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Title: Digital microfluidic biosensors

Authors: Yuye Wang^{1,2}, Shuwen Zeng^{2,3*}, Georges Humbert², Ho-Pui Ho¹,

ABSTRACT:

Over the recent years, digital microfluidics (DMF) has emerged as a powerful tool for the manipulation over individual droplets in integrated microfluidic devices. In DMF, discrete droplets with low volume can be individually controlled to merge, mix, split and dispense from reservoirs through an array of electrodes. Apart from possessing the advantages of conventional microfluidics including minimized reagent use and reaction time, prevention of cross contamination as well as high level of integration, DMF is also capable of conducting precise control over unit droplets without the use of propulsion devices such as pumps or mechanical mixers, which cannot be realized in traditional channel-based microfluidics. The flexibility of DMF also makes it an ideal platform for integration with biosensors, which allows for in-line detection in a fully integrated system. These unique properties have allowed DMF for potential multiplexed detection of biological and chemical reactions at microscale or nanoscale. In fact, it has been exploited in a large variety of application scenarios including cell-based applications, DNA-based applications and protein-based applications. DMF devices have also opened many new possibilities in point-of-care diagnostics. In this book chapter, we first introduce the basic theory of DMF devices and analyze the mechanism of droplet actuation. Two common configurations (parallel-plate and single-plate) of DMF devices as well as their fabrication methods have also been described. Furthermore, a detailed discussion focusing on the integration of biosensors into the DMF systems is given for biological applications, including both electrochemical biosensors and optical biosensors. Various application scenarios based on these devices have also been summarized. Finally, we summarize the advantages of digital microfluidic devices and their future challenges.

1 Introduction

Digital microfluidics (DMF) is a novel technology which has the capability of individual droplet manipulation [1-4]. Since the precise definition of DMF may differ in literature [5-7], we only focus on studying and reviewing the recent progress of integrated DMF systems in which droplet manipulation is carried out on an array of electrodes here. In these systems, the transporting, mixing, splitting and dispensing of individual droplet can be realized through applying a series of potentials to the electrodes. DMF systems can provide numerous advantages such as reduced reagent consumption and reaction time, fast heat transfer

as well as ease of integration. These systems can also effectively reduce cross contamination due to the use of individual droplets as microreactors. Moreover, the DMF systems do not require external networks including pumps, tubing, valves or mechanical mixers. In addition, unlike conventional microfluidic chips which have specialized channel designs for each specific reaction, droplet operations are reconfigurable and highly-adjustable to fit the requirements of different applications in DMF platform. Those unique characteristics of DMF platform have made it a promising tool in a variety of chemical and biological applications.

In the following chapters, the basic mechanism of droplet manipulation using DMF devices has been introduced. Two typical configurations including parallel-plate and single-plate have been explained in detail. We have also summarized several fabrication methods for DMF devices and reported the recent development in exploring new substrates. Further, we have focused on the integration of various types of sensing techniques into the DMF systems, namely electrochemical and optical sensors. Based on these integrated systems, a variety of biological applications including cell-based, DNA-based as well as protein-based applications have been demonstrated. Finally, the current challenges and possible future research direction have been presented.

2 Basics of digital microfluidic devices

2.1 Theory

Droplets can be individually controlled to move across an array of actuation electrodes in DMF devices when applying electrical potentials [8-10]. This technique has been named as “electrowetting-on-dielectric” (EWOD). In early attempts to explain this phenomenon, thermodynamic theory was proposed. In this theory, the change in contact angle is considered as the cause of interface moving of the droplets [11-13]. In another words, the droplet movement can be regarded as the consequence of the capillary pressure resulted from

considered as the cause of interface moving of the droplets [11–13]. In another words, the droplet movement can be regarded as the consequence of the capillary pressure resulted from asymmetric contact angles. In this paradigm, surface tension is considered as the driving force. As a result, electrowetting is described as the electrical control of surface tension. Theoretical analysis of relating the applied voltage to the contact angle is shown in Eq. (6.1) using the Young–Lippmann equation [14,15]:

$$\cos \theta(V) = \cos \theta_0 + \frac{\epsilon_r \epsilon_0}{2\gamma d} V^2 \quad (6.1)$$

where θ_0 is the contact angle without applied voltage, $\theta(V)$ is the contact angle with applied voltage V , ϵ_r denotes the relative permittivity of the dielectric, ϵ_0 denotes the vacuum permittivity, γ is the surface tension of the surrounding liquid media, and d is the thickness of dielectric layer. The EWOD force can then be calculated as [16]

$$F = \gamma w (\cos \theta(V) - \cos \theta_0) = \frac{w \epsilon_r \epsilon_0}{2d} V^2 \quad (6.2)$$

where w is the contact length between the droplet and the actuated electrode. However, there are some limitations with the thermodynamic theory. For example, this theory has difficulty in explaining the movement of dielectric droplets [11,12,17] or droplet motion where no apparent changes in contact angle can be observed [18]. Moreover, phenomena such as contact angle saturation (CAS) cannot be adequately explained using this theory [19].

