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From prototype to point-of-care: Digital microfluidics for scalable biomedical applications

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ABSTRACT

Digital microfluidics (DMF) has emerged as a powerful platform for automating and miniaturizing biomedical assays, enabling high-precision, low-volume workflows. However, its transition from research laboratory to industrial-scale manufacturing faces persistent barriers. These include the need for high actuation voltages, material degradation, lack of standardization, and the complexity of integrating diverse detection systems. This review explores and critically discusses recent advances in integrating sensors, hybrid device architectures, and programmable controllers to enhance DMF reliability, portability, and scalability. Emphasis is placed on interdisciplinary efforts that bridge engineering, materials science, and diagnostics to address fabrication challenges, support real-world applications, and establish DMF as a viable technology for clinical diagnostics and point-of-care platforms.

1. Introduction

Over the past decades, digital microfluidics (DMF) has become a leading technology for clinical diagnostics, especially point-of-care (POC) platforms [1]. DMF operates at microliter to nanoliter volumes, enabling precise, programmable droplet movement by applying electrical potentials to electrodes coated with hydrophobic dielectric layers [2]. The key benefits of this miniaturized and integrated approach include faster analysis, substantial reductions in sample and reagent consumption, improved automation of complex workflows, significant cost savings, and enhanced operational efficiency compared to conventional laboratory methods.

Complementing these improvements, the integration of artificial intelligence algorithms for interpreting DMF-generated data has significantly improved analytical robustness and reliability. These computational tools enhance the suitability of DMF for resource-limited settings by reducing the need for highly trained personnel and minimizing additional data processing requirements [3].

From an engineering perspective, achieving large-scale industrial deployment of DMF will require addressing challenges such as fabricating robust dielectric and hydrophobic layers on the electrode matrix, standardizing modular interfaces, integrating optical and electronic sensors, and scaling fabrication. As a result, long-term operational stability is essential for manufacturing reliability [4].

To address these needs, integrated 'all-in-one' architectures combining DMF, single-board computers, and electrochemical detection modules have been successfully proposed [5]. Through such combinations, promising solutions for limited infrastructure environments and applied research can be achieved, thus enabling the automation of complex processes, such as impedance spectroscopy monitoring. This progress supports the decentralization and broader accessibility of DMF technologies [6].

Despite the advantages of DMF in automation, miniaturization, and cost reduction, its clinical adoption remains limited, in contrast to the widespread use of established, often more expensive technologies [7]. This difference is not only due to cost, but also to technological maturity. Instruments such as nuclear magnetic resonance (NMR) already have regulatory validation, consolidated protocols, and integration into laboratory workflows [8]. DMF, while promising, still faces challenges in standardization, material robustness, and compatibility with industrial

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processes. Thus, its advancement depends less on functional innovation and more on achieving regulatory confidence, operational stability, and integration with existing clinical practice [9,10].

This review assesses recent developments and challenges in bridging the Lab-to-Fab gap currently affecting DMF technology adoption. It identifies key barriers to commercialization and clinical translation and discusses prospects for DMF as a core technology in medical diagnostics.

1.1. Instrumentation and adoption - challenges and opportunities

The commercialization of DMF technology has focused on electrowetting-on-dielectric (EWOD) devices, highlighting both the commercial potential and the barriers to industrial adoption. Certain operating and manufacturing characteristics are crucial to the use of DMF platforms and guide the transition from prototyping to point-of-care. Fig. 1 shows some of these characteristics.

Initially, universities and startups developed DMF technology. Later, multinational corporations such as Corning, Amazon, and Illumina became interested in their use, from liquid lenses to molecular diagnostics. Although some devices earned regulatory approval, reliability challenges remained. While optical applications of EWOD technology have achieved millions of actuation cycles thanks to advances in materials and equipment, biomedical liquid-handling DMF devices still face premature degradation of the dielectric and hydrophobic layers when challenged with complex biological matrices. Even though DMF technology has been used as a versatile analytical platform that integrates sample preparation, separation, and detection in a single device [11], as shown in the example in Fig. 2, issues related to fabrication and operation must be addressed to achieve industrial viability [12].

Fig. 2 shows an example configuration of the DMF system for automated preparation and transfer of proteomic samples. The study uses a common device configuration with two plates (top and bottom) that confine the droplet. The bottom plate is equipped with actuating electrodes, a dielectric layer, and a hydrophobic layer. In contrast, the top plate contains a transparent, hydrophobic-coated indium tin oxide (ITO) electrode that serves as a counter electrode. These two plates are separated by spacers, forming the volume in which the droplets are manipulated over an electrode array. However, this application also

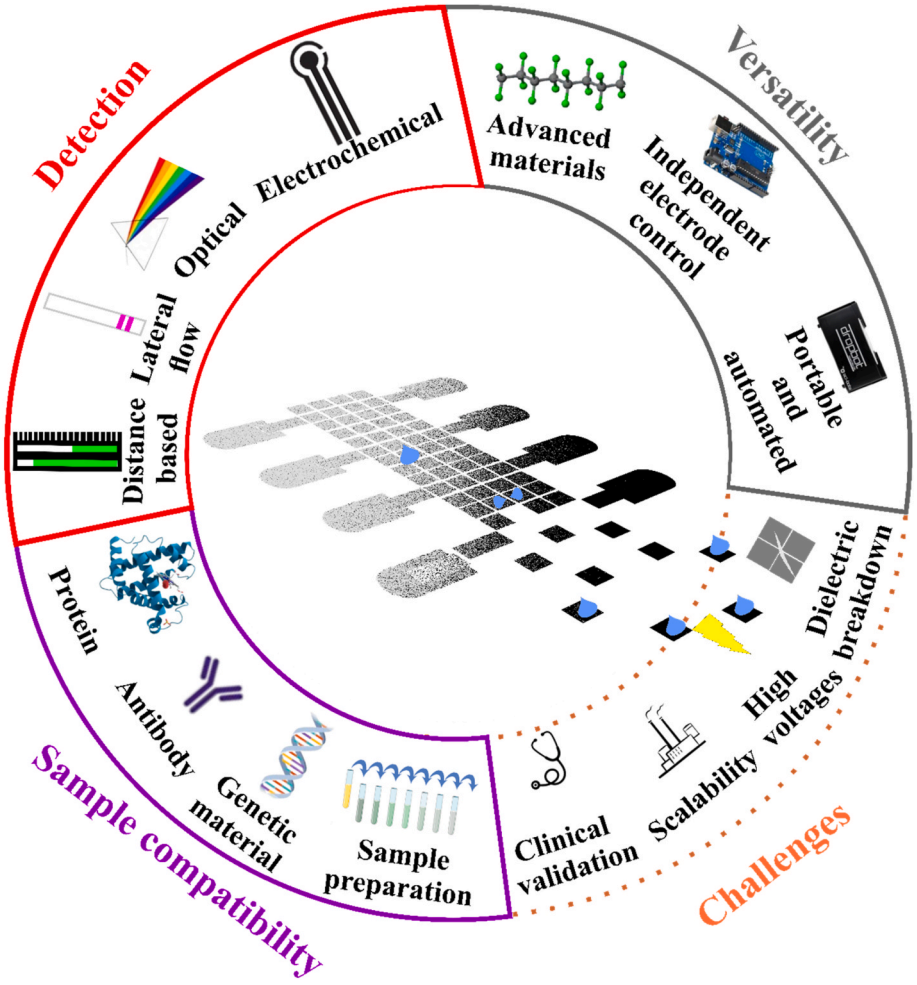

Fig. 1. Conceptual overview of the main dimensions shaping the development of DMF for bioanalytical applications. The central region illustrates a typical EWOD-based electrode array used for programmable droplet manipulation, including transport, splitting, and merging. Surrounding sectors highlight three key aspects influencing DMF analytical performance and applicability. The "Detection'' section (red) presents representative detection strategies that can be coupled with DMF platforms, including electrochemical sensing, lateral-flow assays, distance-based detection, and paper-based analytical devices (μPADs). The "sample compatibility'' section (blue) emphasizes the broad range of biomolecular targets that can be processed on DMF devices, such as proteins, antibodies, genetic material, and samples requiring on-chip preparation steps. The "challenges'' section (orange) summarizes critical technological barriers affecting large-scale implementation, including biofouling, high actuation voltages, scalability of fabrication, and long-term reliability. The outer axis labeled "versatility'' highlights enabling technological advances, such as advanced materials, independent electrode control, portable automated instrumentation, and digital image-based detection, that expand the analytical capabilities and practical deployment of DMF platforms. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

