantifying the free steroids at a concentration of pg/mL in urine.

Compared with microchannel-based format, DMF is particularly suitable for microscale chemistry synthetic applications, because it can individually handle discrete droplets containing samples that facilitate the total process control. This emerging technology is opening up the possibility of performing radiochemistry at the microliter scale to produce tracers for positron emission tomography (PET) labeled with fluorine-18 or other isotopes. For example, Keng et al. [52] designed an all-electronic DMF platform to synthesize [18F]-FDG, which is the the most common tracer in the PET imaging technique. They designed a reaction site consisting of four concentric heating rings so that the DMF device can have multiple functions (e.g., resistance to Joule heating, thermodynamic temperature sensing, and transporting droplets). The design enabled multistep chemical synthesis reactions, in which different solvents are required at different steps. The biodistribution *in vivo* was checked by imaging the mice bearing lymphoma xenograft with micro-PET-CT after on-chip synthesis to validate the quality of [18F]-FDG. The same research group [53] presented an improved Cerenkov imaging system to optimize the PET tracer synthesis on a DMF chip. This imaging system not only optimizes the mixing protocol, but also identifies and corrects for the loss of radioactivity because radioactive vapor moves out of the EWOD heater, which significantly increases the crude radiochemical yield (Fig. 2(b)). Witters et al. [54] utilized a parallel-plate DMF device to attain the synthesis of monodispersed single metal-organic frameworks (MOFs) crystals with high-throughput generation. In their proposed method, HKUST-1[$Cu_3(BTC)_2$] crystals were printed on the hydrophilic patches due to the wettability relative to the surrounding hydrophobic region when mother droplets of the HKUST-1 precursor solution was dispensed from the fluid reservoir and actuated over 20 μm × 20 μm hydrophilic-in-hydrophobic micropatches on the ITO-coated top plate (Fig. 2(c)). This method could flexibly synthesize a large array of MOF crystals with high parallelism and does not require any complicated and expensive equipment unlike other approaches.

4.2 Immunoassays

Immunoassay is an analytical method for different substances (e.g., drugs, hormones, proteins, and micro-organisms) with specific binding reactions between antigens and antibodies. The successful implementation of immunoassays based on DMF has been reported in the literature. To date, almost all DMF-based immunoassay studies rely on custom antibodies attached to the solid surface (either micro-particles [29,55,56] or a nanotextured solid surface of the device [57]). Hence, these immunoassays are generally referred to as "heterogeneous immunoassays." Sista et al. [58] successfully performed the first immunoassay based on magnetic microparticles on a DMF device. In the study, a droplet containing magnetic microparticles was actuated to shuttle across an array of actuation electrodes for dispensing, mixing, and resuspension. Magnetic particles were dispersed completely from the droplet by utilizing an external magnet. Multiple parameters that influenced the immobilization of the magnetic microparticles were optimized to enhance the immunoassay performance. The droplets in this example were driven in the surrounding oil. In this case, oil is helpful for DMF devices because it can decrease the actuation voltage and friction force during droplet movement, as well as significantly lower the evaporation rate of droplets [59]. However, several drawbacks also exist: In such a DMF device, proteins from the droplets may adsorb onto the fluidic-fluidic interface [60]; special packaging is

