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Review

Miniaturized lensless imaging systems for cell and microorganism visualization in point-of-care testing

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Low-cost, robust, and user-friendly diagnostic capabilities at the point-of-care (POC) are critical for treating infectious diseases and preventing their spread in developing countries. Recent advances in micro- and nanoscale technologies have enabled the merger of optical and fluidic technologies (optofluidics) paving the way for cost-effective lensless imaging and diagnosis for POC testing in resource-limited settings. Applications of the emerging lensless imaging technologies include detecting and counting cells of interest, which allows rapid and affordable diagnostic decisions. This review presents the advances in lensless imaging and diagnostic systems, and their potential clinical applications in developing countries. The emerging technologies are reviewed from a POC perspective considering cost effectiveness, portability, sensitivity, throughput and ease of use for resource-limited settings.

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1 Introduction

The challenges associated with delivering healthcare in point-of-care (POC) are significant in developing countries [1–4]. Prevention and treatment of diseases require accurate diagnosis, which is generally achieved with trained personnel and equipment readily available in developed countries. However, the limited availability of qualified personnel, adequate infrastructure and costly medical instruments present significant challenges in treating and preventing the spread of especially the communicable diseases in resource-limited countries [5–7]. There is a significant need for affordable and simple diagnostic technologies for infectious diseases and for monitoring patients' health conditions in these countries [6, 8].

World Health Organization (WHO) has recognized the critical requirements and outlined the standards for diagnostic instruments for resource-limited settings [7, 9–11]. According to WHO standards, these devices need to be inexpensive, disposable and easy to use [12]. They should also be functional under heat and humidity, given the lack of refrigeration, reliable electricity, and clean water resources in many developing countries. The diagnostic devices that can be utilized in POC generally require analysis equipment, which needs to be automated and operable within clinically acceptable sensitivity without extensive technical training. A comparison between the needs for resource-limit-

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Abbreviations: **FOV**, field of view; **LUCAS**, lensless ultra wide-field cell monitoring array platform; **POC**, point-of-care

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Table 1. The differences in needs for resource-limited point-of-care (POC) diagnosis and conventional diagnosis based on World Health Organization (WHO) standards

	Resource-limited POC diagnosis	Conventional diagnosis
Cost	Inexpensive, disposable	Expensive and costly to maintain
Functionality	Single diagnosis readout per unit	Multiple readouts possible with one unit
Personnel	Minimally trained personnel can operate, user-friendly operation	Requires highly trained personnel
Environmental conditions	Functional at high temperature and high humidity environments	Not suitable for high temperature and high humidity environments
Infrastructure	Does not require an infrastructure and a constant electrical supply	Requires advanced infrastructure and vulnerable to blackouts
Flexibility of operation	Can perform multiple diagnosis of pathogens and strains with minimal alteration	Requires different platforms for different diagnostic applications
Accessibility	Deliverable to end users without a need for centralized hospitals or clinics	Generally performed at established hospitals and clinics
Accuracy and precision	Moderate-high (based on application)	High
Throughput	High	High

ed POC diagnosis and conventional diagnosis has been presented in the light of WHO standards in Table 1, which clearly displays the differences and the specific requirements in these two settings. These specific needs impose challenges and require innovative approaches for efficient delivery of healthcare in POC in resource limited settings [3].

Lab-on-a-chip technologies have been emerging for detection and monitoring of infectious diseases at resource-limited settings [3, 5, 13–24]. Healthcare personnel including technicians, nurses and physicians can use these devices with minimum training to diagnose patients for infectious diseases or monitor the progress of a disease [2, 5]. Thus, these technologies will allow the healthcare workforce to deliver medical services more efficiently without the need for expensive equipment or extensive training [1, 13, 24, 25]. Miniaturization and integration of diagnostic devices would allow rapid and reliable high-throughput chemical and biomedical imaging and analysis from a tiny amount of sample such as a fingerprick volume of blood [7, 26–30]. Optofluidics takes advantage of integrating microfluidics and microelectronic optical components onto the same platform [31, 32]. Such a platform, with fluidics for sample delivery/capture and lensless optics for sensing and detection, can be applied to areas such as ultra wide-field cell monitoring array [14, 33, 34], digital in-line holography [35–41], optofluidic microscopy [42–45], and lensless on-chip microscopy [15, 41, 42, 46]. In this review, we provide an overview of the recent literature on lensless imaging technologies with a POC perspective in resource-limited settings. We present the current state of lensless imaging, its advantages, application challenges and the potential for use in POC.