As a result, a more generalized and complete explanation based on electromechanical theory has been proposed [11,20,21]. Unlike thermodynamic theory, the electromechanical perspective considers the contact angle change as the consequence of the electromechanical force rather than the cause of droplet motion. Also, electrostatic force instead of surface tension is regarded as the driving force. It can be calculated through integrating the Maxwell–Stress tensor T_{ij} over any arbitrary surface surrounding the droplet using the following equations [22,23]:

$$\mathbf{F} = \int T_{ij} \mathbf{n} dS \quad (6.3)$$

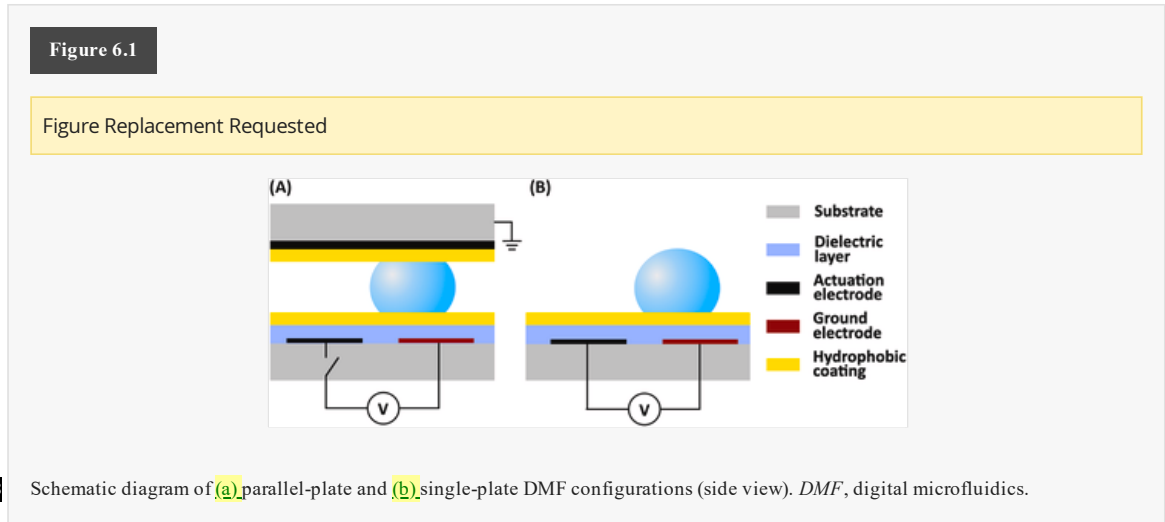
$$T_{ij} = \epsilon \left(E_i E_j - \frac{1}{2} \delta_{ij} \mathbf{E}^2 \right) \quad (6.4)$$

where \mathbf{n} denotes the unit normal vector, i and j represent the pairs of x , y , and z axes, ϵ is the dielectric constant of the surrounding fluid media, \mathbf{E} stands for the electric field around the droplet, and δ_{ij} refers to the Kronecker delta. The electromechanical theory can provide a convincing explanation for the movement of dielectric droplets as well as droplets that do not experience a contact angle reduction. It may also provide some useful insights to a full explanation of CAS [19,20,24].

6.2.2 Device configuration and fabrication

Generally, there are two typical configurations of DMF systems [3,10,15]: parallel-plate format (also known as closed systems) and single-plate format (open systems). As shown in Fig. 6.1, in the parallel-plate configuration, droplets are placed between two plates, which are both patterned with electrodes. The top plate is composed of a ground electrode, which is usually made of indium tin oxide (ITO), while the lower one includes an array of actuation electrodes. In the single-plate configuration, droplets are situated on top of a single substrate, which is patterned with an array of ground and actuation electrodes. Both systems have the configuration of depositing a dielectric layer on top of the electrodes. Moreover, all the surfaces in contact with the droplets are covered with a layer of hydrophobic coating in order to increase the contact angle and prevent sample adhesion as well as contact angle hysteresis. The parallel-plate system has the ability of performing multiple functional units, namely transporting, splitting, mixing, merging, and dispensing, which provides more possibilities in developing multiplex bioassays in need of various droplet manipulation skills

compared to single-plate systems [2,14]. On the other hand, despite the limitation that droplet splitting and dispensing can hardly be achieved in single-plate systems, open systems can offer the advantages of rapid droplet mixing [25], as well as the flexibility for accommodating pipette-based liquid extraction [26] and analytical instruments such as fluorescence detectors [27,28]. It should also be noted that droplet evaporation happens at a faster rate in a single-plate configuration than parallel-plate configuration, which should be prevented in most applications such as polymerase chain reaction (PCR) [29,30], but may also appear advantageous in specific applications where drying analytes are needed [31].



