



Lab on a smartphone (LOS): A smartphone-integrated, plasmonic-enhanced optoelectrowetting (OEW) platform for on-chip water quality monitoring through LAMP assays

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ABSTRACT

We present a low-cost lab-on-a-smartphone (LOS) platform for rapid and in-situ monitoring of fecal indicator bacteria (FIB) such as *Escherichia coli* (*E. coli*) to examine the presence and concentration levels of fecal contamination in environmental waters. To realize the LOS, three main components, a plasmonic-enhanced optoelectrowetting (OEW) device, a transparent heater, and a smartphone, are compactly assembled for on-chip water quality monitoring through loop-mediated isothermal amplification (LAMP) assays. The LOS integration with the OEW device enables pumpless and tubeless droplet manipulations for on-chip water sample preparation using a plasmonic-enhanced OEW technology. The integration with a transparent heater provides isothermal heating at 65 °C, where low-cost, portable LAMP assays are conveniently implemented on the LOS platform. Finally, a smartphone is used as an integrated optical detector to take digital images of target water samples during the LAMP reaction, while a time-dependent red-green-blue (RGB) analysis is performed for quantitative and accurate colorimetric study using a smartphone's image processing app. Experimentally, we have successfully demonstrated on-chip sample processing of water samples spiked with *E. coli* DNA and LAMP mixtures, and conducted in-situ isothermal LAMP amplifications of *E. coli* DNA, while obtaining the LAMP test results within 30 min, followed by an accurate time-dependent colorimetric assessment. The LOS platform truly offers a low-cost, portable, and fully integrated system for rapid, on-site detection for the presence of FIB and their associated pathogens in environmental water without the need for sophisticated laboratory equipment or skilled personnel.

1. Introduction

A lab-on-a-chip (LOC) refers to a miniaturized device that enables one or multiple laboratory functions such as sample preparation, assay, and detection on a single integrated system [1–3]. By manipulating a small volume of reagents in the range of nano- or pico-liters, LOC devices can provide several benefits, including less reagent consumption, fast response time, effective process control, and low-cost chip fabrication [4–6]. Using LOC techniques, numerous biological and chemical applications have been demonstrated such as single-cell analysis [7–9], polymerase chain reaction (PCR) [10,11], environmental detection [12,13], and clinical diagnostics [14,15]. Despite the benefits and numerous applications, conventional LOC devices typically require other auxiliary

optical and mechanical components such as fluidic flow controllers (e.g., pumps or valves), messy and complex tubes to feed individual reagents, microscope and CCD camera for microscopic analyses, a computer monitor to display the captured images, and so on [16,17]. These additional components make LOC systems bulky, complex, and expensive as well as largely consume both space and power, which truly defeats the main purpose of LOC systems.

To address this issue, our group previously presented a pumpless and tubeless droplet-based LOC platform, called single-sided continuous optoelectrowetting (SCOEW) [18]. With the illumination of optical patterns onto a photoconductive surface, the SCOEW device can optically modify the voltage drop across a dielectric layer to induce an electrowetting effect and manipulate oil-immersed aqueous droplets without the need for bulky

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and complex components such as pumps, valves, microchannels, and tubes. Using dynamic optical modulators, various droplet-based microfluidic functions such as droplet injection, transportation, merging, mixing, spitting, and parallel processing have been experimentally demonstrated [18]. Furthermore, a single-sided and continuous configuration of the SCOEW device could also offer several advantages over conventional electrowetting devices [19–22]. First, a fabrication process of the SCOEW device is relatively simple. It only requires two electrodes connected by a photoconductive layer without any complex electrode patterning and wiring issues often encountered by electrowetting devices. Another advantage is that optical droplet manipulation in an oil medium can fully eliminate cross-contamination issues on a continuously deposited photoconductive surface (i.e., no patterned electrodes). More importantly, a single-sided open chamber configuration of the device provides a flexible interface for easy integration with other microfluidic components like optical detectors and reagent reservoirs, which is very beneficial for LOC applications.

To perform optical droplet manipulation, one critical requirement for previous optoelectrowetting (OEW) devices [18,23–26] was the use of a high-quality photoconductive material like amorphous silicon (a-Si) to provide a high photosensitive property. However, its thin-film layer deposition typically requires complex and expensive laboratory facilities such as chemical vapor deposition (CVD) or plasma enhanced chemical vapor deposition (PECVD), leading to device complexity and cost ineffectiveness. To offer low-cost LOC devices, our group has utilized a polymer-based photoconductive material, titanium oxide phthalocyanine (TiOPc) [27]. With the TiOPc, its thin-film photoconductive layer can be simply fabricated via a low-cost, spin-coating (or drop-casting) method without the need for complex and expensive facilities like CVD and PECVD, while remaining the limitation in its photoconductive performance (i.e., a few orders lower than that of the a-Si). To overcome this limitation, we have further presented plasmonic-enhanced OEW, where plasmonic nanoparticles were used as light scattering elements on top of the TiOPc layer to dramatically enhance its light absorption performance by a few orders, resulting in the photoconductivity improvement comparable with the a-Si [28]. Moreover, our recent study presented a smartphone-integrated optoelectrowetting (SiOEW) device for microscopic detection of water quality [29]. The smartphone was integrated to serve as a low-intensity light source for OEW-driven dropwise sample preparation, as well as to operate as a fluorescent microscope for on-chip quantification of target harmful algae cells in water. To show the device portability for on-site water quality detection, all optical and electrical components, including smartphone, lens module, and voltage source, were compactly assembled and housed into a three-dimensional (3D) printed hand-held platform [30].

With the advantages of device simplicity, cost effectiveness, integrability, and portability, our study herein presents a lab-on-a-smartphone (LOS) platform for rapid and in-situ detection of fecal contamination in environmental water through loop-mediated isothermal amplification (LAMP) assays. The LOS was designed as a fully integrated system, where three main components such as a plasmonic-enhanced OEW device, a transparent heater, and a smartphone are compactly assembled into a low-cost miniaturized platform. Firstly, the OEW device enables light-driven droplet manipulations for on-chip water sample preparation with plasmonic-enhanced OEW performance. Secondly, the single-sided open configuration of the OEW device provides a flexible interface for the easy integration with a transparent heater, thus equipping the LOS with portable LAMP testing capability for DNA amplification. Lastly, an integrated smartphone works as a low-intensity light source to provide optical patterns onto the photoconductive surface of the OEW device for on-chip sample preparation. At the same time, the smartphone functions as an optical detector to allow real-time colorimetric analysis of the LAMP tests using its built-in digital camera and image processing app. We have experimentally demonstrated these LOS's capabilities for (1) on-chip *E. coli* sample processing using the OEW-based device operation, (2) portable LAMP assay of the *E. coli* samples at an isothermal heating condition of

65 °C using an integrated transparent heater, and (3) a real-time colorimetric analysis of the *E. coli* samples through the entire duration of the LAMP assay using a smartphone's digital camera. The fully integrated LOS platform can provide the potential for in-situ microbiological monitoring of environmental water by delivering rapid and reliable test results even without the need for bulky and sophisticated laboratory equipment or skilled personnel. In addition, using the smartphone's capabilities for wireless communications and global positioning system (GPS), the LAMP test information (e.g., the location and the time the test was conducted, the assay results, etc.) can be rapidly shared with a central host such as an environmental regulation agency for real-time monitoring and further management of water quality.

