An Imaging Platform for Mammalian Cell Migration Monitoring

N. Moscelli[1], W. Witarski[2], S. van den Driesche[1], M.J. Vellekoop[1]

[1]Institute of Sensor and Actuator Systems, Vienna University of Technology, 1040 Vienna, Austria
[2]Institute of Virology, Slovak Academy of Sciences, 84245 Bratislava, Slovak Republic

Here we present an imaging platform that operates in standard lab incubators for the real-time investigation of multiple cell cultures in disposable multi-well plates. The system consists of four CCD (Charge-Coupled Devices) image sensors built into a custom-made holder, in which the multi-well plate is fixed. Above the multi-well plate, white light emitting LEDs provide vertical illumination on the cell samples and the sensors. The system allows simultaneous motility measurements of individual adherently grown cells over four wells, enabling high throughput and the extraction of statistically relevant biological data. In order to test it, we have monitored stimulated (with Hepatocyte Growth Factor) and control samples of MDCK (Madin-Darby Canine Kidney) cells.

Introduction

For the understanding of tumor progression, cell migration studies are of great importance [1]. Normally, optical cell investigation is carried out by microscopy. Nevertheless, in order to preserve optimal mammalian cell cultivation conditions (37 °C, 5% CO_2 concentration, high humidity level), the only alternative to time-lapse observations out of the incubator is to provide optical microscopes with expensive additional incubation stages for temperature and carbon dioxide control [2]. This way, real-time cell monitoring is achieved. In this work we present a compact and cost-effective alternative to conventional optical microscopy: it operates in standard lab incubators and it operates on multiple in-vitro assays from conventional multi-well plates. In a previous work, we have demonstrated that our system can successfully detect individual and aggregated adherent cells [3]. In this contribution, we prove that our system can simultaneously monitor multiple cell samples in real-time, enabling the extraction of statistically relevant biological parameters. The imaging platform was tested by observing HGF-stimulated (Hepatocyte Growth Factor) and control samples of MDCK (Madin-Darby Canine Kidney) cells and therefore extracting typical migration parameters, such as trajectories and mean cell velocities.

Sensor System

In Fig. 1, the setup of the mammalian cell imaging platform is depicted. The illumination is provided by vertically-aligned white light emitting LEDs, placed above the cell samples and the image sensors. The 10° focused radiation beam is further collimated by 8 mm diameter-apertures. Four CCD image sensors (VGA frame format, 640 x 480 pixels, 5.6 x 5.6 μm^2 pixel size) achieve near-field focusing by employing mini lenses featuring *f* 2.0 and 4.3 mm focal length. The distance between the sensor array and the mini lens was adjusted in order to position the focal plane exactly on the substrate of the well under investigation. In order to withstand the incubation conditions, the sen-

sors and the electronics have been protected by possible humidity condensation by hermetically sealing them with insulating polymeric films and tapes.

