

Well-Designed Smartphone-Based Imaging Biosensor

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Abstract. With the development of hardware and software for smartphones, more and more well-designed smartphone-based imaging biosensors have been created and broadly applied in point-of-care testing (POCT). Imaging biosensors can get clear images through the high pixel density of smartphones' camera systems. And smartphones also provide a chance for imaging processing thanks to smartphones' central processing units (CPUs) and graphics processing units (GPUs). Different approaches have extensively explored smartphone-based imaging biosensors. The commonly used imaging methods are generally implemented by the bright field with the light source or by fluorescence with a fluorescence microscope. Smartphones have enabled the widespread application of imaging-based methods in clinical chemistry, environmental monitoring, flow cytometry, food analysis, drug screening, and medical diagnostics. In detail, this article discusses various imaging biosensors and specific applications of smartphone-based imaging biosensors for bright-field imaging and fluorescence bioimaging. Meanwhile, the opportunities and challenges of smartphone-based imaging biosensors are also analyzed here.

Keywords: Smartphone, Imaging biosensor, Point-of-care, Microfluidics, Fluorescence.

1. Introduction

In order to adapt to complex diagnostic environments and urgent diagnostic tasks, biosensors often need to be portable, low-cost and have short turnaround times. The combination of microfluidics and biosensors presents an opportunity to miniaturize biosensor devices. In addition, microfluidics can also effectively reduce sample analysis costs and the required sample volume, which is advantageous in harsh diagnostic environments. For example, microfluidics wearable biosensors for non-invasive sweat diagnostics are now widely used in health monitoring and disease detection. Microfluidics impedance biosensor chips are currently being used for labeling-free detection using a DNA-BASED self-assembled single-layer sensor layer [1]. The smartphone-based biosensor has been proposed to expand the applicability further and enhance biosensors' portability. Smartphones can assist the biosensor in performing imaging, light absorption, reflection, surface plasmon resonance, and other functions by using the camera as a "smart detector," a complementary metal oxide semiconductor (CMOS) image sensor as a "smart recorder," and a particular optical APP as a "smart reading."

Smartphones are extensively used nowadays, and this portable device provides new possibilities for promoting biomedical sensors. Its advanced imaging devices, powerful computing and outstanding analytical ability present low-cost opportunities for biosensor applications. Biosensors combine cellphones, microfluidics components, and sensing elements to enable the usage of smartphone-based microfluidics biomedical sensing devices. Smartphone-based microfluidics imaging biosensors are one of the common sensing modalities. The total performance of imaging components, central processing units (CPUs), and graphics processing units (GPUs) in smartphones has improved with the advent of superior integrated circuit (IC) design and manufacturing technologies. These tools enable computational microscopy without needing additional PC processing by instantly rectifying, enhancing, and evaluating acquired images. This offers a complete framework for real-time computation and analysis of biomedical pictures.

Moreover, objects' morphological features, like the volume, shape, shade, brightness and tint, can be quantified into recognizable signals by smartphone-based imaging biosensors systems. These unique characteristics have promoted the development of smartphone-based microfluidics imaging biosensors. Smartphone-based imaging biosensors typically use bright field-of-view or fluorescence

techniques for biomedical diagnostics. This paper will first summarize the advantages smartphones bring to developing imaging biosensors. Then it will focus on describing different imaging methods based on bright field and fluorescence techniques. The working principles of the different imaging methods and the devices or software of the smartphones used will be explained in detail, and current applications will be described in detail. Finally, the prospects and challenges of smartphone-based imaging biosensors will be analyzed.

2. The distinctive superiorities of smartphone-based imaging biosensors

With the popularity of smartphones worldwide, the idea of using smartphones to monitor and diagnose health conditions has been spreading. How to achieve early diagnosis and real-time monitoring through the smartphone platform has become a concern for health care stakeholders. Smartphones have played a non-negligible role in facilitating the development of imaging biosensors, and their unique advantages have made the combination of imaging biosensors with them inevitable.

