Recent development of portable imaging platforms for cell-based assays

Ke Yang[a,b,1], Jiandong Wu[b], Susy Santos[c], Yong Liu[a], Ling Zhu[a,1], Francis Lin[b,d,e,f,1,*]

[a] Institute of Applied Technology, Hefei Institutes of Physical Science, Chinese Academy of Sciences, Hefei, Anhui, PR China
[b] Department of Physics and Astronomy, University of Manitoba, Winnipeg, MB, Canada
[c] Victoria General Hospital and River Heights/Fort Garry Community areas, Winnipeg, MB, Canada
[d] Department of Biosystems Engineering, University of Manitoba, Winnipeg, MB, Canada
[e] Department of Immunology, University of Manitoba, Winnipeg, MB, Canada
[f] Department of Biological Sciences, University of Manitoba, Winnipeg, MB, Canada

ABSTRACT

Cell-based assays including both cell identification assays and cell functional assays are broadly used in life science research and for medical applications. Traditionally, cell-based assays are performed in professional laboratories. Although such an approach is reliable, it significantly limits the accessibility of cell-based assays by many researchers without specialized cell imaging facilities and does not permit on-site use at most clinics. Over recent years, growing efforts have been made to overcome this limitation by developing various portable cell imaging platforms. Herein, we provide a timely review on the recent development of portable imaging systems and their biomedical applications. We describe the respective applications of three main classes of portable imaging technologies including miniaturized microscope, lens-free imaging device and smartphone-based microscope for cell-based assays. We conclude by discussing our views on the opportunities, problems and future directions of this emerging field.

1. Introduction

Biological cells with their rich functions and properties are important players in various physiological and pathological processes (Hartwell et al., 1999). Various cell-based assays have been developed and are widely used in life science research and for medical applications (Kim et al., 2007; Yang et al., 2016a). In general, cell-based assays can be classified into two groups: cell identification assays and cell functional assays. Specific cell identification and counting is an important method for biomedical diagnosis (Holmes and Morgan, 2010; Katz et al., 2014). For example, circulating tumor cells (CTC) detection in human whole blood allows early cancer diagnosis (Fatih et al., 2015; Kang et al., 2017). Similarly, CD4 + T cell count is widely used for assessing the immune function of HIV-infected patients (Boyle et al., 2012; Glynn et al., 2013). Advanced cell functional assays are often done to qualitatively or quantitatively measure the presence or level of functional phenotypes of live cells (e.g. cell migration, cell growth, cell

morphology) for disease assessment (Albini and Benelli, 2007; Hong and Zu, 2013; Liang et al., 2007; Lipinski, 2011). To name a few examples: cell migration assays were employed for neutrophil migration measurement to evaluate asthma (Sackmann et al., 2014), sepsis (Hoang et al., 2013) and chronic obstructive pulmonary disease (COPD) (Wu et al., 2015); morphology analysis of red blood cells (RBC) was used for sickle cell anemia diagnosis (Finch et al., 1973); bacterial culture and testing are commonly applied for studying drug responses and diagnosis of infection (Lagier et al., 2015).

Many cell-based assays require specialized optical microscope systems, which are only available in research labs or centralized clinical labs. Portable imaging platforms are therefore desirable for cell-based point-of-care (PoC) biomedical applications. In this direction, new developments in optoelectronics such as micro-optics, solid-state lasers and advanced optical fibers offered the enabling technologies for cost-effective and miniaturized versatile imaging devices (Isa Navruz et al., 2013; Mudanyali, 2010; Sanz et al., 2017). In general, portable imaging

Fig. 1. Illustration of different portable imaging platforms for cell identification assays and cell functional assays. Portable imaging platforms was grouped into three categories: 1) miniaturized microscope, 2) lens-free microscope and 3) smartphone microscope. Novel cell-based assays that integrated traditional culture dishes and microfluidic devices with these portable imaging systems have been developed to enable cell identification or functional assay.

platforms can be grouped into three categories (Fig. 1): 1) miniaturized light microscopes (Table 1) (Balsam et al., 2014; Lynch et al., 2014; Sang et al., 2012); 2) lens-free imaging devices (Table 2) (Coskun et al., 2010; Isikman et al., 2013; Khademhosseinieh et al., 2010; Ozcan and Mcleod, 2016); and 3) smartphone-based imaging systems (Table 3) (Liu et al., 2014b; Skandarajah et al., 2014; Steinhubl et al., 2015; Yang et al., 2016b; Zhu et al., 2013). Miniaturized light microscopes provide reasonable image resolution and magnification and thus are suitable for imaging. Lens-free devices are advantageous for imaging large cell samples at good resolution. Smartphone-based imaging systems enable integrated imaging, data processing and communication capabilities in a universally accepted communication device. These innovative imaging systems require compatible cell assays to enable imaging-based cell identification or functional measurements. As such, novel cell-

based assays that integrated microfluidic devices and portable imaging systems have been increasingly developed (Fig. 1) (Ke et al., 2017; Yang et al., 2016b).

In the rest of this article, we will review the specific applications of portable imaging platforms for the two main classes of cell assays. For cell identification, we will review different assays such as cell counting and cell size detection for diagnosis applications like HIV, cancer, anemia. For cell functional tests, we will review different assays such as cell migration, cell viability and cell culture. We will conclude by discussing our views on the opportunities, problems and future directions of this emerging field.

2. Miniaturized light microscopes for cell-based assays

Traditional light microscopes are invaluable tools for cell biology research and cell-based biomedical applications (Huang et al., 1991; Sharpe et al., 2002; Stephens and Allan, 2003), but are typically limited in specialized research labs due to their large size, high cost and operation skill requirements. Thus, one effective approach to enable portable imaging for cell-based assays is by inheriting the principle of traditional light microscope but assembling it using cost-effective and miniaturized optical and electronic components to achieve cell level imaging resolution.

2.1. Miniaturized light microscope for cell identification assays

Balsam et al. (2014) reported a low-cost flow cytometer by integrating a microfluidic device and webcam imaging to enable continuous cell count (Fig. 2A). This portable flow cytometer was configured with three main components: a commercial webcam coupled with an objective lens for cell imaging; a laser module for cell excitation; and a microfluidic device with a 4 mm wide flow channel for cell sample testing. Cells were pre-loaded in the syringe and injected through the 4 mm wide flow. Flow videos were captured using the webcam at 187 frames s^{-1}. This method was effective to recognize individual cells at a maximal concentration of 100 cells mL^{-1} with a 95% confidence interval, but failed at higher cell densities due to significant overlapping of cells. This webcam-based flow cytometer was successfully validated by counting THP-1 human monocytes labeled by SYTO-9 dye.