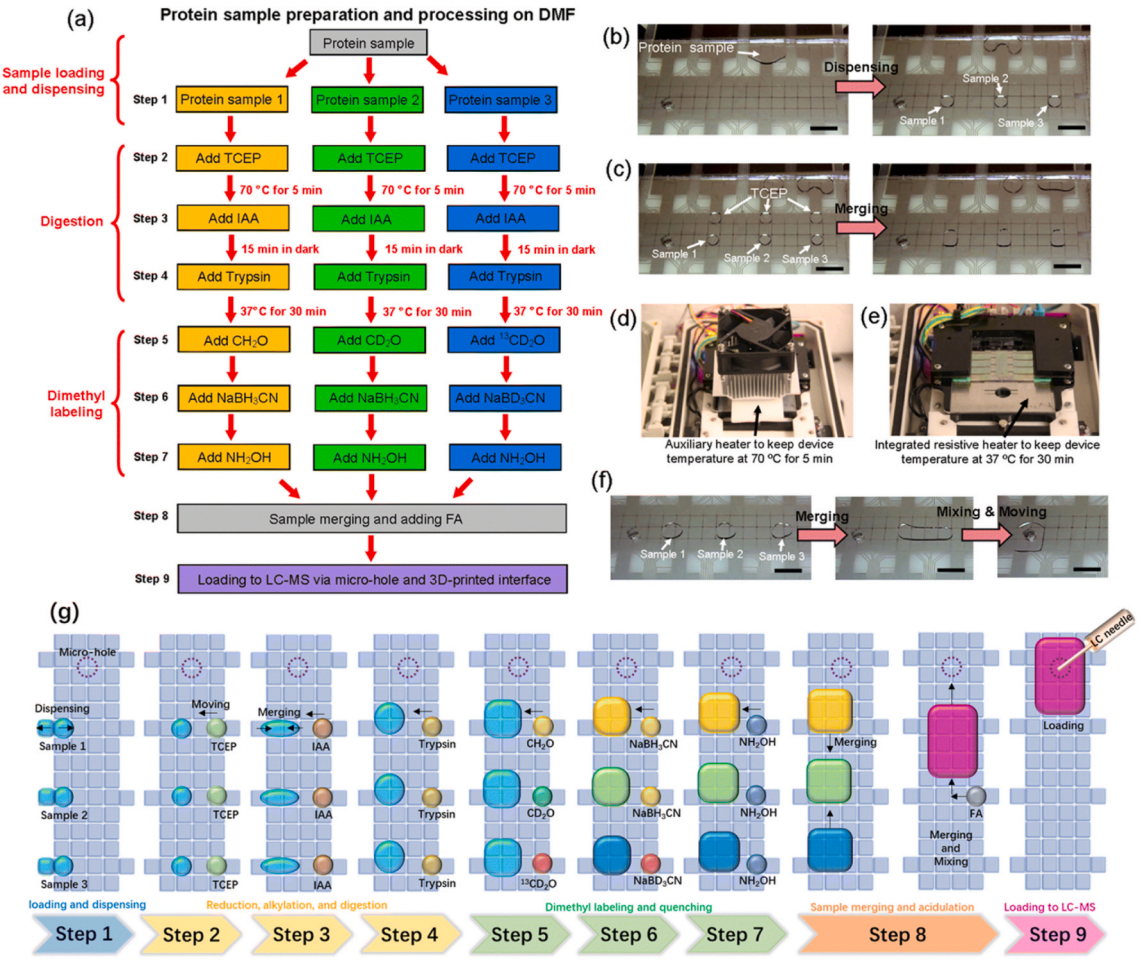

Fig. 2. The All-in-One DMF proteomic pipeline. (a) Workflow illustrating the All-in-One pipeline that includes sample loading (step 1), reduction (step 2), alkylation (step 3), digestion (step 4), isotopic labeling and quenching (steps 5–7), processed sample pooling (step 8) and loading into the HPLC-MS (step 9). Samples labeled with light, medium, and heavy mass-tags are illustrated in orange, green, and blue, respectively. Photographs illustrate various steps in the pipeline, including (b) step 1, (c and d) step 2, (e) step 4, and (f) step 8. (g) Schematic illustrating the entire workflow. Reproduced with permission from Ref. [11]. Copyright 2023 Royal society of chemistry. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

incorporates specific device modifications. The top plate has a micro-hole that allows for sample extraction after processing, constituting an important structural element of the device, and the platform features a DMF-autosampler, composed of a 3D-printed manifold that positions the DMF device in relation to the autosampler needle, allowing direct suction of the sample into the injection loop of the chromatographic system. This ensures that, throughout the loading process, the needle contacts the manifold, the capillary needle extends, and the liquid is aspirated from the DMF device into the analytical system. This example system combines a classical DMF liquid-handling implementation with customized device fabrication (top-plate microholes) and the integration of auxiliary equipment to access an off-device analysis system, highlighting how device fabrication and operational standardization challenges can arise based on the target application.

Moreover, as biomedical applications grow more complex, DMF must process challenging matrices, such as blood and bodily fluids, efficiently and reproducibly, while minimizing cross-contamination. Furthermore, a key limitation remains, as EWOD devices require high actuation voltages, typically tens to hundreds of volts, which increases power consumption, hinders miniaturization, and accelerates electrode degradation [13]. The use of novel dielectric materials, such as polymeric films, can help reduce the voltage requirements while also potentially providing a scalable fabrication method [14]. Additionally, the use of surfactant- and oil-based DMF systems has improved device stability by reducing biofouling, one of the most common causes of failure in older DMF devices, but implementing these systems entails additional requirements. The use of surfactants, such as Tetronic 90R4, typically at 0.1% (v/v), is a well-accepted strategy in operations. Still, it requires a protocol for mixing reagents before loading them into the devices, creating sample-result challenges and introducing non-automated complexity. Some approaches have been proposed to optimize sample delivery to DMF systems, including the use of membranes [15], however, these efforts have increased device fabrication and assembly complexity by adding filtering materials to the design.

On the other hand, oil-based DMF systems have been employed commercially [16] to maintain device simplicity while introducing a new reaction medium that must be injected into the device, which can cause analyte partitioning between phases and often hinders device reuse, potentially increasing analysis costs. Therefore, a significant gap remains between academic innovation and industrial reproducibility, caused by the complexity of materials and the technical requirements of DMF devices. Moreover, standardization issues in chip fabrication and reliance on high-voltage actuation slow industrial adoption.

In addition to the potential and challenges related simply to the processing of liquid biological samples, DMF's versatility and compatibility with detection integration make the technique a powerful analytical and clinical tool, while introducing different challenges depending on the chosen application. In fact, DMF has been integrated with several detection technologies, ranging from big benchtop instruments, such as NMR [17] and mass spectrometers [11,18], to more

compact alternatives, such as electrochemical sensors [19], colorimetric detection systems [20], and distance-based readers [21]. Among these, optical methods are particularly prevalent due to their seamless integration with optical microscopy setups commonly used to monitor microfluidic operations. However, despite their ubiquity, optical techniques often require bulky, expensive instrumentation and high reagent consumption, limiting their applicability in point-of-care and resource-limited settings. Electrochemical sensors are a natural fit for DMF integration, given similar fabrication protocols. Still, integrated devices often require external electrode access, modifications to device architecture, and sensor functionalization compatible with device-layer materials [22]. NMR requires device operation in high electromagnetic fields, and sample ionization requirements often limit MS integration. Regardless of the chosen technique, reliable device integration and standardized architecture remain technical bottlenecks. These issues compromise robustness and reproducibility in manufacturing, highlighting the need for an interdisciplinary approach that aligns the technique's analytical capabilities with industrial demand [23]. Moreover, attempts to integrate DMF into ultra-sensitive digital immunoassays, such as Simoa, reveal conflicting demands between sensitivity and manufacturability. For instance, achieving high sensitivity often requires minimal magnetic bead use, however, the efficient manipulation of such small quantities in confined droplets demands high magnetic fields and optimized geometries. These parameters are still difficult to scale reproducibility [24].

There are, however, examples in the literature that aim to address these analytical challenges, including strategies to address inconsistencies in fabrication, adoption, sampling, and sensor integration. Examples include a roll-to-roll-compatible low-cost DMF fabrication method [25], increased electrode-array fabrication [26], development of field-ready, portable instrumentation [27], anti-fouling surfactant optimization [28], and many sensor-integration methods [1–3].

These are important advances towards scalability and wide technology adoptions that highlight that the obstacles preventing DMF from crossing the gap between laboratory and fab environments are multifaceted, ranging from materials and fabrication processes to new paradigms in automation, sensing integration, and regulatory compliance. Addressing these challenges is essential for unlocking the full potential of DMF in clinical diagnostics, biopharmaceutical production, and real-scale environmental monitoring.