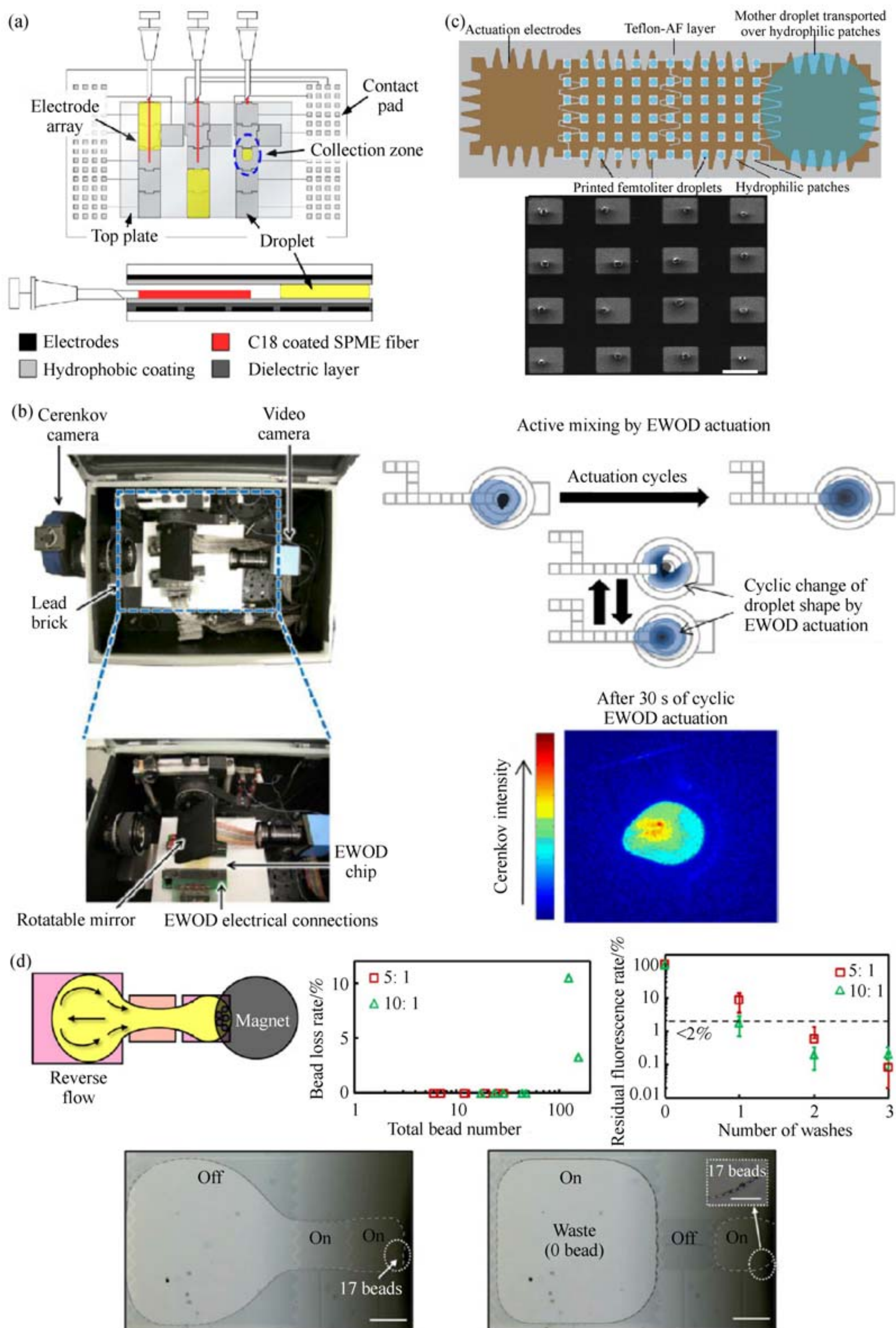

Fig. 2 DMF applications in chemical/enzymatic reactions and immunoassays. (a) Top-view (upper) and side-view (lower) schematics of the SPME-DMF interface; (b) photo (left) that overlooks the EWOD radiosynthesis chip positioned below a pivoting mirror, which directs light to either the video camera or Cerenkov camera inside a light tight box of a Cerenkov imaging system, schematic (top right) of the faster mixing process through the circulating-actuation of EWOD electrodes; and Cerenkov image (bottom right) after 30 s of the mixing based on EWOD actuation; (c) top-view schematic (left) of femtoliter droplets printed inside hydrophilic patches after a mother droplet moves across micro-patches, and scanning electron microscope image (right) of single MOF crystal arrays formed by the DMF technique; (d) schematic (top left) of the three-electrode design with two different sizes for the micromagnetic particle extraction, measured particle loss rates (top middle) with different starting particle numbers and comparison (top right) of the washing efficiency in the case of two different electrode-area ratios; and images (bottom) of a mother droplet that split into two daughter droplets with different sizes by the improved double-side EW method

required to avoid oil leakages [61]; and oil as a medium is unsuitable for certain applications (e.g., cell culture [62]).

In some ways, oil as a medium has affected the development of DMF devices in biochemical applications to some extent. Therefore, the Wheeler research group has developed several new DMF platforms applied to immunoassays, in which droplets were all suspended in the air. In the first platform, they first reported the magnetic microparticle-based immunoassays implemented on DMF within the air to actuate the droplet motion [29]. Given that this new method realized the separation and resuspension of magnetic microparticles on-chip, over 90% of the unbound reagent could be removed in one step. By this technique, noncompetitive and competitive immunoassay methods have been performed, thus demonstrating the universality of the new technique. Unlike traditional techniques, this new approach significantly lowers the reagent consumption and analysis time as well as attains the analytical performance level requied in clinical screening. In the second platform, the same research group utilized a DMF device to accomplish magnetic particle-based immunoassays, which could carry out chemiluminescent detection, to implement the optimization of the design of experiments (DOE) [63]. The new format optimized a three-level, full-factor DOE, which resulted in a five-fold decrease in the limit of the detection and a two-fold reduction of sample incubation time. In the third platform, they similarly introduced the DMF-based immunoassay system integrated with electrochemical detection [55]. The system was employed to detect thyroid stimulating hormone on-chip. In the fourth platform, they designed a new DMF immunoassay system integrated with the sample preparation to detect rubella virus (RV) IgG and IgM [56]. This DMF system could automatically implement three basic sample process steps, namely, average sample distribution for four assays in parallel, dilution, and IgG blocking. More importantly, it can integrate these operations on demand in the immunoassay workflow. The authors also performed a multi-step RV IgG immunoassay on the paper-based DMF device, in which the inkjet printing was integrated with a roll-coating process to fabricate the driving electrode, dielectric, and hydrophobic layers [45]. They reported a detection limit of 0.02 IU/mL, much lower than the diagnostic cut-off value of 10 IU/mL set by the World Health Organization for RV infection and immunity.

Two magnetic particle extraction methods (i.e., single-side and double-side EW methods) are commonly implemented in these microparticle-based immunoassays. They have their own advantages and disadvantages. The former has higher washing efficiency, but requires many magnetic particles. The latter can lower the particle amount required, but the washing efficiency is low when multiple washes is required. Moreover, magnetic particles loss can inevitably occur during the washing process in both methods. Huang et al. [64] utilized a series of asymmetric electrodes to improve the conventional double-side EW method to separate micro-particles in the DMF-based immunoassay. In the improved particle extraction technique, the electrode series consisted of a large electrode and two small electrodes. The mother droplet containing a certain number of magnetic particles was first placed on the large electrode and then stretched along the two small electrodes by turning off the large electrode while activating the two small electrodes. The magnetic particles were attracted and fixed onto the outermost small electrode by the external magnet. Finally, the mother droplet was split into two unequal daughters by turning on the two outermost electrodes. Furthermore, the small daughter droplet retained all the particles without particle loss, because the attraction force generated by the magnetic was sufficient. The authors applied the protocol into the immunoassay for 1 pg/mL concentrations of human sTNF-RI. They demonstrated that the improved particle extraction technique not only resulted in a DMF-based immunoassay with a better detection limit (3.14 pg/ml), but also lowered the reagent consumption (200 nL) and analysis time ($<$ 1 h) compared with the previous off-chip technique in the on-chip immunoassay (Fig. 2(d)).

Although the use of micro-particles ensures that the contact surface area between the sample and surface coated with antibodies is higher and that faster mass transfer conditions are employed at the microscale, an external permanent magnet is still necessary to separate the magnetic micro-particles from the supernatant to perform the corresponding washing procedures. However, this requirement makes the design and fabrication of the device and droplet manipulation more complicated. Therefore, another option is to immobilize the captured antibody directly onto a nanotextured solid surface of the device. Miller et al. [57] designed a DMF device to detect and quantify proteins by heterogeneous immunoassay, in which the captured antibody was immobilized onto the unpatterned ITO-coated top plate. Pluronic F-127 was applied to eliminate the BSA blocking on the DMF device and prevent the non-specific adsorption of proteins to the device surface. Although the sample consumption was decreased 100-fold compared with that in the well-plate method, the IgG molecules were consequently detected and quantified, and the total analysis time required in each assay was significantly lowered.

4.3 Cell-based applications

Cell-based assays are also some of the most important application fields of DMF chips in biology. In most cases, the linear dimensions of cells are in the range of 10 to 100 μm, which is the same as the size of a microfluidic chip. The cell-based assays can also be coupled with polydimethylsiloxane and other materials with low toxicity, biocompatibility, and permeability for oxygen and carbon dioxide. These advantages have turned many DMF chips

into important platforms for cell research. Many cell-based analysis processes can be implemented on DMF chips, which mainly include cell culture, stimulation, sorting, and splitting.

