Interdisciplinary research in field of biomedical image processing

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ABSTRACT

The paper describes the up-to-date interdisciplinary research that has been established in Slovakia in recent years. It requires the close cooperation between specialists from medical fields as well as technicians as it deals with creating the supporting tool for physicians to evaluate a patient's diagnosis. The research is based on the need of doctors to create a system reliable enough, that would help them to assess the health status of human respiratory system. This task first comprises the design of a working station that could serve for capturing series of images (video sequences) of investigated sample obtained by doctors, including a light microscope and a high-speed camera with appropriate software. Then is comprises also development of sophisticated methodologies for analysis of obtained images and detection of objects of interest within them, and finally the evaluation of behavior of investigated structures which determines the health status of a patient. This interdisciplinary cooperation is as unique as it is the only one of this kind in our country as there does not exist any other way how to evaluate the health status of human respiratory system using smart technological approach.

Keywords: interdisciplinary research, cilia of respiratory epithelium, high-speed imaging, image segmentation

1. INTRODUCTION

The cooperation between two universities from various fields of study was established several years ago (in 2009) as a response to real needs of doctors from medical practice. The team of medical specialists from Clinics of Children and Adolescents in Jessenius Faculty of Medicine of Comenius University in Martin, Slovakia, has started to deal with the problematics of research of human airways. Respiratory system is inside covered by structures, which determine its proper function and are responsible for airways' clearing, called cilia of respiratory epithelium. As the malfunction of cilia can cause several serious diseases, they have become the objects of interest of medical research, focused on study of their structure, function and movement.

The first thing that had to be overcome when studying cilia was the question of taking the images from inside the human respiratory system as well as videos which would capture the ciliary movement. This could be done by using sophisticated microscopic techniques and high-speed cameras.

Another task was the processing of obtained images or series of images (video sequence), extracting the useful information, its representation and evaluation. This could serve as supporting tool for a patient's diagnosis determination and proper treatment initiation.

Such difficulties made the doctors to contact technical specialists from the University of Zilina, Department of Mechatronics and Electronics and so started the interdisciplinary research between technical and medical specialists in field of human respiratory system.

2. INVESTIGATED IMAGES

Processed images are usually obtained as a video sequence from a high-speed camera which captures the scene of a certain microscopic specimen. The specimen consists of respiratory epithelium, usually taken from nasal mucous membrane or trachea, with oscillating cilia (in a short time after subduction from the organism – approximately 10 minutes). Example image of obtained specimen is shown below (Fig. 1).

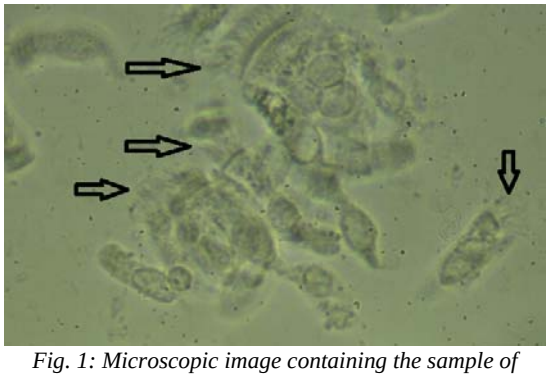

Fig. 1: Microscopic image containing the sample of respiratory epithelium, ciliary areas are marked with arrows

Cilia – objects of interest

Cilia are hair-like microscopic structures with length approximately 6-10 μm and width less than 1 μm. Every cell of respiratory epithelium is covered with 200 – 300 cilia (see Fig. 2). Cilia oscillate in a synchronous mode, with frequency 18 – 30 Hz (see Fig. 3), called Ciliary Beat Frequency (CBF) and this is the underlying factor when appointing ciliary pathologies.

