

## Implementation of image analysis techniques in gastric cancer cell motility analysis

Robertas Petrolis, Rima Ramonaitė, Gediminas Kiudelis, Greta Varkalaitė, Limas Kupčinskas, Algimantas Kriščiukaitis

Lithuanian University of Health Sciences

Gastric cancer research studies are focusing on early detection of cancer, screening, and metastatic indicators, and monitoring therapeutic responses. Previous studies on gastric cancer research have shown that the regulation of biological processes, molecular mechanisms and signaling ways in cancer cells are altered when compared to healthy cells. Migration of cancer cells is a feature, considered as important, reflecting metastasis formation process and contributes to the whole process of gastric cancer. The aim of this research was to develop a method for evaluation of cultured cell migration, based on long-term continuous cell imaging. We investigated cultured gastric cancer cells MKN28. The series of phase-contrast cell culture images were taken during a period of 3-4 hours every 30 seconds. Mathematical morphology methods and structural analysis of image series was used to determine estimates reflecting individual and generalized cell motility estimates.

*Cell migration, Long term cultured cell imaging, mathematical morphology, gastric cancer cell line*

### Introduction

Gastric cancer represents a major cancer burden worldwide, and remains the second leading cause of cancer-related death. [1,2]. The overall survival rate is 15-20% [3]. Gastric cancer is typically diagnosed at advanced stage accompanied by extensive invasion and diagnosed metastasis. Successful therapeutic strategies in most cases are unfortunately limited. The recurrence and metastasis are the major challenges during treatment [4,5]. Previous studies have shown that the regulation of biological processes, molecular mechanisms and signaling pathways in cancer cells are altered when compared to healthy cells. Tumor cell invasiveness and migration to the healthy tissue leads to the emergence of metastases. Cancer cells migrate in various ways, according to cell type and degree of differentiation. Cell motility is an important feature reflecting tumor cell invasiveness and process of metastasis formation. Investigation of this process can give valuable results and information for development of new therapeutic methods [6-8].

The aim of this study was to develop a method for evaluation of cultured cell migration, based on long-term continuous non-stained gastric cancer cells imaging.

### Methods

*Cell culture.* The human gastric cancer cell line MKN28 was maintained in F-12 medium supplemented with 10% fetal bovine serum, and 1% antibiotics (50 IU/ml penicillin, 50 mg /ml streptomycin and 0.5 mg/ml gentamicin) in a humidified air containing 5% CO<sub>2</sub> at 37°C. The cells were trypsinized and 50 µl of the cell suspension was added to 75 cm<sup>2</sup> flasks with medium for the migration testing. Continuous long-term cell culture imaging was performed three times for each flask. After imaging cell culture plates were put back to incubator.

*Image acquisition.* Phase-contrast images of cells (see fig.1) were taken using OLYMPUS IX71 light microscope equipped with Q IMAGING EXI aqua camera at 1392x1040 pixels resolution. We used x10 magnification, suggested by histology specialist, at which each pixel was representing 0.6 µm x 0.6 µm real area. Images were taken

every 30 seconds during 3-4 hours while cell cultures where mounted on microscope and stored as series.

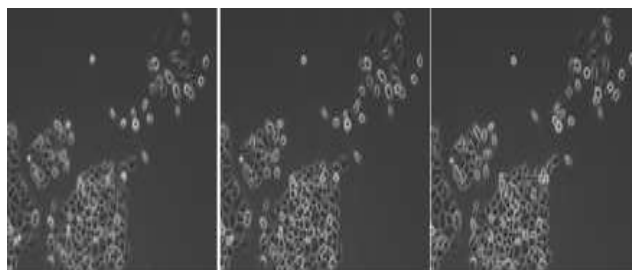


Fig. 1. Microscopic images of gastric cancer cell line MKN28

*Image analysis.* Image analysis algorithms were realized in MATLAB computing environment. Migration pathways of selected individual cells were tracked registering position of center of weight of identified cell body in each image in series. Identification of individual cell body was done by conversion of selected image fragment into binary format (Otsu automatic clustering-based image threshold [9]) followed by mathematical morphology based filtering using methods “imfill” and “imopen” with circle structural elements with optimal diameter [10,11]. According to Otsu's method threshold  $t$  for image conversion into binary format is found by search for such threshold that minimizes the intra-class variance (the variance within the class), defined as a weighted sum of variances of the two classes (1):

$$\sigma_{\omega}(t) = \omega_0 \sigma_0^2(t) + \omega_1 \sigma_1^2(t) \quad (1)$$

where  $\sigma_{\omega}(t)$  - is the intra-class variance of pixel values;

$\omega_{0,1}$  - are the respective probabilities of pixel values to be classified into two classes (the background and the cell) by threshold  $t$ ;

$\sigma_{0,1}^2$  - are the respective variances of these two classes (background and cell).