2 Optical and fluidic concepts and considerations for POC testing

Microscale investigations in life sciences have so far been largely carried out by conventional light microscopes using lenses and visible light (i.e., geometrical optics) [46–48]. These technologies and instruments are difficult to miniaturize and require highly trained personnel. Hence, they are mostly confined to laboratory settings, and currently they have limited practical use for POC testing at resource-limited settings [45]. However, these technologies can be integrated and supplemented with the emerging nano- and microscale methods in fluidics (i.e., nano- and microfluidics), which would allow new and feasible approaches in POC testing and diagnosis. In this section, we review the current status of optics and fluidics with a resource-limited POC perspective, and highlight the associated challenges, needs and future directions.

2.1 Optical aspects and components for imaging

Conventional microscopic imaging and detection technologies primarily consist of four main compo-

nents: (i) a light source, (ii) optical modulators, (iii) lenses, and (iv) a detector, which are sequentially positioned on the spatial beam path [49]. Here, we discuss each of these components, their roles and relevance for POC applications.

2.1.1 Light sources

Microscopes use various light sources ranging from incandescent light bulbs to solid-state light-emitting devices (i.e., light-emitting diodes, LEDs) [50]. LEDs are miniaturized light sources that are commonly used in compact optical devices without high-power demands. However, LEDs emit non-collimated light, whereas high-resolution imaging applications require collimated light sources that minimize diffraction. On the other hand, laser is an intense coherent light source with a narrow bandwidth, high spatial coherence and insignificant chromatic aberration. Laser can be easily focused and facilitates high-resolution imaging [51]. Therefore, laser diodes that combine the portability, low cost and low-power consumption of LEDs and the coherent emission characteristics of lasers would be ideal for on-chip POC diagnostic platforms [52, 53].

2.1.2 Optical modulators

The properties of light (e.g., intensity, path, and wavelength) change as it travels through a medium (e.g., sample of interest), which acts as an optical modulator. The medium reflects, diffracts, and deflects the incident light. Sample characteristics and incident light wavelength affect the sample-light interaction and the observed outcome. For instance, in high-resolution fluorescence imaging, fluorescent molecules transfer energy from the incident light to the emitted light at a different (longer) wavelength. Due to this phenomenon, fluorescence-based detection requires expensive optical filters to eliminate the background noise and the wavelengths other than the emission that carries the relevant information. Therefore, it would be challenging to adapt fluorescence imaging for on-chip microscopy for POC-oriented applications. However, advances in LED and CCD technologies may overcome these challenges and facilitate feasible utilization of fluorescencent luminescence in POC [54, 55].

High-resolution imaging can also be achieved without lenses or filters by employing surfaces as optical modulators (i.e., surface plasmon resonance) [56–60]. Fabrication of nanoscale structures and features on surfaces has been well established enabling the surface plasmon methods for imaging [61–68]. This imaging method is based on optical energy changes, converting incident light into a surface plasmon resonance through interactions between nanoscale structures and incident waves [56, 58–60, 69]. Metals are commonly used for surface-plasmon optical systems as they support surface electric charge waves. Non-metallic materials can also have similar energy transformation properties when used as nanoscale hole arrays on metallic films [70]. High-resolution imaging can also be achieved through light-phase images that differentiate features according to their geometry using a collimated light source [34, 35, 37]. Since these methods do not require expensive filters and can operate in a lensless setting, they may be considered for applications in POC detection.

2.1.3 Lenses

Lens-based imaging is an optical method in which a lens is situated away from the object. Lenses condense and modulate light resulting from the sample of interest (e.g., cells). A lens can be realized by an aperture or using convex or concave optically clear materials. The term aperture in optics refers to an opening that can determine the diffraction angle of a bundle of rays, focusing them on an image plane. The aperture can also determine how much light reaches the image plane. Although the resolution increases with decreased aperture size, images become darker as apertures become narrow due to the constriction of light. Challenges exist regarding both the diffraction limited resolution and practical use of lens-based optical microscopy because of the narrow field of view at high magnifications [71, 72]. Lens-based methods are not capable of imaging nanofeatures (i.e., less than 100 nm) that are smaller than the imaging wavelength (i.e., diffraction limit) [73]. One solution is to send light through a tiny aperture on an opaque screen (e.g., metallic or non-metallic films) without using conventional lenses (i.e., near optical detection approaches). In this method, the target must remain at a sub-wavelength distance (less than the wavelength of the incident light) to the detector to eliminate diffraction effects caused by incoherent light [71, 72, 74]. However, these approaches present challenges in terms of their applications in POC due to requirements such as: expensive high-energy light sources, sensitive detection systems and complex peripheral equipment. Lensless approaches hold great promise in POC testing due to simpler operation and low cost [13, 25, 41, 42].

