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Analysis of Cell Motility with a Successive Sequence of Images

Analiza gibanja celic s serijo zaporednih fotografij

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Abstract. Analysis of cell motility usually requires several hours of continuous video recording of a single view field. Consequently, the method is very much time consuming and only a small number of cells is usually included into analysis. To increase the number of cells monitored in an experiment we developed a simple and expeditious method for analysing the cell motility. A combination of successive series of images of individual cells relocated on CELLocate coverslips and vector graphic editing software Adobe Illustrator 9.0 enabled simultaneous monitoring of locomotion for up to ten times more cells as with previous methods.

Keywords: Cell motility, digital camera, successive sequence of images, software Adobe Illustrator 9.0.

Izvleček. Analiza celičnega gibanja ponavadi zahteva večurno snemanje enega vidnega polja z video kamero. Metoda je zato zamudna in le malo celic se lahko vključi v analizo. Z namenom povečanja števila celic v določenem poizkusu, smo razvili preprosto in hitro metodo za analizo celičnega gibanja. S kombinacijo zaporednega slikanja istega vidnega polja, kar nam je omogočila uporaba CELLokate krovnih stekelc, in vektorskega grafičnega programa Adobe Illustrator 9.0, smo lahko v analizo gibanja celic vključili do desetkrat več celic, v primerjavi z dosedanjimi metodami.

Ključne besede: Celično gibanje, digitalni fotoaparat, zaporedna serija posnetkov, Adobe Ilustrator 9.0

Introduction

Monitoring of cell motility provides information about the level of cell differentiation, its responsiveness to growth factors and can determine at which phase of cell cycle the cell is (BONNETON & al. 1999). The motility of cells is involved in many physiological (embryonic development) and pathological processes (invading malignant cells, immune response and wound repair). Epithelial cells are very much restricted in their motility, because of their attachment to the neighbouring cells and to the extracellular matrix. In order to move from their original place to the final destination, the

epithelial cells have to go through epithelio-mesenchymal transformation (EMT). Cells, that undergo EMT, become motile and assume fibroblast appearance. Mesenchymal cells have a specialized ability to move through the extracellular matrix, they have front-to-back end polarity and form only transient contacts with their neighbours and the extracellular matrix (HAY 1995).

For the measurement of cell motility, various methods have been used recently, mainly using video microscopy and analysis of a sequence of images taken at specific intervals (MORTON & TCHAO 1994, WICK & al. 2003). The common problem for all these methods is that they require monitoring of the same view field for a prolonged period of time due to slow motility of cells. Recently, cover slips with a microgrid became available. They enable relocation of individual cells and consecutive monitoring of several view fields within the time frame of a single experiment.

We have developed a method for measurement of cell motility, where a series of consecutive shots with digital camera can be taken on several view fields within the time frame of a single experiment. Series of images, analysed with vector graphic editing software, precisely show the path, direction and speed of individual cell.

Materials

Cell culture

Cultures of mice urothelial cell line (g/G) were maintained in 1 : 1 mixture of cell culture medium DMEM and HAM F12 with 5 µg/ml insulin, 5 µg/ml transferrin, 5 ng/ml selenit, 100 ng/ml hydrocortisone in 10 % fetal calf serum. All chemicals were obtained from SIGMA Chemical Co. (St. Louis, MO). Cells were grown on microgrid cover slips (Eppendorf CELLocate Coverlips, square 55 µm) in plastic dishes (radius = 2 cm). Motility was stimulated with FGF1 (20 ng / ml) for 48 hours.

Between photographic expositions, the cell cultures were maintained in incubator at 37 °C. The cells were outside the incubator, during taking images, for the maximum of 5 minutes. The room temperature was between 28 °C and 30 °C.

CELLocate coverslips

CELLocate is a round coverslip made of glass in accordance with ISO 8255. The diameter of the coverslip is 12 mm. CELLocate is suitable for light, fluorescence and electron microscopy. Coverslips are available in two different grid sizes: the 55 µm grid size (measured between the inner edges of the measuring square) is suitable for relocation of individual cells, while the 175 µm grid size is more suitable for relocation of cell groups. In this study, we have used coverslips with 55 µm grid size. The grid is composed of 4 x 6 squares and marked alphanumerically.

