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Review article

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Smartphone videoscopy: Recent progress and opportunities for biosensing

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Abstract: Smartphone is emerging as a portable analytical biosensing platform in many point-of-care (POC) applications such as disease diagnostics, environmental monitoring, and food toxin screening. With the recent advancement of imaging technologies on the smartphone, the manual control of acquisition settings (e.g., exposure time, frame rate, focusing distance, etc.) has already been expanded from the photo to the video capturing mode. In modern smartphone models, high frame rate (above 100 fps) can be achieved to bring in a new temporal dimension to the smartphone-supported POC tests by recording high-definition videos. This opens up a new analytical method defined as smartphone videoscopy. In this review, the recent development of smartphone videoscopy is summarized based on different POC applications. Representative examples of smartphone videoscopy systems and how these time-dependent measurements could open up new opportunities for POC diagnostics are discussed in detail. The advances demonstrated so far illustrate the promising future of smartphone videoscopy in biosensing, POC diagnostics, and time-resolved analysis in general.

Keywords: biosensing; fluorescence microscopy; point-of-care diagnostics; smartphone videoscopy; time-resolved.

1 Introduction

Routine laboratory-based diagnostic or analytical tests require trained personnel to operate, and the instruments for highly sensitive biosensing and measurements are usually bulky and costly. It has therefore posed significant challenges to the broad access to healthcare outside of centralized laboratories and well-resourced regions [1]. Portable and cost-effective readout devices are in increasing demand for point-of-care (POC) diagnostics and biosensing, leading to alternate ways for medical diagnostics and personal health monitoring. Since the initial launch in the late 1990s, the smartphone has experienced a significant evolution. The smartphone devices now can be considered as advanced mobile minicomputers, which integrate fast multicore processor, open-source operation system, large memory, adequate power storage, user-interactive touch screen interface, and multiple sensors such as high-quality image sensors, all in a single platform [2]. The significantly improved computing and imaging capabilities have made the smartphone a promising candidate as the new-generation all-in-one diagnostic platform. The multifunctional smartphone device can act as the optical detector, data processor and storage device, as well as wireless telemedicine communicator with only a handheld size [3]. Over the past decades, the smartphone has been widely explored as a sensitive analytical sensing platform in many POC applications, such as disease diagnostics, environmental monitoring, and food toxin screening [4–8].

With the recent development and popularity of 3D printing technologies, optomechanical components such as optical lenses, filters, gratings, optical fibers, LEDs, and batteries can be easily attached to smartphone camera systems to create various imaging and sensing tools. Integrated with POC molecular assays such as microfluidic or paper-based lateral flow tests through 3D-printed attachments, a plethora of smartphone-based sensor and measurement devices, such as light microscope [9], cytometer [10], and spectrometer [11] have recently been developed. These pocket-size reader devices provide optical-based measurement ranging from brightfield [12], colermetric [13], fluorescent [14], to luminescent (i.e. chemiluminescence (CL) [15], electrochemiluminescence (ECL) [16], and photoluminescence [17]) modes, as well as non-optical measurement such as electrochemical detection

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[18]. As the drastic improvement of complementary metal-oxide semiconductor (CMOS) technologies and optical lens systems on the smartphone in recent years, high-resolution megapixel images can be readily generated on smartphones for accurate and rapid quantification of POC tests; making the optical detection mode the most commonly applied analytical modality on smartphone-based biosensors.

Moreover, because of the high-speed readout circuit on CMOS, the frame rate of smartphone videos now can be controlled ranging from <1 fps to >100 fps, providing a tunable temporal resolution that can benefit various smartphone-enabled POC tests. In particular, a high temporal resolution can be achieved by recording optical signals in video mode without significantly sacrificing image resolution. It opens up many promising biosensing applications, such as micro object tracking [19] and real-time monitoring of chemical kinetics [20], which cannot be achieved by still imaging methods before. We define the emerging smartphone biosensing systems that involve video capturing function to detect and monitor analytes as ***Smartphone Videoscopy***. In this Review article, the recent progress on smartphone videoscopy has been summarized and classified into six applications, namely smartphone videoscopy for cytometric measurement, micro object tracking, real-time assay analysis, fluorescence lifetime measurement, spectroscopic analysis, and single-molecule imaging. The article is closed with the discussion of current challenges and future development of smartphone videoscopy at the end. To the best of our knowledge, there are no other review articles on the same topic before. A list of representative smartphone videoscopy systems developed in recent years and their applications are summarized in Table 1 and also shown in a timeline in Figure 1.

Table 1: Summary of representative smartphone videoscopy systems.

Applications	Detection modality	Frame rate	Year	Reference
White blood cell counting	Imaging cytometry	7 fps	2011	[10]
Blood cell counting	Imaging cytometry	NA	2017	[21]
Cell and microbeads counting	Imaging cytometry	200 fps	2014	[22]
Blood cell counting	Imaging cytometry	NA	2016	[23]
Spermatozoa counting and motility analysis	Imaging cytometry	30 and 60 fps	2016	[24]
Semen concentration and motility analysis	Imaging cytometry	30 fps	2017	[25]
Sperm maturity determination	Imaging cytometry	30 fps	2019	[26]
Salmonella typhimurium counting	Imaging cytometry	NA	2019	[27]
Microdroplet megascale detector (μMD)	Imaging cytometry	30 and 60 fps	2017	[28]
Protein detection in droplets	Imaging cytometry	NA	2019	[29]
Observations and measurement of cell nucleus	Scattering imaging	10 fps	2014	[30]
Monitoring growth of cultured cells	Lens-free imaging	NA	2014	[31]
Quantification of filarial parasites in whole blood	Brightfield imaging	NA	2015	[32]
Detection of HIV-1 RNA	Colorimetric LAMP	30 fps	2018	[19]
Detection of Zika virus	Colorimetric LAMP	30 fps	2018	[33]
Detection of waterborne pathogens	Fluorescent LAMP	15 fps	2020	[34]
Monitoring of glucose in the whole blood	Colorimetric	25 fps	2019	[35]
Real-time detection of HPV DNA in saliva and HIV RNA in plasma	Colorimetric LAMP	1 fps	2020	[36]
Real-time detection of mouse IgG antibodies	SPR imaging	30 fps	2017	[37]
Multiplex determination of three cancer biomarkers (CEA, AFP, and PSA)	Chemiluminescent	NA	2020	[38]
H_2O_2 detection	Chemiluminescent	NA	2015	[39]
H_2O_2 detection	Electrochemiluminescent	30 fps	2019	[40]
Detection of human chorionic gonadotropin (hCG)	Time-gated luminescence	30 fps	2017	[41]
Fingerprint imaging	Time-gated luminescence	NA	2019	[42]
Temperature mapping	Time-resolved luminescence	30 fps	2020	[43]
Detection of oxygen	Time-resolved phosphorescence	NA	2019	[44]
Detection of glucose and human cardiac troponin I	Spectroscopic	19 fps	2016	[45]
Chemical kinetics monitoring	Spectroscopic	30 fps	2019	[20, 46]
Absorption, fluorescence, and resonant reflection spectrum	Spectroscopic	60 fps	2017	[47, 48]
Single blink events detection	SERS	30 fps	2013	[49]
Observing of single-molecule blinking and photobleaching	Fluorescence imaging	12.5 fps	2021	[50]

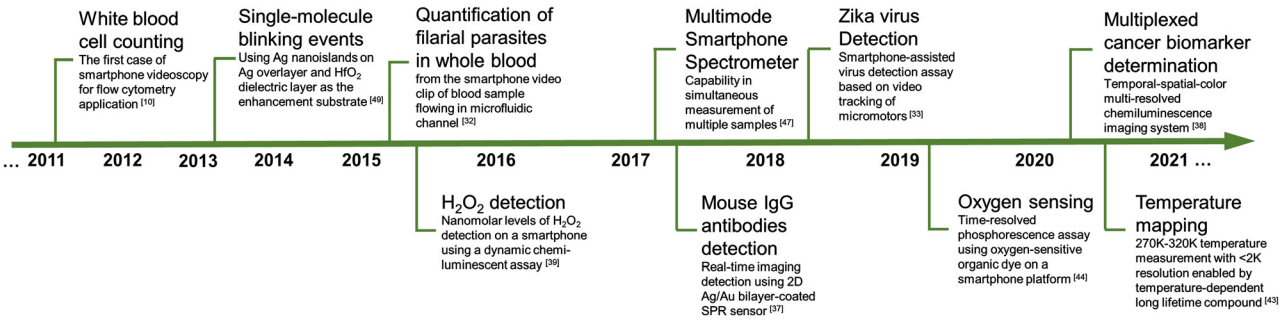

Figure 1: Timeline of representative technological achievements and applications of smartphone videoscopy.

2 Smartphone videoscopy for cytometric measurement

With the constant advancement of smartphone imaging technology in recent years, single-cell resolution has been achieved on smartphone imaging devices while maintaining a relatively large field-of-view [51]. As a result, a number of imaging-based cytometry platforms have been developed by using smartphones as the imager. Smartphone-assisted cytometric sensors can be constructed based on varieties of imaging modalities such as brightfield imaging [12, 52, 53], fluorescence imaging [12, 53, 54], and lens-free imaging [55, 56]. On these platforms, single cells will be identified and counted from single-frame smartphone images. As an alternative, by combining microfluidic chips with smartphone microscopes, flow-based cytometry measurements have also been conducted on several smartphone videoscopy platforms. Compared to smartphone-based imaging cytometry methods, smartphone video cytometry records videos of flowing liquids containing target cells of interest. The continuous workflow allows a higher measurement throughput and easier sample preparation than imaging-based cytometry platforms. The throughput of the smartphone video cytometry system can be adjusted by the flow control system to adapt to different cell types. Meanwhile, additional information such as motility could be extracted from the video clips which is generally unavailable in imaging cytometers. Monitoring of such parameters will provide more clinically relevant information in addition to cell counts.

