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Lens-free shadow image based high-throughput continuous cell monitoring technique

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ABSTRACT

A high-throughput continuous cell monitoring technique which does not require any labeling reagents or destruction of the specimen is demonstrated. More than 6000 human alveolar epithelial A549 cells are monitored for up to 72 h simultaneously and continuously with a single digital image within a cost and space effective lens-free shadow imaging platform. In an experiment performed within a custom built incubator integrated with the lens-free shadow imaging platform, the cell nucleus division process could be successfully characterized by calculating the signal-to-noise ratios (SNRs) and the shadow diameters (SDs) of the cell shadow patterns. The versatile nature of this platform also enabled a single cell viability test followed by live cell counting. This study firstly shows that the lens-free shadow imaging technique can provide a continuous cell monitoring without any staining/labeling reagent and destruction of the specimen. This high-throughput continuous cell monitoring technique based on lens-free shadow imaging may be widely utilized as a compact, low-cost, and high-throughput cell monitoring tool in the fields of drug and food screening or cell proliferation and viability testing.

1. Introduction

A continuous and high-throughput cell monitoring technique has been required in a broad range of cell analysis applications, including cytotoxicity assays, pharmacokinetics, and comparative drug studies. For continuous and high-throughput cell monitoring, measurements of cell proliferation and viability, which can provide quantitative information on cellular growth and apoptosis, are necessary but currently labor intensive. To measure the cell proliferation, researchers have developed many direct and indirect methods such as ELISA (Enzyme-Linked Immuno-Sorbent Assay) (Perros and Weightman, 1991), Western blots (Awasthi and Wagner, 2006), immunohistochemistry (Yu et al., 1992), and cyclin-dependent kinase assays (Ortega et al., 2003). Also, for the determination of cell viability, metabolic activity or membrane integrity is monitored by staining cells with various dyes, e.g. trypan blue, and tetrazolium salt or fluorescence dye, under a conventional microscope (Tennant, 1964; Roehm et al., 1991; Calich et al., 1978; Heo et al., 2003). The invention of the microscope made it possible for humans to start exploring the small world that could not be seen by the naked eye. The microscope, which increases the spatial resolution by magnifying objects based on the optical lenses, has evolved into an essential instrument for monitoring and analyzing biological cells and molecules in modern laboratories. While we can observe small objects through the microscope in detail, we also have to sacrifice a critical item, "field-of-view (FOV)", which directly corresponds to the throughput of an imaging system.

Recently, a lens-free imaging modality, which does not utilize any conventional optical lenses or scanning stage, was successfully demonstrated (Seo et al., 2008, 2009; Su et al., 2009). In this approach, the cells or micro-objects are located close to an optoelectronic device, e.g. a CCD (Charge-Coupled Device) or CMOS (Complementary Metal-Oxide-Semiconductor) image sensor, and illuminated with a low-cost partially coherent light source, e.g. an LED (Light Emitting Diode) conjugated with a micro pin-hole. The shadow or diffraction patterns of the cells or any other micro-objects that were generated by this simple imaging platform were recorded with the optoelectronic device and analyzed to detect and characterize various biological cells or molecules including yeasts (Seo et al., 2009), human blood cells (Seo et al., 2010), and antibodies (Stybayeva et al., 2010). This lens-free imaging platform, of course, has low optical resolution due to the lack of the optical lenses; however, the resulting shadow or diffraction patterns of the target cells can provide

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enough information for their detection and characterization. Also, the FOV of this platform can be extended up to several thousand times greater than that of the conventional microscope by simply selecting a suitable optoelectronic device. Furthermore, due to the simple and compact physical structure of this platform, this technique could be also useful in developing a point-of-care (POC) microscope (Mudanyali et al., 2010) or a miniaturized portable photospectrometer (Kim et al., 2011).

In this study, we firstly demonstrate a high-throughput continuous cell monitoring technique based on lens-free shadow imaging. This technique does not require any labeling reagents or destruction of the specimen. More than 6000 human alveolar epithelial A549 cells are monitored simultaneously and continuously for up to 72 h within a custom built cell incubator integrated with the lens-free shadow imaging platform. In this manuscript, single cell viability and nucleus division are quantified and characterized by calculating the signal-to-noise ratio (SNR) and shadow diameter (SD) of the cell shadow patterns. All the experimental procedures and results for the cell preparation, cell culture, lens-free shadow imaging platform, quantification of cell shadow images, and live cell count are described in the following sections.

2. Materials and methods

2.1. Cell preparation

To demonstrate the high-throughput continuous cell monitoring technique, we used A549 (human alveolar epithelial) cells at a concentration of 250,000 cells/ml maintained in an RPMI-1640 (HyClone) medium. The medium was supplemented with 10% fetal bovine serum (HyClone), penicillin (100 units/ml), and streptomycin (0.1 mg/ml). The cells were initially cultured on the surface of a type 1 (h=0.15 mm) cover glass within a commercial air-jacketed CO_2 incubator for 24 h at 37 °C. Before the cell preparation, all the components directly contacting the cells in Fig. 1(a), including the cover glass, petri dish, polydimethylsiloxane (PDMS) wall, silicon tubes, and quartz flow cell, were autoclaved at 121 °C for 30 min to sterilize them.

