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Interfacing Pathogen Detection with Smartphones for Point-of-Care Applications

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Graphical Abstract

Infectious diseases are clinically relevant, transmissible illnesses caused by microorganisms, such as bacteria, viruses, fungi, and parasites. According to a recent report, between 1980 and 2014 more than four million people in the USA have died from various infectious diseases.¹ Accurate and timely detection of infection and identification of the causative pathogens are crucial in disease prevention, treatment and monitoring.^{2,3} Although reliable methods of pathogen detection are well and long established, many such detection and diagnostics techniques are still limited in use to clinical laboratories due to the need for trained and skilled personnel, and sophisticated diagnostic instruments. Accordingly, there is a pressing need for simple, affordable, and easy-to-use diagnostic tools for the specific detection of pathogens at the point of care (POC), e.g., doctors' offices, clinics, infirmaries, and particularly in resource-limited areas where medical infrastructure is lacking.

Technological developments have created a new “smart” world of mobile communications, pervasive and inexpensive computer resources, communication and sensor networks, and

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low-cost electronic and optical devices in accessible formats, of which smartphones are the most prominent example. According to Statista⁴, an estimated 62.9 percent of the world's population already owned a mobile phone in 2016, and the number of mobile phone users across the world is expected to number 5 billion by 2019. As personal and portable communication devices, smartphones have advanced computing capability, high-resolution image capture and processing (via a built-in smartphone camera), and an open-source operating system, all of which can be utilized in POC diagnostic systems for use at home, in the clinic, and at the doctor's office. Since smartphones are widely available, even to those with modest incomes, their use in a POC device doesn't incur additional costs. Additional capabilities of smartphones enable wireless transmission of test results to the patient's doctor, healthcare and disease-monitoring networks, and access to third-party "cloud" computing and data storage. The ubiquitous smartphones and internet connections offer unprecedented opportunities for remote disease diagnostics, monitoring and management in new paradigms of healthcare, including telemedicine, mobile health (mHealth).⁵⁻⁷ In particular, recent advancements in microfluidics, 3D printing technology, nanotechnology, and the Internet of Medical Things (IoMT),^{7,8} combined with smartphone-based platforms foster intelligent, low-cost, effective testing systems for POC diagnostic applications. Such mobile POC tests are envisioned for a wide variety of applications ranging from disease screening, diagnostics and monitoring to detection of foodborne pathogens and bioterrorism agents.

Here we review recent efforts to adopt smartphone technology to the disease diagnostics, monitoring and management. Since 2014, the number of publications in this field, as adjudged by literature searches on keywords "smartphone" and "disease" (Figure 1), has greatly expanded. Recently, there have been several instructive reviews focused on the various medical applications of smartphone technology, such as cardiovascular diseases (CVDs) detection, food safety monitoring, and biosensing.⁹⁻¹³ In this review, the recent advances in smartphone-based diagnostic technologies are surveyed under various themes, and discussed with a focus of pathogen detection applications at the point of care. In addition, the perspectives are provided for future development of smartphone-based pathogen detection.

PATHOGEN DETECTION

Conventional Technologies.

Microbiological culture^{14,15} is a long-standing and still widely-used method to confirm the identity of pathogens in clinical specimens. However, culture-based pathogen detection is laborious and time-consuming (e.g., several days or weeks), and thus cannot provide timely diagnosis for fast-acting and rapidly spreading infections. For example, if the pathogen is not quickly identified in children (aged < 5 years) with hand, foot and mouth disease (HFMD), fatal complications (e.g., brainstem encephalitis) can often result in as early as two days.^{16,17} Also, identification of pathogen species in culture often depends on microscopic examination, which is somewhat subjective even for experts, who, moreover, may not be available in resource-limited settings. As an alternative to culturing, *in vitro* diagnostics tests based on biochemical assays (e.g., immunoassay, polymerase chain reaction (PCR)) have

been developed for clinical laboratories, and increasingly, for POC testing outside of laboratories.

Immunoassays are biochemical detection techniques that identify or even quantify proteins and other biomolecules based on affinity reactions between antigen (Ag) and antibody (Ab). Immunoassays are made effective and economic by the availability of monoclonal antibodies and various protein labeling and reporter modalities. One widely-used protein detection method is enzyme-linked immunosorbent assay (ELISA)^{18,19}, in which a signal amplification is realized by enzymatic generation of reporters, such a production of chromogenic molecules. In the realm of POC diagnostics, the lateral flow strip represents an elegantly simple, low cost, non- or minimally-instrumented format for immunoassays. Unfortunately, immunoassays may not provide the needed sensitivity nor specificity for many medical needs. Also, immunoassays cannot measure viral loads. For example, many diseases such as human immunodeficiency virus (HIV) have serological windows when neither pathogen-specific antigen nor infection-related antibody can be detected prior to host seroconversion.²⁰ Moreover, immunological tests based on host antibodies do not indicate acute infections. The latter is problematic with Zika infection, where the timeframe of infection with regard to pregnancy is needed.

Molecular diagnostics based on sequence-specific detection of nucleic acids (e.g., bacterial or viral DNA or RNA) have considerable advantages over culture-based detection and immunoassays with regard to sensitivity, specificity, and test time. Nucleic acid based tests typically utilize an enzymatic amplification process that enables limits of detection as low as 1 to 10 molecules. Nowadays, nucleic acid amplification tests (NAATs) have become the primary technology for the detection and control of some newly-discovered or emerging pathogens, such as H7N9 influenza virus^{21–23}, Ebola virus^{24–26}, and Zika virus^{27–30}. NAATs can be categorized as follows: i) PCR method requiring thermal cycling and precise temperature programming, and ii) isothermal amplification method working at a constant temperature. Isothermal nucleic acid amplification (INAA) includes loop-mediated isothermal amplification (LAMP)³¹, isothermal multiple-self-matching-initiated amplification (IMSA)^{32,33}, cross-priming amplification (CPA)^{34,35}, recombinase polymerase amplification (RPA)³⁶, and helicase-dependent amplification (HDA).³⁷ INAA methods are advantageous for POC applications since they require only constant temperature processes which greatly simplifies instrumentation requirements. Further, some INAA techniques such as LAMP give faster results, and are more robust with regard to inhibitors, thus allowing less stringent sample preparation.

Emerging Technologies.

Recent developments and progress in microfluidics^{38,39}, nanotechnology^{40,41}, and 3D printing technology^{42–44}, have enabled and supported advances and novel implementations of POC pathogen detection. Microfluidics technology has provided miniaturized biochemical assays compatible with minimal and/or compact instrumentation. Microfluidic chips can host microculture and microscale biochemical reactions in millimeter and sub-millimeter sized channel or chamber to minimize the consumption of reagents, reduce required sample size, and shorten testing times.^{45–47} Furthermore, integrated microfluidic

chips (combining sample preparation, biochemical reaction and detection on a single chip) can significantly streamline processing, allowing more automated operation in “sample-in and answer-out” formats.^{48,49} To expand detection options, enhance sensitivity and enable multiplexing, nanotechnology provides an increasing number of new materials such as chromogenic^{50–52} and electrochemical^{53–55} substrates. Nowadays, widely available 3D printing technology and computer aided design (CAD) software not only shorten development cycle times in the conception, design, fabrication and testing of diagnostic devices or companion instrumentation, but also ready for customization.^{56–58} In particular, smartphone technology integrated with these emerging technologies have been endowed great potential to transform traditional uses of imaging, sensing and diagnostic systems, even for POC testing applications, which will likely foster adaptation of POC testing and offer new paradigms for healthcare.

SMARTPHONE AND ITS ACCESSORIES

Progress in mobile and portable electronics technology has produced inexpensive, consumer-grade smartphones with computing capabilities on par with desktop and notebook computers. On one hand, smartphone-based pathogen detection may rely primarily on the intrinsic hardware (e.g., smartphone camera), as well as many Apps (software application programs). On the other hand, the addition of external accessories (e.g., optical filters, electrochemical sensors) not only significantly extends the application of smartphone-based POC diagnostics but improves detection sensitivity and reproducibility.

Built-in Functional Modules and Programs.

In current smartphones, the built-in function modules include camera, speaker, customized applications (Apps) software, global position system (GPS), global system for mobile communication (GSM), wireless fidelity (Wi-Fi), and Bluetooth. By taking advantage of these built-in function modules, smartphone can be used as controller, analyzer, and displayer for rapid, real-time disease monitoring, which can significantly simplify instrument design and reduce the cost of detection systems. More specifically, the smartphone camera first captures two-dimensional (2D) color images of samples. Then, the images are analyzed to indicate the testing results using customized Apps. For more professional interpretation and guidance, the image signals and testing results can be transmitted from the smartphone to remote sites including centralized facilities *via* communication links, optionally incorporating or using GSM, Bluetooth, text message and Wi-Fi. Further, the GPS can enable spatiotemporal disease mapping and epidemiological analyses.⁵⁹

Accessories.

Extrinsic (add-on) capabilities, which are not typically available on consumer smartphones can be incorporated to enable and expand the capabilities of POC diagnostics. These accessories may include additional sensors (e.g. electrochemical transducers such as pH sensors, and also photodetectors, and temperature probes), as well as interfacing with or coupling to components for sample processing and microfluidic control. Further, smartphone electronics may also provide control for electric heaters, fluid actuators (e.g., micropumps),

flow control (e.g., valves), optical excitation sources (e.g., LEDs) and various additional optical components (e.g., lenses, filters, collimators) and accessories such as supplemental batteries, displays, and indicators. All these peripheral components and devices can be integrated into a smartphone-based diagnostics platforms, and can be automatically controlled using custom Android or iOS Apps. To this end, these components need to be housed in a user-friendly, compact case that seamlessly couples to the smartphone. The advent of 3D printing technology for rapid prototyping enables fast, cost-effective and easy fabrication of reliable, inexpensive accessories that connect with smartphones for highly sensitive and reliable pathogen detection. Recently, a variety of 3D printing technologies for microfluidic chips, smartphone adaptors, microscope casing and other supportive add-ons have been described for detection of pathogens.^{60–62}

SMARTPHONE-BASED OPTICAL DETECTION

Optical detection/read-out can either be done at some designated endpoint of the test where the reactions involved in generating a signal have effectively gone to completion, or in *real time* where reaction progress is monitored at intervals. Because many of the reactions associated with amplification or signaling saturate, endpoint detection is best suited for a qualitative (positive/negative) test result, whereas real-time detection can often be used to quantitate the amount of target by correlating an increase in signal intensity with analyte concentration. Still, semi-quantitative endpoint detection is possible in some endpoint detection methods using colorimetric, turbidity, pH, or luminescence-based signaling.

Endpoint Detection.

For endpoint detection, its testing signal is “read” at some designated or nominal completion time such that reactions that indicate presence of an analyte have progressed sufficiently to create a signal over and above the noise or background level. According to the types of signals, smartphone-based endpoint optical detection may be based on color change, reflection, light emission, turbidity or fluorescence intensity.

Endpoint Colorimetric Detection.—In this detection method, a bright-field color change is caused by the reporter reactions in tubes, capillaries, microfluidic chips, or on filter paper, nitrocellulose strips. The substrates that are transformed by a reporter reaction may be composed of peroxidases⁶³, peptides that can be hydrolyzed by proteolytic enzymes⁶⁴, metal ion indicators^{65,66}, nucleic acid intercalating dyes^{67,68}, or functionalized gold nanoparticles^{69,70}. Colorimetric or other modes of detection based on a single sampling of the reaction generally only qualitatively determines the pathogen (positive/negative). However, recent work has utilized the smartphone capability to capture images in red, green, and blue (RGB) format, with the averaged grayscale (AG) or weighted average grayscale (WAG)⁷¹ of the pixel intensities of each of the RGB channels. This allows more detailed characterization of the optical signal intensity and spectral characteristics and can enable quantitative analysis. The associated image processing and analysis can be programmed into the smartphone as an App. Also, the smartphone can function as a spectrometer for analysis of biomarkers.⁷² Such approaches assume, for example, that the extent of color change resulting from assay reactions can be correlated with the amount of pathogen.