2.1.4 Detectors

A detector is an electronic sensor that converts incident light into an electrical current as a function of the light intensity. Photomultiplier tubes (PMTs), avalanche photodiodes (APDs), and PIN (p-type-

intrinsic type-n-type) photodiodes are also used as optical detectors. Traditionally, PMTs and APDs have been used in commercial cytometers that measure fluorescence intensity because of their high sensitivity, including the ability to count photons. PMTs are typically chosen for very-low-intensity light applications because they have large internal gains, and low noise [75]. However, they are difficult to use for POC systems, as they are expensive and require peripheral circuitry and heavy equipment (e.g., high-voltage power supply). APDs are more intrinsically sensitive than PMTs (typically three to four orders of magnitude higher). However, they are sensitive to temperature changes. The disadvantage of both types of detectors is their cost. When more light is available, PIN photodiodes can be well-suited for detection and integration in a miniaturized device [76]. In contrast to a single sensor, imaging arrays using CCDs and complementary metal oxide semiconductors (CMOSs) are preferable for wide field of view (FOV) detection since they do not require a peripheral apparatus to scan the entire sample area. In lensless microscopy, CMOS (high speed and low light sensitivity) and CCDs (low speed and high light sensitivity) can be used as light detectors based on the speed and sensitivity needs. For wide FOV applications, CCD and CMOS imaging arrays have been employed in lensless systems and have proven to be effective and feasible. Recently, a wide FOV system using an arrayed CCD image sensor was adopted for high-speed imaging by sacrificing the resolution using sequential frame detection [15, 77].

2.2 General microfluidics for biological applications

Applications of fluidics in the form of nano- and microfluidic systems have been emerging in biomedicine [78–89]. In biological applications, the size and operational regimes of fluidic devices can be adjusted to match that of the target, e.g., ~10 μm for cells, ~10–100 nm for macromolecules, and ~10 nm for small single molecules [90–94]. In these systems, the flow is driven by pressure generated by peripheral syringe pumps, integrated peristaltic pumps, electrokinetic drive, or gravity [42, 95–97]. Optical forces can also be used in microfluidic channels such as optical tweezers, which can manipulate cells [98–100]. Integration of fluidic systems and lensless optics can offer platforms for a wide range of cell manipulation processes, including capture [13], separation, isolation, high-resolution imaging [13, 42, 101], and probing cellular functions at the single-cell level [102].

3 Integration of optics and microfluidics

Optofluidic approaches eliminate the need for lenses and filters by benefiting from low-resolution ultra-wide FOV approaches with CCD-based detection. In these methods, fluidics have been used to immobilize cells and to maintain a single layer of cells within confined flow geometry. For example, the lensless ultra wide-field cell monitoring array platform (LUCAS) has been used with microfluidic channels to detect cells in whole blood [13, 15].

Table 2. Comparison of lensless on-chip imaging platforms for POC applications: Optofluidic microscope (OFM), digital holography and lensless ultra wide-field cell monitoring array platform (LUCAS)

	OFM	Digital holography	LUCAS
Function	Images sample shape in a dynamic fluidic environment with high resolution	Detects and images microscale objects in 3D space	Rapidly detects and counts thousands of target particles in real time
Method	Interchanges fluids to manipulate the fluid medium optical properties	Sample is imaged with a spherical wavefront using a focused laser beam	Rapid pattern analysis with captured target particle shadow images
Image capture	CCD	CCD/CMOS	CCD/CMOS
Illumination	Halogen lamp	LED/Xenon lamp	LED
Image properties	2D	3D with phase information	2D
Size of smallest feature	Submicrometer level resolution is possible	Micrometer level resolution	Micrometer level resolution
Window size	Assembles partial images to create a full image	Physical scanning is needed to create wide field of view images	Rapid ultra-wide field of view imaging
Portability	Requires peripheral equipment such as pumps to mobilize fluid	Requires peripheral equipment and computational capabilities	Requires minimal equipment, portable