The cell cultures implemented on DMF include suspension cultures, 2D cultures on planar substrates, and 3D cultures in hydrogels. As DMF is a tool utilized to control droplets, it is highly suitable for cells to culture in the culture media. In an example of suspension culture, Au et al. [65] designed a DMF platform to culture cells and analyze the cell density of microorganisms inside discrete droplets. Bacteria, algae, and yeasts were cultured for five days through automatic semi-continuous mixing protocol and precise temperature control. The cell concentration was measured by cell absorbance through transparent windows on the DMF platform. This technique of utilizing DMF to culture and analyze microbes was versatile, so it could be extended to a broad range of microbial applications. Shih et al. [66] recently reported a novel droplet-to-DMF system to assess the effects of the type and concentration of ionic liquids on yeast cell growth and its ethanol production. Yeast cells were first encapsulated into droplets in microchannels and were then transferred to the DMF region for long-term cell culture and ethanol production. The automatic hybrid microfluidic system could be employed for cell culture in different conditions (Fig. 3(a)). By this approach, the reagent volume was decreased 600-fold compared with well-plate formats, whereas the total reagent consumption did not exceed 120 nL. Except for suspension cells, many techniques for

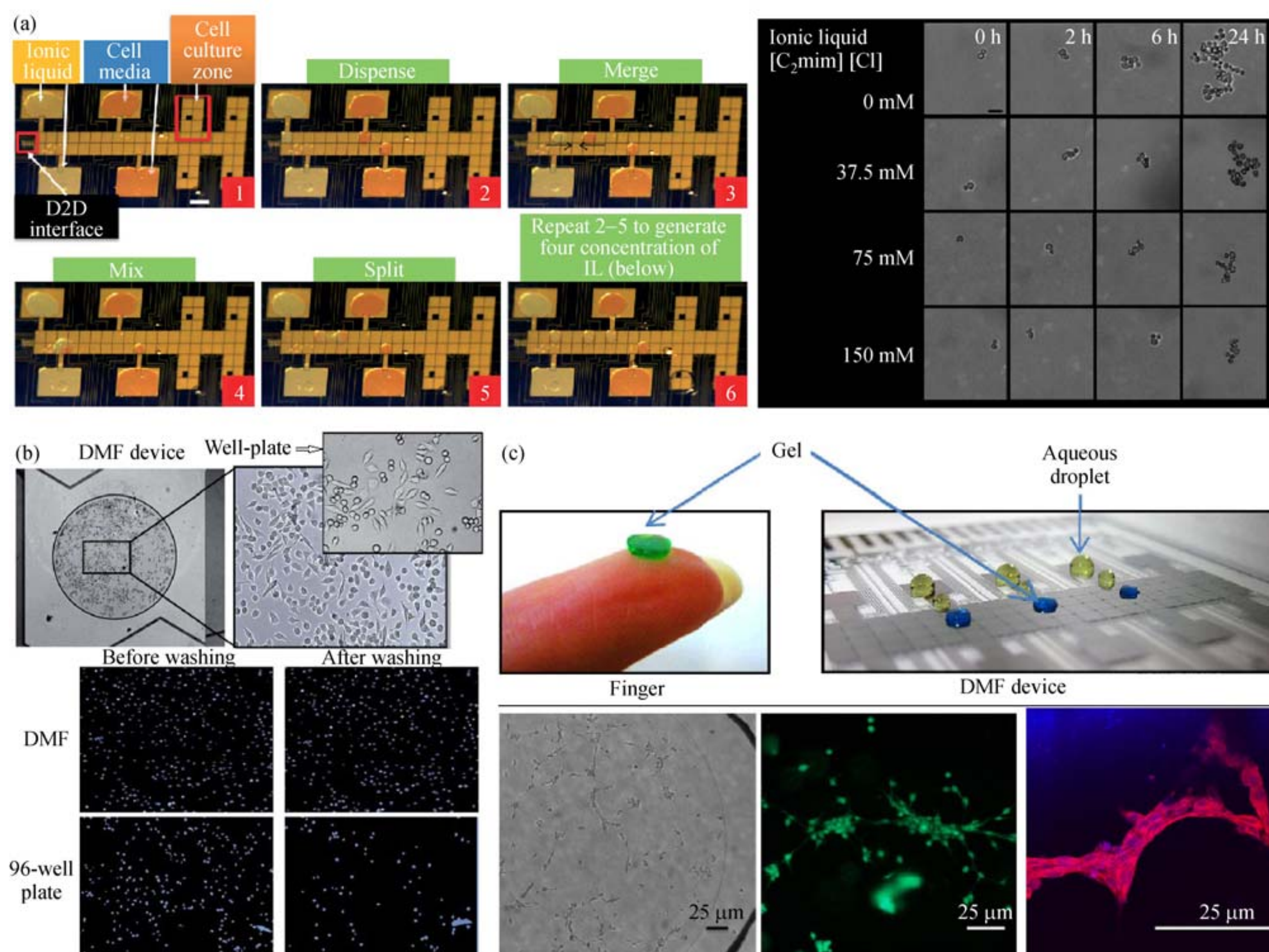

Fig. 3 Cell-based applications in DMF. (a) Frames (1)–(6) of a movie (left) show the driving protocol for generating a droplet with 150 mM (1 mM = 1 mmol/L) IL. Repeating Frames (2)–(5) for the other IL concentrations to generate four droplets with different ionic liquid concentrations at 0, 37.5, 75, or 150 mM (Frame 6). Each droplet first captured a single cell and was then actuated into their respective culture regions. Photo (right) of a single *S. cerevisiae* cell incubated at four different concentrations of [C_2mim][Cl]. (b) Photos (upper) of HeLa cells cultured on a hydrophilic region of a DMF device and a photo (inset) of cells on a contradional 96-well plate; photos (lower) of cells that undergo apoptosis before and after two sequential wash steps on the two devices. (c) Photos (upper) of gel discs on a finger (left) and on the DMF device without the upper plate (right); photos of a 3D cell culture: NIH-3T3 cells were cultured in 0.58 wt% agarose gel discs on DMF devices, and imaged through transparent windows on DMF devices utilizing bright field (left), fluorescence (middle) with calcein AM dye, and confocal fluorescence (right) with 4', 6-diamidino-2-phenylindole (shown in red) and phalloidin (shown in blue) microscopies

cultivating cells on DMF can also be compatible with adherent cells that must adhere to the surface of a support, which rely on the attachment factor to grow and proliferate. However, the DMF configuration must be improved as the hydrophobic coating on DMF is unsuitable for the attachment and cultivation of adherent cells thereon. Eydelnant et al. [67] designed a novel DMF-based approach for cell cultures. A Teflon-AF liftoff protocol is implemented in this device without the addition of biomolecules or proteins to pattern hydrophilic regions on the DMF upper plate for accurate cell dispensing that form virtual microwells. This mechanism is called "passive dispensing (surface tension)." Furthermore, by using this technique, epithelial cell cultures of marbin Darby canine kidney have been proven to have a similar morphology and growth rate compared with those cultivated on the well-plate. DMF has been regarded as a promising new platform for multiplexed screening assays based on cells due to its many advantages, such as reconfigurability, compatibility with traditional high-throughput screening equipment, and non-requirement of moving parts.

