Biotechnology Advances 34 (2016) 291–304

Contents lists available at ScienceDirect

Biotechnology Advances

journal homepage: www.elsevier.com/locate/biotechadv

Research review paper

Medical diagnostics with mobile devices: Comparison of intrinsic and extrinsic sensing

L. Kwon [b,1], K.D. Long [b,1], Y. Wan [c,1], H. Yu [a,1], B.T. Cunningham [a,b]

[a] Department of Electrical and Computer Engineering, University of Illinois at Urbana-Champaign, Micro and Nanotechnology Laboratory, 208 North Wright Street, Urbana, IL, United States
[b] Department of Bioengineering, University of Illinois at Urbana-Champaign, Micro and Nanotechnology Laboratory, 208 North Wright Street, Urbana, IL, United States
[c] School of Electronic and Information Engineering, Beihang University, 37 Xueyuan Road, Beijing, China

ARTICLE INFO

Article history:
Received 1 October 2015
Received in revised form 27 February 2016
Accepted 28 February 2016
Available online 4 March 2016

Keywords:
Biosensors
In vitro diagnostics
Mobile biosensors
Point of care sensors
Point of use sensors

ABSTRACT

We review the recent development of mobile detection instruments used for medical diagnostics, and consider the relative advantages of approaches that utilize the internal sensing capabilities of commercially available mobile communication devices (such as smartphones and tablet computers) compared to those that utilize a custom external sensor module. In this review, we focus specifically upon mobile medical diagnostic platforms that are being developed to serve the need in global health, personalized medicine, and point-of-care diagnostics.

Contents

1. Introduction

Since the first successful commercial introduction of smartphones in 2004, it is estimated that 6 billion mobile phones are in use worldwide (Laksanasopin et al., 2015), with nearly 1.2 billion smartphones sold in 2014 alone (Lunden, 2015). Their combination of technologies that

E-mail address: bcunning@illinois.edu (B.T. Cunningham).
[1] Co-authors contributed equally to preparation of this review.

http://dx.doi.org/10.1016/j.biotechadv.2016.02.010
0734-9750/

Table 1
Summary technologies and applications for intrinsic and extrinsic sensing approaches for smartphone-based medical diagnostics.

Intrinsic methods								
Label	SYTO16	SYTO16	Alexa488	Cy3	None	None	None	None
Analyte	Microparticle, white blood cell, pathogenic protozoan parasite	Red and white blood cell, hemoglobin	Nanoparticle, human cytomegalovirus	microRNA-21	Eggs of soil-transmitted helminth	Porcine immunoglobulin G	β_2 microglobulin	Hepatitis B, HIV
Source	LED array ($\lambda = 470$ nm)	LEDs ($\lambda =$ white, 430, 470 nm)	Laser diode ($\lambda = 450$ nm)	Laser pointer ($\lambda = 532$ nm)	Incandescent flashlight	Incandescent light bulb	Smartphone screen	Smartphone flash
Assay format	Capillary tube, Slide glasses	Plastic cuvette, cytometric chamber	coverslips	Plastic cuvette	Kato–Katz thick smear slides	Photonic crystal sensor	Fluidic device	Plastic cuvette
Readout method	Fluorescence microscopy	Fluorescence/bright field microscopy, absorption	Fluorescence microscopy	Fluorescence spectroscopy	Bright field microscopy	Resonance transmission spectroscopy	Reflection dip of angle-resolved SPR	Reflected light intensity
Authors	Zhu et al.	Zhu et al.	Wei et al.	Yu et al.	Bogoch et al.	Gallegos et al.	Preechaburana et al.	Giavazzi et al.

Sample type	Solid phase									Liquid phase		
Analyte	Thrombin	*Salmonella*	Cholesterol, total bile acid	*Salmonella*, TSH	pH for urinalysis	Urinalysis (multiple analytes)	Urinalysis (multiple analytes)	Malaria, tuberculosis, HIV	Blood type	PSA		Mumps, Measles, HSV
Source	LED ($\lambda = 470$ nm)	LED ($\lambda = 475$ nm)	Biochemilu-minescence	Smartphone flash	Ambient light	Ambient light	Ambient light	LED array ($\lambda = 565$ nm)	Ambient light	LED ($\lambda = 450$ nm)	UV lamp ($\lambda = 340 \sim 400$ nm	LED array ($\lambda = 464$ nm)
Assay format	Paper in glass/ PDMS wells	Paper microfluidic	Paper microfluidic	Paper microfluidic	Paper test strip	Paper test strip	Paper test strip	Lateral flow-based RDT strip	Paper microfluidic	Microcapillary strip		96-well plate
Readout method	Fluorescent light intensity	Fluorescent light Intensity	Biochemilu-minescence light intensity	Mie- Scattered light intensity	Color change	Color change	Color change	Spatial distance	Spatial distance	Absorption	Fluorescent light intensity	Fluorescent light intensity
Authors	Petryayeva et al.	Fronczek et al.	Roda et al.	Park et al.	Shen et al.	Hong et al.	Yetisen et al.	Mudanyali et al.	Guan et al.	Barbosa et al.		Berg et al.

Extrinsic methods						
Connection	USB			Audio jack	Bluetooth	WiFi
Analyte	*pf*HRP2 antigen	DNA from *Bacillus cereus*	DNA from Kaposi's sarcoma herpesvirus	HIV, syphilis protein antigens	Horseradish peroxidase	DNA from *Escherichia coli* and *Staphylococcus aureus*
Source	Powered by smartphone	Powered by smartphone	LED ($\lambda = 520$ nm)	Powered by smartphone	Powered by external Li-ion battery (3.7 V)	Addressable green LEDs
Assay format	Microfluidic chip	Microfluidic chip	Microfluidic cartridge	Disposable cassette	Electrochemical cells cartridge	Microfluidic chip
Readout method	Electrical signal	Electrical signal	Optical density	Quantitative optical density	Electrical signal	Fluorescent light intensity
Authors	Lillehoj et al.	Velusamy et al.	Mancuso et al.	Laksanasopin et al.	Salomón et al.	Stedtfeld et al.

include wireless voice or data communication, internet connectivity, computation, video display, data storage, and audio playback has enabled smartphones to find broad acceptance throughout our society, representing a technological capability that many people carry with them throughout the day, wherever they travel. Intense competition for the smartphone market has led to ever-broadening arrays of features, while technologies for manufacturing and packaging the large variety of components has resulted in devices that are compact and low cost. In order to serve the many segments of the smartphone market, we have experienced a proliferation of models, with many operating system platforms, storage capacities, camera pixel densities, and screen sizes. The emergence of tablet computers may be considered to be an extension of the smartphone market, representing the largest size segment of what we refer to as "mobile devices." (See Table 1.)

Not long after the introduction of mobile devices and the open-source market for innovative software applications that could take advantage of their capabilities, the potential for mobile devices to be utilized for medical diagnostic instruments was considered as a possibility. Nearly every mobile device contains several "intrinsic" sensors: CMOS imaging camera, audio input jack, vibration sensor, rotation sensor, GPS sensor, microphone, and light level sensor. While these sensor inputs to a mobile device were initially conceived for non-medical uses such as recreational digital photography (for the camera) or adjustment of the screen brightness (for the light level sensor), it has been shown that these same sensors, with the aid of special-purpose interfaces and software, could be used for microscopy, readout of electrochemical sensors, readout of assay test strips, spectroscopy, and image/pattern recognition in the context of medical diagnostics.

The advantages of using the existing sensor infrastructure within mobile devices for medical tests are compelling. By utilizing a device that patients have already purchased for other purposes, whose operation is already deeply familiar for people with non-technical backgrounds, the potential for millions of medical instruments to breach an important market entry barrier is eliminated. The potential need for special purpose "cradles" that help make a physical interface between a mobile device and inexpensive external components is a widely accepted strategy for smartphones or tablets to operate in conjunction with automobiles and entertainment systems. A product developer who chooses to utilize an existing mobile device platform as the core of their diagnostic system can leverage the enormous investment in packaging, component performance optimization, manufacturing volume, manufacturing efficiency, and software development tools that exist for creating mobile device hardware and operating system software. Without the need to re-engineer the optics of an imaging camera, the audio processing circuitry that interfaces with a microphone, or the spectral output of a white LED, a product designer may focus on the unique aspects of their diagnostic instrument, while making use of a wide array of pre-existing software tools that enable control, data extraction, and data processing from integrated sensors.

The choice to use the integral sensing components of mobile devices for medical diagnostic instruments also brings several challenges. The most obvious challenge is posed by the rapid rate of mobile device technology development, which results in a "state-of-the-art" phone of today becoming obsolete in 2–3 years, as newer models with improved features become available. While rapid technology development generally will make mobile medical devices even more powerful (through cameras with higher pixel density, faster computation, and availability of more memory, for example), a diagnostic technology product will need to flexibly adapt to ever-changing specifications of its underlying sensor technology. Making this situation even more complex is the lack of standards between competing mobile device manufacturers. Thus, a diagnostic instrument that relies upon the operation of an integral component such as the back-facing imaging camera may need to be available in several variants, for compatibility with the camera resolution, optical filters, software control, lens magnification, lens position, and operating system interface for a wide range of brands and models. This situation is already the case for non-medical apps and hardware for mobile devices, where companies must create cases, mounts, and software updates for a wide range of devices — sometimes choosing to support one platform over another. A second challenge involves the fundamental technical limitations of the sensors used within mobile devices, which have been selected solely for their utility in mass market consumer applications. Using the camera as an example, the CMOS imaging chips used in mobile devices are engineered only for detection of wavelengths in the visible part of the spectrum. If an application requires detection of ultraviolet or infrared wavelengths, it may not be possible to use the integrated image sensor instead, an external detection system that can meet the requirement would have to be developed.

Therefore, the main advantage of using sensors that are extrinsic to a mobile device is flexibility. Without the constraints of sensors that are contained within commercially available smartphones, the product designer may select from a much wider array of sensing components. By developing a diagnostic device with extrinsic sensors, the product designer becomes responsible for integrating the sensor with circuitry for driving the electronics, modulating power from a battery, amplifying output signals, integration, packaging, heat management, and software development. Thus, product development becomes more expensive and time consuming, while requiring more dedicated expertise. These challenges pose a substantial barrier to entry, due to the requirement for engineering time, product development experience, and, importantly, funding required to develop a product. The resulting detection system will be extrinsic to the mobile device and is likely to carry a higher price than a conventional smartphone due to the development cost being spread over a relatively smaller number of units.

While diagnostic systems using extrinsic sensors may not be as portable as those that use integral, or intrinsic, sensors, they may still fully take advantage of the communication capabilities and computational power of mobile devices. The ability for a "standalone" instrument to communicate data with a mobile device via a wireless network, Bluetooth, or a wired connection through a USB data port is now common for many consumer device technologies, and can be implemented very inexpensively. Therefore, an extrinsic sensor system is not required to be in physical contact with a mobile device in order for the mobile device to control the instrument, gather data from it, process the data, and share the data with external servers or data management systems.