First, smartphones are ubiquitous. According to a report published by Strategy Analytics, half of the world's population will have a smartphone by June 2021, or about four billion people. Biosensors contacted with a smartphone can be broadly spread among people. Patients with a smartphone can diagnose themselves at anytime and anywhere. This provides a solid foundation for the widespread use of imaging biosensors.

Second, smartphones are portable. Smartphones are light and small. People now often carry their phones with them. So, when there is a need for biomedical testing, people can directly take out their phones for diagnosis. Bio-imaging sensors combined with smartphones can also be applied to various complex environments. Compared to traditional huge and heavy biochemical analyzers, this portable sensing device is convenient to carry around and diagnose in time.

Besides, smartphones have excellent imaging equipment. With the improved electronic science, the manufacturing of integrated circuits has become further mature. The resolution of smartphone-embedded CMOS image sensor (CIS) cameras now exceeds 20 million pixels, and the pixel pitch has been reduced to about 1 μm . Recently, smartphone optical sensors using this high pixel density have been extensively used for bioimaging medical diagnosis in microfluidics [2-11]. For example, an optical fiber surface plasmon resonance (SPR) sensor system was reported by Bremer et al. They used the flash and camera on the back of a smartphone to excite and interrogate the SPR sensor system [12]. In addition, Sibasish et al. designed a localized SPR (LSPR) sensor for a smartphone camera that combines lightweight, simple laboratory optics with a cell phone camera module [13].

Moreover, smartphones also have image processing capabilities that enable the integration of image acquisition and analysis. Current smartphones have embedded CPUs and GPUs, allowing them to perform real-time biomedical image computation and analysis. This powerful feature enables smartphones to quantify shape properties like object volume, shape, shade, brightness and tint, which can be used for medical diagnosis, flow cytometry, clinical chemistry, drug screening, food analysis, and environmental monitoring. In addition, smartphones have complementary features such as touch screen displays, advanced connectivity, and an ideal platform for developing imaging biosensors with versatility.

What is more, smartphones can be combined with microfluidic chip technology. Microfluidic biosensors with microfluidic bio-integration have a broad application prospect and a wide development space due to their huge computing power and low cost. The system enables rapid, portable and convenient high-sensitivity, high-throughput analysis of miniature biological samples, especially for blood sample diagnosis and monitoring. The situation of smartphones allowing microfluidic chips combined provides new ideas for innovation in imaging, sensing and diagnostic systems.

3. Bright-field imaging biosensors

The development of smartphone-based imaging sensors is divided into two types based on bright-field imaging and based on fluorescent imaging techniques. Firstly, bright-field imaging biosensors are deliberated.

Bright-field imaging is a widely and commonly used method. Imaging biosensors by bright-field commonly analyze the biological samples by irradiating uniformly with a white light source, and then the CIS camera of the smartphone is used to obtain the image.

The 3D print optomechanical accessory is a major component in bright-field microscopes but also includes sample tray, lamp holders, area of view, outer lenses, and focus adjustment [14], among other related units. To improve the resolution of samples, the way that magnifies the sample image or super-resolution processing is usually adopted. Additionally, an external lens can be used with the built-in smartphone lens to improve the image's resolution.

Shade-based imaging is the most basic imaging method of bright field microarray. A microfluidic channel is usually used as a flow space for biological samples, and a CIS chip is integrated near the channel. As the detected biological sample passes through the microfluidic channel under a uniform white light source, the smartphone's imaging sensor takes a series of images with diffraction shadow and super-resolves the resulting images to improve image resolution. In this imaging system, light-emitting diodes (LEDs) are required to function as a white light source, which will increase the complexity and incompleteness of the system and make it difficult to carry. This kind of smartphone-based imaging method has high requirements on light sources, requiring high intensity or monochromatic light. Smartphones cannot meet this requirement, so extra batteries or light sources are usually chosen, such as LEDs or semiconductor lasers, leading to inefficient smartphone use.