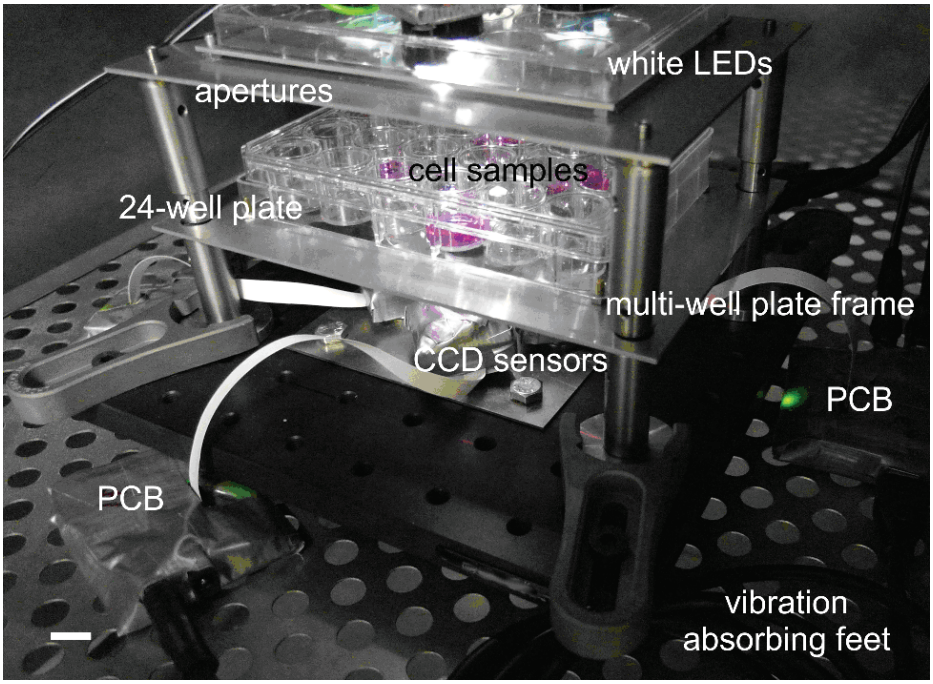

Fig. 1: The imaging platform setup for mammalian cell monitoring placed in a conventional lab incubator with a standard 24-well plate (well diameter: 16 mm).

Experimental

Sample Preparation

Four MDCK cell colonies (ATCC CCL-34) have been seeded in four different wells of a disposable 24-well plate (Greiner Bio-One GmbH, Germany) and incubated in standard conditions (37 °C, 5% CO_2, and high humidity level). The cells have been cultivated in DMEM (Dulbecco's Modified Eagle Medium), 4.5 g/L glucose, 2 mM glutamine, 10% FCS (Fetal Calf Serum) and antibiotics. After 12 hours incubation, all cell samples have been set to starvation with a medium containing 0.5% FCS.

Measurements

In order to test the imaging platform, cell motility experiments have been simultaneously carried out on four MDCK cell colonies. Two of them have been supplemented with HGF in a concentration of 20 ng/mL, while the remaining two cell samples have been left without any stimulation to investigate differences in cell activity. In mammalian organisms, HGF has a major role in embryonic organ development, in adult organ regeneration and in wound healing. Thus, a marked cell motility promotion is expected in the exposed cell colonies. The cell samples have been grown all as described above. After cell sedimentation and adhesion to the bottom of the wells, we have recorded cell activity of each population for 8 hours. The frames have been recorded every 3 minutes from the four sensors. Magnified portions of the acquired frames showing stimulated individual MDCK cells are shown in Fig. 2. To determine the individual cell motility, the *xy* coordinates of each cell in consecutive frames have been semi-automatically

tracked using ImageJ (NIH, USA). From the extracted coordinates, the cell trajectories have been derived in terms of Euclidean distance between two points. Four samples of stimulated and control cell migration trajectories are shown in Fig. 3. The difference in terms of covered paths between stimulated and non-stimulated cells is clearly visible. The distances covered by the monitored cells have been then used to calculate the mean velocity of *n* cells of the same population [4]. This parameter statistically quantifies the effects of drug exposure on cell motility.

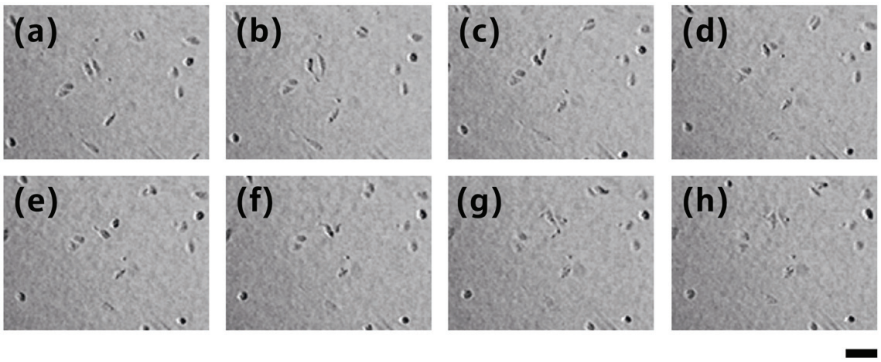

Fig. 2: Frames showing individual HGF-stimulated MDCK cells. Scale bar: 100 µm.