Several review papers have discussed the role of mobile devices on the personalization, globalization, and democratization of medical diagnostics and health monitoring (Erickson et al., 2014; Ozcan, 2014; Vashist et al., 2015a, 2015b, 2013). The vast array of medical-purpose "smart" accessories and applications (not necessarily interfacing with a smartphone or tablet) has previously been categorized based on the bioanalytical application and the stage of development and/or commercialization. In this paper, we review the recent development of medical diagnostics that incorporate a mobile device, such as a smartphone or tablet computer, as a central enabling component of their hardware. We have broadly categorized the highlighted approaches as using either the "intrinsic" sensing capabilities of the mobile device itself, or an add-on "extrinsic" sensor that communicates with the mobile device. Among the "intrinsic" approaches, we found that utilization of the mobile device's imaging camera to be the predominant capability, where the internal CMOS imaging camera may be used to either take photographs of a solid or liquid phase assay, or may be adapted to perform microscopy, spectroscopy, or label-free biodetection with the inclusion of a custom interface. Among the extrinsic approaches, we highlight efforts in which an external instrument transfers information to a mobile device via a hard-wired USB connector, wireless Bluetooth interface, or through the earphone connector. While the majority of the detection approaches and applications are currently in the research/development phase and some have not yet risen to the level of truly functioning as a medical diagnostic, commercial instruments are starting to emerge, and are also briefly discussed in this review.

2. Biodetection approaches using intrinsic sensors

2.1. Measuring solid-phase assays using intensity information from the camera

Humans have evolved with sight as their dominant sense. Therefore, it should come as no surprise that many ways in which we have traditionally measured our surroundings provide visual readouts. Light provides a myriad of tools used in medicine and human health: the color of urine, the emission of fluorescent proteins, the interaction of x-rays with human tissues—each provides information that is clinically and scientifically of interest. Thus, a majority of the first demonstrations of smartphone biosensing utilize the camera to transmit information between the experiment and the sensor.

One of the first demonstrated examples utilized colorimetric assays that could be performed by paper-based microfluidics for quantification of glucose and protein content within simulated urine (Martinez et al., 2008). Since then, a broad range of tests have been demonstrated using readily available bodily fluids and many different biological analytes. All of these tests rely on the same basic principles: a biological liquid sample, containing an unknown concentration of analyte, is introduced to a solid phase with immobilized chromogen. Upon reaction between the biological molecules and the chromogen, a colored region is produced on the solid support (often comprised of paper), where the amount of color produced is directly correlated to the quantity of the analyte. The colors are captured via the on-board camera of the smartphone where a variety of image processing techniques are used to quantify the color change and to provide a calibrated measurement of the biomolecule of interest. Many such tests have been demonstrated in the literature, particularly with gold-based nanoparticles (Lee et al., 2014; Oncescu et al., 2014; Oncescu et al., 2013; Veigas et al., 2012).

An example of a fluorescence-based modality was recently demonstrated for detection of analytes within whole blood (Petryayeva and Algar, 2015), representing a complicated diagnostic fluid due to the high concentration of cellular material, the presence of clotting factors, and high viscosity. The detection setup consisted of a LED source illuminating a microfluidic channel with a bandpass filter between the rear-facing camera and the sample (Fig. 1A). To take this detection modality and apply it to red, opaque whole blood, a quantum-dot based Forster resonant energy transfer (FRET) assay was developed that would allow for maximum transmission of light through blood (high visible wavelength), use affordable LEDs as the source, and maintain appropriate FRET efficiency for meaningful measurements. Thrombin, the analyte of interest, has proteolytic activity, and when present in the liquid sample, cleaves a recognition site separating the FRET acceptor, Alexa Fluor 647 from the quantum dot (QD) FRET donor, recovering the previously quenched QD emission in a manner proportional to the thrombin activity (Fig. 1B). The bandpass filter placed between the sample and the camera removed the light from the FRET acceptor, so the measured intensity of QD-emitted light observed by the camera has a positive correlation to the thrombin activity.

Another fluorescent-based system has been demonstrated in which (Fronczek et al., 2014) capture of DNA from *Salmonella typhimurium* in a paper-based microfluidic device is followed by labeling with a fluorescent intercalating dye that enables detection of *Salmonella* from diluted patient samples with concentrations as low as 10^4 CFU/mL.

Fluorescence emission and optical absorption detection of colored material constitute the most common modalities for colorimetric intensity measurement; however a few additional methods have been demonstrated. A smartphone attachment was designed to illuminate and gather an image from a paper cartridge that contains reagents necessary to perform a biochemiluminescent reaction based upon an enzymatic

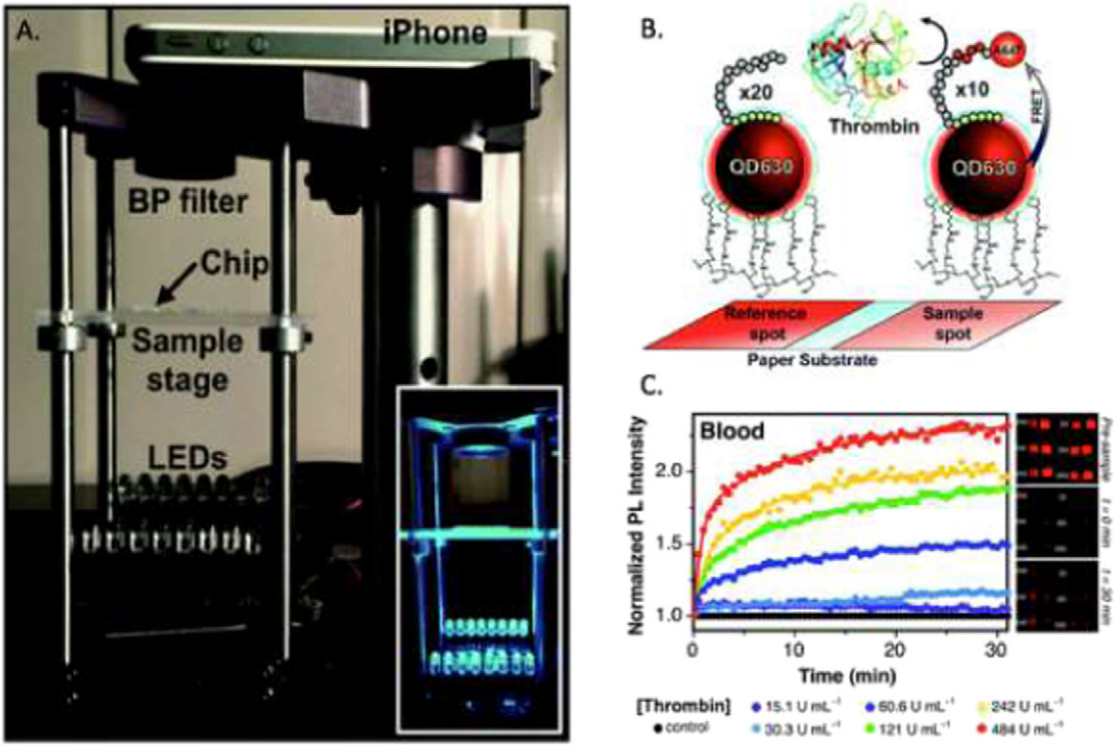

Fig. 1. Fluorescence-based detection of thrombin activity on paper microfluidic devices. A. Photograph of the setup used for smartphone readout of QD-FRET test strip assays with serum and blood samples. The inset shows the setup with LED-based illumination of the sample. B. Design of paper test strips to measure thrombin activity via FRET with immobilized QD donors and A647 acceptor dye-labeled peptide substrates containing a cleavage site recognized by thrombin. The average number of peptides per QD is indicated (10×, 20×). Protease activity was measured through the recovery QD PL with loss of FRET. C. Normalized progress curves for thrombin activity in whole blood samples measured via smartphone imaging. Representative smartphone images are shown for three points in the assays: prior to the addition of sample, immediately after adding sample, and after 30 min. In each image, the spiked thrombin activities were (i) 0, (ii) 15.1 NIH U mL − 1, (iii) 30.3 NIH U mL − 1, (iv) 121 NIH U mL − 1, (v) 242 NIH U mL − 1, and (vi) 484 NIH U mL − 1.
Adapted from Petryayeva and Algar (2015) with permission from The Royal Society of Chemistry.

reaction (Roda et al., 2014). Using a paper microfluidic device, samples of blood and sputum were analyzed for cholesterol and total bile acid. A second unique demonstration of optical-based intensity measurement involves a smartphone-based instrument that quantifies the amount of Mie scattering as a result of antibody-mediated agglutination (Park et al., 2013; You et al., 2013). In an attempt to minimize variation introduced by inconsistencies inherent to paper based microfluidics and wavelength-based lighting sensitivities, the investigators developed an angle-tuned system that quantified *Salmonella* concentrations down to 10^1 CFU/mL.

2.2. Measuring solid-phase assays using color information from the camera

While the paper based assays were each specifically developed for a custom-designed assay protocol, there has also been an effort to utilize a smartphone to quantify solid-phase colorimetric assays that already exist. Such tests are most practical when precise quantification of biological analyte concentration is not important. For example, urine pH fluctuates significantly depending on diet and feeding schedule. While a particularly high or low pH may be diagnostic on its own, more often, a moderately high-or-low pH value may provide information necessary in combination with other tests to suggest a possible pathology. Because precise knowledge of the pH value does not have much clinical utility, the naked eye is often used to estimate the color of an assay test strip. As the human eye is much more effective at discerning differences in color than it is at discerning differences in intensity of a single color, many of these already-developed tests could benefit from the use of a smartphone camera image to differentiate between colors. In 2012 proof-of-concept of this sensing modality with test strips used for urinalysis was demonstrated (Shen et al., 2012). Since then, attempts to develop this concept have focused on measuring multiple analytes simultaneously with a single sample (Fig. 2A), or across multiple smartphone platforms (Fig. 2B) (Hong and Chang, 2014; Yetisen et al., 2014).

2.3. Measuring solid-phase assays using location information from the camera

In addition to intensity and color-based measurements, a third and final modality for solid-phase test strip measurement has been demonstrated: relative and absolute spatial distances. Perhaps the most readily available example of such a test would be a pregnancy test, where a band of immobilized antibody located at a specific position with respect to a control band yields either a positive or negative result. Here the benefit of the smartphone is not based on accurate quantification as most of these tests have a binary positive/negative outcome. Rather, the value of using a mobile device to perform the test is integration with the cloud, ease of transmission to health care providers, and associated diagnostic information that can be delivered via a smartphone app in conjunction with the use of the device. A practical use of such a device—a universal reader for this broad class of rapid diagnostic tests has been demonstrated (Fig. 3A)(Mudanyali et al., 2012). A similar approach was taken to design a smartphone-based blood-typing system (Guan et al., 2014). A custom-made paper microfluidic device is spotted with blood in three places, and a liquid elution buffer is passed over it. By using a parallel set of immunological reactions, blood typing can be 'decoded' from the banding pattern present on the paper device (Fig. 3B). 98 patient samples were correctly typed with the device, demonstrating a device that has relatively few remaining areas of improvement before a potential commercial product could be introduced.