2. An overview of the LOS platform for on-chip detection of fecal contamination in water via LAMP assays

Rapid and in-situ monitoring of waterborne pathogens is important to protect public health from the danger of fecal contamination in water. Due to the cost and difficulty of counting diverse types of pathogens, fecal indicator bacteria (FIB) such as *Escherichia coli* (*E. coli*) and *Enterococcus faecalis* (*E. faecalis*) have been commonly used as proxies to examine the presence and concentration of fecal contamination and its associated pathogens. For microbial detection of FIB, DNA-based techniques such as polymerase chain reaction (PCR) have been typically implemented, however, being confined to advanced laboratory settings with expensive equipment and skilled personnel [31–34]. In recent years, loop-mediated isothermal amplification (LAMP) has been increasingly used as an alternative to PCR-based methods with its benefits of simplicity, cost-effectiveness, high sensitivity, and rapid response [35–38]. In contrast to PCR methods, LAMP reactions can be conducted under an isothermal condition and provide rapid DNA detection without the requirement for expensive equipment (e.g., thermal cyclers), which is useful in resource-limited settings where conventional PCR methods may be impractical [39–42]. Furthermore, the results of LAMP assays can also be easily identified by the naked eye or by adding DNA-intercalating dyes (i.e., skilled personnel is not required) [43,44].

With the benefits of the LAMP approach, we have developed a lab-on-a-smartphone (LOS) platform for on-site and real-time monitoring of fecal contamination in environmental water. The LOS was designed as a fully integrated platform that can conveniently implement on-chip water sample processing and molecular detection of FIB to provide swift response and quick analysis in the event of fecal contamination via LAMP assays. As illustrated in Fig. 1, the LOS mainly consists of three components, a plasmonic-enhanced OEW device, a transparent heater, and a smartphone, which are compactly assembled. The LOS integration with the plasmonic-enhanced OEW device allows on-chip water sample processing on the light-patterned photoconductive surface without the need for bulky and complex microfluidic components like pumps, tubes, and microchannel structures [18,28,29]. Using a plasmonic-enhanced OEW mechanism, various droplet-based manipulation functions such as droplet transportation, merging, mixing, and parallel processing can be automated for water sample preparation on the LOS platform. A transparent heater adheres to the bottom surface of the OEW device. The integration with a transparent heater enables on-chip isothermal nucleic acid amplification at 65 °C for in-situ analysis of water quality via LAMP assays, which can be conveniently carried out on the LOS platform without bulky and expensive equipment. An integrated smartphone can also provide various features to replace auxiliary equipment and components commonly used for a conventional LOC setup. For instance, a smartphone works as a low-intensity, portable light source to provide dynamic light patterns onto a plasmonic-enhanced photoconductive surface of the OEW device through a transparent heater for effective optical manipulation of oil-immersed water samples. Furthermore, the single-sided open-chamber configuration of the LOS allows easy integration with an optical detector. In our study, a smartphone's built-in camera is directly used to capture digital images of target water