2. Versatility of digital microfluidics in biomedical assays

The desire for wider adoption of the DMF industry is warranted by its outstanding versatility as a platform across many fields of research and remarkable examples of field applications. DMF versatility spans three interrelated areas: biological sample types processed on a single DMF device (such as blood, saliva, urine, and tissues), analyte classes addressable (including nucleic acids, proteins, enzymes, and small molecules), and compatible detection methods. DMF's primary benefits include processing diverse samples, analyzing multiple biomolecules simultaneously, and integrating multiple detection techniques onto a single platform. Software-driven protocol changes and cartridge redesigns enable easy workflow adaptation, without the need to rework fluidic architecture. This blend of flexibility and adaptability drives DMF's popularity in clinical diagnostics and POC settings. However, as the versatility of sample matrices and targets grows, so does the need for standardizing surfaces, dielectrics, electronics, and control algorithms [25,26].

In recent years, DMF platforms have been applied to a wide range of clinical matrices, including whole blood, plasma, serum, dried blood spots (DBS), saliva, urine, respiratory fluids, solid tissue digests, and cell suspensions, with a strong focus on POC-compatible formats and integrated workflows [27]. In blood-derived fluids, DMF is particularly advantageous for applications that require minimal volumes and automated steps. Core automated functions across matrices include precise dispensing, droplet transport, mixing, and incubation of reagents [28,29], often coupled with integrated temperature control [30–32].

Validating biosensors in complex clinical matrices is essential for the scalability of DMF devices. Elevated viscosity and high concentrations of blood proteins often cause biofouling and disrupt signal transduction. For example, Chen et al. described a DMF-integrated biosensor for processing low-volume serum and human plasma samples. This method conserves reagents and reduces matrix interferences, thereby enhancing working stability. [26,33]. Similarly, Choi et al. addressed matrix-related challenges by processing crude human serum and fetal bovine serum (FBS) to detect IL-6 and CRP. Their results indicate that automated on-chip washing steps are essential to mitigate matrix effects. Without these steps, the limit of detection (LOD) would increase by an order of magnitude [34].

To validate their platform for diagnosing acute myocardial infarction (AMI), Shen et al. used real patient serum samples, enabling multiplexed detection of cardiac biomarkers. They showed that a thermal module compensates for variations in antibody–antigen binding kinetics inherent to serum samples. This strategy achieved statistical agreement ($p > 0.05$) with gold-standard laboratory chemiluminescence methods [35]. Further illustrating the versatility of DMF, Nsabimana et al. demonstrated system robustness by detecting the H5N1 antigen in human serum. In this study, indium tin oxide (ITO) electrodes on polyethylene terephthalate (PET) films provided the electrochemical stability necessary to operate at potentials that minimize the direct oxidation of serum interferents, such as ascorbic and uric acids [5].

Komatsu et al. introduced a hybrid DMF-paper device for lithium-ion quantification in blood, integrating on-chip plasma separation from undiluted whole blood using dielectrophoresis (DEP) and EWOD (Fig. 3i). This is followed by nanoliter droplet isolation and colorimetric analysis, achieving >90% separation efficiency and ensuring that colorimetric quantification of Li^+ is not compromised by optical interference from blood cells. As a result, diagnostic accuracy in patients with bipolar disorder is preserved. A key advantage of this platform is the elimination of pre-dilution and passive filtration steps, which often limit scalability in point-of-care systems. Unlike passive methods that require larger sample volumes (>50 μL) and are prone to clogging and hemolysis, DEP enables active plasma separation from as little as 5 μL of undiluted whole blood while maintaining temperatures below 39 °C to preserve protein integrity and prevent cell lysis [36]. This integrated approach highlights DMF's capacity to process complex clinical matrices with minimal preprocessing and maintain analytical reliability. However, from an industrial perspective, the system operates within a limited temporal window, as prolonged immersion of paper-based sensors in mineral oil, which is necessary to prevent nanoliter-scale evaporation, reduces sensitivity after 20 min and imposes constraints on multiplexed or repetitive assays.

Yang et al. presented an electroosmotic digital optofluidic (e-DOF) platform for the rapid processing of microliter-scale (2 μL) finger-prick whole-blood samples. This platform addresses the slow reaction kinetics characteristic of conventional DMF systems. Traditional platforms depend on passive molecular diffusion, resulting in incubation times of 2–3 h. In contrast, the e-DOF system utilizes a non-uniform electric field to generate active electroosmotic flow within droplets (Fig. 3ii). This self-circulation mechanism transforms the sensing interface from a passive receptor to an active capture system, thereby reducing diagnostic time to 15 min and achieving a LOD of 0.21 nmol L^{-1}. However, matrix-derived noise and nonspecific adsorption often compromise analytical performance. To address these issues, the authors established a stringent cut-off criterion based on the mean plus 3 standard deviations of matrix noise. As a result, this enabled reliable, multiplexed detection of IgM antibodies to hepatitis A and E with 100% clinical concordance across 17 hospital samples [37]. Notably, integrating active electroosmotic mixing with label-free optical readout reduces assay time, reagent complexity, and biochemical variability, supporting the potential for scalable POC manufacturing. Nevertheless, reliance on

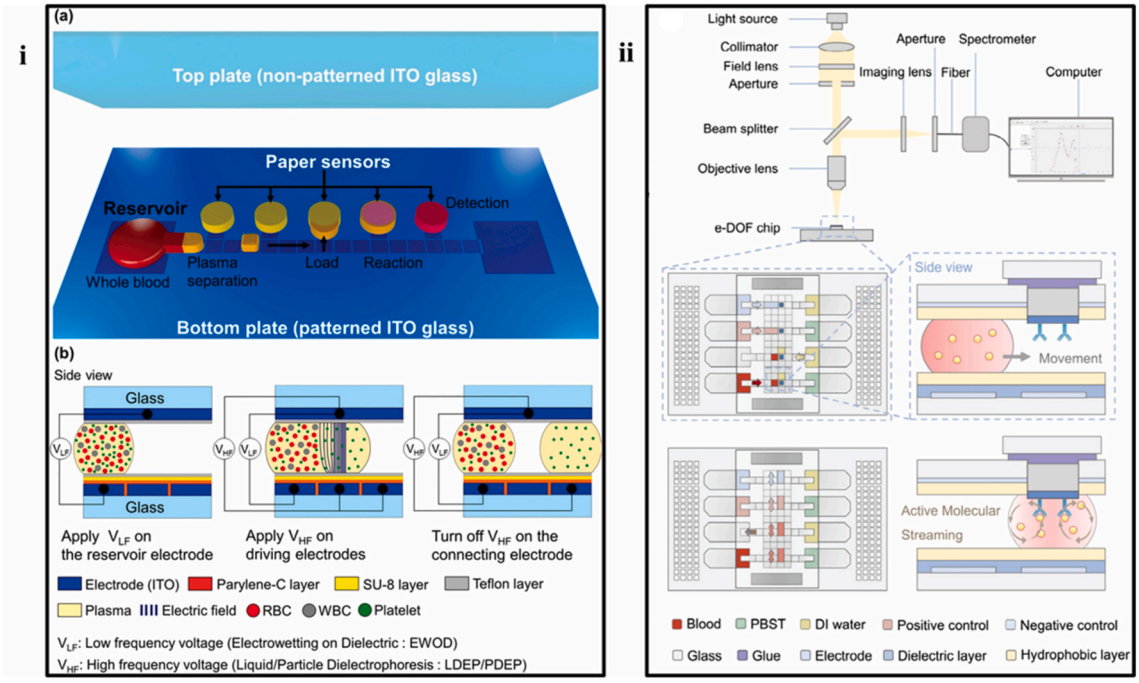

Fig. 3. i - Illustration of DMF–paper hybrid device with preloaded paper sensors to detect Li + concentrations in whole blood (a) and principle of plasma separation via EWOD/DEP (b). After separation, the DEP voltage applied to the connecting electrodes was removed to facilitate droplet splitting [36]. Copyright 2022 Elsevier. **ii - S**chematic diagram of the system construction and detection principle. Schematic diagram of the optical system and detection process on an e-DOF chip. The wafer with a probe is located on the top plate of the e-DOF chip, so that the protein in the blood/saliva sample can specifically bind to the probe when the droplet moves to the corresponding position [37]. Copyright 2025 American Chemical Society.

precise electric-field control and computational optical analysis may pose engineering and standardization challenges that must be addressed for broad industrial adoption.

DMF platforms have also processed DBS extracts from neonatal samples for automated screening of lysosomal storage disorders (LSDs) (IDUA, GAA, GBA, GLA) with total analysis times of a few hours, closely simulating fab/central lab environments [38].

