CHAPTER 1

INTRODUCTION

This chapter gives an overview of the requirement of miniaturization of the traditionally used optical microscopic imaging techniques for life science research and biomedical applications in the regions where adequate facilities are not available. Due to their wide availability, smartphones as a possible solution in the development of many PoC imaging and sensing devices have been discussed thoroughly. Later, this chapter describes the limitations of the demonstrated smartphone-integrated devices and explains the possible solutions for the same. At the end, the chapter summarizes the research motivation, scope of the thesis and the thesis contribution.

1.1 Optical microscopes and their importance for clinical diagnostics and other monitoring applications

The light microscope also referred to as an optical microscope is ubiquitous and has been extensively used for imaging in biology and life science, biomedical, and numerous other applications [1]. It allows the imaging and investigation of cellular and sub-cellular structures and their dynamics. Since its introduction in the 17th century, the microscope has unfolded countless wonders of life and nature. Cutting-edge technological achievements over the years have leveraged the development of a high-resolution light microscope that allows for groundbreaking insights into the nano dimension along with the high-throughput of unprecedented experimental results [2]. Optical microscopy is the vital tool used to access information from various biological specimens in the field of microbiology and life science research. It provides

direct visualization in the insights how the molecular processes work on the cellular level. Microscopy is the gold standard imaging and visualizing tool in the diagnosis of various diseases and is an indispensable component in every clinical and public health laboratory facilities worldwide. It is the most effective method in the surveillance of parasitic infections like malaria, trichomoniasis, giardiasis etc., to infectious diseases like tuberculosis (TB), diarrheal diseases etc.[3]. It is also a widely used imaging technique in the field of other applications like microelectronics, pharmaceutic research, mineralogy etc.[4]. Thus, it has become the basic foundation of scientific imaging and analysis tool in biology, clinical applications and physics sciences.

1.2 Introduction to different conventional microscopic techniques:

Due to technological revolutions in the past few centuries, a tremendous amount of advancements has been made in the development of optical microscope to meet different optical imaging demands. Thus, the optical configurations of a microscope are reaching immense complexity due to the growing number of optomechanical components [5]. A modern optical microscope typically comprises a light source, a condenser, sample stage, objective lens, tube lens (eyepiece or ocular lens), detector and several light conditioning components. These can be categorized into two major parts – the illumination and imaging part. The illumination part is one of the critical determinants of the imaging performance of the microscope. Figure 1.1 shows the photo image of a basic BF microscope. Köhler illumination method is the most predominant technique of all, which was introduced by August Köhler in 1893 [2]. It utilizes a collector lens above the lamp to focus the image of the lamp filament on the condenser diaphragm at the front focal plane of the condenser. By controlling the opening and closing of the condenser diaphragm, the angle of the light rays can be controlled to reach the specimen from all azimuths. Since the light source is not focused at the level of the specimen plane, the illumination happens to be uniform and extended over the entire imaging field-of-view (FoV). The specimen is generally placed on a microscopic glass slide with a coverslip and mounted on the XYZ translational stage, which allows for focusing and positioning of the specimen. In the imaging part, the objective lens is the most important and perhaps the most expensive image-forming component of the microscope. The objective lens determines the various critical imaging parameters such as magnification, resolution and FoV. Modern optical microscopes typically employ infinity-corrected objective lenses, where the rays are projected in parallel bundles and follow the so-called $4f$-configuration. In this configuration, the lenses are aligned such that the focal planes of the adjacent components coincide,

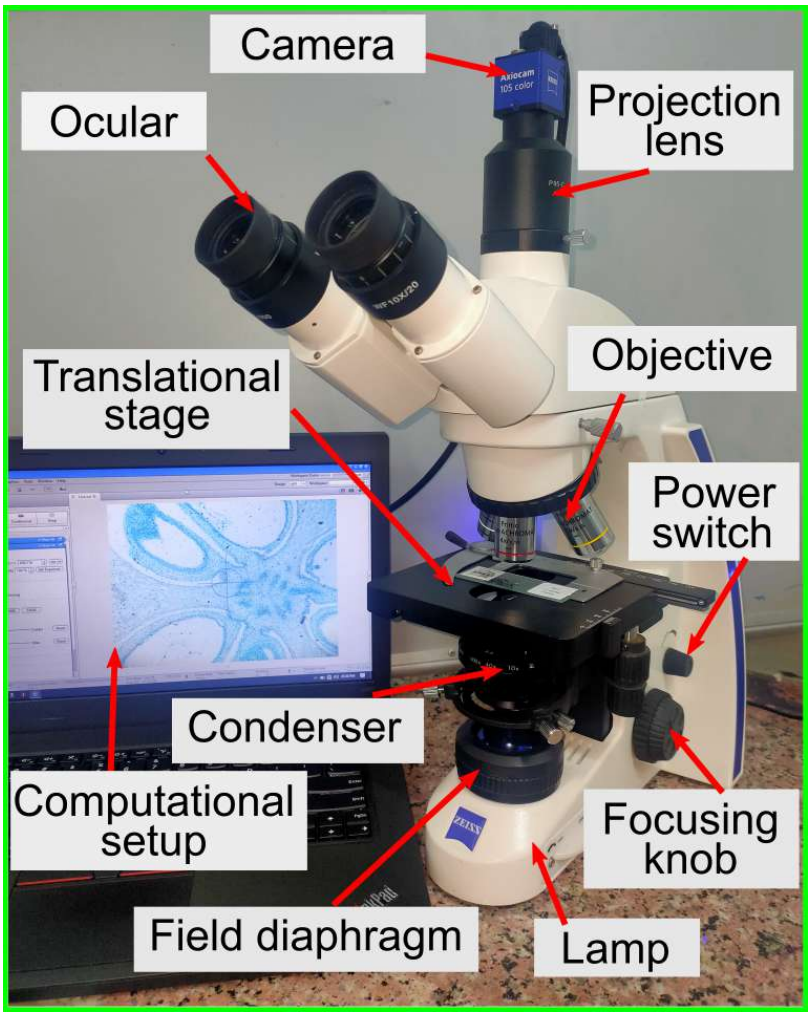

Figure 1.1: A basic BF microscope and its components

reducing the optical aberrations to realize tele-centricity. Here, the system behavior is predicted using the Fourier optics. Finally, the parallel rays projected by the objective lens are focused at the detector plane by an eyepiece lens or tube lens. The $4f$ configuration of the system allows for insertion of additional optical elements, such as filters, polarizers etc., between the parallel optical path between the objective and tube lens [6].

As already mentioned above, the objective lens of a microscope determines several important imaging parameters like magnification and resolution. The ratio of the focal lengths of the tube lens and objective lens represents the optical magnification of an infinity-corrected light microscope. The numerical aperture (NA) of the objective lens is the measure of its ability to collect light and resolve fine features. It is defined as the angle of light collection cone and expressed using the following equation:

$$NA = nsin\theta \tag{1.1}$$

where, n is the refractive index of the medium and θ is the half cone angle. NA

determines the size of the Airy disk pattern, which is formed by imaging a point source through the objective lens due to the diffraction of light. The resolution (r) of the microscope is defined as the smallest resolvable distance between two objects and is expressed by the Rayleigh's criterion:

$$r = \frac{0.61\lambda}{NA} \tag{1.2}$$

where, λ is the wavelength of light. The larger the number of higher diffracted orders registered into the objective lens of the microscope, the smaller the features of the specimen can be resolved clearly. That means the higher the NA of the objective, the better the resolving ability of the system. Similarly, with a shorter wavelength of visible light, the resolution of the system is likely to increase. In order to achieve the full resolution capability in the digital images of a microscope, the projection of the images into the detector should be performed at enough high spatial sampling rate. According to the Nyquist sampling theorem, the projected Airy disk pattern should fit at least two detector pixels [7].