Both the LUCAS and holographic systems use microfluidic channels and flow [13, 15]. Lensless diagnosis systems can be built onto chips using existing fabrication techniques [31, 42, 43, 45, 103]. The portability achieved using lensless optofluidic systems would significantly benefit POC diagnosis devices. On the other hand, the imaging and diagnostic methods targeted for POC need to be robust, user-friendly and operable without extensive training. A comparison of the lensless on-chip imaging technologies is presented in relation to POC applications in Table 2. The advantages and challenges associated with each method in terms of functionality and application in resource-limited POC are highlighted in the next sections.

3.1 Optofluidic microscopy

Optofluidic microscopes (OFM) integrate microscale fluidics and optics in a single system. An earlier application of this approach aimed to investigate the effects of spaceflight on living organisms (i.e., *Caenorhabditis elegans* or round worm) [104]. The samples of interest were transferred through microchannels into a microchamber illuminated by an LED light source (placed above), and imaged with a CMOS sensor array below. When the chamber was illuminated, the samples casted a shadow on the CMOS array, which recorded the images. A challenge in utilization of this system was caused by the convex corners of the microchamber, which reduced the imaging area and necessitated manual recording of the signal spectrum with a vector signal analyzer. In addition, the use of non-collimated LED illumination led to a low resolution, which could be improved by decreasing the distance between the imaging plane and the sensor array or reducing the illumination distance. In an effort to improve the resolution and performance of this approach, masks of nanometer-sized apertures were placed above the CMOS array [42, 43]. This was achieved by coating an opaque metal film layer onto the sensor array with two lines of nano-apertures (Fig. 1a) and encasing them into a microfluidic chip (Fig. 1b). This system could then be operated in an upright position to utilize gravity-driven flow, eliminating the need for external pumps, which is suitable for elongated samples, such as, in this case, round worms (Fig. 1c). However, when spherical or ellipsoidal samples (e.g., cells, bacteria) are imaged, an external pump should be used, e.g., electrokinetic pumps [42]. In the case of imaging cells, a uniform fluid flow created by an external pump would minimize the imaging artifacts due to rotation of the cells. The device is uniformly illuminated from the top with white light (~20 mW/cm^2, approximately the intensity of sunlight) using a halogen lamp. The target object flows across the aperture array, creating a series of image slices that produce a high-resolution image when assembled (Fig. 1d). The flow speed of the specimen can be calculated by tracking the time difference between the detection ob-