2.2. Miniaturized microscopes for cell functional assays

Kim et al. (2012b) reported a mini-microscope using a complementary metal oxide semiconductor (CMOS) imaging module for imaging cell motion. Plastic lens with a 4 mm and 18 mm housing

Table 1
Summary of miniaturized microscope for cell-based assays.

Classification	Using microfluidic	Cell type	Application	Ref.
Miniaturized microscope	Yes	THP-1 human monocytes	Detect fluorescently tagged cells in the micro channel.	(Balsam et al., 2014)
	Yes	3T3 fibroblast cells	Monitor 3T3 fibroblasts migration in three different concentrations of fetal bovine serum.	(Kim et al., 2012b)
	Yes	HepG2 cell	Monitor cell migration and analyze beating of microfluidic liver and cardiac bioreactors.	(Zhang et al., 2015)
		NIH/3T3 fibroblasts		
		Liver cell		
		Cardiac cell		
	No	MCF-7	Perform 12 h time-lapse study on paclitaxel-treated MCF-7 and HEK293T cell lines.	(Jin et al., 2015)
		HEK293T		
	Yes	Neutrophil	Find neutrophil strong chemotax to IL-8 gradient.	(Wu et al., 2014)
	No	Biomphalaria(PLL)	Perform multiple simultaneous time-lapse studies on various cell types.	(Lynch et al., 2014)
		Biomphalaria(Spread)		
		MDA-MB-231		
		SC5 mouse Sertoli		
	No	HEK293 cells	Long-term imaging of recombinant HEK293 cells at varying culture conditions.	(Walzik et al., 2015)

Table 2
Summary of lens-free microscope for cell-based assay.

Classification		Using microfluidic	Cell type	Application	Ref.
Lens-free microscope	Digital holography microscope (DHM)	No	Bacterial	Detect and track prokaryotes.	(Serabyn et al., 2016)
		No	Swine sperm cells	live swine sperm cells and flowing microbeads samples analysis.	(Sanz et al., 2017)
	Partially coherent holography microscope (PCHM)	No	Red blood cells	Provide automated cell and particle size measurement.	(Roy et al., 2015)
			MCF-7, HepG2, HeLa		
		No	NIH 3T3 cells	Track NIH 3T3 cells on polyacrylamide gels over 20 h.	(Pushkarsky et al., 2014)
		No	Human Mesenchymal Stem Cells	Determine cell division orientation of Human Mesenchymal Stem Cells.	(Kesavan et al., 2014)
			Human fibroblast	Observe the effect of transfection of siCellDeath on hMSCs and human Osteo Sarcoma Cells.	
			Human Osteo Sarcoma		
	Incoherent contact imaging microscope (ICIM)	No	Cardiomyocyte	Measure the beating rate and beat-to-beat variations of cardiomyocytes.	(Kim et al., 2011)
		No	Embryonic stem cell	Track embryonic stem cell differentiations over the entire sensor surface.	(Zheng et al., 2011)
		Yes	Euglena gracilis	Automatic cell counting, motion analysis and shape analysis.	(Lee et al., 2012)
		Yes	HeLa cells	Analyze the cells' dynamic response to an anticancer drug, camptothecin.	(Han et al., 2013)
		No	Bacterial	Real-time bacterial microcolony counting.	(Jung and Lee, 2016)
		Yes	Hela cells	Monitor the response of Hela cells to endothelial growth factor by contact fluorescence imaging.	(Takehara et al., 2017)

Table 3
Summary of smartphone microscope for cell-based assay.

Classification		Using microfluidic	Cell type	Application	Ref.
Smartphone microscope	General cell identification and counting	Yes	White blood cells	Measure the density of white blood cells in human blood samples.	(Zhu et al., 2011)
		Yes	Yeast cell	Allow rapid assessment of yeast viability and concentration.	(Jagannadh et al., 2015)
		No	White blood cells, Red blood cells	Count blood cells.	(Zhu et al., 2013)
		Yes	B. anthracis Sterne spores	Detect 50–5000 B. anthracis Sterne spores in 3–5 h.	(Hutchison et al., 2015)
	Cell-based medical diagnosis	No	S. aureus cells	Quantitatively detect and count individual bacteria cells.	(Shrivastava et al., 2018)
		Yes	CD4 + T cell	Automated CD4 testing using a small volume (30 μL) of whole blood.	(Kanakasabapathy et al., 2017a)
		No	Cervical cancer cell, Breast cancer cell, Lung cancer cell, Varian cancer cell, Prostate cancer cells	Screen for precancerous or cancerous cells in cervical specimens.	(Im et al., 2015)
		Yes	Sperm cell	This device could ultimately be applied to rare cell separation and purification.	(Amin et al., 2017)
				Analyze an unwashed, unprocessed liquefied semen sample within < 5 s with ~98% accuracy.	(Kanakasabapathy et al., 2017b)
	Cell functional assay	No	Sickle cell	Distinguish the levitation patterns of sickle versus control red blood cell based on degree of confinement.	(Knowlton et al., 2015)
		No	HepG2 cells	Detect the effect of okadaic acid on the HepG2 cells' viability.	(Su et al., 2017)
		Yes	Neutrophil	Provide an easy and integrated experimental platform for cell migration related research.	(Ke et al., 2017)
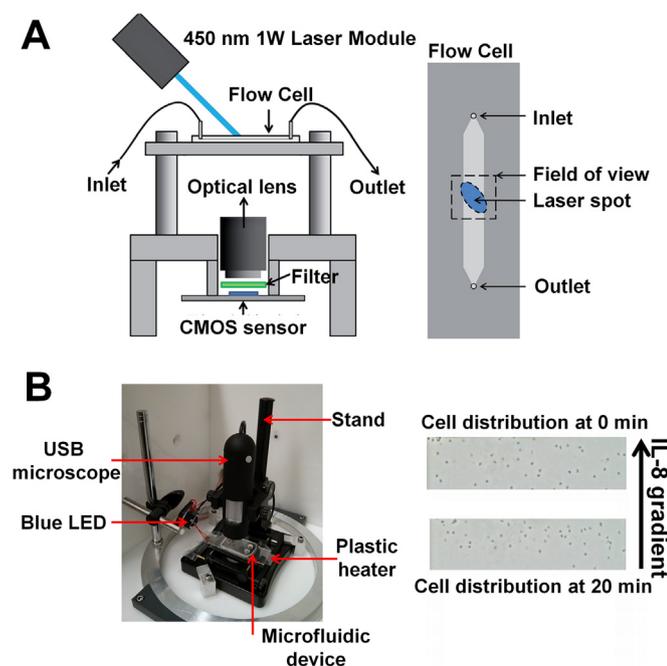
Fig. 2. Examples of miniaturized light microscope for cell-based assays.
(A) Illustration of a miniaturized microscope with an integrated microfluidic device as a wide-field flow cytometer for cell counting. The sensing element consisted of a webcam, a lens, a filter, and a computer for data collecting and analysis. The sample handling module consisted of a microfluidic device and a syringe pump. (B) Illustration of a commercial USB-based microscope combined with a microfluidic device, an environmental control module, and data analysis functions for rapid neutrophil migration and chemotaxis experiments. The system was validated by testing neutrophil chemotaxis to an IL-8 gradient. Images were adapted from ref. (Balsam et al., 2014) and ref. (Wu et al., 2014) with permissions from Royal Society of Chemistry and Springer Publishing.