2.4. Measuring liquid-phase assays with the camera

While many existing colorimetric tests used at the point of care exist as solid phase samples, an even larger set of colorimetric tests have been developed in the liquid phase for use in laboratories where liquid handling technology is readily available. As a majority of biomarkers are detected in liquids, colorimetric detection of liquid-phase samples has been thoroughly developed. Often run in a spectrometer or a 96-well plate reader, these tests serve as fundamental components of contemporary medical diagnostic laboratories.

There are several examples of single-chamber measurement attachments that operate in a similar fashion: light is passed through a sample chamber that is full of liquid, some light is absorbed or fluoresced, and the remaining light is measured by the CMOS camera. As with the intensity-based measurements of solid-phase samples, intensity values are derived from RGB values, whether by direct comparison with

Fig. 2. Multi-analyte analysis and multi-platform analysis of existing solid-phase colorimetric tests. A. Image of a urine analysis test strip consisting of 12 paper-based sensors in array. Image analysis is able to recognize the pattern and location of the tests, and process the results individually. Adapted from Hong and Chang (2014) with permission from The Royal Society of Chemistry. B. Multi-platform compatibility of a smartphone-based system for solid-phase colorimetric tests. Authors demonstrated both Android and iOS compatibility, but only the latter is shown. App was capable of permitting calibration, testing, and test history activities over several sensor types. Detection zones are detected automatically, and test results are displayed to the user before being transmitted to a physician at the user's request. Adapted from Yetisen et al. (2014)) with permission from Elsevier.

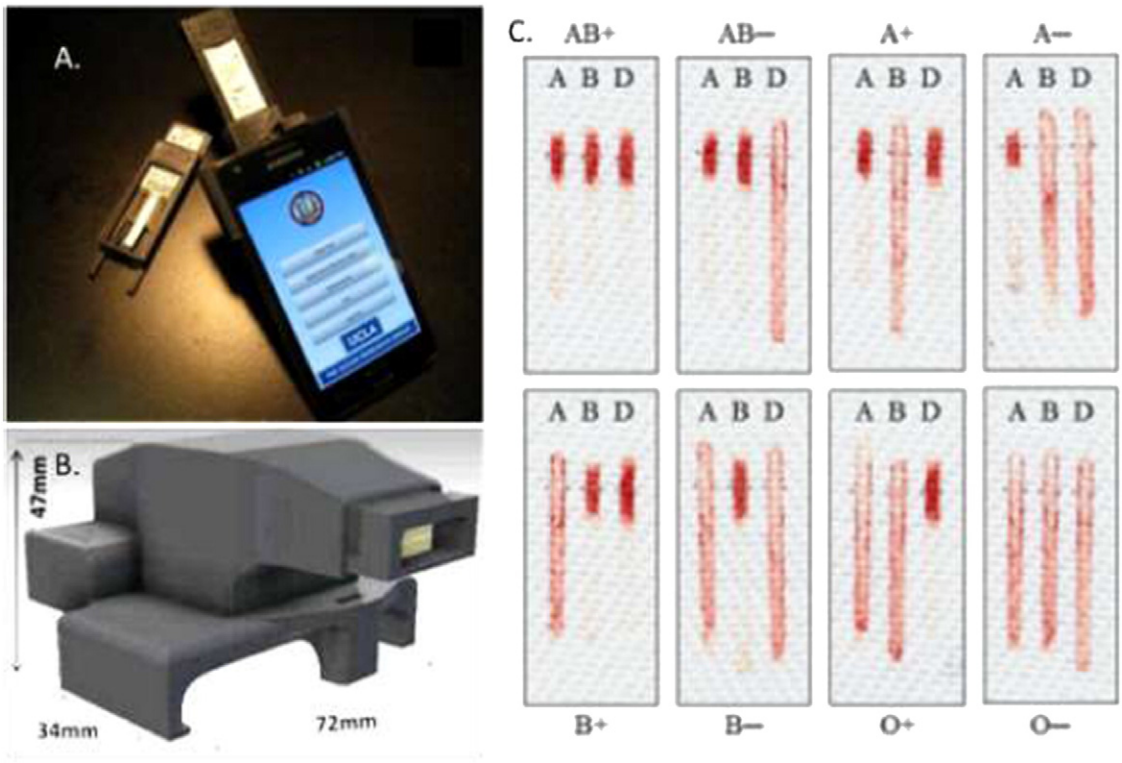

Fig. 3. Distance-based analysis of solid-phase colorimetric tests. A. A smartphone based system for reading a broad range of existing rapid-diagnostic-test strips. To accommodate the many types of RDTs, customized sample trays are used. B. Schematic representation of the cradle attachment in A., demonstrating simplicity and ease of attachment with simple slide. Two AAA batteries are used to power LED arrays for both transmission and reflection modalities, controlled by a small switch. A., B. Adapted from Mudanyali et al. (2012) with permission from The Royal Society of Chemistry. C. Barcode-like readouts for all 8 ABO/RhD bloodtypes. Using hydrophilic bar channels treated with Anti-A, B, and D antibodies, agglutination enables visually-based readout via smartphone. C. Reprinted with permission from (Guan et al., 2014). Copyright 2014 American Chemical Society.

known concentrations (Coskun et al., 2013a; Coskun et al., 2013b), RGB-channel isolation (Awqatty et al., 2014), or RGB conversion to another colorspace (Smith et al., 2014).

While these liquid phase tests open the door for the direct translation of a myriad of laboratory diagnostic tests to a smartphone platform, many such tests require some degree of multiplexing for practical use. Two research groups have arrived at similar solutions for collecting data from multiple wells of liquid simultaneously involving a tablet in addition to a smartphone to provide uniform background illumination (Vashist et al., 2015b; Wargocki et al., 2015). By using a simple spacer (either a box, or a custom 3D printed stand), the phone can be held consistently at a distance that will allow for multiple wells to be measured within the same field-of-view on the smartphone camera. Along the same lines, another research group sought to capture images of entire 96-well plates with a single exposure, but instead did so with a custom-built 96 fiber bundle that allows each well to be mapped to a CMOS camera chip at the same time (Berg et al., 2015) (Fig. 4A). This 3D-printed cradle also contains an array of blue LEDs to provide uniform illumination without the need for a second portable electronic device. Using this system, a substantial number of ELISA tests were run on patient samples for mumps, measles, and HSV showing accuracies over 99%.

Another recent innovation takes a slightly different approach. A microcapillary film (MCF) was utilized to run parallel ELISA tests that can be monitored simultaneously as liquid flows through them (Fig. 4B) (Barbosa et al., 2015). This setup consists of a light source (either broadband or UV) illuminating the MCF, which then is photographed by an iPhone with a 60× lens attachment. The MCF is attached to a plastic syringe that simultaneously controls all 8 channels, so fluid handling is simplified. Using this detection instrument, the smartphone system was able to detect down to 0.9 ng/mL concentrations of prostate specific antigen. While this instrument is not yet held in a casing to make it truly portable, it provides clear proof-of-concept for an alternative viable solution to multiplexing liquid phase tests for intrinsic camera analysis.

2.5. Smartphone-based microscopy

For portable medical diagnostics, there is a strong need for an inexpensive mobile device that can perform microscopy, which can replace a traditional laboratory microscope for observing cell and tissue samples. Nearly all smartphones now incorporate a high-resolution camera and computational power sufficient for image processing, which enable them to function in this role. Although the first uses of a mobile phone for microscopy were limited to simple image capture from the eyepiece of an ordinary laboratory microscope (Frean, 2007) or with a custom-designed microscope attachment including a LED, lenses, filters, an objective, and an eyepiece (Breslauer et al., 2009). The applications for microscopy are evolving rapidly using simplified optics and the mobile phone's capabilities for digital image capture, image processing/storing/sharing, and on-screen data display (Bogoch et al., 2013; Wei et al., 2013; Zhu et al., 2013, 2011). A custom-designed microscope attachment for a mobile phone, integrating several optical elements, is configured to facilitate sample loading and to improve the diagnostic specificity. The attachment incorporates the illumination source, holds the test samples, and filters out unwanted signal proceeding toward phone's camera, while the mobile device gathers and processes images within the field-of-view. Most smartphone-based microscope systems are designed to be implemented on specific phone models, with small modifications required to adapt to a different types of phones.

An example of mobile device microscopy is the compact (35 × 55 × 24 mm) and light-weight (~28 g) wide-field fluorescence imaging system (Zhu et al., 2011). The attachment includes 3 battery-powered light emitting diodes (LEDs) for illumination, a lens to gather

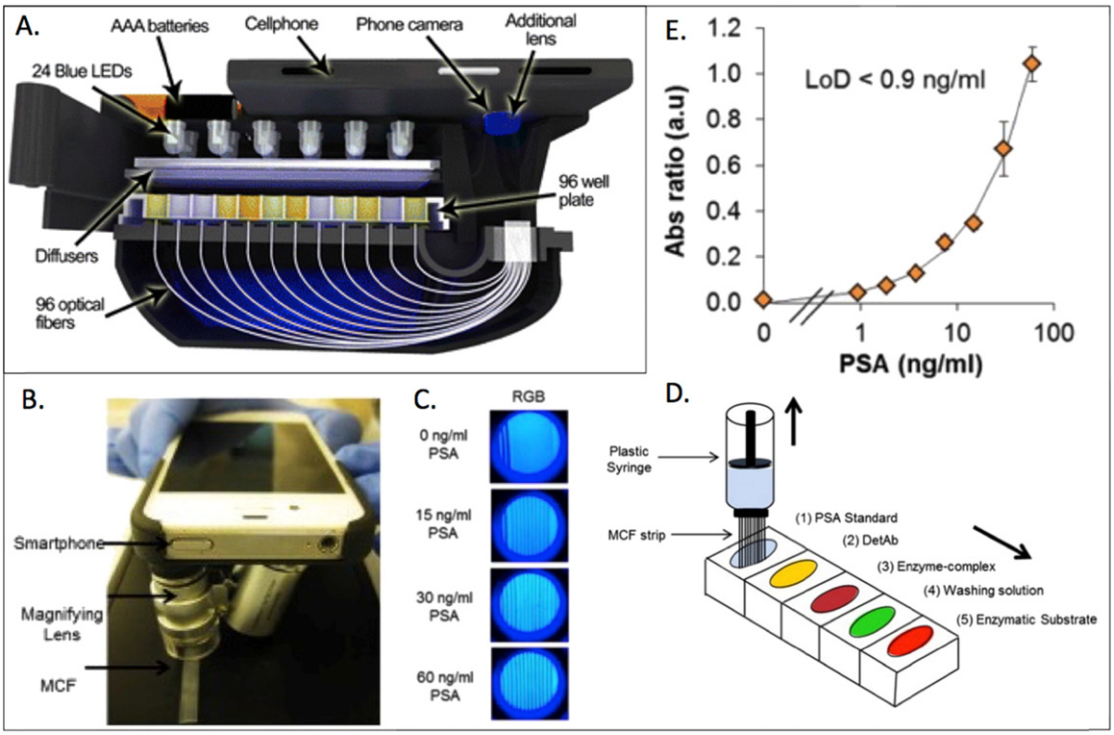

Fig. 4. Demonstrations of simultaneous colorimetric measurement of multiple liquid samples. A. Schematic overview the cellphone-based ELISA reader showing array of optical fibers used to consolidate images of a 96-well plate for interfacing with a smartphone camera. Adapted from (Berg et al., 2015) governed by ACS AuthorChoice open access, doi: 10.1021/acsnano.5b03203. B. MCF-based system for simultaneous analysis of 8 microfluidic channels. C. Images captured from the system in B. demonstrating concentration-based dependence in measured fluorescence across the channel cross-section. D. Assay steps for PSA test. E. Response curves for PSA in buffer via the system demonstrating LOD below 0.9 ng/mL, including concentrations imaged in C. Adapted from (Barbosa et al., 2015) governed by http://creativecommons.org/licenses/by/4.0/, doi:10.1016/j.bios.2015.03.006.