Based on the illumination and detection technique, optical microscopy can be roughly divided into BF, DF, fluorescence and phase-contrast (PC) microscopy. Their schematic representations are shown in figure 1.2. BF is the most common and widely used microscopy technique where the specimen is illuminated from below using a white light source, as shown in figure 1.2(a). Here, the optical contrast is absorption-based because it is created due to greater light absorption in the denser regions of the specimen. Thus, the biological specimens that have no intrinsic absorption cannot be visualized in the BF setup. In such a situation, other microscopic techniques like DF may become more useful. In DF imaging, the specimen is illuminated at an oblique angle greater than the maximum NA of the objective lens, which is achieved using a DF condenser lens, as shown in figure 1.2(b). Consequently, only the scattered light from the specimen can reach to the objective lens creating a dark background. This technique emphasizes the high spatial frequency components of the specimen, and therefore, in some scenarios, it can provide optical resolution beyond the diffraction limit. Fluorescence microscopy is also another very important technique that allows selective tracking of cellular and intracellular activities against a dark background by staining the specimen with exogenous contrast agents. Figure 1.2(d) illustrates the working of a fluorescence microscope. It provides high specificity and sensitivity for detecting and tracking cells, proteins, and other components in a specimen. Another widely used label-free microscopic technique is the PC microscopy which was first introduced by Frits Zernike. Figure 1.2(c) represents the working of a basic PC microscope. This technique generates image contrast by shifting the relative phase of the transparent objects by a quarter wavelength into the intensity distribution of

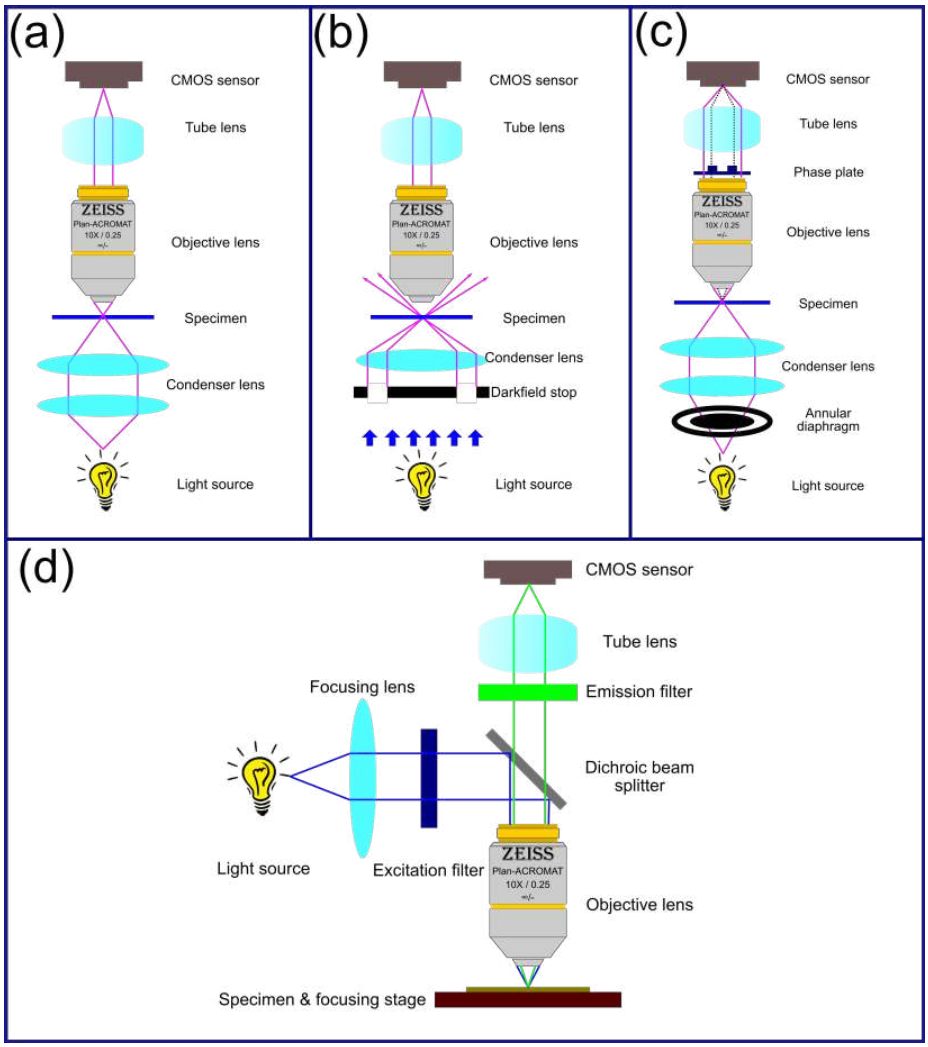

Figure 1.2: Schematic of different microscopic techniques; (a) BF setup, (b) DF setup, (c) PC setup, and (d) fluorescence imaging setup.

the final image. This optical phase shift through the phase objects can be considered a powerful endogenous contrast agent since it reveals morphometric features without the need for any exogenous contrast agents like fluorophores. Quantifying the phase value also allows for the extraction of many important biophysical parameters like cell shapes and their dynamics such as growth and kinetics, dry mass and even intracellular refractive index [2, 6, 8, 9].

1.3 Problem statement of traditional methods with respect to resource-constrained settings and possible solutions

Good health is essential for the livelihood and wellbeing of any human. However, globalization, urbanization, climate change and many other factors contribute to

many disease emergence. The disease burden is not equally distributed around the world. Low and middle-income countries are likely to contribute more to the global burden of disease than developed nations. Due to contamination in agricultural items and water, there is a significant threat to health which is the primary cause of global health burden in many underdeveloped countries. Because of the contamination of water, 1.3 million deaths occur due to diarrheal diseases every year [10]. Vector-borne diseases caused by bacteria, parasites or viruses account for more than 17% of all infectious diseases. Annually, there are more than 700,000 deaths occur due to these diseases. One of the life-threatening parasitic diseases is malaria, which is prevalently occurred in tropical countries. According to World Health Organization (WHO) report, in the year 2020, 627,000 people were dead due to malaria, and 241 million new cases were reported globally [11]. Tuberculosis (TB) is the 13th leading cause of death, infecting an estimated of 10 million people, and 1.5 million people were dead by 2020 [12]. One of the most common cancers among women worldwide is cervical cancer. By 2020, 342,000 women were dead, with an estimated 604,000 new cases due to cervical cancer. More importantly, 90% of the cases are from low and middle-income countries [13].

Diagnosis and monitoring of diseases are critical issues to be addressed for clinical management. Generally, monitoring and diagnosis of fatal diseases are being carried out in the government-approved centralized laboratory facility conditions with the traditional laboratory methods and benchtop instruments. Optical microscopy is one of the most commonly and gold-standard instruments to diagnose and monitor diseases in these laboratory facilities. Almost all kinds of diseases such as malaria, sickle cell anemia, oral cancer, cervical cancer, tuberculosis, infertility issues etc., can be diagnosed and monitored using an optical microscope [14]. It can provide precise results with high sensitivity and specificity. Samples from different locations are brought to the central laboratory for analysis; however, if the laboratory is far from where samples were being collected, this process may become tedious and costly. Besides these logistics issues, there are many other technical factors due to which traditional monitoring methods in centralized laboratory facilities fail with respect to the resource-constrained settings. Modern microscopes are reaching immense complexity in the design of the optical setup with the growing number of optical and photomechanical components, which is making the instrument very expensive. For instance, in India, a basic BF microscope can cost more than USD 2,000.00 (Carl Zeiss's Primo Star, Germany), and a multimode microscope that has the ability of imaging in BF, DF, fluorescence and phase-contrast would be four to five times higher than that. Assembling and maintaining this costly instrument as well as analyzing and verifying the produced results require a specialized human resource having the necessary knowledge and skills to operate the instrument. To resolve some of these

issues, low-cost rapid diagnostic test (RDT) kits are being introduced for those remote areas where access to quality benchtop instruments are limited [15]. However, according to the report, their performances degrade in tropical areas having low detection capabilities in sensitivity and specificity compared to traditional laboratory diagnostic methods [16].

Establishing sophisticated laboratory facilities in many underdeveloped and developing countries where the burden of the disease is high is impossible due to financial and political issues. Moreover, due to cost, size, weight and requirement of external power supply restrict the use of optical microscopes in resource-poor areas. Also, because of many geographical constraints, mediocre connectivity and lack of governmental authorization, still, there are many remote regions even in India where conventional laboratory monitoring facilities are not available. In developing nations like India, due to slow acquisition and unreliable supply chains further inflate the prices, making it extremely challenging to use it as a PoCT in resource-constrained settings.

Early diagnosis and continuous monitoring of diseases can save thousands, even millions of lives, from being suffered. Thus, it is apparent to develop portable, cost-effective microscopic imaging systems that could facilitate its deployment as a PoCT device in the regions where facilities are inadequate. Novel and innovative solutions are the requirement for an inclusive, sustainable and affordable healthcare system. These devices or solutions should be accessible by anyone with any economical background. For infield deployment in the rural and remote regions, these devices should be robust and field-portable. It should also have a proper communication facility so that produced data can be shared immediately with an expert in the centralized laboratory facilities for their opinion to initiate effective monitoring on time. With all these capabilities, the system should also be user-friendly so that any individual can easily operate without prior scientific knowledge. This kind of optical imaging device could be useful not only for applications in resource-poor regions but also immensely beneficial for research and education in optics. It would provide a hands-on experience allowing the students to understand and reconfigure an optical setup, substantially reducing the effort within minutes. This would lead everyone to perceive optics simply as a playground by exploring new design approaches.