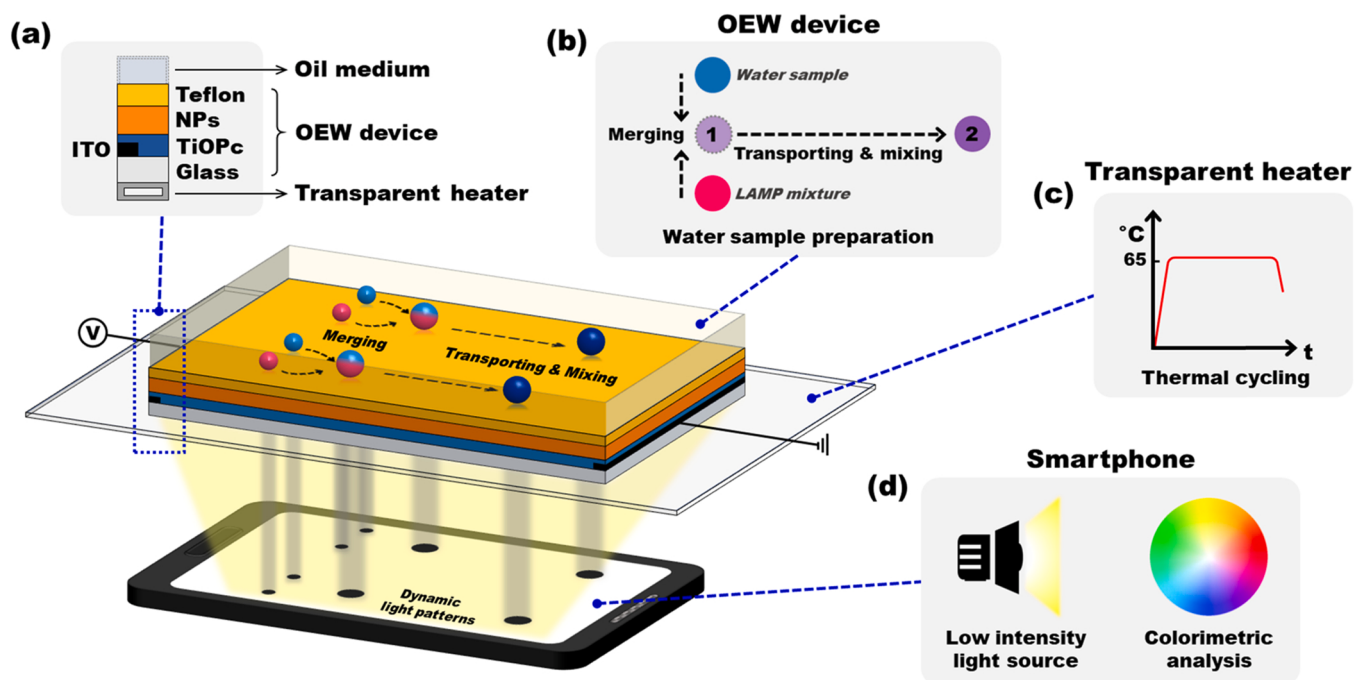


Fig. 1. Schematic illustrations of the LOS platform for on-site detection of water quality through portable LAMP testing. (a) and (b) A LOS platform mainly consists of 3 components, plasmonic-enhanced OEW, transparent heater, and smartphone. The OEW device is composed of the patterned ITO substrate coated with a 1.0 μm thick TiOPc photoconductive layer. To enhance the OEW performance of the device, a 1.8 μm thick layer of plasmonic nanoparticles (NPs) is deposited on the TiOPc layer, after which being covered with a 1.0 μm thick Teflon layer to provide a hydrophobic and dielectric property. A single-sided open configuration of the device easily allows to house water sample droplets in an oil medium. Next, a smartphone is integrated below the OEW device to serve as a low-intensity, portable light source to perform droplet-based microfluidic operations such as droplet merging, mixing, and transporting for on-chip water sample preparation. (c) A transparent heater allows light patterns from the smartphone to pass through itself and reach the OEW device for light-driven droplet manipulations. Upon completion of the water sample preparation process, the transparent heater is switched on to perform an isothermal nucleic acid amplification for LAMP assays. (d) A smartphone allows dual functions of acting as a low-intensity light source for on-chip droplet manipulation as well as serving as a portable biosensor for colorimetric analysis during the LAMP reaction. The LOS platform was implemented for on-chip water sample processing and rapid assessment of the presence of *E. coli* in environmental water samples via LAMP assays.

samples and conduct a real-time colorimetric assessment of the LAMP assay results. A smartphone's image processing app can be further used for quantitative analysis of LAMP-based time-dependent color change of the target samples by counting their red-green-blue (RGB) values. All these components are compactly housed together to form the portable LOS platform. Each component of the LOS will be detailed in the following sections.

3. Descriptions of the LOS components

3.1. Device fabrication and its working principle

Fig. 1(a) shows a schematic of the plasmonic-enhanced OEW device that enables optical manipulation of oil-immersed droplets for on-chip water sample processing. Device fabrication begins with patterning of two indium tin oxide (ITO) electrodes at the edges of a glass substrate with a gap of 40 mm. To offer low-cost and simple device fabrication, a current study has utilized a polymer-based TiOPc photoconductive material, as reported in our previous study [27]. For TiOPc layer deposition, titanyl phthalocyanine powder (Sigma-Aldrich, USA) was dissolved in a chlorobenzene solvent (1.0 wt%) at 80 $^{\circ}\text{C}$ for 2 h. Then, the solution was spin-coated at 100 rpm on top of the electrode layer for 30 s, before curing at room temperature for another 2 h to obtain a 1.0 μm TiOPc layer. To allow plasmonic-enhanced OEW performance, a layer of aluminum (Al) nanoparticles was subsequently provided on top of the TiOPc layer [28]. These nanoparticles serve as plasmonic light scattering elements to effectively increase the optical path length of incoming light rays over a wide angular spread and redirect them back onto the TiOPc layer. As a result, light absorption of the TiOPc can be

significantly enhanced to provide its high photo-state conductivity, leading to an enhanced OEW performance. For the preparation of a nanoparticle solution, 2.0 wt% of Al nanoparticles (sizing ~ 50 nm) were first dissolved in a 6% Teflon solution (AF 1601S, DuPont) and then diluted to 3% with Fluorinert Liquid (FC-72, 3 M) for a better dispersion of nanoparticles. After a spin-coating step (at 1000 rpm for 90 s), followed by curing at 110 $^{\circ}\text{C}$ for 2 h, a 1.8 μm thick nanoparticle layer was obtained. Next, to provide hydrophobic and dielectric properties, a 1.0 μm thick Teflon layer (AF 1601S, DuPont) was also spun-coated (at 800 rpm for 30 s) above and cured at 110 $^{\circ}\text{C}$ for another 2 h. Lastly, droplets of water samples and LAMP reagents were placed gently on top of the Teflon layer filled with mineral oil (Sigma-Aldrich, USA). Not only does this oil-immersed dropwise microfluidic platform prevent water samples and LAMP reagents from evaporation during a LAMP-based isothermal amplification process, but also isolates each of the reagent samples into an oil-immersed droplet to minimize cross-contamination issues [35,45,46].

A droplet actuation principle on the OEW device has been well presented in our previous works [18,27–29]. In brief, the droplet's contact angle change can be modified by illuminating a specific light pattern onto a photoconductive layer of the device. This OEW phenomenon can be explained by the Young-Lippmann equation [47]:

$$\cos \theta - \cos \theta_0 = \frac{c}{2\gamma} (\Delta V)^2 \quad (1)$$

where c represents the specific capacitance, γ is the surface tension between the droplet and its surrounding medium, and θ_0 indicates the droplet's initial contact angle under uniform light illumination onto the device. The droplet's contact angle change (i.e., $\cos \theta - \cos \theta_0$) can be

determined by optical modulation of the voltage drop (ΔV) across a dielectric layer. In this study, a Teflon layer works as a dielectric layer coated on the nanoparticle layer (see Fig. 1a).

To achieve droplet actuation, a direct current (DC) bias voltage is applied between the two ITO electrodes positioned at the edges of the device, which creates a uniform electric field along a lateral direction. This forms a shunt circuit, where the photoconductive (TiOPc) and dielectric (Teflon) layers are modelled as a series connection of photo-resistors and parallel connection of capacitors, respectively [18]. Initially, when a uniform light pattern illuminates onto a photoconductive layer of the device, the voltage drops across the dielectric capacitors at both left and right sides of the droplet are equal ($\Delta V_L = \Delta V_R$) such that it is symmetrical in shape with the same contact angles at both edges of the droplet, according to the Young-Lippmann Eq. (1). This is due to the photo-resistance of the TiOPc layer constantly remained across the device, resulting in no droplet movement with zero net force around the droplet. However, when a dark pattern illuminates onto one edge of the droplet, the photo-resistance of the TiOPc layer increases only at the dark illuminated region where the voltage drop across the dielectric layer becomes enlarged ($\Delta V_L \neq \Delta V_R$). Correspondingly, the droplet contact angle decreases only at the area where a dark pattern illuminates to form the advancing angle (θ_{adv}), while another side of the droplet forms the receding angle (θ_{rec}). This asymmetric shape of the droplet causes the pressure gradient inside and gives rise to its actuation in the direction of the dark illuminated region, achieving OEW-based droplet manipulation. To further improve OEW performance of the device, our previous study has utilized Al nanoparticles as light scattering elements to induce plasmonic field enhancement, where light absorption of the TiOPc layer can be significantly improved by a few orders [28]. As a result, the conductivity switching performance ($b = \sigma_{photo} / \sigma_{dark}$) between the photo and dark states of the TiOPc was considerably enhanced, leading to larger contact angle change of the droplet to generate stronger OEW forces for effective droplet manipulation. Such plasmonic-enhanced OEW performance allows to provide large enough OEW forces even using a smartphone as a low-intensity light source.