the fluorescence emission, and a plastic color filter to create the necessary dark-field background. The CMOS sensor of a mobile phone camera takes fluorescent images of specimens, and the platform achieves an imaging field-of-view of ~81 mm^2 with a raw spatial resolution of ~20 μm. The resolution can be further doubled (~10 μm) through digital signal processing based on compressive sampling. One of the important features of the system is the lens-free butt-coupled LEDs, which illuminate the sample from the side and excite it uniformly over the illumination area. Because the excitation light is guided perpendicular to the detection path, a simple plastic color filter can be used to create the dark-field background. The authors demonstrate the system capabilities by imaging fluorescent microparticles (*dia.* = 10 μm) emitting two different colors (λ_1 = 515 nm, λ_2 = 605 nm), white blood cells (WBCs) labeled with nucleic acid staining, and pathogenic protozoan parasites such as *Giardia lamblia* cysts. Due to its large field-of-view, the system can screen large sample volumes (>0.1 mL) for rapid screening of blood, urine, and saliva. While low cost and portability are advantages of the phone-based fluorescent microscopy platform, its main limitation is low spatial resolution, which remains approximately 10 μm. The spatial resolving power is sufficient to detect micro-beads, WBCs, or protozoan parasites, although smaller objects like single nano-particles and nanoscale viruses cannot be imaged with this approach. The same research group announced the extension of the capabilities of mobile phone based microscopy by adopting a cytometry platform, which performs blood analyses such as density measurements of white blood cells, red blood cells (RBCs), and hemoglobin (Zhu et al., 2013). The system requires very small sample volume (~10 μL of whole blood/test). The process is fast and provides cell or hemoglobin concentration information in less than 10 s for each measurement. The attachment, fabricated by a 3D printer, consists of a universal port for holding three add-on components in addition to separate optical components specifically for WBC counting, RBC counting, and hemoglobin concentration measurement. Fluorophore-labeled WBCs are illuminated by a butt-coupled blue LED (λ = 470 nm) and counted using emission along a perpendicular optical path through a plastic filter installed in front of the camera, while unlabeled RBCs are counted using bright field illumination/imaging. The hemoglobin concentration of blood samples is determined by measurement of light absorbance using a blue LED (λ = 430 nm) through a cuvette. A custom Android app processes the captured image from the three modalities. The app computes the selected sample's concentration using two different algorithms, one for the blood cells and the other for the hemoglobin, based on pixel-by-pixel analysis, and reports the results as a number of cells/μL (WBC, RBC) and as grams/dL (hemoglobin) on the smartphone screen.

Recently, smartphone based fluorescence microscopy has been extended toward detection of isolated nanoscale objects, as shown in Fig. 5A (Wei et al., 2013). A compact and portable opto-mechanical attachment interfaces with a smartphone camera, which enables users to detect individual fluorescent nano-particles and viruses. The attachment's body, fabricated by a 3D printer, holds a blue laser diode to excite fluorophores, a lens to collect the fluorescence for the specimen, a mechanical translation stage to aid alignment of optical components, and a long-pass (LP) thin film interference filter to prevent the excitation source from reaching the smartphone camera. The systems weighs ~186 g. When the test sample is excited and emits fluorescence, the smartphone camera works as a fluorescence detector with field-of-view of 0.6 × 0.6 mm. The authors validate the performance of the system by imaging isolated nano-objects like fluorescent polystyrene (PS) beads (*dia.* = 100 nm) and human cytomegaloviruses (HCMVs) labeled with fluorescent PS particles (λ_{ex} = 580 nm, λ_{em} = 605 nm). The adoption of a high-power (~75 mW) laser diode, oblique incidence (~75°) of the laser beam, and a high-performance LP filter contribute to its performance (Fig. 5B). Fig. 5C summarizes the arrangement of components within the attachment with respect to the mobile phone camera. This approach provides high resolution and sensitivity, but the active field-of-view is smaller than the full field-of-view (~3 × 3 mm) due to the

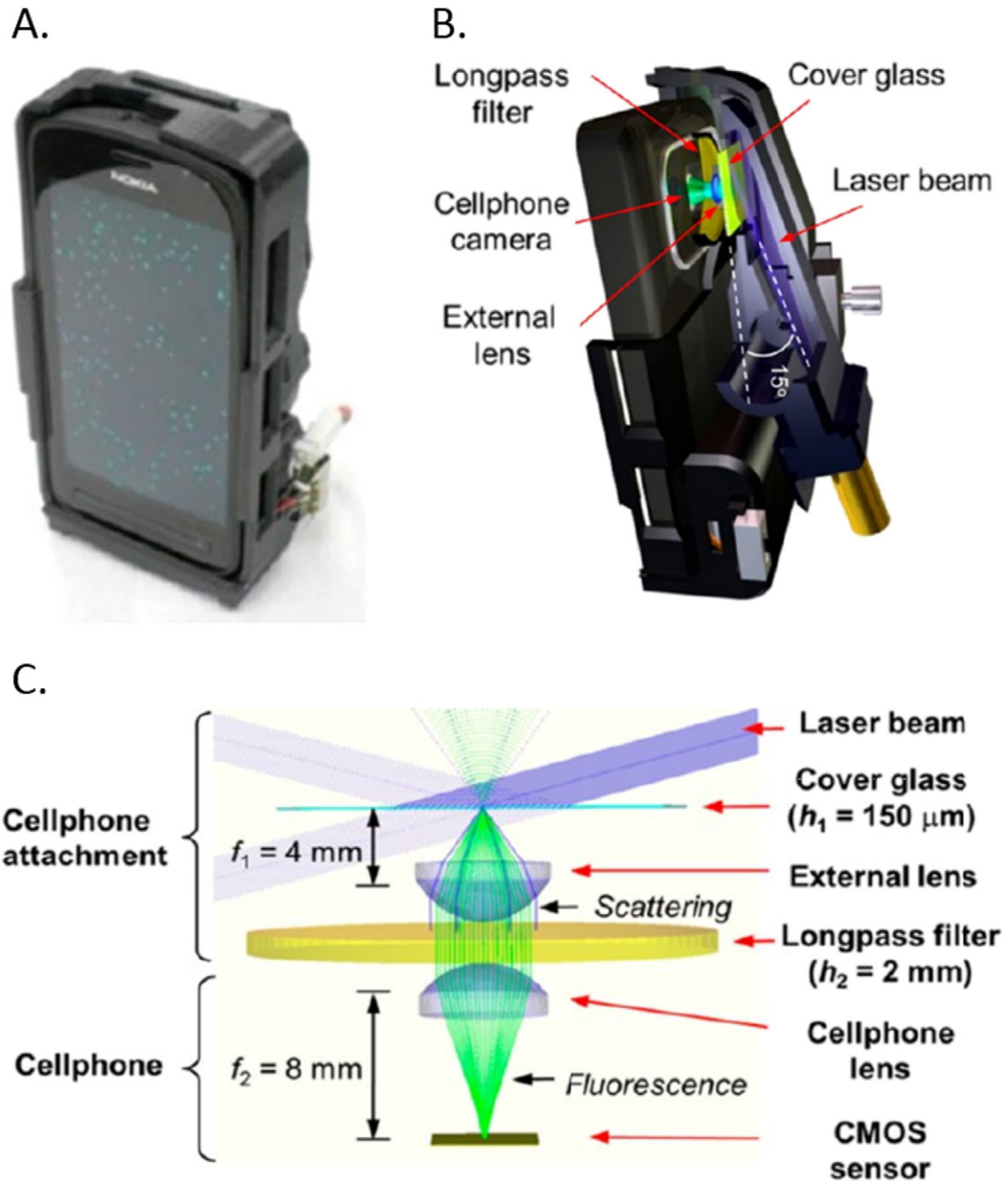

Fig. 5. Smartphone-based fluorescence imaging system for detection of nanometer-scale objects (Wei et al., 2013). A. Microscope attachment installed on the smartphone shows the fluorescence image of green fluorescent beads (*dia.* = 1 μm). B. Schematic illustration of the system's back side. C. Ray-tracing diagram of the system near the camera. The fluorescence is depicted with solid green rays, while excitation and scattered beams are highlighted with solid blue and weak blue rays, respectively.
Reprinted with permission from the American Chemical Society.

small beam size of the laser diode (*dia.* = ~1.8 mm) and the aberration of the low NA imaging optics.

Another approach that further simplifies mobile phone microscopy for the practical diagnosis of parasitic worms was tested in the field (Bogoch et al., 2013). A mobile phone was transformed into a microscope by mounting a 3-mm ball lens onto the center of the camera using double-sided tape. The system can be easily assembled in a few minutes at a cost of ~$15. The samples of interest were illuminated by a battery-powered incandescent flashlight, and the intrinsic digital zoom function enabled detection of eggs of soil-transmitted helminth such as *Ascaris lumbricoides* and *Trichuris trichiura*. The system is capable of up to 60× magnification, and registered a sensitivity of 69.4% for detecting soil-transmitted helminth infection.

2.6. Smartphone-based spectroscopy

While gathering color images with a smartphone camera in which the spectral information is partitioned into red, green, and blue (RGB) pixels with distinct wavelength bands can provide rudimentary color spectra, the ability to truly measure the spectral characteristics of liquid absorption, fluorescence emission, or scattered light can allow a phone to perform functions of sophisticated laboratory instruments. Mobile spectroscopy can be achieved by dispersing incident light over the pixels of the intrinsic CMOS detector, which can separate light's spectral components with greatly higher fidelity than possible with RGB pixels. This capability is critical for biological assays that result in color change of a liquid, or those that involve photon emission by chemical

fluorescence, quantum dot emission, bioluminescence, or phosphorescence. For example, Enzyme Linked Immunosorbent Assays (ELISA) represent a predominant assay format in diagnostics in which an enzyme-substrate interaction generates a chromophore, whose concentration is directly related to the concentration of the target analyte by the chromophore's absorption. Likewise, the most common assays for detection of specific nucleic acid sequences, including molecular beacons and polymerase chain reaction (PCR) generate fluorescence emission as the sensed quantity. Therefore, many DNA and RNA-related diagnostic applications, such as infectious disease diagnostics, microbial pathogen detection in food products, monitoring for antibiotic-resistant bacterial, and genotyping can be performed at the point-of-use through smartphone fluorescence spectroscopy.