Endpoint colorimetric detection of pathogen-related proteins are based on the interaction of antigens and antibodies, as for example, ELISA-based colorimetric detection. In ELISA, as shown in Figure 2A, the development of blue color change is produced using H_2O_2 and 3,3', 5,5'-tetramethylbenzidine (TMB) chromogen with antibody-linked horseradish peroxidase (HRP).⁷³ However, for some pathogens, antibody-based assays have not achieved the needed sensitivity and specificity.^{74,75} Recently, aptamers⁷⁶ have been used in place of antibodies for the detection of *Mycobacterium tuberculosis* (*Mtb*) in direct and indirect dot-blot assays (Figure 2B), with advantages⁷⁷ stemming from comparatively inexpensive synthesis, ready modification, small size leading to more favorable kinetics, and higher specificity, stability and affinity. In the aptamer-based assay, a smartphone captured the color change and the image was analyzed using a customized App (*Mtb* Sensor) to quantify the *Mtb* (Figure 2C). To enhance the HRP-mediated signal amplification, a magnetic-nanocomposite based ELISA has been developed. As shown in Figure 2D, the antibody-labeled magnetic beads first captured the *Salmonella* Enteritidis by binding surface antigens in the bacteria, from which antibody-HRP-inorganic “nanoflowers” were then formed to initiate the TMB-based color change.⁷⁸ Analogous to conventional ELISA assays, a smartphone microplate reader was used quantify the color intensity. This smartphone-based microplate reader used a custom-designed microprism array to compensate for the limited field-of-view (FOV) between the smartphone camera and the microplates (Figure 2E).⁷⁹ To enable portable and low-cost immunoassay, smartphones was used for data collection and analysis. Figure 2F shows a plastic microchip coupled with the mobile phone for the detection of HIV capsid p24 antigen.⁸⁰

Nucleic acids-targeting endpoint colorimetric detections are mainly achieved using metal ion indicators, pH-sensitive dyes, nucleic acids intercalating dyes, and oligonucleotides-labeled gold nanoparticles. Since the amounts of pathogenic nucleic acids are very small, *in vitro* amplification is crucial to obtain a detectable level of nucleic acid target. During the DNA synthesis by polymerase, dNTPs are consumed to produce pyrophosphate ions (PPi^-) that can chelate free metal ions (e.g., Mg^{2+} and Mn^{2+}).^{33,66} Simultaneously, hydrogen ions are also generated and change the pH value in non-buffered reaction solution.^{81,82} Thus, metal ion indicators and pH-sensitive dyes can monitor the changes of metal ions and pH, enabling colorimetric detection of nucleic acids. Currently, metal ions indicators such as hydroxynaphthol blue (HNB)⁶⁵ and calcein⁶⁶ are typically used in INNA-based endpoint colorimetric detection methods (mainly in LAMP and IMSA). pH-sensitive dyes such as phenol red, cresol red, neutral red, and m-Cresol purple can be used in both PCR and INAA colorimetric detection.⁸² In addition, many of metal ion indicators and pH-sensitive dyes are compatible with amplification reaction solution, thereby enabling one-pot reaction systems without need for opening reaction tubes to add the dyes at the end of amplification, while for many of nucleic acid intercalating dyes^{67,68,83} and oligonucleotides-labeled gold nanoparticles^{84–86}, the color detection step is post amplification, and is implemented by adding a relatively high concentration of dyes or nanoparticles. However, this opening of the reaction tubes containing amplified products can cause carryover contamination,^{87,88} wherein false positives in subsequence testing is due to amplicons from previous tests. To reduce the risk of contamination, microcrystalline wax⁸⁹ can be used to encapsulate and separate the nucleic acid dyes from the reaction mixture during the amplification. Another

approach uses modified nanoparticles⁹⁰ which are compatible with amplification buffers such that nucleic acids-targeting endpoint colorimetric reporters can be inspected by the naked eye, in which case, a smartphone is not necessary. However, image capture with a smartphone can improve sensitivity, remove subjective interpretation, permit some degree of quantitation, and enable data archiving and transmission.

Endpoint Lateral Flow Assay.—Endpoint lateral flow assays (LFAs) are often performed in a strip with or without a plastic cartridge. As shown in Figure 3A, each strip contains four parts: i) the sample pad (the area for loading sample), ii) a conjugate pad (where labeled biomolecule conjugates are pre-stored), iii) wicking membrane (e.g., nitrocellulose) on which antibody or oligo test and control lines are striped to capture the conjugate-analyte complex, and iv) an adsorption pad to create capillary action and collect the waste.^{91–93} According to the types of analytes, lateral flow assays may be divided into two categories: lateral flow immunoassays (LFIAs) and nucleic acid lateral flow assays (NALFAs).

In LFIAs, either sandwich or competitive format is employed according to the number of epitopes available on the analytes. Pathogen targets with more than one epitopes allow a sandwich format where the analyte bridges a capture antibody and reporter antibody that each bind on distinct epitopes. As shown in Figure 3A, the conjugation pad is preloaded with primary antibodies labeled with the reporter. These bind an epitope of the target protein and form a target-reporter-conjugated complex that migrates to the test line where it can be captured by another antibody with affinity for a second distinct epitope immobilized on the test line forming the sandwich complex. Note that in the absence of target protein, the sandwich complex cannot form and therefore there is no accumulation of reporter at the test line. For a negative sample, the reporter is instead captured at a second line where antibodies with affinity for the reporter are immobilized, as are some portion of reporters in positive samples. The second line thus serves as a control. The conjugated reporters include enzymes, gold nanoparticles, magnetic particles, quantum dots, and carbon nanotubes. In the case of the pathogens with just one epitope or otherwise not exhibiting two epitopes, such as with relatively small molecule analytes, a competitive format is adopted (Figure 3B). Distinct from the sandwich assay configuration, in the competitive format the test line contains pre-immobilized antigen (the same as analyte) which can bind specifically to label conjugate. Smartphone used for LFIAs can provide and store the images of strips. Based on the lines indicated in the strips, qualitative test results can be read out. Currently, due to the advances of 3D printing technology, smartphone-based detection device is coupled with the LFIAs. As shown in Figure 3C, dual LFIAs were carried out in a 3D printed device integrated with a smartphone detector to simultaneously detect *Salmonella* Enteritidis and *Escherichia coli* O157:H7 in food samples.⁹⁴ Such portable smartphone-based detection device shows great promise for foodborne pathogen identification or in-field food safety tracking.

For NALFAs, the analytes are usually the pathogenic nucleic acids or the amplified products after the nucleic acid amplification. As shown in Figure 3D, there are four different detection strategies in NALFAs.⁹⁵ In many pathogen molecular diagnostics, nucleic acid amplification is necessary since the amount of pathogen nucleic acid in clinical sample is usually too low

for direct detection. Therefore, NALFAs are often used to detect amplified products after nucleic acid enzymatic amplification (e.g., PCR, LAMP). If this entails opening reaction tubes or microfluidic chips to transfer the amplicons to NALFA device, there is a considerable risk of carryover contamination. To this end, Choi *et al.*⁹⁶ have developed a fully integrated NALFA device which features “sample-in and answer-out” detection (Figure 3E). In this device, apart from the NALFA strip, the paper-based microfluidic device contained four hydrophobic polyvinyl chloride (PVC) layers, at one of which an FTA cellulose-based nucleic acid binding membrane was placed to extract DNA from samples. After DNA extraction, the middle two PVC layers are combined and covered by LAMP reaction reagent pad, then subjected to incubation. Post amplification, the NALFA strip was combined with the PVC layers to initiate the lateral flow assay of amplicons. Finally, smartphone recorded the images of strip and quantitatively analyzed the testing results. Such fully integrated NALFA platform coupled with a smartphone has great potential for detecting various pathogens.

Endpoint Fluorescence Detection.—Distinct from colorimetric detection, endpoint fluorescence detection requires a UV or blue excitation light to simulate the fluorescence signal and can achieve a higher detection sensitivity. The fluorescent substrates include nucleic acid intercalating dyes (e.g., SYBR® Green and EvaGreen™), calcein, fluorophore-labeled oligonucleotides, and nanoparticles-labeled antibodies. To enable smartphone-based fluorescent detection, optical path design and choice of fluorescence filters are crucial. To achieve highly sensitive fluorescence detection, a variety of 3D-printed optical accessories have been developed for smartphone-based fluorescent detection.

Nucleic acid intercalating or binding dyes can produce a remarkably increased fluorescence after intercalating double-stranded DNA (ds-DNA) (e.g., amplified products) (Figure 4A).⁹⁷ The wavelength of optimal excitation light for SYBR® Green I and EvaGreen™ ranges from 450 to 490 nm and their wavelength of emission light is from 515 to 530 nm. Thus, for fluorescence based endpoint detection with a smartphone, a 485-nm blue LEDs can be used as an excitation light source, and a 525 nm long pass filter is utilized to selectively transmit the emission light to the detector (Figure 4B).⁹⁸ To increase the fluorescence intensity, SYBR® Green I³³ and EvaGreen™⁹⁹ were, respectively, mixed with HNB in the nucleic acid amplification reaction solution and fluorescence signals were recorded by a smartphone.

Calcein fluorescent dye is also commonly used in isothermal amplification (e.g., LAMP). As shown in Figure 4C, before amplification, calcein chelates the Mn^{2+} and the fluorescence is quenched under ultraviolet (UV) excitation light. After amplification, due to the precipitation between Mn^{2+} and PPi^{-} , Mg^{2+} replaces the Mn^{2+} from calcein-Mn complex and recovers the calcein fluorescence.⁶⁶ To detect the fluorescence with a smartphone, a 365-nm LED is used to produce the UV light and a dichroic beam splitter reflects the filtered UV light to excite the calcein in LAMP reaction solution (Figure 4D).¹⁰⁰ Then, the generated fluorescence passes through the splitter and is concentrated by a plano-convex lens, followed by fluorescence imaging with smartphone camera.

The fluorophore-labeled oligonucleotides show higher specificity than nucleic acid intercalating dyes for pathogen detection, because they can discriminate between the specific amplicons and other ds-DNA byproducts (e.g., primer-dimer) resulting from non-specific amplification. Such fluorophore-labeled oligonucleotide probes have TaqManTM probes, molecular beacons, dual hybridization probes, eclipse probes, scorpions PCR primers, LUX PCR primers, and QZyme PCR primers.¹⁰¹ However, due to difference of polymerases employed, most fluorophore-labeled probes for PCR detection are not compatible with INAA methods. Thus, the fluorophore-labeled oligonucleotides used in INAA have their own formats, such as strand displacement probes¹⁰², strand exchange probes¹⁰³, restriction enzyme-based probes¹⁰⁴, and nuclease-based probes¹⁰⁵. Figure 4E shows a smartphone-based endpoint fluorescence detection using reverse-transcription RPA (RT-RPA) in which a fluorophore-labeled probe (i.e., TwistAmpTM exo probe) was bound to the target and cleaved by Exonuclease III to produce target-specific fluorescence.^{36,106} In particular, they used hot water as the heating source and Thermos container as an incubator of RPA reaction.¹⁰⁶ To detect the fluorescence signal, blue LEDs served as an excitation light, and low-cost theater color films were used as an emission filter. The emitted fluorescence from RPA reaction tubes were directly captured by a smartphone camera without need for other optical detector.

To improve detection sensitivity, endpoint fluorescent detection based on nanoparticles-labeled antibodies has recently been used for LFIAs. In addition to traditional fluorophore-labeled nanoparticles¹⁰⁷, near-infrared (NIR)-to-NIR up-conversion nanoparticles (UCNPs)¹⁰⁸ have been adopted to minimize background noise. As shown in the LFIA platform in Figure 4F, the NIR-to-NIR UCNPs were conjugated to the antibodies which can specifically recognize the zoonotic avian influenza viruses and calcium ion serves as an enhancer to improve the NIR-to-NIR up-conversion photoluminescence. Under the excitation at 980 nm (NIR) of light, the UCNPs worked well when dispersed in the virus particles-containing opaque stool samples, showing a good compatibility with cruel biological samples. The test line and control line in the NIR emission images were captured using a smartphone camera, making it possess potential in the POC application.

Real-time Quantitative Detection.

Compared to endpoint detection, real-time detection can reduce testing time, since that the amplification and detection can be done simultaneously, and often the test result can be inferred early in the amplification stage, especially for samples with relatively high concentration of target. Also, and more importantly, real-time detection permits a quantitative detection of pathogens. Real-time detection can be implemented using the optical components described above and a customized App for periodical fluorescence measurements. As a rapid POC detection format, smartphone-based real-time pathogen detection proves an increasingly popular format, and includes both real-time fluorescence detection and real-time bioluminescence detection.