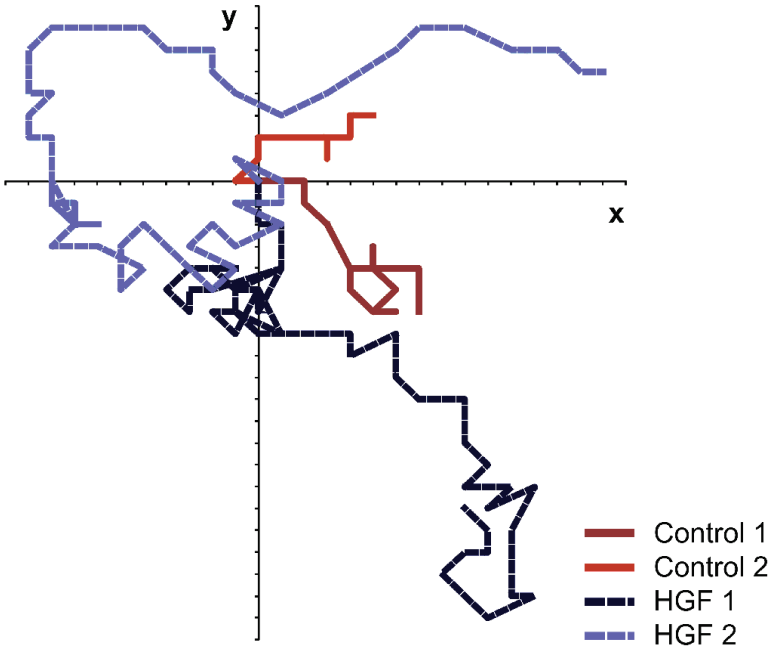

Fig. 3: Individual cell motility plot. The trajectories of HGF-stimulated and control cells are compared over a period of 8 hours.

By tracking and analyzing a population of 10 cells per each of the four colonies, we have extracted the mean cell velocity of stimulated and control cell populations, as shown in Fig. 4. From the plot it is possible to clearly observe that stimulated cell migration results in a mean cell velocity higher than 40 µm/h: approximately 4 times the mean cell velocity of the control samples. These results are in good agreement with the average velocities derived from the cell trajectories shown in Fig. 3.

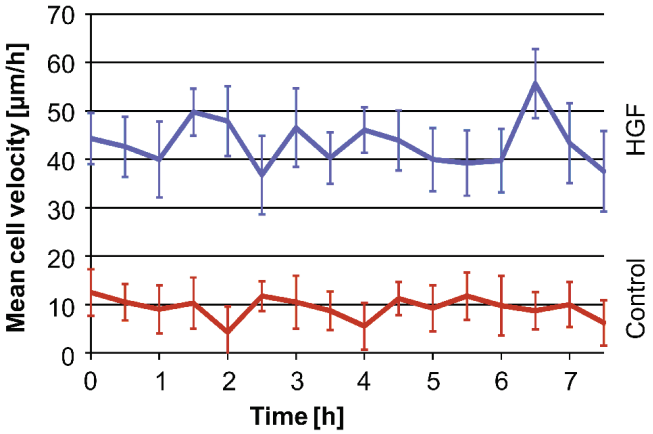

Fig. 4: Mean cell velocity in time. A significant change in the motility of the stimulated cells can be observed after half an hour; after 1 hour, the mean cell velocity reaches its maximum. Error bars: standard deviation.

Conclusions

We have realized, characterized and experimentally tested a live cell imaging platform for the real-time investigation of individual cell motility. The obtained results show that with our live cell monitoring platform we are able to successfully extract and measure every cell motility parameter. Furthermore, the compatibility with lab disposables and standard lab incubators and the ability to simultaneously track up to four biological samples in different conditions make our system attractive to achieve statistically relevant measurements.

Acknowledgements

This project is a part of the EU Marie Curie Research Training Network (MRTN) CellCheck “On-Chip Cell Handling and Analysis”, Project No. 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 during the early testing phase of the device. They also thank Ewald Pirker of the Institute of Sensor and Actuator Systems, Vienna University of Technology, for fabricating the platform holder.

References

[1] S. Suresh: “Biomechanics and biophysics of cancer cells”, Acta Biomater, Vol. 3, 2007, pp. 413 – 438

[2] M. Poujade, E. Grasland-Mongrain, A. Hertzog, J. Jouanneau, P. Chavrier, B. Ladoux, A. Buguin, P. Silberzan: “Collective migration of an epithelial monolayer in response to a model wound”, PNAS, Vol. 104, 2007, pp. 15988 – 15993

[3] N. Moscelli, S. van den Driesche, W. Witarski, S. Pastorekova, M.J. Vellekoop: “An imaging system for real-time monitoring of adherently grown cells”, Sens Act A Phys, accepted for publication, doi: 10.1016/j.sna.2011.05.010

[4] R. Hartmann-Petersen, P.S. Walmod, A. Berezin, V. Berezin, E. Bock: “Individual cell motility studied by time-lapse video recording: influence of experimental conditions”, Cyt A, Vol. 40, 2000, pp. 260 – 270