

# A REAL-TIME CELL PROLIFERATION AND MOTILITY MONITORING SYSTEM

Nicola Moscelli, Sander van den Driesche, Michael J. Vellekoop

*Institute of Sensors and Actuator Systems, Vienna University of Technology, Gusshausstrasse 27-29/E366, Vienna, Austria*

Wojciech Witarski

*Institute of Virology, Slovak Academy of Sciences, Dubravska Cesta 9, Bratislava, Slovak Republic*

**Keywords:** Optical detection, CCD sensor, Real-time monitoring, Epithelial cells.

**Abstract:** In this contribution we present a compact imaging system to monitor the proliferation and the motility of cells in real-time. Our monitoring system is compatible with standard multi-well plates and operates in CO<sub>2</sub>, temperature and humidity controlled cell-culture incubators. Adherent grown epithelial cells in a multi-well plate well, positioned on top of a custom made holder, have been monitored in real-time with a fixed CCD image sensor. As light source an LED is placed above the plate holder. A field of view of 3.3 × 2.5 mm<sup>2</sup> was achieved by using a 4.6 × 4.0 mm<sup>2</sup> image sensor and mini lens system. The image sensor has a resolution of 640 × 480 pixels. Consequently, the obtained sensing resolution of the imaging system is about 5 μm. The cell monitoring system has first been validated by visualizing micro-beads of known dimensions. Then, our system has been successfully tested, tracking the migration paths and proliferation of respectively adherent grown MDCK (Madin-Darby canine kidney) and A549 (human lung carcinoma) epithelial cells.

## 1 INTRODUCTION

The study of cell motility is of importance to understand the mechanisms related to many physiological and pathological processes such as tumour progression and immunological responses (Suresh, 2007). A commonly used technique to track and quantify cell motility is by conducting gold colloidal phagokinetic assays (Niinaka, 2001): because of their movement, the adherently grown cells displace gold monodisperse nanoparticles deposited on the substrate, tracing clearly visible paths. This technique is attractive for time-lapse measurements, where the preservation of optimal cultivation conditions such as fixed temperature, humidity, oxygen and CO<sub>2</sub> levels for biological culture growth is required. In order not to influence the cultivation conditions during cell proliferation and motility experiments, lab microscopes are normally equipped with additional expensive stages to control temperature, humidity, and CO<sub>2</sub> levels (Poujade et al., 2007). Our proposed real-time imaging system is a compact and low-cost alternative to such an optical setup: it can be

operated in standard lab incubators and is also compatible with unmodified lab disposables. Gabriel et al. have presented a real-time cell monitoring solution for incubator comprising a contact imaging device. These devices are based on an image sensor array directly in contact with the sample to be investigated (Gabriel et al., 2009). However, this proposed imaging device needs ad hoc modified lab disposables and an elaborate cleaning step when serial experiments are desired. Moreover, unlike conventional optical imaging systems, where the resolution depends on the pixel number and the lens magnification, contact imaging devices have a resolution which depends on the pixel size together with the distance between the object and the sensor surface (Ji et al., 2007). This limits the suitability of such devices, because the sensor needs to be decoupled by the biological sample to preserve its functionality.

In the following section, we provide a description of our monitoring system setup. In Section 3 we present and discuss the validation tests and the cell monitoring experiments.