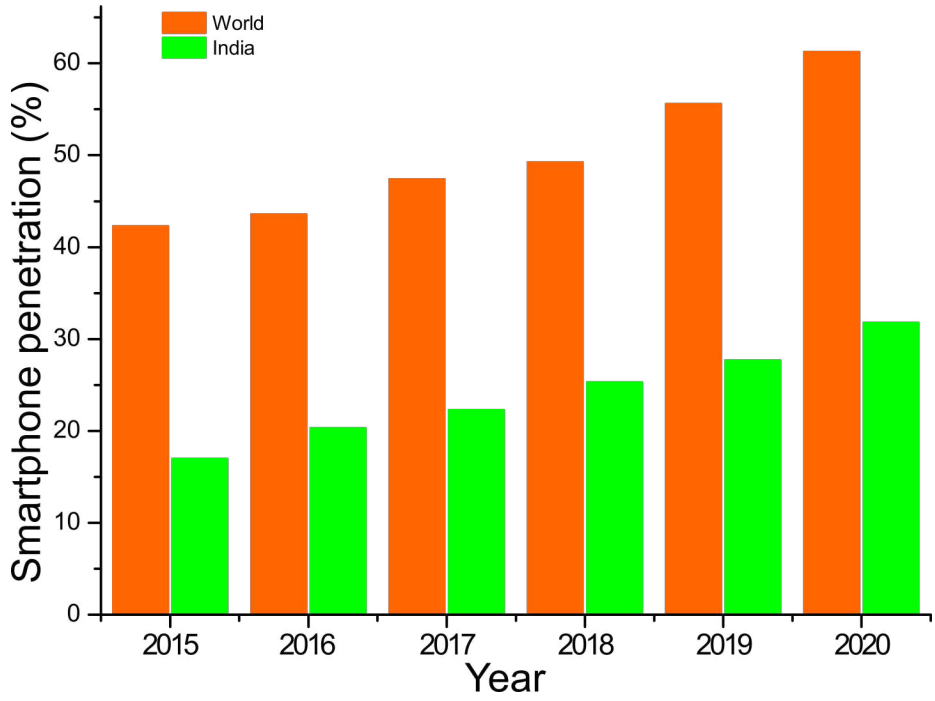

Figure 1.3: Smartphone market penetration during the period 2015-2020 in the world and in India.

1.4 Introduction of smartphones (mobile phones) and 3D-printing technology in the development of low-cost platforms

In modern days, the smartphone is the most loyal companion of our regular day-to-day life, and it is greatly influencing the way we live in our society. It is the most common and widely used mobile device globally than any commercially available electronic device. By 2020, it has been reported that there are 6.05 billion smartphone users with a penetration rate of 61.28% worldwide [17]. In comparison to that, India has the second-highest number of smartphone users of 439.42 million with a penetration rate of only 35.4% [18]. This is shown in figure 1.3 This signifies that irrespective of the economic background of a country, the smartphone is the most accessible mobile device even in developing countries. Figure 1.3 illustrates the growth of the smartphone penetration from 2015 to 2020 in the world and India. Such huge penetration of smartphones to this extent is possible only because of their affordability and consumer-oriented low-cost design approach. Besides, due to cellular network connectivity at an affordable price, smartphones are promoted by connecting people even from isolating regions because of many social and political issues to the mainstream developmental initiatives provided by many governmental and non-governmental organizations (NGOs).

The first functional mobile phone was demonstrated by Martin Cooper from Mo-

torola in 1973 [19]. Since then, due to rapid technological advancements, mobile phones have become one of the fastest-growing innovations shaping human lives. It can be roughly classified into feature phones and smartphones. Smartphones have the advanced functionality capable of displaying photos, playing videos, making emails, surfing the web etc., beyond making phone calls and text messages which are the basic functions of a feature phone. Recent smartphones are the result of continuously developing process of integration of cutting-edge technologies into a small footprint at a very large scale. Now, with the rapid smartphone manufacturing technology, low-cost smartphone devices (also referred to as low-end devices) also offer similar sensing and processing capabilities to those of more expensive (high-end) devices. These are not only provide a better communication facility but also acts as a pocket computing device with ever-improving hardware and sensors, e.g., complementary metal-oxide-semiconductor (CMOS) module, ambient light sensor (ALS), display module, USB port etc. along with user-friendly software interface with optimized battery life. Modern smartphones accommodate a powerful computing unit (central processing unit, CPU) with optimized power consumption. In many smartphone models, to relieve the main CPU for real-time applications like image processing and classification or speech recognition, a dedicated powerful graphics processing unit (GPU) is integrated. The operating system (OS) controls the entire device properly, and the user-oriented graphical user interface (GUI) ensures the intuitive interaction between a user and the mobile applications or app. These apps can be readily developed using open-source platforms such as Android Studio and MIT app inventor, and with those, the sensing data from the built-in sensor hardware of the phone can be accessed directly for scientific applications [20, 21]. To cut a long story short, a smartphone is a perfect pocket computing device for the use in scientific contexts. Researchers around the globe are continuously working in the development of different scientific tools promoting science and technology to the need-based communities removing the economic barrier.

Together with smartphones, disruptive manufacturing technologies such as three dimensional (3D) printing has also gained the momentum [22]. It is a rapid manufacturing technique by creating physical objects through additive manufacturing. Based on the applications of the 3D printing technology methods, these can be classified as fused deposition modelling (FDM), stereolithography (SLA), directly ink writing (DIW), lamination (LOM), laser sintering and laser melting (SLS, SLM), photopolymer jetting (Ployjet) and binder jetting (3DP) [23]. Here, only the extrusion-based FDM 3D printing technology will be discussed. This technique involves three basic steps – the digital design of the 3D model, the conversion of the model into printing instructions required by the printer and, at last, the fabrication of the model by adding patterned layers of the printing material. The commonly used materials

are polymers such as polylactic acid (PLA) and polyethylene terephthalate glycol (PETG). The 3D printing process starts by designing an idea from scratch in digital format using computer-aided design (CAD) software (e.g. Solidworks, ZW3D etc.). After that, the 3D CAD model is exported in the STL (Standard Tessellation Language) file format that encodes the model's surface geometry without any information of color or texture, and this can be shared with anyone. Finally, the created STL file is imported into a slicing software (e.g. Ideamaker, Cura etc.) to convert it into a set of instructions that can be directly interpreted by the 3D printer, called G-code. In scientific research, 3D printing is becoming the most rapidly used critical technique that allows rapid development of inexpensive and robust prototypes with an unprecedented speed for different biological and biomedical applications [24, 25].

1.5 Review of literature

Over the past decades, smartphones have been explicitly used for the development of various miniaturized, portable and inexpensive sensing and imaging systems primarily to be used for PoC clinical and environmental monitoring applications [31–35]. These are the primary choice of interest because of their astounding penetration into the daily living routine of a human being. Moreover, these are the perfect solutions to develop compact and low-cost scientific tools over the existing conventional laboratory confined scientific instruments for applications in the low-resource areas [34]. These are embedded with the cutting-edge advanced miniaturized technological solutions that have been designed to perform regular tasks in a small form factor. Initially, the designing of several analytical devices had been proposed using the feature phones for PoC diagnostic applications outside of the well-established laboratory facilities. However, due to low computational ability and worse interface design, this had limited their performance, whereas laptop computers were always needed to analyze the signals for final results [26, 36, 37]. Smartphones are the latest generation of mobile phones that has advanced built-in technologies. Compared to the earlier versions of mobile phones, these have advanced multicore processors (CPU) along with graphical processing unit (GPU) to fasten the processing speed, superior display technology, CMOS digital camera technology and other useful sensors like ALS, gyroscope etc. and open-source operating software integrated in a very small footprint [20]. Thus, the usage of smartphones for the development of various sensing and imaging devices can simplify the design with minimized volume size and lowered overall fabrication expenses, thus allowing the deployment for onsite healthcare and other monitoring applications. Here, only the latest development on the smartphone based sensing and imaging systems for PoC applications have been briefly summarized. Based on the detection principle and the utilization of the built-in sensor techniques, smart-