length were directly aligned with the CMOS sensor of the mini-microscope to achieve 10 × and 40 × magnification, respectively. Then, the mini-microscope can provide the field-of-view (FOV) of 1.52 mm × 1.13 mm, and 0.21 mm × 0.16 mm, respectively. The resolution was about 1.4 μm. The mini microscope was used to image mouse 3T3 fibroblast migration in the scratch assay and the stem cell aggregation. Zhang et al. (2015) further developed a similar system with added fluorescence imaging capability. This mini-microscope can adjust the magnifications from 8 × to 20 ×, 40 ×, 60 ×, and provide a resolution of 2 μm and imaging rate of 30 frames s^{-1}. Fluorescence detection usually requires effective filtering of the excitation light. In this study, an innovative digital separation method was used to effectively split the red, green, and blue channels from the original image without requiring filtering. This upgraded miniaturized microscope was effectively used to monitor the migration of liver cells and beating of heart cells, and to measure the viability of hepatocyte labeled by calcein/ethidium homodimer-1 before and after acetaminophen treatment.

Jin et al. (2015) reported a mini wireless microscope containing a camera and a series of optical lens for cell culture assay. This low-cost platform included a light emitting diode (LED) for illumination, a group of excitation and emission filters for fluorescence detection, and a camera coupled with an achromatic lens for cell image capturing. The camera was configured with the Wi-Fi function for wireless image transmission so that the whole microscope could be placed in an incubator for long time assays. In addition, the camera contained a set of zooming lenses for FOV adjustment. A maximal 113 mm^2 FOV and 3 μm resolution can be achieved by combining compressive imaging sensing technology. The compression sensing calculation was achieved by combining 6 × interpolation of the original image with an iterative interior point least squares solver. This mini wireless microscope was

validated by evaluating the dynamic response of MCF-7 and HEK293T cell lines to paclitaxel treatment over 12 h.

Many studies have demonstrated the feasibility of using portable imaging platforms for cell functional assays while they often require some common lab facilities such as cell culture incubators. We previously reported a USB microscope-based system with integrated microfluidic device and temperature control module for cell migration test (Fig. 2B) (Wu et al., 2014). Image acquisition and analysis with the USB microscope was operated by a MATLAB program that applied a Gaussian filtering and threshold algorithm to smooth the raw image and extract cells from the background. This USB microscope-based cell migration assay was validated by evaluating the migration of neutrophils in an interleukin-8 gradient or a COPD sputum supernatant gradient at 37 °C. Afterwards, Lynch et al. (2014) further reported a similar system but with three independent USB microscopes to image and track cell migration at higher throughput. The USB microscopes had a maximum magnification of 413.6 × and the spatial resolution was around 10 μm. Although these USB microscope-based imaging platforms allow cell-based assays with temperature control, they are still lacking other required environmental controls such as CO_2 and humidity for long-time cell culture and imaging. Walzik et al. (2015) reported a mini optical microscope that was configured with three environmental control sensors for temperature, gas and humidity, thus allowing long-time cell culture assays. The mini optical microscope used a 2 megapixel CMOS sensor with a resolution of 1.75 μm. A programmable motorized stage was coupled to the mini optical microscope for multi-position imaging. These images of different positions were stitched together to generate a large FOV image. This novel platform was demonstrated by imaging the growth of HEK293 cells under various culture conditions. Average growth areas recorded using the mini optical microscope were in agreement with the measurements using the conventional method (R^2 = 0.99 ± 0.22). Despite the attractive features of this system, the requirement of a motorized stage adds the complexity. Furthermore, image mosaic is essential to assemble multiple small images to a lager FOV. Thus, any failed image acquisition (e.g. mis-alignment or missing frames) would result in the redeployment of the entire experiment (Anon, 2018b).
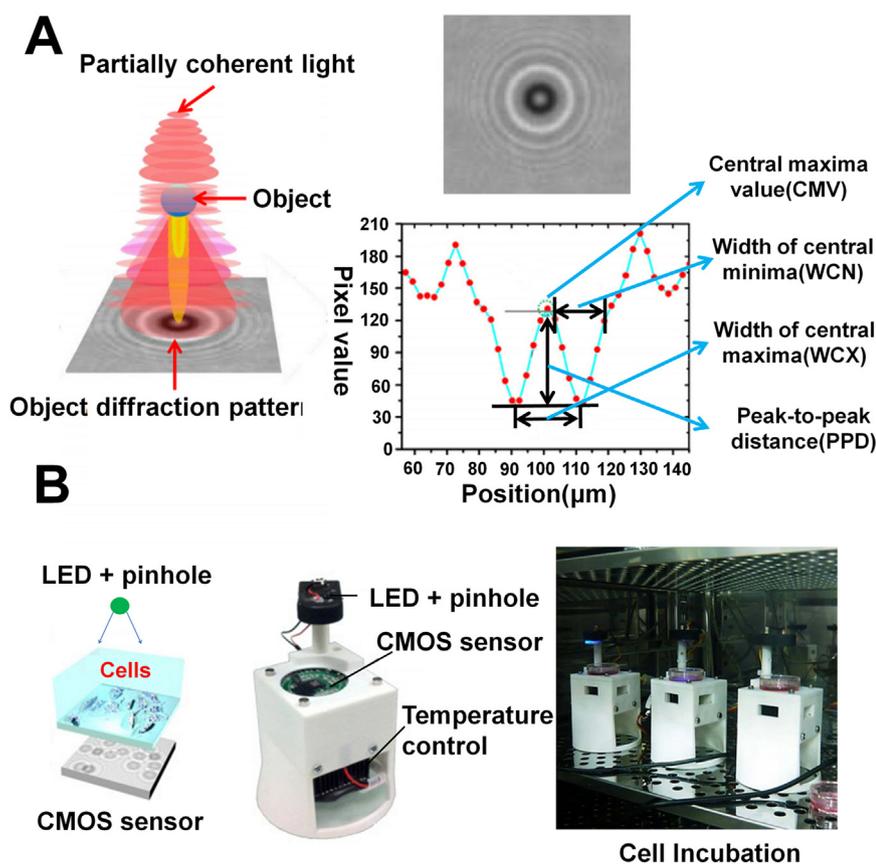
3. Lens-free imaging systems for cell-based assays

In general, lens-free microscopes do not require optical lenses and thus bypass the contradicting features between FOV and resolution as in a traditional compound microscope, which is beneficial for imaging larger samples such as cell cluster motility. We in this review described three lens-free imaging platforms and highlighted their biological applications (Kim et al., 2012a; Ozcan and Mcleod, 2016).