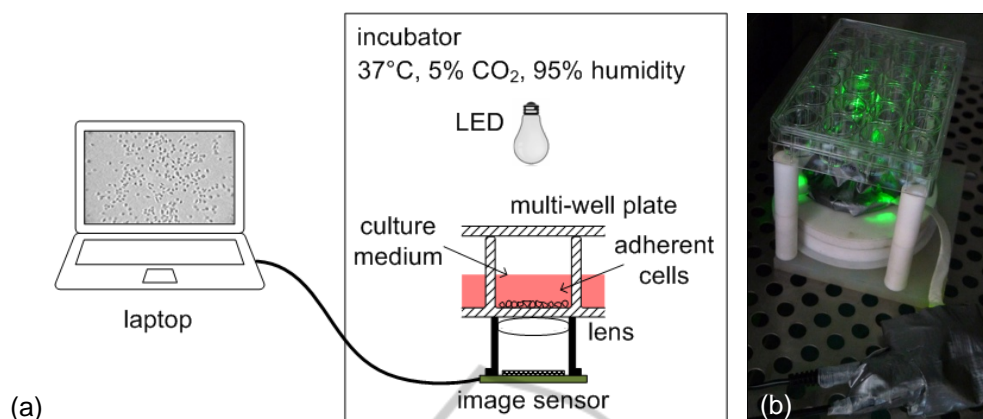


Figure 1: (a) Schematic of the monitoring system setup; (b) Photograph of the system with a 24-well plate in the incubator. To withstand the high humidity condition inside the incubator, the sensor and the printed circuit board have been hermetically sealed with Parafilm and Duct tape.

## 2 SENSOR SYSTEM

Our monitoring system operates with standard disposable multi-well plates. A multi-well plate is positioned on top of a custom made Teflon holder. A CCD sensor (Sony ICX-098BQ with Fire-i Digital Remote Camera Board) is placed underneath the multi-well plate, aligned and fixed in close proximity to the well containing the biological sample. The image sensor features a resolution of  $640 \times 480$  pixels over a surface of  $4.6 \times 4.0$  mm<sup>2</sup>. Together with a mini lens, the sensor is capable to focus over an area of  $3.3 \times 2.5$  mm<sup>2</sup>, yielding to an overall optical resolution of approximately 5  $\mu$ m in both dimensions. The CCD sensor is capable of progressive scanning, and thus full frame non-interlaced acquisition. Typical image acquisition settings (frame rate, shutter, gain, gamma, white balance, black level) are fully adjustable in order to obtain the finest image conditions. Moreover, the sensor is placed on a remote unit which allows minimal area encumbrance under the multi-well plate. A schematic representation and a photo of the optical imaging system setup are depicted in Fig. 1. In order to prevent condensation on the electronics due to high humidity conditions in the incubator, we sealed the sensor and the PCB with several layers of polymeric films and tapes (Parafilm and Duct tape), as depicted in Fig. 1b. A 10° angle focused illumination is provided by a LED light source (Luxeon Star/O) mounted orthogonally at 20 cm distance over the multi-well plate. Vertical illumination from the top of the biological sample is attractive to minimize light scattering through the radiation path (the light radiation has to cross the

cover lid of the multi-well, the cultivation medium, the cells, and the bottom substrate of the well). The cells are almost transparent and therefore hard to be optically detected, if high light scattering occurs. The supplied power for the LED was about 36 mW; during our tests in incubator, no noticeable overheating has been experienced.

## 3 RESULTS AND DISCUSSION

### 3.1 System Validation

In order to characterize our imaging system, we have visualized particles of known shape and size. For this scope, we have selected monodisperse non transparent polystyrene spherical beads with a diameter of 12  $\mu$ m (Sigma Aldrich Fluka 885110). In Fig. 2 the sedimented beads imaged by our system are shown. The pixel distribution has been determined by visualizing four individual beads and two beads in close proximity. The comparable intensity distributions of the four individual beads show that each bead corresponds approximately to a  $2 \times 2$  pixel matrix. This result is consistent with the calculated optical system resolution of  $5 \times 5$   $\mu$ m<sup>2</sup> per pixel. Also, the image shows the feasibility to clearly discriminate between two beads in close proximity, as long as the distance between them measures more than one pixel.

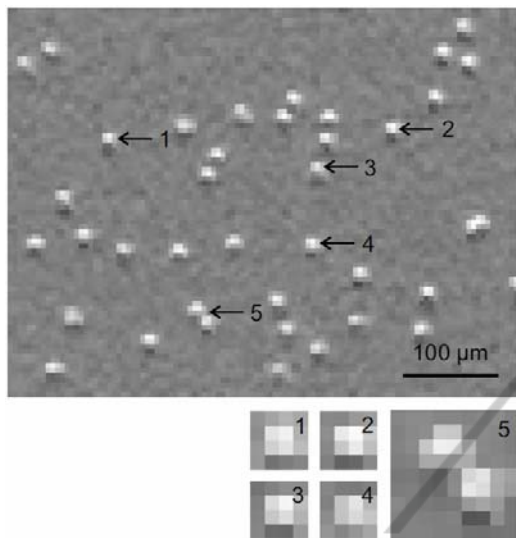


Figure 2: On top, individual polystyrene spherical beads of 12  $\mu\text{m}$  diameter observed with our imaging system; below, magnified pixel distributions of four different single beads (1 to 4) and two beads in close proximity (5).