The first mobile phone spectroscopy for fluorescence-based biomedical assays was introduced in 2014 (Yu et al., 2014). The smartphone fluorimeter was first used to perform molecular beacon Förster resonance energy transfer (MB-FRET) assays for detection of a specific miRNA sequence (22 nts) with 10 pM of limit of detection. The MB probe was synthesized with a Cy3 donor (Cyanine 3, $\lambda_{max.ex} = 550$ nm, $\lambda_{max.em} = 570$ nm) and a BHQ2 acceptor quencher (Black Hole Quencher, $\lambda_{max.ex} = 550$ nm, $\lambda_{max.em} = 570$ nm) at each end, and a loop domain which hybridizes the target miRNA sequence. Fig. 6 shows the arrangement and alignment of system components. The system uses a battery-powered green laser pointer to excite fluorophores, and a collecting lens to gather fluorescence from a small volume (~150 μL) of target sample. Here, the illumination for fluorophore excitation was perpendicular to the axis of light collection to minimize the direct collection of the excitation source. The light is transmitted to a custom designed smartphone attachment via an optical fiber. The attachment holds a pinhole, a collimating lens, a cylindrical lens, and a transmission diffraction grating to receive, collimate, and disperse light signals onto the smartphone's camera that is adjoined the grating that was oriented at a 47° angle with respect to the axis of incident light to disperse the first order diffracted mode across the pixels of the rear-facing CMOS camera of an iPhone4. The mobile phone fluorimeter has a dispersion of 0.338 nm/pixel in the spectral direction, and this high single-pixel wavelength increment, making the system suitable for most fluorescent based biomedical assays.

2.7. Label-free biosensing transducers

Label-free biosensing requires detection of biological analytes through their intrinsic physical properties, such as dielectric permittivity, mass, or conductivity. Because label-free assays do not require fluorescent tags, multiple reagents, or washing steps, they are simple one-step procedures that can be performed at the point of care more readily than label-based approaches. Among the label-free detection approaches that have been demonstrated, those based on optical transducers detecting intrinsic dielectric permittivity have been successfully implemented commercially for their ability to provide real-time, high-throughput and quantitative detection with sufficiently high sensitivity for many applications. For example Surface Plasmon Resonance (SPR) and Photonic Crystal (PC) optical biosensors are products that are used in the pharmaceutical screening process to characterize biomolecular interaction rate constants, and to measure the effects of drug molecules upon immobilized live cells. Detection instruments for SPR biosensing and PC biosensing have been implemented as instruments with a desktop-size footprint for laboratory-based applications. Recently, label-free detection has also been integrated into a smartphone format, utilizing the spectroscopy capability that is enabled by an attachment that disperses light transmitted through a biosensor surface across the pixels of a smartphone CMOS camera (Gallegos et al., 2013; Giavazzi et al., 2014; Preechaburana et al., 2012; Zeng et al., 2014).

The first smartphone-based photonic crystal (PC) label-free biosensor detection instrument was described in 2013 (Gallegos et al., 2013), in which the phone's rear-facing camera is transformed into a spectrometer by placement of a diffraction grating immediately in front of the camera opening. The resulting system measures the resonant transmission spectrum from the PC biosensor, and can measure shifts of the PC resonant wavelength as biomolecular material attaches to the PC surface. An external broadband light source, such as an incandescent

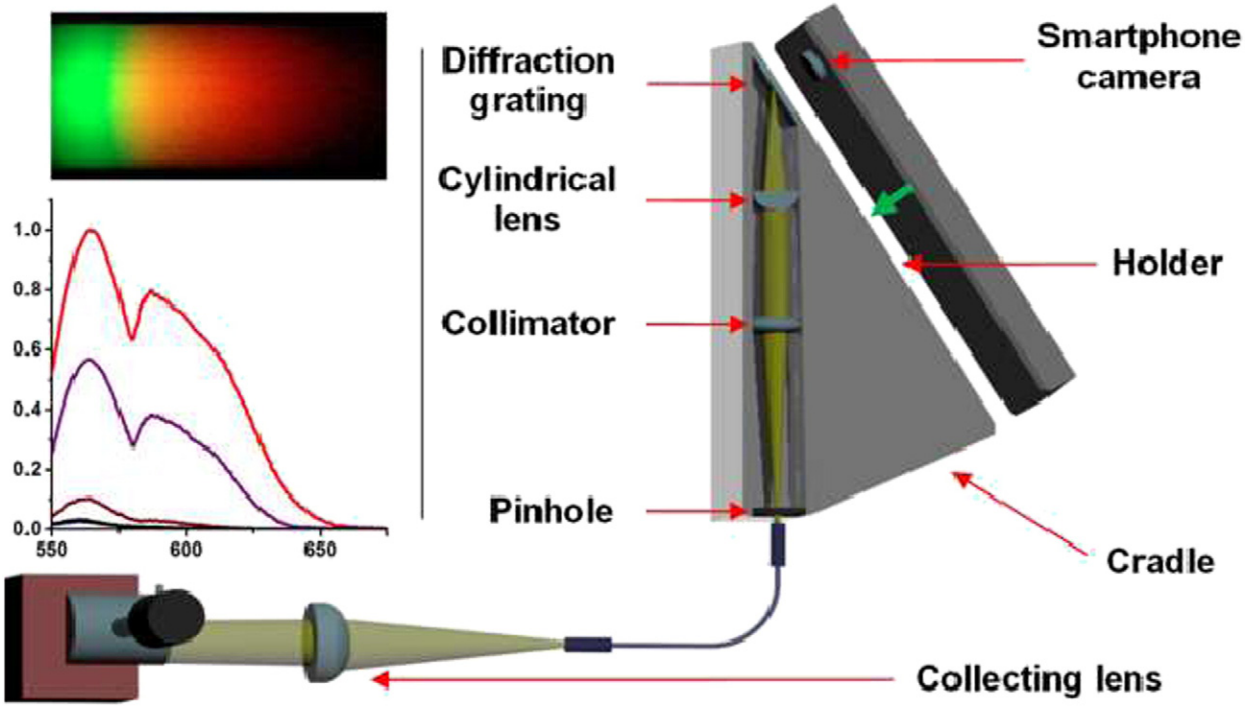

Fig. 6. Illustration of the smartphone fluorimeter system (Yu et al., 2014). Inset: fluorescence spectrum measured (T-miRNA conc. = 1 μM) and intensity distributions as T-miRNA concentration. Varies from 1 μM to 1 pM.
Reprinted with permission from American Chemical Society.

light bulb passes light through a pinhole to produce a semi-collimated point source, which is further collimated by a collimating lens. A polarizing filter selects one specific electric field orientation, perpendicular to the grating direction of the PC biosensor. The PC sensor chip is comprised of one dimensional subwavelength grating structure that supports high efficiency resonant reflection for a narrow wavelength band, and thus a "dip" in the transmitted light spectrum is observed at the resonant wavelength of the PC. The PC is inserted into a small slot in the attachment, which places it in the optical path, so collimated light reaches it at normal incidence. The transmitted light is focused along one axis by a cylindrical lens before it is incident upon a diffraction grating that spatially separates the wavelength components of the beam across the CMOS image sensor of the phone camera. The dip in the transmission spectrum is observed as a dark band in the spectrum, which software analyzes to determine the central wavelength. The cradle attachment was initially designed to interface with an iPhone4. An app was developed to facilitate the gathering of spectra, measuring the shifts of the PC resonant wavelength and wireless transmission of all spectra and measurements.

As a further example of smartphone-based label-free biosensor instrumentation, an angle-resolved surface plasmon resonance (SPR) detection system based on a single disposable SPR coupler made of polydimethylsiloxane (PDMS) has been presented (Preechaburana et al., 2012). For SPR biosensors, the illumination is configured to excite surface plasmon resonance at the surface of a thin gold film, and the resonance can be observed in the reflection as a characteristic dip that occurs when monochromatic light is incident on the surface of the gold film at the angle that matches the surface plasmon coupling condition. As the SPR effect is very sensitive to the optical properties at the metal surface, small changes in the surface refractive index due to surface-adsorbed biomolecules can be transduced by measuring shifts of the angle at which the reflection dip occurs. The SPR coupler is attached directly to the phone screen, where the illumination of the phone screen display within a certain region is coupled in as the divergent light source, and the output of the coupler is directed into the front camera of the smartphone. The optical components in the SPR coupler, including a plano-cylindric lens, a cylindrical lens, a prism and a hosting block, are all fabricated using PDMS and epoxy, resulting in a low-cost disposable sensor. The coupler is independent of the sensor chip and can be used with commercially available SPR chips. The detection of β_2 microglobulin (β_2M), an established biomarker for cancer and other diseases, has been demonstrated with a commercially available Biacore CM5 sensor chip. A detection limit of 0.1 μg/mL is reported, which is comparable to the performance required for clinical utility.

Based on the dielectric permittivity detection, another portable immunosensor using the intrinsic light source and camera of a smartphone is reported (Giavazzi et al., 2014) for diagnosis of hepatitis B and HIV in complex media (bovine fetal serum). The method is based on measurement of the light reflected by a functionalized surface with very low reflectivity. A plastic cradle attachment for the phone is designed to hold the optical components and a measuring cuvette. Multiple spots can be analyzed within one single image from the camera, depending on the immobilization of multiple targets on the sensing surface. As a diagnostic tool, the detection limit of a few ng/mL is estimated for this approach, with an assay time of ~30 min.

3. Biodetection using extrinsic sensors

3.1. USB connection of sensors to a smartphone

Because the intrinsic set of sensors in mobile devices is limited to those in great demand for consumer applications (for example, sensitivity to wavelengths in the visible part of the spectrum by the internal camera), using an extrinsic sensor to perform an advanced measurement and transmitting the data back to the mobile device can be more practical in certain circumstances. An essential component of a smartphone is its ability to interface with many electronic devices, such as computers, keyboards, trackpads, and speakers. The Universal Serial Bus (USB) has emerged as the most widely adapted interface for connection, communication and power supply between electronics devices, and thus provides a natural interface between extrinsic sensors and mobile devices. Usually these external attachments consist of the primary sensor, an interface with samples, and circuitry to both interpret user commands from the smartphone and to transmit data between the sensor and the smartphone for display, analysis and storage. As long as the smartphone is able to serve as a USB host device, the sensor can be powered via the USB port, which eliminates the need for batteries or an external power supply. Both electrochemical detection (Lillehoj et al., 2013; Velusamy et al., 2013) and optical detection (Jiang et al., 2014b; Mancuso et al., 2014) of biomolecules have been demonstrated using USB-connected sensors.