The first case of smartphone videoscopy for flow cytometry application was reported by Zhu et al. (Figure 2a) [10]. On this platform, fluorescently labeled white blood cells (WBC) were diluted before they were continuously delivered into the microfluidic chamber by a syringe pump. Two blue LEDs were placed on both sides of the chamber to illuminate the sample through waveguide coupling. The emission light from the fluorophores on the WBCs was collected by an emission filter and subsequently focused by an external lens placed in front of the smartphone camera. During the test, the fluorescent flow videos of WBCs in the chamber were captured and analyzed by a single-cell tracking algorithm. The concentration of WBCs in the sample was calculated and compared with a commercial flow cytometer. This optofluidic platform demonstrated the feasibility of using smartphone videoscopy for continuous flow cytometry measurement, which opens many opportunities for single-cell detection and counting. In another example of blood cell counting, Moravapalle et al. designed a smartphone-based blood cytometer with a three-dimensional translational stage to cover a large scanning area during each measurement [21]. A video clip that included a 3×3 mm area of blood smear sample was captured and analyzed. Each frame of the video was processed with a cell-counting algorithm that recognizes and counts cells based on pretraining. The cytometer showed a relatively low counting error of 7% [21]. Recently, machine learning has been introduced to process the super-resolved holographic videos captured by a lensless imaging cytometer. Huang et al. introduced an extreme-learning-machine super-resolution processing (ELM-SR) algorithm to perform recognition and cell counting in a continuously flowing solution [23]. After processing, the lensless contact-imaging based microfluidic cytometer reached less than 8% error for counting the absolute number of microbeads [22]. More recently, they also compared the previous ELM-SR method with the convolutional neural-network-based super-resolution (CNN-SR) and found that CNN-SR has a 9.5% improvement over the ELM-SR method on the resolution enhancement. The improved algorithm was also used for blood cell counting and recognition, and the performance was compared with a commercial flow cytometer [23].

Besides blood cells, smartphone videoscopy has been used for counting and analysis of other types of cells.

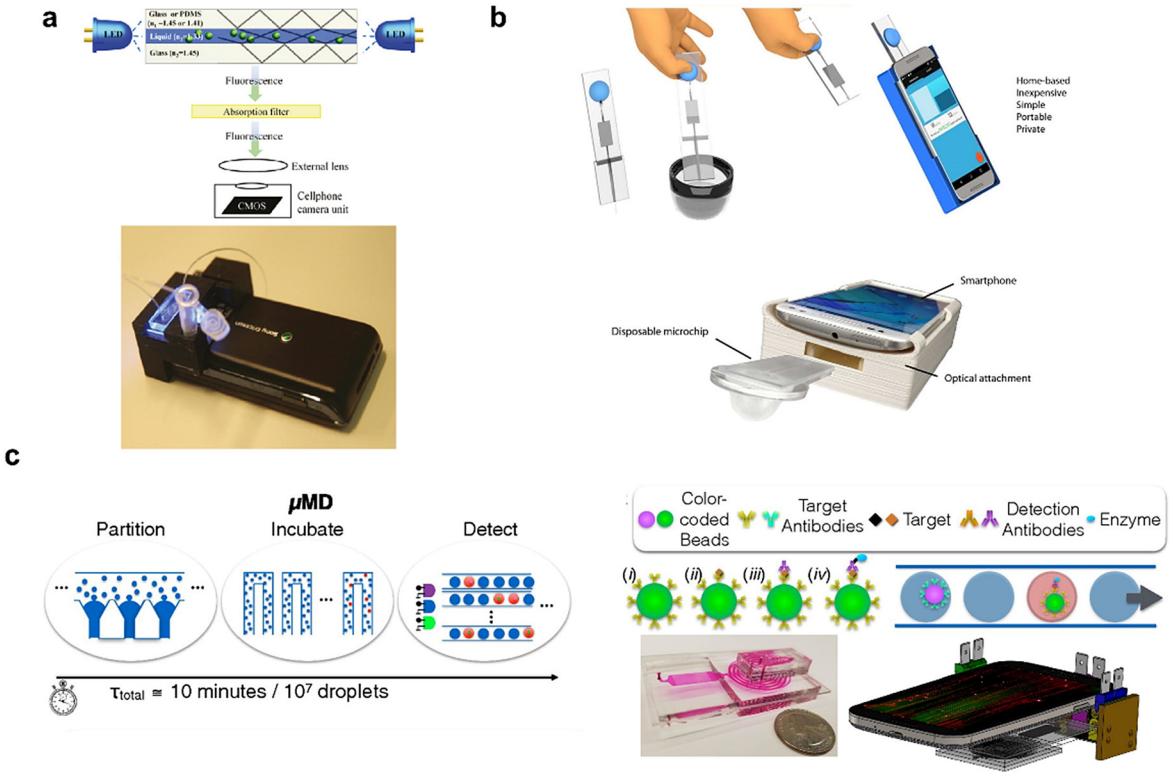

Figure 2: Smartphone-based videoscopic cytometry.
(a) An optofluidic smartphone microscopy device for fluorescence flow cytometry. (b) Point-of-care semen analysis on a smartphone. (c) Mobile phone-based droplet assay for ultrafast and multiplexed detection of protein. Reproduced with permission from Refs. [10, 25, 29], copyright 2011 American Chemical Society and 2017 American Association for the Advancement of Science.

Recently, counting and analysis of spermatozoa have been conducted on smartphone-based imaging cytometer [24]. A simple ball lens-based smartphone microscope was used in the automated semen analysis. By analyzing the smartphone videos of semen samples, the numbers and motilities of spermatozoa could be determined. The system was tested on three smartphones with different models for comparison. Kanakasabapathy et al. reported a POC semen analysis platform based on smartphone video microscopy (Figure 2b) [25]. The platform features a disposable microfluidic chip that has a capillary tip at the inlet and a bulb at the other end. During each use, the user created a negative pressure by pressing the bulb, which drew the semen sample into the microfluidic chip. The chip was then inserted into the smartphone device for brightfield video capturing. Both semen concentration and motility were calculated and were compared with the results from a laboratory-based semen analysis. The simple and user-friendly smartphone platform demonstrated the possibility for accessible male health monitoring at home or in remote areas. More recently, the same group has further extended the application of their system for other sperm quality assessment tests, including hyaluronic binding assay (HBA), sperm viability tests, and sperm DNA fragmentation test [26]. In the HBA assay, videos of spermatozoa in the hyaluronic acid-coated region and nonhyaluronic acid-coated region were differentially compared to calculate HBA scores. The smartphone software was able to process the video in less than 10 s and run the whole assay in less than 1 min [26].

Counting of *Salmonella typhimurium* has been reported using smartphone videoscopy as well. In this platform, the bacteria were immuno-labeled with magnetic nanoparticles and separated from other impurities. Then, the bacteria were labeled with fluorescent nanospheres and separated from unreacted microspheres. Finally, the concentrated, fluorescently labeled bacteria were continuously injected into a microfluidic chip. The number of fluorescent bacteria was finally calculated by processing the captured videos in a smartphone application [27].

Besides cell counting, the concept of smartphone videoscopic flow cytometry has also been combined with microfluidic droplet assays for high-throughput and multiplexed detection. Issadore et al. have recently developed

an ultrafast digital droplet assay (microdroplet Megascale Detector, μMD) on the smartphone [28]. Earlier, they reported the detection of 106 droplets per second using a smartphone camera. The fluorescent and nonfluorescent droplets were generated, mixed, and injected into parallel microfluidic channels on a chip. The videos of droplets flowing in the parallel channels were recorded and analyzed. The high-throughput, ultrafast droplet detection was achieved by the correlation of time-domain modulated excitation with the extracted signal from the video clips. The correlation-based detection algorithm is capable of resolving neighboring droplets regardless of the frame rate of smartphone video recording. More recently, they have further applied the system on protein detection (Figure 2c) [29]. In this assay, color-coded, antibody-functionalized beads were individually encapsulated into droplets if the target protein is captured. Beads processing, mixing, droplet generation, and incubation were all integrated on the microfluidic chip. Droplets with beads were detected using the algorithm discussed above. By using different fluorescent dyes, duplex protein detection with sub-picomolar limit of detection (LOD) was successfully achieved. The system can potentially be applied to high-throughput cell screening as well.

3 Smartphone videoscopy for micro-object tracking

In addition to cell counting, smartphone videoscopy has also received a number of applications in micro object tracking, including micro organism and single-particle tracking (SPT). By combining robust tracking algorithms, these object-tracking platforms could be applied to extract rich information about the targets such as motility, diffusion coefficient, etc., in addition to counts and particle density that are typical for counting applications. This additional information can improve the efficiency of classification of different micro organisms or pathogens. It also provides a noninvasive means to measure the physiological status of the targets. For example, Wu et al. have developed a scattering imaging-based cell analyzer to identify different types of cells from their nuclear structures [30]. In this device, the cells were allowed to flow in a glass chamber. An LED was placed above the sample chamber as the light source with a microlens placed below it for focusing. Between the microlens and the sample is a metal slit to reject most of the directly incident light that hits the sample. A thin light-sheet was generated in this configuration to illuminate passing cells, and the image was focused by an objective lens before the darkfield image of the cell was formed on the CMOS sensor. On the other hand, the diffusive autofluorescence signal from the microlens could also be used as brightfield illumination for the cells [30]. A video clip of cells passing through the chamber was recorded. From the video, both darkfield and brightfield images of passing cells were recorded in the chamber at the single-cell resolution. The nuclear structural characteristics were resolved in the darkfield images, while the cell contours were shown in the brightfield images. By analyzing these images, cells in different proliferation stages as well as different types of WBCs, such as mononuclear and neutrophil cells, could be identified. Time-lapsed lens-free imaging has also been used to monitor the growth of cultured cells [31]. Consisting of an LED, a pinhole, and a CMOS sensor, the lensfree imaging device was relatively simple in structure. The lensfree holograms of the cells were received by the CMOS sensor, and reconstructed to reveal the morphology of the cells. Using this device, a number of events including cell-substrate adhesion, cell spreading, cell division orientation, as well as cell death, could be tracked over a time duration from 2 to 70 h. Moreover, given the large field-of-view of the imaging setup, events from around 3500 cells could be monitored simultaneously. Cell motility was also monitored and recorded over a 65-h test. Although the resolution is not quite comparable with lens-based imaging, this simple lensfree imaging setup can be well used in the large-scale monitoring of cell culture and related biological research. For micro-organism tracking, D'Ambrosio et al. tracked and quantified filarial parasites in the whole blood sample from smartphone-recorded videos (Figure 3a) [32]. In this integrated platform, a microcontroller was used to control the optical illumination on the smartphone microscope, as well as the liquid flow in the chamber. Short smartphone video clips were recorded as the blood sample containing filarial parasite flew through the chamber. The differential images between each frame and an averaged image from all frames were calculated. The presence of the parasite could be effectively identified from these differential images. By imaging blood samples from five different areas of the chamber, which corresponded to a total sample volume of <15 μL, the parasite density can be accurately estimated by using this device. A pilot field study was conducted in Cameron to validate the effectiveness of the smartphone videoscopic diagnostic platform.