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DMF devices are typically fabricated under clean-room procedures, including photolithography and etching. As introduced in the device configuration part, DMF devices are generally composed of substrates, dielectric layers, electrodes, and hydrophobic coatings. The fabrication process is mainly determined by the choice of substrate materials. Among various materials, silicon and glass have been selected as the substrates of DMF devices due to their chemical inertness. However, the complicated fabrication procedures, low throughput, and the high cost of these materials have greatly hindered the widespread use of DMF systems and thus weakening their superiority over traditional channel-based microfluidic systems. To facilitate the widespread application of DMF devices, many researchers have exploited a printed circuit board (PCB) as the substrate [32–34]. PCB substrates have the advantages of low cost and ease of fabrication. Moreover, the multilayer arrangement enabling electrical access of individual electrodes can be easily enabled through using the mature PCB technology [35,36]. Besides PCB substrates, paper-based substrates have also been developed. Paper-based electronics possess the advantages of low cost, portable size and weight, biocompatibility, well-developed fabrication methods as well as ease of disposal [37–39]. Electrodes are commonly made of metals such as gold, aluminum, copper, or chromium. Other conductive materials, including ITO and doped polysilicon, have also been frequently used. The dielectric layer can be fabricated through various techniques depending on the type of materials used [2,7], for example, the vapor deposition method for parylene, amorphous fluoropolymers, and silicon nitride materials, thermal growth method for depositing silicon oxide as well as spin-coating technique for polydimethylsiloxane or SU-8. As for the hydrophobic coating, it is typically formed by Teflon-AF material using the spin-coating method.

6.3 Integrated digital microfluidic devices with sensing technologies

6.3.1 Integration of sensing techniques into digital microfluidic systems

In recent years, many studies have been carried out on integrating various sensing techniques into DMF systems for biosensing applications. In this section, the detailed information of coupling detection techniques, including optical detection and electrochemical detection with DMF systems, will be described.

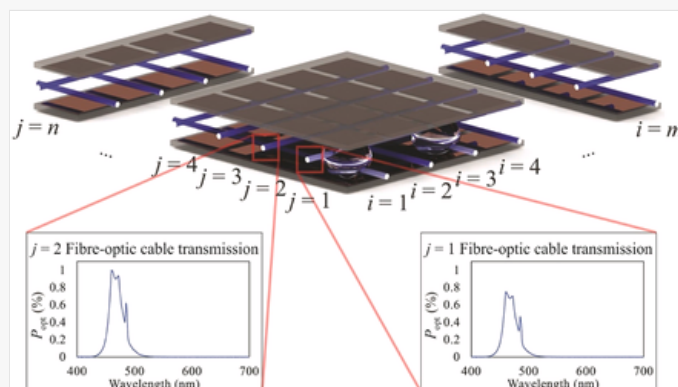
6.3.1.1 Optical sensing in digital microfluidic systems

Among various sensing techniques and systems, optical sensing possesses the merits of extremely high sensitivity, being contact free, and having the capability of multiplexed detection, which has drawn a lot of attention in biological or chemical applications. Over the past few years, many studies have been carried out in developing more advanced optical biosensors as well as exploiting them for point-of-care applications. More particularly, their integration with microfluidics [26,40] has been further investigated. To ensure the integration of optical sensors into a DMF device, first, the substrate material should be carefully selected. Normally, the use of transparent substrates as well as electrodes is needed, for example, ITO, to allow for the smooth observation and detection of droplets within an enclosed microfluidic chamber. Moreover, the optical sensing module needs to be compatible with the hydrophobic coating in DMF systems, which ensures effective sensing without interrupting or affecting the electrowetting transport of droplets.

One of the earliest studies in integrating optical sensors into DMF systems focused on the heterogeneous integration of photodetectors [41,42]. In their work, a semiconductor photodetector in the format of thin-film InGaAs has been bonded onto the glass substrate and integrated with the EWOD platform. Based on this integrated device, the test of chemiluminescent compounds in droplets has been successfully demonstrated. The EWOD system was responsible for mixing of two droplets, while the detection of chemiluminescent optical signal resulted from the mixing was made possible by a thin-film photodetector, which clearly showed the advantages of this integrated platform. Later on, some researchers have coupled surface plasmon resonance sensors into the EWOD devices to enable label-free biodetection [43,44]. Malic et al. have demonstrated the parallel [surface plasmon resonance \(SPR\)](#) detection of different samples through combining SPR sensing techniques with an array-based DMF device. In this system, droplets containing different solutions were manipulated using EWOD and four detection spots were enabled for SPR signal detection [43].

More recently, optical fiber based sensing has also been integrated with DMF systems. Choi et al. have developed an integrated DMF platform for absorbance and fluorescence detection based on with fused silica optical fiber waveguides [45]. The optical fibers were inserted and aligned by a custom manifold in the plane of a DMF device, which enables contact-free detection. This integrating technique also allows for convenient removal and insertion of optical fibers. The system can provide the advantages of multistep sample processing and reaction as well as optical detection with high sensitivity, which can be useful in various analytical applications. Moreover, a fiber-optic sensing architecture that allows for $m \times n$ microdroplet sensing spots with only $(m+n)$ control points has been proposed for DMF devices [46]. As shown in Fig. 6.2, m parallel column fiber-optic cables are orthogonally overlapped with n parallel row fiber-optic cables. The detection of microdroplet was based on the change in the total internal reflection angle in contact with fiber-optic cables through lowered optical transmission. This enables sensing the location of microdroplets with correction abilities as well as distinguishing multiple microdroplets.