An example of a smartphone-based electrochemical detection platform was recently demonstrated for the diagnosis of malaria in human serum (Lillehoj et al., 2013). A sample solution (containing *pf*HRP2 antigen, the biomarker for malaria) and reporter solution (containing *pf*HRP2 antibodies conjugated to horseradish peroxidase in DPBS) are introduced to the microfluidic chip inlets using a pipette. The solutions are mixed and then passed through a tightly-packed serpentine channel situated over the electrochemical sensor. In the sensing region, any *pf*HRP2 antigen present will interact with *pf*HRP2 capture antibodies immobilized in a robust polypyrrole (PPY) matrix. Detection antibodies in the reporter solution are captured in the sensing region only in the presence of antigen from the sample solution. A TMB/H_2O_2 solution is loaded into the chip that serves to wash unbound conjugated *pf*HRP2 antibody and then functions as a substrate for an HRP-catalyzed reaction. When a voltage potential is applied across the sensing region, the oxidation of the TMB substrate generates a small electrochemical current directly proportional to the concentration of the *pf*HRP2 protein in the sample. The rest of the device surface is comprised of a capillary pump that provides the liquid flow for the system, thereby eliminating the need for pumps or peripheral components. This electrical signal is processed and packaged by the sensor circuit and transmitted back to the smartphone via the USB port. An Android application is designed to provide user instructions and data visualization and analyses. The assay can be completed in 15 min with 2–3 loading steps and provides a limit of detection of *pf*HRP2 antigen of 16 ng/mL. A similar electrochemical analyzer system for *Bacillus cereus* DNA detection interfaced to a smartphone through the USB interface (Velusamy et al., 2013) is based on a similar detection principle for DNA, providing a detection limit of 33.3 pg/mL.

Kaposi's sarcoma herpesvirus (KSHV) was also detected using a USB accessory by direct quantification of nucleic acids using optical absorption of gold nanoparticles in solution (Mancuso et al., 2014). Short DNA probes for KHSV DNA were designed and attached to gold nanoparticles. Once introduced to a patient sample, binding between the probes and target KHSV DNA from the sample affects the absorption of the gold nanoparticles, which can be quantified by measuring the optical density of the mixture at a wavelength of 520 nm. A LED ($\lambda = 520$ nm) provides illumination to a microfluidic chip and the transmitted intensity is detected by a photocell connected by a pull-down resistor, where the voltage drop measured is a result of the intensity of the measured transmission. The controlling circuitry controls both the LED power and the photocell measurements. An app was developed in Eclipse for the Android platform, written in Java to provide power to the sensing attachment and to process the data. Additional information for each measurement (including user information, time stamps and locations) is collected and results are either communicated by email or uploaded to the cloud. Further extension of this work has been completed by the researchers to demonstrate solar-powered polymerase chain reaction (PCR) amplification of DNA and analysis of human skin biopsies with KSHV (Jiang et al., 2014b).

3.2. Sensor communication with a smartphone through the audio jack

As stated previously, extrinsic sensors can be designed to interface with smartphones and other mobile devices, conferring a myriad of functions in broader technological contexts. In addition to sensors that communicate with mobile devices using Bluetooth® connection, some extrinsic sensors connect via the standard 3.5 mm audio jack. While different mobile devices may exhibit nonstandard and sometimes proprietary connectivity ports, the audio jack is standardized and accessories utilizing this mode are easily adaptable, making it an attractive choice as a mode of connection.

Recently, the use of a "smartphone dongle" for diagnosing HIV and both treponemal and non-treponemal syphilis at the point of care was demonstrated (Laksanasopin et al., 2015). The dongle integrates fluidic, mechanical, optical, and electronic components to function as an automated, portable, and cost-effective version of a benchtop ELISA instrument, with signal enhancement using gold nanoparticles and silver ions instead of enzyme and substrate. A plastic cassette is inserted into the dongle, which is connected via audio jack to the mobile device—in this case, a 4th generation Apple iPod Touch®. The disposable plastic cassette consists of three components: (1) a test cassette including a waste pad for on-chip fluid waste collection, and five detection zones, each pre-coated with a particular disease-specific protein, positive control, or negative control; (2) a reagent cassette storing wash and silver A and B reagents; and (3) an antibody holder containing lyophilized gold-labeled antibodies. In this assay, the optical density (OD) of the silver enhancement corresponds to the concentration of captured analyte. In the assay process, diluted whole blood from a finger prick is pipetted into the cassette. The target analyte, if present, binds to the corresponding capture protein on the test cassette surface. One push of a mechanically-activated vacuum allows gold-labeled antibody, wash buffer, and silver reagents to flow through the channel, in that order. The dongle contains no internal power source of its own and relies solely on power provided by the smartphone or other mobile device. The smartphone sends a 19-kHz audio signal that is converted to a stable DC 3.0 V which powers the OD readings and frequency shift keying (FSK) data transmission to the smartphone; the dongle requires no power during the fluid flow steps. A mobile app allows the user to enter a "Patient ID", provides step-by-step pictorial instructions, displays assay progress, and records the assay results. The results, displayed as "Positive", "Negative", or "Indeterminate", obviate user interpretation. A field test was performed in Rwanda, where the device achieved sensitivities and specificities of 100% and 87%, respectively, for HIV; 92% and 92% for treponemal syphilis; and 100% and 79% for non-treponemal syphilis. In particular, patient satisfaction surveys indicated 97% of the patients would recommend the smartphone-based assay, citing factors such as shorter turnaround time, panel screening, and ease of sample collection.

Another recent proof-of-concept demonstration of a smartphone accessory, for use as a low-cost electrochemical biosensor (Sun et al., 2014). Consists of a low-power potentiostat that interfaces between a disposable screen-printed electrode (SPE) and a smartphone. Here, cyclic voltammetry measurements using equal amounts of potassium ferrocyanide ($K_4(Fe(CN)_6)$) and potassium ferricyanide ($K_3(Fe(CN)_6)$) run on a printed circuit board (PCB)-based prototype returned results comparable to those obtained using a benchtop potentiostat. While it is not specifically demonstrated for use in medical diagnostic applications, the electrodes can be functionalized with biorecognition molecules, such as nucleic acids coupled to redox molecules, for detecting clinically relevant target molecules (Hsieh et al., 2015; Liu et al., 2015). The module can be improved in terms of portability and functionality, power-consumption, and user-friendliness by the incorporation of well-defined application-specific design requirements, lower-power electronic components, and a smartphone app user interface.

Audio jacks have four channels: the right and left audio channels, ground, and microphone input. The audio channels can be used for sending user input and providing power from the mobile device to the accessory, while data from the accessory can be collected by the mobile device via the microphone input. In particular, the ability to harvest power from the mobile device through the audio jack (Kuo et al., 2010), coupled with carefully chosen electrical components, eliminates the need for an external battery, allowing these accessories to achieve a small form factor. Newer 3.5 mm TRRS audio jacks using the CTIA standard are, for most part, standardized across different mobile device platforms.

3.3. Sensor connection via Bluetooth® communication to a smartphone

Because the intrinsic sensing options within a mobile device are quite limited, detection instruments that require different capabilities must use an extrinsic sensor that can take commands and return sensor readings. Because all sensor information can be digitized, it may be communicated via wireless communication protocols. The most broadly adopted wireless technology standard for exchanging data over short distances is Bluetooth®, which utilizes UHF radio waves in the ISM band from 2.4 to 2.485 GHz. Bluetooth hardware and protocols are managed by the Bluetooth Special Interest Group, which has over 25,000 member companies which license patents for individual qualifying devices. Bluetooth is primarily designed for low power consumption and short range, using low cost transceiver microchips in each device. Because nearly all mobile communication devices now incorporate a Bluetooth transceiver, an extrinsic sensor module with its own transceiver may interact with the mobile device by a secure wireless connection.

An example of such a system is the recently reported smartphone-based system for diagnosis of infectious disease (Salomon et al., 2014), in which a set of electrochemical sensors are measured by an extrinsic desktop instrument that communicates with a smartphone with a Bluetooth connection. The detection principle utilizes antigen-coated magnetic particles that are incubated with patient sera, followed by exposure to secondary antibodies conjugated with horseradish peroxidase (HRP). The presence of specific antibodies in serum that indicate prior exposure to a target pathogen results in immobilization of the magnetic particles on the surface of an electrode, where the HRP generates oxidation products which extract electrons from the electrode, thus generating current. The current magnitude is related to the number of transducer-immobilized HRP molecules, which in turn is linearly related to the number of captured specific antibodies. In this work, the electrochemical cells are assembled in a disposable cartridge that contains eight independent sensor electrodes that is inserted into a detection system that effectively serves as a precision current meter and as a base station for communicating data to a smartphone. The detection instrument has its own analog-to-digital converter for current measurements, a small computer processor, a battery, power management electronics, and a Bluetooth chip. Using this approach, software allows the system to carry out amperometric recording of the sensor arrays using cyclic voltammetry, so the sensors can be monitored continuously in real time. An Android app that resides on the smartphone, developed in Java, initiates voltage step measurements, allows the user to select assay options, and calculates the average value of the work current. The app makes a determination of a positive or negative test result, reports the results on screen, and maintains a record of the entire measurement.

Because electrochemical sensor data can be easily converted to digital values and communicated with brief transmissions (in terms of data size), they have been among the first to be adopted for Bluetooth applications. While not strictly targeted as a medical diagnostic, the impedance-based sensor for TNT detection (Zhang et al., 2015) represents an approach in which specific target-recognizing peptides are immobilized on a working electrode in close proximity to a counter electrode and reference electrode for performing cyclic voltammetry. The compact integrated system is capable of measuring one sensor at

a time while the extrinsic control circuit communicates via Bluetooth with an Android-based smartphone. The mobile system measures impedance of the electrode a ~20 MHz frequencies rather than DC impedance, as high frequency impedance measurements were found to provide greater signal-to-noise measurements. This approach may be amenable to detection of small molecule analytes in related biomedical applications such as sensing drug metabolites, nutrients, and other small molecule biomarkers. The same research group used a similar high frequency impedance approach for detection of bacteria for environmental monitoring of drinking water (Jiang et al., 2014a). Here, an extrinsic electrochemical impedance spectroscopy (EIS) sensor measures changes in the high frequency impedance of a set of interdigitated electrodes fabricated on a silicon chip, and changes in AC impedance were found to be dependent upon the concentration of captured *Escherichia coli* bacteria. Such a system may be adapted to clinical applications such as detection of antibiotic resistant bacteria through the use of specific capture antibodies that bind with proteins displayed on the bacteria outer surface membrane.

A further approach that demonstrates the flexibility of extrinsic sensor approaches that communicate by Bluetooth is a mobile system for breath analysis (Gupta et al., 2010). The approach uses laser absorption spectroscopy (LAS) that measures the absorption coefficient of gas at specific wavelengths in the infrared. The demonstrated system was specifically tuned for measuring oxygen concentration, but could be tuned to other gasses. As with other examples cited here, the system utilized a Bluetooth chip to communicate with an Android-based smartphone app, but in this case reported optical intensity values from a photodiode.

In a similar manner to Bluetooth communication, the WiFi connection of a mobile device can be used for data communication to an extrinsic diagnostic instrument. Common examples of this are readily observable in the consumer arena, with smart-watch or smart-band monitoring of physiological attributes such as pulse, step count, and physical activity monitoring that are then relayed back to a smartphone-based user interface and data repository. Similarly, devices that measure biological samples can be remotely controlled via WiFi, such as the Gene-Z device, a portable, but not handheld, platform for detecting bacterial pathogens via isothermal nucleic acid amplification and detection of genomic DNA from *E. coli* and *Staphylococcus aureus* (Stedtfeld et al., 2012). The Gene-Z prototype amplifies genetic target DNA sequences via loop-mediated isothermal amplification (LAMP) monitored by fluorescence-based optical emission. A WiFi module integrated into the Gene-Z device facilitates communication between the device and a smartphone or similar device with wireless capabilities, such as the iPod Touch. A custom app provides a user interface that facilitates adjustment of experimental conditions, data processing, display, and cloud integration.