Figure 1.4: The development of different inexpensive microscopic imaging platforms on smartphone; (a) a mobile microscopy by utilizing conventional optical elements, reproduced from [26] with permission from Public Library of Science (PLOS), (b) demonstration of smartphone based fluorescent microscope for detection of water-borne parasites like giardia lamblia cysts, reproduced from [27] with permission from Royal Society of Chemistry, (c) prototype of a video microscopy on smartphone for blood-borne filarial parasites detection, reproduced from [28] with permission from American Association for the Advancement of Science, (d) demonstration of a smart-phone microscope using color compound polymer lenses, reproduced from [29] with permission from Nature Publishing Group, and (e) demonstration of a chip scale lensfree microscope using ambient light, reproduced from [30] with permission from Royal Society of Chemistry.

phone based biosensors can be broadly categorized into optical imaging and sensing, Surface Plasmon Resonance (SPR), electrochemical and near-field communication (NFC) based sensors [38–40]. The details of these reported sensing devices have been illustrated below:

Smartphone based optical imaging platforms

Smartphone cameras are primarily designed for regular photography applications; therefore, their operation is limited within the visible region by incorporating an infrared (IR) cut-off filter. The smartphone cameras are the highly integrated electronic assemblies that have mostly back-illuminated (BI) CMOS sensors with densely packed pixel numbers. The number of these pixels has doubled almost every year and in modern smartphones the size of the pixels is reduced to 0.8 μm with increased fill factor and better signal-to-noise ratio (SNR) [34]. Thus, with the proper incorporation of additional optics and illumination condition to the camera module, one can convert a smartphone into a low-cost, portable, yet very powerful microscope with submicron resolution. Efforts have been made by several research groups around the world to demonstrate the working of smartphone microscopic imaging platform for PoC global health applications [35]. Based on the optics and illumination design approach adapted in these platforms, different imaging modalities can be broadly classified as BF, DF, fluorescence, polarized and phase-contrast microscopy. Breslauer et al. have demonstrated the first mobile microscope using a feature phone and conventional microscope objective and eyepiece lenses for global health applications. It can capture the morphologies of blood cells and microorganisms in BF and fluorescence mode, respectively [26]. Smith et al. have reported a 350× smartphone microscope simply using a ball lens for educational applications [41]. Using a simple lens and a proper illumination configuration, Zhu et al. have demonstrated the usability of the smartphone based imaging device for cost-efficient onboard rapid blood analysis [42]. To resolve the trade-off between resolution and FoV inherent to optical imaging systems, Switz et al. have demonstrated an inexpensive mobile phone microscope using reversed mobile phone camera lens for biomedical applications [43]. The first video smartphone microscope has been successfully demonstrated by D'Ambrosio et al. for the detection of blood-borne filarial parasites using onboard computer vision [28]. Recently, Dai et al. have presented the usability of a fluorescence microscope on smartphone utilizing polymer lenses that have dual functionality of both optical imaging and filtering [29]. Similarly, other research groups have demonstrated the usability of the smartphone platform microscope for biomedical applications like detection of malaria, sickle cell anemia, water-borne parasites such as giardia lamblia cysts, Schistosoma haematobium infection, molecular assays such as targeted DNA sequencing

and mutation detection, nanoparticle and virus detection, infertility screening etc. [27, 44–49]. With the more advancement in the computational power of the device, complex microscopy techniques such as phase microscope [50, 51], single shot multi-contrast microscope [52], deep learning microscope [53], Fourier Ptychographic Microscope [54] on a smartphone are becoming a reality now. Researchers have also reported another kind of smartphone microscope that doesn't use any lens system to magnify specimens [30, 55, 56]. These are based on digital holographic technique where the shadow of the specimen is allowed to fall on the CMOS sensor directly by removing the lens system of the phone. The recorded hologram is then reconstructed to obtain the final image using computationally expensive algorithms. Figure 1.4 represents the examples of some of the demonstrated smartphone based microscopic imaging platforms utilizing the device's camera and onboard computational power.

Again, with the rapid advancement in smartphone camera technology, most of the phone's embedded camera has an average pixel size of 1.12 μm. This allows the smartphone to differentiate different wavelengths of light by spatially separating them in different pixel positions by simply using an external dispersive element (e.g. grating) for spectroscopic applications. Many researchers have reported the development of the smartphone based spectroscopic system based on the principle of absorption, fluorescence, and reflectance [57]. Hossain et al. have reported the development of an inexpensive combined dual wavelength smartphone platform spectrometer based on the principle of absorption and fluorescence with a spectral resolution of 0.42 nm/pixel [58]. The same research group has also reported the working of a reflectance spectrometer on smartphone using endoscopic fiber bundle for industrial applications such as production analysis in agriculture [59]. Yu et al. have demonstrated the working of a smartphone integrated spectrometer for fluorescence-based biological assays [60]. Similarly, many other research groups have also demonstrated smartphone platform spectroscopic devices for different applications like biosensing [61, 62], environmental monitoring [63–65]. Figure 1.5 shows the working of different smartphone based spectroscopic platforms.

Apart from microscopic and spectroscopic implementation of smartphone, these are also being widely used for onboard colorimetric and fluorescent based assays for different sensing applications. Initially, the colorimetric detection platform using the built-in camera module of a feature phone for detection of different bioanalytes using paper-based microfluidic device has been demonstrated by Martinez et al. [37]. Due to low computational ability of the phone at that time, the captured images were sent to a centralized facility using network communication for result analysis. Presently, with the boom of the smartphone technology, the colorimetric detection as well as data analysis can be performed within the phone itself by developing custom-coded applications [69]. Thus, the usability of low-cost smartphone colorimetric devices have

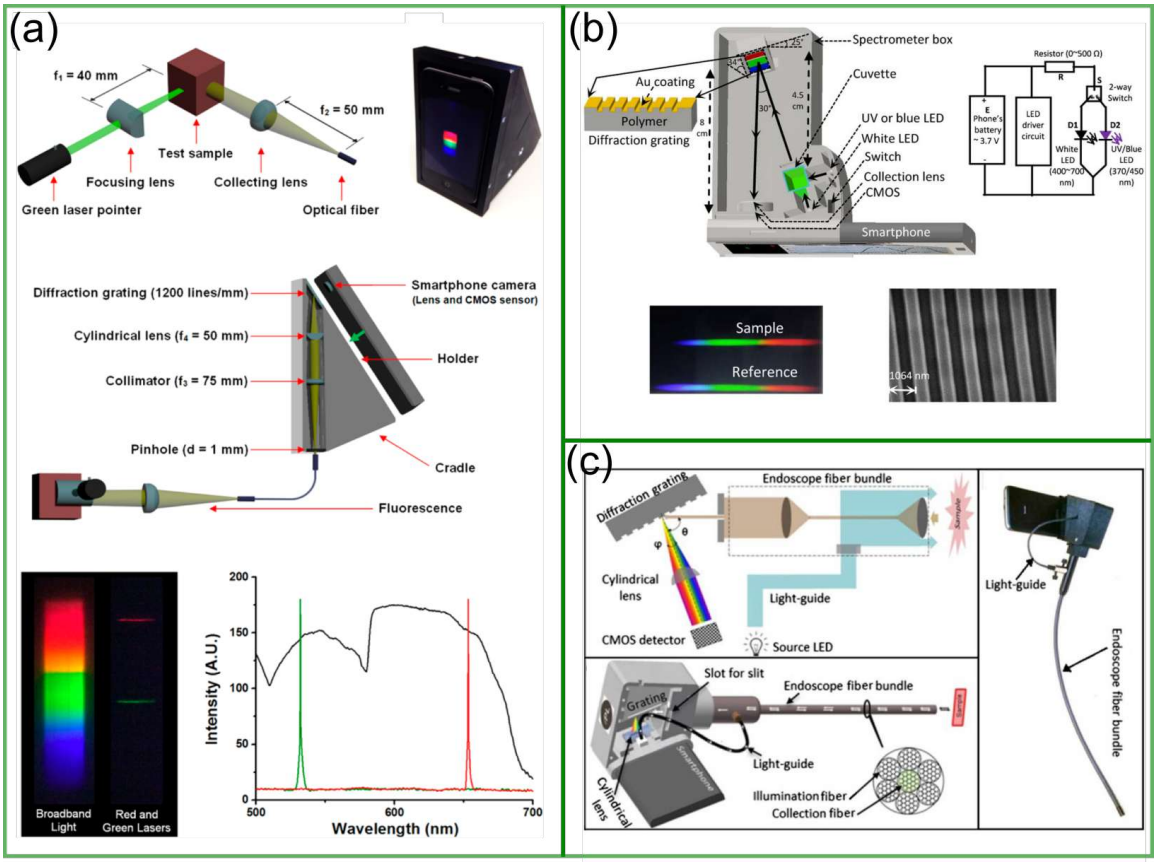

Figure 1.5: The development of different spectroscopic platforms on smartphone; (a) the working principle of a smartphone based fluorescence spectroscope for biological assays, reproduced from [60] with permission from American Chemical Society, (b) demonstration of a smartphone based dual wavelength spectroscope based on absorption and fluorescence, reproduced from [58] with permission from Optica Publishing Group, and (c) prototype of the smartphone platform reflectance spectrometer using optical fiber, reproduced from [59] with permission from Optica Publishing Group.