In addition to use with acellular biofluids, DMF is versatile in processing complex cellular matrices. The move from cell-based analysis to clinical matrices with DMF is driven by the need to characterize cellular heterogeneity, which is often hidden in bulk measurements. DMF technology enables programmable single-cell isolation with efficiencies above 95% and lossless processing of intracellular biomarkers, while maintaining cell viability near 99% after manipulation. This operational flexibility enables advanced protocols, such as the DMF-Bimol system, which automates isolation, lysis, and proximity ligation assay (PLA) workflows for absolute digital quantification of mRNA and proteins. This integrated approach reveals biologically significant differences between transcriptional and translational levels in tumor cells [19,28]. For clinical immune monitoring, DMF enables sensitive detection of peripheral blood mononuclear cells (PBMCs) using integrated electrochemical impedance spectroscopy (EIS) biosensors, providing a portable and cost-effective alternative to conventional flow cytometry. Dynamic control of droplet trajectories during incubation increases cellular capture efficiency and analytical signal intensity by up to 2.4-fold compared to static modes, enabling accurate analysis from as low as 4 μL of sample volume [19,28]. Additionally, DMF platforms are suitable for dynamic secretome profiling, facilitating automated sampling of cytokines such as CCL2, IL-8, and TNF-α from stimulated macrophages at defined intervals [39].

Nevertheless, the large-scale transition from laboratory to fabrication also requires addressing the substantial engineering demands imposed by the diversity of sample matrices on chip and instrumentation design. Viscous, protein-rich samples, such as whole blood and inflammatory fluids, increase biofouling and contact angle hysteresis, thereby accelerating dielectric failure. In contrast, standardized dry matrices, such as DBS, are more compatible with fabrication processes but require additional elution and conditioning modules, thereby increasing cartridge complexity. Therefore, while clinical adaptability is particularly valuable, it introduces significant challenges to scalable DMF production with limited, reusable cartridge designs [40].

The versatility of DMF extends to the analysis of a broad range of analyte classes, including nucleic acids, proteins (antigens and antibodies), enzymes, small molecules, and emerging diagnostic targets such as aptamers, exosomes, and cells [41]. DMF has been pivotal in automating nucleic acid amplification tests (NAATs), integrating sample preparation, isothermal or thermal amplification, and detection on a single chip. Combinations of DMF with PCR, loop-mediated isothermal amplification (LAMP), and recombinase polymerase amplification (RPA) have enabled detection of DNA and RNA from various pathogens, including *Plasmodium falciparum*, respiratory viruses (e.g., influenza, SARS-CoV-2, RSV), and other infectious agents [12,23,27,31,41].

The DMF-RPA-CRISPR/Cas12a system developed by Liu et al., aiming to rapidly detect *H. pylori* and genotype virulence genes (cagA and vacA), represents a significant advance in molecular diagnostics. This method achieved a remarkable LOD of just 10 copies per reaction in under 30 min and enabled parallel multi-target detection on a single chip. Integration with DMF automates the complex workflow, minimizing aerosol contamination risk through silicone oil sealing and reducing reagent consumption by approximately 90% for RPA and 80% for CRISPR-Cas12a. The on-chip automated process (Fig. 4) comprises a series of reagent allocation and mixing steps culminating in the final readout. As shown, the RPA-CRISPR/Cas12a assay provides a naked-eye visual result under UV light: the presence of targets (*H. pylori* or its virulence genes *cagA*, *vacA*, and *ureB*) results in a color change from brown to yellow due to cleavage of a fluorescent ssDNA reporter [31].

In another example, Liang et al. demonstrated the DMF-Bimol platform for digital mRNA quantification in single cells via on-chip isolation, lysis, and RT-ddPCR [28]. Key advantages of DMF in NAATs include reagent miniaturization (up to 40,000-fold volume reduction), faster

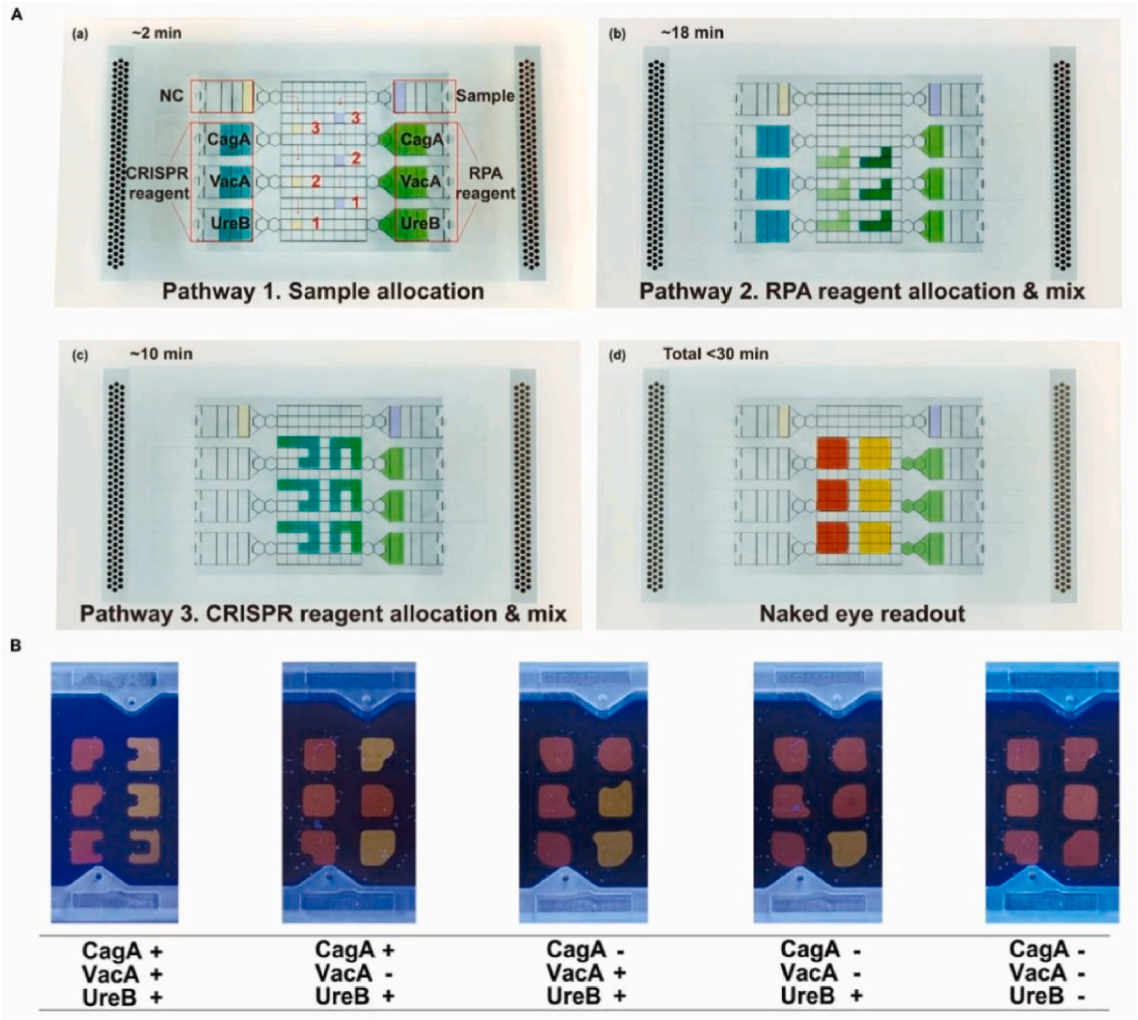

Fig. 4. DMF-based workflow for *H. pylori* detection and genotyping. (A) Diagram of the on-chip workflow: (a) initial sample and CRISPR reagents allocation (~2 min); (b) the addition and mixing of RPA reagents (~18 min); (c) the allocation and mixing of additional CRISPR reagents (~10 min); the entire process was completed within 30 min; (d) visual readout (with the naked eye). Red arrows and associated numbers indicate the direction and order of operations for each step. (B) The panel displays the outcome of the detection process with distinct color changes indicating the presence or absence of the cagA, vacA, and ureB gene. Samples in the left and right columns are the control and clinical groups, respectively [31]. Copyright 2024 Elsevier. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

reactions, partial or complete automation, and prevention of cross-contamination via oil encapsulation [27,31,42]. However, challenges remain regarding nucleic acid extraction efficiency at low volumes and droplet dispensing variability in systems lacking feedback control [31,42].

DMF is also extensively used in immunoassays for cardiac, inflammatory, and cancer biomarkers. It supports automation of heterogeneous immunoassays and multiplexed diagnostics, such as the detection of AMI markers (h-FABP, cTnI, Myo, CK-MB) in 15–30 min [33,35]. The h-FABP detection reached a LOD of 0.14 ng/mL [33]. New approaches such as the Dual-Digital ImmunoAssay (DDA) illustrate the potential of DMF to achieve exceptional levels of automation and sensitivity. By combining digital amplification and CRISPR technologies, DDA platforms have achieved LODs at zeptomolar levels (10^{-21} mol/L) for oncology biomarker detection, with fully automated workflows from sample preparation to signal readout (Fig. 5). These systems demonstrate how precise droplet control can support complex biochemical protocols without manual intervention. Nevertheless, this sophistication demands advanced materials, finely tuned liquid–solid interfaces, multilayer structures, and complex control electronics, all elements that challenge conventional manufacturing processes [43].