By integrating with the plasmonic-enhanced OEW device, the LOS platform enables on-chip sample processing without any bulky and complex mechanical components like pumps, tubes, and microchannels. Furthermore, the LOS platform can be advantageously offered for droplet-based microfluidic sample preparation by eliminating complex

wiring, patterning, and interconnection issues of the electrodes typically encountered by conventional electrowetting and OEW devices [19,23,25,26,48].

3.2. Transparent heater for on-chip LAMP assays

For on-chip LAMP assays, the LOS platform was equipped with a transparent ITO heater below the OEW device, as shown in Fig. 1(a). One interesting feature of the heater is its transparency that allows optical patterns projected from the smartphone to pass through itself and reach the TiOPc surface of the device to induce OEW forces. The transparent heater has an $80 \times 50 \text{ mm}^2$ size as a heating element (Perm Top Co. Ltd., Taiwan) that was connected to a microcontroller (Arduino Nano) through a relay module (JQC-3FF-S-Z, Tongling, China), as shown in Fig. 2(a). By supplying a power of 0.087 W/cm^2 and using a programmed algorithm with the Arduino software, the heater was controlled to switch on and off alternatively to provide isothermal heating necessary for LAMP assays of water samples mixed LAMP reagents. For on-chip temperature monitoring, a Type K thermocouple (Labfacility, UK) was used to measure the temperature of the oil chamber at every 0.25 s. The measured temperature and corresponding time were instantly displayed on an organic light-emitting diode (OLED) display module (SSD1306, Generic, China). This temperature monitoring process has been repeated at several different locations of the oil chamber. Since the heater is relatively larger than the oil chamber, the uniform temperature profile has been obtained in a whole area of the oil chamber. Fig. 2(b) shows a temperature profile at $65.0 \pm 0.25^\circ\text{C}$ measured at a center location of the oil chamber where water sample mixtures were mainly positioned and underwent isothermal amplification over the entire duration of a LAMP reaction. The LOS integration with a transparent heater performs on-chip, portable LAMP assays by providing isothermal heating at 65°C without the need of bulky and expensive equipment such as thermal cyclers. Furthermore, LAMP assays conducted on the LOS only require a small volume of reaction samples (less than $5 \mu\text{L}$), which is significantly lesser than typical tube-based LAMP tests (total reaction volume of $25\text{--}50 \mu\text{L}$) [49–51]. Thus, the LOS platform allows cost-effective LAMP testing for rapid and on-site water quality detection.

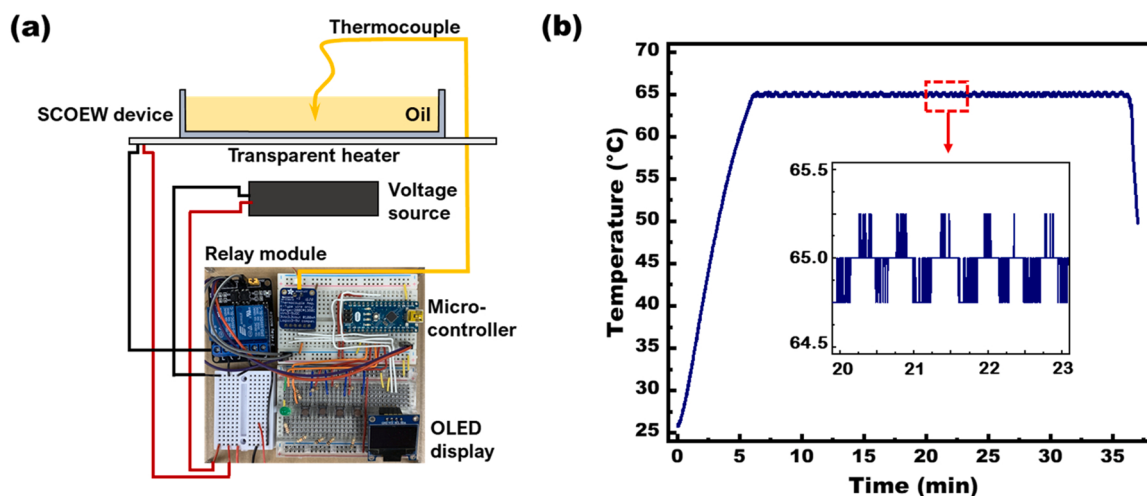


Fig. 2. Integration of a transparent heater for portable LAMP assays and its temperature profile. (a) On-chip LAMP assays are enabled by integrating a transparent heater connected to a microcontroller. A Type K thermocouple was used to periodically monitor the temperature of the oil chamber and the temperature and time are displayed instantly on an OLED display module. Using the Arduino software, the transparent heater was alternatively switched on and off when the temperature of the oil chamber exceeded the predetermined upper and lower limits ($64.75^\circ\text{C} < t < 65.25^\circ\text{C}$). (b) The temperature profile in the oil medium of the SCOEW device measured by the thermocouple over the entire duration of a LAMP reaction. The heater-integrated LOS platform can provide the acceptably constant temperature at $T = 65.0^\circ\text{C}$ for stable LAMP assays.

3.3. Integrated smartphone

The LOS platform is completed with the integration of a smartphone, which has already been equipped with various features to substitute auxiliary equipment and components typically found in conventional LOC systems. Particularly, previous OEW studies [18,23–27] have required a collimated laser or an optical projector as a light source to induce large enough OEW forces. However, the operation of these optical components presented additional complexity and bulkiness to the overall LOC system. To achieve compactly assembled LOC systems, our study has used a commercially available smartphone (VKY-L29, Huawei) as a low-intensity, portable light source to induce OEW forces for droplet-based operations on the OEW device. The smartphone display projects programmed optical patterns onto the photoconductive surface of the device for dynamic manipulation of individual droplets with various droplet-based microfluidic operations (e.g., merging, mixing, and transportation) for on-chip sample preparation (Fig. 1a and b). Another feature of the smartphone used for this study is its built-in digital camera and CMOS sensor, which were used as an integrated optical detector. During an isothermal amplification process, droplets of water samples mixed with LAMP reagents undergo their color changes. To conduct a colorimetric assessment of the LAMP assay results, a smartphone's built-in camera was directly used to capture digital images of target water sample droplets throughout the entire duration of the LAMP reaction (Fig. 1d). Thereafter, a smartphone's image processing app carried out a time-dependent colorimetric analysis of the target water samples for an accurate evaluation of LAMP tests by counting their red-green-blue (RGB) values.

