Sensors and Actuators A 172 (2011) 175–180

Contents lists available at ScienceDirect

Sensors and Actuators A: Physical

journal homepage: www.elsevier.com/locate/sna

An imaging system for real-time monitoring of adherently grown cells

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ARTICLE INFO

Article history:
Available online 18 May 2011

Keywords:
Optical detection
CCD sensor
Real-time cell monitoring
Adherent cells

ABSTRACT

In this contribution a real-time imaging system for cell cultures, adherently grown in standard disposable multi-well plates is presented. The compact system is designed to be operated in a lab incubator. The imaging system consists of a custom made platform in which a CCD image sensor is aligned and fixed directly at the bottom of the well of interest; a LED light source is positioned above the cell sample. The image sensor has a resolution of 640 × 480 pixels. A mini lens mounted on top of the CCD sensor allows close-up focusing. The field of view on the focal plane measures 3.26 mm × 2.45 mm, which corresponds with an overall optical resolution of 5.1 μm × 5.1 μm. The system has been validated by visualizing spherical beads of known size. With the presented imaging system we have successfully monitored collective (wound healing assay) and individual cell migration, as well as epithelial cell proliferation.

1. Introduction

Basic and advanced research in biological and clinical laboratories relies on microscopy. Moreover, several biologically relevant parameters (such as cell morphology, differentiation, division, and viability) are directly or indirectly obtained by optical detection. It is of great importance to keep the optimum cell cultivation conditions stable preserve cell metabolism and viability. For mammalian cells, these conditions are normally 37°C temperature, 5% CO_2 concentration, and high humidity level. Usually, cell events are tracked by means of time-lapse microscopy, which requires taking the sample outside of the controlled incubator. These interruptions cause dramatic changes in environmental conditions that could directly affect the reliability of the experiment. Also, the cell sample might be subjected to pollution and contamination hazards. Furthermore, precise sample alignment is required to allow iterative observations. To overcome these drawbacks and to enable real-time cell observation, microscopes need to be equipped with expensive incubation chambers ensuring controllable temperature, CO_2, and humidity levels [1]. In Fig. 1a, cell sample observation by conventional microscopy is illustrated. So far, the only alternative method for online cell monitoring is based on contact imaging, where the image sensor is in direct contact to the sample of interest (without any intermediary optics). The image is acquired by projecting light through the sample of interest on the sensor surface. Contact imaging has previously been introduced for biomedical applications by different research groups [2–5]. Gabriel et al. have demonstrated that it is feasible to conduct contact imaging based cell investigations in standard incubators [5]: in order to ease cell cultivation and accessibility, a previously modified disposable Petri dish with an aperture for the sensor was used (see Fig. 1b). However, as reported by Ji et al., in near-field conditions the resolution of contact imaging is determined not only by the pixel size of the image sensor, but also by the distance between the object under investigation and the sensor array [3]. Unfortunately, this limits the attractiveness of lens-free imaging, because the sensor needs to be physically separated from the biological sample to preserve its functionality; an intermediate protective layer is therefore strictly required. Furthermore, a thorough cleaning protocol is required when the contact imager has to be reused, which makes the device less attractive.

In the current work, we have designed and realized a cost-effective, easy to use cell monitoring system that is compatible with standard unmodified lab disposables and operates in lab incubators [6]. This way, no change in cell assay protocols is required. The potential of the realized imaging system has been proven by investigating the real-time monitoring of collective cell migration (wound healing assay), and individual cell motility and proliferation.

2. Materials and methods

2.1. System design and realization

The proposed cell monitoring system consists of three main parts: a custom made holder platform, an optical sensing unit, and

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doi:10.1016/j.sna.2011.05.010