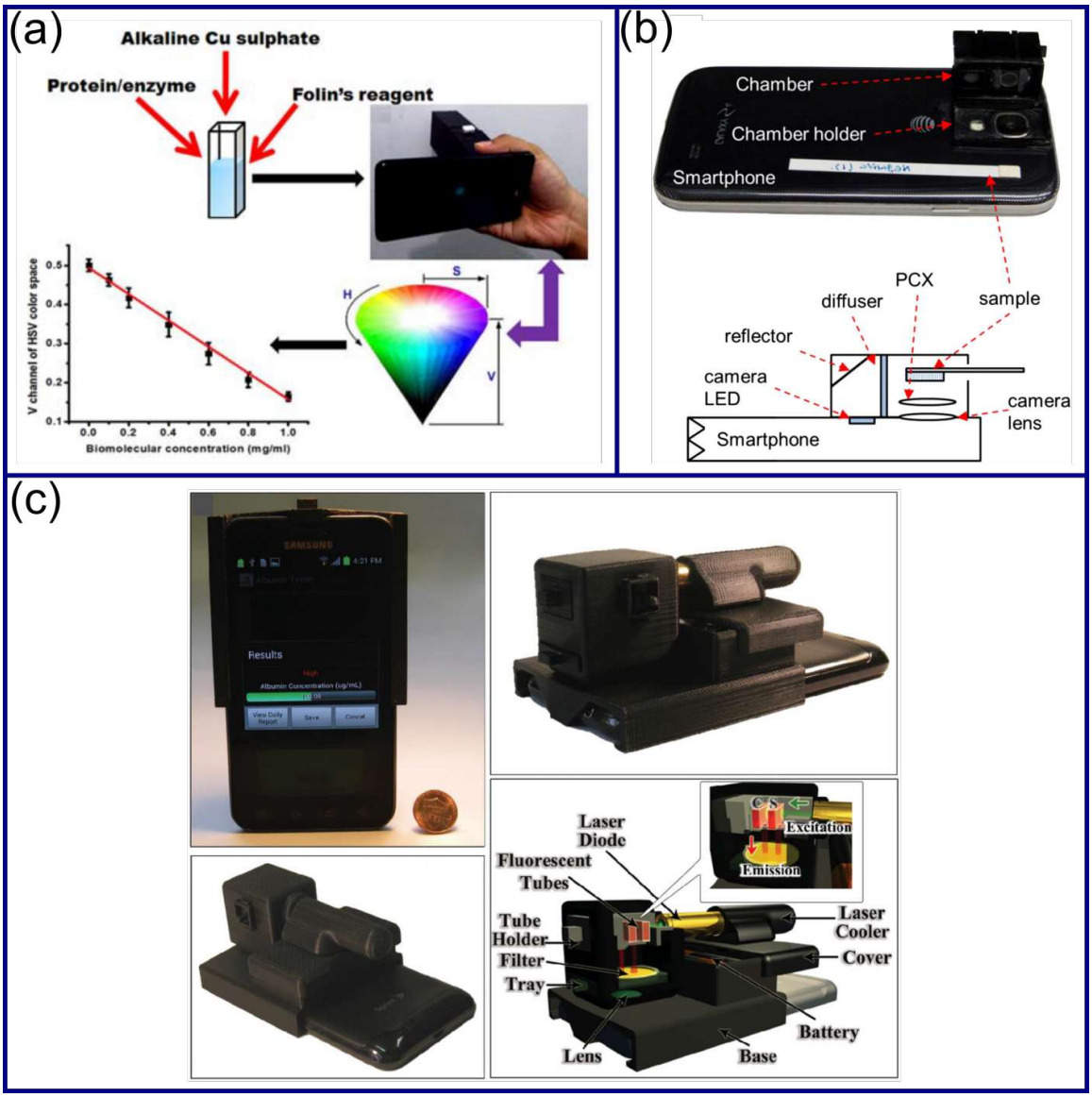

Figure 1.6: Demonstration of smartphone based colorimetric detection platforms; (a) optical scheme of the colorimetric analyzer platform for detection of proteins and enzymes, reproduced from [66] with permission from Wiley Online Library, (b) prototype of the smartphone based colorimetric detection of saliva alcohol concentration, reproduced from [67] with permission from Optica Publishing Group, and (c) demonstration of the smartphone based albumin tester in urine, reproduced from [68] with permission from Royal Society of Chemistry.

been rigorously demonstrated for different PoC applications such as quantification of proteins and enzymes, glucose monitoring, urinalysis, saliva alcohol concentration, food quality analysis etc. [66–68, 70–73]. Some of the reported colorimetric platforms on smartphone are shown in figure 1.6.

Smartphone based optical sensing platforms

All modern smartphones have a very useful built-in sensor which is the ALS that is utilized to optimize the battery consumption by frequently adjusting the brightness of the display screen automatically through sensing the ambient light condition. The ALS is a silicon (Si) photodiode that has a spectral response in the range of 350 nm to 1000 nm and it is capable of sensing both monochromatic and polychromatic light sources [80]. It has a very wide dynamic range which may vary for different manufacturer's phone models. For example, Sony models have a dynamic range of 0-20000 Lux and Motorola models have in the range of 0-27000 Lux [81]. The usability of the ALS sensor can be realized for different optical sensing applications since the light intensity data can be directly retrieved from the sensor using a custom-coded smartphone application without any further processing. Park et al. have demonstrated the working of an immunoblot-based optical sensing platform using the integrated smartphone ALS as detector and white LED flash as light source for detection of osteoarthritis [74]. The schematic of the platform is shown in figure 1.7(a) The research group has further extended their work by demonstrating the similar ALS based bio-sensing system using the ambient light source like outdoor sunlight and indoor fluorescent light [82]. The smartphone ALS based sensing platforms are also being used to monitor the quality of the drinking water onsite. Hussain et al. have reported an inexpensive and field-portable photometric sensor to detect fluoride concentration level in drinking water using the embedded ALS and LED flash light of the smartphone [75]. The sensing platform is based on the famous Beer-Lambert law of absorption and schematic illustration is shown in figure 1.7(b) The same research team has also demonstrated the onsite sensing of turbidity in various natural water resources by employing the Mie-scattering principle and using the integrated IR proximity sensor of the smartphone [76]. The usability of the smartphone ALS has also been demonstrated for education purpose in many Physics laboratory experiments [83, 84].

Smartphone based SPR sensing platforms

SPR can be defined as the resonant oscillation of conduction electrons at a metal/dielectric interface [85]. This is highly sensitive to the change in dielectric function at the metal surface, that's why it is widely used for chemical and bio-sensing applications [86].