In order to make the system more compact, an independent chain-level lens-less microscope has been developed with ambient lighting as the light source [15], as shown in Figure 1. The device is available via Android. The image reconstruction feature of the Android app has been customized to achieve sub-micron resolution. The device imagines blood smears and freshwater microbial smears, greatly improving portability due to the lack of additional light sources. It also highlights promising applications for smartphone microscope devices for healthcare and real-time monitoring of the surrounding environment.

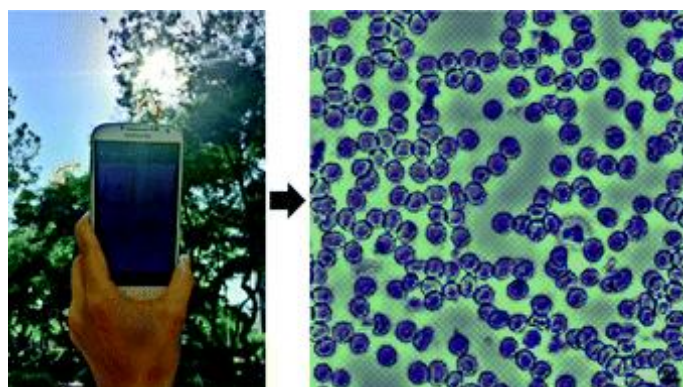


Figure 1. Imaging by a chip-level lens-less microscope device that uses ambient lighting [15].

Another example of smartphone-based microscopy technology uses a tapered optical fiber array that contacts a sample on top of the smartphone's camera system to take an image, as shown in Figure 2 [16]. An Android app on a smartphone computes the sequence of images with transmitted light patterns to improve their resolution.

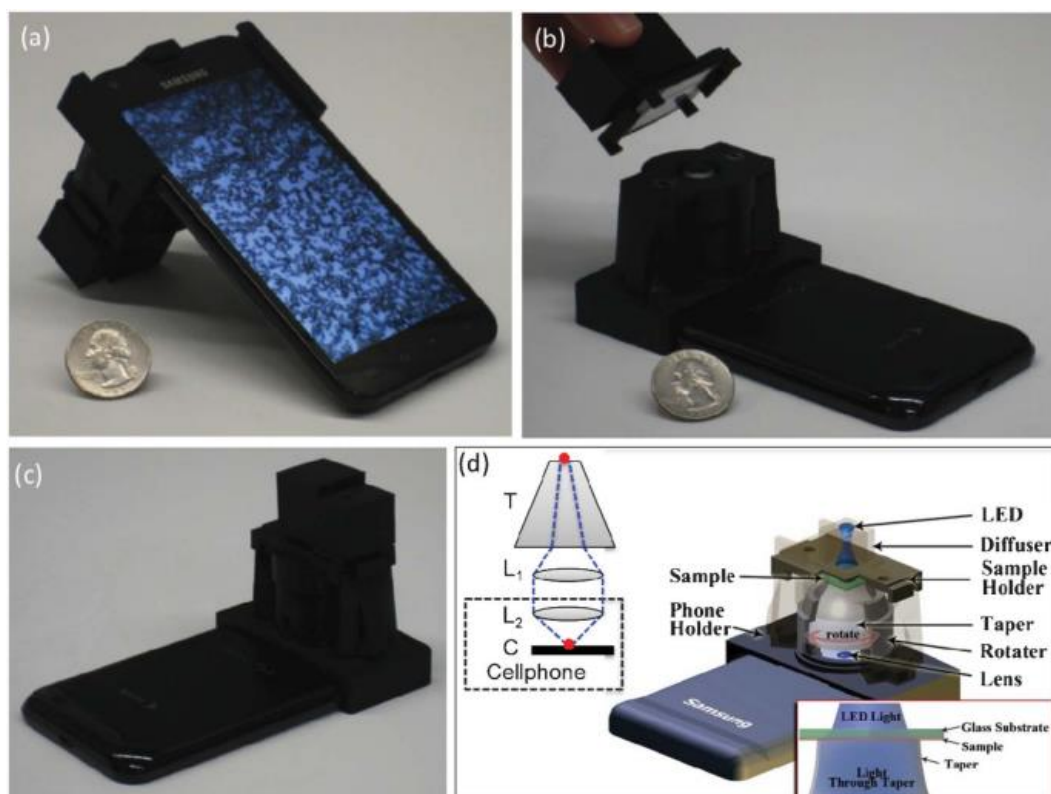


Figure 2. (a-c) The images of the contact range on an Android phone. (d) Schematic diagram of contact range mobile phone accessories [16].