4. Experimental demonstrations

An experimental study has been conducted with water samples spiked with *E. coli* DNA and commercially available LAMP reagents to demonstrate the LOS capabilities for on-chip sample processing and rapid assessment of fecal contamination in water. To prepare environmental water samples spiked with *E. coli* DNA, a single colony of *E. coli* was first purchased from Sigma-Aldrich, USA, and inoculated on a Luria-Bertani (LB) agar plate with 100 mL of LB broth, which was also purchased from Sigma-Aldrich, USA. It was subsequently incubated at 37 °C and simultaneously shaken at 200 rpm. Thereafter, approximately 10⁷ CFU/mL of pure culture of *E. coli* was spiked into sterilized environmental water samples that were collected from freshwater reservoirs in Singapore. Then, *E. coli* DNA from these freshwater samples were extracted using a commercial DNA extraction kit (DNeasy Blood & Tissue Kit) procured from QIAGEN Singapore. Next, LAMP reagents were also purchased, including WarmStart® Colorimetric LAMP 2× Master Mix (M1800) from New England Biolabs and 10× LAMP Primer Mix from Integrated DNA Technologies, Singapore. The LAMP Primer Mix includes 6 LAMP primers (DNA sequences listed in Table 1): 1.6 μM forward inner primer and backward inner primer (FIP and BIP), 0.2 μM forward outer primer and backward outer primer (F3 and B3), 0.4 μM of two loop primers (LoopF and LoopB). In accordance with the manufacturer's instructions, a LAMP mixture was prepared with sterile deionized water. For the experimental test, the LOS platform was developed by compactly assembling three main components, as presented in

Table 1
Primer sequences used in this study.

Primer	Primer Sequence (5'–3')
FIP	TAACGCGCTTTCCACCAACGGCCTGTGGGCATTTCAGTC
BIP	TAACGATCAGTTCCGCGATGCACTGCCCCAACCTTTCGGTAT
F3	CKGTAGAAACCCCAACCCG
B3	AWACGACGACGATACGC
LoopF	TCCACAGTTTTCGCGATCCA
LoopB	ACGTCGTGTATCAGCGCGAAGT

Fig. 1. Using the light-driven OEW operation, the LOS can carry out droplet manipulation functions to perform on-chip sample processing by addressing dynamic light patterns projected from a smartphone. LAMP assays can then be implemented on the *E. coli* samples combined with LAMP reagents at an isothermal temperature of 65 °C for a 30 min duration. Finally, the smartphone was used for the colorimetric assessment of the LAMP assay results. Experimental demonstrations of each of the processes will be detailed in the following sections.

4.1. On-chip *E. coli* sample processing

To demonstrate the LOS's capability for on-chip sample processing, four droplets of water samples and the LAMP mixtures were prepared on the OEW's surface immersed in an oil medium without any interference to prevent premature mixing of the droplets. Fig. 3(a) shows a 1.0 μL freshwater droplet containing *E. coli* DNA and another droplet comprising of 1.5 μL LAMP mixture placed on the LOS for the implementation of the LAMP assays. As a comparative study, another set of two droplets, including 1.0 μL deionized water with no *E. coli* DNA and 1.5 μL LAMP mixture, was prepared for negative control. A bias voltage was applied to the plasmonic-enhanced OEW device at 100 V/mm. With the illumination of the optical patterns from the smartphone, the OEW forces were generated on the device surface to individually manipulate the droplets of water samples and LAMP mixtures for mixing. The droplets were first brought closely together and underwent electrocoalescence resulted from the dipole-dipole interaction between the droplets. Next, they were merged to form 2 sets of the combined droplets. Fig. 3(b) shows the 2 sets of the combined droplets after the electrocoalescence, but before mixing, where a visible divide between the left half (clear) and right half (dark-colored) of both droplets can be observed. Then, these 2 combined droplets were driven back and forth by the optical patterns' movement (Fig. 3c) at an average speed of 1.21 mm/s. During this transportation process, the shear forces from interactions with the bottom surface effectively induced an internal flow inside each of the droplets to enhance mixing of their internal contents (i.e., *E. coli* DNA and LAMP mixture, negative control and LAMP mixture). It is noted that our previous works have experimentally demonstrated this light-induced droplet mixing process on the OEW device [18,29,30]. Fig. 3(d) shows the 2 sets of uniformly dark-colored droplets after completing the mixing process.

4.2. LAMP assays on the portable LOS platform

After the completion of the mixing process of the water samples with LAMP reagents on the LOS platform, an isothermal heating process began for on-chip and rapid detection of fecal contamination in water via LAMP assays. Fig. 4 shows the digital images of the LAMP reaction droplets taken by a smartphone camera without the need for additional bulky optical components. While operating the transparent heater to provide isothermal heating at 65.0 °C, the designated LAMP primers in the mixture target the region of the β-D-glucuronidase (*uidA*) genes in *E. coli* DNA [35,52]. This LAMP reaction successfully completes the amplification of the target DNA. As a result, the color of the water droplet containing *E. coli* DNA changed from dark pink to yellow to indicate the presence of *E. coli* bacteria in the water sample (Fig. 4a). For negative control, on the other hand, the LAMP reaction mixture retains its original dark pink color when the water sample contains no traces of *E. coli* DNA (Fig. 4b). The LAMP assay could be conveniently operated on the LOS platform without the need for bulky and complex equipment such as thermal cyclers. In addition, the LAMP test result could be observed with the naked eye within 30 min, which is suitably applicable for in-situ monitoring of fecal contamination in water.