Other strategies, such as the integration of magnetic microspheres (commonly referred to as magnetic beads, MBs) for the cleanup of complex samples and the development of improved sample-preparation workflows, offer promising solutions to long-standing analytical challenges. MBs function as mobile solid-phase materials that capture and isolate target analytes from complex matrices. Their high surface area-to-volume ratio increases molecular recognition efficiency and enhances analytical sensitivity compared to passive-diffusion-based approaches [4,9]. Importantly, MBs can be manipulated independently of the surrounding droplet by magnetic fields, allowing aggregation, retention during washing, and resuspension in clean buffers [13,23]. This capability enables automated execution of critical cleanup steps, including cell lysis, protein or nucleic acid extraction, immunoassay development, and purification [9,23]. Choi et al. used electromagnetic bead actuation to enhance washing/mixing and processed eight samples in parallel within 40 min in an immunoassay [34]. The use of MBs eliminates the need for centrifugation or external pumping systems, thereby facilitating the development of miniaturized, fully integrated workflows [4]. An additional benefit is that MBs enable novel detection strategies, as exemplified by *Le* et al., who combined MB-based analyte capture with streptavidin detection antibody to enable subsequent

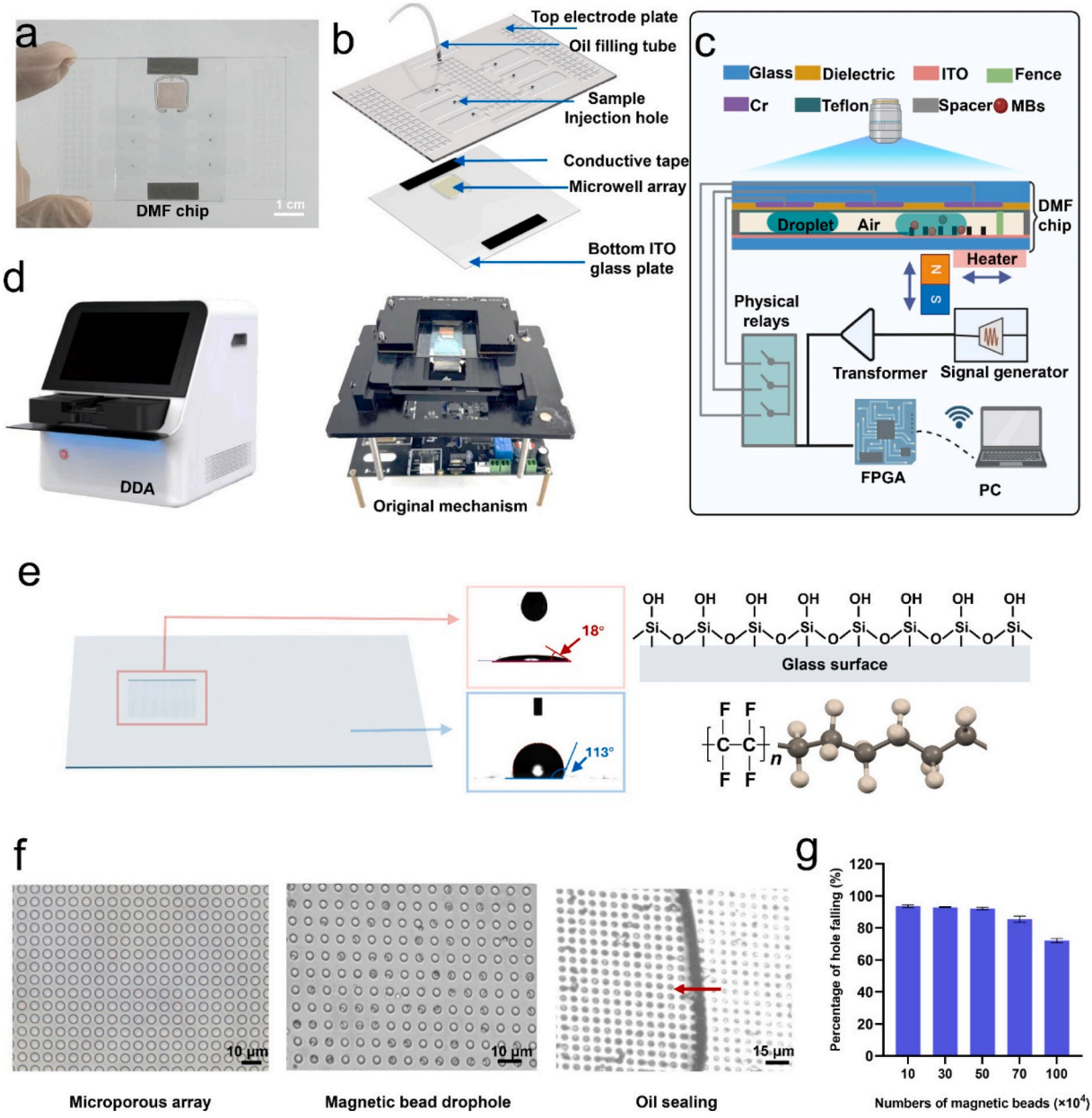

Fig. 5. Construction and characterization of the microwell-array DMF chip. (a) Photograph of the assembled microwell-array DMF chip (b) Exploded-view schematic of the DMF chip assembly, showing the top electrode plate with injection holes and oil filling tube, the bottom ITO glass plate with microwell array, and the conductive tape spacers. (c) Schematic illustration of the droplet actuation mechanism. (d) The left image shows a photograph of the assembled instrument; the right image displays its internal layout, including the DMF chip platform and control module. (e) Spatially controlled wettability on the bottom microwell array plate. A hydrophilic microwell array region (contact angle ~ 18°) is created by combining plasma and hydrophilic solution treatment to facilitate bead and fluid entry. The surrounding Teflon-coated surface remains hydrophobic (~113°) for precise droplet control. (f) Microscopic imaging of the microwell array, magnetic bead loading, and oil sealing process. Left: Bright field micrograph of the microwell array. Middle: Microscopic image showing magnetic beads settling into the microwells under the combined influence of gravity and magnetic force. Right: Image of FC-40 fluorinated oil being applied to seal the microwells after bead loading, forming isolated microreaction units. The red arrow indicates the direction of movement of the oil–water interface during the sealing process. (g) Microwell captures efficiency at different magnetic bead input quantities [43]. Copyright 2025 American Chemical Society. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

localized rolling circle amplification (RCA) biotin-primer amplification [21]. The amplified primer was bound to fluorescently tagged complementary DNA, and positive beads (those that bound the analyte) were counted to generate a highly sensitive digital assay on DMF. While promising, the use of MBs on DMF requires an external magnet (or magnetic lenses) to be incorporated into the instrumentation, which increases the footprint and introduces engineering complexity to the system. However, magnetic separation has demonstrated to be compatible with shoe-sized portable DMF instruments used in real field analysis settings [44–46]. Furthermore, a DMF instrument capable of MBs manipulation is currently being commercialized by the Sci Bots company (Toronto, ON, Canada), using a modular approach that demonstrates its commercial viability.

Adapting analytical protocols to DMF enables miniaturized, automated workflows by reducing reagent volumes. Lu et al. demonstrated this advantage with the DEL-DMF system for H5N1 detection, reducing reagent and washing volumes from 3800 μL in conventional ELISA to 27.3 μL [47]. However, throughput limitations and the need for efficient

fluid handling continue to restrict scalability. To address these issues, Chen et al. developed a DMF workstation featuring an automated ejection mechanism that transfers droplets from the chip to external 384-well plates. This approach eliminates redundant electrode use during prolonged detection, thereby significantly increasing throughput for analytes such as heart-type fatty acid–binding protein (h-FABP) [33]. Maintaining assay integrity also requires effective biofouling control, as biofouling can compromise reproducibility and wettability. While surfactants are frequently employed to prevent biofouling and facilitate droplet manipulation, they may interfere with subsequent assay steps. Alternatively, encapsulating samples in oil, such as silicone or mineral oil, reduces cross-contamination and evaporation, thereby improving assay stability [31,34].

Small biomarkers, such as ions and metabolites, are also detected using colorimetric DMF assays. Komatsu et al. developed a hybrid DMF-paper system to quantify lithium ions using F28 porphyrin sensors, completing each analysis in ~4 min [36]. Velasco et al. reported creatinine detection via automated Jaffé reaction on a DMF-PADs platform with on-chip dilution cycles, reducing manual steps and reagent use [48]. These studies indicate that integrating DMF with PADs minimizes sample and reagent consumption, supports automated dilution, and eliminates the requirement for complex optical instrumentation. This integration facilitates the development of portable, low-cost platforms aligned with green chemistry principles.