3.1. Digital holography microscope (DHM) for cell functional assays

A DHM applies a laser and a micron-sized pinhole to create coherent light. The sample is located between the light source and the CMOS plane, but closer to the light source for illumination and feature recognition (Kreuzer and Manfred, 2006; Micã[3] and Zalevsky, 2010; Serabyn et al., 2016). This way, spherical aberration is avoided without using any optical lenses and can achieve high image resolution (Gabor, 1948).

Sanz et al. (2017) reported the use of DHM for cell migration imaging with comparable resolution to a conventional compound microscope. The DHM used three coherent light beams to illuminate the sample simultaneously and recorded multiplex hologram using a color camera at the video frame rate. Then, a fast convergence algorithm was created for image post-processing. This DHM was also capable of recording 3D trajectories of the moving objects enabled by its large depth of field. This platform was successfully validated by evaluating the swimming behavior of artificial spheres, algae, bacteria and other prokaryotes.

Fig. 3. Examples of partially coherent holography microscope (PCHM) for cell-based assays. (A) Illustration of a compact and low-cost telemedicine device providing automated cell and particle size measurement based on lens-free shadow imaging technology. The left image is the object diffraction pattern; the upper-right image is the diffraction pattern of a 20 μm bead; the lower-right image is the pixel value profile and definitions of the diffraction parameters of a diffraction pattern. (B) Illustration of placing several digital in-line holography microscopes in an incubator for various cell functional assays. This microscope consisted of a LED, a pinhole and a 24 mm² CMOS sensor. Image adapted from ref. (Roy et al., 2015) and ref. (Kesavan et al., 2014) with permissions from Elsevier and Nature Publishing.

3.2. Partially coherent holography microscope (PCHM) for cell functional assays

PCHM can provide large FOV, but resolution is limited due to a lack of time and space consistency. However, the user can fine-tune the spatial and temporal coherence of the light beam by selectively adding an incoherent light source and a 0.5–1 mm pinhole. Then, holographic information of cells can be recorded and digitally reconstructed. In PCHM, the objects are placed far from the light source but closer to the CMOS plane (Seo et al., 2009).

Roy et al. (2015) reported a PCHM for measuring the cell size (Fig. 3A). The FOV of the PCHM was 23.52 mm², which was 25 times larger than that of 100 × desktop optical microscope. In addition, the PCHM had a maximum resolution of 2 μm. Using the fact that the pixel distance between peaks and troughs of a cell's shadow pattern were linearly proportional to the cell size, an image processing algorithm was developed and the PCHM was successfully validated by automated size evaluation of various biological cells such as RBC, MCF-7 cells, HepG2 cells and HeLa cells. The results were compared with it measured by traditional systems such as Coulter counter, flow cytometer and particle size analyzer. In another study, an automatically cell tracking algorithm was developed to grab and save the global coordinates of each detected cell in a cropped image. Using this approach, Pushkarsky et al. (2014) reported a PCHM, which enabled NIH 3T3 cell migration monitoring inside an CO₂ incubator. In cell tracking analysis, more than 120 cells were detected in a wide FOV (24 mm²), which was about 10 times larger than a 10 × objective lens. To perform long-time cell culture monitoring at high-throughput, multiple PCHM can be placed in an incubator. Using this method, Kesavan et al. (2014) reported a PCHM for various cell functional analysis such as cell adhesion, cell spreading, cell division and cell death (Fig. 3B). The lens-free microscope consisted of a 5.7 mm × 4.3 mm CMOS sensor providing a resolution of 2.2 μm. The FOV was 24 mm², which can image 3700 cells. Floating and adherent cells were automatically identified from the raw image. Specifically, floating cells were detected by using normalized cross correlation function, and adherent cells were identified by applying a constant threshold algorithm based on the grayscale pixel value and area of the cell. The effective use of the PCHM was demonstrated by monitoring the Brownian motion and proliferation of human mesenchymal stem cells (hMSCs) and human fibroblasts, as well as the viability of hMSCs and human osteosarcoma (U2OS) cells after transfected with siCellDeath.

3.3. Incoherent contact imaging microscope (ICIM) for cell functional assays

ICIM has the advantage of not requiring coherent light source and other advanced optical parts. In the ICIM system, objects are placed directly on or near a CMOS sensor, and an incoherent light source is used for illumination. The CMOS sensor can record diffraction or shadow patterns of cells.

Kim et al. (2011) reported an ICIM for real-time monitoring of beating behavior of the cardiomyocyte. The ICIM was validated by recording the changes of the beating and beat-by-beat rate of cardiomyocytes treatment by doxorubicin and isoproterenol. Zheng et al. (2011) reported a ICIM for monitoring cell culture and tracking cell growth. The ICIM was demonstrated to image samples in a 6 mm × 4 mm area with a 660 nm resolution. Raster scanning and digital reconstruction algorithm were coupled to the ICIM for capturing multiple low-resolution shadow images and creating high-resolution images (Wu et al., 2010). This platform was used to observe cell motility and morphology during embryonic stem cell differentiation under treatment of anti-cancer drugs. Later, Lee et al. (2012) developed a novel ICIM and combined the pattern recognition algorithm to analyze the motion and morphology of *Euglena gracilis* (Fig. 4A). Various cell parameters such as cell count, size, shape, and migration speed can be obtained
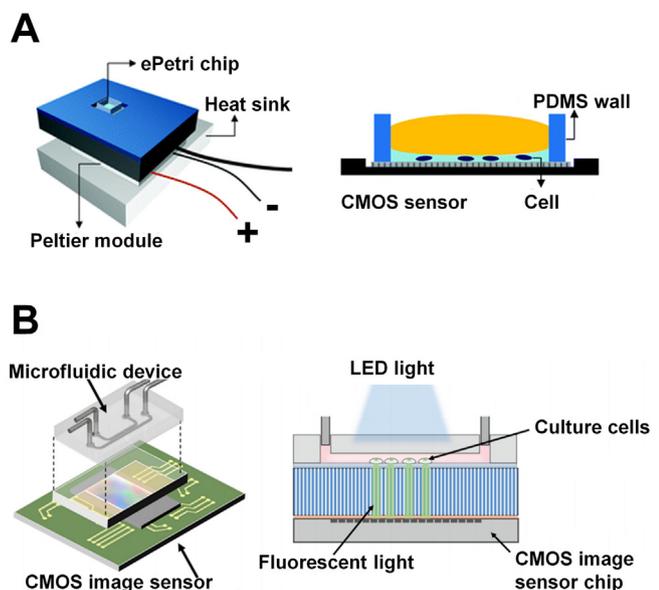
Fig. 4. Examples of incoherent contact imaging microscope for cell-based assays. (A) Illustration of the basic compositions of a single ePetri dish chip. Sample was dispensed into a chip and a drop of oil was used to prevent evaporation of the medium. A Peltier module and a heat sink were used to maintain the temperature of the chip. (B) Schematic diagrams of an on-chip fluorescence imaging platform for cell viability analysis. Cells were cultured in the microfluidic device and illuminated by cost-effective LEDs. The fluorescence images were obtained using the CMOS sensor. Image adapted from ref. (Lee et al., 2012) and ref. (Takehara et al., 2017) with permissions from Royal Society of Chemistry and AIP Advances Publishing.