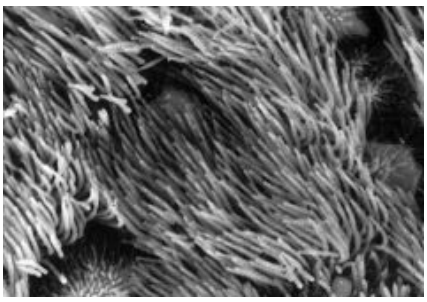

Fig. 2: Detailed microscopic image with cilia of respiratory epithelium, [1]

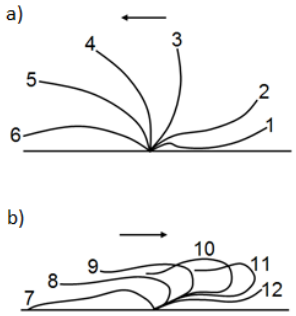

Fig. 3: Illustration of ciliary action, a) power stroke and b) return stroke, [2]

Main role of these structures is to remove undesirable particles – dust spew, viruses, bacteria or some allergens from human airways. Their wrong function of malfunction leads to often or even chronic inflammations of airways and to many serious and life-threatening diseases [3]. Assessment of ciliary functionality using high-speed imaging aims to proper diagnostics of affected patients.

Artifacts within images

Obtained images of respiratory epithelium often contain also unwanted objects, which might cause false evaluation of cilia's vitality. Such objects are for example erythrocytes (i.e. red blood cells) which may occur in the images due to bleeding during obtaining the specimen from airways.

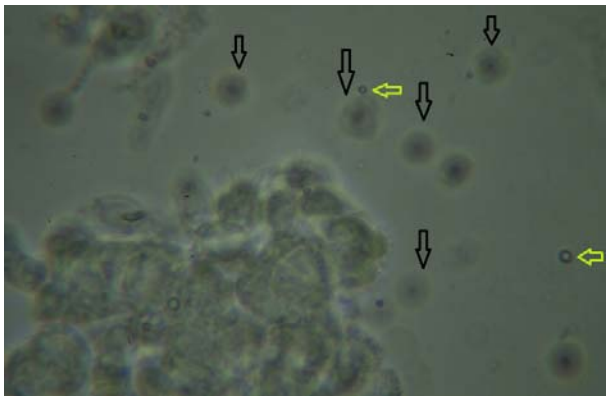

Fig. 4: Microscopic image with specimen of respiratory epithelium affected with artifacts: red blood cells (marked with black arrow) and small air bubbles (marked with green arrow)

Other type of unwanted elements can be smaller or larger air bubbles, small black spots on camera's lens, non-homogenous lighting of the scene, etc. All of them can be in general marked as artifacts in resulting images (see Fig. 4 and 5). Removal of as many artifacts as it is possible is a key factor when analyzing ciliary areas

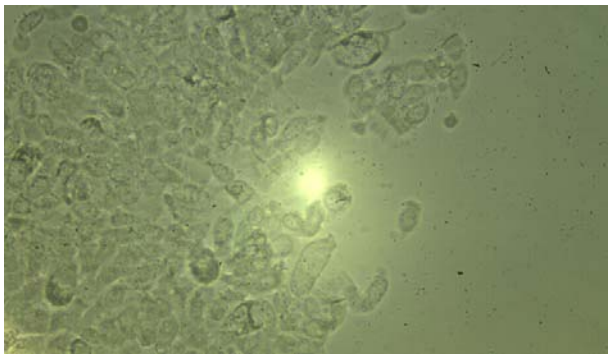

Fig. 5: More artifacts in obtained images: non-homogenous lighting (in the middle) and small black spots in background (in the right part)

3. IMAGE OBTAINING

High-speed imaging

As cilia beat with high frequencies (up to 30 Hz), the FPS (frame ratio, frames per second) factor of camera needs to be at least two times higher as maximal useful frequency (f_{MAX}) from Fourier's spectrum of extracted signal representing the motion of an object, following famous Shanon – Kotelnik theorem, Eq. (1):

$$FPS > 2.f_{MAX} \tag{1}$$

However, in practical application the coefficient 2 is often replaced with 10, 20 or more. Considering this elementary theorem and maximal beating frequency of cilia, digital camera has to support frame rates in range of 300 – 600 fps, [4].