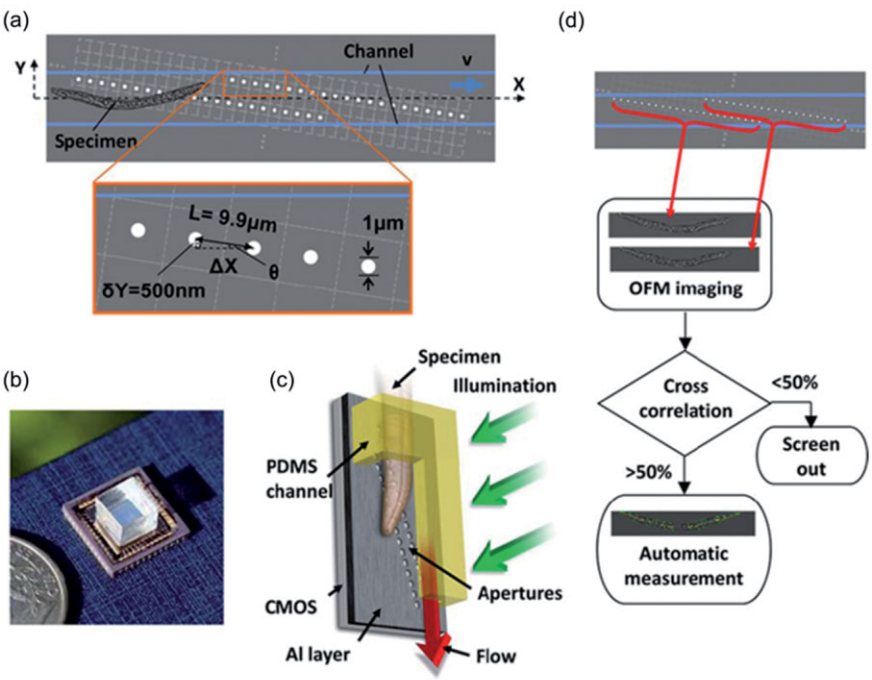

Figure 1. Optofluidic microscope (OFM) system. (a) OFM combines microfluidics with aperture-based optics. The apertures (white circles) are fabricated on a CMOS sensor array, which spans across the microfluidic channel (blue lines). (b) The assembled chip on a CMOS array. (c) The chip can be operated in an upright position to facilitate gravity-driven flow and hence to avoid the use of external pumps. (d) Flow diagram of OFM operation, which involves comparison between two images (red arrows) acquired by the two OFM arrays. The image pair is accepted if the image correlation is greater than 50% (Adapted from [42]).

tained through each aperture, which is then used to reconstruct an image. Since the object passes over each aperture at different time intervals, the time-varying light transmission through the aperture constitutes a line trace. When these line traces from all the apertures are assembled an image is constructed (Fig. 1d).

The challenges associated with the OFM approach include the critical dependence of the imaging outcome on the orientation of the sample and the constant flow rate within the channel. In other words, an acceptable image would not be obtained if the sample orientation during imaging changes (i.e., rotation of the sample during flow). A constant and uniform flow rate needs to be achieved inside the channel for this approach to work effectively, which introduces operational difficulties that make it not suitable for applications in resource-limited POC. In the system, the aperture array needs to be in perfect alignment with the underlying sensor grid and the resolution is limited by the size of the apertures and the spacing in between. If these challenges are addressed or minimized with improved design and fabrication methods, OFM microscopy would be suitable for POC since it allows portable operation and does not require expensive peripheral equipment.

3.2 Digital holography

Digital holography is an emerging phase-contrast imaging technique that offers both qualitative and quantitative information [34, 38, 105]. In holographic imaging (Fig. 2a), samples are illuminated with a spherical wavefront produced by focusing a laser beam from a 1-μm diameter pinhole (Fig. 2b). The focused light serves as a reference beam, while the scattered light serves as an object beam. The CCD sensor array records the interference pattern produced by the superposition of the two wavefronts (the hologram). A subsequent digital elaboration allows retrieving the volume information on the sample from a single 2D hologram (Figs. 2c–h). In this method, multiple focal planes can be achieved and captured mimicking the focus control of conventional optical microscopes. Digital holography records the entire object in 3D space, whereas a conventional microscope has a 2D focal plane and requires mechanical scanning to create a full image. Due to the complex analysis that can be performed with holography, the orientation of asymmetric yeast cells can be determined by the broken symmetry as shown in Figs. 2c–h. The resolving power in this method is a function of the hologram recording geometry, the CCD dimensions and incident wavelength.