Meanwhile, Bogojevic et al. [68] introduced the first DMF device that was utilized to perform multiplex cell-based apoptosis assays via the Teflon-AF liftoff method. Hela cells were incubated at hydrophilic sites located on the DMF upper plate with Teflon-AF coating (Fig. 3(b)). The cell attachment and spreading features on the DMF device surfaces were not only significantly different from those on the polystyrene tissue culture surfaces in the 96-well plates, but this DMF platform also surpassed the multiple well-plate format in implementing an identical assay. For example, it lowered the detection limits, enlarged the dynamic range, and decreased the reagent consumption 33-fold. Although the 2D cell culture remains the most commonly utilized technique in research laboratories, the 3D cell culture has attracted more attention, because it can be designed to simulate the physiological environment *in vivo*, so that the physiological behavior of cells is closer to the actual physical environment. Fiddes et al. [69] first proposed the concept of 3D cell cultures, in which cylindrical hydrogel discs were integrated onto DMF devices. NIH-3T3 fibroblasts were seeded in the ultra-low gelling temperature agarose solution, gelled into hydrogel discs, and then incubated for seven days. The capacity of the cells to form 3D networks largely depended on the agarose concentration. Cells that were embedded in 0.58 wt% agarose discs self-integrated to form tightly packed network structures with cell-cell connections (Fig. 3(c)). George and Moon [70] recently applied DMF into an optimized chemical screening system by employing the 3D cell culture method. In this system, the hydrogel remained in place during the reagent chemicals transport and removal without the need for physical barriers or chemical hydrophilic patterning. They also measured the effect of different concentrations of dimethyl sulfoxide on the viability of 3D cells that were cultured in alginate hydrogels. 3D cell cultures can also be conducted in free-floating, hydrogel-based micro-tissues, which are not constricted to pillars [71]. All these studies show that DMF has a significant potential to be adopted for the automatic development and chemical screening of 3D cell tissues.

Cell sorting and purification are crucial steps in cell-based applications. In DMF devices, different techniques (e.g., electric, optics, or magnetic forces) have been applied to sort and control cells within droplets. Nejad et al. [72] utilized negative dielectrophoresis to realize the sorting and manipulation of cells within discrete droplets, as well as studied the effect of nDEP trap geometry on capturing particles. Regardless of the trap shape, the trap should have a specific size less than which the particles cannot settle down. This approach has been proven to be well suited to designing trap geometries for applications that require high capture efficiency or the immobilization of single particles. Valley et al. [73] designed a DMF device to sort and control cells by optical force. The DMF platform was integrated with optoelectrowetting and optoelectronic tweezers as the manipulation modalities to control both discrete droplets and micro-particles inside droplets with the use of light. Results show that this technique can sort a single Hela cell from a group of cells and then encapsulate it. Kumar et al. [74] manipulated cells inside a droplet by using magnetic forces on DMF devices. The osmotic treatment of a cluster of single protoplasts was automatically implemented by capturing and immobilizing them at a specific position through an external magnetic field. The optimal concentration of magnetic beads was utilized for efficient protoplast isolation. The integrated DMF platform, which employed magnetic forces, offered a solution for the required immobilization of non-adherent cells when monitoring and handling them on-chip.

4.4 DNA-based applications

Manipulating and characterizing DNA samples are critical steps in a wide range of applications. However, conventional laboratory analysis methods commonly require time-consuming manual handing and complicated instrumentation. The emergence of DMF has overcome these limitations. Thus, the use of DMF chips to develop fluidic microprocessors and small-volume microreactors for DNA-based applications has received significant interest. In particular, DMF has been applied in the purification and extraction of DNA samples, DNA amplification and detection, and DNA sequencing.

Before DNA samples are handed and characterized, they must be purified and extracted from complex components, which is one of the most essential steps in DNA-based assays and requires a range of reagent mixing and splitting. For example, Schell et al. [75] reported an improved, real-time polymerase chain reaction (PCR) system based on DMF to extract and purify the rDNA of Candida albicans

from blood samples to perform an early and rapid diagnosis of systemic candidiasis. Hung et al. [76] recently developed a novel DMF-based DNA extraction protocol, in which incubation could be completed at room temperature and the number of the washing cycles was significantly decreased. The authors employed magnetic particles to extract DNA from entire blood samples. The DNA extraction processes mainly included two steps. The first step was to collect magnetic particles onto one side of the droplet, and the second step was to wash off the unbound reagents or DNA from the magnetic particles. To collect the magnetic particles, the authors utilized a curved meniscus to generate sufficient pull forces, which could drag the magnetic particles from the substrate. Once the extracted products on the magnetic particles were obtained, two evaluation techniques (i.e., quantitative and qualitative analysis) were adopted to confirm them (Fig. 4(a)).

DMF has been touted as a key technology for DNA and RNA amplification and detection. PCR is one of the most commonly used techniques for DNA amplification. Specifically, PCR is utilized to selectively amplify a specific region of DNA in vitro by replicating DNA fragments *in vivo*, which results in an exponential increase in the DNA number. DNA samples are commonly amplified through PCR after being purified and extracted. Schell et al. [75] optimized the traditional PCR protocol and utilized it to detect the limit of the yeast cell number. The optimized PCR protocol was also applied to an innovative DMF-based real-time PCR device to improve the diagnosis performance of Candida albicans rDNA by PCR. Comparing the detection of *C. albicans* DNA from human blood samples by tranditional PCR and DMF-based PCR, the result shows that the latter is comparable to the former in its ability to diagnose candidemia. However, the latter shows its significant advantages in several respects; for example, it is more economical and does not require a molecularly trained technologist, consumes less sample elution volume, and the PCR run time is shorter (i.e., 70 min for the former and 45 min for the latter) because the thermocycling times decreased. Yehezkel et al. [77] designed a DMF device with programmable order polymerization (POP) for the cloning of assembled synthetic DNA by using single-molecule PCR (smPCR). smPCR was implemented on the disposable cartridge, in which single molecule templates were obtained through the limited dilution method and amplified by droplets that shuttle between temperature regions to attain cycling (Fig. 4(b)).

