
Proceedings Paper:
Zhang, Z., Bedder, M., Smith, S.L. et al. (3 more authors) (2015) Automated Motion Analysis of Adherent Cells in Monolayer Culture. In: Information Processing in Cells and Tissues. 10th International Conference, IPCAT 2015 Lecture Notes in Computer Science, 9303 . Springer Verlag , pp. 185-194. ISBN 978-3-319-23107-5

https://doi.org/10.1007/978-3-319-23108-2_16

Automated Motion Analysis of Adherent Cells in Monolayer Culture

Zhen Zhang[1], Matthew Bedder[2], Stephen L. Smith[1], Dawn Walker[3], Saqib Shabir[4], Jennifer Southgate[4]

[1]Department of Electronics, University of York, Heslington, York YO10 5DD, UK
zz636@york.ac.uk, stephen.smith@york

[2]Department of Computer Science, University of York, Heslington, York YO10 5GW, UK
mb708@york.ac.uk

[3]Department of Computer Science & Insigneo Institute for in silico Medicine, University of Sheffield, Sheffield S1 4DP, UK
d.c.walker@sheffield.ac.uk

[4]Jack Birch Unit, Department of Biology, University of York, Heslington, York YO10 5DD, UK
jennifer.southgate@york.ac.uk

Abstract

This paper presents a novel method for tracking and characterizing adherent cells in monolayer culture. A system of cell tracking employing computer vision techniques was applied to time-lapse videos of replicate normal human uro-epithelial cell cultures exposed to different concentrations of adenosine triphosphate (ATP), acquired over a 20 hour period. Subsequent analysis, comprising feature extraction, demonstrated the ability of the technique to successfully separate the modulated classes of cell.

1 Introduction

The bladder is lined by urothelium, a remarkable tissue that forms the tightest and most efficient self-repairing barrier in the body. After physical or other damage, the urothelium switches rapidly and transiently from a stable mitotically-quiescent barrier into a highly regenerative state. The mechanisms involved in this switch are poorly understood, but are central to understanding the pathophysiology of the human urinary bladder.

The urothelium is reported to respond to mechanical and chemical stimulation by releasing various transient mediators, including adenosine triphosphate (ATP), which have been proposed to play a role in mediating neuronal signalling [1]. In addition,

the urothelium expresses purinergic P2X and P2Y receptors and channels that are responsive to ATP released from autocrine or paracrine sources [2]. The outcome of such signalling is incompletely understood, as it could have a feedback role in modulating neuronal signalling, but alternatively could play a more direct role in urothelial barrier repair [2]. It has been further suggested that aberant expression of receptors and/or mediator release by the urothelium is involved in dysfunctional diseases of the bladder, including idiopathic detrusor instability and interstitial cystitis [3, 4].

Despite the literature reporting expression of these channels and receptors by the urothelium, consensus has been confounded by contradictions in experimental approaches, including the species, specificity of reagents, and the nature of the tissue preparation (reviewed [5]). Our approach to address these questions has been to develop a cell and tissue culture system for investigating normal human urothelial cells and tissues in vitro. In our work, we have shown that stimulation of P2 receptors with exogenous ATP enhanced scratch wound repair, as did the addition of the ecto-ATPase inhibitor ARL-67156, which prevents the breakdown of autocrine-produced ATP. By contrast, blockade of P2 activity inhibited scratch wound repair in either the presence or absence of ATP [2]. This indicates that ATP is one of the major factors released upon damage and contributes to the regenerative phenotype.

To understand the effect of ATP on urothelial cell phenotype, time-lapse videos have been generated of urothelial cell cultures to which exogenous ATP has been applied. This paper describes the development of an automated method for objective measurement of these videos using computer vision techniques, followed by the extraction of features, with the aim of describing the cell behaviour. These processes are described in detail in Section 2, where measurements have been obtained from the set of videos of urothelial cell cultures with and without exogenous ATP added. The results with statistical analysis are presented in Section 3, verified by manual analysis.

2 Methods

The automated analysis of cell motion comprises the following sequence of analysis: capturing images of the cells at regular intervals using videomicroscopy, tracking cells within the video on a frame-by-frame basis using custom-written software, followed by characterisation of cell movement through the extraction of specially designed features. Each of these stages is considered in further detail in the following sections.