In addition to live-cell tracking and parasite detection, SPT has also been demonstrated on the smartphone for applications related to POC pathogen detection. For instance, Draz and his colleagues designed an assay based on particle mobility to detect HIV-1 RNA on the smartphone

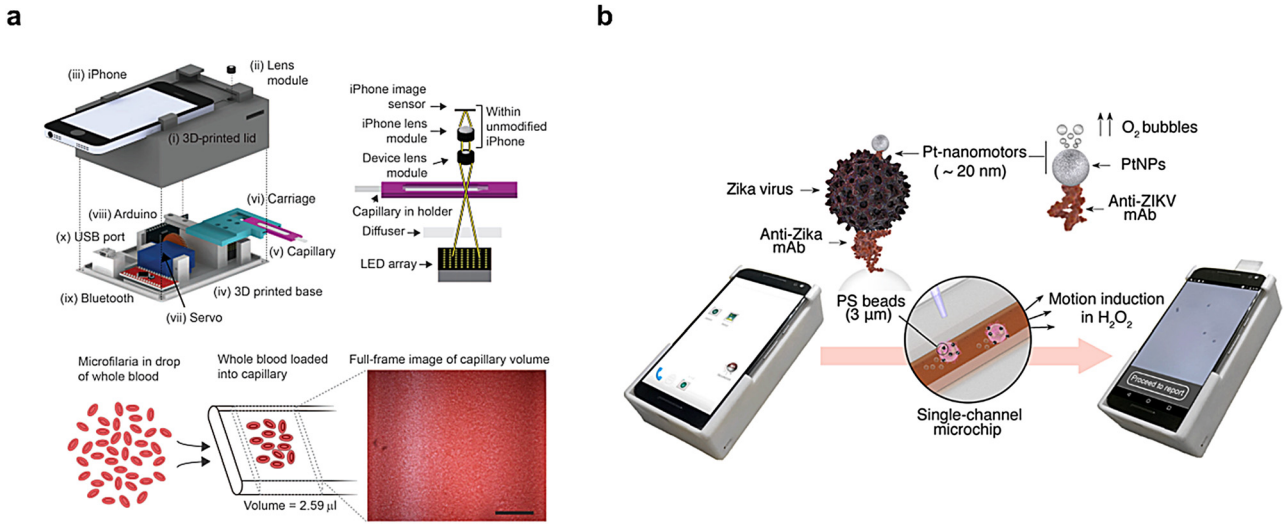

Figure 3: Smartphone videoscopy for object tracking.
(a) Blood-borne filarial parasite detection by using a smartphone-based video brightfield microscope (b) A smartphone-assisted virus detection assay based on video tracking of micromotors. Reproduced with permission from Refs. [32, 33], copyright 2015 American Association for the Advancement of Science and 2018 American Chemical Society.

[19]. The core of the assay, the micromotor, consists of a polystyrene (PS) bead with platinum (Pt) nanoparticles coated on one side and DNA conjugated gold (Au) nanoparticles on the other side. The DNA segment on the Au nanoparticle is able to capture the amplicon from a loop-mediated isothermal amplification (LAMP) assay. When H_2O_2 was added, the Pt nanoparticle would catalyze the decomposition of H_2O_2. Driven by the bubbles from the reaction, the movement of the micromotor was recorded by a smartphone imaging device. A higher copy number of amplicons would reduce the speed of the moving micromotor, which can be finally correlated to the concentration of target HIV-1 RNA. Consequently, the sensitive detection of HIV-1 RNA could be achieved by measuring the motion speed of the Pt-micromotor. In the study, a threshold velocity of micromotor was applied to determine the presence of LAMP DNA from the background noise. A smartphone application interface was developed to record, analyze the video, and provide the user with positive/negative diagnostic results. Based on a similar principle, this assay was also used in the detection of Zika virus (ZIKV) on the smartphone (Figure 3b) [33]. In the presence of ZIKV, an antigen-antibody sandwich structure would form between the catalytic nanoparticle, ZIKV, and the PS bead. The catalysis-induced motion of Pt-PS micromotor indicated the presence of the virus, while unbound PS microspheres would stay relatively still when no virus was present. The LOD was calculated to be as low as one particle per uL in ZIKV-spiked urine samples. Recently, in another work from the same group, they reported another smartphone assay combining catalytic Pt nanoparticle and deep neuron network for ZIKV detection [57]. In this assay, the virus was captured at the bottom of a microfluidic cartridge, followed by labeling of Pt nanoparticles. In the presence of the captured virus, oxygen bubbles were formed because of the catalytic activity of Pt nanoparticles in contact with H_2O_2. The videos of the bubbles were recorded and were used to train the convolutional neuron network (CNN). The system achieved 98.97% sensitivity in detecting ZIKV-infected samples down to 250 copies/mL.

Detection of bacteria with smartphone videoscopy has also been reported. Moehling et al. developed a smartphone-based particle-tracking detection of LAMP amplicon targeting the water-borne pathogen *Vibrio cholerae* [34]. The method quantified the increase of solution viscosity after LAMP reaction by imaging the fluorescent microbeads suspended in the solution. The diffusion coefficient was calculated from the Brownian motion trajectory of the particle in the solution, which is related to the viscosity of the solution. The presence of bacterial DNA could be detected by the change in the diffusion coefficient calculated from particle movement videos. A simple smartphone fluorescent imaging setup based on tilted laser illumination was adopted to capture the smartphone videos. The LOD of this system was determined to be 400 cells/mL, which is relevant to the environmental concentration range. A selectivity test was also conducted in spiked pond water samples.

4 Smartphone videoscopy for real-time assay analysis

Taking advantage of the sensitive CMOS imaging sensors on the smartphone, various biomolecular assays have been quantified on the smartphone as a portable and affordable optical reader device. In the past decades, by combining smartphone with molecular technologies like lateral flow immunoassay (LFIA), enzyme-linked immunosorbent assay (ELISA), and nucleic acid amplification such as LAMP, colorimetric-based [58–60] and fluorescence-based [61–63] detection on the smartphone are widely explored for molecular sensing and diagnosis. Different from single snap-shots, the video recording function of smartphones makes it extremely useful in monitoring assay dynamics in real time, which can help detect the analytes at a much earlier stage.

Video-based colorimetric-based detection on a smartphone was explored on various analytes in conjunction with different assay technologies. For example, glucose in whole blood was monitored by a smartphone video-based colorimetry method in combination with a thread-paper microfluidic device (μTPAD) [35], which can deliver the results in 12 s with only 3 μL of whole blood without any pretreatment. The color change on the cotton thread caused by oxidized 3,3′,5,5′-tetramethylbenzidine (TMB) was monitored by the smartphone video recording (1440 × 1080 pixels, 25 fps) in real-time. The system detected the glucose in the whole blood with a LOD of 12 μM. Yin et al. developed a portable smartphone-based quantitative pathogen detection platform (termed 'SCPT'), by using synergistically enhanced colorimetric LAMP assay and smartphone-based color analysis [36]. The authors claimed for the first time to quantify nucleic acid biomarkers in real-time using an unmodified smartphone by a hue-based LAMP assay. Unlike the most used video frame rate (e.g., 30 fps), the authors set a slow acquisition rate of one frame per minute for 60 min to monitor the whole amplification reaction process by a custom app. HPV DNA in saliva and clinical vaginal swab samples, and HIV RNA in plasma samples were quantitatively detected on this platform, with a detection sensitivity of <100 copies for HPV16 DNA, which is comparable to the results of benchtop PCR machines. To eliminate the influence of light conditions and improve the sensitivity of colorimetric-based biosensing on the smartphone, Coleman et al. adopted a video processing algorithm to select the best inputs from a large set of video frames in 20 s [64]. The algorithm was applied to monitor the NS1-based sandwich ELISA assay for Zika detection, and the LOD was two times lower than snap-shot-based methods when no video-based analysis is used.

Surface plasmon resonance (SPR) is a label-free and real-time biosensing technique with high sensitivity, which is suitable for quantitative analysis and characterization of biomolecular interactions [65]. A few studies have recently reported SPR sensing on image-based smartphone platform [66, 67]. Liu et al. reported using flash LED and rear camera of the cell phone as the light source and imaging sensor for SPR detection. The change of refractive index (RI) was recorded at a frame rate of 2 fps by extracting the light intensities from the individual frames. Moreover, Guner et al. [37]. demonstrated a 2D SPR imaging sensor for high-throughput and multiplexed detection on a smartphone with a 4-by-4 microarray of sensing spots on an Ag/Au bilayer-coated sensor (Figure 4a). Videos at 30 fps were recorded for real-time monitoring biomolecular reactions and the signal-to-noise ratio was enhanced by averaging several consecutive frames. This system is capable of detecting multiple analytes simultaneously by fabricating a microfluidic chip with multiple sensing spots. Utilizing spatially resolved multiplexing and video-based real-time detection, the system was applied to detect mouse IgG antibodies with a nanomolar level LOD.

CL is an important measurement strategy in analytical chemistry and biosensing. Smartphone provides an attractive platform for CL measurement to satisfy the needs of on-site analysis of kinetic curves for reaction [68] and quantitative bioanalysis [69]. Li et al. developed a temporal-spatial-color multiresolved CL imaging system on the smartphone for multiplex immunoassays [38]. The CL signals were recorded by the smartphone camera in video mode. The image frames at different time points of the video were extracted for observing the dynamic process of luminescence reactions. The extracted images were stacked to create a new image with strong and uniform intensities for quantitative analysis. Lebiga et al. demonstrated CL intensity as a function of time following an exponential decay pattern, by recording videos of the reaction of H_2O_2 and bis(2,4,6-tri- chlorophenyl)oxalate in the presence of rubrene and imidazole with a smartphone (Figure 4b) [39]. They also found the concentration of H_2O_2 in the reaction affects not only the peak intensity but also the rate of CL decay against time. The H_2O_2 concentration as low as 250 nM can be detected with only 25 μL samples in the paper–plastic disposable microfluidic device. Differently, Escobedo et al. also designed a smartphone-based POC platform for the determination of H_2O_2 by means of video recording based on ECL emission mechanism, which is an electrogenerated form of CL [40].