Figure 6.2



Fiber-optic sensing architecture integrated with DMF devices. DMF, digital microfluidics.

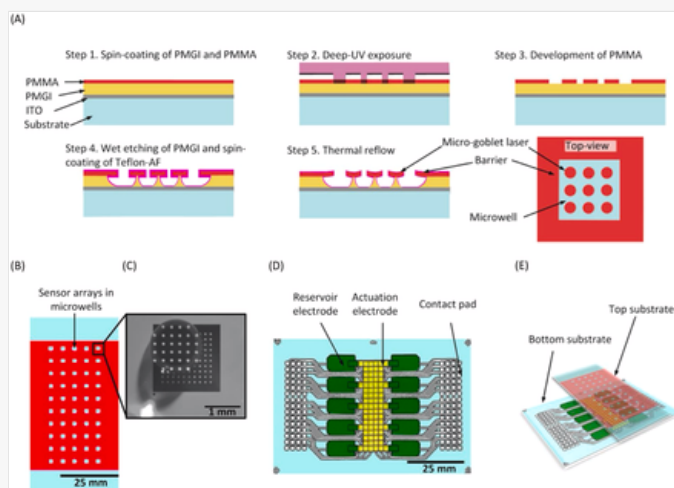
Reproduced with permission I. Spotts, D. Ismail, N. Jaffar, C.M. Collier, *Fibre-optic sensing in digital microfluidic devices*, *Sens. Actuator A Phys* 280 (2018) 164–169, doi:10.1016/j.sna.2018.07.039. Copyright 2018, Elsevier.

To enable multiplexed sensing, optical microresonators have been chosen as an effective sensing tool in DMF systems [47–49]. For the first time, Wondimu et al. have successfully integrated an array of whispering gallery mode (WGM) optical sensors into a single cell of a DMF system to enable label-free and multiplexed sensing [49]. As shown in Fig.

6.3, microgoblet sensors are integrated with the top substrate of the DMF system. The sensor chip contained 5000 integrated WGM sensors in total. The optical sensing and readout were made possible through free-space optics, which allows for large-scale integration of sensor arrays without the need of including extra coupling elements such as prisms or optical fibers. Actuation electrodes were placed on the bottom substrate of the system to manipulate droplets. For demonstration purposes, refractive index sensing with sensitivity up to 28.11 nm per RIU and label-free detection of streptavidin with a sensitivity of 3.83 $\text{pm}/\mu\text{g}/\text{mL}$ were shown using this device. Exploiting the merits of both DMF and WGM resonators, this system has the capability of in situ label-free sensing and analysis of multiple analytes in a single DMF cell.

Figure 6.3

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Q9 Fabrication and device integration of the DMF system integrated with WGM sensors. DMF, digital microfluidics; WGM, whispering gallery mode.

Reproduced with permission S.F. Wondimu, S. von der Ecken, R. Ahrens, W. Freude, A.E. Guber, C. Koos, *Integration of digital microfluidics with whispering-gallery mode sensors for label-free detection of biomolecules*, *Lab. Chip* 17 (2017) 1740–1748, doi:10.1039/c6lc01556e. Copyright 2017, Royal Society Chemistry.

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6.3.1.2 Electrochemical sensing in digital microfluidic systems

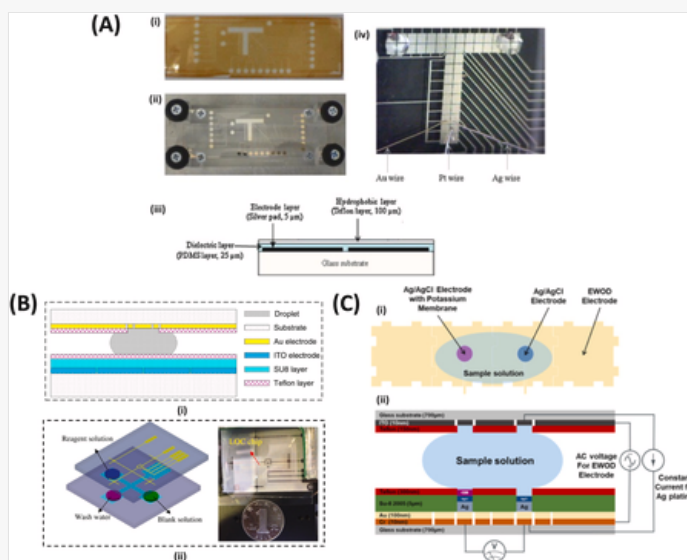
Electrochemical sensors have the ability to detect chemical reactions that happen at the surface of the electrode through measuring certain electrical signals, including current, potential, or capacitance. Compared to optical sensors that require external devices such as light source or detectors, the electrochemical sensors normally require only several electrodes [26,50], which makes them much easier to be integrated with DMF systems. Moreover, due to the similar manufacturing methods of electrochemical sensors and DMF devices, these two are inherently compatible with each other and thus can be easily combined together to form an integrated system [51,52]. Here, we reviewed the recent studies concerning integrating different types of electrochemical sensors with DMF platforms.