from more than 200 cells in one test. Furthermore, Han et al. (2013) incorporated fluorescence imaging unit to the ICIM platform for more advanced cell monitoring of HeLa cells. The ICIM can image at a maximum rate of 1 frame per 115 s, a FOV of 13 mm^2 and a resolution of 1.2 μm. Similar system were also applied to real-time monitoring and counting of viral plaque and bacterial (Han and Yang, 2014; Jung and Lee, 2016). By incorporating a fluorescent ICIM, Takehara et al. (2017) developed a cell viability analysis system (Fig. 4B). As a proof-of-concept, this system was demonstrated by testing the effect of endothelial growth factor on the viability of HeLa cells.
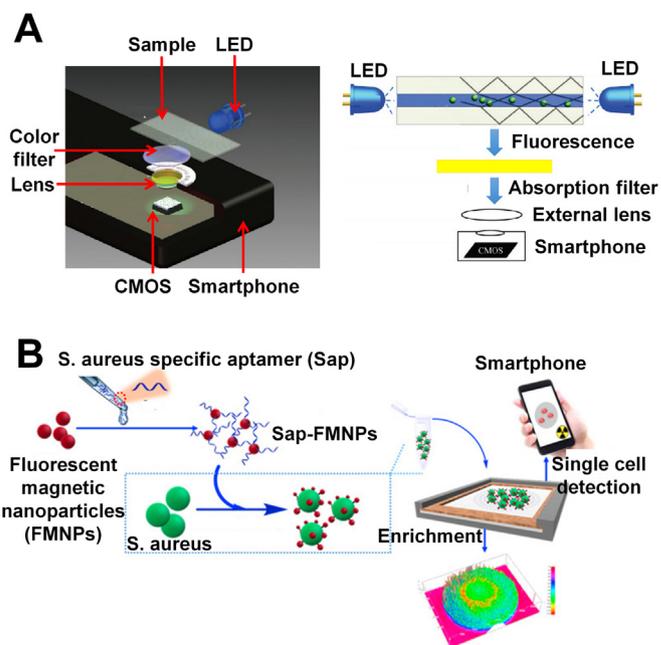
4. Smartphone microscope for cell-based assays

In recent years, significant advancement of smartphone technologies enabled a wide range of smartphone-based imaging application. In particular, smartphone-based imaging systems were used for various cell-based disease diagnosis assays (Liu et al., 2014a; Skandarajah et al., 2014). Various smartphone-based mini microscope (Meng et al., 2016; Navruz et al., 2013), lens-free microscope (Tseng et al., 2010), and contact imaging system (Lee and Yang, 2014; Navruz et al., 2013) were developed for cell identification and functional testing. Importantly, the common features of smartphones and microfluidic devices in miniaturization, ease-of-use and portability have facilitated their integration to harness the potential of mobile sensing applications (Erickson et al., 2014; Hu et al., 2016; Yang et al., 2016b).

4.1. Smartphone microscope for cell identification and counting

4.1.1. WBC, RBC identification and counting

White blood cells (WBC) and RBC counting are most common health indicators in routine blood analysis, which provide a wealth of information for health assessment and diseases diagnosis (Chuang and Hu, 2004). Thus, cost-effective, compact and accurate blood cell analyzers are highly desirable. Zhu et al. (2011) reported a smartphone

Fig. 5. Examples of smartphone microscope for blood cell and bacteria identification and counting. (A) Illustration of a smartphone-based optofluidic fluorescent microscope for blood cell counting application. Each microfluidic device was illuminated from the side by LEDs. The fluorescent images of the flowing cells can be captured using the smartphone camera. B) Schematic representation of bacterial capture and quantitative detection from minimally processed samples by using a smartphone microscope. *S. aureus* specific aptamer (Sap) was covalently attached to fluorescent magnetic nanoparticles (FMNPs). The target bacteria were labeled by Sap-FMNPs and loaded into the bacterial detection cassette for further smartphone imaging and quantification. Image adapted from ref. (Zhu et al., 2011) and ref. (Shrivastava et al., 2018) with permissions from American Chemical Society and Elsevier Publishing.

microscope with integrated microfluidic device for cell counting (Fig. 5A). Fluorescently labeled cells were injected into the inlet of the microfluidic device using an additional pump at a flow rate of 1 μL min^{-1}. Then, two blue LEDs were aligned with the sidewalls of the microfluidic channel to excite the fluorescence labeling and an imaging processing algorithm was developed. Smartphone microscope can record the flow videos at a resolution of 2 μm and a rate of 7 frames s^{-1}. This system was successfully validated by measuring the density of WBC in human blood. The test results were in agreement with the measurements by a commercial hematology analyzer (Sysmex KX-21N) and showed < 5% error. Later, Zhu et al. (2013) further reported a multi-purpose smartphone microscope for measuring WBC and RBC density as well as hemoglobin with low volume human blood samples. The captured images were rapidly processed by the smartphone using a custom developed app to determine cell density or hemoglobin within 10 s. The test results can be either stored on the smartphone or wirelessly sent to a remote server for further analysis. The test results showed a 7% leukocyte counting error, a 5% erythrocyte counting error, and a 5% hemoglobin concentration measurement error, respectively, yielding comparable results with a standard commercial Sysmex KN21 hematology analyzer. Although not for WBC or RBC counting, Jagannadh et al. (2015) reported a similar system with an integrated six-channel microfludic device to evaluate yeast cell viability at higher throughput.