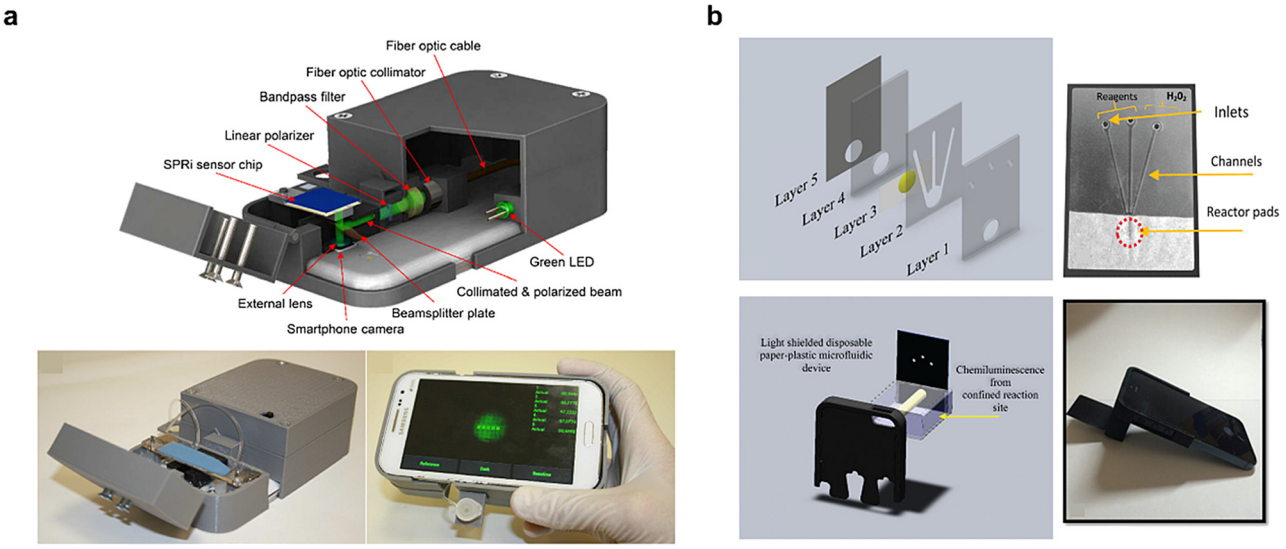

Figure 4: Smartphone videoscopy for time-dependent assay analysis.
(a) A smartphone-based biosensor for surface plasmon resonance imaging. (b) Chemiluminescence detection of nanomolar levels of H_2O_2 on a smartphone. Reproduced with permission from Refs. [37, 39], copyright 2016 Elsevier and 2015 The Royal Society of Chemistry.

5 Smartphone videoscopy for fluorescence lifetime measurement

Paper-based lateral flow assay (LFA) is a widely used POC testing technology since it is inexpensive, rapid, and easy to fabricate. Popular examples include the rapid tests for pregnancy [70] and HIV [71]. The existing LFA devices can be categorized into qualitative and quantitative tests. The latter requires an optical reader device that can accurately and quantitatively measure the signals of the positive lines of the LFA assay. Due to their imaging performance and portability, smartphones demonstrated significant advantages in quantifying LFA testing [72]. However, one of the remaining challenges of the conventional LFA is that the autofluorescence from the cellulose-based paper substrate and also the biological reagents such as the proteins significantly interferes with the fluorescent emission of report molecules, resulting in a decreased signal-to-noise ratio and detection sensitivity [41, 73].

One approach that can address autofluorescence interference is through time-resolved fluorescence/luminescence detection. The time-resolved method takes advantage of long-lived luminescence probes, whose luminescent signals are detected in a delayed time window following the short excitation pulse, whereas scattering and short-lived autofluorescence interference will be diminished by the time delay [74, 75]. The smartphone is an emerging platform for time-resolved detection by capturing image sequences or videos of long-lived luminescence immediately after the excitation pulse. Only simple optomechanical components such as UV LED and focusing lens are needed to construct a time-resolved smartphone microscope in most cases. Depending on different strategies of video processing, the smartphone can do either time-gated [41, 42] or time-resolved lifetime detection [43, 44, 76]. The time-gated detection on the smartphone can be achieved by extracting the first post-excitation frame from the smartphone videos after the excitation is turned off. On the other hand, luminescence lifetime can be calculated by fitting the intensities of postexcitation frames into an exponential decay function: $I = A \cdot \exp\left(-\frac{t}{\tau}\right)$, where I is the intensity of a region of interest (ROI) at time point t, A is the intensity of the same ROI at the first frame after turning off the excitation, and τ is the luminescent lifetime to be calculated. The frame rate of most smartphone cameras (30 fps or above) provides enough temporal resolution for time-resolved lifetime detection with properly chosen long-lived luminescent reporters.

Paterson et al. [41]. adapted time-gated imaging on an iPhone 5s to capture continuous videos at a frame rate of 30 fps to detect human chorionic gonadotropin (hCG) with an LFA and persistent luminescent strontium–aluminate nanophosphors ($SrAl_2O_4$:Eu^{2+}, Dy^{3+}) as the reporter. To simplify the device, the authors used the smartphone's camera flash as the excitation light. The phosphors were first excited by a 3 s torch followed by a 300 ms flash, during which the frames of the captured videos were

extracted to determine the exact frame of flash-off. Then, the first postflash frame was captured and stored for processing and signal quantitation. The whole process was controlled and implemented by a programmed application running on the phone. A consistent time delay between the flash and first postflash image was achieved by a gap of approximately 100 ms, which is efficient in removing the background autofluorescence. The detection limit for hCG on this time-gated smartphone imaging platform is determined to be around 1.2 pM, which is 10- to 50- fold better than commercial LFA-based tests [77–81]. Tian et al. implemented a similar time-gated strategy on a Mi 4 phone to detect fingerprints with a different persistent luminescent reporter (1 wt% CzDPS in cross-linked TRPGDA (tripropylene glycol diacrylate)), which also effectively eliminated the interference from background autofluorescence [42].

Luminescent lifetime in the range of hundreds of milliseconds can also be resolved from the analysis of video frames recorded by the smartphone camera, which usually operates at a frame rate of 30 fps [43, 44]. By using long-lifetime luminescent probes, which are sensitive to the microenvironment [82, 83], analytes i.e. pH [84], temperature [43], and oxygen [44] can be quantitatively detected. In a recent work by Katumo et al. [43], they demonstrated a phosphor, europium-doped gadolinium oxysulfide ($Gd_2O_2S:Eu^{3+}$), which exhibits a temperature-dependent long lifetime on the order of a few hundreds of milliseconds (Figure 5a). Such a long lifetime allowed thermal maps to be created via the analysis of videos captured with a smartphone (Galaxy A5 2017) at a rate of 30 fps. After a pulsed 1000 ms excitation by a UV LED, the lifetime is determined by exponentially fitting the red-channel luminescence intensities of postexcitation frames over time. By establishing the delayed luminescence lifetime as a function of temperature, the smartphone-based thermometry allows reliable temperature measurement in the range of 270–320 K with a temperature resolution better than 2 K, which is comparable with many commercial devices used in thermal imaging. With a similar strategy, Zhou et al. used a polymer film doped with an oxygen-sensitive and long-lived (lifetime about 350 ms) unconventional organic molecule with asymmetric noncoplanar D-p-A system, namely TBBU for quantitative detection of oxygen (Figure 5b) [44]. For shorter lifetime probes, Zhu et al. [76] effectively resolved the lifetime in the microseconds range by tracing the luminescence decay in the spatial domain instead of the conventional time domain with a smartphone and motor-driving turntable.

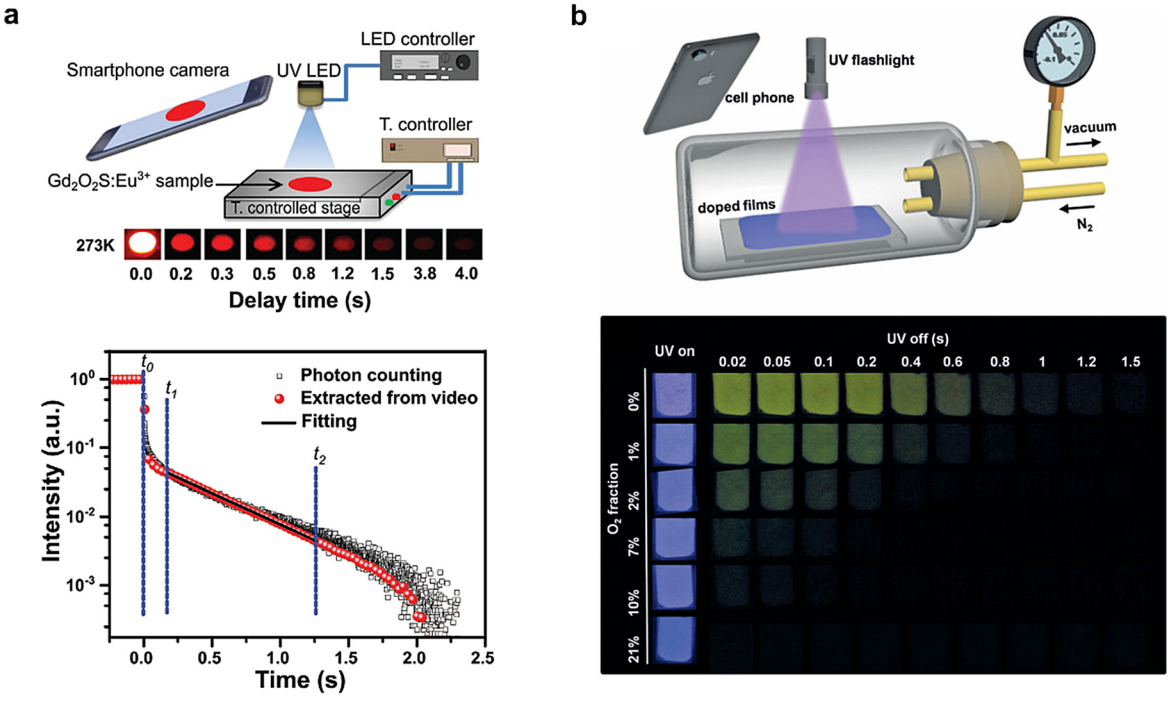

Figure 5: Smartphone videoscopy for luminescent lifetime measurement.
(a) Smartphone-based luminescent thermometry. (b) Quantitative detection of oxygen in real time based on persistent lifetime measurement on a smartphone platform. Reproduced with permission from Refs. [43, 44], copyright 2019 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