2.2. Cell culture

For continuous cell monitoring with the shadow imaging platform, the cell containing cover glass, enclosed with a quartz flow cell (49-Q-0.1, Starna Cells Inc.), was relocated to the continuous cell culture apparatus and assembled. A custom designed PDMS wall (h=1 mm), functioning as an oxygen permeable spacer, was sandwiched between the cover glass and the quartz flow cell as illustrated in Fig. 1(a). Since the total oxygen consumption rate of the cells growing in the channel of the quartz flow cell with the initial cell concentration of 250,000 cells/ml was estimated as $\sim 5.795 \times 10^{-7}$ mol/day, which is less than one-third of the total rate of oxygen permeation through the PDMS wall, i.e. $\sim 1.771 \times 10^{-6}$ mol/day (Leclerc et al., 2003), a separate oxygen supply system was not considered in this work. A CO_2 independent medium (18045, GIBCO) was continuously provided at a speed of 5–10 μl/min through a silicon tube connected to a dual channel syringe pump (M200, KD Scientific) for the whole observation period, i.e. up to 72 h. To keep the incubation temperature constant at ~37 °C, the entire cell monitoring system was designed to be covered by a custom built heating block which is connected to a programmable logic controller (PLC) as shown in Fig. 1(b). To minimize local temperature changes in the incubator, the CMOS image sensor was turned on only for the moment of image capture.

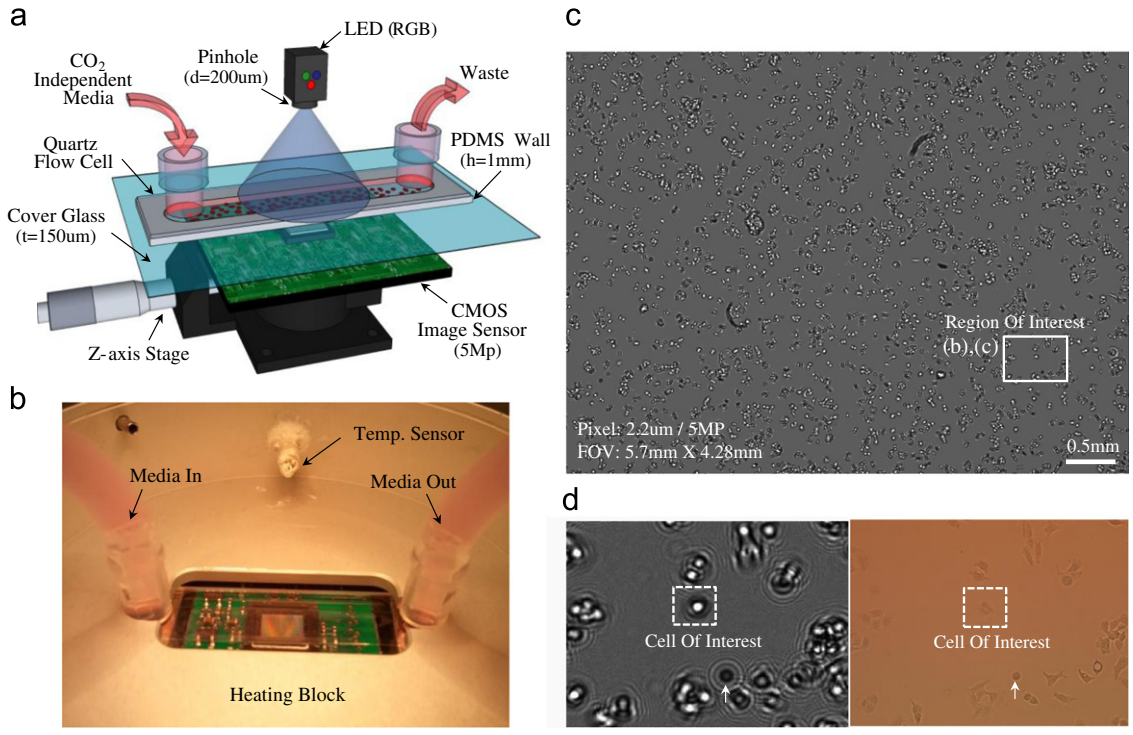

Fig. 1. Experimental apparatus of high-throughput continuous cell monitoring platform using lens-free shadow imaging. (a) Schematic diagram of the set-up. (b) Experimental apparatus equipped with a custom built incubator. (c) Lens-free shadow image of A549 cells captured with a 5 megapixel CMOS image sensor. Note that the FOV of the shadow image is 5.7 mm × 4.28 mm which is more than 100 times larger than that of a conventional optical microscope. (d) Magnified cell shadow image for region of interest (ROI). (e) Corresponding microscope image for ROI.