4.3. Smartphone-based colorimetric analysis

For the further quantitative and accurate colorimetric study, both the

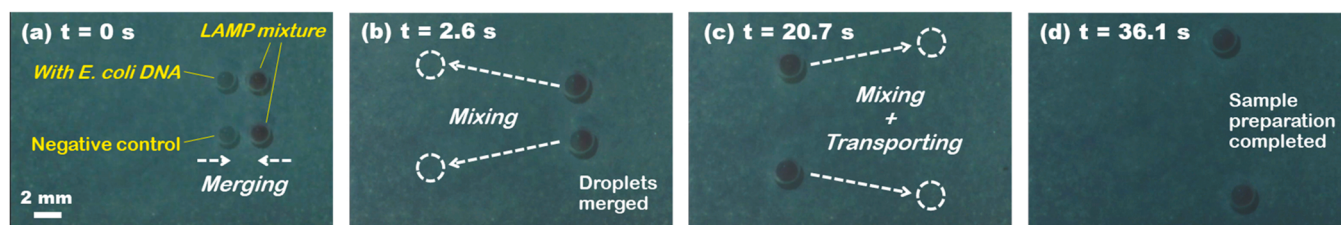


Fig. 3. Video snapshots of on-chip *E. coli* sample processing. (a) Two sets of the droplets (1.0 μL freshwater spiked with culture of *E. coli* and 1.5 μL LAMP mixture, 1.0 μL deionized water and 1.5 μL LAMP mixture for negative control) are prepared on the OEW device surface surrounded by an oil medium. (b) While projecting dynamic optical patterns from the smartphone, these droplets come closely and merged after experiencing electrocoalescence due to their dipole-dipole interaction. (b) and (c) As the optical patterns continue to be projected across the device, the OEW forces generated on the droplets caused them to move back and forth with the optical patterns. This process enhanced the droplets to mix their internal contents due to the internal flow within the droplet caused by shear forces from the bottom surface. (d) The water sample droplets were thoroughly mixed and transported back to the center of the SCOEW device, where LAMP assays can commence. A scale bar indicates 2 mm.

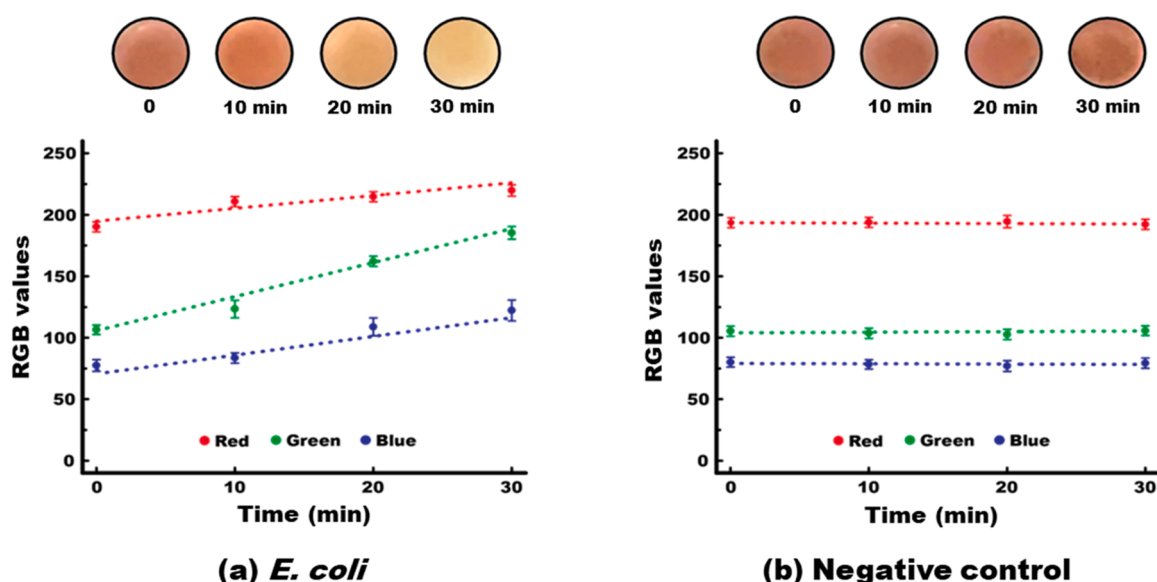


Fig. 4. Time-dependent RGB analysis of LAMP assay results by smartphone-based colorimetric detection. By analyzing the digital images taken by a smartphone camera at 10 min intervals throughout the LAMP reaction, (a) the color of the LAMP reaction mixture containing traces of *E. coli* DNA gradually changed from dark pink to yellow due to the successful amplification of the target DNA sequence, indicating a positive LAMP assay. Through colorimetric analysis, the RGB values before and after LAMP reaction are plotted in the graph, validating an apparent difference in its RGB values. (b) On the other hand, the LAMP reaction mixture containing no traces of *E. coli* DNA (negative control) has no visible color change and retained its dark pink color. This is verified by the RGB graph showing unchanged RGB values through the 30 min duration of the LAMP reaction. The above smartphone-based colorimetric tests were repeated, and the color change results were consistently observed over three times in trials.

smartphone's built-in digital camera and its image processing app were used. The digital images of the LAMP reaction droplets were taken at 10 min intervals for the entire 30 min duration of the LAMP reaction. In Fig. 4(a), the color of the droplet containing traces of *E. coli* DNA changed visibly from dark pink to yellow after the LAMP reaction, indicating a successful DNA amplification. This colorimetric observation was quantitatively validated by a time dependent RGB analysis of the droplet during this LAMP reaction using the smartphone app, Colorimeter. An apparent difference in its RGB values is shown in the graph of Fig. 4(a). On the other hand, the digital images taken for the droplet containing no traces of *E. coli* DNA (negative control) present no color change and retain its original dark pink color (Fig. 4b). Similarly, this colorimetric observation was compared with the droplet's RGB values through the 30 min duration of the LAMP reaction. The graph in Fig. 4 (b) verifies that the droplet's RGB values remain almost unchanged. The above color change results were consistently observed over three times in trials, demonstrating the portability and convenience of performing LAMP assays on the LOS with reliable results and fast analysis.

5. Conclusion

With the ubiquity and vast capabilities of a smartphone, we presented a lab-on-a-smartphone (LOS) platform for rapid and on-site monitoring of fecal indicator bacteria (i.e., *E. coli*) through portable LAMP testing. The LOS platform featuring a smartphone-integrated and plasmonic-enhanced OEW device has been developed as an effective droplet actuation approach for on-chip water sample preparations. In addition, the single-sided open configuration of the plasmonic-enhanced OEW device provides a flexible interface for the easy integration with a transparent heater, thus equipping the LOS with portable LAMP testing capability. With the LOS platform, we have successfully performed on-chip *E. coli* sample preparation by optically manipulating droplets of water samples and LAMP mixture on the plasmonic-enhanced OEW device. In addition, on-chip LAMP assays have been experimentally demonstrated by integrating a transparent heater that can provide isothermal heating at 65.0 $^{\circ}\text{C}$ for the LAMP reaction of *E. coli* DNA on the LOS platform. The water sample containing traces of *E. coli* DNA was successfully amplified, where a visible color change can be observed

with a naked eye. Finally, the integrated smartphone was used to verify this colorimetric assessment of the LAMP assay results by using its built-in camera to take digital images of the water droplets at 10 min intervals for the 30 min duration of the LAMP reaction. Subsequently, using the smartphone's image processing app, the digital images were quantitatively validated by a time-dependent analysis of their RGB values. With the capability of performing LAMP assays conveniently with reliable results and rapid analyses, the LOS platform truly offers a low-cost, portable, and fully integrated system for rapid on-site monitoring of FIB without the need for sophisticated laboratory equipment or skilled personnel.