6 Smartphone videoscopy for spectroscopic analysis

The concept of "Cell Phone Spectrometer" was first raised in 2010 by A. Scheeline et al. [85]. by attaching a light source and a grating in front of the phone camera as a demonstration of low-cost optical instrument in teaching. Later, Cunningham et al. greatly matured this area and developed a series of smartphone-based spectrometers as label-free biosensing instrument based on photonic crystals (PC) [86]. Since then, many other smartphone-based spectrometers have been developed, which are integrated with different types of light sources, e.g., laser [87], LED [88], sunshine [89], phone flash [90], and varied grating [11, 91–94] for promising applications ranging from absorption/fluorescence measurement [92], to POC biosensing [91, 95], and diagnostics [88, 93]. For diagnostic applications, most of the smartphone spectrometers are designed for colorimetric or fluorescent assays that emit visible light between 400 and 700 nm. The signals therefore can be readily captured by the color CMOS camera on the smartphone. To obtain spectral information, the emission or transmission light from the sample is dispersed into a rainbow band after passing the diffraction grating, which projects a spectrum image over the smartphone image sensor. After calibration by correlating pixel position with specific wavelength values, the spectrum image can be converted into a spectral intensity profile. The video function integrated on the smartphone can further add a temporal resolution to the spectrum measurements for real-time or time-dependent detection.

A smartphone spectrometer was developed by Wang et al. by using the build-in flash LED as the light source and a compact disk (CD) as the grating substrate (Figure 6a) [45]. Smartphone videos with a rate of 19 fps were recorded for real-time colorimetric detection of glucose and human cardiac troponin I. The spectrum images were first extracted from the recorded videos and then converted to intensity spectra in the wavelength range from 430 to 650 nm with a resolution of 0.386 nm per pixel. The developed smartphone spectrometer was used to monitor the whole spectrum change over time and provided a two-fold higher detection sensitivity compared to that of commercial plate-readers. Bogucki and Greggila et al. also used a smartphone-based spectrometer to collect time-dependent data using the video mode at a frame rate of 30 fps (Figure 6b) [20, 46]. By engineering a dual-beam geometry inside a 3D-printed adaptor that can hold two sample cuvettes, the smartphone spectrometer device can collect the sample and background spectra simultaneously to significantly increase the signal-to-noise ratio and reproducibility of the data.

While the above-mentioned systems utilized the smartphone videos to monitor the absorption spectra, Cunningham et al. demonstrated a smartphone-based handheld spectrometer using video recording to extend the multiplexing capacity by performing simultaneous measurement of multiple samples (Figure 6c) [47, 48]. The team fabricated a cartridge comprised of a series of linear compartments containing sample replicates or experimental controls to achieve spatial multiplexing on the smartphone spectrometer. The spectra of all samples in the cartridge were recorded successively by the smartphone videos at 60 fps, when the cartridge was swiped across the examination spot. Customized software was developed that can automatically select representative spectra with the best light alignment of each compartment from the video. This device was also reported as one of the early examples of a smartphone-based spectroscopic sensor that can measure three distinct spectral modalities on the same platform, i.e., colorimetric absorption spectrum, fluorescence emission spectrum, and resonant reflection spectrum.

7 Smartphone videoscopy for single-molecule imaging

Single-molecule imaging plays an increasingly important role in biological science, such as protein and RNA detection [96, 97], protein structure determination [98], and DNA sequencing [99]. Traditional microscopy and spectroscopy approaches for single-molecule imaging require bulky and expensive instrument, which limits its applications in advanced laboratory settings. Although many sensing and imaging systems have been demonstrated on the smartphone in recent years, performing single-molecule imaging on the smartphone remains challenging and is considered the next milestone for researchers. Limited by the sensitivity of the smartphone sensor and the low numerical aperture (NA) of the smartphone imaging system, observing single-molecule events was difficult without proper enhancement of the fluorescence signals. In earlier attempts, a thin film of silver was used as the plasmonic substrate to enhance the fluorescence signal in smartphone-based fluorescence microscopy [100]. DNA-origami nanobeads with predefined numbers of fluorophores were used as brightness standards. The limit of sensitivity was estimated to be ~80 fluorophores per diffraction-limited spot for the silver film-enhanced smartphone fluorescence microscopy. Using an upgraded

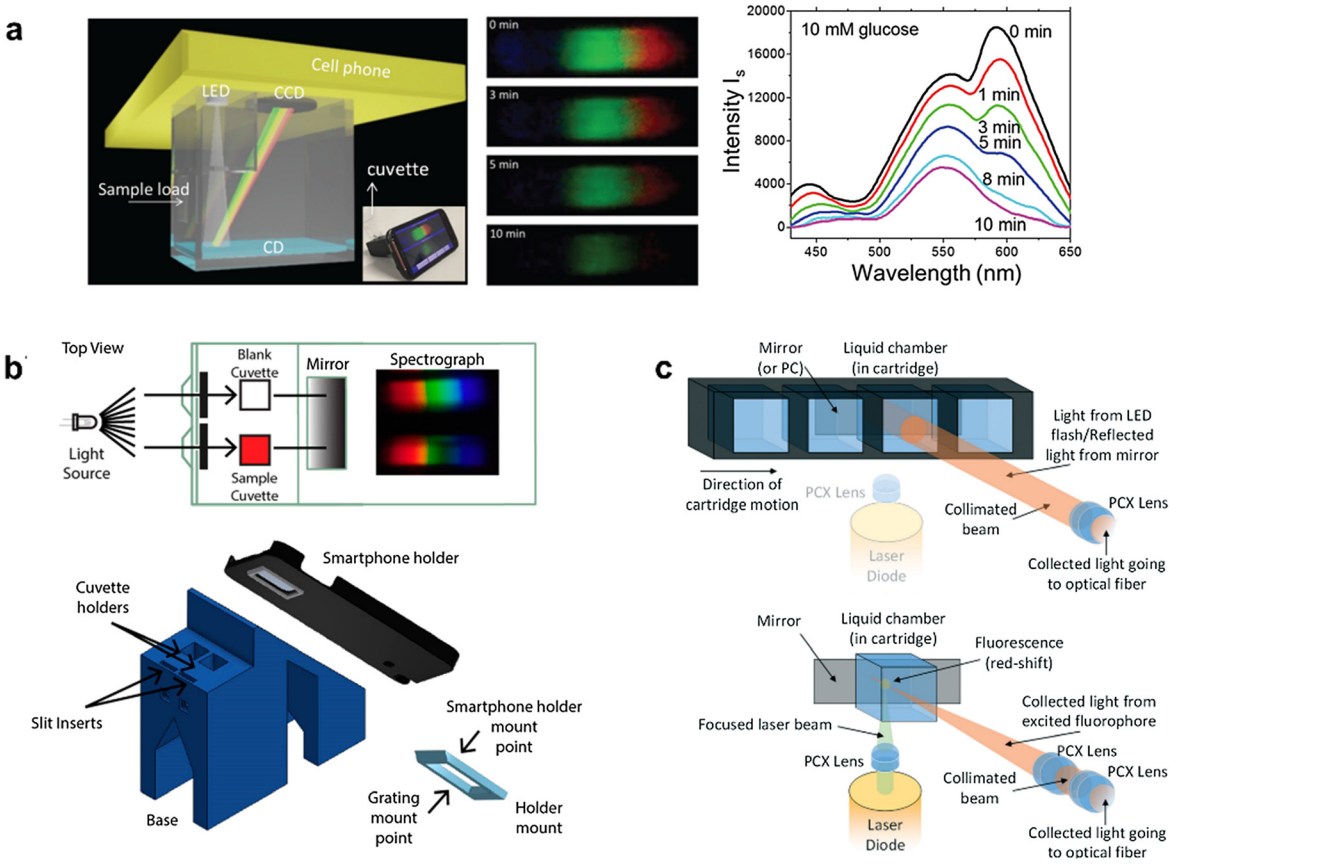

Figure 6: Smartphone videoscopy-enhanced spectrometer.
(a) Smartphone spectrometer for colorimetric biosensing. (b) A 3D-printed dual-beam spectrophotometer on a smartphone. (c) Smartphone-based multimode spectrometer for transmission, reflection, and fluorescence spectral biosensing. Reproduced with permission from Refs. [45–47], copyright 2016 The Royal Society of Chemistry, 2019 American Chemical Society and 2017 The Royal Society of Chemistry.

phone model with a monochromic image sensor, this limit of sensitivity was further reduced down to ~10 fluorophores per diffraction-limited spot on the phone [101]. However, either the enhancement from the substrate or the sensitivity of the imaging system is not sufficient to observe single-molecule events directly on a smartphone in the previous studies.

Significant progress has been made on the single-molecule imaging on the smartphone recently. Ayas et al. observed single-molecule blinking events on the smartphone by using Ag nanoislands on Ag overlayer and HfO_2 dielectric layer as the enhancement substrate [49]. The thickness of the Ag overlayer and dielectric layer was optimized to produce the highest blinking rates and intensities. The smartphone was placed in front of the eyepiece of a benchtop microscope to complement the imaging apparatus. With the optimized plasmonic substrate, blinking events could be observed from smartphone videos recorded at a frame rate of 30 fps. Raman spectrum and surface-enhanced Raman scattering (SERS) blinking events were also studied using the custom-built low-resolution spectrometer. In the spectrometer configuration, the collection fiber from the objective was collimated and dispersed with a transmission grating before entering the smartphone camera detector. By comparing the time series of Raman spectrum between a smartphone detector and a cooled charge coupled device (CCD) detector, a similar single-molecule blinking event was observed on the smartphone. More recently, single-molecule blinking and photobleaching events were observed on a standalone, portable smartphone microscope for the first time (Figure 7) [50]. In this work, a strong amplification of fluorescence signals from the emitters was achieved by using silver nanoparticle dimers assembled by DNA origami pillars, also known as NanoAntennas with Cleared HOtSpots (NACHOS) (Figure 7a). Using this smartphone microscopy platform, up to 461-fold fluorescence enhancement was observed. Finally, single-molecule imaging was conducted

on this smartphone microscope by using a portable laser diode as the light source and an inexpensive lens module as the objective lens (Figure 7b). From the fluorescence time trace extracted from the smartphone video clips, hallmarks of single-molecule events such as blinking and single-step photobleaching were observed, confirming the direct visualization of single fluorescent molecules on the smartphone (Figure 7c). A sandwich DNA detection assay was also developed to demonstrate the potential application of such as handheld single-molecule imaging device. The strong, enhanced fluorescence signals were only observed in the presence of target DNA sequences, demonstrating the future application of this system for POC diagnostics.