4. Commercially available smartphone-based diagnostics

The majority of commercially available smartphone-based health management platforms are mobile applications, or “apps” which utilize the mobile device for logging data or alerting the user about personal health trends. As these apps are purposed for health management and tracking rather than diagnosis or treatment, they do not require explicit FDA approval. Besides these, several diagnostic accessories interfacing with a mobile device have been commercialized within the past decade. While a number of commercial smartphone-based diagnostic products have been reviewed previously (Vashist et al., 2014) we review the iBGStar® blood glucose monitor and provide some updates on the recent diagnostic applications of the AliveCor® heart monitor and CellScope digital home health kit.

iBGStar, by Sanofi-Aventis LLC, is an extrinsic biosensor that connects to an Apple iPhone or iPod touch via the 30-pin proprietary dock connector. Like other glucose monitors, the iBGStar quantifies blood glucose levels by making amperometric measurements. Additionally, a phasor transform algorithm derived from dynamic electrochemistry corrects for non-specific signaling from other molecules, resulting in highly accurate tests (Pfutzner et al., 2013). Based on the iBGStar website, while the sensor itself is able to store up to 300 test results (including the date and time), it can be coupled with the iBGStar Diabetes Manager app for additional storage, better tracking of blood glucose levels, and data-sharing.

The AliveCor Mobile ECG, which also uses an extrinsic sensor, is available for a number of Android and iOS devices. The attachment price ($99 US at the time of this review) includes two plates that communicate wirelessly with the free AliveECG app on the smartphone. The app not only stores the ECG, but enables a patient to grant access to the ECG results to the physician. The AliveCor Mobile ECG has recently been reported in the context of monitoring patients after atrial fibrillation ablation (Tarakji et al., 2015), early identification of atrial fibrillation (Williams et al., 2015), and diagnosing tachycardia (Richley and Graham, 2015).

Aside from using extrinsic sensors for gathering data, some commercialized devices such as the CellScope Oto utilize intrinsic sensors, such as the smartphone camera, for performing diagnostic tests. The Oto is a cone-shaped attachment containing the necessary optics to allow a cellphone to function as an otoscope ($79 US). It aligns to the back-facing camera, is used to take images of the inside of one's ear, and can be useful in detecting ear infections. A specialized mobile app helps identify significant landmarks such as the tympanic membrane, records images, and allows data-sharing with a physician. In addition to the Oto, other camera-based diagnostic systems are currently being developed by CellScope for a comprehensive home health kit. Recently reported prototypes include attachments for detecting ophthalmologic abnormalities (Maamari et al., 2014), blood-borne parasites (D'Ambrosio et al., 2015), and fluorescence imaging of sputum smears (Chaisson et al., 2015) in developing countries.

5. Regulatory challenges for mobile diagnostics

Regulatory approval is an important commercialization hurdle for any diagnostic technology that includes a mobile phone or tablet as part of its hardware, whether the sensor is intrinsic or extrinsic. For diagnostic applications that are subject to the regulatory requirements of the FDA, a smartphone becomes part of an instrument that must pass a rigorous approval process. Aside from the current FDA requirements for diagnostic tests used for a medical purpose to be performed and supervised by certified personnel, the mobile device and its related hardware, manufacturing quality control, and product development methodology must meet stringent requirements that are not ordinarily considered for consumer electronics. For diagnostic systems that use an extrinsic sensor, the hardware regulatory requirements may be performed upon the sensor module, while the mobile device simply serves the role of a computer and communication device (which must still pass regulatory approval for software validation). The regulatory landscape in less-regulated markets may result in the emergence of commercial products first in China, India, and Africa, where there are large populations with pressing healthcare needs that can be addressed with mobile diagnostic platforms. For any diagnostic technology, false positive and false negative results can have dire consequences, and there is a likelihood that error rates will increase when tests are performed by non-specialists in uncontrolled conditions, using materials that may not have been shipped or stored under prescribed conditions. Therefore, it will be important for commercially available implementations of mobile diagnostic technologies to be extremely robust through a combination of engineering design, incorporation of built-in experimental controls, replicate tests, and detection of user errors.

6. Conclusion

As the highlighted set of examples show, the internal sensing capabilities of modern mobile devices are sufficiently powerful to perform

many of the same tasks as laboratory instruments. Through the broad introduction of high resolution CMOS cameras with excellent low-light sensing capabilities that are intended for digital photography which may occur indoors, the same capability may be adapted to new purposes that are potentially impactful for medical diagnostics. While the most straightforward intrinsic sensing approaches involve capturing an image of a test strip and subsequent analysis of the position or color of assay spots in the context of lateral flow assays, more sophisticated approaches may involve incorporation of a special-purpose optical excitation source (such as an LED) and optical filters that allow measurement of fluorescent or chemiluminescent outputs. Likewise, the ability to deconstruct an image into its RGB component colors may be used as a crude form of spectroscopy that can be applied to perform color analysis of solid or liquid-based assays. The intrinsic sensing capabilities of mobile devices may be further extended through the addition of optical elements that can be packaged within an add-on module that allow the camera to perform high-magnification microscopy, highly accurate spectroscopy, and label-free biosensor analysis.

While the internal CMOS cameras of mobile devices are powerful, they are not suited to all diagnostic sensing approaches. A growing family of sensors that utilize external electrochemical sensors use highly customized instruments that can easily communicate with a mobile device either through a hard-wired connection or radio communication. The “extrinsic” sensing approach has the potential to extend many types of medical diagnostics, regardless of the sensed parameter, to a mobile format. While relatively simple devices, such as physiological monitors for heart rate and blood oxygen have been commercially available for several years, more complex diagnostic instruments that include ultrasound imaging transducers and otoscopes are now being introduced to the market.

The characteristic that all the approaches in this review have in common is the ability to use the computation and communication capabilities of mobile devices. As wireless communication availability has become pervasive and as the computing power of mobile devices has surpassed that of the desktop computers of only a few years ago, it is possible to perform sophisticated image and signal analysis with a handheld device. The open software development tools that are available for mobile device platforms facilitate development of applications and publishing them for use by a broad audience, which further encourages development of new approaches. While we are witnessing the emergence of mobile sensing approaches for medical diagnosis, there are parallel efforts for development of “personalized medicine” and “electronic health” platforms that are aimed at making a greater variety of medical information available to the patient from tests that can be performed in the home, in health clinics, in pharmacies, or in remote locations. Such systems are comprised of information networks and systems for storing medical records that will allow certified clinicians or technicians to interpret diagnostic tests that are performed remotely, and with the aid of software tools, identify important trends. Such systems may be aimed at identifying a medical condition while it is in the development stage in order to preempt acute episodes that lead to visits to the emergency room. Another envisioned application is frequent monitoring of patients with a known medical condition, such as heart disease or cancer, in order to track the progress of treatment. Yet another application enabled by wide adoption of mobile diagnostics is diagnosis of infectious disease and combination with geolocation information to inform epidemiological understanding of disease spread. These types of analyses are enabled by the availability of compact, inexpensive, and simple-to-operate diagnostic instruments, which are very likely to take advantage of the available communication and computational infrastructure available through mobile devices and the data networks they communicate with.

Acknowledgement

The authors are grateful for financial support from the National Science Foundation (CBET 1512043, CBET 1264377, IIP 1534126). The views and opinions presented in this paper do not reflect those of the National Science Foundation. HY receives partial financial support from the NSF Center for Innovative Instrumentation Technology (CiiT). LK gratefully acknowledges financial support from the NSF IGERT for Cellular and Molecular Mechanics and BioNanotechnology (CMMB). YW acknowledges the support from China Scholarship Council and National Science Foundation China (NSFC) (61205078). Co-authors KDL, HY, LK, and YW contributed equally to the literature research and writing of the paper. BTC provided overall organization, writing, and editing of the manuscript.

References

Awqatty, B., Samaddar, S., Cash, K.J., Clark, H.A., Dubach, J.M., 2014. Fluorescent sensors for the basic metabolic panel enable measurement with a smart phone device over the physiological range. Analyst 139, 5230–5238.

Barbosa, A.I., Gehlot, P., Sidapra, K., Edwards, A.D., Reis, N.M., 2015. Portable smartphone quantitation of prostate specific antigen (PSA) in a fluoropolymer microfluidic device. Biosens. Bioelectron. 70, 5–14.

Berg, B., Cortazar, B., Tseng, D., Ozkan, H., Feng, S., Wei, Q., et al., 2015. Cellphone-based hand-held microplate reader for point-of-care testing of enzyme-linked immunosorbent assays. ACS Nano 9, 7857–7866.

Bogoch, I.I., Andrews, J.R., Speich, B., Utzinger, J., Ame, S.M., Ali, S.M., et al., 2013. Mobile phone microscopy for the diagnosis of soil-transmitted helminth infections: a proof-of-concept study. Am. J. Trop. Med. Hyg. 88, 626–629.

Breslauer, D.N., Maamari, R.N., Switz, N.A., Lam, W.A., Fletcher, D.A., 2009. Mobile phone based clinical microscopy for global health applications. PLoS ONE 4, e6320.

Chaisson, L.H., Reber, C., Phan, H., Switz, N., Nilsson, L.M., Myers, F., et al., 2015. Evaluation of mobile digital light-emitting diode fluorescence microscopy in Hanoi, Viet Nam. Int. J. Tuberc. Lung Dis. 19, 1068–1072.

Coskun, A.F., Nagi, R., Sadeghi, K., Phillips, S., Ozcan, A., 2013a. Albumin testing in urine using a smart-phone. Lab Chip 13, 4231–4238.

Coskun, A.F., Wong, J., Khodadadi, D., Nagi, R., Tey, A., Ozcan, A., 2013b. A personalized food allergen testing platform on a cellphone. Lab Chip 13, 636–640.

D'Ambrosio, M.V., Bakalar, M., Bennuru, S., Reber, C., Skandarajah, A., Nilsson, L., et al., 2015. Point-of-care quantification of blood-borne filarial parasites with a mobile phone microscope. Sci. Transl. Med. 7 (286re4).

Erickson, D., O'Dell, D., Jiang, L., Oncescu, V., Gumus, A., Lee, S., et al., 2014. Smartphone technology can be transformative to the deployment of lab-on-chip diagnostics. Lab Chip 14, 3159–3164.

Frean, J., 2007. Microscopic images transmitted by mobile cameraphone. Trans. R. Soc. Trop. Med. Hyg. 101, 1053.

Fronczek, C.F., Park, T.S., Harshman, D.K., Nicolini, A.M., Yoon, J.-Y., 2014. Paper microfluidic extraction and direct smartphone-based identification of pathogenic nucleic acids from field and clinical samples. RSC Adv. 4, 11103–11110.