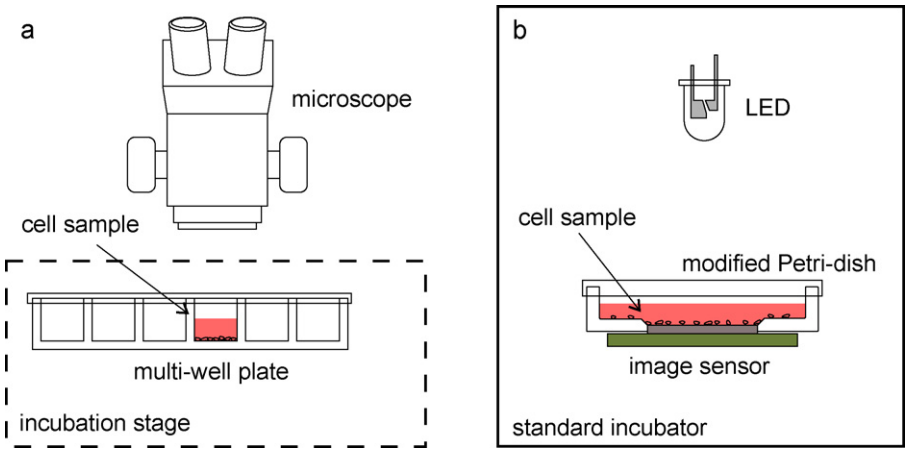

Fig. 1. (a) Conventional observation of a cell sample through an optical microscope; in order to enable real-time measurements, an additional incubation chamber is required. (b) Contact imaging setup for in incubator real-time adherent cell monitoring: the cell sample is placed on the image sensor without any intermediary optics.

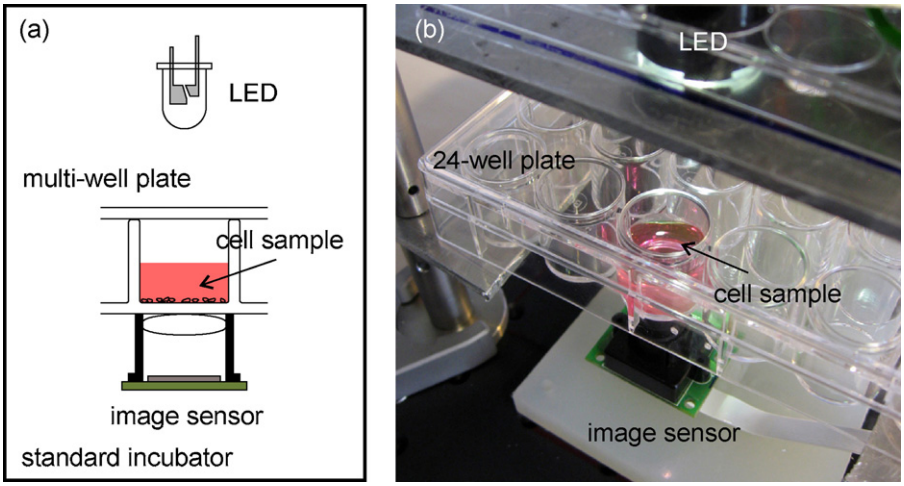

Fig. 2. (a) Schematic representation of our imaging system setup. (b) Photograph of the imaging system operating with a 24-well plate; the diameter of the well under test measures 16 mm.

a light source (Fig. 2). The holder platform (components obtained from Thorlabs GmbH, Germany) has been designed to provide mechanical support to a multi-well plate, free access to the optical sensor on the bottom of the multi-well plate, and vertical alignment for the light source. An optical image sensor, designed for industrial and robotic applications (Fire-i Digital Board Camera Remote CCD, Unibrain Inc., CA, USA), was selected for its compactness and image quality (progressive scanning, and thus full frame non-interlaced acquisition). It features a compact sensing unit that is connected to the signal processing board via a flexible cable. This way, an area of only 3.0 cm × 2.8 cm is required under the multi-well plate, allowing the observation of multiple wells when more sensing units are placed. The sensor settings are fully adjustable: typical parameters such as frame rate, shutter, gain, gamma, white balance, and black

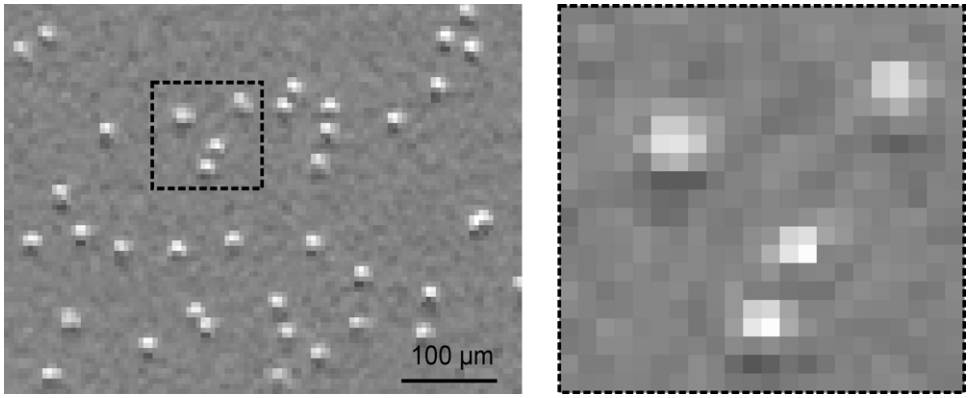

Fig. 3. On the left: individual polystyrene spherical beads of 12 μm diameter acquired by the imaging system. On the right: magnified pixel distributions of four different single beads.