### 3.2 Cell Monitoring

We have tested our optical system with two different epithelial cell lines: human carcinomic alveolar basal (A549, ATCC CCL-185) and Madin-Darby canine kidney cells (MDCK, ATCC CCL-34). 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. The cells have been incubated at 37°C and with 5% CO<sub>2</sub> concentration (a NAPCO CO<sub>2</sub> 1000 incubator was used).

In a first experiment, we have tracked the motility of adherently grown individual MDCK cells. In order to promote cell movement, HGF (Hepatocyte Growth Factor) in a concentration of 50 ng/ml has been added to the cell sample under investigation. The frame-capture rate of the sensor system has been set to one frame each 3 minutes, which was sufficient enough to detect changes of cell position within the parameters of this experiment. After the cells have sedimented and attached to the bottom of the well, their activity has been recorded for 2.5 hours. In Fig. 3a-d, four frames highlight the movement of two cells. The pictures illustrate only a section of the detected area. Also the migration paths covered by the two cells during the 2.5 hours experiment time frame are plotted (see Fig. 4). The trajectories of the cells have been obtained with MATLAB software (MathWorks Inc.) by extracting the coordinates of the cells per

each recorded frame. During the 2.5 hours observation, both cells have covered a distance of about 220  $\mu\text{m}$ , which corresponds to a velocity of about 1.5  $\mu\text{m}/\text{min}$ . The detected migration paths shown demonstrate that the achieved pixel resolution of our system is adequate for epithelial cell motility studies.

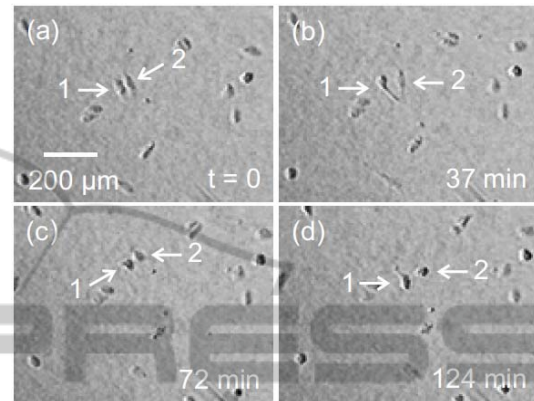


Figure 3: (a) – (d) Frames showing sparse MDCK cells in movement: cell positions as well as morphological changes during motion are clearly detectable.

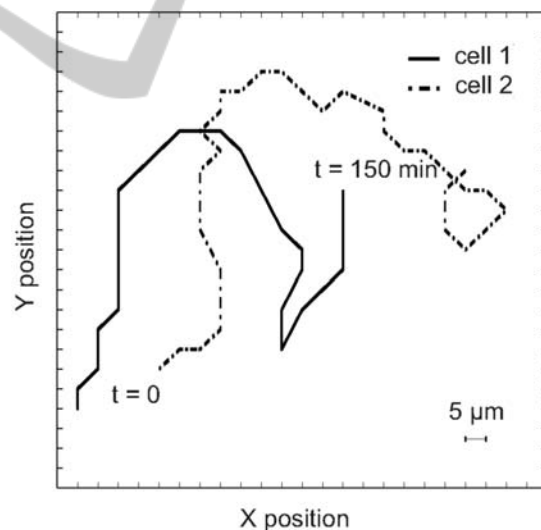


Figure 4: Migration trajectories of the two selected cells during the 2.5 hours experiment; in that period both cells covered a distance of about 220  $\mu\text{m}$ , which corresponds to a velocity of about 1.5  $\mu\text{m}/\text{min}$ .