4.1.2. Bacteria identification and counting

Specific bacterial identification and counting provide critical diagnostic information for various diseases especially infectious diseases. Traditional bacteria detection methods are time-consuming as they rely on centralized lab facilities. Smartphone-based imaging platforms

provide useful tools for rapid bacteria detection thereby accelerating disease diagnosis and treatment plan. Hutchison et al. (2015) reported a smartphone microscope coupled with a microfluidic device to monitor the entire growth process of bacillus anthracis spores. Later, Shrivastava et al. (2018) reported another smartphone microscope for capturing and quantifying fluorescently labeled bacterial colonies (Fig. 5B). In this system, the smartphone microscope was configured with a white LED, a 10 × objective, a cassette and a dichroic mirror to excite the fluorescently labeled bacteria. The smartphone microscope had a FOV of 1.76 mm^2 and can resolve 1 μm beads. A mini adjustable stage was coupled to the smartphone microscope for focusing. However, the images were still analyzed using ImageJ in an external PC. The smartphone system was demonstrated by imaging and counting *S. aureus* cells over a concentration range of 50–2000 cfu mL^{-1}, and the results were statistically comparable with the conventional plate counting method.

4.1.3. CD4 + T cell identification and counting

On a global scale, HIV caused the death of more than 35 million people and infection of another 36.9 million people, thus resulting in a high healthcare burden, especially in the developing countries and regions (Anon, 2018a). HIV infected patients have the significantly lower CD4 + T cell count in the blood compared to healthy subjects. Thus, blood CD4 + T cell count provides a useful measure of HIV infection. Smartphone imaging systems offered the enabling tools for rapid and easy CD4 + T cell count-based HIV diagnosis, particularly in medical resource limited areas (Shafiee et al., 2015). Kanakasabapathy et al. (2017a) reported a smartphone microscope with an integrated microfluidic device for automated CD4 + T cells counting with minute volume of whole blood (Fig. 6A). CD4 + cells were selectively captured from whole blood in cell identification and counting from the images acquired using the smartphone microscope was controlled by an open-source OpenCV database and thresholding algorithm in combination with other image processing algorithms to extract cells from the background. Using this smartphone microscope, CD4 + T cell count from whole blood was tested for HIV-positive patients and compared with healthy volunteers. The smartphone microscope was validated by testing the CD4 + T cells below the threshold of 200 cells μL^{-1}, and the sensitivity, specificity and accuracy were 100%, 91.30%, and 93.3%. At the threshold of 500 cells μL^{-1}, the sensitivity, specificity, and accuracy were 100%, 92.31% and 96.66%. The performance of this platform was comparable to the commercially available Alere PIMA™ test and Cy-Flow® miniPOC cytometer.

4.1.4. Cancer cell identification and counting

The global incidence of cancers has been consistently increasing over recent years and the WHO predicted the new cancer incidence to reach 19.3 million in 2025 (Cadet et al., 2016). Cancer can be better controlled by early diagnosis and evidence-based prevention strategies (Shah et al., 2016). Cancer cell detection is one of the most important methods for cancer diagnosis, prognosis and treatment decision (Chiyu et al., 2018; Rajesh et al., 2015). Smartphone-based imaging systems started to be applied for cancer cell detection. Im et al. (2015) reported a smartphone-based imaging systems for rapid cancer cell counting (Fig. 6B). The FOV and spatial resolution of the smartphone were 14 mm^2 and 2 μm, respectively. For a single image acquisition, more than 100,000 individual objects can be detected. In this study, cancer cells were labeled with specific detection microbeads and the labeled cells were placed on a glass slide for imaging. The diffraction images of cancer cells and microbeads were obtained by the smartphone camera. Image reconstruction was operated by a cloud server that applied the Rayleigh-Sommerfeld diffraction principle to identify and count the microbead labeled cells based on their own transmittance and phase (Kreis, 2002; Latychevskaia and Fink, 2007). The clinical applicability of the platform was validated by screening precancerous or cancerous cells in cervical specimens.
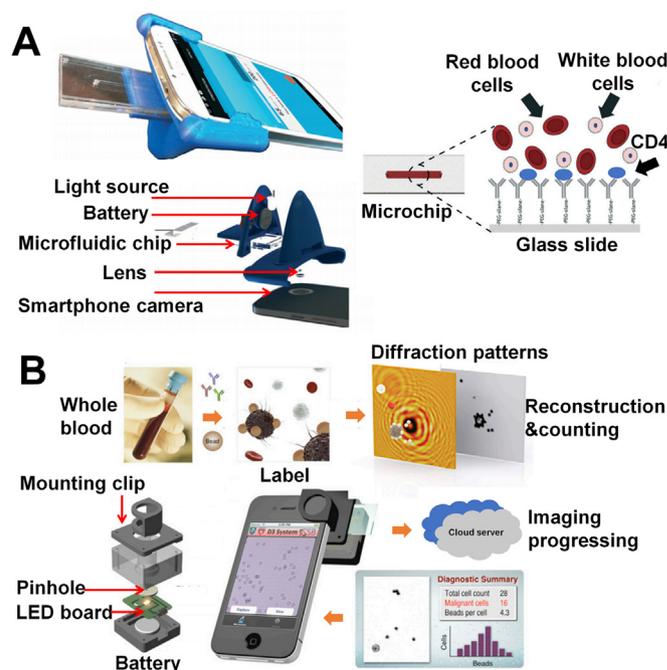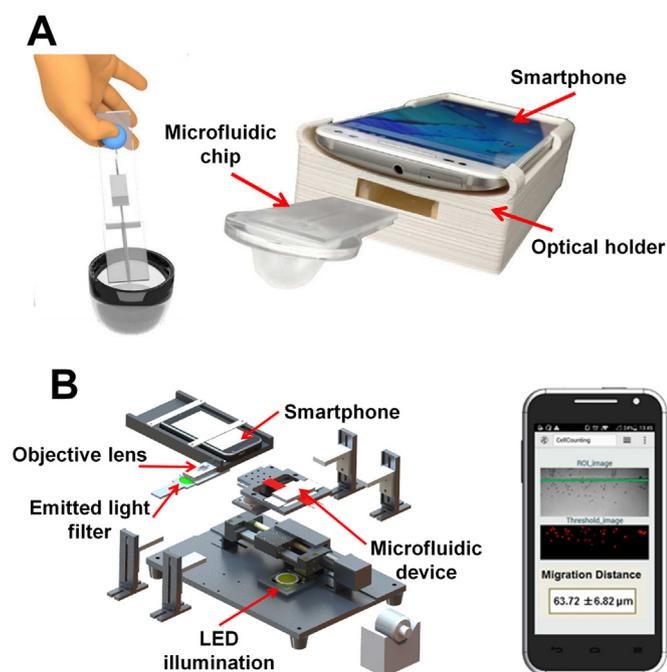