2.1 Cell Culture and Videomicroscopy

Normal human urothelial (NHU) cells were established in culture as finite (non-immortalised) cell lines and maintained as detailed elsewhere [6]. For ATP experiments, cells were seeded in a 12 well plate and the growth medium was supplemented with 0, 10 or 50 μM ATP in replicates of four. Cultures were observed by differential interference contrast videomicroscopy (Olympus IX81 microscope) in

an environmental chamber with an automated mechanical stage. Timelapse videos were compiled from individual images captured digitally every 10 minutes over a 20 hour time period. A sample frame from one such video is illustrated in Fig.1.

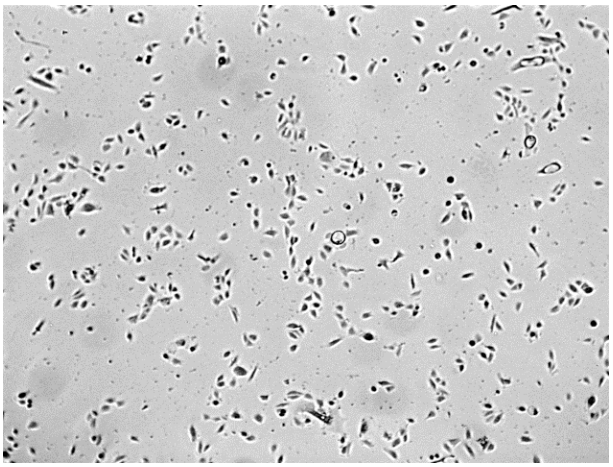


Fig. 1. Sample frame from timelapse video of NHU cells in culture.

2.2 Cell Tracking

Custom-written software was developed to undertake automated cell tracking using the OpenCV computer vision programming library [7]. In order to track the relative movement of cells within a video, each frame undergoes processing to identify the likely locations of cells. This process takes the raw videos as an input, performs common preprocessing to each frame, and then either tries to identify the likely location of cells, or track the location of previously located cells.

Each video frame initially undergoes Gaussian blurring to remove noise, followed by simple thresholding against a predetermined fixed value, resulting in a binary image separating the foreground and background (i.e. the cells from the frame background). A distance transform is then applied to this binary image, resulting in frames where the centre of large cells (or masses of cells) have a larger value, the edge of cells have a low value, and the background has a value of 0.

In order to efficiently estimate the locations of the centres of cells, the local maxima of the distance images are computed. Local maxima are then selected from the highest to lowest scoring, with a small area around each selected maxima being filtered out to reduce the number of selections made within the body of a cell. The (x,y) coordinates of the selected maxima are then used as estimations of the locations of cells within the frame.

To estimate the location of a cell within a frame, given the location within the previous frame, the distance image around the previous cell location is first multiplied

by a simple Gaussian filter. The maximal pixel value in this region can then be used to estimate the new cell location. This approach, although simplistic, is demonstrated to be effective. The usage of the distance image promotes matches with the centre of cells, whilst the application of the Gaussian filter means that matches are preferred that are close to the original location of the cell.

Although the process for tracking cells works well over the videos so far tested, it is unable to consistently identify and track cell locations for the duration of the videos. In order to detect as many cells as possible, a large number of potential cell locations are initially calculated, with many of these quickly converging into the same locations. Similarly, the cell tracking process can occasionally fail to track the location of cells within frames, meaning that if cell detection were only to be performed on the first frame of the video then many cells would not be tracked in the latter parts of the video. These difficulties associated with tracking cells can be due to cell proliferation - giving rise to new cells, cell death – the loss of cells, and cells moving in and out of the field of view.

In order to cope with these issues, an approach was adopted where duplicated cell locations are removed from the tracking process and cell detection is performed at regular intervals to find new candidate locations. This approach is found to be effective and results in location data for a sufficient number of cells over the duration of the video to adequately describe the cell population behavoir. The entire cell tracking process is summarised in Fig. 2.

