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Staining-free cell viability measurement technique using lens-free shadow imaging platform

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ABSTRACT

Tests for cell viability, i.e., an assay quantifying the ratio of viable cells or tissues over the total cells or tissues within an index between 0 and 1 (or 0 and 100%), play an important role in cell or tissue culturing procedures. The viability test result, varying with several biological factors such as mechanical activity, motility, contraction, or mitotic activity of cells or tissues, is a crucial indicator in cell related research protocols including toxicity and anabolic activity assays. There are several well-established methods for evaluating cell viability, such as trypan blue assay, propidium iodide assay, 7-aminoactinomycin D assay and resazurin and formazan (MTT/XTT) assay. However, most of these methods determine viability using stained cell samples, which intern affect the cells morphology eventually making it unable to keep culturing the specimen. To address this issue, we have developed a novel shadow imaging technique to capture the diffraction patterns (shadow patterns) of micro objects without the use of any staining reagent. In this paper, we introduce a shadow imaging platform that can determine cell viability of more than 3000 human cancer cells immediately with a single digital image. Our custom-built lens-free shadow imaging platform consists of a compact, cost-effective light source, i.e., a light-emitting diode, and an optoelectronic image recording device, i.e., a complementary metal-oxide semiconductor image sensor. Three types of human cancer cell lines (Caco-2, HepG2, and MCF7) were incubated in 24-well plates, and H_2O_2 was added to track and compare the cell viability at each concentration tested. We obtained high correlation indices, with a minimum of 0.94, between the MTT assay and the shadow imaging platform. All these characterizations were done by custom developed automated detection algorithm. This algorithm analyzes the various elements of the diffraction pattern (shadow image), such as pixel intensity and connected pixel numbers, and counts the viable cells automatically, allowing the cell viability to be determined easily and immediately in a staining-free manner.

1. Introduction

Many biological and pharmaceutical research laboratories worldwide have made concentrated efforts to discover more effective drugs. Recent technological breakthroughs have accelerated these efforts especially in cancer research. Tests for cell viability play a key role in the drug screening protocols [1]. A range of viability assays are available. The commonly used techniques, such as the trypan blue dye assay [2] and the hydrogen acceptor 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) assay, rely on staining to differentiate between live and dead cells [3]. However, the staining of cells is a time-consuming process and susceptible to subjective error. Eventually, the fragile, stained cells become an impediment to the continuous monitoring used in drug screening [4]. To address this issue, we utilize the lens-free shadow imaging technique which has been widely studied by various research groups worldwide recently. From a historic article of Ozcan in 2008 [5], the lens-free imaging has been evolved to count cells [6,7], differentiate cells [8–10], detect the cell and particle sizes [11], measure the protein and enzyme concentration [12,13], and analyze the number of human epithelial cells continuously [14]. Moreover, combining with image

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reconstruction process, applications of this technique has been even extended into the lens-free microscopy [15], nano particle analysis [16], and on-line cell monitoring [17]. Details about the achievements and remaining challenges of the lens-free imaging technique are also available in a reference work [18]. However, so far, staining-free cell viability testing based on the lens-free shadow imaging platform has not been demonstrated yet.

In this paper, we firstly demonstrate that the lens-free shadow imaging platform can provide the staining-free cell viability measurement within a single cell level. The proposed platform is composed of a low-cost, light-emitting diode (LED) as the light source and a lensless complementary metal-oxide semiconductor (CMOS) image sensor as the imaging system. A schematic of the system is shown in Fig. 1(a). The lights from the LED are diffracted by the objects in the sample plane, producing a shadow image of the objects below. The CMOS sensor below the sample plane captures an image of the diffraction pattern (shadow image) of the objects, which conveys the characteristics of the cells. We have developed an image processing algorithm to analyze the diffraction pattern of the cells. This algorithm quantitatively reports on the cell viability by analyzing the different elements in the diffraction pattern, such as the intensity value of the pixel and the number of connected pixels.

The experiments in this study were performed using human cancer cell lines. Three types of cancer cell lines, such as Caco-2 (human epithelial colorectal adenocarcinoma cell lines developed by the Sloan-Kettering Institute for Cancer Research), HepG2 (human liver carcinoma cell line derived from the liver tissues), and MCF7 (Michigan Cancer Foundation-7, human breast adenocarcinoma cell line), were incubated in 24-well plates. Various concentrations of these cell lines with various H_2O_2 concentrations were studied. All the experimental results from the shadow imaging system were compared with the results from a standard MTT assay enabling the agreement evaluation of two modalities.