Real-Time Fluorescence Quantitative Detection.

A typical workflow for real-time fluorescence detection in a smartphone is shown in Figure 5A.¹⁰⁹ First, the images of fluorescence were captured by the smartphone camera at some specified time interval (e.g., 1 minute). Then, the custom-made App cropped the regions of

interest and the RGB bitmap data were extracted. Subsequently, the RGB bitmap data were processed with a gamma transformation (gRGB). According to the specific fluorescent substrates, the pixel matrix of the corresponding channel intensity was extracted to calculate the average fluorescence intensities. For example, the green channel intensity was selected for SYBR® Green I and 6-carboxyfluorescein (6-FAM)-labeled probes. Lastly, a nonlinear sigmoid function was adopted to fit the time-course averaged intensities collected from the series of raw images. This workflow is also applicable for endpoint fluorescence detection using a single frame or averaged frames.

In real-time nucleic acid amplification detection, relatively low concentrations of SYBR® or SYTO® intercalating dyes are usually employed, given that high concentrations can significantly inhibit nucleic acid amplification. However, using low concentrations of dyes will lead to low fluorescence intensities, thereby impacting the sensitivity of smartphone's camera. So, for smartphone-based real-time detection, the optimization on the concentration of nucleic acid dyes is required. Recently, newly-developed nucleic acid intercalating dyes such as EvaGreen™ have claimed higher fluorescence intensity and lower inhibition. Figure 5B shows a smartphone-based real-time LAMP detection platform (dubbed “Smart Cup”), for detection of herpes simplex virus type 2 (HSV-2).¹¹⁰ In the Smart Cup-based detection, EvaGreen™ dye was used and the heating for on-chip isothermal amplification reaction was supplied by exothermic chemical reaction (e.g., water-triggered Mg-Fe alloy). During the amplification, a series of fluorescence images were obtained in real-time by a smartphone. In particular, to eliminate the need of external LED excitation light, the smartphone's flashlight was used for dye excitation. Next, the average fluorescence intensity signals from each image were extracted and normalized using a MATLAB to plot real-time fluorescence curves. As a minimally-instrumented POC detection device, Smart Cup is very suitable for use in resource-limited settings, in the field and especially in areas without reliable electric power. In addition, smartphone-based real-time LAMP assay using EvaGreen™ dye is also reported for multiple pathogen diagnostics. As shown in Figure 5C, an “all-in-one” multiple detection platform was assembled to simultaneously detect Zika, Chikungunya, and Dengue viruses.¹¹¹ The platform was capable of performing on-chip extraction, on-chip amplification, and smartphone-based real-time fluorescence detection. According to the predefined time interval, smartphone can capture the time-course raw fluorescence images of the diagnostic chip. Similarly, real-time fluorescence change for each target was analyzed using MATLAB to automatically calculate the average fluorescence intensity.

Theoretically, real-time detection based on fluorophore-labeled probes is more specific to the target than nucleic acid intercalating dyes. As shown in Figure 5D, smartphone-based real-time PCR platform has been developed to detect viral RNA of influenza A (H1N1) using TaqMan probes.¹¹² In this platform, convection PCR was adopted and accomplished in a capillary tube heated by a resistive heater. The capillary tube was inserted in a smartphone-based detection system to monitor in real-time the fluorescence change. The real-time curve fluorescence can be plotted with a customized program in the smartphone.

Real-time Bioluminescence Quantitative Detection.—Bioluminescence is generated from chemical reactions, catalyzed by luciferase, luciferin, and other enzyme components.

¹¹³ Such real-time bioluminescence assays don't need an excitation light source and optical

filters, and avoid autofluorescence and background effects which limit detection in fluorescence assays. Figure 6A illustrates schematic biochemical reactions of a bioluminescent assay coupled with LAMP.¹¹⁴ In reaction 1, the inorganic pyrophosphate (PPi^-) is produced as byproduct during nucleic acid amplification (e.g., LAMP). In reaction 2, the PPi^- is transformed to adenosine 5'-triphosphate (ATP) by ATP sulfurylase in the presence of adenosine 5'-phosphosulfate (APS). Then, ATP can provide the energy for luciferase to initiate the oxidation reaction of luciferin to produce bioluminescence (equation 3). In real-time bioluminescence-based LAMP assays, a typical bioluminescence intensity curve with a sharp peak occurs for positive reactions, whereas relatively flat curve is observed for negative reaction (Figure 6B). To enable POC detection of such bioluminescence-based LAMP assay, Song *et al.* developed a smartphone-based real-time bioluminescence LAMP detection platform (dubbed “smart-connected cup”) (Figure 6C).¹¹⁵ The inexpensive smart connected cup (SCC) consisted of a thermos cup body, a 3D-printed holder and a smartphone with a customized App for bioluminescence imaging capture, data processing and pathogen quantification. Similar to their previous Smart Cup,¹¹⁰ the heating for on-chip isothermal amplification was provided by the water-triggered exothermic chemical reaction of Mg-Fe alloy. To quantify the pathogen in samples, the averaged bioluminescence intensity was recorded and analyzed, and real-time bioluminescence reverse-transcription LAMP (RT-LAMP) curves were plotted for quantification using the customized App (Figure 6D). Further, to demonstrate the connectivity and spatial disease mapping capability of their smart connected cup, a website was designed to map GPS locations of the tests (Figure 6E). Such smart connected devices have considerable potential to serve as an IoMT device for pathogen detection and disease monitoring.

SMARTPHONE-BASED ELECTROCHEMICAL DETECTION

Electrochemical detection has been extensively employed for determining the presence of pathogens and other biochemical molecules in clinical samples. In principle, compared to the relatively bulky optical detector needed for light sources, camera, lenses and filters, electrochemical sensors offer a relatively compact means of detection, using a microscale sensor and associated electronics. However, in practice, many electrochemical measurements still rely on large and sophisticated electrochemical instruments, which limit them to laboratory settings. Alternatively, recently miniature electrochemical sensors have been interfaced with smartphones, enabling electrochemical-based POC diagnostics. According to the electrochemical detection mechanism, smartphone-based electrochemical detection can be classified into three types: amperometric, potentiometric, and impedimetric.¹¹⁶ Because potentiometric detection is mainly used to measure ions by ion-selective electrodes, amperometric and impedimetric methods for pathogen detection applications are reviewed below.

Amperometric Detection.

In amperometric methods, there are many different detection strategies, such as square wave voltammetry (SWV), differential pulse voltammetry (DPV), chronoamperometry, and cyclic voltammetry (CV). As shown in Figure 7A, a smartphone-based potentiostat platform was developed to detect the core antibody of hepatitis C virus (HCV).¹¹⁷ In this assay, yeast cell

lines were modified as a dual-affinity yeast chimera to display HCV core antigen that was concatenated to gold binding peptide (GBP). Once anti-HCV core IgG is present, the substrate of *p*-aminophenyl phosphate (*p*APP) was converted to *p*-aminophenol (*p*AP) by alkaline phosphatase (ALP) conjugated anti-anti-HCV-core IgG. The conversion can be monitored *via* cyclic voltammetry (CV) that was then demodulated and reconstructed in the smartphone-based potentiostat. As shown in Figure 7B, a smartphone-based handheld device has been developed to measure the malarial antigen, *Plasmodium falciparum* histidine-rich protein 2 (PfHRP2) through chronoamperometry using “sandwich” electrochemical ELISA.¹¹⁸ This device can be coupled with other mobile phones and is compatible with communication networks. Recently, a smartphone-based open-source potentiostat has been designed based on “universal wireless electrochemical detector” (UWED), in which Bluetooth communication was used to interface the detector with the smartphone (Figure 7C).¹¹⁹ The smartphone screen can display the real-time detection results after data storing, processing, and transmission. The results obtained with such smartphone-based electrochemical detector was comparable to that of the commercialized potentiostats.

When determining proteins (or small molecules), aptamers are commonly used in electrochemical detection. As shown in Figure 7D, the electrode surface is first modified using EDC/NHS, thiol-amide, or glutaraldehyde to conjugate the aptamers. In the presence of protein analytes, direct binding took place at the terminals of aptamers.¹²⁰ Moreover, in order to improve the detection performance, secondary aptamers or antibodies which are labeled with functionalized materials are adopted to recognize the analytes in a “sandwich” format.¹²⁰ Apart from aptamers, some antibodies are also immobilized on electrode surfaces. Currently, to further improve the detection sensitivity, nanomaterials or nanocomposite- electrodes are applied to either increase the surface area, or serve as catalysts of oxidation-reduction reactions.

In electrochemical DNA detection, oligonucleotides complementary to target DNA are immobilized on the electrode surface. Figure 7E shows the general design for electrochemical DNA detection.¹²¹ The target DNA is captured through DNA hybridization to produce the electrochemical signals. The direct electrochemical detection of DNA is established based on the reduction and oxidation of DNA (e.g., the oxidation of purine bases) which can be achieved using a variety of electrodes such as indium tin oxide (ITO), carbon, gold, and other polymer-coated materials. However, the signals in direct DNA electrochemistry are often accompanied by high background, compromising detection sensitivity. To circumvent this, indirect DNA electrochemistry using electrochemical mediators has been developed to detect attomoles of DNA target. For example, the oxidation of guanine can be mediated by the polypyridyl complexes of Ru²⁺ and Os²⁺. To facilitate the signal transductions, the electrode surfaces are modified with polymers. Also, DNA can mediate the double helix-associated oxidation-reduction of reporter molecules. Compared to most other chemical labeling strategies, DNA-mediated electrochemistry is simple, sequence-independent, and well suited for mismatch and multiple sequence detection. As shown in Figure 7F, if the DNA sequence is perfectly matched, the methylene blue (MB⁺) molecules intercalate in the duplex DNA, reduced by the unobstructed current and transformed to leucomethylene blue (LB).¹²¹ Then, the LB can reduce the ferricyanide and reproduce the MB⁺, thereby realizing the signal amplification of DNA hybridization. Due to

its low cost and ease of miniaturization, smartphone-based amperometric detection holds great promise for applications at point-of-care diagnostics and in field testing.

Impedimetric Detection.

Impedimetric detection, also known as electrochemical impedance spectroscopy (EIS), is a simple and sensitive electrochemical technology to analyze impedance change caused by biorecognition events, such as antigen-antibody interaction, nucleic acid hybridization. To enable portable and low-cost electrochemical detection, EIS biosensors can be integrated on a microfluidic chip. Figure 7G shows a smartphone-based microfluidic EIS pre-concentrator and sensor for detecting *Escherichia coli* (*E. coli*).¹²² The custom Android App was designed to record and visualize the detection results, and the Bluetooth circuit module was used to enable real-time detection. Recently, an EIS-based immunosensor has been applied for the detection of Zika-virus protein (Figure 7H).¹²³ The electrochemical immunosensor was fabricated using self-assembled dithiobis (succinimidyl propionate) (DTSP) monolayer on the micro-electrode of gold (IDE-Au) array, and the Zika-virus-envelope protein antibody (Zev-Abs) was immobilized at the monolayer. Such EIS immune-sensing chips can be integrated with a miniaturized potentiostat and a smartphone for rapid detection of infectious diseases.

SMARTPHONE-BASED DIGITAL DETECTION

Real-time PCR using serial dilutions and calibration standards has been the ‘gold standard’ of quantitative nucleic acid detection for about two decades. Digital PCR (dPCR) and related ‘digital’ methods based on highly multiplexed qualitative (positive/negative) analysis of samples divided into microvolume reactions have emerged as a practical way of absolute quantification of sample components. While digital NAATs are generally associated with sophisticated and expensive benchtop instruments, recently, digital detection in microfluidic chips has been demonstrated and offers prospects for POC applications. Further, expensive CCD camera used in conventional digital PCR detection can be replaced with a smartphone camera. To date, two main formats have been proposed for smartphone-based digital detection, namely, microfluidic chip-based digital detection and droplet-based digital detection.

Chip-Based Digital Detection.