The main purpose of DNA amplification is to prepare for DNA sequencing, which has an important practical value in disease diagnose and genotyping. DNA sequencing has become an extremely critical part of modern molecular biology research since the emergence of next-generation sequencing (NGS) more than 10 years ago. The NGS technique utilizes massively parallel processing capabilities to perform genome, transcriptome, and epigenome sequencing with several outstanding advantages (e.g., high throughput, high sensitivity, and relative lower cost). Pyrosequencing is the earliest NGS technique employed for DNA sequencing. This is an enzyme cascade chemiluminescence reaction catalyzed by four enzymes in the same reaction system, which achieves the real-time DNA sequencing by detecting the release and intensity of the light generated during the reactions. Welch et al. [78] implemented a pyrosequencing process on an EWOD-based DMF device at the picoliter scale for the first time. Droplets containing adenosine triphosphate (ATP) were combined with luciferase, and the produced light intensity was recorded, which determined the bases number added to the DNA strand. By changing the ATP concentration in the droplet, the detection limit was calculated to be approximately 7 nM (1 M = 1 mol/L), much lower than the expected concentration of a single nucleotide added to the DMF device. Changing the luciferase concentration in the droplet containing enzymes also contributed to the optimization of the reaction conditions to provide the maximum possible luminescence. When the luciferase concentration was higher, the initial light was brighter because most of the ATP was consumed immediately, and then the intensity decreased rapidly. Hence, a higher luciferase concentration offers better detection limits to optimize the reaction. Although DNA sequencing was successfully performed in the said study, the DNA preparation for the sequencing must be implemented manually, thus limiting the applications of NGS techniques in time-critical biological defense and public health that lack rapid, reliable, and efficient automatic DNA sample preparation approaches. To overcome this limitation, Kim et al. [79,80] developed a DMF chip utilized as the central hub for fluid distribution, which integrated a multiplex external DNA sample processing subsystem by in-plane Teflon-coated capillary tubes to perform NGS. The capillary tubes enabled the samples to be interconverted between the continuous-flow and discrete droplets to perform automated DNA sample preparation operations, including dispensation, fractionation, separation, mixing, and reagent metering on a single device (Fig. 4(c)). In the said system, magnetic beads were first applied for repeated buffer exchange, sample clean-up, and DNA concentration operations between DNA fragmentation, ligation, PCR amplification, and quantification, as well as for DNA size selection. The NGS libraries from the bacterial and human genomic DNA samples were prepared on-chip, which matched the reference genome with an alignment of up to 99%, to verify the performance of this automated system. The genome coverage was relatively uniform with no distinct spikes and drops in the relative coverage depth.

4.5 Protein analysis

Given that multistep tedious sample processing is generally required in proteomic experiments before the

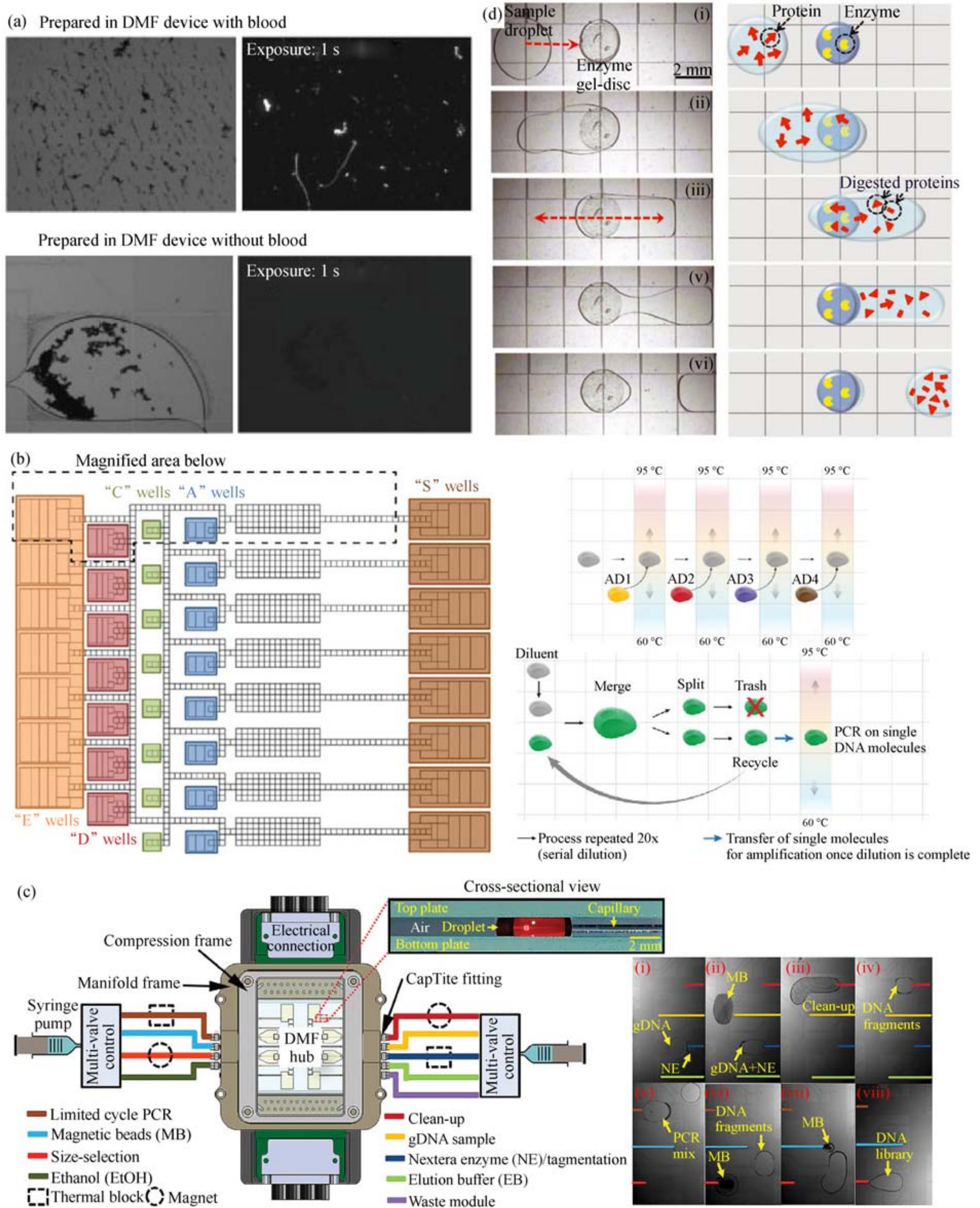

Fig. 4 DMF applications in DNA-based and protein assays. (a) Photos of SYBR-Green added to double-stranded DNA and generated fluorescence light; photos (left and right) indicate the optical and fluorescent images, respectively. (b) Overview (left) of a DMF cartridge design, and POP assembly schematics (upper right) of DNA synthesis utilizing DMF. A droplet with a DNA template (gray) was mixed with the assembly droplet 1 (AD1), which contained the primers and assembly mixture to generate a droplet (gray) that contained assembly product 1 (AP1); the AP1 droplet was then mixed in sequence with the assembly droplet 2 (AD2), 3 (AD3), and 4 (AD4), which all contained the primers and assembly mixture until generating the full-length molecule (AP4) 2 (AD2). Scheme (lower right) of in vitro cloning by smPCR on DMF. (c) Top view of the integrated microfludics system and side view of the interface that connects the DMF hub and capillary (left); sequence of frames (right) from a movie that describes the automated library preparation process for a sample that contains human gDNA from peripheral blood mononuclear cells. (d) A series of images from a video (left) and schematic (right) that show the digestion process for a proteomic sample on a hydrogel disc microreactor (2 mm in diameter)