In the case of high speed imaging and light microscopy, suitable illumination and its parameters are key elements for generating and acquiring good images. Exposition time for high speed camera is often too short and the intensity of light source inversely depends on sensor exposition time, Eq. (2):

$$I \sim \frac{1}{\Delta T_{EXP}} \sim \text{FPS} \tag{2}$$

where ΔT_{EXP} is a time 1/FPS decreased by the time of reading the digital image from sensor (non-integration time – dark time) (Fig. 6) [5]. When camera captures video sequence with 256 fps, sensor saturation and image reading must be done in the time less than 4 ms. These conditions are crucial in designing high speed imaging workstation and supporting hardware conditioning.

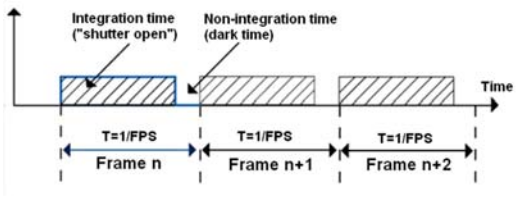

Fig. 6: Basic camera sensor timing for sequence acquisition

During last years the teams in Martin and Žilina have designed workstation with light microscope and high speed camera (Basler A504kc) for recording and analyzing main motion parameters of microscopic objects in respiratory system, see Fig. 7. Designed high speed workstation consists of various parts integrating itself variety of technical branches: light microscope, high speed camera, illumination and supplying devices, communication interfaces and acquisition computer. Used high speed camera Basler A504kc contains Progressive Scan CMOS sensor with resolution 1280 x 1024 pixels for color or monochrome imaging. Maximal frame ratio for full resolution is 500 fps, for 32-line region 16 000 fps can be used. Amount of data per second can reach level of 650 MB/s. Data transfer between camera and acquisition computer is provided via Full CameraLink and Bitflow interface. Storage device on the side of acquisition computer is compound from 8 stripped HDDs with full capacity of 8 TB. Camera is linked with microscope through C-Mount-to-F-Mount adaptor.

Fig. 7: Acquisition of video sequences by a doctor – specialist using designed workstation

4. IMAGE ANALYSIS

The aim of analysis of obtained images is to reliably answer the question of vitality of cilia of each patient and so help doctors to reveal the right diagnosis and launch early treatment if possible. This means the need of software tool, that would be first able to detect and find motile cilia and identify if they move with proper frequency as well as if their mode is synchronous, and then to find also the static (non-motile) cilia. When static cilia are found in an image (i.e. dead cilia in fact), this could indicate the high probability of ciliary pathology on one hand, or some kind of mistake on the other hand (either during the image analysis or even when taking the sample from patient's airways). However, as this diagnosis is often crucial for a patient, it is important to minimize the failures, i.e. to identify and exclude artifacts in an image that might lead to wrong detections.

Identification of moving objects

Older methods of investigation of respiratory epithelium's cilia and their kinematics were based mainly on subjective observation of structures in slow mode. Methods were based on manual counting of frames needed for several beating cycles (motion periods) of an object [6], [7]. However, such approach required continuous attention and presence of a specialist, and moreover, this was very time consuming and contained subjective error of an observer. Many times these methods brought only simple answer: beating (motion) appears slower / higher than normal (compared to physiological reference). In addition, observer often was not able to see all important regions in image with naked eye.

This was the reason why the technical specialists proposed automated segmentation method, which is able to search for important regions in entire video sequence, highlight and quantify them. Time sequence of two dimensional images is input information to designed algorithm (Fig. 8). In addition, such algorithm allows to segment any video sequence to areas with or without motion not only in medical applications, but also in other applications. Results of this algorithm applied to selected video are shown in Fig. 9.