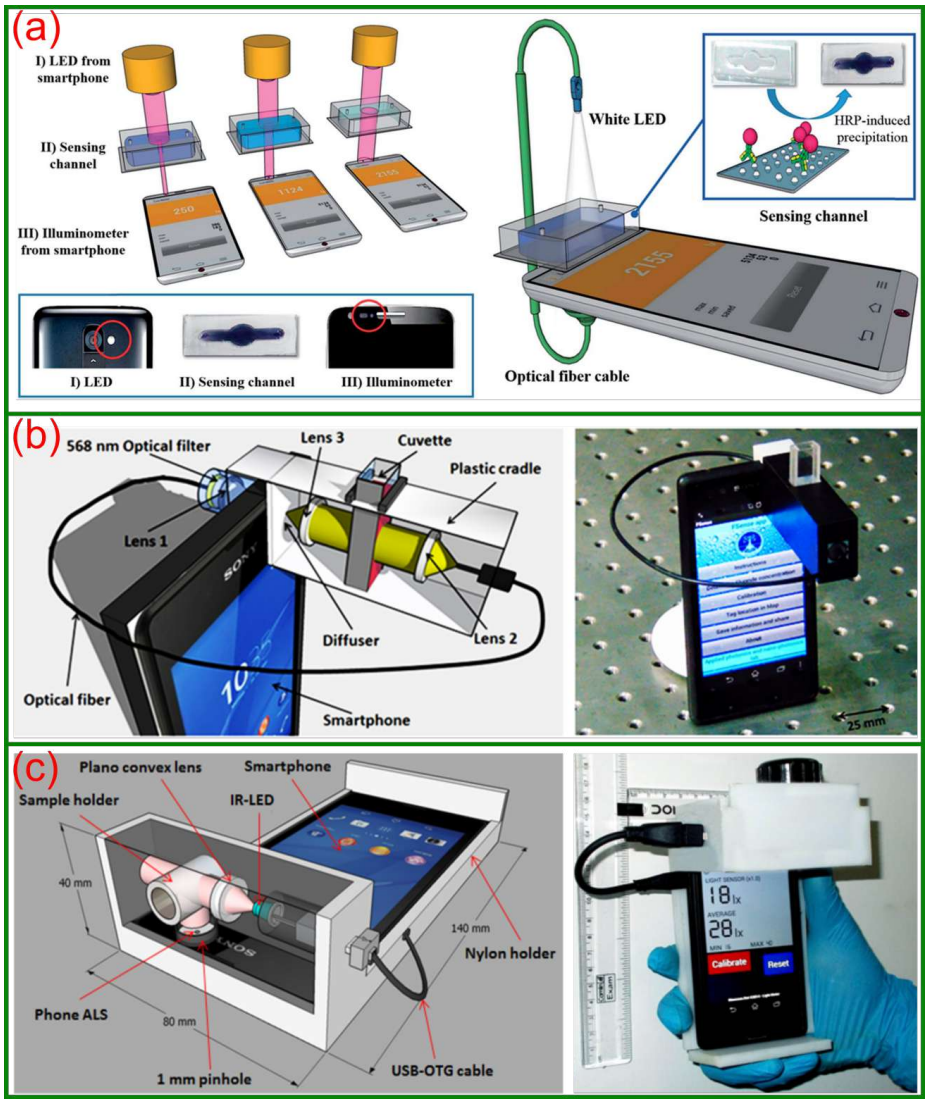

Figure 1.7: Different smartphone based optical sensing platform; (a) an optical biosensor for screening of osteoarthritis, reproduced from [74] with permission from Royal Society of Chemistry, (b) demonstration of a photometric sensor for fluoride level detection in drinking water, reproduced from [75] with permission from American Chemical Society, and (c) turbidity measurement using smartphone, reproduced from [76] with permission from Royal Society of Chemistry.

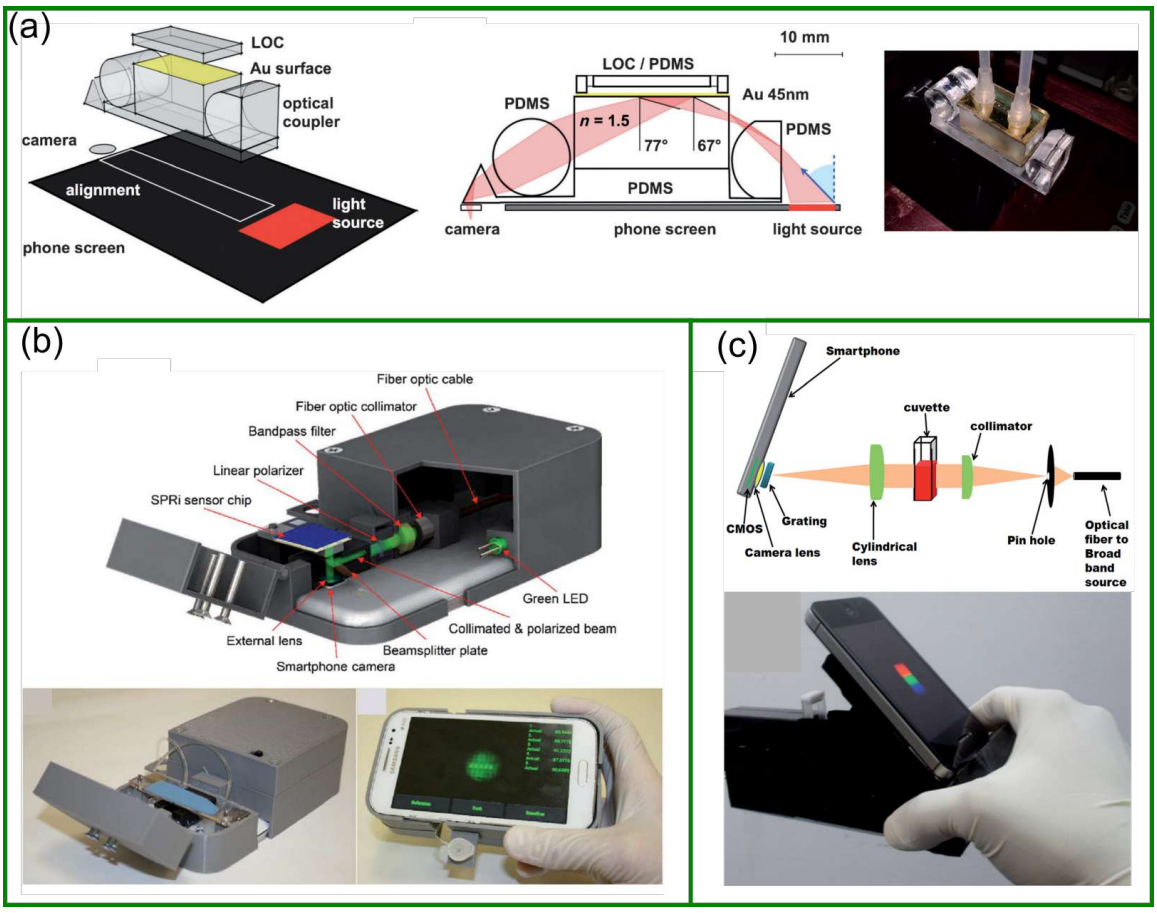

Figure 1.8: Demonstration of smartphone based SPR sensing platforms; (a) an illustration of the SPR sensing device on smartphone for chemosensing, reproduced from [77] with permission from Wiley Online Library, (b) a prototype of the SPR imaging platform for on-site bio-detection, reproduced from [78] with permission from Elsevier, and (c) demonstration of the smartphone based LSPR sensing platform for bio-conjugation detection, reproduced from [79] with permission from Royal Society of Chemistry.

Several groups have been continuously working on the combination of this strong SPR sensing system with smartphone to develop a powerful mobile sensing platform for onsite biomedical and environmental detection in resource-constrained settings. Here, only few literatures on the development of SPR sensors using the onboard smartphone hardware have been discussed. Preechaburana et al. have reported a cell phone based lab-on-a-chip SPR sensor to detect β_2 microglobulin (β_2M) using the phone camera and screen illumination [77]. Figure 1.8(a) represents the schematic and prototype of the proposed sensing device. The detection of β_2M is critical since it is an important biomarker for cancers, kidney diseases and inflammatory diseases. Guner et al. have reported a cost-effective and portable smartphone based SPR imaging (SPRi) platform for biological assay and this is shown in figure 1.8(b) [78]. Dutta et al. have reported a localized SPR (LSPR) sensing system using the smartphone camera by creating a visible spectrophotometer for the detection and quantification of bio-conjugation [79]. The designed sensing platform record the LSPR peak absorption wavelength shift from the conjugation of analyte such as protein, enzyme etc. with gold nanoparticles (AuNPs). The working of the platform is shown in figure 1.8(c).

Smartphone based electrochemical sensing platforms

The mini USB port of a smartphone is utilized to charge the device and also it can be used to connect additional electronic elements such as pen-drive. It can act as both host and connector, thus provide powering to the phone and simultaneously control the data communication with the peripheral electronic devices using USB OTG protocol. We can also make use of the audio jack of the smartphone to communicate with electronic devices. It can send and receive audio signals. These features of the smartphone can be used to develop field-portable, inexpensive electrochemical sensing platforms. For instance, Lillehoj et al. have presented a compact and rapid amperometric electrochemical platform on smartphone to detect Plasmodium falciparum histidine-rich protein 2 (PfHRP2), which is an important biomarker for malaria [87]. This is shown in figure 1.9(a). In the same manner, Zhang et al. have demonstrated a portable smartphone based potentiometric electrochemical biosensor for POC applications [92]. Delaney et al. have reported a smartphone based device using the audio jack that emits electro-chemiluminescence (ECL) signal from the electrochemical reaction. This is controlled and polarized by an external electrode via playing appropriate sound file. Then, the emitted ECL signal was being detected by the smartphone camera to quantify the analyte content [88]. Recently, Xu et al. have demonstrated a wireless, compact smartphone based electrochemical platform for onsite monitoring of nitrite contamination in water as shown in figure 1.9 (c) [89].