analysis by MS or other detectors, the capability of DMF to address many reagents and phases simultaneously enables DMF to be highly suitable for different applications related to proteomics. In early DMF-based applications in proteomics, DMF devices were mostly utilized to purify peptides and proteins from heterogeneous mixtures by MALDI-MS [81,82]. Fiddes et al. [69,83] recently integrated DMF with hydrogels for proteomic encapsulation and digestion, in which hydrogels serve as a microreactor to immobilize the enzyme (e.g., trypsin or pepsin). Hydrogel discs were placed between the two plates of the closed DMF, with each disc spanning two electrodes on the lower plate (Fig. 4(d)). The said authors modified hydrogel discs to bear covalently attached proteolytic enzymes in order to validate the capability of the hydrogel-based DMF device for proteome profiling applications. Therefore, the enzymatic cleavage of the proteins occurred once the droplets carrying the proteomic analytes contacted the modified gel discs. Given that the number of aldenhyde groups produced on the hydrogels determined the ultimate immobilized enzyme density, which directly affected the overall reaction efficiency in the process, a gel series was exposed to different concentrations of sodium periodate to attain the best digestion rate. When hydrogels were exposed to 3.5 mM sodium periodate, the digestion rate reached its highest value. The parallel digestion scheme also allowed a single sample to be simultaneously digested by multiple hydrogel microreactors with different enzymes. This system facilitated the establishment of automated platforms for proteome analysis. MS is generally the last procedure in proteomic analysis. Matrix-assisted laser desorption and ionization (MALDI) is a technique for sample ionization in MS, and is applied to the analysis of biological molecules and particularly well suited for proteomic research. In 2012, Aijian et al. [84] applied DMF into protein processing and crystallization to form a complete sample preparation technique for protein analysis based on MALDI-MS. In the said study, a small amount of fluorinated surfactant was added to the matrix solution of MALDI to provide a hydrophilic region to promote the crystallization of the matrix on the hydrophobic layer. Protein adsorption was monitored by the measurement of the contact angle and fluorescence microscopy. The MS obtained from the DMF sample preparation by this technique were comparable to those obtained from conventional and manual protocols.

5 Conclusions and future outlook

Since its emergence in the early 2000s as a novel microfluidic technique, DMF has already been applied to different fields, including chemistry, biology, and medical diagnosis. This review summarizes the basic principles of droplet actuation, configuration design, and fabrication of a DMF device, as well as discusses the latest progress about DMF applications from the perspective of biochemistry. DMF has become cheaper and more accessible for industry and research laboratories with the rapid development of PCB- or paper-based chips and rapid prototyping manufacturing techniques. Specifically, the DMF chip is fabricated on a paper substrate by the printing technique, which largely expands the application range of DMF. This advancement can be particularly suitable for complicated, automatic, and multistep assays, which are difficult to accomplish with the capillary-driven technique. Therefore, even if few reports exist about paper-based DMF chip, we anticipate that more researchers will study this fabrication method, and paper-based DMF devices will be more widely utilized in different applications. The review shows that, among all related fields, biology has been the most active one in investigating DMF applications, in which multiplex complicated samples are individually pretreated and analyzed simultaneously on a single device.

Although DMF has many advantages, several challenges must still be resolved. First, this microfluidic technique is still in its infancy, so it has only been applied in a few laboratories around the world. Second, some phenomena (e.g., CAS) are poorly understood because of the many controversies on the interpretation of this issue, and no uniform view exists about the mechanism that completely explains CAS. Third, even if the DMF chip size is in the micro scale, it is still equipped with many complex peripheral apparatuses, which limit its application (i.e., POCT) to a certain extent.

Despite these challenges, we propose that the increasing number of DMF researchers can solve several practical problems in the next decade. The DMF performance will certainly continue to improve in the future, along with the introduction of new technologies of droplet actuation, fabrication, and detection. DMF will continue to have significant potential in promoting its applications in chemistry, biology, and medical diagnosis. Furthermore, we anticipate that DMF can have broader applications in the future, particularly in more extensive fields, such as forensic science, environmental monitoring, and food safety.

Acknowledgements This work was supported by the Research Fund for the Doctoral Program of Higher Education of China (Grant No. 20133201110009).

References

1. Terry S C, Jerman J H, Angell J B. A gas chromatographic air analyzer fabricated on a silicon wafer. IEEE Transactions on Electron Devices, 1979, 26(12): 1880–1886
2. Reyes D R, Iossifidis D, Auroux P A, et al. Micro total analysis system. 1. Introduction, theory, and technology. Analitical Chemistry, 2002, 74(12): 2623–2636
3. Mugele F, Baret J C. Electrowetting: From basics to applications.