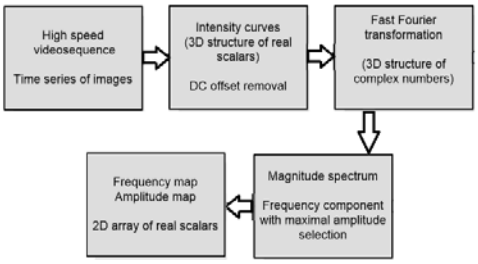

Fig. 8: Block diagram of algorithm for searching of regions with active ciliary motion

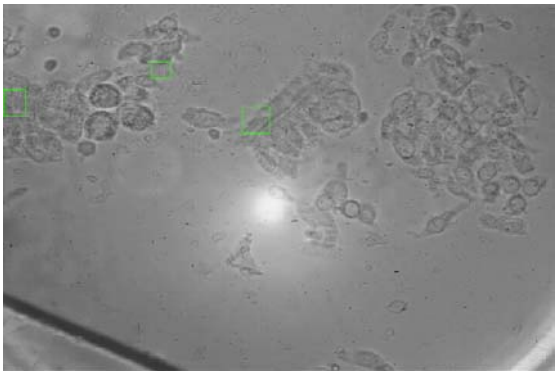

Fig. 9: Results of identified moving cilia within one image of a video sequence (green rectangles indicate detected ciliary areas)

Another approach to identification of moving ciliary areas is using the differential methods. One can use either simple extraction of two neighbouring video frames, or more sophisticated ones, e.g. background extraction method [8], [9]. The extraction of moving objects from static background is based on time as well as on space domain. Each video frame is subtracted from the following one, i.e. pixel intensities on particular positions are subtracted from corresponding intensities on that same positions in next frame. Then the frame difference mask (FDM) can be created using Eq. (3).

$$FDM = \begin{cases} 255; if \left| d_{mn}(x,y) \right| > k \\ 0; else \end{cases} \quad (3)$$

where $d_{mn}(x,y) = f_m(x,y) - f_n(x,y)$ is the difference between two following frames (f_m and f_n) and k is set thresholding value. Results obtained from differential method usage is illustrated in following figures, Fig. 10 and Fig. 11, the method was tested in the same image as the method using frequency maps, so the results can be compared.

Fig. 10: Obtained differential image after applying the differential method to original image

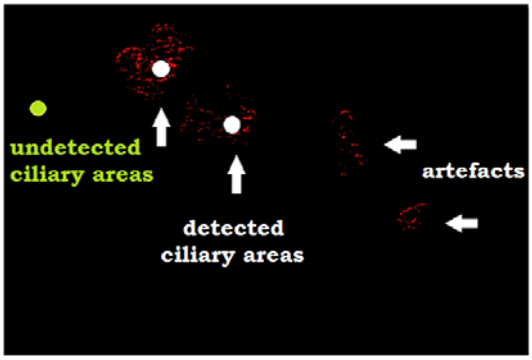

Fig. 11: Segmentation result using differential method: two ciliary areas are detected correctly (white dots), one ciliary area is not detected (green dot) and false-positive detections also occurred (artifacts)

Searching for static cilia

Identification of non-motile cilia is currently a very demanding task. This is so due to several reasons: the images are of a low contrast, the searched structures are very small, often a bit blurred, and there are also other, much larger structures in the image – epithelial cells, erythrocytes, air bubbles and other artifacts might occur, too.

Segmentation of an image means partitioning of its content into several disjoint sets, e.g. separation of the objects of interest from the background (or unimportant structures in the background) [10]. Segmentation of above described images will separate ciliated cells from artefacts or other background structures and this might significantly contribute to better and more precise evaluation of their vitality (movement). There is a vast range of ways how to approach to image segmentation. Many of them were tested, however, they did not give satisfactory results – mainly due to the specificity of given microscopic images and this is the reason why the approach of texture analysis is discussed below.