The third dimension of DMF versatility involves compatible detection techniques, ranging from optical to electrochemical. Optical detection, particularly fluorescence, chemiluminescence, and colorimetry, is predominant. Fluorescence measurements span from bulk droplet readings to single-molecule digital assays, balancing sensitivity with instrumentation complexity [35,43]. Chemiluminescence is a common method for immunoassays that use an enzymatic reaction to generate light. Portable DMF-ELISA systems, such as the MR Box, exemplify the effective use of chemiluminescence, enabling the monitoring of IgG/IgM immunity to measles and rubella in remote settings [44].

Colorimetric DMF systems, with naked-eye detections such as RPA-

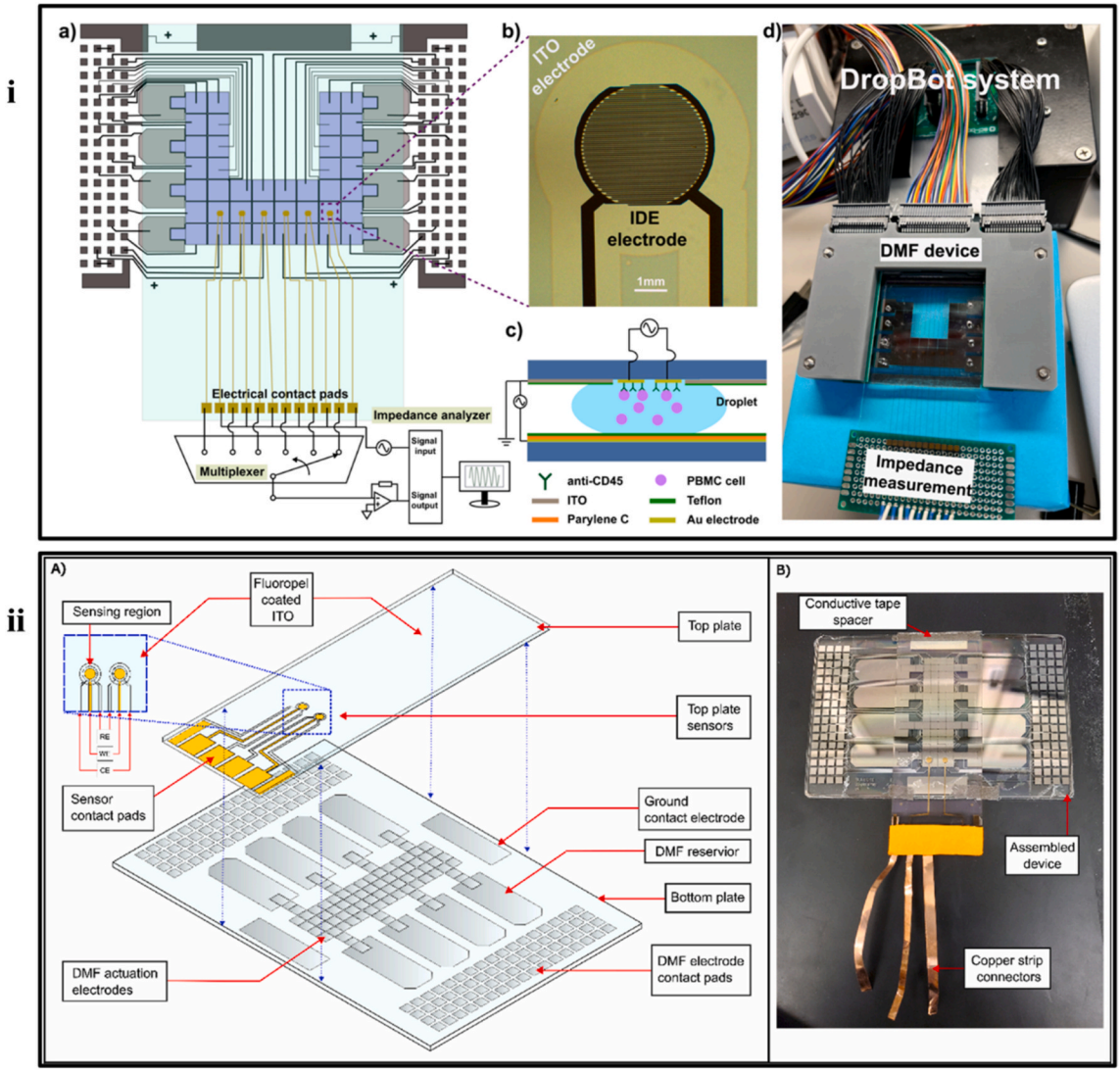

Fig. 6. i - (a) Overview of DMF device with integrated interdigitated electrodes (IDEs). Schematic illustration of the device showing 40 actuation electrodes on the bottom plate and 6 sets of sensing electrodes on the top plate. The sensing electrodes were connected to a multiplexer and an impedance analyzer for the detection; (b) Microscopic image of IDE electrodes deposited on the top plate. (c) Cross-section diagram of the device, with a droplet of cells on the sensing electrodes (not to scale); (d) A photo demonstrating the assembly of an integrated DMF device. DropBot system was used to operate the device; the impedance measurement was conducted by connecting electrical contact pads to a digital impedance analyzer [19]. Copyright 2022 MDPI. **ii -** Electrode configuration and DMF design. A) Schematic cartoon of DMF device and top plate containing two Au/ITO electrochemical sensors. Inset: Detailed view of sensors, DMF region (coated with Fluoropel) and sensing regions (not coated with Fluoropel). B) Photograph of fully assembled device [51]. Copyright 2024 Elsevier.

CRISPR/Cas12a for *H. pylori* detection with UV light mentioned earlier [31], or Digital Image Colorimetry (DIC) detection used for lithium detection [36], and H5N1 antigen quantification [27] using a digital camera, and creatinine quantification using a scanner [48], and protein concentration using a smartphone [49], support low-cost POC setups. These methods sacrifice some sensitivity but are robust with proper contrast and lighting, often requiring image-processing algorithms [36, 48].

Another possible application of DMF is the indirect colorimetric analysis of S-nitrosocysteine (CySNO) in biological samples, as reported by Rocha and coworkers. The authors explored the technique as a platform for developing an integrated analytical system based on a mini-studio equipped with a 3D-printed holder and UV-LEDs. The developed assembly enabled the photolytic degradation steps of the sample, reagent mixing, colorimetric reaction, and webcam detection directly on the chip. The method showed 96% degradation of CySNO to nitrite, and results obtained in synthetic serum and human plasma were statistically equivalent to those obtained by spectrophotometry, demonstrating the potential of integrating DMF with simple optical systems for complete analysis of low-molecular-weight compounds [50].

Electrochemical detection is increasingly important for fab integration due to low-cost instrumentation, portability, and compatibility with embedded electronics. Nsabimana et al. used square-wave voltammetry (SWV) on ITO electrodes integrated into PET films, achieving a LOD of 0.6 ng/mL for the H5N1 antigen [5]. Zang et al. performed EIS measurements on interdigital electrodes (IDEs) integrated into the top plate of the DMF (Fig. 6i) to quantify PBMC abundance [19]. Both platforms used the same electrodes for droplet actuation and detection (e.g., SWV and EIS), enabling miniaturized, low-power designs [19,23]. *Campos* et al. have also demonstrated the use of EIS sensors integrated into DMF for broad-spectrum detection of pathogen molecular patterns (PAMPs) associated with gram-negative bacteria (Fig. 6ii) [51]. By functionalizing gold electrodes fabricated on a DMF top plate with Toll-like receptor proteins, the authors demonstrated the detection of PAMPs associated with the bacterial class rather than specific bacteria, a finding that is paramount for environmental screening applications. In this example, sensor versatility enhances DMF capabilities by enabling the detection of different types of pathogens through functionalized protein changes. However, this approach relied on labor-intensive DMF top-plate redesign and cleanroom fabrication, which limits scalability.

Although sensor regeneration mitigates cost and time issues associated with fabrication, the approach would greatly benefit from simplified alternative fabrication methods. Challenges to the incorporation of electrochemical detection to DMF include the complexity of fabricating electrodes into the chip, the need for strict electrical isolation between actuation and sensing units, compatibility with sensor functionalization protocol and device materials, and susceptibility to interference from surfactants and electroactive species in biological matrices, which can compromise charge transfer and signal fidelity. Additionally, electrode degradation at high actuation voltages and the challenges associated with nonspecific biofouling limit the analytical stability and reproducibility of such systems [5,23]. An alternative to simplifying electrode fabrication is the use of external sensing systems coupled to DMF. This has been demonstrated by *Campos* et al., who successfully reported that the integration of commercial plug-in-play sensors (such as glucose and β-ketone test strips), screen-printed electrodes, and bespoke paper-based sensors with the DMF device [22]. The authors demonstrated multi-model detection using electrochemistry and chemiluminescent detection in a split-sample droplet. Since microfluidic devices and sensors are produced separately, this solution eliminates fabrication constraints while requiring minimal changes to the DMF architecture. Although promising, more investigation is required to fully assess the advantages and limitations of coupling external sensors to DMF.