2. Materials and methods

2.1. Cell preparation

To demonstrate the staining-free cell viability measurement technique, we used three types of human cancer cell lines, i.e., HepG2, Caco-2, and MCF7. All these selected cell lines have been widely studied for the development of new drugs of colorectal (Caco-2), liver (HepG2), and breast (MCF7) cancers which are notorious for their high lethality. Two sets of cell lines were prepared for the experiment. In the first set, HepG2 cell lines, at a concentration range of 2000 cells/mL to 20,000 cells/mL (at intervals of 2000 cells/mL), were prepared in a 24-well plate in high glucose growth medium (Grand Island, NY). In the second set, Caco-2, HepG2, and MCF7 cell lines were prepared in three separate 24-well plates at a concentration of 18,000 cells/mL and maintained in high glucose growth medium. For both sets, the medium was

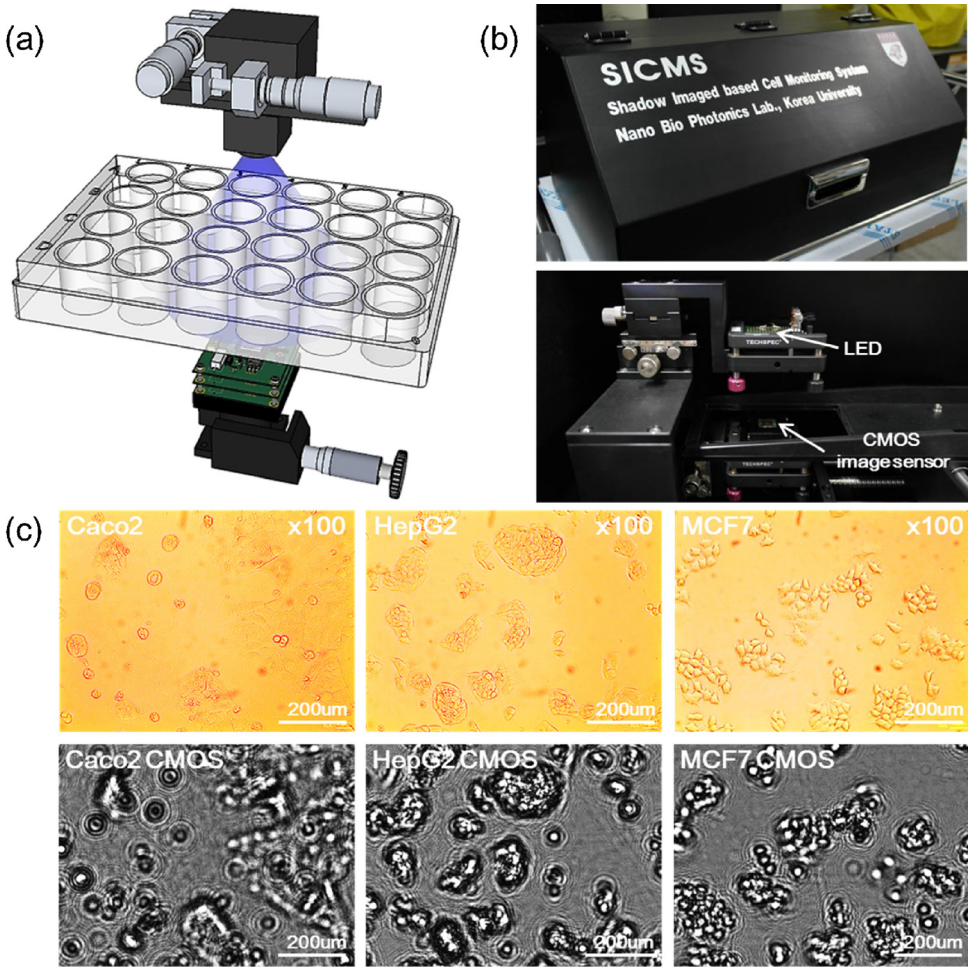

Fig. 1. Experimental setup of the shadow imaging platform. (a) Schematic diagram of the setup. (b) Actual shadow imaging system used in this paper. (c) Three types of human cancer cell lines (Caco-2, HepG2, and MCF7), magnified with a microscope 100× image, and the CMOS shadow image for the same field of view.