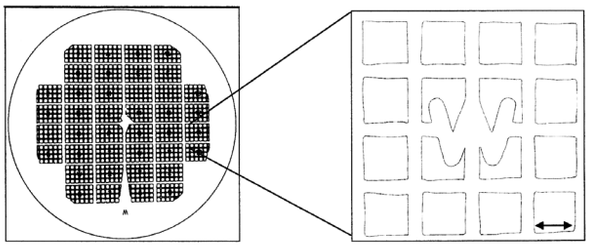


Figure 1: A microgrid on a CELLocate coverslip. The arrow indicates a 55 µm distance.
Slika 1: Mikromreža na CELLocate krovnem stekelcu. Puščica označuje 55 µm razdaljo.

Consecutive photography with digital camera

Digital camera NIKON COOLPIX 950 attached to inverted phase contrast microscope (NICON TE 300) was used.

A low-density culture of g/G cells grown on CELLocate coverslips, with the measuring grid, was used for the analysis of cell motility. The areas with 3 – 5 cells per square were selected. The same area on CELLocate coverslips was relocated every 30 minutes for 5 hours and 30 minutes and as a result, 12 images of an individual view field were obtained.

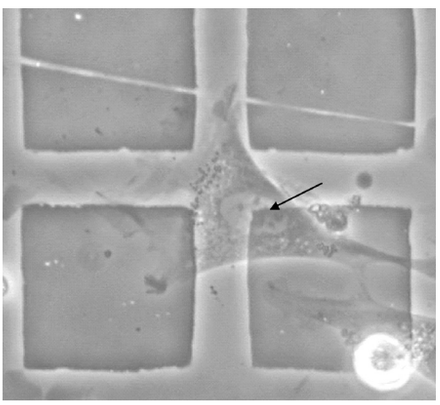


Figure 2: A cell grown on a microgrid of CELLocate coverslip. An arrow points to the nucleus.
Slika 2: Celica na mreži CELLocate krovnega stekelca. Puščica kaže jedro celice.

Results

The analysis of a sequence of consecutive images with Adobe Illustrator 9.0

The sequence of images, taken with digital camera, were copied to the hard disk of a personal computer. For the analysis of the motility a vector graphic editing software Adobe Illustrator 9.0 was used.

The analysis of cell motility was performed as follows.

The working sheet was resized to 55 x 55 mm in order to adjust it to the size of CELLocate microgirds square, which was 55 x 55 μm (Fig. 3)

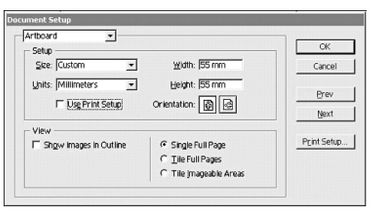


Figure 3: Resizing the working sheet: File > New > Document Set-up > Units = mm > Width = 55 mm > Height = 55 mm.
Slika 3: Umerjanje velikosti lista: Datoteka > Nova > Nastavitev dokumenta > Enote = mm> Širina = 55 mm > Višina = 55 mm

During the next step, a sequence of images were added using the »new layer« for each photograph in the sequence and borders of the microgrid square were aligned to the borders of the working sheet (Figs. 4 and 5).