The early investigations on incorporating electrochemical sensors into DMF devices were usually based on the external electrodes. For example, Dubois et al. have demonstrated a device containing two catenaries to enable both electrowetting and electrochemical measurements [53]. The electrochemical sensing was based on voltammetric measurements conducted between these two catenaries through connecting the chip to a potentiostat. Using this device, the ionic liquid droplets can be effectively mixed and then transported to the sensing area for on-line analysis of droplets. The validity of results has been successfully confirmed through comparing the electrochemical measurements with those obtained in microvolume, which shows its potential for enabling convenient chemical synthesis and analysis. Another group has also proposed an external three-electrode system coupled with an EWOD microfluidic device [54]. This system includes a working electrode (Au) where the biochemical reaction takes place, a reference electrode (Ag)

to eliminate any external disturbance, and an auxiliary electrode (Pt). Detailed information on this integrated DMF system with electrochemical sensors is given in Fig. 6.4A. In this system, two droplets containing Tris buffer and potassium iodide solution, respectively, were mixed through a T-junction EWOD device and then transported to the three-electrode system suspended at the end of the T junction. The concentration of iodide in the analyte droplets can then be quantitatively analyzed through cyclic voltammetry. The successful analysis of different analyte concentrations in droplets has proven the great potential of the combination of DMF and electrochemical sensing in rapid chemical analysis.

Figure 6.4

Figure Replacement Requested



(A) (i) Glass substrate with photoresist pattern. (ii) Glass chip with EWOD silver electrodes. (iii) Cross-sectional structure of EWOD chip. (iv) EWOD chip with a three-electrode system. (B) (i) Schematic of DMF-based biochip. (ii) Assembly and picture of completed microfluidic chip. (C) Schematics of an electrochemical cell integrated with EWOD electrodes. DMF, digital microfluidics; EWOD, electrowetting-on-dielectric.

(A) Reproduced with permission C. Karuwan, K. Sukthang, A. Wisitsoraat, D. Phokharatkul, V. Patthanasettakul, W. Wechsato, et al., *Electrochemical detection on electrowetting-on-dielectric digital microfluidic chip*, *Talanta* 84 (2011) 1384–1389, doi:10.1016/j.talanta.2011.03.073. Copyright 2011, Elsevier. (B) Reproduced with permission Y. Yu, J. Chen, J. Zhou, *Parallel-plate lab-on-a-chip based on digital microfluidics for on-chip electrochemical analysis*, *J. Micromech. Microeng.* 24 (2014), doi:10.1088/0960-1317/24/1/015020. Copyright 2014, IOP Publishing. (C) Reproduced with permission A. Farzod, H. Moon, *Integration of reconfigurable potentiometric electrochemical sensors into a digital microfluidic platform*, *Biosens. Bioelectron.* 106 (2018) 37–42, doi:10.1016/j.bios.2018.01.048. Copyright 2018, Elsevier.

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Rather than using external electrodes, in recent years, many researchers have focused on developing on-chip electrochemical measurements integrated with DMF systems [55,56]. Yu et al. have proposed a fully integrated DMF system with electrochemical sensing by transferring the sensor to the top plate [55]. The top plate consists of patterned ground electrodes with an optimized configuration of the electrochemical cell, as shown in Fig. 6.4B, which has the capability of achieving rapid and sensitive electrochemical response. The fabrication processes, including reactive ion etching as well as lift-off patterning, were **integrated circuit (IC)** compatible, which facilitates mass manufacturing. This integrated platform has been successfully exploited to conduct automatic analysis of ferrocenemethanol and dopamine at different concentrations. More recently, Farzod et al. have proposed an integrated DMF platform with potentiometric electrochemical sensors [51]. Instead of building the platform with isolated prefabricated electrochemical sensor elements, this group has proposed a novel on-demand fabrication method in which Ag/AgCl electrodes can be fabricated during the operation of a DMF platform. Fig. 6.4C shows the schematic diagram of the integrated DMF electrochemical platform. In this configuration, the top plate not only acts as the ground electrode for the DMF device but also plays a role in electroplating of Ag. Similarly, the bottom plate serves as both an electrode for the DMF system

and the seed layer for electrochemical sensor electrodes. This on-chip electroplating and fabrication technique allows for seamless integration of biosensors with the DMF platform. Moreover, the reconfiguration of sensors can be achieved without the need of disassembling or interrupting the device thanks to the on-demand fabrication method. This study also provides an efficient way to enhance the lifespan of reference electrodes of the sensing device. The proposed platform has been successfully demonstrated to be a useful tool in measuring potassium concentration, which shows great potential in low-cost and portable home-use sensing applications.