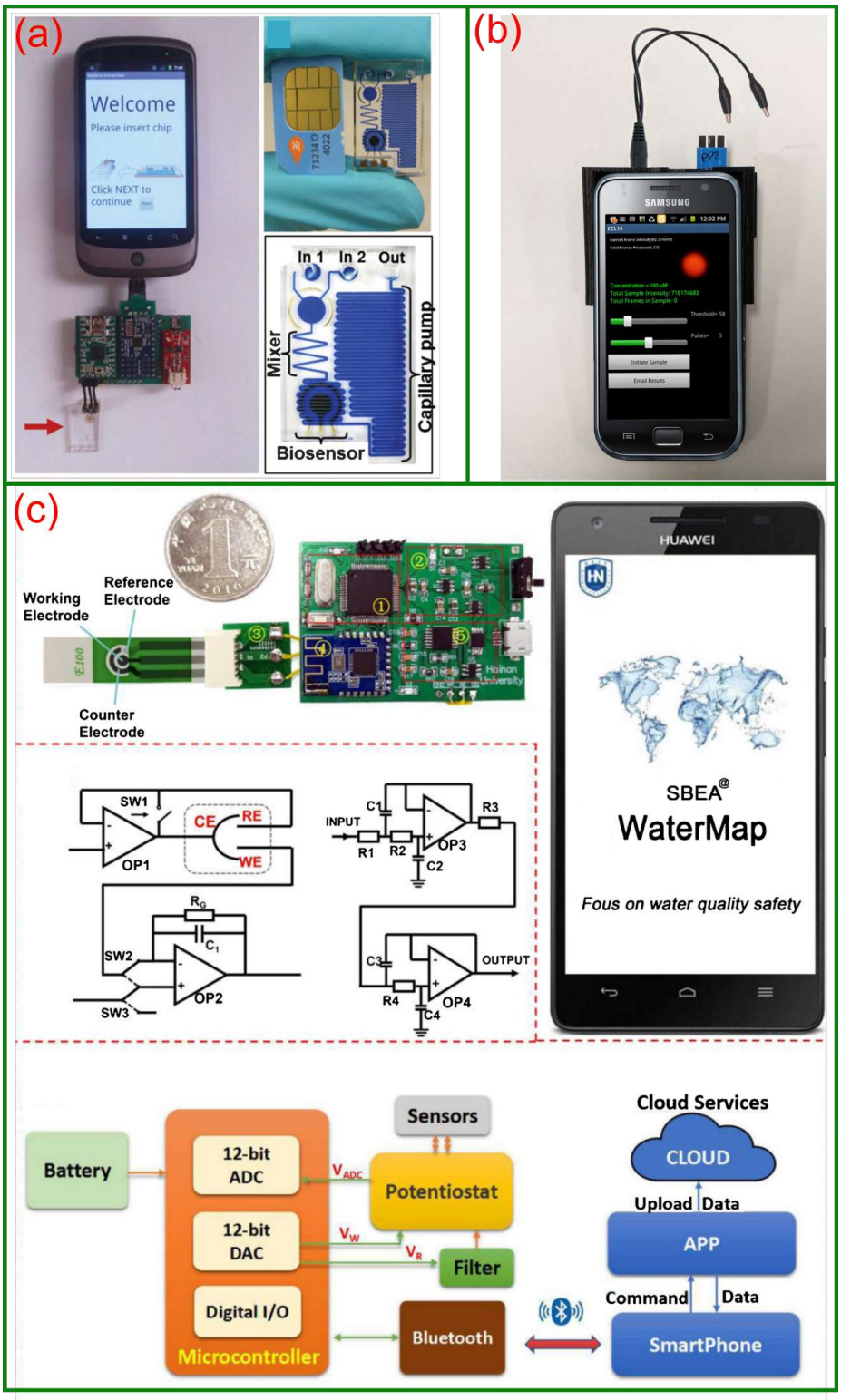

Figure 1.9: Smartphone based electrochemical biosensor; (a) demonstration of a rapid electrochemical analyzer using the USB port of a smartphone for quantitative biomolecular detection, reproduced from [87] with permission from Royal Society of Chemistry, (b) potentiometric electrochemical biosensor for PoC applications using audio jack of smartphone, reproduced from [88] with permission from Elsevier, and (c) an smartphone electrochemical sensor for nitrite contamination in water, reproduced from [89] with permission from Elsevier.

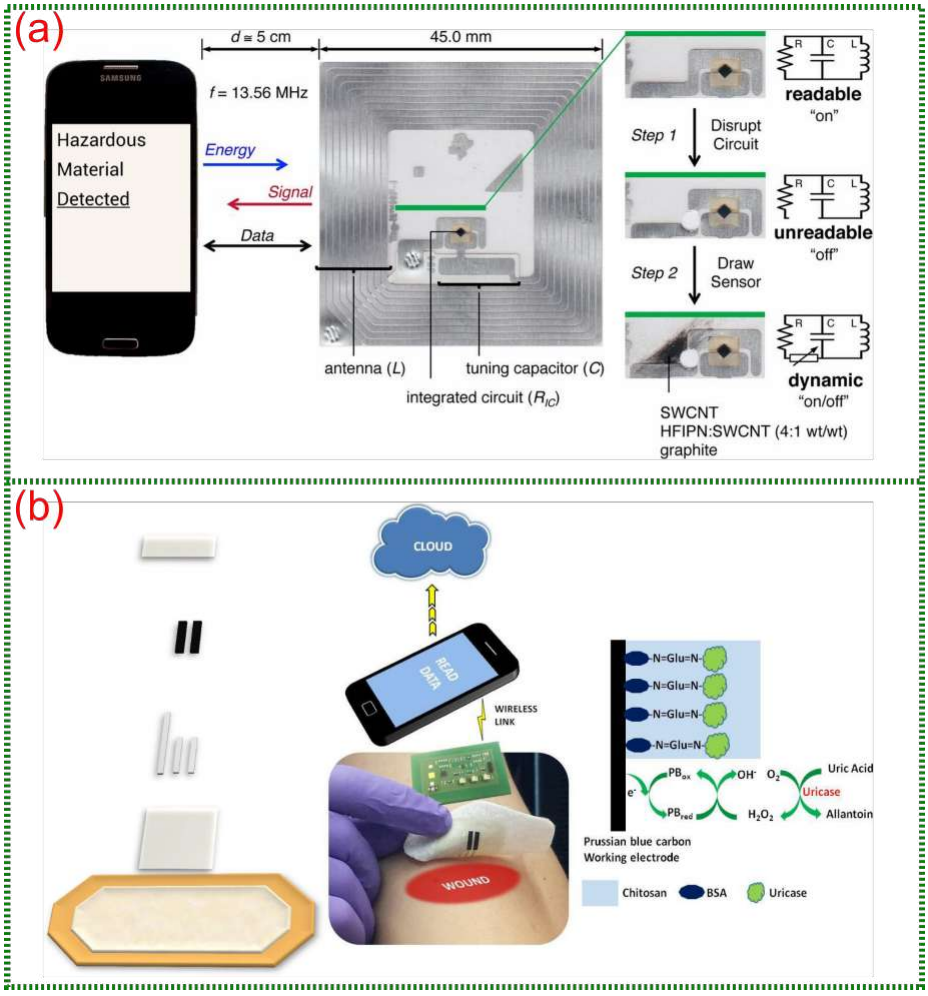

Figure 1.10: Smartphone platform NFC sensing systems; (a) demonstration of a smartphone based NFC sensing device for gas sensing, reproduced from [90] with permission from National Academy of Sciences, and (b) a smartphone based NFC wound monitoring device, reproduced from [91] with permission from Elsevier.

Smartphone based NFC sensing platforms

Now a day, in many smartphones NFC hardware is integrated which is configured to communicate wirelessly with NFC tags that are simple resonant electrical circuits comprising of elements like inductance (L), capacitance (C) and resistance (R) on a plastic substrate [90]. NFC which is actually a subset of radio-frequency identification (RFID) technology provides a short range wireless connectivity between two NFC-enabled electronic devices. These devices can work either as NFC reader or as NFC tag which can send energy signal to a nearby NFC tag and read the received signal [93]. Due to availability of NFC technology, it is now opening new opportunities to develop smartphone based wireless sensing platforms for different applications. Azzarelli et al. have demonstrated the first wireless gas detection platform using the embedded NFC technology in smartphone [90]. They converted a commercially available NFC tag into a programmable chemically actuated resonant device (CARD) by replacing single-walled carbon nanotubes (SWCTs) in place of a portion of the resonant circuit. This is shown in figure 1.10(a). The presence of the particular gas can be reliably detected from the interaction of the hazardous chemical gases with SWCTs as when the electrical resistance increases, feedback signal from the NFC to the phone decreases. Following the similar detection mechanism, Zhu et al. have also demonstrated NFC based oxygen sensors using a smartphone. They have simply replaced the SWCTs with Fe(II)-Polymer Wrapped Carbon Nanotubes (P4VP-SWCNTs) [94]. Similarly, Kassal et al. have demonstrated a smart bandage biosensor interface that provides on-demand wireless data transfer of uric acid status to a computer, tablet or smartphone by RFID or NFC technology (figure 1.10(b)) [91]. Recently, Teengam et al. have demonstrated the development of a smartphone-enabled electrochemical sensor using NFC technology to create a simple label-free immunoassay platform for the detection of Hepatitis B Virus (HBV) [95].