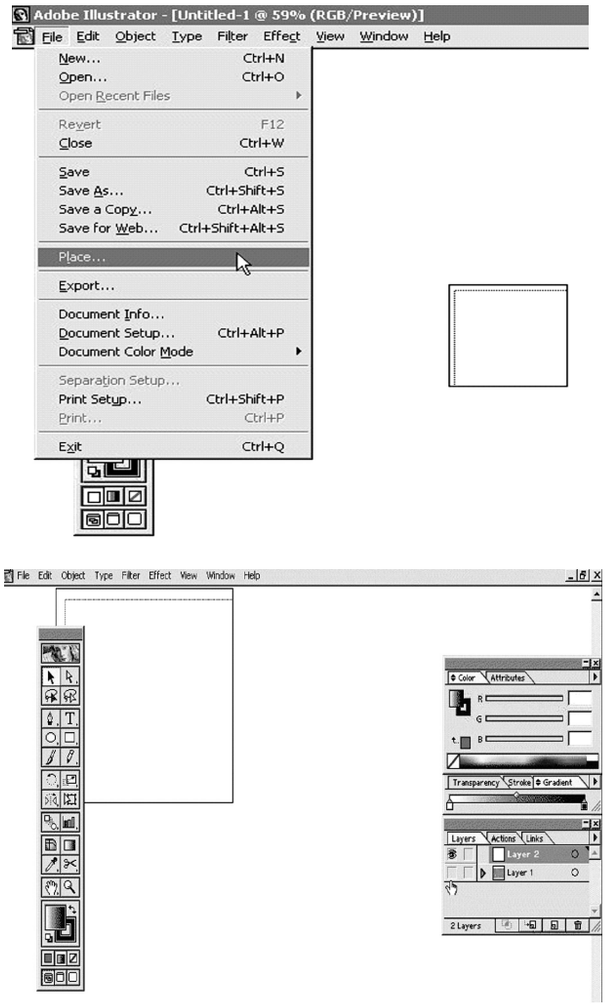


Figure 4 and 5: Serial addition of the pictures: Window > Show Layers > Layer 1 > File > Place (place 1st picture) (Fig. 4) > New Layer > Layer 2 (place second picture) (Fig 5).
Sliki 4 in 5: Zaporedno dodajanje slik: Okno> Pokaži plasti > Plast 1 > Datoteka > Postavi (postavimo prvo sliko) (Slika 5) > Nova plast > Plast 2 (postavimo drugo sliko) (Slika 5).

A contour of a nucleus of individual cell was made on each picture by using graphic tools (Fig.6). Each picture in a sequence was labelled and listed (Fig. 7 and 8).

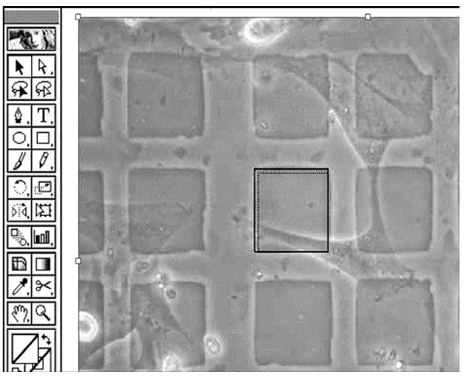
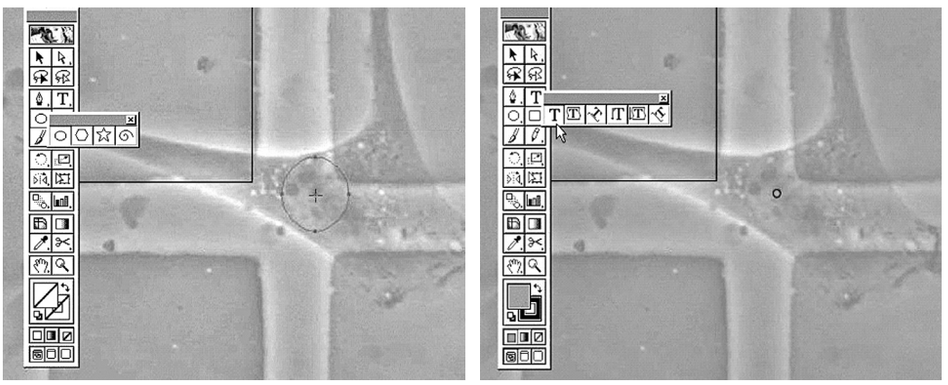


Figure 6: Borders adjustment: Window > Show Tools > Selection Tool (an arrow) > press and hold »shift« button and draw the corner of the picture with an arrow to adjust the borders of microgrid and working sheet.
Slika 6: Prilagoditev robov: Okno > Orodja > Izbrano orodje (puščica) > ob sočasnem držanju tipke »shift« s puščico vlečemo vogal fotografije, da se pokrijeta robova delovnega lista in kvadrata mikromreže