8 Summary and future perspective

The recent decades have witnessed the rapid development and popularization of smartphone as a sensitive analytical sensing platform in many POC applications. After the first example of video-based smartphone biosensing reported in 2011 [10], smartphone videoscopy has been widely explored for the detection of various analytes in conjunction with different assay technologies. The number of related publications has grown dramatically especially after 2017. The video function of smartphone has been used to record the flow of cells or particles in microfluidic chips, track the motion of micro organism or single particles, monitor the trace of chemical kinetics in real-time, detect long luminescent lifetime, measure the spectra during reactions, and visualize the single-molecule blinking events. The smartphone videoscopy with a controllable temporal resolution has significantly advanced the conventional smartphone POC tests by extracting useful diagnostic information in the time domain.

Although many related works have been reported, smartphone videoscopy is still in the early stage of exploration with high potential in the future. There are still several challenges necessary to overcome in the design of next-generation video-based smartphone biosensors. First, the temporal resolution of smartphone videoscopy is still limited by the available frame rate of the smartphone video. The most common acquisition setting is 30 fps, and many video-based mobile biosensing applications have been reported to use this frame rate. However, for some emerging applications such as single-molecule imaging

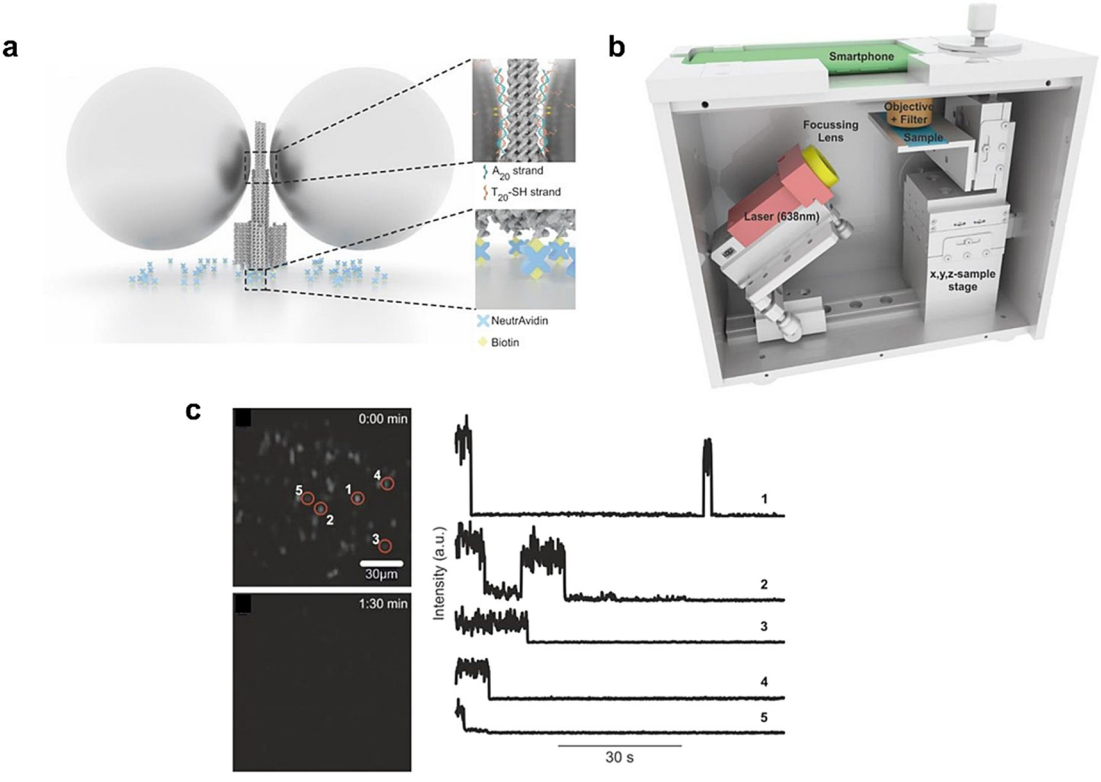

Figure 7: Single-molecule imaging on a portable smartphone microscope using DNA origami-assisted plasmonic nanoantenna.
(a) Schematics of NACHOS assembly (b) Sketch of the portable smartphone microscope (c) Fluorescence image of a single Alexa Fluor 647 in NACHOS (left) and exemplary fluorescence transients (right) showing single bleaching steps of dyes and long-time blinking events measured on the portable microscope setup. Reproduced with permission from Ref. [50].

and luminescent lifetime detection, 30 fps is far too slow to capture the quick decay signals. To this end, several latest smartphone models on the market such as Google Pixel 5 and Sony Xperia 1 II are capable of recording videos at 120 fps with a 1080 p resolution. In addition, the slow-motion mode on Samsung Galaxy S20 Ultra, Xiaomi Mi 10 Pro, or Huawei P40 Pro allows an even higher frame rate of 960 fps for a short duration, which is able to provide a much better time resolution down to 1 ms. However, the higher frame rate will be companioned by shorter exposure time, which represents the second challenge to detect signals with very faint fluorescent intensity. A smartphone image sensor with higher detection sensitivity will be needed for these applications and a convex lens with higher NA is required to increase the throughput of light transmission to the smartphone imaging sensor. Finally, high-frame-rate video recording also demands more efficient data transmission/processing protocols to achieve real-time or near-real-time data analysis and visualization. A cloud-based data communication framework and processing algorithms will be needed to shorten the data processing time and increase the data analysis accuracy. In addition, smartphones with higher computational power are desired to provide on-phone video data processing capability. Overall, smartphone videoscopy is promising to play a crucial role in future POC biosensing and applications.

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References

[1] K. E. McCracken and J.-Y. Yoon, "Recent approaches for optical smartphone sensing in resource-limited settings: a brief review," *Anal. Methods*, vol. 8, pp. 6591–6601, 2016.
[2] X. Huang, D. Xu, J. Chen, et al., "Smartphone-based analytical biosensors," *Analyst*, vol. 143, pp. 5339–5351, 2018.
[3] D. Xu, X. Huang, J. Guo, and X. Ma, "Automatic smartphone-based microfluidic biosensor system at the point of care," *Biosens. Bioelectron.*, vol. 110, pp. 78–88, 2018.
[4] A. Roda, E. Michelini, M. Zangheri, M. Di Fusco, D. Calabria, and P. Simoni, "Smartphone-based biosensors: a critical review and perspectives," *TrAC – Trends Anal. Chem.*, vol. 79, pp. 317–325, 2016.
[5] I. Hernández-Neuta, F. Neumann, J. Brightmeyer, et al., "Smartphone-based clinical diagnostics: towards democratization of evidence-based health care," *J. Intern. Med.*, vol. 285, pp. 19–39, 2019.
[6] D. Zhang and Q. Liu, "Biosensors and bioelectronics on smartphone for portable biochemical detection," *Biosens. Bioelectron.*, vol. 75, pp. 273–284, 2016.
[7] L. Neubeck, N. Lowres, E. J. Benjamin, S. B. Freedman, G. Coorey, and J. Redfern, "The mobile revolution—using smartphone apps to prevent cardiovascular disease," *Nat. Rev. Cardiol.*, vol. 12, pp. 350–360, 2015.
[8] S. Kanchi, M. I. Sabela, P. S. Mdluli, Inamuddin, and K. Bisetty, "Smartphone based bioanalytical and diagnosis applications: a review," *Biosens. Bioelectron.*, vol. 102, pp. 136–149, 2018.
[9] I. Navruz, A. F. Coskun, J. Wong, et al., "Smart-phone based computational microscopy using multi-frame contact imaging on a fiber-optic array," *Lab Chip*, vol. 13, p. 4015, 2013.
[10] H. Zhu, S. Mavandadi, A. F. Coskun, O. Yaglidere, and A. Ozcan, "Optofluidic fluorescent imaging cytometry on a cell phone," *Anal. Chem.*, vol. 83, pp. 6641–6647, 2011.
[11] C. Zhang, G. Cheng, P. Edwards, M.-D. Zhou, S. Zheng, and Z. Liu, "G-fresnel smartphone spectrometer," *Lab Chip*, vol. 16, pp. 246–250, 2016.
[12] D. N. Breslauer, R. N. Maamari, N. A. Switz, W. A. Lam, and D. A. Fletcher, "Mobile phone based clinical microscopy for global health applications," *PloS One*, vol. 4, 2009, Art no. e6320.
[13] Z. Xie, H. Ge, J. Du, T. Duan, G. Yang, and Y. He, "Compartmentalizing incompatible tandem reactions in pickering emulsions to enable visual colorimetric detection of nitramine explosives using a smartphone," *Anal. Chem.*, vol. 90, pp. 11665–11670, 2018.
[14] Q. Wei, H. Qi, W. Luo, et al., "Fluorescent imaging of single nanoparticles and viruses on a smart phone," *ACS Nano*, vol. 7, pp. 9147–9155, 2013.
[15] A. Roda, M. Guardigli, D. Calabria, M. Maddalena Calabretta, L. Cevenini, and E. Michelini, "A 3D-printed device for a smartphone-based chemiluminescence biosensor for lactate in oral fluid and sweat," *Analyst*, vol. 139, pp. 6494–6501, 2014.
[16] J. L. Delaney, C. F. Hogan, J. Tian, and W. Shen, "Electrogenerated chemiluminescence detection in paper-based microfluidic sensors," *Anal. Chem.*, vol. 83, pp. 1300–1306, 2011.
[17] A. I. Barbosa, P. Gehlot, K. Sidapra, A. D. Edwards, and N. M. Reis, "Portable smartphone quantitation of prostate specific antigen (PSA) in a fluoropolymer microfluidic device," *Biosens. Bioelectron.*, vol. 70, pp. 5–14, 2015.
[18] P. B. Lillehoj, M. C. Huang, N. Truong, and C. M. Ho, "Rapid electrochemical detection on a mobile phone," *Lab Chip*, vol. 13, pp. 2950–2955, 2013.
[19] M. S. Draz, K. M. Kochehbyoki, A. Vasan, et al., "DNA engineered micromotors powered by metal nanoparticles for motion based cellphone diagnostics," *Nat. Commun.*, vol. 9, p. 4282, 2018.
[20] M. Greggila, *Chemical Kinetics Using a Smartphone Spectrometer*, Diss., The University of Akron, 2019.
[21] U. P. Moravapalle, A. Deshpande, A. Kapoor, R. Ramjee, and P. Ravi, "Blood count on a smartphone microscope: challenges," in *Proc. of the 18th International Workshop on Mobile Computing Systems and Applications*, *Sonoma, CA, USA*, 2017, pp. 37–42. https://doi.org/10.1145/3032970.3032986.
[22] X. Huang, J. Guo, X. Wang, M. Yan, Y. Kang, and H. Yu, "A contact-imaging based microfluidic cytometer with machine-learning for