Fig. 6. Examples of smartphone microscope for HIV and cancer diagnosis. (A) Illustration of the smartphone-based microscope combined with a microfluidic device for CD4 + T cell counting. CD4 + T-cells were first isolated from whole blood and settled in the channel of the microfluidic device, and then analyzed using the smartphone application. (B) Illustration of a smartphone-based microscope for screening the precancerous or cancerous cells in cervical specimens. The system consists of a LED, a pinhole and a sample mount. Target cells in patient samples were labeled with microbeads, and the diffraction patterns were recorded by the smartphone camera and reconstructed via a remote cloud service. Image adapted from ref. (Kanakasabapathy et al., 2017a) and ref. (Im et al., 2015) with permissions from Royal Society of Chemistry and PNAS publishing.

Recently, Amin et al. (2017) reported the use of a smartphone microscope for real-time separation and quantification of cancer cells. The detection principle was based on the magnetic focusing, which has validated its useful for cell sorting and cytometry, including diagnosis of sickle cell disease, separation of lymphocytes, leukocytes, and RBC, and monitoring leukocyte activation (Tasoglu et al., 2015; Yenilmez et al., 2016a, 2016b). The smartphone microscope was configured with a square capillary and two magnets. The square capillary was placed between the two magnets, of which the same polar faces were placed oppositly. The smartphone camera was aligned with the microcapillary to obtain the cell images. The performance of the smartphone microscope was validated by demonstrating the separation of breast, lung, ovaria and prostate cancer cells mixed with blood cells. The separation distance of cancer and blood cells was around 100 μm, allowing the two cell types to be easily distinguished. In addition, Knowlton et al. (2015) verified the feasibility of the smartphone microscope for sickle cell detection.

4.2. Smartphone microscope for cell functional assays

In addition to cell identification and counting, smartphone-based imaging platforms have also been used for various cell functional assays. One of the application areas is to enable new infertility test. Previous studies showed that infertility affects 15–20% of couples, of which 50% are caused by the male (Agarwal et al., 2015). Thus, relevant sperm properties such as density, motility and viability can be measured using computer-aided sperm analysis (CASA) such as the FertilMARQ SpermCheck or the Trak Male Fertility Testing System, which provide useful information to determine infertility. However, the

Fig. 7. Examples of smartphone microscope for infertility diagnosis and cell migration assay. (A) Illustration of a smartphone microscope combined with a microfluidic device for measuring the concentration and motility of sperm cells. During detection, semen sample was loaded into a microfluidic device. The sperms motion videos were captured and analyzed by the smartphone. (B) Illustration of a smartphone microscope for on-site cell migration evaluation. The imaging platform consists of a U-shape holder, a white LED for illumination, and a transparent heater for temperature control. Image adapted from ref. (Kanakasabapathy et al., 2017b) and ref. (Ke et al., 2017) with permissions from Science and Elsevier Publishing.

current sperm tests are laborious, expensive, and require specialized lab facilities and skills. PoC sperm analysis systems are therefore highly desirable, and the smartphone-based imaging offers a promising enabling technology. Kanakasabapathy et al. (2017b) reported a smartphone microscope with an integrated disposable microfluidic device for measuring the density and motility of sperms (Fig. 7A). The smartphone microscope can record sample videos at a rate of 30 frames s^{-1} with a maximum effective FOV of 886 pixel × 886 pixel. The semen sample was loaded at the entrance of the microfluidic device, which was subsequently transported to the detection region by manually pressing the rubber ball integrated with the microfluidic device. Image analysis with the smartphone microscope was operated by a smartphone app that applied a self-adaptive filtering algorithm to extract the sperms from the background and map the trajectories of the sperms. Then various quantitative parameters such as density and velocity of sperm were calculated. The system was successfully used to test unprocessed patient semen samples with sperm concentrations between 0 and 408 million sperm mL^{-1} and had a 97.71% accuracy.

Su et al. (2017) reported a smartphone microscope to evaluate the effect of okadaic acid on the HepG2 cell line's viability. HepG2 cells were first cultured in a 96-well plate (MTP) for 4 h, followed by adding 10 μL cell counting kit-8 (CCK-8) to each well. The CCK-8 contains WST-8 (i.e. 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfonic acid) benzene-2 h-tetrazole monosodium salt), which can be reduced to a highly water-soluble yellow formazan dye by the dehydrogenase in the cell. The amount of formazan produced was proportional to the number of living cells, which can be measured using the smartphone microscope.

We previously reported a smartphone microscope integrated with a double-channel microfluidic device for cell migration test (Fig. 7B) (Ke et al., 2017). The maximal usable FOV of the smartphone microscope

was about 0.5 mm^2 and the resolution was around 2.76 μm. Image acquisition and processing was operated by a custom developed Android app that applied a filtering algorithm to smooth the raw image and extract cells from the background. Chemotaxis measurement was done by calculating the cell migration distance along the gradient direction without tedious single cell tracking analysis. This smartphone microscope was validated by evaluating the migration of human blood neutrophils and a breast cancer cell line in their chemoattractant gradients (i.e. N-formyl-met-leu-phe (fMLP) for neutrophils; epidermal growth factor for breast cancer cells). The smartphone microscope-based blood neutrophil chemotaxis test was further performed in a local clinic demonstrating its applicability for on-site clinical test.

5. Concluding remarks

Recent technological advances of portable imaging platforms as well as their applications for cell-based assays were highlighted in this review article. Among the three main classes of imaging platforms, the miniaturized microscope can be built with an integrated CMOS sensor and a small objective lens. It offers reasonable image resolution while the main limitation is its small detection FOV. By contrast, the lens-free microscope allows large FOV (e.g. up to 24 mm^2), which enables imaging many more cells and gaining rich biological information. The main drawback of lens-free microscope is its requirement of separate computing units due to time consuming and computationally intensive image reconstruction. Finally, the popularity and potential of the smartphone platform is supported by the globally increasing users of smartphones (i.e. The number of smartphone users was forecasted to grow from 2.1 billion in 2016 to around 2.5 billion in 2019 (Anon 2018c)). With CMOS cameras and fully integrated smartphone apps, smartphone microscope could achieve high image resolution and allows local image processing and data rendering. Similar to the miniaturized microscope, the main limitation of the current smartphone platform is the generally small FOV. A more detailed parallel comparison of the key technical parameters among these three classes of imaging platforms is summarized in Table 4.