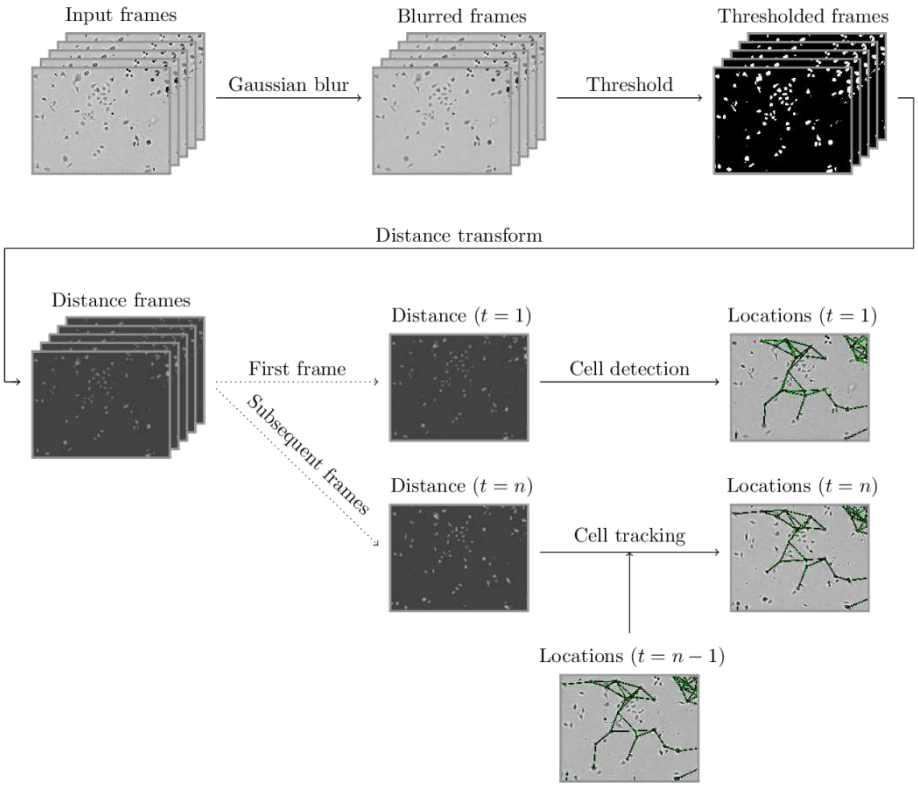


Fig. 2. The process used for detecting cell locations within a video, and tracking detected cells between video frames

2.3 Feature Extraction

Once the location of cells has been identified for each frame of the video in the form of (x,y) coordinate pairs, it is possible to extract features with the aim of describing the cell population behaviour. This was undertaken using the MATLAB programming environment [8] and to illustrate the processing applied, a single cell from a video analysed is taken as an example to demonstrate how features of interest are calculated. The cell selected has been tracked from a video of NHU cell culture with 50 μM exogenous ATP. As this cell was successfully tracked from the beginning to the end of the video, its path can be shown graphically as depicted in Fig. 3.