In summary, electrochemical sensors possess the merits of high sensitivity, rapid detection speed, excellent portability, and simplicity. Another big advantage is their ease of fabrication and integration with DMF devices, which makes them a promising tool for biological and chemical sensing applications [57,58]. In the future, more efforts should be made into integrating electrochemical sensors with DMF platforms using simpler fabrication and integration methods, which is of vital importance to facilitate large-scale integration as well as mass production and further promote the widespread use of integrated DMF systems.

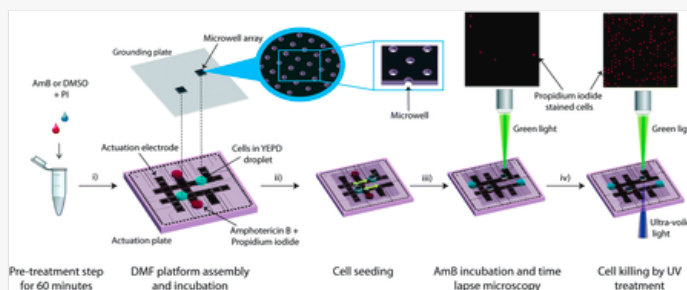
6.3.2 Applications of digital microfluidic biosensors

The excellent sample manipulation abilities of DMF devices with the remarkable sensitivity of several sensing technologies built into it have made DMF biosensors a useful tool in a wide variety of applications, including cell-based applications, DNA-based applications, and protein-based applications.

6.3.2.1 Cell-based applications

Cell-based applications include cell culture, cell sorting, and purification. To date, various types of cell culture such as suspension culture [59,60], 2D cell culture on planar substrates [61,62], and 3D culture in hydrogels [63,64] have been demonstrated on a DMF platform. To enable single-cell culture and analysis, Kumar et al. have exploited a DMF platform to analyze isolated yeast cells [65]. Fig. 6.5 shows the procedures of conducting a cytotoxicity assay using a DMF platform, including pretreatment, cell incubation, and seeding manipulated through a DMF system and the subsequent detection. In this platform, five different concentration levels of antifungal drug amphotericin B have been successfully tested with real-time cell responses monitored through fluorescence microscopy. The experimental results show that a DMF platform has great potential in performing single-cell analysis. Recently, Zhai et al. have also developed a DMF platform for single-cell isolation and stable cell culturing through engineering 3D microstructures on chip [66]. In this work, innovative micropatterned arrays were constructed to efficiently trap a single cell and thus enable long-term cell culturing. After culturing breast cancer cells at different concentration levels, drug toxicity test was performed using a DMF system in comparison with the off-chip experimental condition. The comparable results have proven the efficiency of single-cell trapping and culturing using the designed DMF platform, which can be a useful tool in biological applications at a single-cell level.

Figure 6.5



Flowchart of using a DMF system for conducting cytotoxicity assays on single *Saccharomyces cerevisiae* cells. DMF, digital microfluidics.

Reproduced with permission P.T. Kumar, K. Vriens, M. Cornaglia, M. Gijs, T. Kokalj, K. Thevissen, et al., *Digital microfluidics for time-resolved cytotoxicity studies on single non-adherent yeast cells*, *Lab. Chip* 15 (2015) 1852–1860, doi:10.1039/c4lc01469c. Copyright 2015, Royal Society Chemistry.

Many investigations have been carried out concerning cell sorting and purification, which are also of vital importance in cell-based applications. There are a variety of techniques such as utilizing electrical, optical, or magnetic forces to

control the cells inside the microdroplets [1,67,68]. For example, the typical binary separation scheme was developed based on electrophoresis [69,70]. The sorting procedures normally consist of three steps, namely the separation of cells inside the mother droplet through electrophoresis, the splitting of mother droplet into daughter droplets through EWOD, and the manipulation of splitted daughter droplets individually for the subsequent operations. Nejad et al. have also studied the effect of negative dielectrophoresis trap geometry on cell sorting and manipulation based on DMF systems [71]. This study provides insight into designing and optimizing the geometry of trapping sites, which can largely increase the efficiency of cell capturing and sorting. Kumar et al. have recently integrated DMF devices with optical tweezers to enable effective single cell trapping, relocation as well as proliferation [72]. The DMF platform was composed of microwell arrays on the ground substrate. The optical tweezers can offer the ability of selective trapping and relocation of cells into the microwell array based on the expression of fluorescent proteins. Thus, this platform has been successfully demonstrated to be capable of selective retrieving and trapping of single bacteria as well as the subsequent proliferation at a single-cell level. In another study, this group has also demonstrated the use of magnetic force for manipulating cells inside the microdroplet [73]. In this system, an external magnet was placed above the top plate of the DMF device to induce the magnetic field, which was exploited to immobilize and manipulate the individual magnetic particle-conjugated protoplast droplet. An average of seven protoplasts can be manipulated and analyzed in every field of view through DMF manipulation. This integrated platform provides a useful solution to immobilize nonadherent cells and perform efficient analysis at a single-cell level.