In this format, digital detection is carried out on microfluidic chips where an array of microwells is fabricated to spatially partition the original samples into separate microreactions. As shown in Figure 8A, a handheld smartphone-based digital PCR detection system has been developed to quantify DNA with high accuracy.¹²⁴ This digital PCR reaction was run on a self-priming fractal branching microchannel net dPCR (SPF dPCR) chip. The portable detection system was constructed of a miniaturized PCR thermocycler and a smartphone-based optical imaging setup. Through a custom App, thermal cycling control, on-chip dPCR, data acquisition, and analysis were automated. In addition to digital PCR, digital LAMP and RPA were also interfaced with a smartphone.^{125,126} In particular, results of on-chip digital LAMP has been demonstrated for direct readout using unmodified camera phones, eliminating the need for expensive fluorescence imaging detection.¹²⁷

Droplet-Based Digital Detection.

Compared with microwells-based digital detection, droplet-based digital detection greatly reduces the fabrication challenge of microfluidic chip with millions of microreactions, since a huge number of droplets can be easily generated through the disaggregation of bulk liquid in a very simple microfluidic chip. However, for POC diagnostics, droplet-based digital detection platforms reported previously are limited to be used at laboratory settings, because they require separate instrumentation for droplet generation, droplet control, and signal measurement. To overcome this challenge, a microdroplet megascale detector (μ MD), requiring only a smartphone camera has been developed to achieve both generation and detection of droplets.¹²⁸ In the μ MD (Figure 8B), droplets were parallelly generated at a frequency (f) of over 10^6 droplets per second, and time-domain encoded detection (f is about 10^6 droplets per second) was achieved in 120 parallel microchannels using a smartphone camera video recording. The excitation light was modulated based on pseudorandom sequence, such that this handheld format enables the resolution of single droplets, addressing the resulting overlap from the digital cameras' limited frame rate.

SMARTPHONE-BASED MICROSCOPY

As an alternative to conventional microscopy, smartphone-based microscopy allows portable, inexpensive, and user-friendly microscopic examinations of pathogen samples for POC applications. This streamlined microscopy platform takes advantage of the smartphone's built-in lens system and complementary-metal-oxide-semiconductor (CMOS) image sensors. Moreover, some external accessories can assist the microscopy to achieve a high sensitivity and resolution. According to the type of light source, smartphone-based microscopy is mainly composed of two categories: bright-field microscopy and fluorescence microscopy.

Bright-field Microscopy Imaging.

For bright-field microscopy detection with smartphone, the imaging of pathogen specimens is usually achieved without optical filters. For imaging with adjustable FOV and resolution, both lens-based and lens-free microscopes have been used. As shown in Figure 9A, a smartphone-based clinical microscopy incorporated the eyepiece and objectives of standard microscope, in which condenser and collector lens were combined.¹²⁹ For example, for examination of Giemsa-stained smears of red blood cells in malaria infection detection, a smartphone-based microscope provided bright-field color images for both thick and thin smears, sufficient for clinical diagnosis. Figure 9B shows a smartphone-based microscopic device to count cells.¹³⁰ The portable platform consists of a customized slider-scale microfluidic chip to preprocess the samples and a smartphone as a detector. In the system, a single-ball lens was set to realize compact microscope, and the detection results were in agreement with the results using a commercial microscope and flow cytometry.

Lens-free cellphone-based microscopy has been first reported by Tseng *et al.*¹³¹ As shown in Figure 9C, a 587-nm LED served as the light source and a 100- μ m aperture was set in front of the LED. The cellphone-based lens-free microscopy possessed a spatial resolution ranging from 1.5 to 2.0 μ m with a FOV of about 24 mm². By eliminating lenses and filters,

the weight of entire device was only about 38 g. The microscope demonstrated utility for the inspection of a waterborne parasite, *Giardia lamblia*. Ambient illumination can be also used as a light source, eliminating the need for an external light source. As shown in Figure 9D, the lens on the back camera of an Android smartphone was removed and the sample was placed directly on the camera surface.¹³² A custom-built App indicated the sub-pixel shifts for imaging process. Due to its simplicity and robustness, this smartphone-based microscope is suitable for the POC pathogen detection in the resource-limited areas.

Fluorescence Microscopy Imaging.

To realize a smartphone-based fluorescence microscope, the smartphone should be integrated with opto-mechanical attachments including an excitation light source, excitation filter, emission filter, and external lens. Laser diodes or LEDs can serve as the excitation source, and the choice of filters depends on the type of fluorophores (“stain”) used. For pathogen detection, specific nucleic acids, antigens, and proteins are either directly labeled with the fluorophores or conjugated with the fluorophore reporters, then subjected to smartphone-based fluorescence microscope imaging.

Nanoparticles are increasingly used as substrates or mediators since they can improve both resolution and detection sensitivity. Figure 9E shows a smartphone-based quantitative fluorescence microscopy to image and detect the *Staphylococcus aureus* (*S. aureus*) cells tagged by aptamer-functionalized fluorescent magnetic nanoparticles.¹³³ First, a 100-nm-thick silver-coated PMMA sheet was combined with imaging gasket to form an imaging chamber. Then, the imaging chamber was inserted into a magnetic holder, followed by adding the bacterial cells. Finally, the captured cells were inspected using the smartphone-based fluorescence microscope device. The quantitative detection capability of the platform was as good as 10 CFU/mL and it was also applicable for direct detection from a peanut milk spiked with *S. aureus* cells. Figure 9F shows a portable smartphone-based microscope was assembled using exchangeable 3D printed accessories to detect *E. coli* O157:H7 in foods (e.g., yoghurt and eggs).¹³⁴ A sandwich structure was formed due to the binding of the pathogenic antigens and the antibodies conjugates of magnetic nanoparticle and FITC fluorophore. Through this 3D-printed imaging system, the fluorescence signals from a cuvette were collected by the smartphone camera. The proposed system showed 106.98% and 107.37% recovery for the yogurt and egg samples with 10³ CFU/mL *E. coli* O157:H7, respectively, providing a highly sensitive fluorescence microscopy for the detection of *E. coli* O157:H7 in real matrixes.

APPLICATIONS EXAMPLES

In this section, we review recent applications on pathogen detection at the POC using smartphone or smartphone-based detection platform.

HIV Detection.

HIV infection causes acquired immunodeficiency syndrome (AIDS) which impairs the human immune system, increasing susceptibility to other infections.¹³⁵ As there is no vaccine, rapidly identifying HIV infection is a crucial step in control and anti-viral

treatment. Periodic (3 to 6 months) measurement of viral load (virions per ml plasma) is needed to assess treatment efficacy. To date, several different strategies have been developed for HIV infection detection based on smartphone platforms. By using plastic micro-pit array chips, Li *et al.*⁸⁰ developed a smartphone-based HIV detection for p24 antigen. The LODs (limits of detection) reached 650 pg/mL and 190 pg/mL p24 antigen for spiked human serum and buffer, respectively. Allan-Blitz *et al.*¹³⁶ described a smartphone-based electronic reader to rapidly detect both HIV and syphilis at the POC. The antibodies specific to HIV were taken as the targets and the sensitivity of this immunoassay can reach 98% concordance to reference 'gold standard' testing of 283 specimens. Damhorst *et al.*¹³⁷ detected HIV virus from minimally-processed HIV-spiked whole blood samples by RT-LAMP on their smartphone-based detection platform. They have demonstrated that the platform is capable of detecting as few as 670 virus particles per microliter of whole blood. Coupling bioluminescent assay in real-time with LAMP (BART-LAMP), Song *et al.*¹¹⁵ designed a smart-connected cup to detect various viruses (e.g., HIV virus in blood) and demonstrated spatiotemporal disease mapping function.

Zika Virus Detection.

Zika virus can be rapidly spread by infected *Aedes* species mosquitos. In recent years, zika virus (ZIKV) infection has caused global health concerns because it is linked to congenital microcephaly, Guillain- Barré syndrome (GBS), and other neurological defects in newborns.¹³⁸ Immunoassay (e.g., lateral flow immunoassay) for ZIKV detection often suffers from low sensitivity and specificity, and doesn't indicate acute infections. To achieve accurate ZIKV detection at the POC, INAA methods such as RT-LAMP have been employed in combination with smartphone-based detection platform, with expected advantages related to rapid test results, high sensitivity and specificity, and visual detection of products. For example, through targeting the envelope protein coding region in Zika virus, Song *et al.*¹¹⁵ developed a smartphone-based BART-LAMP platform to rapidly and quantitatively detect the Zika virus spiked in urine. Kaarj *et al.*¹³⁹ adopted smartphone to detect Zika virus on paper-based LAMP chip with the limit of detection of down to 1 copy/ μ L. Priye *et al.*¹⁴⁰ coupled RT-LAMP with QUASR (quenching of unincorporated amplification signal reporters) technique to detect Zika virus in a "LAMP box" equipped with a smartphone. This platform was able to identify ZIKV directly from crude biological matrices including blood, urine, and saliva. To enable POC detection, Ganguli *et al.*¹¹¹ built up a hands-free smartphone-based Zika diagnostics system, which can detect Zika virus in whole blood as low as 1.56×10^5 PFU/ml.

HPV Detection.

As one of the most common sexually transmitted pathogen, human papillomavirus (HPV) has caused serious health problems including genital warts and some cancers (e.g., cervical and oropharyngeal cancers).¹⁴¹ HPV has more than 100 subtypes, but less than 15% of them are high malignancy risk subtypes such as HPV16, HPV18, and HPV52.¹⁴² To achieve sensitive HPV DNA detection, the conserved or unique regions of HPV-specific genes of E1, E6, E7, and L1 are often used as the targets. Ho *et al.*¹⁴³ devised target hybridization-based visual HPV detection without need of nucleic acid amplification, and implemented the assays in a modular microfluidic platform. The enzymatically produced optical signals were

readily quantified using a smartphone. This HPV assay could be completed in 2 hours, and the sensitivity was better than 10 amol of target molecules. To develop low-cost molecular diagnostics of HPV, Im *et al.*¹⁴⁴ proposed a diffraction-based approach in which microbeads produce unique diffraction patterns. A smartphone connected with a remote server could acquire and process the patterns. Without any nucleic acid amplification, this smartphone-based detection platform was able to detect down to attomole HPV16 and HPV18 DNA targets.

Influenza A Virus Detection.

Influenza A virus infection is widely prevalent among humans. The influenza A viruses are classified by different subtypes according to the varieties of two surface proteins: the hemagglutinin (H) and the neuraminidase (N).¹⁴⁵ Moreover, some subtypes of influenza A virus with high fatality rates have emerged, for instance, H5N1 and H7N9.^{146,147} To rapidly identify the virus, immunoassay and molecular diagnosis methods have been respectively developed to detect the H and N proteins and their coding genomic regions. Yeo *et al.*¹⁴⁸ applied a smartphone-based fluorescent diagnostic device for the detection of H5N1. In this device, a LFIA detection strip was used and the test lines were immobilized with anti-H5N1 nucleocapsid (NP) antibody. In a clinical comparative trial, the smartphone-based diagnostic device possessed 96.55% sensitivity and 98.55% specificity, respectively. The testing results from their distributed individual smartphones can be wirelessly transmitted *via* short messaging service and collected by a centralized database system for further information processing and data mining, which enables rapid identification of patients and efficient control of avian influenza (AI) dissemination. Wu *et al.*¹⁴⁹ reported a low-cost paper-based microfluidic Dot-ELISA system for influenza A virus detection by taking advantage of a smartphone as a detector and processor, where a custom Java App automated the multiple steps for the Dot-ELISA.

CONCLUSION AND FUTURE PRESPECTIVES

In this review, recent advances in smartphone-based POC pathogen detection, specifically in terms of technical principles, detection strategies, and selected applications are surveyed. Based on reports to date, smartphones can greatly improve pathogen detection in terms of cost, convenience, and functions, fostering a new generation of “smart” POC tests where pathogen detection is simple, low-cost, easy-to-use, portable, and highly sensitive and specific. This streamlined detection format frees a wide range of powerful diagnostics from the centralized laboratory, and provides a variety of miniaturized handheld detection devices for screening, monitoring, assessing disease progression and efficacy of therapy at the POC. Furthermore, interfacing pathogen detection with smartphone facilitates new paradigms of healthcare such as personal diagnostics and customized therapy, and offers a sustainable technology for resource-limited areas of the world.