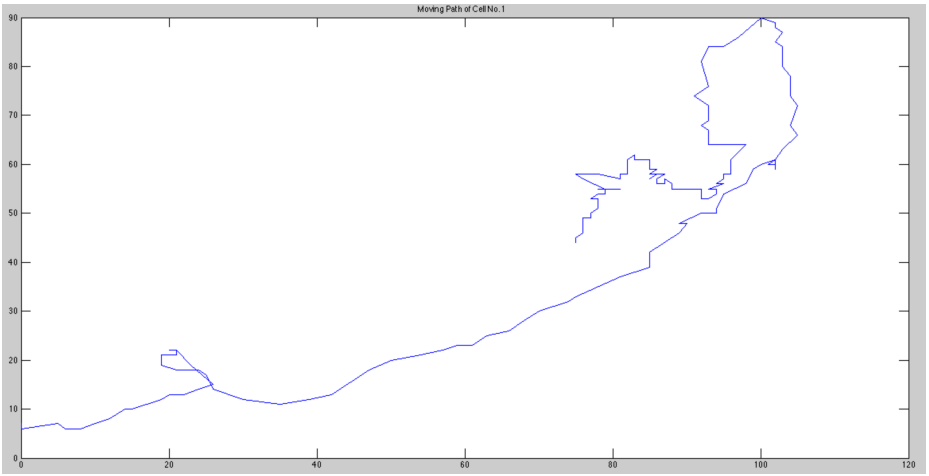


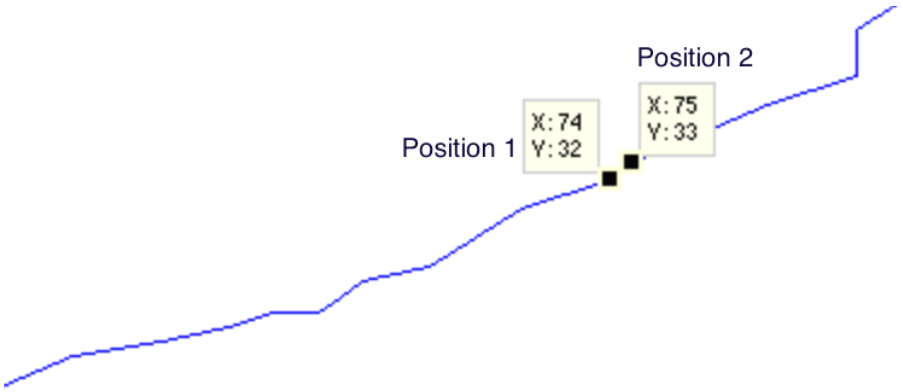
Fig. 3. Example tracking of a single cell over a 20 hour video sampled every 10 minutes.

2.4 Choice of Features

The choice of features to extract from the videos was made on the basis of their subsequent use in describing cell behaviour. For this reason features of (i) cell migration speed and (ii) migration persistence were defined as described below.

2.4.1 Cell Migration Speed

Speed of an object is the rate of change of its position. In this case, the aim is to obtain the migration speed of a cell from a video, which can be determined by calculating the number of pixels travelled over a certain time interval. The time interval applicable in this context is that between two consecutive video frames, at a frame rate of one every 10 minutes. The migration speed is therefore simply obtained by calculating the Euclidean distance between the two pairs of coordinates for the cell between consecutive frames. This is shown graphically in Fig. 4 where the initial position of the cell is at coordinates (74,32) and in the subsequent frame, coordinates (75,33). Hence, the distance travelled by this cell over time *dt* (10 minutes), and subsequently, its speed, can be calculated. The migration speed of all cells tracked during the entire video was calculated in the same way.

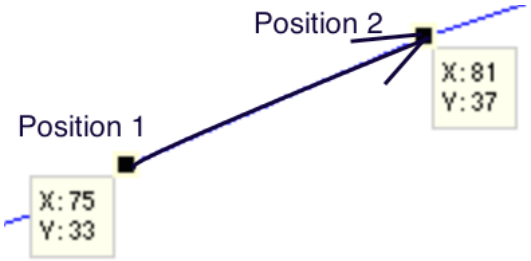


$$\text{Cell migration speed} = \frac{\sqrt{(x'-x)^2-(y'-y)^2}}{dt}$$

Fig. 4. Example calculation of cell migration speed.

2.4.2 Cell Migration Persistence

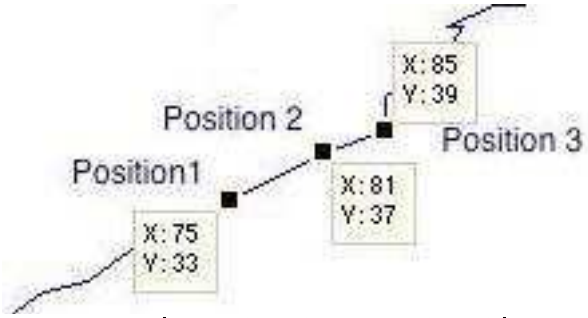
In cell migration, persistence is one of the features in which biologists are most interested and can be described as the tendency of cells to change direction. Hence, obtaining the direction of travel of the cell in each frame of the video is essential for calculating migration persistence.



$$\text{Direction of travel} = \tan^{-1}\left(\frac{y'-y}{x'-x}\right) \text{ radians}$$

Fig. 5. Direction of travel of cell migration.

Fig. 5 shows how the angle of the vector formed from the coordinates of the cell in consecutive frames of the video can be used to determine the direction of travel. Angular Velocity is defined here as the rate of change of the direction of travel of a cell over subsequent frames. Fig. 6 illustrates an example calculation over two consecutive frames.



$$\text{Angular Velocity} = \left|\tan^{-1}\left(\frac{37-33}{81-75}\right) - \tan^{-1}\left(\frac{39-37}{85-81}\right)\right| \text{ radians/frame}$$

Fig. 6. Example calculation of cell migration persistence over two consecutive frames.

3 Results

Four time-lapse videos have been generated from three classes of NHU cell cultures: (i) a control culture with no ATP; (ii) a culture with 10 μM ATP; and (iii) a culture with 50 μM ATP. The average cell migration speeds and average angular velocity for each video is presented in Table 1.

Table 1. Automated average migration speed and average angular velocity values for a control culture with no ATP, a culture with 10 μM ATP and a culture with 50 μM ATP.