Journal of Physics Condensed Matter, 2005, 17(28): R705–R774
4. Pollack M G, Shenderov A D, Fair R B. Electrowetting-based actuation of droplets for integrated microfluidics. Lab on a Chip, 2002, 2(2): 96–101
5. Washizu M. Electrostatic actuation of liquid droplets for micro-reactor applications. IEEE Transactions on Industry Applications, 1998, 34(4): 732–737
6. Cho S K, Fan S K, Moon H, et al. Towards digital microfluidic circuits: Creating, transporting, cutting and merging liquid droplets by electrowetting-based actuation. In: Proceedings of the Fifteenth IEEE International Conference on Micro Electro Mechanical Systems. Las Vegas: IEEE, 2002, 32–35
7. Cho S K, Moon H, Kim C J. Creating, transporting, cutting, and merging liquid droplets by electrowetting-based actuation for digital microfluidic circuits. Journal of Microelectromechanical Systems, 2003, 12(1): 70–80
8. Berthier J. Microdrops and Digital Microfluidics. Norwich: William Andrew Inc., 2008
9. Wang W, Jones T B. Moving droplets between closed and open microfluidic systems. Lab on a Chip, 2015, 15(10): 2201–2212
10. Wheeler A R. Putting electrowetting to work. Science, 2008, 322 (5901): 539–540
11. Hsieh T H, Fan S K. Dielectric droplet manipulations by electropolarization forces. In: Proceedings of the IEEE International Conference on Micro Electro Mechanical Systems. Piskataway: IEEE, 2008, 641–644
12. Jones T B, Wang K L, Yao D J. Frequency-dependent electro-mechanics of aqueous liquids: Electrowetting and dielectrophoresis. Langmuir, 2004, 20(7): 2813–2818
13. Mugele F, Baret J C. Electrowetting: From basics to applications. Journal of Physics Condensed Matter, 2005, 17(28): R705–R774
14. Gupta R, Sheth D M, Boone T K, et al. Impact of pinning of the triple contact line on electrowetting performance. Langmuir, 2011, 27(24): 14923–14929
15. Chen L Q, Bonaccurso E. Electrowetting—From statics to dynamics. Advances in Colloid and Interface Science, 2014, 210: 2–12
16. Kang K H. How electrostatic fields change contact angle in electrowetting. Langmuir, 2002, 18(26): 10318–10322
17. Peykov V, Quinn A, Ralston J. Electrowetting: A model for contact-angle saturation. Colloid & Polymer Science, 2000, 278(8): 789–793
18. Darhuber A A, Chen J Z, Davis J M, et al. A study of mixing in thermocapillary flows on micropatterned surfaces. Philosophical Transactions. Series A, Mathematical, Physical, and Engineering Sciences, 2004, 362(1818): 1037–1058
19. Darhuber A A, Valentino J P, Troian S M. Planar digital nanoliter dispensing system based on thermocapillary actuation. Lab on a Chip, 2010, 10(8): 1061–1071
20. Heron S R, Wilson R, Shaffer S A, et al. Surface acoustic wave nebulization of peptides as a microfluidic interface for mass spectrometry. Analytical Chemistry, 2010, 82(10): 3985–3989
21. Jin H, Zhou J, He X, et al. Flexible surface acoustic wave resonators built on disposable plastic film for electronics and lab-on-a-chip applications. Scientific Reports, 2013, 3: 2140
22. Pang H, Fu Y, Garcia-Gancedo L, et al. Enhancement of microfluidic efficiency with nanocrystalline diamond interlayer in the ZnO-based surface acoustic wave device. Microfluidics and Nanofluidics, 2013, 15(3): 377–386
23. Shilton R J, Mattoli V, Travagliati M, et al. Rapid and controllable digital microfluidic heating by surface acoustic waves. Advanced Functional Materials, 2015, 25(37): 5895–5901
24. Seemann R, Brinkmann M, Pfohl T, et al. Droplet based microfluidics. Reports on Progress in Physics, 2012, 75(1): 016601
25. Gu H, Duits M H G, Mugele F. Droplets formation and merging in two-phase flow microfluidics. International Journal of Molecular Sciences, 2011, 12(12): 2572–2597
26. Renaudot R, Agache V, Daunay B, et al. Optimization of liquid dielectrophoresis (LDEP) digital microfluidic transduction for biomedical applications. Micromachines, 2011, 2(4): 258–273
27. Renaudot R, Daunay B, Kumemura M, et al. Optimized micro devices for liquid-dielectrophoresis (LDEP) actuation of conductive solutions. Sensors and Actuators B: Chemical, 2013, 177: 620–626
28. Timonen J V I, Latikka M, Leibler L, et al. Switchable static and dynamic self-assembly of magnetic droplets on superhydrophobic surfaces. Science, 2013, 341(6143): 253–257
29. Ng A H C, Choi K, Luoma R P, et al. Digital microfluidic magnetic separation for particle-based immunoassays. Analytical Chemistry, 2012, 84(20): 8805–8812
30. Witters D, Knez K, Ceyssens F, et al. Digital microfluidics-enabled single-molecule detection by printing and sealing single magnetic beads in femtoliter droplets. Lab on a Chip, 2013, 13(11): 2047–2054
31. Shi D, Bi Q, He Y, et al. Experimental investigation on falling ferrofluid droplets in vertical magnetic fields. Experimental Thermal and Fluid Science, 2014, 54: 313–320
32. Choi K, Ng A H C, Fobel R, et al. Digital microfluidics. Annual Review of Analytical Chemistry, 2012, 5(1): 413–440
33. Kumar A, Williams S J, Chuang H S, et al. Hybrid opto-electric manipulation in microfluidics—Opportunities and challenges. Lab on a Chip, 2011, 11(13): 2135–2148
34. Takinoue M, Takeuchi S. Droplet microfluidics for the study of artificial cells. Analytical and Bioanalytical Chemistry, 2011, 400 (6): 1705–1716
35. Vergauwe N, Witters D, Atalay Y T, et al. Controlling droplet size variability of a digital lab-on-a-chip for improved bio-assay performance. Microfluidics and Nanofluidics, 2011, 11(1): 25–34
36. Yaddessalage J B. Study of the capabilities of electrowetting on dielectric digital microfluidics (EWOD DMF) towards the high efficient thin-film evaporative cooling platform. Dissertation for the Doctoral Degree. Arlington: The University of Texas at Arlington, 2013
37. Elvira K S, Leatherbarrow R, Edel J, et al. Droplet dispensing in digital microfluidic devices: Assessment of long-term reproducibility. Biomicrofluidics, 2012, 6(2): 022003
38. Yafia M, Najjaran H. High precision control of gap height for enhancing principal digital microfluidics operations. Sensors and Actuators B: Chemical, 2013, 186: 343–352
39. Chang J H, Pak J J. Twin-plate electrowetting for efficient digital microfluidics. Sensors and Actuators B: Chemical, 2011, 160(1): 1581–1585