For the future development of POC pathogen detection with smartphone technology, we anticipate the following directions. One is to further improve the detection accuracy of assay chemistry, particularly in reporter reactions.^{150,151} For instance, reactions that provide higher contrast fluorescence or color change will be beneficial for improving the detection

sensitivity of smartphone-based imaging. Another direction is to simplify or modularize the diagnostic device fabrication. 3D printing technology is compressing design, prototyping, and test cycles, but, there is still room for improvement on the printing precision and surface characteristics (e.g., smoothness). In addition, more materials compatible with 3D printing or other rapid prototyping methods should be developed to provide more options and flexibility with regard to optical functions, filtering, conjugation with biomolecules, heating and thermal insulation, and electrical and sensor components. For sample preparation, new materials, such as nucleic acid and protein binding media that facilitate simpler sample loading, washing and elution steps, would simplify sample processing needed to extract the nucleic acids or purify the pathogenic proteins with high efficiency.^{152,153} Novel enzymes, probes, dyes, and indicators should be explored to minimize the nonspecific reactions and maximize signal-to-noise ratio. Furthermore, more sophisticated algorithms compatible with smartphones are needed for image processing and data analysis. For the smartphone itself or smartphone-based diagnostic platform, more powerful optical components and sensors that can readily interface with a smartphone would expand functionality and application areas. With the development of IoMT and mobile health,^{5–8,154,155} a smartphone-based detection platform can transmit test results to the doctor's office and communicate test information (i.e., location, test time, and deidentified results) to public health officials, providing critical data to decision and policy makers as well as epidemiologists. Further, together with global positioning, such systems can track in real time the geo-spatial distribution of the epidemic diseases and predict disease transmission risk. Since this is a highly interdisciplinary research area including clinical microbiology, analytical chemistry, microfluidics, and computer science, close collaboration among engineers, chemists, clinicians and industry partners is crucial to develop next generation smartphone-based POC pathogen detection technology, adequately addressing issues such as patient privacy and security with diagnostics test data. We envision that POC pathogen detection coupled with smartphone technology will offer a great promise of a variety of practical applications in addition to detection of infectious diseases, such as companion diagnostics for cancer and other diseases, environmental monitoring, detection of bioterrorism agents, as well as tests for food quality, water contamination, and veterinary work.

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Biographies

Xiong Ding obtained his B.S. degree in veterinary medicine from South China Agriculture University in 2011, M.S. degree in sugar engineering from South China University of Technology in 2014, and Ph.D. in biochemistry and molecular biology from Zhejiang University, China in 2017. After that, he started his first postdoctoral research at the Iowa State University in 2017. He joined the group of Dr. Changchun Liu for postdoctoral position at the University of Connecticut Health Center in 2018, focusing on developing isothermal nucleic acid amplification techniques and nucleic acids associated biosensors for pathogen detection.

Michael G Mauk received his BEE (Electrical Engineering), BChE (Chemical Engineering) and PhD (Electrical Engineering) from the University of Delaware, as well as MS (Microbiology, Univ. Florida), MS (Biochemistry, University of the Sciences, Philadelphia), and MS (Biotechnology, Johns Hopkins University). He worked for fifteen years in industry as a researcher in the optoelectrics, and is now a researcher at the University Pennsylvania in the area of microfluidics for POC diagnostics, as well as a Professor of Engineering Technology at Drexel University.

Kun Yin obtained his Ph.D. at the University of Chinese Academy of Sciences in 2016 under the supervision of Prof. Lingxin Chen. After that, he did his first postdoctoral training at the Ohio State University and worked alongside Prof. Mingjun Zhang to develop optical cyclic peptide nanoparticles for Alzheimer's disease diagnosis. Then, he joined Prof. Changchun Liu's Lab as a postdoc researcher at the University of Pennsylvania in 2017 and the University of Connecticut in 2018, where his research has focused on developing smartphone-based molecular detection platform for disease diagnostics.

Karteek Kadimisetty obtained his Ph.D. in analytical chemistry from University of Connecticut in 2017 under the supervision of Dr. James F. Rusling. His research in graduate studies mainly focused on developing modular microfluidic platforms integrated with nanomaterials aimed at early cancer screening via simultaneous multiple protein detection. Then he moved to University of Pennsylvania as a Post-Doctoral research fellow where he worked alongside of Dr. Changchun Liu in developing state-of-the-art miniaturized 3D printed microfluidic molecular diagnostic platforms for infectious disease diagnostics at point of need.

Changchun Liu received his B.S. and M.S. degree in Chemistry from Yunnan University, China, and Ph.D. in Physical Electronics from the Institute of Electronics, Chinese Academy of Sciences, China in 2005. After a postdoctoral training at the University of Pennsylvania, he worked as a research assistant professor in 2012 and was promoted to research associate professor in 2017 in the Department of Mechanical Engineering and Applied Mechanics at the University of Pennsylvania. He joined the Department of Biomedical Engineering at the University of Connecticut Health Center as an associate professor in 2018. His research lab is interested in applying interdisciplinary approaches to develop new medical devices and systems for disease diagnostics, mobile health, as well as personalized medicine.

REFERENCES

- (1). El Bcheraoui C; Mokdad AH; Dwyer-Lindgren L; Bertozzi-Villa A; Stubbs RW; Morozoff C; Shirude S; Naghavi M; Murray CJ. JAMA 2018, 319, 1248–1260. [PubMed: 29584843]
- (2). Caliendo AM; Gilbert DN; Ginocchio CC; Hanson KE; May L; Quinn TC; Tenover FC; Alland D; Blaschke AJ; Bonomo RA. Clin. Infect. Dis 2013, 57, S139–S170. [PubMed: 24200831]
- (3). Dong J; Olano JP; McBride JW; Walker DH. J. Mol. Diagn 2008, 10, 185–197. [PubMed: 18403608]
- (4). The Statistics Portal, 2018 <https://www.statista.com/statistics/274774/forecast-of-mobile-phone-users-worldwide/>
- (5). Weinstein RS; Lopez AM; Joseph BA; Erps KA; Holcomb M; Barker GP; Krupinski EA. Am. J. Med 2014, 127, 183–187. [PubMed: 24384059]
- (6). Bastawrous A; Armstrong MJ. J. R. Soc. Med 2013, 106, 130–142. [PubMed: 23564897]

- (7). McKay FH; Cheng C; Wright A; Shill J; Stephens H; Uccellini M. J. Telemed. Telecare 2018, 24, 22–30. [PubMed: 27760883]
- (8). Joyia GJ; Liaqat RM; Farooq A; Rehman S. J. Commun 2017, 12.
- (9). Xu X; Akay A; Wei H; Wang S; Pingguan-Murphy B; Erlandsson B-E; Li X; Lee W; Hu J; Wang L. Proc. IEEE 2015, 103, 236–247.
- (10). Yang K; Peretz-Soroka H; Liu Y; Lin F. Lab Chip 2016, 16, 943–958. [PubMed: 26899264]
- (11). Quesada-González D; Merkoçi A. Biosens. Bioelectron 2017, 92, 549–562. [PubMed: 27836593]
- (12). Rateni G; Dario P; Cavallo F. Sensors 2017, 17, 1453.
- (13). Hu J; Cui X; Gong Y; Xu X; Gao B; Wen T; Lu TJ; Xu F. Biotechnol. Adv 2016, 34, 305–320. [PubMed: 26898179]
- (14). Perry JD. Clin. Microbiol. Rev 2017, 30, 449–479. [PubMed: 28122803]
- (15). Eisfeld AJ; Neumann G; Kawaoka Y. Nat. Protoc 2014, 9, 2663. [PubMed: 25321410]
- (16). Huang C-C; Liu C-C; Chang Y-C; Chen C-Y; Wang S-T; Yeh T-F. N. Engl. J. Med 1999, 341, 936–942. [PubMed: 10498488]
- (17). Wong S; Yip C; Lau S; Yuen K. Epidemiol. Infect 2010, 138, 1071–1089. [PubMed: 20056019]
- (18). Engvall E; Perlmann P. Immunochemistry 1971, 8, 871–874. [PubMed: 5135623]
- (19). Lequin RM. Clin. Chem 2005, 51, 2415–2418. [PubMed: 16179424]
- (20). Branson BM. Clin. Infect. Dis 2007, 45, S221–S225. [PubMed: 18190290]
- (21). Yu X; Jin T; Cui Y; Pu X; Li J; Xu J; Liu G; Jia H; Liu D; Song SJ. Virol. 2014, JVI 02059–02013.
- (22). Zhang J; Feng Y; Hu D; Lv H; Zhu J; Cao M; Zheng F; Zhu J; Gong X; Hao L. J. Clin. Microbiol 2013, JCM 01907–01913.
- (23). Nie K; Zhao X; Ding X; Li X; Zou S; Guo J; Wang D; Gao R; Li X; Huang W. Clin. Microbiol. Infect 2013, 19, E372–E375. [PubMed: 23718218]
- (24). Towner JS; Rollin PE; Bausch DG; Sanchez A; Crary SM; Vincent M; Lee WF; Spiropoulou CF; Ksiazek TG; Lukwiya M. J. Virol 2004, 78, 4330–4341. [PubMed: 15047846]
- (25). Leroy E; Baize S; Lu C; McCormick J; Georges A; Georges-Courbot MC; Lansoud-Soukate J; Fisher-Hoch S. J. Med. Virol 2000, 60, 463–467. [PubMed: 10686031]
- (26). Kurosaki Y; Takada A; Ebihara H; Grolla A; Kamo N; Feldmann H; Kawaoka Y; Yasuda J. J. Virol. Methods 2007, 141, 78–83. [PubMed: 17194485]
- (27). Faye O; Faye O; Dupressoir A; Weidmann M; Ndiaye M. J. Clin. Virol 2008, 43, 96–101. [PubMed: 18674965]
- (28). Pardee K; Green AA; Takahashi MK; Braff D; Lambert G; Lee JW; Ferrante T; Ma D; Donghia N; Fan M. Cell 2016, 165, 1255–1266. [PubMed: 27160350]
- (29). Wang X; Yin F; Bi Y; Cheng G; Li J; Hou L; Li Y; Yang B; Liu W; Yang L. J. Virol. Methods 2016, 238, 86–93. [PubMed: 27793644]
- (30). Song J; Mauk MG; Hackett BA; Cherry S; Bau HH; Liu C. Anal. Chem 2016, 88, 7289–7294. [PubMed: 27306491]
- (31). Notomi T; Okayama H; Masubuchi H; Yonekawa T; Watanabe K; Amino N; Hase T. Nucleic Acids Res. 2000, 28, e63–e63. [PubMed: 10871386]
- (32). Ding X; Nie K; Shi L; Zhang Y; Guan L; Zhang D; Qi S; Ma X. J. Clin. Microbiol 2014, JCM 03298–03213.
- (33). Ding X; Wu W; Zhu Q; Zhang T; Jin W; Mu Y. Anal. Chem 2015, 87, 10306–10314. [PubMed: 26383158]
- (34). Fang R; Li X; Hu L; You Q; Li J; Wu J; Xu P; Zhong H; Luo Y; Mei J. J. Clin. Microbiol 2009, 47, 845–847. [PubMed: 19116359]
- (35). Xu G; Hu L; Zhong H; Wang H; Yusa S.-i.; Weiss TC; Romaniuk PJ; Pickerill S; You Q. Sci. Rep 2012, 2, 246. [PubMed: 22355758]
- (36). Piepenburg O; Williams CH; Stemple DL; Armes NA. PLoS Biol. 2006, 4, e204. [PubMed: 16756388]
- (37). Vincent M; Xu Y; Kong H. EMBO Rep. 2004, 5, 795–800. [PubMed: 15247927]
- (38). Tang M; Wang G; Kong S-K; Ho H-P. Micromachines 2016, 7, 26.