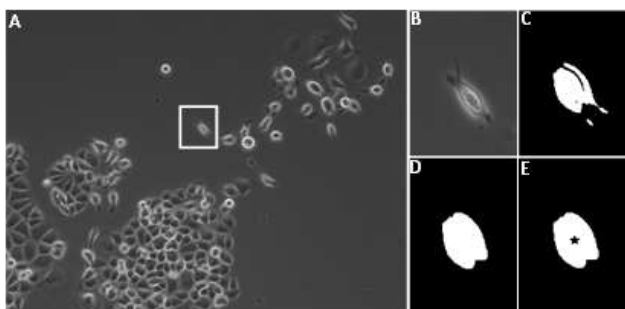
Coordinates of weight center of identified cell body was determined using special mathematical morphology

method realized in MatLab function 'regionprops' [12]. Difference in coordinates of such weight centers determined in neighboring images of the series represents single step of identified cell migration. Sum of the lengths of all steps of identified cell migration represents length of pathway of the cell. The ratio of length of pathway of the cell to time, during which whole series of images was taken gives an estimate of cell motility. From which other quantitative estimates can be calculated.

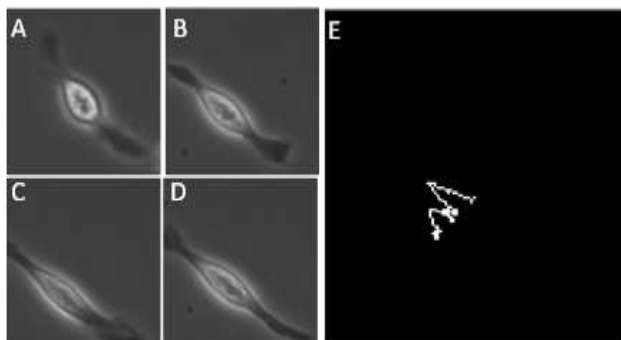
## Results and discussion

The ability of cells to migrate is essential for physiological functions such as immune surveillance, wound healing, and tissue morphogenesis during development. Pathological processes such as cancer invasion and metastasis also rely on the ability of malignant cells to acquire invasive and migratory capabilities. In this study, we presented the image processing algorithm which performs detailed cell motility analysis of phase-contrast illumination microscope images of non-stained gastric cancer cells [6, 13-17].

Illustration of steps of image processing algorithm detecting coordinates of center of weight of selected cell is presented on fig.2. Migrating cell is changing its shape, what we can see on fig.3.



**Fig. 2.** Illustration of steps of image processing algorithm detecting coordinates of center of weight of selected cell. A - raw image with marked area of interest; B - image excerpt with selected cell; C - binary version of the image excerpt after application of Otsu threshold; D - result of mathematical morphology filtering. E- detected center of weight of cell body.



**Fig. 3.** A, B, C, D - Images of the observed cell during its migration; E- pathway of cell movement.

However as reference point we take center of weight, determined as described above. The detected pathway of selected cell migration is shown on fig.3 E. In this case length of the pathway was 112 pixels, what corresponds to  $112 \times 0.6 = 67.2 \mu\text{m}$  during  $119 \times 30 \text{ sec.}$  (the series

contained 119 images, taken every 30 seconds). So, estimate of cell motility in this case was  $67,8 \mu\text{m/h}$ . We found cell motility estimate ranging from  $44.2 \mu\text{m/h}$  till  $92.6 \mu\text{m/h}$  in our processed image series. At the moment we have only few good quality series of images of each cell types, so statistical evaluation of results is left for near future works. However these first results illustrate the promising idea of evaluation of cultured cell motility to be used in studies of invasiveness of cancer cells, possible intensity of metastasis formation or monitoring of possible therapeutic responses.

## Conclusions

Long-term continuous cell imaging method, based on image structural analysis and mathematical morphology was elaborated for evaluation of cultured cell migration. Cell motility estimates could be used in studies of invasiveness of cancer cells, possible intensity of metastasis formation or monitoring of possible therapeutic responses.