CRedit authorship contribution statement

Si Kuan Thio: Investigation, Formal analysis, Validation, Data curation, Writing – original draft. **Sung Woo Bae:** Validation, Writing – review & editing, Funding acquisition, Supervision. **Sung-Yong Park:** Conceptualization, Methodology, Writing – review & editing, Funding acquisition, Project administration, Supervision.

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Declaration of Competing Interest

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

References

- [1] R. Daw, J. Finkelstein, Lab on a chip, *Nature* 442 (2006) 367, <https://doi.org/10.1038/442367a>.
- [2] J.K.S. Tan, S.Y. Park, H.L. Leo, S. Kim, Continuous separation of white blood cells from whole blood using viscoelastic effects, *IEEE Trans. Biomed. Circuits Syst.* PP (99) (2017) 1–7.
- [3] S.-Y. Park, T.-H. Wu, Y. Chen, M.A. Teitell, P.-Y. Chiou, High-speed droplet generation on demand driven by pulse laser-induced cavitation, *Lab Chip* 11 (2011) 1010–1012.
- [4] A.-N. Cho, et al., Microfluidic device with brain extracellular matrix promotes structural and functional maturation of human brain organoids, *Nat. Commun.* 12 (1) (2021) 4730.
- [5] B. Namgung, et al., Vibration motor-integrated low-cost, miniaturized system for rapid quantification of red blood cell aggregation, *Lab Chip* 20 (21) (2020) 3930–3937.
- [6] S.-Y. Park, P.-Y. Chiou, Light-driven droplet manipulation technologies for Lab-on-a-Chip applications, *Adv. Optoelectron.* 2011 (2011), 909174, <https://doi.org/10.1155/2011/909174>.
- [7] K. Liu, et al., Generation of disk-like hydrogel beads for cell encapsulation and manipulation using a droplet-based microfluidic device, *Microfluid. Nanofluidics* 13 (5) (2012) 761–767.
- [8] W. Shi, J. Qin, N. Ye, B. Lin, Droplet-based microfluidic system for individual *Caenorhabditis elegans* assay, *Lab Chip* 8 (9) (2008) 1432–1435.
- [9] S.-Y. Park, T.-H. Wu, Y. Chen, S. Nisperos, J.F. Zhong, E.P.-Y. Chiou, A pulse laser-driven microfluidic device for ultra-fast droplet generation on demand and single-cells encapsulation, in: *Proceeding of the 14th International Conference on Miniaturized Systems for Chemistry and Life Sciences (μTAS 2010)*, Groningen, Netherlands, 2010.
- [10] Z. Guttenberg, et al., Planar chip device for PCR and hybridization with surface acoustic wave pump, *Lab Chip* 5 (3) (2005) 308–317.
- [11] Y.-H. Chang, G.-B. Lee, F.-C. Huang, Y.-Y. Chen, J.-L. Lin, Integrated polymerase chain reaction chips utilizing digital microfluidics, *Biomed. Microdevices* 8 (3) (2006) 215–225.
- [12] A. Golberg, et al., Cloud-enabled microscopy and droplet microfluidic platform for specific detection of *Escherichia coli* in water, *PLoS One* 9 (1) (2014), e86341.
- [13] D.M. Tourlousse, F. Ahmad, R.D. Stedfeld, G. Seyrig, J.M. Tiedje, S.A. Hashsham, A polymer microfluidic chip for quantitative detection of multiple water- and foodborne pathogens using real-time fluorogenic loop-mediated isothermal amplification, *Biomed. Microdevices* 14 (4) (2012) 769–778.
- [14] C.H. Ahn, et al., Disposable smart lab on a chip for point-of-care clinical diagnostics, *Proc. IEEE* 92 (1) (2004) 154–173.
- [15] V. Srinivasan, V.K. Pamula, R.B. Fair, An integrated digital microfluidic lab-on-a-chip for clinical diagnostics on human physiological fluids, *Lab Chip* 4 (4) (2004) 310–315.
- [16] T.-H. Wu, et al., Pulsed laser triggered high speed microfluidic fluorescence activated cell sorter, *Lab Chip* 12 (7) (2012) 1378–1383.
- [17] J.F. Zhong, et al., A microfluidic processor for gene expression profiling of single human embryonic stem cells, *Lab Chip* 8 (2008) 68–74.
- [18] S.-Y. Park, M.A. Teitell, E.P.-Y. Chiou, Single-sided continuous optoelectrowetting (SCOEW) for droplet manipulation with light patterns, *Lab Chip* 10 (2010) 1655–1661.
- [19] S.-K. Fan, H. Yang, W. Hsu, Droplet-on-a-wristband: chip-to-chip digital microfluidic interfaces between replaceable and flexible electrowetting modules, *Lab Chip* 11 (2011) 343–347.
- [20] Y.-Y. Lin, E.R.F. Welch, R.B. Fair, Low voltage picoliter droplet manipulation utilizing electrowetting-on-dielectric platforms, *Sens. Actuators B: Chem.* 173 (2012) 338–345.
- [21] A.R. Wheeler, Putting electrowetting to work, *Science* 322 (2008) 539–540.
- [22] S.-Y. Park, Y. Nam, Single-sided digital microfluidic (SDMF) devices for effective coolant delivery and enhanced two-phase cooling, *Micromachines* 8 (1) (2017) 3.
- [23] H.-S. Chuang, A. Kumar, S.T. Wereley, Open optoelectrowetting droplet actuation, *Appl. Phys. Lett.* 93 (2008), 064104.
- [24] P.-Y. Chiou, S.-Y. Park, M.C. Wu, Continuous optoelectrowetting for picoliter droplet manipulation, *Appl. Phys. Lett.* 93 (22) (2008), 221110.
- [25] P.-Y. Chiou, Z. Chang, M.C. Wu, Droplet manipulation with light on optoelectrowetting device, *J. Microelectromech. Syst.* 17 (2008) 133–138.
- [26] P.-Y. Chiou, H. Moon, H. Toshiyoshi, C.-J. Kim, M.C. Wu, Light actuation of liquid by optoelectrowetting, *Sens. Actuators A: Phys.* 104 (3) (2003) 222–228.
- [27] D. Jiang, S.-Y. Park, Light-driven 3D droplet manipulation on flexible optoelectrowetting devices fabricated by a simple spin-coating method, *Lab Chip* 16 (2016) 1831–1839.
- [28] S.K. Thio, S.W. Bae, S.-Y. Park, Plasmonic nanoparticle-enhanced optoelectrowetting (OE) for effective light-driven droplet manipulation, *Sens. Actuators B: Chem.* 308 (2020), 127704.
- [29] D. Jiang, S. Lee, S.W. Bae, S.-Y. Park, Smartphone integrated optoelectrowetting (SiOE) for on-chip sample processing and microscopic detection of water quality, *Lab Chip* 18 (2018) 532–539.
- [30] S. Lee, S. Thio, S.-Y. Park, S. Bae, An automated 3D-printed smartphone platform integrated with optoelectrowetting (OE) microfluidic chip for on-chip monitoring of viable algae in water, *Harmful Algae* 88 (2019), 101638.
- [31] S. Bae, S. Wuertz, Rapid decay of host-specific fecal *Bacteroides* cells in seawater as measured by quantitative PCR with propidium monoazide, *Water Res.* 43 (19) (2009) 4850–4859.
- [32] V.J. Harwood, C. Staley, B.D. Badgley, K. Borges, A. Korajkic, Microbial source tracking markers for detection of fecal contamination in environmental waters: relationships between pathogens and human health outcomes, *FEMS Microbiol. Rev.* 38 (1) (2014) 1–40.
- [33] S. Siefing, M. Varma, E. Atikovic, L. Wymer, R. Haugland, Improved real-time PCR assays for the detection of fecal indicator bacteria in surface waters with different instrument and reagent systems, *J. Water Health* 6 (2) (2008) 225–237.
- [34] E.C. Chern, K.P. Brenner, L. Wymer, R.A. Haugland, Comparison of fecal indicator bacteria densities in marine recreational waters by qPCR, *Water Qual. Expo. Health* 1 (3–4) (2009) 203–214.
- [35] S. Lee, V.S.L. Khoo, C.A.D. Mediano, T. Lee, S.-Y. Park, S. Bae, Rapid and in-situ detection of fecal indicator bacteria in water using simple DNA extraction and portable loop-mediated isothermal amplification (LAMP) PCR methods, *Water Res.* 160 (2019) 371–379.
- [36] T. Notomi, Y. Mori, N. Tomita, H. Kanda, Loop-mediated isothermal amplification (LAMP): principle, features, and future prospects, *J. Microbiol.* 53 (1) (2015) 1–5.
- [37] R. Martzy, et al., A loop-mediated isothermal amplification (LAMP) assay for the rapid detection of *Enterococcus* spp. in water, *Water Res.* 122 (2017) 62–69.
- [38] N.A. Tanner, T.C. Evans Jr., Loop-mediated isothermal amplification for detection of nucleic acids, *Curr. Protoc. Mol. Biol.* 105 (1) (2014), 15.14. 1–15.14. 14.
- [39] J. Tomlinson, N. Boonham, Detection of *Botrytis cinerea* by loop-mediated isothermal amplification, *Lett. Appl. Microbiol.* 51 (6) (2010) 650–657.
- [40] K. Nagamine, T. Hase, T. Notomi, Accelerated reaction by loop-mediated isothermal amplification using loop primers, *Mol. Cell. Probes* 16 (3) (2002) 223–229.
- [41] Y. Mori, T. Notomi, Loop-mediated isothermal amplification (LAMP): a rapid, accurate, and cost-effective diagnostic method for infectious diseases, *J. Infect. Chemother.* 15 (2) (2009) 62–69.
- [42] T. Iwamoto, T. Sonobe, K. Hayashi, Loop-mediated isothermal amplification for direct detection of *Mycobacterium tuberculosis* complex, *M. avium*, and *M. intracellulare* in sputum samples, *J. Clin. Microbiol.* 41 (6) (2003) 2616–2622.
- [43] C.S. Ball, Y.K. Light, C.-Y. Koh, S.S. Wheeler, L.L. Coffey, R.J. Meagher, Quenching of unincorporated amplification signal reporters in reverse-transcription loop-mediated isothermal amplification enabling bright, single-step, closed-tube, and multiplexed detection of RNA viruses, *Anal. Chem.* 88 (7) (2016) 3562–3568.
- [44] M. Goto, E. Honda, A. Ogura, K.-I. Hanaki, Colorimetric detection of loop-mediated isothermal amplification reaction by using hydroxy naphthol blue, *Biotechniques* 46 (3) (2009) 167–172.
- [45] Y. Mori, M. Kitao, N. Tomita, T. Notomi, Real-time turbidimetry of LAMP reaction for quantifying template DNA, *J. Biochem. Biophys. Methods* 59 (2) (2004) 145–157.
- [46] H. Zhang, Y. Xu, Z. Fohlerova, H. Chang, C. Iliescu, P. Neuzil, LAMP-on-a-chip: Revising microfluidic platforms for loop-mediated DNA amplification, *TrAC Trends Anal. Chem.* 113 (2019) 44–53.