- (39). Zhang D; Bi H; Liu B; Qiao L; ACS Publications, 2018.
- (40). Kishore P; Panda SK; Minimol V; Mohan C; Ravishankar C. In Research Methodology in Food Sciences; Apple Academic Press, 2018, pp 21–36.
- (41). Kumar M; Das A. Adv. Colloid Interface Sci 2017.
- (42). Ho CMB; Ng SH; Li KHH; Yoon Y-J. Lab Chip 2015, 15, 3627–3637. [PubMed: 26237523]
- (43). Chan HN; Tan MJA; Wu H. Lab Chip 2017, 17, 2713–2739. [PubMed: 28702608]
- (44). Waheed S; Cabot JM; Macdonald NP; Lewis T; Guijt RM; Paull B; Breadmore MC. Lab Chip 2016, 16, 1993–2013. [PubMed: 27146365]
- (45). Schoepp NG; Schlappi TS; Curtis MS; Butkovich SS; Miller S; Humphries RM; Ismagilov RF. Sci. Transl. Med 2017, 9, eaal3693. [PubMed: 28978750]
- (46). Wan L; Chen T; Gao J; Dong C; Wong AH-H; Jia Y; Mak P-I; Deng C-X; Martins RP. Sci. Rep 2017, 7, 14586. [PubMed: 29109452]
- (47). Bian X; Jing F; Li G; Fan X; Jia C; Zhou H; Jin Q; Zhao J. Biosens. Bioelectron 2015, 74, 770–777. [PubMed: 26226346]
- (48). Oh SJ; Park BH; Choi G; Seo JH; Jung JH; Choi JS; Seo TS. Lab Chip 2016, 16, 1917–1926. [PubMed: 27112702]
- (49). Park BH; Oh SJ; Jung JH; Choi G; Seo JH; Lee EY; Seo TS. Biosens. Bioelectron 2017, 91, 334–340. [PubMed: 28043075]
- (50). Fu X; Cheng Z; Yu J; Choo P; Chen L; Choo J. Biosens. Bioelectron 2016, 78, 530–537. [PubMed: 26669705]
- (51). Singh J; Sharma S; Nara S. Food Chem. 2015, 170, 470–483. [PubMed: 25306373]
- (52). Quesada-González D; Merkoçi A. Biosens. Bioelectron 2015, 73, 47–63. [PubMed: 26043315]
- (53). Liu G; Zhang Y; Guo W. Biosens. Bioelectron 2014, 61, 547–553. [PubMed: 24953841]
- (54). Kavosi B; Salimi A; Hallaj R; Moradi F. Biosens. Bioelectron 2015, 74, 915–923. [PubMed: 26257183]
- (55). Liu G; Qi M; Zhang Y; Cao C; Goldys EM. Anal. Chim. Acta 2016, 909, 1–8. [PubMed: 26851079]
- (56). Kadimisetty K; Song J; Doto AM; Hwang Y; Peng J; Mauk MG; Bushman FD; Gross R; Jarvis JN; Liu C. Biosens. Bioelectron 2018, 109, 156–163. [PubMed: 29550739]
- (57). Kadimisetty K; Mosa IM; Malla S; Satterwhite-Warden JE; Kuhns TM; Faria RC; Lee NH; Rusling JF. Biosens. Bioelectron 2016, 77, 188–193. [PubMed: 26406460]
- (58). Lee W; Kwon D; Choi W; Jung GY; Au AK; Folch A; Jeon S. Sci. Rep 2015, 5, 7717. [PubMed: 25578942]
- (59). Larocca A; Visconti RM; Marconi M. Malar. J 2016, 15, 520. [PubMed: 27776516]
- (60). Liu C; Liao S-C; Song J; Mauk MG; Li X; Wu G; Ge D; Greenberg RM; Yang S; Bau HH. Lab Chip 2016, 16, 553–560. [PubMed: 26732765]
- (61). Berg B; Cortazar B; Tseng D; Ozkan H; Feng S; Wei Q; Chan RY; Burbano J; Farooqui Q; Lewinski M. In CLEO: Applications and Technology; Optical Society of America, 2016, p ATu1O. 4.
- (62). Laksanasopin T; Guo TW; Nayak S; Sridhara AA; Xie S; Olowookere OO; Cadinu P; Meng F; Chee NH; Kim J. Sci. Transl. Med 2015, 7, 273re271–273re271.
- (63). Sun L; Ghosh I; Barshevsky T; Kochinyan S; Xu M-Q. Methods 2007, 42, 220–226. [PubMed: 17532508]
- (64). Vickers C; Hales P; Kaushik V; Dick L; Gavin J; Tang J; Godbout K; Parsons T; Baronas E; Hsieh F. J. Biol. Chem 2002.
- (65). Goto M; Honda E; Ogura A; Nomoto A; Hanaki K-I. Biotechniques 2009, 46, 167–172. [PubMed: 19317660]
- (66). Tomita N; Mori Y; Kanda H; Notomi T. Nat. Protoc 2008, 3, 877. [PubMed: 18451795]
- (67). Peng J; Zhang J; Xia Z; Li Y; Huang J; Fan Z. J. Virol. Methods 2012, 185, 254–258. [PubMed: 22771738]
- (68). Njiru ZK; Mikosza ASJ; Armstrong T; Enyaru JC; Ndung'u JM; Thompson ARC. PLoS Negl. Trop. Dis 2008, 2, e147. [PubMed: 18253475]

- (69). Verma MS; Rogowski JL; Jones L; Gu FX. *Biotechnol. Adv* 2015, 33, 666–680. [PubMed: 25792228]
- (70). Baetsen-Young AM; Vasher M; Matta LL; Colgan P; Alocilja EC; Day B. *Biosens. Bioelectron* 2018, 101, 29–36. [PubMed: 29031887]
- (71). Wong JX; Liu FS; Yu H-Z. *Anal. Chem* 2014, 86, 11966–11971. [PubMed: 25420202]
- (72). Long KD; Yu H; Cunningham BT. *Biomed. Opt. Express* 2014, 5, 3792–3806. [PubMed: 25426311]
- (73). Bos E; Van der Doelen A; Rooy N. v.; Schuurs AH. *J. Immunoassay Immunochem* 1981, 2, 187–204.
- (74). Steingart KR; Henry M; Laal S; Hopewell PC; Ramsay A; Menzies D; Cunningham J; Weldingh K; Pai M. *PLoS Med.* 2007, 4, e202. [PubMed: 17564490]
- (75). McNerney R; Daley P. *Nat. Rev. Microbiol* 2011, 9, 204. [PubMed: 21326275]
- (76). Li L; Liu Z; Zhang H; Yue W; Li C-W; Yi C. *Sens. Actuators B Chem* 2018, 254, 337–346.
- (77). Gopinath SC; Lakshmipriya T; Chen Y; Phang W-M; Hashim U. *Biotechnol. Adv* 2016, 34, 198–208. [PubMed: 26876017]
- (78). Zeinhom MMA; Wang Y; Sheng L; Du D; Li L; Zhu M-J; Lin Y. *Sens. Actuators B Chem* 2018, 261, 75–82.
- (79). Wang L-J; Sun R; Vasile T; Chang Y-C; Li L. *Anal. Chem* 2016, 88, 8302–8308. [PubMed: 27434250]
- (80). Li F; Li H; Wang Z; Wu J; Wang W; Zhou L; Xiao Q; Pu Q. *Sens. Actuators B Chem* 2018, 271, 189–194.
- (81). Toumazou C; Shepherd LM; Reed SC; Chen GI; Patel A; Garner DM; Wang C-JA; Ou C-P; Amin-Desai K; Athanasiou P. *Nat. Meth* 2013, 10, 641.
- (82). Tanner NA; Zhang Y; Evans TC Jr *Biotechniques* 2015, 58, 59–68. [PubMed: 25652028]
- (83). Hill J; Beriwal S; Chandra I; Paul VK; Kapil A; Singh T; Wadowsky RM; Singh V; Goyal A; Jahnukainen TJ. *Clin. Microbiol* 2008, 46, 2800–2804.
- (84). Fu Z; Zhou X; Xing D. *Methods* 2013, 64, 260–266. [PubMed: 23948710]
- (85). Tan E; Erwin B; Dames S; Voelkerding K; Niemz A. *Clin. Chem* 2007, 53, 2017–2020. [PubMed: 18030699]
- (86). Kong C; Wang Y; Fodjo EK; Yang G.-x.; Han F; Shen X.-s. *Microchim. Acta* 2018, 185, 35.
- (87). Tang Y; Chen H; Diao Y. *Sci. Rep* 2016, 6, 27605. [PubMed: 27270462]
- (88). Hsieh K; Mage PL; Csordas AT; Eisenstein M; Soh HT. *Chem. Commun* 2014, 50, 3747–3749.
- (89). Zhang M; Liu Y; Chen L; Quan S; Jiang S; Zhang D; Yang L. *Anal. Chem* 2012, 85, 75–82. [PubMed: 23181490]
- (90). Wong JK; Yip SP; Lee TM. *Small* 2014, 10, 1495–1499. [PubMed: 24623485]
- (91). Sajid M; Kawde A-N; Daud M. *J. Saudi Chem. Soc* 2015, 19, 689–705.
- (92). Bahadır EB; Sezgentürk MK. *Trends Analyt. Chem* 2016, 82, 286–306.
- (93). Anfossi L; Baggiani C; Giovannoli C; D’Arco G; Giraudi G. *Anal. Bioanal. Chem* 2013, 405, 467–480. [PubMed: 22543716]
- (94). Cheng N; Song Y; Zeinhom MM; Chang Y-C; Sheng L; Li H; Du D; Li L; Zhu M-J; Luo Y. *ACS Appl. Mater. Interfaces* 2017, 9, 40671–40680. [PubMed: 28914522]
- (95). Posthuma-Trumpie GA; Korf J; van Amerongen A. *Anal. Bioanal. Chem* 2009, 393, 569–582. [PubMed: 18696055]
- (96). Choi JR; Hu J; Tang R; Gong Y; Feng S; Ren H; Wen T; Li X; Abas WABW; Pingguan-Murphy B. *Lab Chip* 2016, 16, 611–621. [PubMed: 26759062]
- (97). Dragan A; Pavlovic R; McGivney J; Casas-Finet J; Bishop E; Strouse R; Schenerman M; Geddes C. *J. Fluoresc* 2012, 22, 1189–1199. [PubMed: 22534954]
- (98). Chen W; Yu H; Sun F; Ornob A; Brisbin R; Ganguli A; Vemuri V; Strzebonski P; Cui G; Allen KJ. *Anal. Chem* 2017, 89, 11219–11226. [PubMed: 28819973]
- (99). Kong JE; Wei Q; Tseng D; Zhang J; Pan E; Lewinski M; Garner OB; Ozcan A; Di Carlo D. *ACS Nano* 2017, 11, 2934–2943. [PubMed: 28234452]