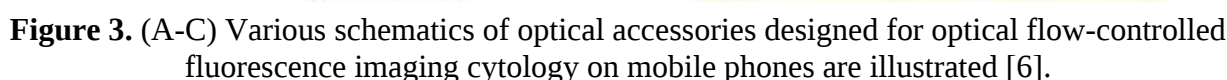
Similarly, optical fiber arrays are used to achieve imaging with a smartphone-based fiber SPR biosensor [17]. The SPR test system uses the optical fiber SPR sensor and the smartphone as the light source and detector. Inside the mobile phone housing, the optical element and the sensor element are connected by fibers. The mobile phone LEDs emit the measurement, control and reference channels into the fiber, while the mobile phone camera detects the end point from the fiber. The SPR sensing element was fabricated with a photoconductive silicon capillary, stripped and coated with a 50-nanometer gold film. If the sample is invaded into the flow cell, light generated with the sensor is absorbed due to SPR resonance. An Android software application was developed to specify the camera's exposure and allow video and LED flash. The smart application can extract MC, CC and RC light intensity information from 2 Hz frequencies. Since the end surfaces of the measuring pass, controlling the pass and referencing the pass obtained by the camera shows the three points of light, which are calculated and compared. Since the light intensity fluctuation of flash with the LED has similar effects on the measuring, controlling, and referencing pass, we use the relative intensity to eliminate the error caused by the LED power fluctuation.

The advantage of this detection method is that it can be easily installed or removed from a smartphone. All elements of optics and senses are linked with optical fiber and fastened to the smartphone case. Small size, simple design, more suitable for smartphones, easy to install or remove. Second, sensing elements with high sensitivity and good portability and optocouplers based on fiber optic components enable precise detection of SPR instruments without the need for complex, special and fragile optical elements or precise optical calibration. But this test system realizes a single-point or one-dimensional spatial resolution SPR sensor, which cannot detect many interesting samples in a short time, limiting its possibility in multi-channel and high-throughput detection.

Nevertheless, SPR imaging technology can be applied to high-throughput biological detection. The system uses inexpensive grating-coupled SPR sensing wafers using existing memory disks. In addition, they also used a 3D printing device to design a small optical system consisting of an LED light source, a collimator, a bandpass filter, a linear polarizer, a beam splitter, and an outer lens. A surface plasmon is a kind of electromagnetic wave propagating along the metal/medium interface and

Because of its multi-sensor structure, it can detect analytes at the dilution of multiple samples. Reveals the potential of multianalyte detection and the use of low-cost, integrated platforms for advanced array-based biochemical analysis. A microfluidic channel is placed between the metal layers to control the flow of the liquid. The usage of Blu-ray disks and regular metal deposition technologies, and low-cost microfluidic channels have greatly reduced costs.

Fluorescence imaging is another common microfluidic imaging method. Compared to bright-field imaging biosensors, fluorescent imaging biosensors are more sensitive and specialized, providing better detection of biological targets even at the nanoscale. So far, smartphone-based fluorescent imaging biosensors have been commonly utilized to analyze fluid body samples and detect biological targets such as mycotoxins, cells, bacterial and viral antigens, nucleic acids and proteins. Imaging biosensors with fluorescence technique commonly work with an excitation filter, an LED or laser diode as a source to stimulate the 3D printing camera in the fluorescence microscope to achieve fluorescence imaging.