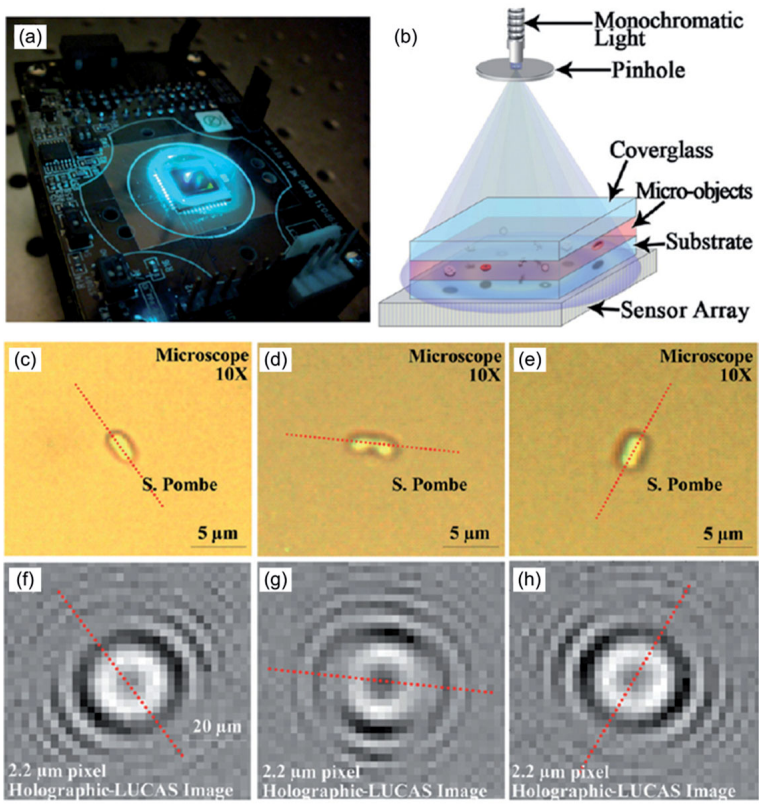

Figure 2. Digital holography with lensless ultra wide-field operation. (a) The photograph of a digital holographic microscope platform. (b) Schematic drawing of the Holographic-microscope platform. (c–e) Microscope images of asymmetric *S. pombe* yeast cells imaged under 10× objective-lens. (f–h) Detection of the 2D orientation of the cells using the holographic microscope, which are in the same field of view as in c–e, respectively (Adapted from [34]).

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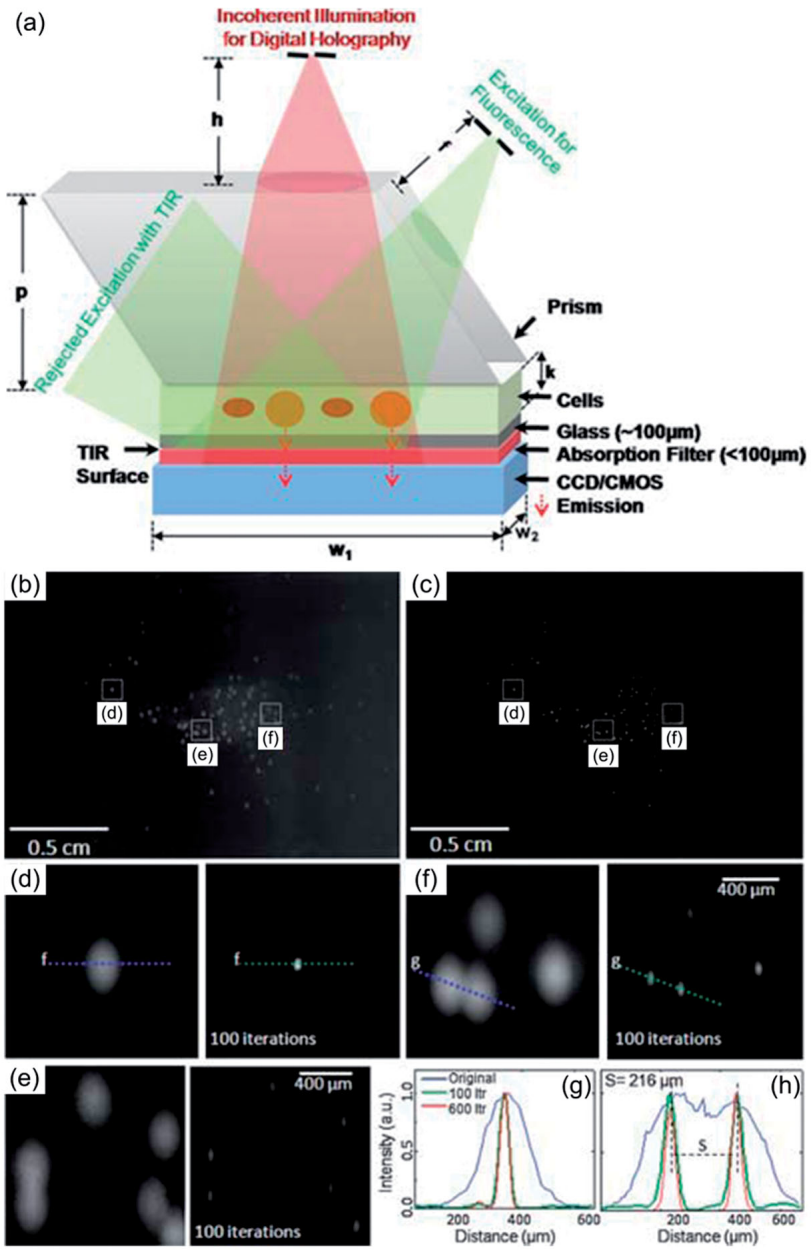

Figure 3. Digital holography integrated with fluorescent imaging. (a) Wide-field lensless fluorescence imaging platform with digital holography. (b–e) The fluorescence imaging of calcein-labeled white blood cells and corresponding results using iterative deconvolution algorithm. The cross-sections of fluorescent signatures for the raw lensless image (blue curve) as well as for 100 (green curve) and 600 (red curve) iterations of deconvolution are shown in (g) and (h), respectively (Adapted from [102]).