The common attractive features of these imaging platforms in portability, low-cost and integrated functions promised their potential to be widely adopted for cell-based biological and biomedical applications in the future. However, challenges are to be addressed in order to fully realize such a potential, which point to the directions of future development of this emerging area.

On the technical side, the portability feature of these imaging platforms is comprised by their generally lower image quality (i.e. generally > 1 μm) compared to sophisticated microscope facilities. In addition, the image quality also significantly varies among different portable imaging platforms (Table 4). Thus, efforts to further improve the imaging performance of these portable platforms are critically needed for their wider adoption by the research community. As such, rapid development of electronic technology has the potential to enable higher pixel density, faster sampling speed and higher quality of the imaging units thereby enhancing the overall image quality of the portable systems. For example, pixel super-resolution has been successfully combined with lens-free microscope to obtain half-pitch resolution as fine as 225 nm on commercially available CMOS sensors with a pixel size of 1.12 μm (Greenbaum et al., 2013; McLeod et al., 2013).

In addition, most portable imaging platforms still rely on cell culture facilities for various cell-based assays. While these facilities are commonly available in many research labs, it hinders the use of the portable systems in resource-limited areas and for clinical applications. Integrated cell preparation and environmental control functions are needed for the portable system to meet the standalone assay requirement. Although some integrated systems have been demonstrated, more sophisticated yet cost-effective components are highly desirable. In this direction, miniaturized heating units and control modules and advanced microfluidic devices are expected to provide promising

Table 4
Comparison of the three main portable imaging platforms.

Imaging platform	System	Resolution	Other features (FOV, magnification and frame rate)	Reference
Miniaturized microscope	A Sony PlayStation® Eye webcam	–	187 frames s^{-1}	(Balsam et al., 2014)
	A commercial webcam (Logitech, c160)	1.4 μm	1 frame per 2 min 100 × and 400 × magnification	(Kim et al., 2012b)
	Logitech C160m USB web camera	2 μm	30 frames per second 8 ×, 20 ×, 40 ×, and 60 × magnification	(Zhang et al., 2015)
	a QX10 camera (Sony)	3 μm	–	(Jin et al., 2015)
	A USB microscope (VMS-004D)	–	400 × magnification	(Wu et al., 2014)
	Three microscopes (VMS-004D)	1 μm	400 × magnification	(Lynch et al., 2014)
	A webcam-based microscope	1.75 μm	–	(Walzik et al., 2015)
Lens-free microscope	Ethernet camera assay (Baumer model TGX50-P)	1.5 μm	–	(Serabyn et al., 2016)
	Multi-Illumination Single-Holographic-Exposure Lensless Fresnel–MISHELF microscopy	1.7 μm	–	(Sanz et al., 2017)
	A CMOS sensor	2 μm	23.52 mm^2 FOV	(Roy et al., 2015)
	A CMOS sensor	–	24 mm^2 FOV	(Pushkarsky et al., 2014)
	A CMOS sensor(12-bitAPTINA MT9P03)	2.2 μm	24 mm^2 FOV; 3700 cells	(Kesavan et al., 2014)
	A CMOS sensor	–		(Kim et al., 2011)
	A CMOS sensor	2.2 μm	24 mm^2 FOV	(Zheng et al., 2011)
	Aptina MT9P031 5 megapixel image sensor	2.2 μm	27.36 mm^2 FOV; > 200 cells	(Lee et al., 2012)
	A CMOS sensor chip (MT9M001C12STM)	1.2 μm	13 mm^2 FOV; 1 frame per 115 s	(Han et al., 2013)
	Aptina MT9P031 5 megapixel image sensors	2.2 μm	27.36 mm^2 FOV	(Jung and Lee, 2016)
	A CMOS sensor	–	–	(Takehara et al., 2017)
Smartphone microscope	a Sony-Erickson U10i Aino	2 μm	450 cells s^{-1}; 7 frames s^{-1}	(Zhu et al., 2011)
	–	535 nm	0.035 mm^2; 30 frames s^{-1}	(Jagannadh et al., 2015)
	Samsung Galaxy SII-	–	–	(Zhu et al., 2013)
	iPhone 5 s	1 μm	350 × magnification	(Hutchison et al., 2015)
	–	1 μm	–	(Shrivastava et al., 2018)
	MotoX-XT1575, Motorola	–	0.3 mm^2 FOV	(Kanakasabapathy et al., 2017a)
	iPhone 4 S	2 μm	14 mm^2 FOV; > 10,000 cells	(Im et al., 2015)
	Motorola, IL	–	–	(Amin et al., 2017)
	Moto X, Moto G4, and LG G4	–	30 frames/s; 0–408 million sperm mL^{-1}	(Kanakasabapathy et al., 2017b)
	Motorola, IL	–	–	(Knowlton et al., 2015)
	–	–	–	(Su et al., 2017)
	HUAWEI	2.76 μm	0.56 mm^2 FOV	(Ke et al., 2017)

solutions. For example, a portable live-cell imaging system equipped with an invert-upright-convertible architecture and a mini-bioreactor was recently developed for long-term cell imaging (Rajan et al., 2018). Specifically, the mini-bioreactor comprises of a transparent Indium-Tin-Oxide (ITO) cell heater, a polydimethylsiloxane (PDMS)-based cell chamber and the gas supply for CO_2 buffering.

From the business perspective, the lengthy, expensive and time-consuming regulatory approval process is a major barrier for the wider adoption of these portable cell assays as practical products by the healthcare community. For example, medical devices are only allowed to be sold in the USA and Europe after being cleared by the USA Food and Drug Administration and Conformité Européenne (European Conformity), which generally takes more than two years. This regulatory approval requirement is a significant challenge faced by timely translation of new medical devices, especially those PoC diagnostic assays to clinical practice. Continued efforts to validate the effective use of these new PoC assays with comparable or even higher performance against the conventional gold standard methods will facilitate the commercialization of the new assays. Interdisciplinary collaborations between the engineering and medical research communities, healthcare professionals and the relevant industrial sectors are the key to enable further development of these PoC assays and their timely translation to practical products.

In conclusion, the fast-developing field of portable imaging platforms has the potential to enable new ways of performing various cell-based assays outside traditional lab setups thereby advancing life science research and personalized medicine. As Bill Gates once envisioned "A computer on every desk and in every home", which is much of a reality now, we foresee the rapid realization of the portable imaging system as a common tool at the hands of every researcher and clinical professional in the near future.

Competing financial interests

The authors declare no competing financial interests.

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