Cell Culture Video	Average Migration Speed (pixels/frame)	Average Angular Velocity (rads/frame)
Control1	3.52	1.31
Control2	3.75	1.35
Control3	3.45	1.37
Control4	3.56	1.36
10uM ATP1	3.04	1.52
10uM ATP2	3.09	1.39
10uM ATP3	3.08	1.52
10uM ATP4	3.01	1.46
50uM ATP1	2.00	1.79
50uM ATP2	2.22	1.74
50uM ATP3	2.06	1.77
50uM ATP4	1.83	1.85

By applying analysis of variance (ANOVA), it can be seen in Fig. 7 that the separation between the three classes for migration speed are statistically significant. Verification of these results was confirmed by comparing with manual tracking of 15 random cells for each experimental condition as shown in Fig. 7. Similarly, results

for angular velocity, shown in Fig. 8, also demonstrate good separation between the three sets of culture conditions.

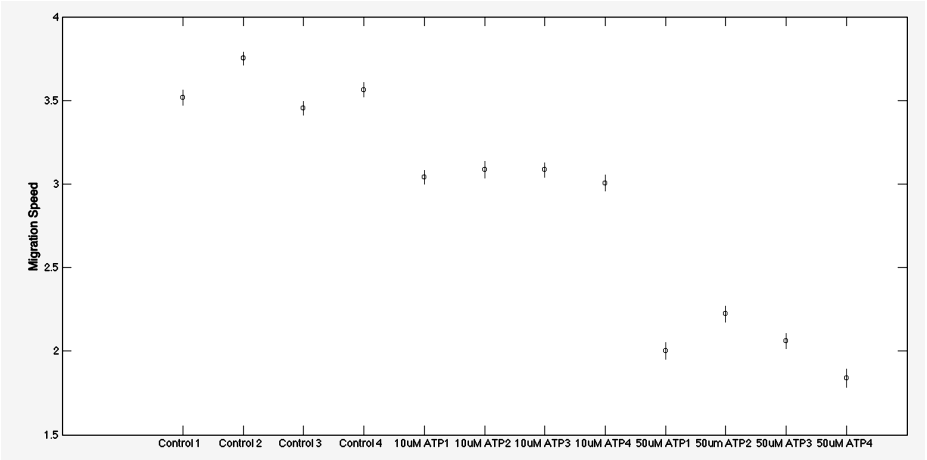


Fig. 7. Automated calculation of cell migration persistence. Average migration speeds are shown in F-distribution form for Control, 10uM ATP and 50uM ATP Videos: small circles mark the mean of the group and the bars the 95% confidence interval.

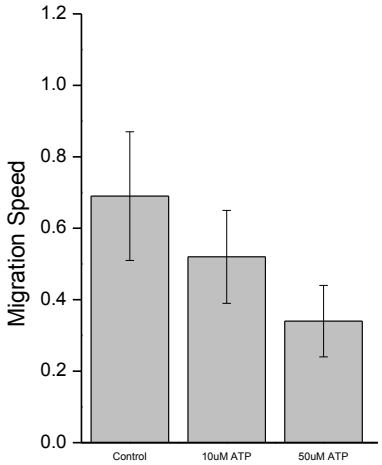


Fig. 8. Calculation of cell migration persistence using manual tracking.

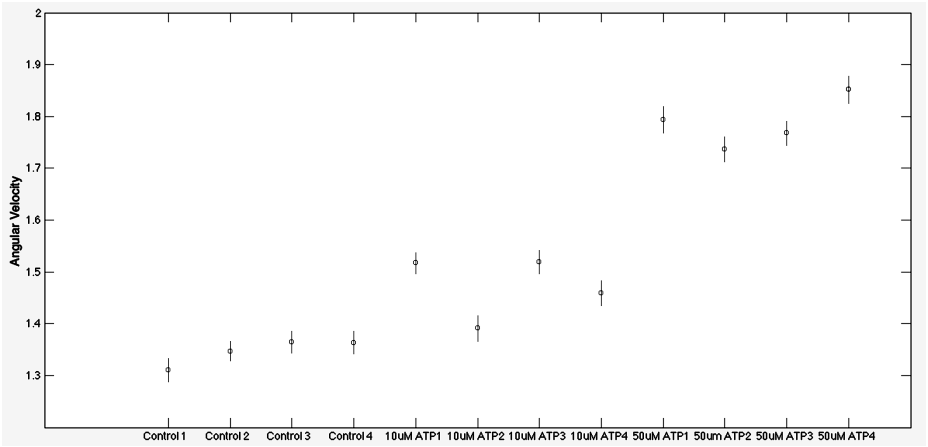


Fig. 9. Automated calculation of cell angular velocity. Average migration speeds are shown in F-distribution form for Control, 10uM ATP and 50uM ATP Videos: small circles mark the mean of the group and the bars represent 95% confidence intervals.