Digital holography has been integrated with fluorescence imaging to achieve lensless fluorescence detection without any thin-film filters or mechanical scanning with an ultra-wide FOV (2.5 cm × 3.5 cm) [102]. In this approach, the sample was illuminated with an incoherent excitation beam from an LED or a Xenon lamp source located directly above (Fig. 3a). Fluorescence excitation was provided from the side utilizing a rhomboid prism. The incoherent light beam interacted with the sample, undergoing total internal reflection (TIR) at the bottom (Fig. 3a). Detection of the fluorescence emission from the excited cells/particles over the entire FOV of the sensor array (CCD or CMOS) was achieved without using any lenses. Unlike the traditional fluorescence microscopy, the need for expensive interference filters was not a limiting factor for this method. An inexpensive plastic-based absorption filter was used between the sample and detector planes to achieve a better dark-field background. The issue of potential overlapping of diverged fluorescence emission of cells or particles at the sensor plane was addressed by deconvolving the acquired images, which provided a resolution of ~40–50 μm over the entire sensor FOV without the use of a lens. The system was val-

idated by imaging white blood cells as shown in Figs. 3b–h.

The digital holography and lensless fluorescence imaging platforms discussed above are innovative approaches that are transforming microscopy techniques dramatically by simplifying and by reducing the cost of imaging equipment. These methods can provide valuable information about the sample, such as the 3D structure and orientation. Although they are of relatively more complex nature, they offer higher computational needs compared to other on-chip imaging technologies (Table 2). Therefore, the aptness and feasibility of utilizing digital holography-based approaches in resource-limited POC is questionable and warrants further investigation.

3.3 LUCAS

A wide FOV imaging platform would be an alternative to optical microscopes for detection and counting applications, where high resolution is not critical. Therefore, a "lensless, ultra wide-field cell monitoring array platform – LUCAS" has been developed that can be used to detect and count cells on-chip with a two orders of magnitude wider FOV than conventional light microscopes (Figs. 4a and b) [13, 15]. This system has been shown to detect and count thousands of individual cells in real time in seconds. LUCAS system is based on recording the "shadow images" of microscale objects onto an optoelectronic sensor array plane. Microscopic objects (e.g., cells) are placed between two microscope glass slides (in a microfluidic device) and are uniformly illuminated with an incoherent white-light source or a laser beam (Fig. 4a). The cell shadow pattern is digitally recorded using a CCD sensor array. A CMOS chip, depending on the application type, can also be used as the optoelectronic sensor array. While the former is preferred for applications where image signal-to-noise (S/N) ratio is important, the latter is the sensor of choice for high-speed image acquisition. Each cell's shadow falling onto the sensor array is recorded as object-specific "signatures". The cell types, assigned with specific signatures, can easily be differentiated and counted (Fig. 4c). The distance between the active region of the sensor array (i.e., the active surface of the CCD chip) and the microscopic object location (e.g., cells) is important for optimizing the LUCAS operation. The shadow diameter is estimated from diffracted light intensity at the sensor plane. Final-