In a second experiment, we have observed the proliferation of adherently grown A549 epithelial cells in sparse concentration. Identical sensor recording settings were kept for this second measurement. In Fig. 5 a 2 hours time lapse of dividing A549 cells is depicted in 8 frames. The pictures clearly illustrate cell division in all its

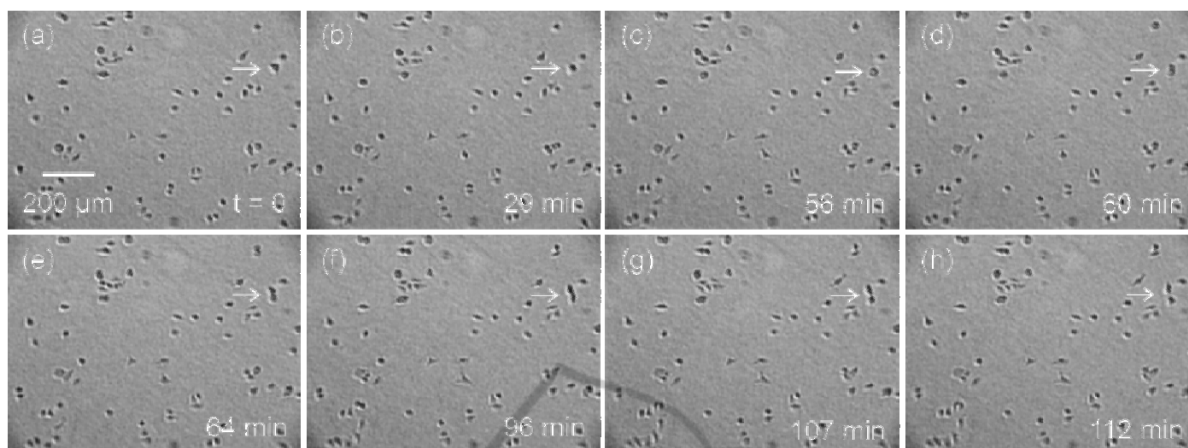


Figure 5: An A549 cell before, during and after the division process. (a) The cell is visible with its protrusions; (b) The cell assumes a round shape; (c) The cell (brighter) partially detaches from the substrate and starts the division process; (d) – (f) The cell is in the cytokinetic phase; (g) End of the mitosis; (h) The two daughter cells get separated. The whole division process (from (c) to (g)) takes place in about 50 minutes.

phases and prove that epithelial cell events related to morphological cell changes can be well monitored in time by our system.

In addition, from both experiments other biologically relevant parameters such as the amount of cells and their position in real-time can be obtained. The shown frames have not been processed with any software image manipulation technique.

## 4 CONCLUSIONS

The successful results of the shown experiments prove the suitability of our optical monitoring system for real-time adherent grown cell observation. Furthermore, by utilizing multiple sensors the simultaneous recording and tracking of different cell samples in the same multi-well plate is feasible. This would allow real-time observation of multiple cells exposed to diverse analytes (e.g.: growth factors, motility inhibitors, and toxins). Finally, the compatibility of our system with unmodified lab disposables together with standard incubators makes it an attractive and versatile analysis tool for biomedical applications.

## ACKNOWLEDGEMENTS

This project is part of the EU Marie Curie Research Training Network “On-Chip Cell Handling and Analysis” CellCheck (MRTN-CT-2006-035854). The authors gratefully acknowledge Filippo Iuliano

of the Institute of Virology of the Slovak Academy of Sciences in Bratislava for his valuable help in the early stage of the device development.

## REFERENCES

- Suresh, S., 2007. *Biomechanics and Biophysics of Cancer Cells*. Acta Materialia, Vol. 55, Elsevier.
- Niinaka, Y., Haga, A., Raz, A., 2001. *Quantification of Cell Motility: Gold Colloidal Phagokinetic Track Assay and Wound Healing Assay*. Methods in Molecular Medicine, Vol. 58, Springer.
- Poujade, M., Grasland-Mongrain, E., Hertzog, A., Jouanneau, J., Chavrier, P., Ladoux, B., Buguin, A., Silberzan, P., 2007. *Collective Migration of an Epithelial Monolayer in Response to a Model Wound*. PNAS, Vol. 104, No. 41.
- Gabriel, M., Picollet-D'hahan, N., Block, M., Haguët, V., *Monitoring Adherent Cells by Contact Imaging*, 2009. In Proc. MicroTAS 2009.
- Ji, H., Sander, D., Haas, A., Abshire, P. A., *Contact Imaging: Simulation and Experiment*, 2007. IEEE Trans Circ Sys, Vol. 54, No. 8.