4 Conclusion and Discussion

The application of an automated approach for tracking and characterizing adherent cells in monolayer culture has been presented that compensates for the loss and gain of cells during the course of the video – a commonly encountered problem in tracking systems of this type. This opens the possibility of lineage-tracking within mixed populations, where the automated approach removes the labour intensive nature of manual tracking. Here, the classification of cells from cultures with and without exogenous ATP has identified statistically significant effects on cell behaviour that will contribute to understanding of urothelial tissue repair mechanisms and the role of ATP.

We do not yet understand the implications of the results for urothelial biology as we have not previously had the capability to measure these aspects of cell behaviour. Purinergic receptor activation by ATP as a result of cellular release upon injury has been implicated in cellular migration during the restitution of cornea [9], airway [10] [11] and bladder [2] epithelial tissues, suggesting common effects. The underpinning mechanisms are poorly understood, although amplification of TGFβ1-induced actin remodelling associated with cell migration has been suggested [11]. The results of our study were unexpected in that we found ATP to give increased angular velocity and reduced migration speeds. However, we studied the effect of exogenous application of ATP rather than local endogenous ATP release and although speculative, it may be that the precise mode (concentration/locality) of ATP

stimulation might affect cell migration response including directionality, for recruitment to the wound.

Current and future work is focused on further characterisation of the behaviour of cells using additional extracted features that relate to *cohesivity* – the tendency of cells to form and stay in clumps. The nature of this contact can be described in terms of the duration of the contact and number of cells that form a clump, as well as the effect on post-contact migration speed and angular velocity of individual cells. These characteristics relate to the physical nature of the contact that forms between cells and provides critical information as to what extent they are transient or more sustained in character.

References

1. Birder, L.A.: Urothelial signaling. Handbook of experimental pharmacology 207-231 (2011)
2. Shabir, S., Cross, W., Kirkwood, L.A., Pearson, J.F., Appleby, P.A., Walker, D., Eardley, I., Southgate, J.: Functional expression of purinergic P2 receptors and transient receptor potential channels by the human urothelium. American Journal of Physiology-Renal Physiology 305, F396-F406 (2013)
3. Birder, L.A., de Groat, W.C.: Mechanisms of disease: involvement of the urothelium in bladder dysfunction. Nature clinical practice. Urology 4, 46-54 (2007)
4. Sun, Y., Chai, T.C.: Up-regulation of P2X3 receptor during stretch of bladder urothelial cells from patients with interstitial cystitis. The Journal of urology 171, 448-452 (2004)
5. Yu, W., Hill, W.G.: Defining protein expression in the urothelium: a problem of more than transitional interest. American journal of physiology. Renal physiology 301, F932-942 (2011)
6. Southgate, J., Hutton, K.A., Thomas, D.F., Trejdosiewicz, L.K.: Normal human urothelial cells in vitro: proliferation and induction of stratification. Laboratory investigation; a journal of technical methods and pathology 71, 583-594 (1994)
7. Bradski, G.: The opencv library. Doctor Dobbs Journal 25, 120-126 (2000)
8. MATLAB R2014A. The MathWorks, Inc., Natick, Massachusetts, United States.
9. Boucher, I., Rich, C., Lee, A., Marcincin, M., Trinkaus-Randall, V.: The P2Y2 receptor mediates the epithelial injury response and cell migration. American Journal of Physiology-Cell Physiology 299, C411-C421 (2010)
10. Wesley, U.V., Bove, P.F., Hristova, M., McCarthy, S., van der Vliet, A.: Airway epithelial cell migration and wound repair by ATP-mediated activation of dual oxidase 1. Journal of Biological Chemistry 282, 3213-3220 (2007)
11. Takai, E., Tsukimoto, M., Harada, H., Sawada, K., Moriyama, Y., Kojima, S.: Autocrine regulation of TGF-β1-induced cell migration by exocytosis of ATP and activation of P2 receptors in human lung cancer cells. Journal of cell science 125, 5051-5060 (2012)