Gallegos, D., Long, K.D., Yu, H., Clark, P.P., Lin, Y., George, S., et al., 2013. Label-free biodetection using a smartphone. Lab Chip 13, 4053–4064.

Giavazzi, F., Salina, M., Ceccarello, E., Ilacqua, A., Damin, F., Sola, L., et al., 2014. A fast and simple label-free immunoassay based on a smartphone. Biosens. Bioelectron. 58, 395–402.

Guan, L., Tian, J., Cao, R., Li, M., Cai, Z., Shen, W., 2014. Barcode-like paper sensor for smartphone diagnostics: an application of blood typing. Anal. Chem. 86, 11362–11367.

Gupta, S., Breen, P., Wu, D., Sabharwal, A., 2010. In: ACM (Ed.), Breath Analysis With Laser Sensors on an Android Platform. Wireless Health, San Diego, CA.

Hong, J.I., Chang, B.-Y., 2014. Development of the smartphone-based colorimetry for multi-analyte sensing arrays. Lab Chip 14, 1725–1732.

Hsieh, K., Ferguson, B.S., Eisenstein, M., Plaxco, K.W., Soh, H.T., 2015. Integrated electrochemical microsystems for genetic detection of pathogens at the point of care. Acc. Chem. Res. 48, 911–920.

Jiang, J., Wang, X.H., Chao, R., Ren, Y.K., Hu, C.P., Xu, Z.D., et al., 2014a. Smartphone based portable bacteria pre-concentrating microfluidic sensor and impedance sensing system. Sensors Actuators B Chem. 193, 653–659.

Jiang, L., Mancuso, M., Lu, Z., Akar, G., Cesarman, E., Erickson, D., 2014b. Solar thermal polymerase chain reaction for smartphone-assisted molecular diagnostics. Sci. Rep. 4, 4137.

Kuo, Y.-S., Verma, S., Schmid, T., Dutta, P., 2010. Hijacking power and bandwidth from the mobile phone's audio interface. Proceedings of the First ACM Symposium on Computing for Development. ACM, London, United Kingdom, pp. 1–10.

Laksanasopin, T., Guo, T.W., Nayak, S., Sridhara, A.A., Xie, S., Olowookere, O.O., et al., 2015. A smartphone dongle for diagnosis of infectious diseases at the point of care. Sci. Transl. Med. 7 (273re1).

Lee, S., Oncescu, V., Mancuso, M., Mehta, S., Erickson, D., 2014. A smartphone platform for the quantification of vitamin D levels. Lab Chip 14, 1437–1442.

Lillehoj, P.B., Huang, M.-C., Truong, N., Ho, C.-M., 2013. Rapid electrochemical detection on a mobile phone. Lab Chip 13, 2950–2955.

Liu, Y., Liu, Y., Matharu, Z., Rahimian, A., Revzin, A., 2015. Detecting multiple cell-secreted cytokines from the same aptamer-functionalized electrode. Biosens. Bioelectron. 64, 43–50.

Lunden, I., 2015. 1.2B Smartphones Sold In 2014, Led By Larger Screens And Latin America. (http://techcrunch.com/2015/02/16/1-2b-smartphones-sold-in-2014-led-by-larger-screens-and-latin-america/2015).

Maamari, R.N., Ausayakhun, S., Margolis, T.P., Fletcher, D.A., Keenan, J.D., 2014. Novel telemedicine device for diagnosis of corneal abrasions and ulcers in resource-poor settings. JAMA Ophthalmol. 132, 894–895.

Mancuso, M., Cesarman, E., Erickson, D., 2014. Detection of Kaposi's sarcoma associated herpesvirus nucleic acids using a smartphone accessory. Lab Chip 14, 3809–3816.
Martinez, A.W., Phillips, S.T., Carrilho, E., Thomas, S.W., Sindi, H., Whitesides, G.M., 2008. Simple telemedicine for developing regions: camera Phones and paper-based microfluidic devices for real-time, off-site diagnosis. Anal. Chem. 80, 3699–3707.
Mudanyali, O., Dimitrov, S., Sikora, U., Padmanabhan, S., Navruz, I., Ozcan, A., 2012. Integrated rapid-diagnostic-test reader platform on a cellphone. Lab Chip 12, 2678–2686.
Oncescu, V., Mancuso, M., Erickson, D., 2014. Cholesterol testing on a smartphone. Lab Chip 14, 759–763.
Oncescu, V., O'Dell, D., Erickson, D., 2013. Smartphone based health accessory for colorimetric detection of biomarkers in sweat and saliva. Lab Chip 13, 3232–3238.
Ozcan, A., 2014. Mobile phones democratize and cultivate next-generation imaging, diagnostics and measurement tools. Lab Chip 14, 3187–3194.
Park, T.S., Li, W., McCracken, K.E., Yoon, J.-Y., 2013. Smartphone quantifies *Salmonella* from paper microfluidics. Lab Chip 13, 4832–4840.
Petryayeva, E., Algar, W.R., 2015. Single-step bioassays in serum and whole blood with a smartphone, quantum dots and paper-in-PDMS chips. Analyst 140, 4037–4045.
Pfutzner, A., Schipper, C., Ramljak, S., Flacke, F., Sieber, J., Forst, T., et al., 2013. Evaluation of the effects of insufficient blood volume samples on the performance of blood glucose self-test meters. J. Diabetes Sci. Technol. 7, 1522–1529.
Preechaburana, P., Gonzalez, M.C., Suska, A., Filippini, D., 2012. Surface plasmon resonance chemical sensing on cell phones. Angew. Chem. Int. Ed. 51, 11585–11588.
Richley, D., Graham, A., 2015. Diagnosing symptomatic arrhythmia via mobile phone. Br. J. Cardiac Nurs. 10, 130–131.
Roda, A., Michelini, E., Cevenini, L., Calabria, D., Calabretta, M.M., Simoni, P., 2014. Integrating biochemiluminescence detection on smartphones: mobile chemistry platform for point-of-need analysis. Anal. Chem. 86, 7299–7304.
Salomon, F., Tropea, S., Brengi, D., Hernandez, A., Alamon, D., Parra, M., et al., 2014. Smartphone controlled platform for point-of-care diagnosis of infectious diseases. IEEE 9th Ibero-American Congress on Sensors (ibersensor). Bogota 2014, pp. 1–4.
Shen, L., Hagen, J.A., Papautsky, I., 2012. Point-of-care colorimetric detection with a smartphone. Lab Chip 12, 4240–4243.
Smith, J.E., Griffin, D.K., Leny, J.K., Hagen, J.A., Chávez, J.L., Kelley-Loughnane, N., 2014. Colorimetric detection with aptamer-gold nanoparticle conjugates coupled to an android-based color analysis application for use in the field. Talanta 121, 247–255.
Stedtfeld, R.D., Tourlousse, D.M., Seyrig, G., Stedtfeld, T.M., Kronlein, M., Price, S., et al., 2012. Gene-Z: a device for point of care genetic testing using a smartphone. Lab Chip 12, 1454–1462.
Sun, A., Wambach, T., Venkatesh, A.G., Hall, D.A., 2014. A low-cost smartphone-based electrochemical biosensor for point-of-care diagnostics. IEEE Biomedical Circuits and Systems Conference, pp. 312–315.
Tarakji, K.G., Wazni, O.M., Callahan, T., Kanj, M., Hakim, A.H., Wolski, K., et al., 2015. Using a novel wireless system for monitoring patients after the atrial fibrillation ablation procedure: the iTransmit study. Heart Rhythm. 12, 554–559.
Vashist, S., Schneider, E., Luong, J., 2014. Commercial smartphone-based devices and smart applications for personalized healthcare monitoring and management. Diagnostics 4, 104.
Vashist, S.K., Luppa, P.B., Yeo, L.Y., Ozcan, A., Luong, J.H.T., 2015a. Emerging technologies for next-generation point-of-care testing. Trends Biotechnol. 33, 692–705.
Vashist, S.K., Mudanyali, O., Schneider, E.M., Zengerle, R., Ozcan, A., 2013. Cellphone-based devices for bioanalytical sciences. Anal. Bioanal. Chem. 406, 3263–3277.
Vashist, S.K., van Oordt, T., Schneider, E.M., Zengerle, R., von Stetten, F., Luong, J.H.T., 2015b. A smartphone-based colorimetric reader for bioanalytical applications using the screen-based bottom illumination provided by gadgets. Biosens. Bioelectron. 67, 248–255.
Veigas, B., Jacob, J.M., Costa, M.N., Santos, D.S., Viveiros, M., Inacio, J., et al., 2012. Gold on paper-paper platform for Au-nanoprobe TB detection. Lab Chip 12, 4802–4808.
Velusamy, V., Arshak, K., Korostynska, O., Al-Shamma'a, A., Hristoforou, E., Vlachos, D.S., 2013. A novel handheld electrochemical analyzer system interfaced to a smartphone. Materials and Applications for Sensors and Transducers Ii, pp. 47–50.
Wargocki, P., Deng, W., Anwer, A., Goldys, E., 2015. Medically relevant assays with a simple smartphone and tablet based fluorescence detection system. Sensors 15, 11653.
Wei, Q., Qi, H., Luo, W., Tseng, D., Ki, S.J., Wan, Z., et al., 2013. Fluorescent imaging of single nanoparticles and viruses on a smart phone. ACS Nano 7, 9147–9155.
Williams, J., Pearce, K., Benett, I., 2015. The effectiveness of a mobile ECG device in identifying AF: sensitivity, specificity and predictive value. Br. J. Cardiol. 22, 70–72.
Yetisen, A.K., Martinez-Hurtado, J.L., Garcia-Melendrez, A., da Cruz Vasconcellos, F., Lowe, C.R., 2014. A smartphone algorithm with inter-phone repeatability for the analysis of colorimetric tests. Sensors Actuators B Chem. 196, 156–160.
You, D.J., Park, T.S., Yoon, J.-Y., 2013. Cell-phone based measurement of TSH using Mie scatter optimized lateral flow assays. Biosens. Bioelectron. 40, 180–185.
Yu, H., Tan, Y., Cunningham, B.T., 2014. Smartphone fluorescence spectroscopy. Anal. Chem. 86, 8805–8813.
Zeng, X., Gao, Y., Ji, D., Zhang, N., Song, H., Gan, Q., et al., 2014. On-chip plasmonic interferometer array for portable multiplexed biosensing system. CLEO: 2014. Optical Society of America, San Jose, California (p. FM3K.3).
Zhang, D.M., Jiang, J., Chen, J.Y., Zhang, Q., Lu, Y.L., Yao, Y., et al., 2015. Smartphone-based portable biosensing system using impedance measurement with printed electrodes for 2,4,6-trinitrotoluene (TNT) detection. Biosens. Bioelectron. 70, 81–88.
Zhu, H., Sencan, I., Wong, J., Dimitrov, S., Tseng, D., Nagashima, K., et al., 2013. Cost-effective and rapid blood analysis on a cell-phone. Lab Chip 13, 1282–1288.
Zhu, H., Yaglidere, O., Su, T.W., Tseng, D., Ozcan, A., 2011. Cost-effective and compact wide-field fluorescent imaging on a cell-phone. Lab Chip 11, 315–322.