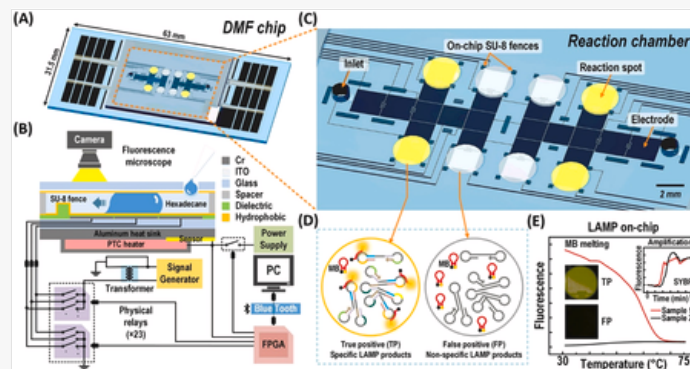
6.3.2.2 DNA-based applications

DNA-based applications normally cover fields like DNA extraction, DNA amplification, as well as DNA sequencing. Normally, a DNA sample needs to be extracted and purified in advance of further processing and detection. Microfluidic technology has become a useful tool in performing automatic DNA extraction through integrating multiple functional units on chip. In DMF platforms, Hung et al. have developed a magnetic beads-based protocol for DNA extraction with reduced washing cycles in the room-temperature environment [74]. In this protocol, a series of droplet manipulation strategies were adopted. Broadly, two steps were included: collecting the magnetic beads into one side of the droplet through the meniscus-aid collection technique and washing out the unbound DNA from the magnetic beads. The results have also been verified through both SYBR-Green dye staining and gel electrophoresis, which confirms its validity in performing DNA extraction with minimized reagent use and reaction time.

Loop-mediated isothermal amplification (LAMP) and PCR are two most frequently adopted methods for DNA amplification. DMF platforms can serve as an important tool to implement these two methods [1,29,75]. As shown in Fig. 6.6, Wan et al. have developed an integrated DMF platform for LAMP-based pathogen nucleic acid detection [76]. They have integrated a temperature sensor as well as a heater with the control unit of a DMF platform for real-time thermal regulation and actuation signal generation. This DMF LAMP system was capable of both sample loading and detection of amplification products. Moreover, this platform has the potential of realizing fully integrated point-of-care diagnostics when further integrated with sample preparation and fluorescence detection modules. Hua et al. have exploited DMF devices for real-time multiplexed PCR with an extremely high amplification efficiency up to 94.7% [77]. In this system, the thermocycling was effected through transporting target droplets cyclically between different temperature zones at a temperature required for PCR. Moreover, by separating the reactions in space, that is, using a set of droplets containing different primer sets as reactors for parallel experiments, the DMF cartridge can be configured to perform multiplexed PCR analysis of both multiple genetic targets and multiple DNA samples in parallel. These works have proven that integrated DMF devices can be an excellent platform for DNA amplification due to its flexibility, droplet manipulation ability as well as thermocycling capability.

Figure 6.6

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Overview of the DMF system for LAMP reaction and analysis. DMF, digital microfluidics; LAMP, loop-mediated isothermal amplification.

Reproduced with permission L. Wan, T. Chen, J. Gao, C. Dong, A.H. Wong, Y. Jia, et al., A digital microfluidic system for loop-mediated isothermal amplification and sequence specific pathogen detection. *Sci. Rep.* 7 (2017) 14586, doi:10.1038/s41598-017-14698-x. Copyright 2017, Springer Nature.

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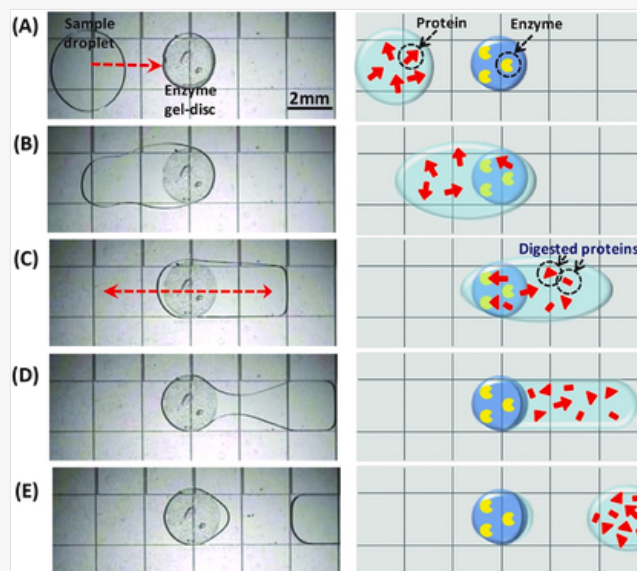
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After DNA extraction, purification, and amplification, one extremely important step is DNA sequencing, which plays an important role in genotyping and clinical diagnostics. Many studies related to performing DNA sequencing on a DMF platform have been reported. Welch et al. have provided a groundwork for performing pyrosequencing on EWOD devices at a picoliter scale [78]. To carry out the pyrosequencing process, droplets containing ATP and luciferase solutions were mixed using DMF techniques. Afterward, the light intensity produced during the reaction was recorded to determine the number of bases added to the DNA strand. The detection limit can reach approximately 7 nM. This work has made efforts in decreasing the reagent volume and reducing the cost of DNA sequencing. Later on, Kim et al. have further improved the integration level of the DMF devices by adding sample preparation processes to the system [79]. Through integrating the distinct capillary-based modules into the DMF platform, a fully integrated device for sample preparation and analysis has been built. More recently, a DMF sequencing platform capable of real-time, automatic analysis of DNA mutations has been proposed by Zou et al. [80]. This portable system is composed of 48 electrodes, a **photomultiplier tube (PMT)** module, and a circular magnet assembly. Experimental results showed that a DNA template with up to 53 bp can be sequenced by this system with 100% accuracy within 2 hours. These studies show that an integrated DMF platform can prove to be a powerful tool in next-generation sequencing.