- (100). Hui J; Gu Y; Zhu Y; Chen Y; Guo S.-j.; Tao S.-c.; Zhang Y; Liu P. *Lab Chip* 2018, 18, 2854–2864. [PubMed: 30105321]
- (101). Didenko VV. *Biotechniques* 2001, 31, 1106. [PubMed: 11730017]
- (102). Ding X; Wang G; Sun J; Zhang T; Mu Y. *Chem. Commun* 2016, 52, 11438–11441.
- (103). Jiang YS; Bhadra S; Li B; Wu YR; Milligan JN; Ellington AD. *Anal. Chem* 2015, 87, 3314–3320. [PubMed: 25708458]
- (104). Wang Y; Wang Y; Lan R; Xu H; Ma A; Li D; Dai H; Yuan X; Xu J; Ye C. *J. Mol. Diagn* 2015, 17, 392–401. [PubMed: 26094089]
- (105). Jung C; Chung JW; Kim UO; Kim MH; Park HG. *Anal. Chem* 2010, 82, 5937–5943. [PubMed: 20575518]
- (106). Chan K; Wong P-Y; Parikh C; Wong S. *Anal. Biochem* 2018, 545, 4–12. [PubMed: 29339059]
- (107). Jiang H; Wu D; Song L; Yuan Q; Ge S; Min X; Xia N; Qian S; Qiu X. *SLAS Technol.* 2017, 22, 122–129. [PubMed: 27899699]
- (108). Kim J; Kwon JH; Jang J; Lee H; Kim S; Hahn YK; Kim SK; Lee KH; Lee S; Pyo H. *Biosens. Bioelectron* 2018, 112, 209–215. [PubMed: 29709831]
- (109). Priye A; Ugaz VM. In *Biosensors and Biodetection*; Springer, 2017, pp 251–266.
- (110). Liao S-C; Peng J; Mauk MG; Awasthi S; Song J; Friedman H; Bau HH; Liu C. *Sens. Actuators B Chem* 2016, 229, 232–238. [PubMed: 26900258]
- (111). Ganguli A; Ornob A; Yu H; Damhorst G; Chen W; Sun F; Bhuiya A; Cunningham B; Bashir R. *Biomed. Microdevices* 2017, 19, 73. [PubMed: 28831630]
- (112). Qiu X; Ge S; Gao P; Li K; Yang S; Zhang S; Ye X; Xia N; Qian S. *Microsyst. Technol* 2017, 23, 2951–2956.
- (113). Haddock SH; Moline MA; Case JF. *Annual Review of Marine Science* 2010, 2, 443–493.
- (114). Gandelman OA; Church VL; Moore CA; Kiddle G; Carne CA; Parmar S; Jalal H; Tisi LC; Murray JA. *PLoS ONE* 2010, 5, e14155. [PubMed: 21152399]
- (115). Song J; Pandian V; Mauk MG; Bau HH; Cherry S; Tisi LC; Liu C. *Anal. Chem* 2018, 90, 4823–4831. [PubMed: 29542319]
- (116). Zhang D; Liu Q. *Biosens. Bioelectron* 2016, 75, 273–284. [PubMed: 26319170]
- (117). Aronoff-Spencer E; Venkatesh A; Sun A; Brickner H; Looney D; Hall DA. *Biosens. Bioelectron* 2016, 86, 690–696. [PubMed: 27472403]
- (118). Nemiroski A; Christodouleas DC; Hennek JW; Kumar AA; Maxwell EJ; Fernández-Abedul MT; Whitesides GM. *Proc. Natl. Acad. Sci. U.S.A* 2014, 111, 11984–11989. [PubMed: 25092346]
- (119). Ainla A; Mousavi MP; Tsaloglou M-N; Redston J; Bell JG; Fernández-Abedul MT; Whitesides GM. *Anal. Chem* 2018, 90, 6240–6246. [PubMed: 29658268]
- (120). Mishra GK; Sharma V; Mishra RK. *Biosensors* 2018, 8, 28.
- (121). Drummond TG; Hill MG; Barton JK. *Nat. Biotechnol* 2003, 21, 1192. [PubMed: 14520405]
- (122). Jiang J; Wang X; Chao R; Ren Y; Hu C; Xu Z; Liu GL. *Sens. Actuators B Chem* 2014, 193, 653–659.
- (123). Kaushik A; Yndart A; Kumar S; Jayant RD; Vashist A; Brown AN; Li C-Z; Nair M. *Sci. Rep* 2018, 8, 9700. [PubMed: 29946074]
- (124). Gou T; Hu J; Wu W; Ding X; Zhou S; Fang W; Mu Y. *Biosens. Bioelectron* 2018, 120, 144–152. [PubMed: 30173010]
- (125). Song B; Jin W; Song Q; Jin Q; Mu Y. *Chem. Res. Chin. Univ* 2015, 31, 519–525.
- (126). Yeh E-C; Fu C-C; Hu L; Thakur R; Feng J; Lee LP. *Sci. Adv* 2017, 3, e1501645. [PubMed: 28345028]
- (127). Rodriguez-Manzano J; Karymov MA; Begolo S; Selck DA; Zhukov DV; Jue E; Ismagilov RF. *ACS Nano* 2016, 10, 3102–3113. [PubMed: 26900709]
- (128). Yelleswarapu VR; Jeong H-H; Yadavali S; Issadore D. *Lab Chip* 2017, 17, 1083–1094. [PubMed: 28225099]
- (129). Breslauer DN; Maamari RN; Switz NA; Lam WA; Fletcher DA. *PLoS ONE* 2009, 4, e6320. [PubMed: 19623251]

- (130). Zeng Y; Jin K; Li J; Liu J; Li J; Li T; Li S. *Sens. Actuators A Phys* 2018, 274, 57–63.
- (131). Tseng D; Mudanyali O; Oztoprak C; Isikman SO; Sencan I; Yaglidere O; Ozcan A. *Lab Chip* 2010, 10, 1787–1792. [PubMed: 20445943]
- (132). Lee SA; Yang C. *Lab Chip* 2014, 14, 3056–3063. [PubMed: 24964209]
- (133). Shrivastava S; Lee W-I; Lee N-E. *Biosens. Bioelectron* 2018, 109, 90–97. [PubMed: 29533818]
- (134). Zeinhom MMA; Wang Y; Song Y; Zhu M-J; Lin Y; Du D. *Biosens. Bioelectron* 2018, 99, 479–485. [PubMed: 28822314]
- (135). Bandura A. *Eval. Program Plann* 1990, 13, 9–17.
- (136). Allan-Blitz L-T; Vargas SK; Konda KA; de Cortina SH; Cáceres CF; Klausner JD. *Sex. Transm. Infect* 2018, sextrans-2017–053511.
- (137). Damhorst GL; Duarte-Guevara C; Chen W; Ghonge T; Cunningham BT; Bashir R. *Engineering* 2015, 1, 324–335. [PubMed: 26705482]
- (138). Petersen LR; Jamieson DJ; Powers AM; Honein MA. *N. Engl. J. Med* 2016, 374, 1552–1563. [PubMed: 27028561]
- (139). Kaarj K; Akarapipad P; Yoon J-Y. *Sci. Rep* 2018, 8, 12438. [PubMed: 30127503]
- (140). Priye A; Bird SW; Light YK; Ball CS; Negrete OA; Meagher RJ. *Sci. Rep* 2017, 7, 44778. [PubMed: 28317856]
- (141). Muñoz N; Bosch FX; De Sanjosé S; Herrero R; Castellsagué X; Shah KV; Snijders PJ; Meijer CJ. *N. Engl. J. Med* 2003, 348, 518–527. [PubMed: 12571259]
- (142). Bouvard V; Baan R; Straif K; Grosse Y; Secretan B; El Ghissassi F; Benbrahim-Tallaa L; Guha N; Freeman C; Galichet L. *Lancet Oncol.* 2009, 10, 321–322. [PubMed: 19350698]
- (143). Ho NR; Lim GS; Sundah NR; Lim D; Loh TP; Shao H. *Nat. Commun* 2018, 9, 3238. [PubMed: 30104566]
- (144). Im H; Castro CM; Shao H; Liong M; Song J; Pathania D; Fexon L; Min C; Avila-Wallace M; Zurkiya O. *Proc. Natl. Acad. Sci. U.S.A* 2015, 201501815.
- (145). Suarez DL. *Animal Influenza* 2016, 1–30.
- (146). Subbarao K; Klimov A; Katz J; Regnery H; Lim W; Hall H; Perdue M; Swayne D; Bender C; Huang J. *Science* 1998, 279, 393–396. [PubMed: 9430591]
- (147). Gao R; Cao B; Hu Y; Feng Z; Wang D; Hu W; Chen J; Jie Z; Qiu H; Xu K. *N. Engl. J. Med* 2013, 368, 1888–1897. [PubMed: 23577628]
- (148). Yeo S-J; Choi K; Cuc BT; Hong NN; Bao DT; Ngoc NM; Le MQ; Hang NLK; Thach NC; Mallik SK. *Theranostics* 2016, 6, 231. [PubMed: 26877781]
- (149). Wu D; Zhang J; Xu F; Wen X; Li P; Zhang X; Qiao S; Ge S; Xia N; Qian S. *Microfluid. Nanofluidics* 2017, 21, 43.
- (150). Xu S; Ouyang W; Xie P; Lin Y; Qiu B; Lin Z; Chen G; Guo L. *Anal. Chem* 2017, 89, 1617–1623. [PubMed: 28208287]
- (151). Li J; Liu Q; Xi H; Wei X; Chen Z. *Anal. Chem* 2017, 89, 12850–12856. [PubMed: 29120162]
- (152). Liu C; Geva E; Mauk M; Qiu X; Abrams WR; Malamud D; Curtis K; Owen SM; Bau HH. *Analyst* 2011, 136, 2069–2076. [PubMed: 21455542]
- (153). Varona M; Ding X; Clark KD; Anderson JL. *Anal. Chem.* 2018, 90 (11), 6922–6928 [PubMed: 29757616]
- (154). Yuehong Y; Zeng Y; Chen X; Fan Y. *J. Ind. Inform. Integ* 2016, 1, 3–13.
- (155). Jusak J; Pratikno H; Putra VH. In *IEEE Int. Conference on Communication, Networks and Satellite (COMNETSAT 2016)*, Surabaya, Indonesia, 2016, pp 75–79.

Figure 1. Number of publications as year in the field of smartphone technology for disease-related applications. The data were collected through searching the PubMed database using the keywords of “smartphone” and “disease”.

Figure 2.

Smartphone-based endpoint colorimetric detection of proteins associated with pathogens. (A) Color change (from colorless to blue) developed by using H_2O_2 and 3,3',5,5'-tetramethylbenzidine (TMB) chromogen to antibody-conjugated horseradish peroxidase (HRP). (B) Direct and indirect dot-blot assays for colorimetric detection of *Mycobacterium tuberculosis* (*Mtb*). Reprinted from Sens. Actuators B Chem., Vol. 254, Li, L.; Liu, Z.; Zhang, H.; Yue, W.; Li, C.-W.; Yi, C. A Point-of-need Enzyme Linked Aptamer Assay for Mycobacterium Tuberculosis Detection Using A Smartphone, pp. 337–346 (ref 76). Copyright 2018, with permission from Elsevier. (C) Interfaces of custom-made App displaying the image processing and quantitative testing results for *Mtb* detection in (B). Reprinted from Sens. Actuators B Chem., Vol. 254, Li, L.; Liu, Z.; Zhang, H.; Yue, W.; Li, C.-W.; Yi, C. A Point-of-need Enzyme Linked Aptamer Assay for Mycobacterium Tuberculosis Detection Using A Smartphone, pp. 337–346 (ref 76). Copyright 2018, with permission from Elsevier. (D) Schematic illustration of ultrasensitive detection of *Salmonella Enteritidis* using conjugation of magnetic beads-antibody and enzyme-antibody-inorganic nanoflowers. Reprinted from Sens. Actuators B Chem., Vol. 261, Zeinhom, M. M. A.; Wang, Y.; Sheng, L.; Du, D.; Li, L.; Zhu, M.-J.; Lin, Y. Smart Phone Based Immunosensor Coupled with Nanoflower Signal Amplification for Rapid Detection of Salmonella Enteritidis in Milk, Cheese and Water, pp. 75–82 (ref 78). Copyright 2018, with permission from Elsevier. (E) Wide field-of-view (FOV) achieved by using a microprism array for smartphone imaging of multiple wells. Reproduced from Wang, L.-J.; Sun, R.; Vasile, T.; Chang, Y.-C.; Li, L. Anal. Chem. 2016, 88, 8302–8308 (ref 79). Copyright 2016 American Chemical Society. (F) Schematic illustration of a POC immunoassay in a plastic microchip with micro-pit array (μPAC) for detection of HIV p24 antigen. Reprinted from Sens. Actuators B Chem., Vol. 271, Li, F.; Li, H.; Wang, Z.; Wu, J.; Wang, W.; Zhou, L.; Xiao, Q.; Pu, Q. Mobile Phone Mediated Point-of-care Testing of HIV p24 Antigen Through Plastic Micro-pit Array Chips, pp.189–194 (ref 80). Copyright 2018, with permission from Elsevier.

Figure 3.

Smartphone-based endpoint lateral flow assays (LFAs). (A) Sandwich format in lateral flow immunoassays (LFIsAs). (B) Competitive format in LFIsAs. (C) Dual LFIsAs in a 3D printed cartridge to simultaneously detect *Salmonella Enteritidis* and *Escherichia coli* O157:H7 in food samples by a smartphone. Reproduced from Cheng, N.; Song, Y.; Zeinhom, M. M.; Chang, Y.-C.; Sheng, L.; Li, H.; Du, D.; Li, L.; Zhu, M.-J.; Luo, Y. *Anal. Chem.* 2017, 9, 40671–40680 (ref 94). Copyright 2017 American Chemical Society. (D) Four different strategies in nucleic acid lateral flow assays (NALFAs). Reprinted by permission from Springer Nature: ANALYTICAL AND BIOANALYTICAL CHEMISTRY, Posthuma-Trumpie, G. A.; Korf, J.; van Amerongen, A. *Anal. Bioanal. Chem.* 2009, 393, 569–582 (ref 95). Copyright 2009. (E) A “sample-in and answer-out” NALFA device using smartphone-based signal readout. Reproduced from Choi, J. R.; Hu, J.; Tang, R.; Gong, Y.; Feng, S.; Ren, H.; Wen, T.; Li, X.; Abas, W. A. B. W.; Pingguan-Murphy, B. *Lab Chip* 2016, 16, 611–621 (ref 96), with permission of The Royal Society of Chemistry.

Figure 4.

