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Procedia Engineering 5 (2010) 492–495

Procedia Engineering

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Proc. Eurosensors XXIV, September 5-8, 2010, Linz, Austria

A real-time monitoring system for adherently grown cells

Nicola Moscelli[a,*], Sander van den Driesche[a], Wojciech Witarski[b], Filippo Iuliano[b], Michael J. Vellekoop[a]

[a]*Institute of Sensor and Actuator Systems, Vienna University of Technology, Gusshausstrasse 27-29, 1040, Vienna, Austria*
[b]*Institute of Virology, Slovak Academy of Sciences, Dubravska Cesta 9, 84245, Bratislava, Slovak Republic*

Abstract

We present a real-time, compact and low-cost imaging system for cell cultures grown attached in standard disposable multi-well plates. The system comprises a custom made platform to position a standard multi-well plate and a 1/4" CCD image sensor aligned, fixed and focused directly at the bottom of the well of interest. The image sensor has a resolution of 640 × 480 pixels. With a lens mounted on top of the CCD sensor we obtained a 3.26 × 2.45 mm^2 field of view, corresponding to a pixel size of about 5 μm. With our imaging system we have successfully observed adherent epithelial cells (A549): individually, in spheroid shaped cluster, and in a wound healing assay.

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Keywords: Optical detection, CCD sensor, cell monitoring

1. Introduction

Real-time monitoring of adherent grown cells is of importance for cell proliferation (drug screening and toxicology) and cell motility experiments. The standard method to observe cell cultures is by microscopy. Unfortunately, the samples should then be taken out of the incubator influencing the cultivation conditions such as temperature, humidity and CO_2 concentration and therefore not allowing long real-time measurements. A method to overcome this problem is by equipping the microscope with an expensive stage for controlled thermal, humidity and CO_2 regulation [1]. Our proposed solution for adherently grown cell monitoring comprises a compact and low-cost alternative which can be used in standard incubators. Furthermore, our system is compatible with unmodified lab disposables, which is not the case for contact imaging systems [2]: these devices rely on the direct interaction between the biological sample and the sensor without any intermediary optics, which means that a cleaning protocol is then required for serial experiments. In order to characterize our optical imaging system, we have tested it by monitoring adherent grown epithelial cells in different culture conditions which are typical for *in-vitro* experiments in biological laboratories. In the next section, a description of the optical imaging system setup is provided. In Section 3, the results of system validation and cell monitoring experiments are shown and discussed.

*Corresponding author: tel.+43-1-58801-36625; fax +43-1-58801-36699
Email address: `nicola.moscelli@tuwien.ac.at` (Nicola Moscelli)

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doi:10.1016/j.proeng.2010.09.154

2. System description

A schematic representation together with a picture of the optical imaging system are shown in Fig. 1. A custom made platform provides placement for a standard multi-well plate. Under this multi-well plate, a 1/4" CCD image sensor with 640 × 480 pixels resolution (Sony ICX-098BQ with Fire-i Digital Remote Camera Board) is aligned and fixed directly at the bottom of the well of interest. A lens mounted on top of the CCD sensor allows close-up focusing with a field of view of $3.26 \times 2.45\ mm^2$, yielding a resolution of about 5 µm.

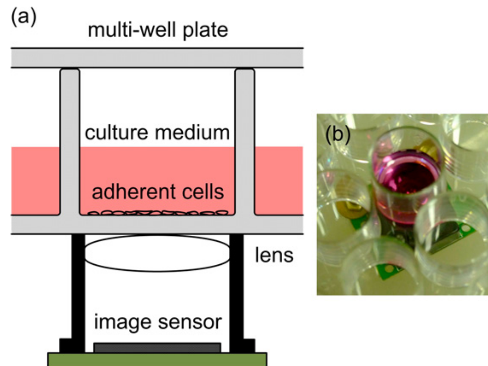

Figure 1: (a) Schematic representation of the adherent cell monitoring system setup; (b) photograph of the optical system with a disposable standard 24-well plate (the well under investigation is filled with the cell sample and the culture medium).

3. Results and discussion

3.1. System validation

Before testing attached growing mammalian cells, we have first validated our monitoring system by observing particles with known shape and size. For this purpose, we selected monodisperse non transparent polystyrene spherical beads with a diameter of 12 µm (Sigma Aldrich Fluka 885110). In Fig. 2a, a frame of the beads taken with our monitoring system is shown. As depicted in Fig. 2b, the intensity distributions of four single 12 µm particles correspond to areas of approximately 2 × 2 pixels, which are consistent with the calculated resolution of about $5 \times 5\ \mu m^2$ per pixel.

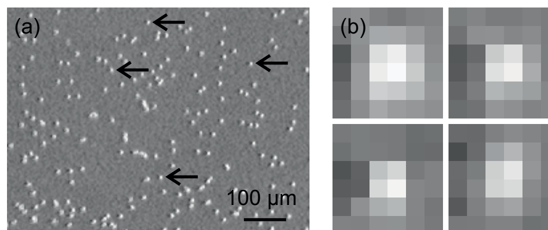

Figure 2: (a) Individual polystyrene beads of 12 µm diameter observed with our monitoring system; (b) magnified pixel distributions of four different single beads.