DMF assay compatibility across matrices, analytes, and detection modalities supports its growing adoption in biomedical and multi-test platform development [12,49]. However, this flexibility creates challenges for fabrication integration. Expanding combinations of matrices, analytes, and detection methods increase operational variability, including higher risks of biofouling [10,34], contact angle hysteresis [49], variability in detectability levels [24,33], and instability of surface coatings [35]. These factors complicate the establishment of operating windows and qualification protocols required for scalable production and regulatory approval [12,13,33].

Therefore, the versatility of DMF in biomedical assays should be regarded not simply as a collection of use cases, such as transitioning between NAATs [13], digital protein assays [24,52], or enzymatic assays [29,38], but as a fundamental factor influencing design, testing, and standardization requirements. Systems with high versatility that can alternate between NAATs (such as LAMP or RPA), digital immunodiagnostics (such as Simoa and DDA), and enzymatic assays across diverse matrices highlight both the promise of DMF and the considerable advancements still needed in materials, electronics, algorithms, and regulatory frameworks to achieve reproducible, scalable, and clinically validated products [12,13,29].

3. Field applications for digital microfluidics

The application of DMF to routine disease diagnostics has advanced significantly in both molecular assays [53] and serological testing [54]. The versatility of the technique allows for customized configurations that incorporate different detection methods for analysis across a range of samples and matrices of varying complexity. Table 1 presents relevant examples of two-plate DMF and several detection configurations for analytical applications.

Although not currently produced at large scale, DMF demonstrates maturity in executing biochemical reactions and sample preparation using minimal reagent volumes, in an automated fashion and without reliance on a complex infrastructure. Fig. 7 shows some recent applications of DMF in POC settings, highlighting the technique's unique automation capabilities and motivating efforts to overcome challenges in crossing laboratory borders.

In a recent study [55], a DMF platform was developed to integrate RPA with colorimetric detection using SYBR Green I for the identification of the *PfLDH* (L-lactate dehydrogenase) gene in *Plasmodium falciparum*. The system allowed simultaneous testing of up to 5 samples, achieving sensitive detection under UV (365 nm) with a 15-fold signal enhancement. The estimated costs were $150 per chip and $0.09 per assay droplet, substantially lower than for other routine tests such as antigen rapid tests, microscopy, or PCR ($0.50–$50), offering a path toward democratizing molecular diagnostics.

In the same vein, a low-cost DMF platform integrated with LAMP and direct colorimetric readout was developed to be used in low-resource settings. Targeting the 18S rRNA gene of *Plasmodium falciparum*, the assay was performed at 65 °C over 45 min using only 1.5 μL of reagents. While the exact test costs were not disclosed, the DMF device, fabricated on a printed circuit board (PCB), required minimal installation. This study demonstrated the feasibility of malaria diagnostics via DMF but highlighted the need for more robust clinical validation under field conditions [42].

Knipes et al. reported an innovative field deployment of a DMF-based lab system (MR Box v2) with disposable cartridges during a measles and rubella outbreak in the Democratic Republic of Congo. The platform enabled detection of IgM and IgG antibodies via chemiluminescent capture immunoassays using whole blood samples from vaccinated and unvaccinated individuals, achieving high sensitivity and specificity (respectively), for measles IgG (82% and 78%), measles IgM (88% and 89%), rubella IgG (85% and 85%), rubella IgM (81% and 82%). Comparative studies using gold-standard methods showed that DMF reduced turnaround time relative to conventional ELISA, especially in outbreak settings, where samples are collected and often sit in storage for days before analysis due to high volumes. This reinforced DMF viability for real-world epidemiological deployment in settings with

Table 1
DMF platform configurations in biomedical diagnostics.

Detection	Device integration	Matrix/Sample	Analyte/Target	Main Advantages	Ref.
HPLC–MS/MS	Off-chip detection – HPLC–MS/MS analysis after DMF sample preparation	Proteomic samples	Proteins/ proteome	Integrates complete sample preparation with proteomic analysis	[11]
(SWV)	On-chip detection – electrochemical (square-wave voltammetry)	Human serum/ plasma	H5N1 antigen	High electrochemical stability and reduced matrix interference	[5]
Electrochemical impedance spectroscopy (EIS)	On-chip detection – electrochemical impedance spectroscopy	PBMCs/cells	Cellular abundance	Portable cellular monitoring and improved capture efficiency	[19]
Colorimetry	On-chip detection – colorimetric sensor integrated in paper	Whole blood	Li^+ ions	Active plasma separation and rapid analysis without pre-dilution	[36]
Colorimetry (Jaffé reaction)	On-chip detection – colorimetric reaction on paper device	Clinical samples	Creatinine	Reduced manual steps and reagent consumption	[48]
Nucleic acid RPA-CRISPR)	On-chip detection – optical sensing interface integrated in the chip	Pathogen DNA/ RNA	Pathogen genes	Integrates preparation, amplification, and detection on a single chip	[31]
Optical immunoassay	On-chip detection – optical immunoassay measurement	Human serum	Cardiac biomarkers	Compensation for kinetic variations in serum samples	[35]
Luminescence with CCD camera	On-chip reaction with optical detection using CCD camera	Serum	AFP, AFP-L3, DCP	Rapid multiplexed analysis with low sample volume	[32]

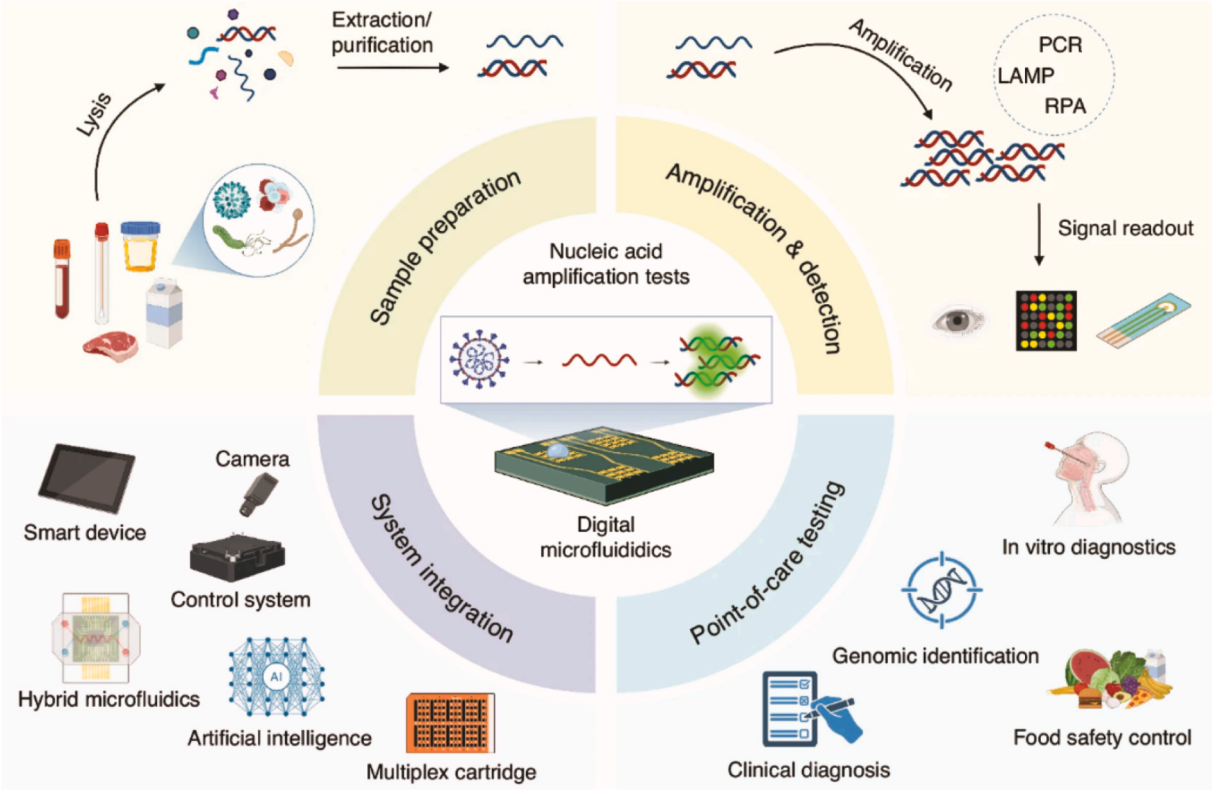

Fig. 7. Overview of nucleic acid amplification tests at the point of care in digital microfluidics. Created at https://BioRender.com. Reproduced with permission from Ref. [53] Copyright 2026 Springer Nature Limited.

limited infrastructure [44].