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fluorescent markers when connected to it, and a smartphone can capture a series of images and videos of fluorescent-labeled particles flowing through microfluidic channels. The fluorescent video frames are digitally processed. Each obvious fluorescent particle is detected by a contour detection algorithm and automatically calculated to provide the user with the density of a given cell sample. This static microscope mode allows the images by the technique of fluorescence obtained during the optical flow phase to be stored in JPG format in the phone's memory and allows the smartphone's screen to view these images directly. Its advantage is that the connection units are compact, lightweight and economical.

Another that uses fluorescence bioimaging is a system of sensors based on machine learning. The system automatically detects and enumerates cysts in *Giardia lamblia* cysts, as shown in Figure 4 [23]. The handheld, compact, and cost-effective platform show that about 12 cysts per 10 ml of cyanobacteria can be detected, with about 95% accuracy in classifying various water samples. Sample collection, marking, filtering, optical inspection, data transmission, and automatic counting using the server take 1 hour.

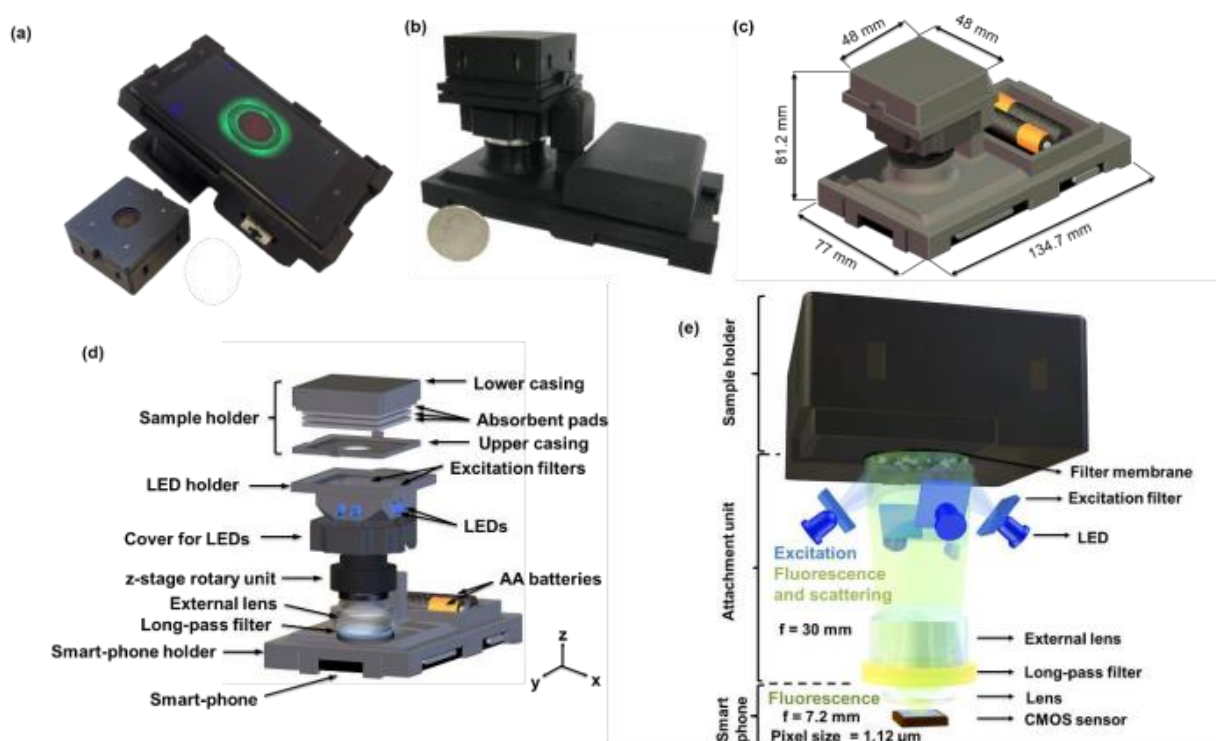


Figure 4. (a-b) Smartphone-based digital photos of fluorescence microscopes include a throwaway sample tape. (c) An illustrative diagram presenting the size of the test platform. (d) Extended design drawings (e) Schematic lighting/excitation paths [23].