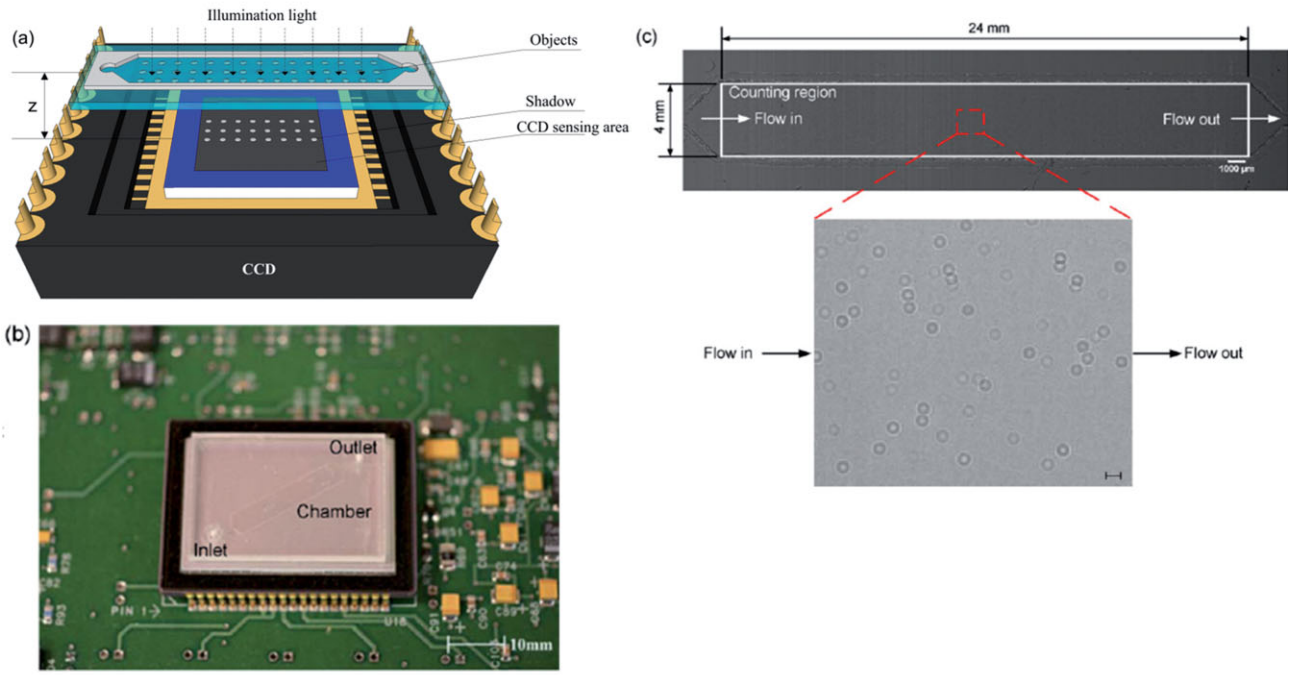

Figure 4. Integration of microfluidics with lensless ultra wide-field cell monitoring array platform (LUCAS). (a) Schematic representation of a microfluidic channel placed on a CCD sensor array. When light is incident on the sample (e.g., cells), the transmitted light is diffracted and the shadows of the samples are captured by the CCD sensor in less than 1 s. (b) Picture of the microfluidic chip placed on a CCD imaging platform. Field of view of the CCD sensor is 35 mm × 25 mm and, therefore, the entire microfluidic device can be imaged without the need for alignment of positioning by simply placing the microfluidic channel on the sensor. (c) Shadows of the captured $CD4^+$ T lymphocytes captured with the lensless CCD imaging platform. Scale bar is 100 μm (Based on [13]).

ly, individual cells are computationally modeled as uniform circular objects with a reduced field-transmission coefficient. This technology has recently been modularized with microfluidic devices, paving the way for lensless cell counting technologies for POC application. A recent study has also demonstrated the potential of this lensless technology for microfluidic-based HIV monitoring applications [13].

4 Conclusions and perspectives

The needs and challenges in resource-poor POC diagnosis are distinct from the needs and challenges of conventional diagnostics that can be performed at well-equipped settings by well-trained personnel (Table 1). The essential characteristics of resource-limited POC detection and diagnostic platforms are: low cost, functionality, portability, robustness, simplicity, flexibility, ease of use, and sensitivity. Recent advances in on-chip approaches have enabled and facilitated the integration of optics and microfluidics technologies to create new and more effective platforms (Table 2) that are needed at the POC in developing countries. The lensless imaging technologies are best suited for deployment to areas with limited resources. These platforms are promising since they enable rapid detection and counting of thousands of cells of interest. They have already been utilized for rapid $CD4^+$ tests to monitor HIV [13]. The technology has so far been used for HIV diagnostics as this is one of the greater problems of the world. Such technologies combined with surface chemistry can also be used for multiple other diseases including capture of circulating tumor cells [106], detection of other infectious diseases, such as sepsis [107] and bacterial pathogens including *E. coli* [34]. The advances and approaches discussed in this review may help improve healthcare in developing countries by allowing healthcare personnel to rapidly diagnose and disseminate information and, therefore, prevent the spread of communicable diseases. These technologies will potentially shape the future of clinical medicine and provide solutions for global health problems with potential positive influence on the developed world healthcare systems.

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