## Acknowledgments

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### Skaitmeninių vaizdų analizės metodų taikymas skrandžio navikinių ląstelių migracijos tyrimuose

Santrauka

Moksliniai tyrimai parodė, kad biologinių procesų reguliavimo mechanizmai navikinėse ląstelėse yra pakitę lyginant su sveikomis ląstelėmis. Vėžinių ląstelių invazyvumas bei migracija į sveikus audinius lemia metastazių atsiradimą ir navikinių susirgimų sunkumą. Todėl atliekami ląstelių kultūrų funkciniai tyrimai, vertinamas terapijos priemonių ar biologinių veiksnių poveikis navikinių ląstelių migracijai. Darbo tikslas – sukurti ilgalaikio nuolatinio ląstelių kultūrų vaizdų registravimo ir analizės metodą skirtą skrandžio navikinių ląstelių kultūrų judrumui bei judėjimo trajektorijai rasti. Tyrime MKN28 ląstelių kultūras, registruodami fazių kontrasto vaizdus kas 30 sekundžių 1-4 valandų bėgyje. Matematinės morfologijos ir struktūrinės vaizdų analizės metodai taikyti ląstelių individualiems ir globaliems judrumo įverčiams rasti.

Šis straipsnis aprašo algoritmą skirtą vėžinių skrandžio ląstelių migracijai tirti.

*Ląstelių migracija, vaizdų analizė, matematinės morfologijos modeliai, vėžinės ląstelių kultūros*

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**Robertas PETROLIS.** Lietuvos sveikatos mokslų universiteto Neuromokslų instituto mokslų daktaras, jaunesnysis mokslų darbuotojas. Adresas: Eivenių g. 4, LT-50009 Kaunas. Tel. (8 37) 302966, el. paštas: robertas.petrolis@ismuni.lt

**Robertas PETROLIS.** Lithuanian University of Health Sciences Neuroscience Institute, doctor of sciences, junior researcher. Address: Eivenių st. 4, LT-50009 Kaunas. Tel (+370 37) 302966, e-mail: robertas.petrolis@ismuni.lt

**Rima RAMONAITĖ.** Lietuvos sveikatos mokslų universiteto Virškinimo sistemos tyrimų instituto mokslų daktarė, jaunesnioji mokslų darbuotoja. Eivenių g. 2, LT-50009, Kaunas. Tel. (8 37) 326092, el. paštas: rima.ramonaite@ismuni.lt

**Rima RAMONAITE.** Institute for Digestive Research, Lithuanian University of Health Sciences, doctor of sciences, junior researcher. Address: Eivenių st. 2, LT-50009 Kaunas. Tel. (8 37) 326092, el. paštas: rima.ramonaite@ismuni.lt

**Gediminas KIUDELIS.** Lietuvos sveikatos mokslų universiteto Gastroenterologijos klinikos mokslų daktaras, vyriausiasis mokslų darbuotojas. Eivenių g. 2, LT-50009, Kaunas. Tel. (8 37) 326264, el. paštas: gediminas.kiudelis@ismuni.lt

**Gediminas KIUDELIS.** Department of Gastroenterology, Lithuanian University of Health Sciences, doctor of sciences, senior researcher. Eivenių st. 2, LT-50009 Kaunas. Tel. (8 37) 326264, e-mail: gediminas.kiudelis@ismuni.lt

**Greta VARKALAITĖ.** Lietuvos sveikatos mokslų universiteto Virškinimo sistemos tyrimų instituto magistrantė, laborantė. Eivenių g. 2, LT-50009, Kaunas. Tel. (8 37) 326092, el. paštas: grevark@gmail.com

**Greta VARKALAITĖ.** Institute for Digestive Research, Lithuanian University of Health Sciences, master student, laboratory technician. Address: Eivenių st. 2, LT-50009 Kaunas. Tel. (8 37) 326092, e-mail: grevark@gmail.com

**Limas KUPČINSKAS.** Lietuvos sveikatos mokslų universiteto Virškinimo sistemos tyrimų instituto habilituotas mokslų daktaras, vyriausiasis mokslų darbuotojas. Eivenių g. 2, LT-50009, Kaunas. Tel. (8 37) 326896, el. paštas: limas.kupcinskas@ismuni.lt

**Limas KUPČINSKAS.** Institute for Digestive Research, Lithuanian University of Health Sciences, habilitated doctor of sciences, senior researcher. Eivenių st. 2, LT-50009 Kaunas. Tel. (8 37) 326896, e-mail: limas.kupcinskas@ismuni.lt

**Algimantas KRIŠČIUKAITIS.** Lietuvos sveikatos mokslų universiteto Fizikos, matematikos ir biofizikos katedros profesorius. Adresas: Eivenių g. 4, LT-50009 Kaunas. Tel. (8 37) 302966, el. paštas: Algimantas.Krisciukaitis@ismuni.lt

**Algimantas KRIŠČIUKAITIS.** Lithuanian University of Health Sciences Department of Physics, Mathematics and Biophysics professor. Address: Eivenių st. 4, LT-50009 Kaunas. Tel (+370 37) 302966, e-mail: Algimantas.Krisciukaitis@ismuni.lt