1.6 Research motivation

The working of the handheld smartphone based microscopic imaging platforms has been demonstrated extensively for various applications. These are proved to be useful as an affordable and reliable alternative platform over the existing laboratory confined conventional microscopes [96]. Many great steps in this context have already been made. In fact, few of them are already available in the consumer market [97, 98]. However, most of the reported phone-based microscopes use a singlet lens that has a very short focal length, which forms a finite-conjugate imaging system with the phone's internal camera-lens system. It is relatively simple to construct and provides pixel-limited resolution rather than diffraction-limited resolution. This means the

resolution is fully dependent on the pixel pitch of the imaging sensor of the phone due to the low optical magnification of the finite-conjugate system. A fairly high magnification along with a good degree of resolution is often necessary to visualize and conduct morphological studies in many biological applications. Besides, the usable FoV is limited due to the aberrations and distortions present in the imaging system. Because smartphone cameras, in general, are not designed for microscopic imaging, thus need additional good quality optical elements to reduce aberrations and distortions due to the high angle collection and multiple wavelengths of light from the specimen. Again, existing smartphone microscopes are specifically optimized for a single imaging type, such as BF, DF or fluorescence. However, the imaging system often requires being dynamically adaptable based on the specimen properties. For instance, BF imaging may not be suitable to visualize the specimens that are translucent in nature. Other contrast-enhancing techniques such as DF or PC imaging could be more useful for such purposes. On the other hand, some specimens may find certain wavelengths phototoxic or require narrow-band excitation to produce better contrast. This kind of imaging mode generally requires special illumination configuration by adapting additional optomechanical hardware such as DF condenser annuli and specialized PC objective lenses, thus making the optical system relatively restrictive to fit within a small form factor and budget of a smartphone microscope.

Still, there is substantial room for improvement in the design permitting effortless interfacing between the elements of the low-cost smartphone microscopes, including sources, optics, optomechanics and detector. Smartphone multi-mode imaging platform with a simple optimized design could facilitate easy adaptation to almost any imaging task on the go. Hence, changing one imaging mode to another could be simply a mere reconfiguration rather than a new optomechanical design. This kind of optical imaging device could be useful not only for PoC applications but also immensely beneficial for research and education in optics. It would provide a hands-on experience allowing the students to understand and reconfigure an optical setup, substantially reducing the effort within minutes. This would lead everyone to perceive optics as a new avenue by exploring new design approaches.

1.7 Scope of the thesis and thesis contribution

Optical microscopy is the basic foundation of scientific imaging and analysis that allows the visualization of cellular and sub-cellular structures and their dynamics. It is also the gold standard tool for various disease diagnosis. Commercially available standard microscopes can deliver precise imaging with high resolution. However, their bulkiness, cost, fragility, and need for skilled personal to operate, limit their usability within well-established and advanced laboratory facility conditions. Such an

important and useful instrument should not be limited within a centralized laboratory facility. Thus, efforts have been made to develop affordable and portable different microscopic imaging platforms on a smartphone for various biological, biomedical and other monitoring applications so that everyone can access the tool without any economic barrier even in resource-poor regions. Both $3f$ and $4f$ optical configurations have been implemented to develop the imaging platforms. The CMOS camera module has been primarily exploited as a detector in the present study. Other hardware parts such as LED flashlight of the phone, USB port (for powering and establishing communication between the smartphone and the external peripheral modules) etc., have also been utilized to make a complete standalone smartphone based microscopic system. Rapid prototyping of the proposed systems has been performed using the 3D-printing technology. Along with the optics design of the proposed imaging system, efforts have been made to make the system more versatile by developing custom-coded smartphone applications for image parameter analysis such as image enhancement, cell recognition etc.

The contributions of the present thesis work in the field of microscopic imaging on a smartphone are outlined with the motivations as stated below:

1. In the first step of the thesis, a high resolution, wide-field multi-modal finite-conjugate microscopic imaging on a single platform using a smartphone has been developed by integrating off-the-shelf optical components. The designed system utilizes the built-in camera for recording of the images and the LED flash of the phone as an optical source. Three dynamically adaptable modes of imaging, namely transmission BF, OIDF and TIRDF, have been demonstrated on a single platform. The design parameters such as magnification, resolution etc. and construction of the device have been discussed thoroughly. The applicability of the platform has been evaluated by comparing the performances with a standard laboratory microscope.

2. One of the major drawbacks of a finite-conjugate imaging system is that the spatial resolution of a microscopic platform depends on the pixel pitch of the CMOS sensor of the smartphone due to low optical magnification. To tackle this, in the second step, a low-cost 3D-printed smartphone microscopic system with a very high optical magnification has been developed. The imaging system is based on the $4f$ optical configuration where an objective lens and a tube lens are stacked adjacently such that their focal planes coincide. Modern optical microscopes that are based on infinity-corrected objectives follow this configuration [10]. This allows the utilization of the numerical aperture at the full potential delivering enhanced resolution. An image processing algorithm has been proposed by using cloud-based services, which can be accessed anytime

through a mobile broadband network. Using this facility, the quality of the captured images can be enhanced further, thus obviating the need for dedicated computational tools for post-processing of the images. Since, the primary intention is to design affordable and portable technological solutions that are meant to be used in regions where resources are very limited, hence further optimization in the design of the platform has been implemented. In the next phase, the microscopic system has been modified to operate in high-throughput BF and fluorescence modes at three different optical magnification levels that perform at par with that of a laboratory-grade microscope. The versatility of the device has been demonstrated through imaging of standard microbeads and human blood samples both in BF and fluorescence modes of imaging. Furthermore, the designed imaging platform is equipped with an on-board cell recognition feature which has been obtained through developing a smartphone application for automatic cell counting with high precision.

3. In the third step of the thesis, a programmable illumination smartphone microscopy (PISM) as a flexible multimodal imaging platform for contrast enhancement of various samples has been proposed using different low-cost consumer optoelectronics parts. Usually, different microscopic imaging modes require spatially modulated illumination systems, which can be obtained by introducing optical filters and physical masks into the condenser lens of the microscope. For instance, in a DF microscope, a specialized opaque disc is inserted into the aperture focal plane of the condenser lens to create a hollow cone of light. It would produce a numerical aperture larger than the objective lens so that only the scattered light from the specimen can enter the objective lens. Similarly, for phase-contrast imaging, a phase ring is inserted into the condenser's aperture plane to fit the ring-shaped phase plate inside the objective lens. To integrate all the imaging modes together in a single platform is either extremely laborious and expensive or impracticable. Herein, a miniature (0.96 inches), programmable organic light-emitting diode (OLED) display has been used as an optical source to develop the proposed PISM system. By displaying different color and binary patterns on the OLED display, six well-established imaging modes, namely BF, DF, OI, FI, RI, and DPC imaging have been demonstrated on a single optical setup. Furthermore, by incorporating additional optical components such as an optical polarizer into the setup, another imaging mode-polarized imaging has been realized with the proposed PISM system.

4. Finally, the thesis ends with the demonstration of a versatile one-shot BF, DF and DPC imaging solution using the off-the-shelf optical and electronics com-

ponents for infield applications. Currently, bulky and complex optomechanical design are being implemented to obtain various label-free multi-contrast microscopic setups. The basis for economical and versatile microscopic imaging applications is the cost-effective engineering of the illumination component. The work details the design of a one-shot multi-contrast microscopic imaging platform on a smartphone by generating a multiplexed color pattern on an OLED panel used as an optical source for the proposed system. The same color pattern has also been adapted to a basic laboratory microscope to transform it into a multi-contrast imaging platform. The color channel of the final image has been decomposed and subsequently computed to obtain the different contrast enhancing imaging modes. Both the systems provide three imaging modalities for every single shot, namely BF, DF, and DPC imaging on a single optical setup.

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