The platform uses a smartphone-based fluorescence microscope to catch fluorescent images of fluorescent-labeled *Giardia lamblia* cysts through a filter and transmit them via smartphone to a server for remote digital processing. Smartphone-based fluorescence microscopy combined with machine learning allows for quick imaging, effective test and quantification of *Giardia lamblia* cysts. The fluorescent microscope kit includes a 3D print case aligned with the smartphone's existing camera module, an outside lens, an excitation filter, an emission filter, eight LEDs, a mechanical Z-class, and two batteries. Structurally, the smartphone's camera unit is aimed at a handheld fluorescence microscope, and the imaging is performed on a specially designed disposable tape of water samples. The advantage of such systems is that the images can be quickly captured and displayed by smart apps running on the smartphone. It implements real-time imaging and sensing platforms for mobile phone operations in the field, providing rapid and quantitative detection of microorganisms. In addition, this sensor can detect water quality and perform temporal analysis even in resource-poor

areas without too much money. In addition, this machine learning-based portable fluorescence imaging and detection device can screen target biological fluids, such as blood and urine.

A smartphone-based cell classifier is another design that combines magnetic focusing and fluorescence imaging [24]. The device images cells in brilliant, dark or fluorescent imaging mode with permanent magnets and a smartphone's CIS and separates cells according to their density, reflecting cell activity and cell types such as cancer and white blood cells. The combination of magnetic focusing and fluorescence microscopy, two highly versatile techniques, enables the system to provide instant care. Many cells usually expensive separation technology and fluorescence microscope, using the people need to receive professional training, to some extent, limits the availability of developing countries. But the platform is low cost and relatively easy to use for clinical application under the condition of natural environment resources and shortage of human resources while analyzing cell types. Further, it helps improve access to medical diagnostics worldwide.

5. Conclusion

In this paper, some typical imaging biosensors that are easy to use in smartphones are discussed, and their structures, analysis methods, sensing methods and advantages and disadvantages of various applications are introduced. These reports suggest that smartphone-based imaging biosensor systems can make POCT in resource-constrained settings low-cost, compact, portable, and accurate, as well as having the ability to solve the cost, bulky, and other problems of biomedical analytical instruments. Smartphones' high pixel count, sensitive camera and light source provide a suitable platform for developing imaging biosensors. In addition, smartphones enable the incorporation of other accessories such as additional optical sensors and chips, providing additional possibilities for extending the functions of medical systems to achieve more sophisticated sensory systems. Sensors can detect small molecules, nucleic acids, and even bacteria and viruses. All those show the flexibility of sensors. Smartphone-based imaging biosensor is a hybrid biosensor that can analyze and transmit analyte images more efficiently and save diagnostic time by combining smartphone applications.

In conclusion, smartphone-based imaging biosensors are essential for biomedical sensing applications, particularly in the POCT field. However, there are still some challenges in integrating and designing smartphone-based imaging biosensor systems. Firstly, the imaging biosensor system requires accessories, such as extra light sources, to be installed on a smartphone. For easy operation, these accessories need to be designed to be small and easy to install and remove. This makes the design more difficult, and too many accessories can affect the flexibility and accuracy of the sensor system and complicate the diagnosis process. In addition, the sensitivity and accuracy of smartphone-based imaging biosensors still need to be improved compared to traditional laboratory imaging instruments. Imaging biosensors for image processing, we still need sensitive and portable professional instruments and more sophisticated algorithms to improve image resolution. So far, the popularity of smartphones has created unprecedented opportunities for the development of hybrid biosensor systems based on smartphones. However, their integration is still not fully developed, and many efforts still need to be made in synchronous detection. In terms of smartphone-based imaging biosensors, future research should focus on optimizing the structure of smartphones to simplify their necessary accessories and improving the processing capacity of smartphones for images and other information to improve the accuracy of results.

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