A similar approach employed a DMF system incorporating polydopamine-coated magnetic surfaces for ELISA-based detection of hepatitis B surface antigen (Fig. 8). Although the LOD achieved (67.1 ng/mL) was higher than that obtained with traditional ELISA (3.75 ng/mL), the method required smaller sample volumes and was qualitatively described as having a faster response time, without specifying the exact analysis time. However, the protocol was proof-of-concept and lacked validation with real samples. Moreover, the reductive nature of polydopamine may induce false positives, requiring further validation before clinical use [56].

In Brazil, a DMF platform integrated with fluorimetric detection was used to evaluate enzymatic activities of four LSDs: α-L-iduronidase (IDUA, MPS I), α-glucosidase (GAA, Pompe), β-glucosidase (GBA, Gaucher), and α-galactosidase (GLA, Fabry) in over 10,000 newborns. The system demonstrated high multiplexing capacity, completing analyses within 4 h and achieving 456 tests/day with 4 fluorimeters. Cut-off values were 5.1 μmol/L/h (IDUA), 5.9 μmol/L/h (GAA), 3.9 μmol/L/h (GBA), and 5.7 μmol/L/h (GLA). All assays exhibited satisfactory linear behavior (correlation coefficients ≥0.98) [57].

A similar study in Campania, Italy, validated the commercial, FDA-approved SEEKER® DMF platform in a pilot program that screened over 7000 newborns between 2022 and 2023. Regional cut-offs were established for IDUA (2.43 μmol/L/h), GAA (4.50 μmol/L/h), GBA (3.20 μmol/L/h), and GLA (3.00 μmol/L/h), and integration with confirmatory tests (e.g., genetic and ELISA) was demonstrated. A low false-positive rate of 0.049% was reported across 6050 DBS samples. Despite strong performance, the study acknowledged the dependency on

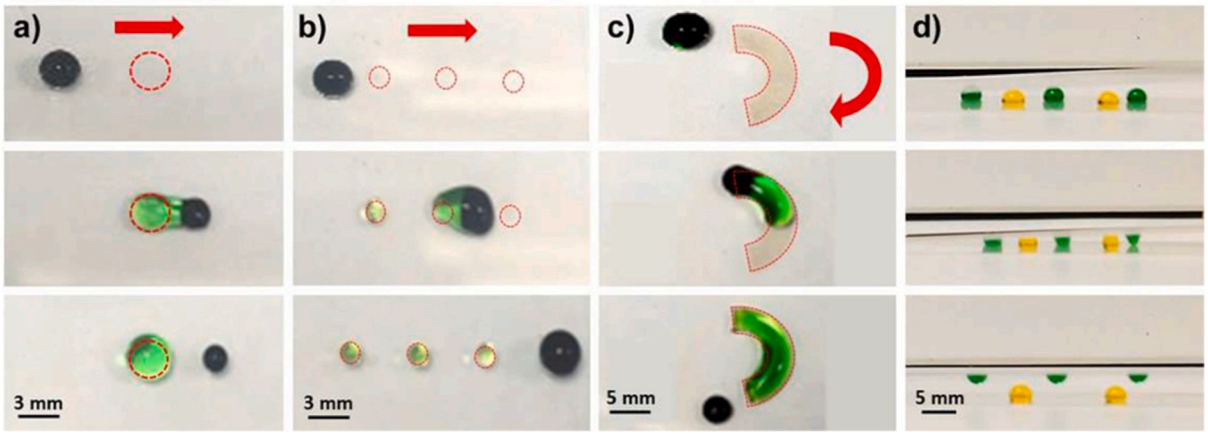

Fig. 8. Droplet manipulation on the polydopamine surface-enabled polydopamine energy trap digital microfluidic magnetic platform in a single-plate configuration. (A) particle extraction, (B) liquid dispensing, (C) liquid shaping, and (D) transfer between platforms. Dash lines on all panels indicate the polydopamine surface energy trap. Red arrows indicate the direction of movement of the permanent magnet. Reproduced with permission from Ref. [56] Copyright 2026 John Wiley & Sons. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

disposable cartridges and highlighted the need for protocol standardization [29].

In another study, a luminescent DMF-EWOD platform with CCD camera detection was developed for early diagnosis of hepatocellular carcinoma (HCC) via three biomarkers: alpha-fetoprotein (AFP), AFP-L3, and des-gamma-carboxy prothrombin (DCP) [32]. The protocol automated AFP-L3 fractionation in 10 min, with a total assay time of 15 min. The protocol allowed multiplexed analysis of 15 targets using only 2.4 μL of serum per marker. The LODs obtained were 0.24 ng/mL (AFP) and 1.89 ng/mL (AFP-L3 and DCP). However, the small sample size (27 serum samples) and lack of statistical comparison with standards limit diagnostic conclusions, although the platform shows strong potential for scale-up.

To illustrate the practical applicability of digital microfluidics in various diagnostic contexts, Table 2 summarizes representative studies, highlighting examples that reinforce the versatility of digital microfluidics platforms.

While DMF applications in diagnostics have expanded outside of the laboratory, their broader adoption remains limited by fabrication challenges and integration with low-cost platforms. Nonetheless, commercial systems such as SEEKER® (used in neonatal screening) and the MR Box (deployed in outbreak settings) exemplify how DMF can indeed support both high-precision laboratory automation and portable diagnostics, underscoring its versatility across diverse clinical scenarios.

The consolidation of DMF as a clinically validated technology adopted on an industrial scale will depend primarily on overcoming manufacturing-related challenges. Advances in the fundamental stages of developing more robust dielectric materials and hydrophobic layers capable of withstanding prolonged operating cycles without degradation, as well as reducing the high operating voltages typical of EWOD systems, which currently increase energy consumption and accelerate electrode wear, are crucial for the popularization of the technique. Furthermore, it will be necessary to advance chip manufacturing standardization and scalability. Another essential point for transforming DMF into complete and reliable analytical platforms is the efficient integration of optical and electrochemical sensors, along with programmable control systems and portable instrumentation, to enable implementation in resource-limited locations. We believe that the transition from the academic environment to clinical and commercial applications will require an interdisciplinary effort involving engineering, materials science, and the medical field. This joint action can facilitate regulatory validation and enable its implementation in routine clinical analysis.

4. Conclusion

Digital microfluidics is establishing itself as a promising platform for analytical automation in clinical diagnostics and point-of-care

Table 2
Representative applications of digital microfluidics (DMF) in biomedical diagnostics.

Disease	Target	Detection method	Sample	LOD	Ref.
Hepatitis B	HbsAg	Colorimetric ELISA (TMB – HRP) in magnetic DMF	HBsAg with Dynabeads	67.1 ng/ml	[56]
Malaria	18S rRNA of *Plasmodium falciparum* parasites	Colorimetric LAMP	*Plasmodium* DNA	Not specified	[42]
Malaria	*Plasmodium falciparum*	Colorimetric RPA with SYBR Green I	Plasmid with LDH gene	15x 365 nm (UV)	[55]
Hepatocellular Carcinoma (Hcc)	AFP, AFP-L3% and DCP	Luminescence by CCD camera	Serum	0.24 ng/ml; 1.89 ng/ml	[32]
LSDS	IDUA, GAA, GBA and GLA	Fluorimetric enzymatic reaction	DBS	IDUA: 5.1 μmol/L/h GAA: 5.9 μmol/L/h GBA: 3.9 μmol/L/h GLA: 5.7 μmol/L/h	[57]
LSDS	IDUA, GAA, GBA and GLA	Fluorescence enzymatic reaction	DBS	IDUA: 2.43 μmol/L/h GAA: 4.50 μmol/L/h GBA: 3.20 μmol/L/h GLA: 3.00 μmol/L/h	[29]
Rubella And Measles	Immunoglobulin M (IgM) and G (IgG)	Immunoenzymatic reaction with chemiluminescence signal output	Fresh whole blood	Sensitivity and specificity reported	[44]

applications. However, its transition from the laboratory to industrial scale faces barriers, including high drive voltages, material degradation, biofouling, and a lack of standardization. In recent years, several strategies have been implemented to mitigate these limitations, including the development of cheaper fabrication methods, integration of detection strategies, replacement of unstable materials such as ITO with printed or paper electrodes, and oil encapsulation to reduce evaporation and contamination. The incorporation of accessible components, such as Raspberry Pi boards, and the integration of optical and electrochemical sensors point to more portable, modular, and scalable solutions. Examples such as μChemLab and hybrid paper-DMF platforms demonstrate compatibility with clinical workflows and robustness in resource-limited environments. Consolidating DMF as an industrial technology will require modularization, reliable automation, and regulatory compliance, supported by an interdisciplinary approach.

CRediT authorship contribution statement

Daniel J.A. dos Santos: Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft. **Danyanne K. da Silva:** Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft. **Larissa G. Velasco:** Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft. **Richard P.S. de Campos:** Investigation, Methodology, Validation, Writing – review & editing. **Wendell K.T. Coltro:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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