single-frame super-resolution processing," *PloS One*, vol. 9, 2014, Art no. e104539.
[23] X. Huang, Y. Jiang, X. Liu, et al., "Machine learning based single-frame super-resolution processing for lensless blood cell counting," *Sensors*, vol. 16, p. 1836, 2016.
[24] Y. Kobori, P. Pfanner, G. S. Prins, and C. Niederberger, "Novel device for male infertility screening with single-ball lens microscope and smartphone," *Fertil. Steril.*, vol. 106, pp. 574–578, 2016.
[25] M. K. Kanakasabapathy, M. Sadasivam, A. Singh, et al., "An automated smartphone-based diagnostic assay for point-of-care semen analysis," *Sci. Transl. Med.*, vol. 9, 2017, Art no. eaai7863.
[26] I. L. Dimitriadis, C. Bormann, M. K. Kanakasabapathy, et al., "Automated smartphone-based system for measuring sperm viability, DNA fragmentation, and hyaluronic binding assay score," *PloS One*, vol. 14, 2019, Art no. e0212562.
[27] S. Wang, L. Zheng, G. Cai, et al., "A microfluidic biosensor for online and sensitive detection of Salmonella typhimurium using fluorescence labeling and smartphone video processing," *Biosens. Bioelectron.*, vol. 140, p. 111333, 2019.
[28] V. R. Yelleswarapu, H.-H. Jeong, S. Yadavali, and D. Issadore, "Ultra-high throughput detection (1 million droplets per second) of fluorescent droplets using a cell phone camera and time domain encoded optofluidics," *Lab Chip*, vol. 17, pp. 1083–1094, 2017.
[29] V. Yelleswarapu, J. R. Buser, M. Haber, J. Baron, E. Inapuri, and D. Issadore, "Mobile platform for rapid sub–picogram-per-milliliter, multiplexed, digital droplet detection of proteins," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 116, pp. 4489–4495, 2019.
[30] T. F. Wu, T. M. Yen, Y. Han, Y. J. Chiu, E. Y. S. Lin, and Y. H. Lo, "A light-sheet microscope compatible with mobile devices for label-free intracellular imaging and biosensing," *Lab Chip*, vol. 14, pp. 3341–3348, 2014.
[31] S. V. Kesavan, F. Momey, O. Cioni, et al., "High-throughput monitoring of major cell functions by means of lensfree video microscopy," *Sci. Rep.*, vol. 4, p. 5942, 2014.
[32] M. V. D'Ambrosio, M. Bakalar, S. Bennuru, et al., "Point-of-care quantification of blood-borne filarial parasites with a mobile phone microscope," *Sci. Transl. Med.*, vol. 7, 2015, Art no. 286re4.
[33] M. S. Draz, N. K. Lakshminaraasimulu, S. Krishnakumar, et al., "Motion-based immunological detection of Zika virus using Pt-nanomotors and a cellphone," *ACS Nano*, vol. 12, pp. 5709–5718, 2018.
[34] T. J. Moehling, D. H. Lee, M. E. Henderson, et al., "A smartphone-based particle diffusometry platform for sub-attomolar detection of Vibrio cholerae in environmental water," *Biosens. Bioelectron.*, vol. 167, 2020, Art no. 112497.
[35] M. M. Erenas, B. Carrillo-Aguilera, K. Cantrell, et al., "Real time monitoring of glucose in whole blood by smartphone," *Biosens. Bioelectron.*, vol. 136, pp. 47–52, 2019.
[36] K. Yin, V. Pandian, K. Kadimisetty, et al., "Real-time colorimetric quantitative molecular detection of infectious diseases on smartphone-based diagnostic platform," *Sci. Rep.*, vol. 10, pp. 1–9, 2020.
[37] H. Guner, E. Ozgur, G. Kokturk, et al., "A smartphone based surface plasmon resonance imaging (SPRi) platform for on-site biodetection," *Sensor. Actuator. B Chem.*, vol. 239, pp. 571–577, 2017.
[38] F. Li, L. Guo, Z. Li, J. He, and H. Cui, "Temporal-spatial-color multiresolved chemiluminescence imaging for multiplex immunoassays using a smartphone coupled with microfluidic chip," *Anal. Chem.*, vol. 92, pp. 6827–6831, 2020.
[39] E. Lebiga, R. E. Fernandez, and A. Beskok, "Confined chemiluminescence detection of nanomolar levels of H2O2 in a paper-plastic disposable microfluidic device using a smartphone," *Analyst*, vol. 140, pp. 5006–5011, 2015.
[40] P. Escobedo, M. M. Erenas, A. Martínez-Olmos, et al., "General-purpose passive wireless point–of–care platform based on smartphone," *Biosens. Bioelectron.*, vol. 141, 2019, Art no. 111360.
[41] A. S. Paterson, B. Raja, V. Mandadi, et al., "A low-cost smartphone-based platform for highly sensitive point-of-care testing with persistent luminescent phosphors," *Lab Chip*, vol. 17, pp. 1051–1059, 2017.
[42] D. Tian, Z. Zhu, L. Xu, H. Cong, and J. Zhu, "Intramolecular electronic coupling for persistent room-temperature luminescence for smartphone based time-gated fingerprint detection," *Mater. Horiz.*, vol. 6, pp. 1215–1221, 2019.
[43] N. Katumo, G. Gao, F. Laufer, B. S. Richards, and I. A. Howard, "Smartphone-based luminescent thermometry via temperature-sensitive delayed fluorescence from Gd 2 O 2 S:Eu^{3+}," *Adv. Opt. Mater.*, vol. 8, 2020, Art no. 2000507.
[44] Y. Zhou, W. Qin, C. Du, H. Gao, F. Zhu, and G. Liang, "Long-lived room-temperature phosphorescence for visual and quantitative detection of oxygen," *Angew. Chem.*, vol. 131, pp. 12230–12234, 2019.
[45] Y. Wang, X. Liu, P. Chen, et al., "Smartphone spectrometer for colorimetric biosensing," *Analyst*, vol. 141, pp. 3233–3238, 2016.
[46] R. Bogucki, M. Greggila, P. Mallory, et al., "A 3D-printable dual beam spectrophotometer with multiplatform smartphone adaptor," *J. Chem. Educ.*, vol. 96, pp. 1527–1531, 2019.
[47] K. D. Long, E. V. Woodburn, H. M. Le, U. K. Shah, S. S. Lumetta, and B. T. Cunningham, "Multimode smartphone biosensing: the transmission, reflection, and intensity spectral (TRI)-analyzer," *Lab Chip*, vol. 17, pp. 3246–3257, 2017.
[48] B. T. Cunningham, K. D. Long, H. Yu, et al., "Mobile biosensing using the sensing capabilities of smartphone cameras," in *2017 IEEE MTT-S Int. Microw. Work. Ser. Adv. Mater. Process. RF THz Appl. (IMWS-AMP), Pavia, Italy*, 2017, pp. 1–3.
[49] S. Ayas, A. Cupallari, O. O. Ekiz, Y. Kaya, and A. Dana, "Counting molecules with a mobile phone camera using plasmonic enhancement," *ACS Photonics*, vol. 1, pp. 17–26, 2014.
[50] K. Trofymchuk, V. Glembockyte, L. Grabenhorst, et al., "Addressable nanoantennas with cleared hotspots for single-molecule detection on a portable smartphone microscope," *Nat. Commun.*, vol. 12, no. 1, p. 950, 2021.
[51] S. Zhang, Z. Li, and Q. Wei, "Smartphone-based cytometric biosensors for point-of-care cellular diagnostics," *Nanotechnol. Precis. Eng.*, vol. 3, pp. 32–42, 2020.
[52] M. K. Kanakasabapathy, H. J. Pandya, M. S. Draz, et al., "Rapid, label-free CD4 testing using a smartphone compatible device," *Lab Chip*, vol. 17, pp. 2910–2919, 2017.