40. Cui W, Zhang M, Zhang D, et al. Island-ground single-plate electrowetting on dielectric device for digital microfluidic systems. Applied Physics Letters, 2014, 105(1): 013509
41. Ko H, Lee J, Kim Y, et al. Active digital microfluidic paper chips with inkjet-printed patterned electrodes. Advanced Materials, 2014, 26(15): 2335–2340
42. Fobel R, Kirby A E, Ng A H C, et al. Paper microfluidics goes digital. Advanced Materials, 2014, 26(18): 2838–2843
43. Fobel R, Kirby A E, Wheeler A R. Paper microfluidics goes digital. In: Proceedings of 17th International Conference on Miniaturized Systems for Chemistry and Life Sciences, MicroTAS. Freiburg: Chemical and Biological Microsystems Society, 2013, 708–710
44. Dixon C, Kirby A E, Fobel R, et al. Paper digital microfluidics and paper spray ionization mass spectrometry. In: Proceedings of 18th International Conference on Miniaturized Systems for Chemistry and Life Sciences, MicroTAS. San Antonio: Chemical and Biological Microsystems Society, 2014, 2196–2198
45. Dixon C, Ng A H C, Fobel R, et al. An inkjet printed, roll-coated digital microfluidic device for inexpensive, miniaturized diagnostic assays. Lab on a Chip, 2016, 16(23): 4560–4568
46. Yafia M, Shukla S, Najjaran H. Fabrication of digital microfluidic devices on flexible paper-based and rigid substrates via screen printing. Journal of Micromechanics and Microengineering, 2015, 25(5): 057001
47. Taniguchi T, Torii T, Higuchi T. Chemical reactions in microdroplets by electrostatic manipulation of droplets in liquid media. Lab on a Chip, 2002, 2(1): 19–23
48. Ito T, Torii T, Higuchi T. Electrostatic micromanipulation of bubbles for microreactor applications. In: Proceedings of IEEE the Sixteenth Annual International Conference on Micro Electro Mechanical System. Kyoto: IEEE, 2003, 335–338
49. Sista R S, Eckhardt A E, Wang T, et al. Digital microfluidic platform for multiplexing enzyme assays: Implications for lysosomal storage disease screening in newborns. Clinical Chemistry, 2011, 57(10): 1444–1451
50. Boles D J, Benton J L, Siew G J, et al. Droplet-based pyrosequencing using digital microfluidics. Analytical Chemistry, 2011, 83(22): 8439–8447
51. Choi K, Boyacı E, Kim J, et al. A digital microfluidic interface between solid-phase microextraction and liquid chromatography—Mass spectrometry. Journal of Chromatography A, 2016, 1444: 1–7
52. Keng P Y, Chen S, Ding H J, et al. Micro-chemical synthesis of molecular probes on an electronic microfluidic device. Proceedings of the National Academy of Sciences of the United States of America, 2012, 109(3): 690–695
53. Dooraghi A A, Keng P Y, Chen S, et al. Optimization of microfluidic PET tracer synthesis with Cerenkov imaging. Analyst (London), 2013, 138(19): 5654–5664
54. Witters D, Vergauwe N, Ameloot R, et al. Digital microfluidic high-throughput printing of single metal-organic framework crystals. Advanced Materials, 2012, 24(10): 1316–1320
55. Shamsi M H, Choi K, Ng A H C, et al. A digital microfluidic electrochemical immunoassay. Lab on a Chip, 2014, 14(3): 547–554
56. Ng A H C, Lee M, Choi K, et al. Digital microfluidic platform for the detection of rubella infection and immunity: A proof of concept. Clinical Chemistry, 2015, 61(2): 420–429
57. Miller E M, Ng A H C, Uddayasankar U, et al. A digital microfluidic approach to heterogeneous immunoassays. Analytical and Bioanalytical Chemistry, 2011, 399(1): 337–345
58. Sista R S, Eckhardt A E, Srinivasan V, et al. Heterogeneous immunoassays using magnetic beads on a digital microfluidic platform. Lab on a Chip, 2008, 8(12): 2188–2196
59. Fair R B. Digital microfluidics: Is a true lab-on-a-chip possible? Microfluidics and Nanofluidics, 2007, 3(3): 245–281
60. Yoon J Y, Garrell R L. Preventing biomolecular adsorption in electrowetting-based biofluidic chips. Analytical Chemistry, 2003, 75(19): 5097–5102
61. Shah G J, Kim C J. Meniscus-assisted high-efficiency magnetic collection and separation for EWOD droplet microfluidics. Journal of Microelectromechanical Systems, 2009, 18(2): 363–375
62. Barbulovic-Nad I, Au S H, Wheeler A R. A microfluidic platform for complete mammalian cell culture. Lab on a Chip, 2010, 10(12): 1536–1542
63. Choi K, Ng A H C, Fobel R, et al. Automated digital microfluidic platform for magnetic-particle-based immunoassays with optimization by design of experiments. Analytical Chemistry, 2013, 85(20): 9638–9646
64. Huang C Y, Tsai P Y, Lee I C, et al. A highly efficient bead extraction technique with low bead number for digital microfluidic immunoassay. Biomicrofluidics, 2016, 10(1): 011901
65. Au S H, Shih S C C, Wheeler A R. Integrated microbioreactor for culture and analysis of bacteria, algae and yeast. Biomedical Microdevices, 2011, 13(1): 41–50
66. Shih S C C, Gach P C, Sustarich J, et al. A droplet-to-digital (D2D) microfluidic device for single cell assays. Lab on a Chip, 2015, 15 (1): 225–236
67. Eydelnant I A, Uddayasankar U, Li B, et al. Virtual microwells for digital microfluidic reagent dispensing and cell culture. Lab on a Chip, 2012, 12(4): 750–757
68. Bogojevic D, Chamberlain M D, Barbulovic-Nad I, et al. A digital microfluidic method for multiplexed cell-based apoptosis assays. Lab on a Chip, 2012, 12(3): 627–634
69. Fiddes L K, Luk V N, Au S H, et al. Hydrogel discs for digital microfluidics. Biomicrofluidics, 2012, 6(1): 014112
70. George S M, Moon H. Digital microfluidic three-dimensional cell culture and chemical screening platform using alginate hydrogels. Biomicrofluidics, 2015, 9(2): 024116
71. Au S H, Chamberlain M D, Mahesh S, et al. Hepatic organoids for microfluidic drug screening. Lab on a Chip, 2014, 14(17): 3290–3299
72. Nejad H R, Chowdhury O Z, Buat M D, et al. Characterization of the geometry of negative dielectrophoresis traps for particle immobilization in digital microfluidic platforms. Lab on a Chip, 2013, 13 (9): 1823–1830
73. Valley J K, Ningpei S, Jamshidi A, et al. A unified platform for optoelectrowetting and optoelectronic tweezers. Lab on a Chip, 2011, 11(7): 1292–1297
74. Kumar P T, Toffalini F, Witters D, et al. Digital microfluidic chip technology for water permeability measurements on single isolated plant protoplasts. Sensors and Actuators B: Chemical, 2014, 199: 479–487

75. Schell W A, Benton J L, Smith P B, et al. Evaluation of a digital microfluidic real-time PCR platform to detect DNA of Candida albicans in blood. European Journal of Clinical Microbiology & Infectious Diseases, 2012, 31(9): 2237–2245
76. Hung P Y, Jiang P S, Lee E F, et al. Genomic DNA extraction from whole blood using a digital microfluidic (DMF) platform with magnetic beads. Microsystem Technologies, 2015, 21: 1–8
77. Yehezkel T B, Rival A, Raz O, et al. Synthesis and cell-free cloning of DNA libraries using programmable microfluidics. Nucleic Acids Research, 2015, 44: 1–12
78. Welch E R F, Lin Y Y, Madison A, et al. Picoliter DNA sequencing chemistry on an electrowetting-based digital microfluidic platform. Biotechnology Journal, 2011, 6(2): 165–176
79. Kim H, Bartsch M S, Renzi R F, et al. Automated digital microfluidic sample preparation for next-generation DNA sequencing. Journal of Laboratory Automation, 2011, 16(6): 405–414
80. Kim H, Jebrail M J, Sinha A, et al. A microfluidic DNA library preparation platform for next-generation sequencing. PLoS One, 2013, 8(7): e68988
81. Wheeler A R, Moon H, Bird C A, et al. Digital microfluidics with in-line sample purification for proteomics analyses with MALDI-MS. Analytical Chemistry, 2005, 77(2): 534–540
82. Wheeler A R, Moon H, Kim C J, et al. Electrowetting-based microfluidics for analysis of peptides and proteins by matrix-assisted laser desorption/ionization mass spectrometry. Analytical Chemistry, 2004, 76(16): 4833–4838
83. Luk V N, Fiddes L K, Luk V M, et al. Digital microfluidic hydrogel microreactors for proteomics. Proteomics, 2012, 12(9): 1310–1318
84. Aijian A P, Chatterjee D, Garrell R L. Fluorinated liquid-enabled protein handling and surfactant-aided crystallization for fully in situ digital microfluidic MALDI-MS analysis. Lab on a Chip, 2012, 12 (14): 2552–2559