Figures 7 and 8: A contour of a nucleus: Tool > Ellipse Tool > (Fig.7) draw a contour of a nucleus > click in the centre of the ellipse > (Fig. 8) choose the width and length of the ellipse (2 x 2 mm) > Window > Show Colour > Selection Tool > choose the new ellipse (circle) > colour the circle > Type Tool > write the serial number of the picture
Sliki 7 in 8: Obrisovanje jedra: Orodja > Elipsa > (Slika 7) nariši obris jedra > klikni v sredino elipse > (Slika 8) izberi širino in višino elipse (2 x 2 mm) > Okno > Pokaži barve > Izbrano orodje > izberemo novo elipso > obarvamo elipso > Črkovno orodje > vpišemo zaporedno številko slike

The use of different colours (Window > Show Colour), transparency (Window > Show Transparency) and/or darker outlines (Window > Show Colour) is recommended to distinguishing partly overlapping nucleus positions in slow moving cells.

The procedure was repeated for all twelve images taken. Each photograph was deleted after contouring the nucleus and only contours were retained on the layers to reduce the size of the file (Fig. 9). In the end, all the layers were made visible on the monitor and the path of cells was analysed.

While the distance between the two points, showing position of the nucleus in 30 minutes intervals, was short (7-9 μm), the path between the points was estimated to be straight.

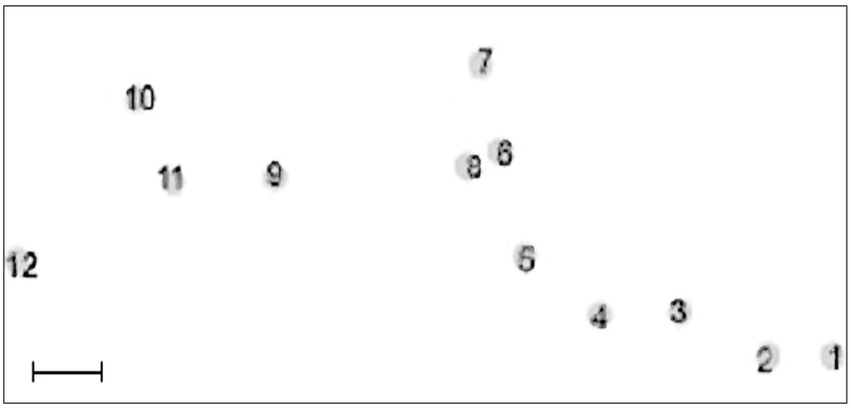


Figure 9: Result of 12 consecutive positions of a cell nucleus. Bar = 10μm
Slika 9: Prikazanih je 12 zaporednih položajev jedra gibajoče se celice. Merilna črtica = 10 μm

The velocity of an individual cell was calculated from the distance the cells reached in a definite time interval. The path was manually measured on a printed cumulative image, containing the positions of the nuclei at 30 minutes intervals. The system error was calibrated by 12 measurements of the distance between the same two points (from the centre to the centre of two points). The error in measurements of the path between the two points was ± 0.3 μm.

Discussion

The aim of the present study was to find an exact and quick method for the measurement of individual cell migration. The system of consecutive images and vector graphic editing software Adobe Illustrator 9.0 was used.

The problem in analysing epithelial cell migration is that an average speed of motility is approximately 20 μm per hour, so at least 5 hours of monitoring is required for the analysis of the motility of cell moving in a single view area (BRAY 1992). Thus, in most studies the number of monitored cells was limited less than eighth cells within an experiment (MORTON & TCHAO 1994, WICK & al. 2003). With our method, using series of successive images of cells on defined areas of a coverslip with a micro-grid, motility of more than 80 cells (20 cells per experimental group) was analysed within the time frame of a single experiment. The increased number of monitored cells makes the results of measurements more accurate.

In our experiments, where the velocity of urothelial cell line g/G was measured after stimulation with FGF1, we used 30 minutes long intervals, because we ascertained that this is the optimal interval for the velocities close to 20 μm / h. In 30 minutes, the centre of gravity of the fastest cells

changed their position for 10 μm, which is approximately half of the cell length. Therefore, the 30 minutes long interval between snap-shots is long enough for monitoring of five view fields with minimal error. Because of various velocities of cell motilities in different cell types and frequent changes of direction, shorter intervals are appropriate for the analysis of higher velocity. However, shorter intervals reduce the number of experimental groups, which can be monitored simultaneously.