6.3.2.3 Protein-based applications

Microfluidic technology is a promising and advantageous tool in protein-based applications due to the fact that these applications usually require multiple sample processing procedures before being analyzed by mass spectrometry or any other methods. Among various microfluidic technologies, DMF techniques have been exploited in applications related to proteomics such as protein extraction [81,82], protein crystallization [83,84], and mass spectrometry [85–87] due to their advantages of efficient sample manipulation and minimized reagent use. In 2012, Luk et al. have integrated DMF devices with hydrogels to perform proteomic sample processing, including encapsulation and digestion [88]. In DMF systems, gel disc microreactors can be integrated directly with the relevant electrodes. As shown in Fig. 6.7, sample droplets containing protein can be manipulated to merge with the stationary gel discs for sample digestion. Afterward, the digested droplets containing digest peptides can be split from gel discs for subsequent sample processing or analysis. This platform has the potential of parallel sample processing based on multiple gel discs and thus can play an important role in proteome profiling.

Figure 6.7



Images and schematic that show the operation of hydrogel proteolytic enzyme microreactors on a DMF device. DMF, digital microfluidics.

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In a recent study, Leipert et al. developed an efficient sample preparation workflow for proteomics based on DMF platforms, which was also compatible with the subsequent LC-M analysis [89]. By introducing a single-pot, solid-phase-enhanced sample preparation approach on-chip, the polymeric detergent removal and magnetic bead-enabled protein clean-up can be made possible, which enables the whole workflow, including cell lysis, protein extraction, reduction, alkylation as well as proteolytic digestion. Exploiting this system, they have successfully conducted proteome analysis on Jurkat T cells and identified up to 2500 proteins from approximately 500 cells. The results have shown the capability of this system in handling and analyzing a small volume of protein input, which provides insights into developing miniaturized protein-based applications.


6.4 Conclusions and outlooks

Exploiting the advantages of DMF techniques, over the years, various applications in chemical and biological fields have been presented based on DMF platforms. In particular, since DMF systems can provide a convenient way to sample transporting, mixing and splitting, significant progress has been made in sample preparation steps. Despite its numerous advantages, DMF technology still faces some challenges. To begin with, although a sophisticated electromechanical theory has been proposed, there are still some phenomena such as CAS that cannot be fully explained. Moreover, DMF systems are normally equipped with other peripheral modules and need a complicated fabrication process, which may hinder its widespread application to some extent. In addition, most of the current DMF systems only addressed part of the issues in a complete set of bioassays or biodetection process. There are still quite a few challenges facing the integration of all the modules and developing a fully automated on-chip device. For example, the compatibility of the sensing module with the hydrophobic coatings and its influence on the electrowetting-based sample manipulation should be further studied.

More efforts should be made toward addressing these challenges of DMF platforms and promoting its applications in point-of-care diagnostics in the future. First, more studies should be carried out on exploring a more comprehensive and solid theory of DMF mechanisms, which will help gain a deeper understanding of certain phenomenon and promote their practical applications. Second, it is highly desired to further lower the cost and facilitate large-scale fabrication of DMF devices to promote its widespread applications. PCB-based and paper-based DMF substrates have already been explored over the years with more accessibility in terms of cost and fabrication. These emerging substrates have provided more possibilities in DMF-based applications. More importantly, despite the current developments in the integration of various detection techniques, there is still much room for developing integrated DMF platforms coupled with more sensitive and miniaturized sensing systems. The multiplexing abilities as well as real-time monitoring capabilities should be further explored. We believe an ideal DMF platform integrated with all the necessary steps,

including sample loading, manipulation, detection, and analysis, will be a reality in the near future, which will play an important role in empowering chemical and biological applications.

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 The corrections made in this section will be reviewed and approved by master copier.

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