- [47] M. Vallet, B. Berge, L. Vovelle, Electrowetting of water and aqueous solutions on poly (ethylene terephthalate) insulating films, *Polymer* 37 (12) (1996) 2465–2470.
- [48] J. Li, C.-J.C. Kim, Current commercialization status of electrowetting-on-dielectric (EWOD) digital microfluidics, *Lab Chip* 20 (10) (2020) 1705–1712.
- [49] Y. Tang, X. Yu, H. Chen, Y. Diao, An immunoassay-based reverse-transcription loop-mediated isothermal amplification assay for the rapid detection of avian influenza H5N1 virus viremia, *Biosens. Bioelectron.* 86 (2016) 255–261.
- [50] Y. Chen, N. Cheng, Y. Xu, K. Huang, Y. Luo, W. Xu, Point-of-care and visual detection of *P. aeruginosa* and its toxin genes by multiple LAMP and lateral flow nucleic acid biosensor, *Biosens. Bioelectron.* 81 (2016) 317–323.
- [51] J.-E. Lee, H. Mun, S.-R. Kim, M.-G. Kim, J.-Y. Chang, W.-B. Shim, A colorimetric Loop-mediated isothermal amplification (LAMP) assay based on HRP-mimicking molecular beacon for the rapid detection of *Vibrio parahaemolyticus*, *Biosens. Bioelectron.* 151 (2020), 111968.
- [52] F. Ahmad, et al., Most probable number-loop mediated isothermal amplification (MPN-LAMP) for quantifying waterborne pathogens in < 25 min, *J. Microbiol. Methods* 132 (2017) 27–33.

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