During motility, cells undergo dramatic phenotype changes that are mainly based on the dynamic assembly and disassembly of actin filaments underlying the plasma membrane (BRAY 1992). Cells that become motile usually assume a fibroblastic appearance with lamellipodia adjusting the shape by each change of the motility direction (BONNETON & al. 1999, BRAY 1992). Such changes of the cell shape make motility of different parts of the cell inhomogeneous and so difficult to analyse. The problem is usually solved by determining nucleus as the centre of gravity. Because this most prominent organelle of eukaryotic cells, lies in the area, where usually most of the cell mass is concentrated (BONNETON & al. 1999). In our experiments, a contour of nucleus was drawn with Adobe Illustrator 9.0 software. The nuclei of our cells are usually round, which simplifies drawing a contour. The monitoring of an individual cell nucleus displacement was enabled by the use of CELLocate coverslips with a microgrid.

Until now, the displacement of each individual cell has usually been followed using recording tape of time-lapse video recorder, tracing paper and curvimeter. Due to better resolution compared to most video cameras, which were mainly used in such studies (BONNETON & al. 1999, MORTON & TCHAO 1994, WICK & al. 2003), makes digital camera better choice for the analysis of cell motility. Better resolution is important for the accurate localization of the nuclei taken as the centre of cell gravity.

During cell division, the nuclear envelopes become fragmented and reorganization of cytoskeleton appears. Cells become rounded, reduce the attachment surface and become immobile until the end of mitosis (BONNETON & al. 1999). Occasionally such dividing cells detach from the surface and become lost. Changing of cell morphology and motility during cell division represents an obstacle in measurements of velocity. For this reason, it is of benefit to monitor as many cells as possible.

Certain cell lines do not adhere well to glass and therefore, do not grow well on CELLocate. In such cases, Eppendorf Company recommends that CELLocate is coated with collagen. Using collagen is not appropriate for the coverslips with 55 μm grid size, because it reduces the clarity and visibility of cell nucleus. The margins of 175 μm grid size coverslips are deeper, which enhance the contrast and so enable the use of collagen coating.

This method for analysing the cell motility with a combination of successive series of images of individual cells and vector graphic editing software Adobe Illustrator 9.0 has revealed itself as very successful, while being quick and exact. Most of all it enables simultaneous monitoring of locomotion for much larger number of cells in comparison with previously used methods.

Povzetek

V dosedanjih raziskavah so za spremljanje gibanja celic uporabljali predvsem videomikroskopijo. Vse dosedanje metode omogočajo spremljanje gibanja celic le v enem vidnem polju za vsak eksperiment. Zaradi majhne hitrosti gibanja celic je spremljanje celic zamuden proces, zato je večina analiz omejena na majhno število celic, kar zmanjša verodostojnost dobljenih rezultatov. Z namenom, da bi lahko hkrati spremljali gibanje celic na več vidnih poljih, smo razvili metodo

zaporednega fotografiranja z digitalnim fotoaparatom in analizo hitrosti gibanja z grafičnim vektorskim programom Adobe Illustrator 9.0.

Med celičnim gibanjem se oblika celice spreminja, zato smo spremljali spremembe položaja težišča izbranih celic. Za težišče celice smo določili vedno bolj ali manj okroglo jedro, ki ponavadi leži v področju, kjer je zbrana glavnina mase celice.

Namesto videokamere smo uporabili digitalni fotoaparat, ki zaradi boljše ločljivosti omogoča natančnejše meritve. V določenih časovnih zamikih smo izbrana polja s celicami zaporedoma fotografirali in tako dobili serijo slik, ki ponazarjajo smer in hitrost gibanja določene celice. Ponovna lokalizacija celic in merjenje premika celice je omogočila uporaba CELLocate krovnih stekelc z jedkano mrežo. Serija dobljenih posnetkov individualnih celic predstavlja prepotovano pot celice, iz katere lahko izračunamo hitrost potovanja. Predstavljena metoda omogoča spremljanje celic na več vidnih poljih istočasno, kar lahko bistveno poveča število opazovanih celic in s tem relevantnost rezultatov merjenja hitrosti gibanja celic.

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