Smartphone-based endpoint fluorescent detection. (A) SYBR Green I (SG) (above) and EvaGreen™ (below) as ds-DNA intercalating fluorescent dyes. (B) A smartphone-based fluorescence detection platform for LAMP amplification detection with EvaGreen™ fluorescence dye. Reproduced from Chen, W.; Yu, H.; Sun, F.; Ornob, A.; Brisbin, R.; Ganguli, A.; Vemuri, V.; Strzebonski, P.; Cui, G.; Allen, K. J. *Anal. Chem.* 2017, 89, 11219–11226 (ref 98). Copyright 2017 American Chemical Society. (C) Schematic diagram of calcein-based fluorescent detection in LAMP assay. Reprinted by permission from Springer Nature: NATURE PROTOCOLS, Tomita, N.; Mori, Y.; Kanda, H.; Notomi, T. *Nat. Protoc.* 2008, 3, 877 (ref 66). Copyright 2008. (D) A smartphone-based imaging system for multiple DNA detection by LAMP assay with calcein fluorescent dye. Reproduced Hui, J.; Gu, Y.; Zhu, Y.; Chen, Y.; Guo, S.-J.; Tao, S.-C.; Zhang, Y.; Liu, P. *Lab Chip* 2018, 18, 2854–2864 (ref 100) with permission of The Royal Society of Chemistry. (E) TwistAmp exo probes used for smartphone-based RPA detection. Reprinted from *Anal. Biochem.*, Vol. 545, Chan, K.; Wong, P.-Y.; Parikh, C.; Wong, S. Moving Toward Rapid and Low-cost Point-of-care Molecular Diagnostics with a Repurposed 3D Printer and RPA, pp.4–12 (ref 106). Copyright 2018, with permission from Elsevier. (F) Fluorescence-based LFIA using NIR-to-NIR up-conversion nanoparticle and a smartphone as a fluorescence reader for the detection of avian influenza virus in opaque stool samples. Reprinted from *Biosens. Bioelectron.*, Vol. 112, Kim, J.; Kwon, J. H.; Jang, J.; Lee, H.; Kim, S.; Hahn, Y. K.; Kim, S. K.; Lee, K. H.; Lee, S.; Pyo, H. Rapid and Background-free Detection of Avian Influenza Virus in Opaque Sample using NIR-to-NIR Up-conversion Nanoparticle-based Lateral Flow Immunoassay Platform, pp.209–215 (ref 108). Copyright 2018, with permission from Elsevier.

Figure 5. Smartphone-based real-time fluorescence detection. (A) A typical workflow of real-time fluorescence detection of nucleic acid amplification by a smartphone. Reprinted by permission from Springer Nature: BIOSENSORS AND BIODETECTION, Priye, A.; Ugaz, V. M. In Biosensors and Biodetection; Springer, 2017, pp 251–266. (ref 109) Copyright 2017. (B) Smart Cup for herpes virus detection at the point of care. Reprinted from Sens. Actuators B Chem., Vol. 229, Liao, S.-C.; Peng, J.; Mauk, M. G.; Awasthi, S.; Song, J.; Friedman, H.; Bau, H. H.; Liu, C. Smart Cup: A Minimally-instrumented, Smartphone-based Point-of-care Molecular Diagnostic Device, pp.232–238 (ref 110). Copyright 2016, with permission from Elsevier. (C) Workflow illustration of an “all-in-one” diagnostic platform for multiplex detection of Zika, Chikungunya, and Dengue. Reprinted by permission from Springer Nature: BIOMEDICAL MICRODEVICES, Ganguli, A.; Ornob, A.; Yu, H.; Damhorst, G.; Chen, W.; Sun, F.; Bhuiya, A.; Cunningham, B.; Bashir, R. Biomed. Microdevices 2017, 19, 73 (ref 111). Copyright 2017. (D) Real-time convection PCR detection by a smartphone using TaqMan probes. Reprinted by permission from Springer Nature: MICROSYSTEM TECHNOLOGIES, Qiu, X.; Ge, S.; Gao, P.; Li, K.; Yang, S.; Zhang, S.; Ye, X.; Xia, N.; Qian, S. Microsyst. Technol. 2017, 23, 2951–2956 (ref 112). Copyright 2017.

Figure 6.

Smartphone-based real-time bioluminescence detection. (A) Biochemical reactions for bioluminescent assay in real-time LAMP amplification. Reproduced with permission from Public Library of Science, Gandelman, O. A.; Church, V. L.; Moore, C. A.; Kiddle, G.; Carne, C. A.; Parmar, S.; Jalal, H.; Tisi, L. C.; Murray, J. A. *PLoS ONE* 2010, 5, e14155 (ref 114). Copyright 2010. (B) A typical bioluminescence intensity curve for real-time bioluminescence detection using LAMP. Reproduced with permission from Public Library of Science, Gandelman, O. A.; Church, V. L.; Moore, C. A.; Kiddle, G.; Carne, C. A.; Parmar, S.; Jalal, H.; Tisi, L. C.; Murray, J. A. *PLoS ONE* 2010, 5, e14155 (ref 114). Copyright 2010. (C) Smart connected cup platform and its microfluidic chip for real-time bioluminescence detection of LAMP amplification. Reprinted from Song, J.; Pandian, V.; Mauk, M. G.; Bau, H. H.; Cherry, S.; Tisi, L. C.; Liu, C. *Anal. Chem.* 2018, 90, 4823–4831 (ref 115). Copyright 2018 American Chemical Society. (D) Real-time bioluminescence curves of RT-LAMP for ZIKV detection on smart connected cup shown in (C). Reprinted from Song, J.; Pandian, V.; Mauk, M. G.; Bau, H. H.; Cherry, S.; Tisi, L. C.; Liu, C. *Anal. Chem.* 2018, 90, 4823–4831 (ref 115). Copyright 2018 American Chemical Society. (E) Spatiotemporal mapping of disease detection on a Google Map using smart connected cup shown in (C). Reprinted from Song, J.; Pandian, V.; Mauk, M. G.; Bau, H. H.; Cherry, S.;

Tisi, L. C.; Liu, C. *Anal. Chem.* 2018, 90, 4823–4831 (ref 115). Copyright 2018 American Chemical Society.

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Figure 7.

Smartphone-based real-time electrochemical detection platforms. (A) Smartphone-based potentiostat platform for detection of the core antibody of hepatitis C virus (HCV). Reprinted from *Biosens. Bioelectron.* Vol. 86, Aronoff-Spencer, E.; Venkatesh, A.; Sun, A.; Brickner, H.; Looney, D.; Hall, D. A. Detection of Hepatitis C Core Antibody by Dual-affinity Yeast Chimera and Smartphone-based Electrochemical Sensing, pp. 690–696 (ref 117). Copyright 2016, with permission from Elsevier. (B) Smartphone-based handheld electrochemical detection system to measure the malarial antigen. Reproduced with permission from *Proceedings of the National Academy of Sciences USA* Nemiroski, A.; Christodouleas, D. C.; Hennek, J. W.; Kumar, A. A.; Maxwell, E. J.; Fernández-Abedul, M. T.; Whitesides, G. M. *Proc. Natl. Acad. Sci. U.S.A.* 2014, 111, 11984–11989 (ref 118). (C) Universal wireless electrochemical detector (UWED) coupled with a smartphone. Reprinted from Ainla, A.; Mousavi, M. P.; Tsaloglou, M.-N.; Redston, J.; Bell, J. G.; Fernández-Abedul, M. T.; Whitesides, G. M. *Anal. Chem.* 2018, 90, 6240–6246 (ref 119). Copyright 2018 American Chemical Society. (D) Schematic illustration of different strategies to immobilize the analyte-specific aptamers and various sandwich-type strategies to construct electrochemical aptasensor. Reproduced with permission from *Molecular Diversity Preservation International and Multidisciplinary Digital Publishing Institute*. Mishra, G. K.; Sharma, V.; Mishra, R. K. *Biosensors* 2018, 8, 28 (ref 120). Copyright 2018. (E) General design for electrochemical DNA detection. Reprinted by permission from Springer Nature: NATURE BIOTECHNOLOGY, Drummond, T. G.; Hill, M. G.; Barton, J. K. *Nat. Biotechnol.* 2003, 21, 1192 (ref 121). Copyright 2003. (F) DNA-mediated electrochemical detection using methylene blue (MB⁺) molecules. Reprinted by permission from Springer Nature: NATURE BIOTECHNOLOGY, Drummond, T. G.; Hill, M. G.; Barton, J. K. *Nat. Biotechnol.* 2003, 21, 1192 (ref 121). Copyright 2003. (G) Smartphone-based microfluidic pre-concentrator and EIS sensor for *E. coli* detection. Reprinted from *Sens. Actuators B*

Chem. Vol. 193, Jiang, J.; Wang, X.; Chao, R.; Ren, Y.; Hu, C.; Xu, Z.; Liu, G. L. Smartphone Based Portable Bacteria Pre-concentrating Microfluidic Sensor and Impedance Sensing System, pp. 653–659 (ref 122). Copyright 2014, with permission from Elsevier. (H) Smartphone-based EIS electrochemical sensor for the detection of Zika-virus protein. Reprinted by Reprinted by permission from Springer Nature: SCIENTIFIC REPORTS, Kaushik, A.; Yndart, A.; Kumar, S.; Jayant, R. D.; Vashist, A.; Brown, A. N.; Li, C.-Z.; Nair, M. Sci. Rep. 2018, 8, 9700 (ref 123). Copyright 2018.

Figure 8.

Smartphone-based digital detection platforms. (A) Handheld smartphone-based digital PCR system using a self-priming fractal branching microchannel net chip for digital PCR. Reproduced from Biosens. Bioelectron., Vol. 120, Gou, T.; Hu, J.; Wu, W.; Ding, X.; Zhou, S.; Fang, W.; Mu, Y. Biosens. Bioelectron. 2018, 120, pp. 144–152 (ref 124). Copyright 2018, with permission from Elsevier. (B) Smartphone-based microdroplet megascale detector (μ MD). Reproduced from Yelleswarapu, V. R.; Jeong, H.-H.; Yadavali, S.; Issadore, D. Lab Chip 2017, 17, 1083–1094 (ref 128) with permission of The Royal Society of Chemistry.

Figure 9.

Smartphone-based microscopy imaging platforms. (A) Smartphone-based clinical microscopy with eyepieces and objectives of standard microscope. Reproduced with permission from Public Library of Science, Breslauer, D. N.; Maamari, R. N.; Switz, N. A.; Lam, W. A.; Fletcher, D. A. PLoS ONE 2009, 4, e6320 (ref 129). Copyright 2009. (B) Smartphone-based microscopy for cell counting. Reprinted from Sens. Actuators A Phys. Vol. 274, Zeng, Y.; Jin, K.; Li, J.; Liu, J.; Li, J.; Li, T.; Li, S. Sens. A Low Cost and Portable Smartphone Microscopic Device for Cell Counting, pp. 57–63 (ref 130). Copyright 2018, with permission from Elsevier. (C) Lens-free cellphone-based microscopy. Reproduced from Tseng, D.; Mudanyali, O.; Oztoprak, C.; Isikman, S. O.; Sencan, I.; Yaglidere, O.; Ozcan, A. Lab Chip 2010, 10, 1787–1792 (ref 131) with permission of The Royal Society of Chemistry. (D) Smartphone-based microscopy using ambient illumination as a light source. Reproduced from Lee, S. A.; Yang, C. Lab Chip 2014, 14, 3056–3063 (ref 132) with permission of The Royal Society of Chemistry. (E) Smartphone-based quantitative fluorescence microscopy for detecting *S. aureus* cells. Reprinted from Biosens. Bioelectron. Vol. 109, Shrivastava, S.; Lee, W.-I.; Lee, N.-E. Culture-free, Highly Sensitive, Quantitative Detection of Bacteria from Minimally Processed Samples Using Fluorescence Imaging by Smartphone, pp. 90–97 (ref 133), with permission from Elsevier. (F) Portable smartphone-based microscope with exchangeable 3D printed accessories for detection of *E. coli* O157:H7 in foods. Reprinted from Biosens. Bioelectron. Vol. 99, Zeinhom, M. M. A.; Wang, Y.; Song, Y.; Zhu, M.-J.; Lin, Y.; Du, D. A Portable Smart-phone Device for Rapid and Sensitive Detection of *E. coli* O157:H7 in Yoghurt and Egg, pp. 479–485 (ref 134), with permission from Elsevier.