[53] H. Zhu, I. Sencan, J. Wong, et al., "Cost-effective and rapid blood analysis on a cell-phone," *Lab Chip*, vol. 13, pp. 1282–1288, 2013.
[54] S. Knowlton, A. Joshi, P. Syrrist, A. F. Coskun, and S. Tasoglu, "3D-printed smartphone-based point of care tool for fluorescence-and magnetophoresis-based cytometry," *Lab Chip*, vol. 17, pp. 2839–2851, 2017.
[55] S. A. Lee and C. Yang, "A smartphone-based chip-scale microscope using ambient illumination," *Lab Chip*, vol. 14, pp. 3056–3063, 2014.
[56] H. Im, C. M. Castro, H. Shao, et al., "Digital diffraction analysis enables low-cost molecular diagnostics on a smartphone," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 112, pp. 5613–5618, 2015.
[57] M. S. Draz, A. Vasan, A. Muthupandian, et al., "Virus detection using nanoparticles and deep neural network–enabled smartphone system," *Sci. Adv.*, vol. 6, 2020, Art no. eabd5354.
[58] V. Oncescu, D. O'Dell, and D. Erickson, "Smartphone based health accessory for colorimetric detection of biomarkers in sweat and saliva," *Lab Chip*, vol. 13, p. 3232, 2013.
[59] L. Shen, J. A. Hagen, and I. Papautsky, "Point-of-care colorimetric detection with a smartphone," *Lab Chip*, vol. 12, pp. 4240–4243, 2012.
[60] S. C. Kim, U. M. Jalal, S. B. Im, S. Ko, and J. S. Shim, "A smartphone-based optical platform for colorimetric analysis of microfluidic device," *Sensor. Actuator. B Chem.*, vol. 239, pp. 52–59, 2017.
[61] K. E. McCracken, T. Tat, V. Paz, and J. Y. Yoon, "Smartphone-based fluorescence detection of bisphenol A from water samples," *RSC Adv.*, vol. 7, pp. 9237–9243, 2017.
[62] S. Cho, A. Islas-Robles, A. M. Nicolini, T. J. Monks, and J. Y. Yoon, "In situ, dual-mode monitoring of organ-on-a-chip with smartphone-based fluorescence microscope," *Biosens. Bioelectron.*, vol. 86, pp. 697–705, 2016.
[63] A. K. Yetisen, N. Jiang, A. Tamayol, et al., "Paper-based microfluidic system for tear electrolyte analysis," *Lab Chip*, vol. 17, pp. 1137–1148, 2017.
[64] B. Coleman, C. Coarsey, M. A. Kabir, and W. Asghar, "Point-of-care colorimetric analysis through smartphone video," *Sensor. Actuator. B Chem.*, vol. 282, pp. 225–231, 2019.
[65] J. Homola, "Surface plasmon resonance sensors for detection of chemical and biological species," *Chem. Rev.*, vol. 108, pp. 462–493, 2008.
[66] S. Dutta, K. Saikia, and P. Nath, "Smartphone based LSPR sensing platform for bio-conjugation detection and quantification," *RSC Adv.*, vol. 6, pp. 21871–21880, 2016.
[67] P. Preechaburana, M. C. Gonzalez, A. Suska, and D. Filippini, "Surface plasmon resonance chemical sensing on cell phones," *Angew. Chem. Int. Ed.*, vol. 51, pp. 11585–11588, 2012.
[68] T. H. Fereja, S. A. Kitte, W. Gao, et al., "Artesunate-luminol chemiluminescence system for the detection of hemin," *Talanta*, vol. 204, pp. 379–385, 2019.
[69] M. Zangheri, L. Cevenini, L. Anfossi, et al., "A simple and compact smartphone accessory for quantitative chemiluminescence-based lateral flow immunoassay for salivary cortisol detection," *Biosens. Bioelectron.*, vol. 64, pp. 63–68, 2015.
[70] G. A. Posthuma-Trumpie, J. Korf, and A. Van Amerongen, "Lateral flow (immuno)assay: its strengths, weaknesses, opportunities and threats. A literature survey," *Anal. Bioanal. Chem.*, vol. 393, pp. 569–582, 2009.
[71] M. K. Haleyur Giri Setty and I. K. Hewlett, "Point of care technologies for HIV," *AIDS Res. Treat.*, vol. 2014, pp. 1–20, 2014.
[72] K. H. Foysal, S. E. Seo, M. J. Kim, O. S. Kwon, and J. W. Chong, "Analyte quantity detection from lateral flow assay using a smartphone," *Sensors*, vol. 19, p. 4812, 2019.
[73] T. H. Ulep and J. Y. Yoon, "Challenges in paper-based fluorogenic optical sensing with smartphones," *Nano Converg.*, vol. 5, pp. 1–11, 2018.
[74] Y. Wang, N. Sayyadi, X. Zheng, et al., "Time-resolved microfluidic flow cytometer for decoding luminescence lifetimes in the microsecond region," *Lab Chip*, vol. 20, pp. 655–664, 2020.
[75] Y. Lu, J. Lu, J. Zhao, et al., "On-the-fly decoding luminescence lifetimes in the microsecond region for lanthanide-encoded suspension arrays," *Nat. Commun.*, vol. 5, pp. 1–8, 2014.
[76] Z. Zhu, "Smartphone-based apparatus for measuring upconversion luminescence lifetimes," *Anal. Chim. Acta*, vol. 1054, pp. 122–127, 2019.
[77] L. A. Cole, J. M. Sutton-Riley, S. A. Khanlian, M. Borkovskaya, B. B. Rayburn, and W. F. Rayburn, "Sensitivity of over-the-counter pregnancy tests: comparison of utility and marketing messages," *J. Am. Pharm. Assoc.*, vol. 45, pp. 608–615, 2005.
[78] S. Johnson, M. Cushion, S. Bond, S. Godbert, and J. Pike, "Comparison of analytical sensitivity and women's interpretation of home pregnancy tests," *Clin. Chem. Lab. Med.*, vol. 53, pp. 391–402, 2015.
[79] H. Alfthan, C. Haglund, J. Dabek, and U. H. Stenman, "Concentrations of human choriogonadotropin, its β-subunit, and the core fragment of the β-subunit in serum and urine of men and nonpregnant women," *Clin. Chem.*, vol. 38, pp. 1981–1987, 1992.
[80] J. Hampl, M. Hall, N. A. Mufti, et al., "Upconverting phosphor reporters in immunochromatographic assays," *Anal. Biochem.*, vol. 288, pp. 176–187, 2001.
[81] L. A. Cole, "The utility of six over-the-counter (home) pregnancy tests," *Clin. Chem. Lab. Med.*, vol. 49, pp. 1317–1322, 2011.
[82] D. L. Ma, V. P. Y. Ma, D. S. H. Chan, K. H. Leung, H. Z. He, and C. H. Leung, "Recent advances in luminescent heavy metal complexes for sensing," *Coord. Chem. Rev.*, vol. 256, pp. 3087–3113, 2012.
[83] A. Thibon and V. C. Pierre, "Principles of responsive lanthanide-based luminescent probes for cellular imaging," *Anal. Bioanal. Chem.*, vol. 394, pp. 107–120, 2009.
[84] M. P. Lowe and D. Parker, "PH switched sensitisation of europium(III) by a dansyl group," *Inorg. Chim. Acta.*, vol. 317, pp. 163–173, 2001.
[85] A. Scheeline, "Teaching, learning, and using spectroscopy with commercial, off-the-shelf technology," *Appl. Spectrosc.*, vol. 64, pp. 256A–268A, 2010.
[86] D. Gallegos, K. D. Long, H. Yu, et al., "Label-free biodetection using a smartphone," *Lab Chip*, vol. 13, p. 2124, 2013.
[87] H. Yu, Y. Tan, and B. T. Cunningham, "Smartphone fluorescence spectroscopy," *Anal. Chem.*, vol. 86, pp. 8805–8813, 2014.

[88] L.-J. Wang, Y.-C. Chang, R. Sun, and L. Li, "A multichannel smartphone optical biosensor for high-throughput point-of-care diagnostics," *Biosens. Bioelectron.*, vol. 87, pp. 686–692, 2017.
[89] D. Jian, B. Wang, H. Huang, et al., "Sunlight based handheld smartphone spectrometer," *Biosens. Bioelectron.*, vol. 143, 2019, Art no. 111632.
[90] M. A. Hossain, J. Canning, K. Cook, and A. Jamalipour, "Optical fiber smartphone spectrometer," *Opt. Lett.*, vol. 41, p. 2237, 2016.
[91] L.-J. Wang, Y.-C. Chang, X. Ge, et al., "Smartphone optosensing platform using a DVD grating to detect neurotoxins," *ACS Sens.*, vol. 1, pp. 366–373, 2016.
[92] M. A. Hossain, J. Canning, K. Cook, S. Ast, P. J. Rutledge, and A. Jamalipour, "Absorption and fluorescence spectroscopy on a smartphone," in *Fifth Asia-Pacific Optical Sensors Conference*, vol. 9655, B. Lee, S.-B. Lee, and Y. Rao, Eds., Jeju, Korea, SPIE, 2015, p. 96551Z.
[93] A. Das, T. Swedish, A. Wahi, et al., "Mobile phone based mini-spectrometer for rapid screening of skin cancer," in *Next-Generation Spectroscopic Technologies VIII*, vol. 9482, M. A. Druy, R. A. Crocombe, and D. P. Bannon, Eds., Bellingham, WA, USA, SPIE, 2015, p. 94820M.
[94] L. Kong, Y. Gan, T. Liang, et al., "A novel smartphone-based CD-spectrometer for high sensitive and cost-effective colorimetric detection of ascorbic acid," *Anal. Chim. Acta*, vol. 1093, pp. 150–159, 2020.
[95] K. D. Long, H. Yu, and B. T. Cunningham, "Smartphone instrument for portable enzyme-linked immunosorbent assays," *Biomed. Opt. Express*, vol. 5, p. 3792, 2014.
[96] D. M. Rissin, C. W. Kan, T. G. Campbell, et al., "Single-molecule enzyme-linked immunosorbent assay detects serum proteins at subfemtomolar concentrations," *Nat. Biotechnol.*, vol. 28, pp. 595–599, 2010.
[97] L. Cohen, M. R. Hartman, A. Amardey-Wellington, and D. R. Walt, "Digital direct detection of MicroRNAs using single molecule arrays," *Nucleic Acids Res.*, vol. 45, p. e137, 2017.
[98] K. O. Nagata, C. Nakada, R. S. Kasai, A. Kusumi, and K. Ueda, "ABCA1 dimer–monomer interconversion during HDL generation revealed by single-molecule imaging," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 110, pp. 5034–5039, 2013.
[99] J. Eid, A. Fehr, J. Gray, et al., "Real-time DNA sequencing from single polymerase molecules," *Science*, vol. 323, pp. 133–138, 2009.
[100] Q. Wei, G. Acuna, S. Kim, et al., "Plasmonics enhanced smartphone fluorescence microscopy," *Sci. Rep.*, vol. 7, pp. 1–10, 2017.
[101] C. Vietz, M. L. Schütte, Q. Wei, et al., "Benchmarking smartphone fluorescence-based microscopy with dna origami nanobeads: reducing the gap toward single-molecule sensitivity," *ACS Omega*, vol. 4, pp. 637–642, 2019.