3.2. Cell monitoring

After validation, we have tested our system with adherent grown carcinomic human alveolar basal epithelial cells (A549 ATCC CCL-185). The cells have been incubated at 37°C and with 5% CO_2 concentration. The cultivation media consisted of DMEM (Dulbecco's Modified Eagle Medium) with 4.5 g/L glucose, 10% fetal calf serum, 2 mM L-glutamine, and antibiotics. These cells have been monitored in three different culturing conditions: individually grown, in a cluster and in a wound healing assay. During the experiments, the illumination was provided by a halogen lamp with an aperture of 6 mm, mounted orthogonally over the well of interest.

In Fig. 3 the individual cells are shown in sparse and higher concentration: in both pictures, it is possible to clearly discriminate between two adjacent cells. Also, cell activity such as attachment/detachment and movement processes is detectable. Moreover, parameters such as cell number, position and morphology are clearly obtained.

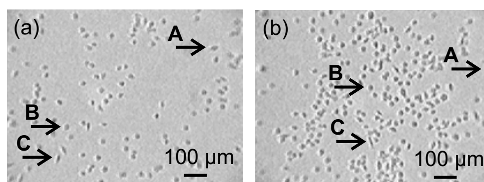

Figure 3: Sparse A549 cells (a) and in higher concentration (b); in both pictures, round attaching or detaching cell (A), mesenchymal like phenotype of fully attached cells (B,C), also in moving process (C) are recognizable.

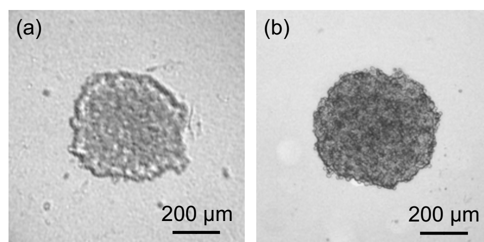

Figure 4: A spheroid of A549 cells with a diameter of about 400 μm pictured with (a) the imaging system and (b) a phase-contrast microscope with 5x magnification (a time lapse of half an hour occurs between the two images).

We have also observed the cells in a spheroidal cluster (Fig. 4) by our monitoring system and phase-contrast microscopy. The shape and the contour of the cell cluster frame taken with our system are well defined and similar to the photograph taken with the microscope at 5x magnification.

The real-time collective cell motility in a wound healing experiment has been monitored over a confluently grown cell monolayer. A scratch of about 500 μm has been made by gently scraping the cell monolayer with a sterile pipette tip; the cell sample was then rinsed with medium to remove cellular debris. In Fig. 5a-d, cell migration in time is depicted: in each frame the cell fronts of the wound are clearly detectable. From these frames we have quantified the closure of the wound in terms of ratio of the original wound area (Fig. 5a) and calculated the wound repair velocity. Initially, in a time lapse of 1 hour and a half the wound area has decreased to 86% of its original area (Fig. 5b) with a velocity of 135 μm^2/min. With the same velocity, the wound has been reduced to 67% in the subsequent 2 hours (Fig. 5c). After 1 hour and 45 minutes, the wound area was covering 46% of the initial area (see Fig. 5d), which corresponds to an increment of the healing velocity to 175 μm^2/min. The obtained results show a nonlinear wound closure kinetics which is in agreement with the studies presented by Poujade et al. [1].

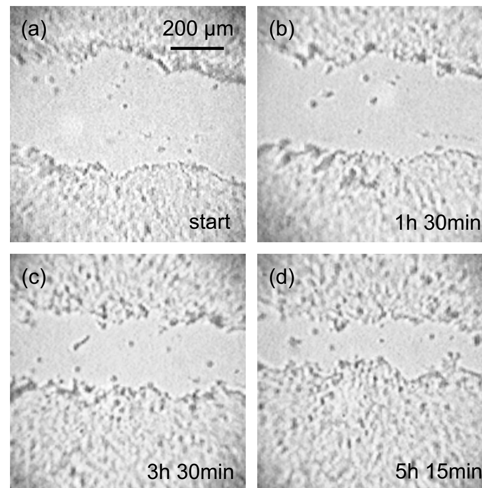

Figure 5: Captured frames of the wound healing experiment with adherent grown A549 cell monolayer. The progression of the opposite edges of the wound in time is clearly visible: due to cell migration, the wound area gradually decreases to 86% of its initial value (a) in (b), 67% in (c), and 46% in (d).

4. Conclusions

By the shown measurement results we have proven that our real-time optical imaging system enables the observation of single epithelial cells as well as cell aggregates such as spheroidal clusters and wound healing assays. Therefore, we have successfully demonstrated the potential of our system for adherent cell cultures investigation. Moreover, its compatibility with unmodified lab disposables and incubators makes this system an attractive and versatile analysis tool for biological laboratories.

Acknowledgments

This project is part of the EU Marie Curie Research Training Network "On-Chip Cell Handling and Analysis" CellCheck (MRTN-CT-2006-035854).

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