Within the microscopic images of respiratory epithelium there can be recognized five basic types of textures: cilia, air bubbles, epithelial cells (called also epithelium), erythrocytes (red blood cells) and image background. Examples of these structures are shown in Fig. 12. Every image is then considered to contain only these kinds of texture, although not necessarily all five. Using texture analysis within an image, each texture is characterized by a certain feature vector and according to it, all image pixels are separated into defined number of groups, e.g. five. Some of the first results of static cilia identifying obtained using such approach are shown in Fig. 13.

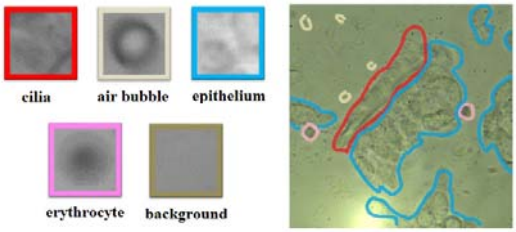

Fig. 12: Five different types of texture within microscopic image, labelled with matching colors

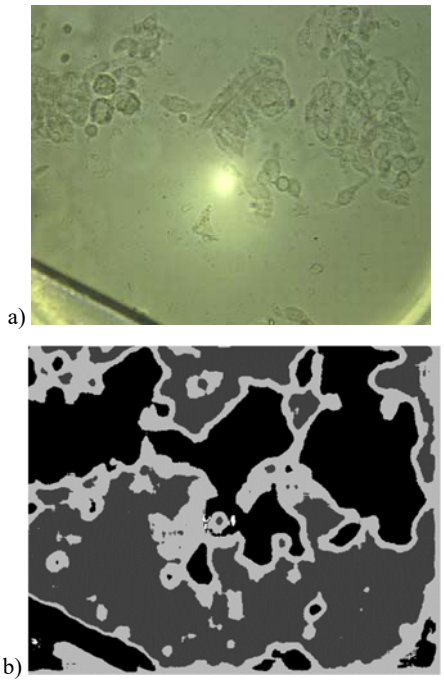

Fig. 13: a) Original image, b) image segmented into three different groups using texture analysis

5. CONCLUSIONS AND FUTURE WORK

Described cooperation between the Jessenius Medical Faculty of Comenius University and the technical experts from the University of Zilina is a living system which is still improving. The subjects of research – cilia of respiratory epithelium – bring many difficulties during their identification and reliable detection. Such difficulties are connected with image obtaining, the size and movement of cilia, presence of artifacts, etc., and all this is what makes the research to go forward. The current issues are consulted among doctors and technicians often right after a patient's examination or obtaining the sample form respiratory system and so the solutions are designed right for the actual demands. Many software tools and their designing or improvement are also solved within university as a part of diploma or dissertation theses of excellent students and PhD. students.

Planned future work will mainly consist of refinement of the methods of sample obtaining (i.e. shorter time of taking the sample, more comfort for patients, less potential bleeding of respiratory tissue, etc.) as well as of improvement of the image

analysis methods (faster processing or possibility of real-time processing and diagnosis evaluation, more precise image segmentation and texture analysis and not so computationally demanding software tools).

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7. REFERENCES

[1] **Structure and function of cilia**, online, available at <http://www.ciliopathyalliance.org/cilia/structure-and-function-of-cilia.html>.

[2] **Eucaryotic cilia and Flagella**, online, available at <http://cronodon.com/BioTech/Cells_motility_2.html>.

[3] Kamil Javorka, et al., **Lekárska fyziológia (Medical physiology)**. Martin: Osveta, 2001, ISBN: 80-8063-023-2.

[4] L.Tan, **Digital Signal Processing – Fundamentals and Applications**, Elsevier, 2008, ISBN 978-0-12-374090-8.

[5] P. Carellas, **Fundamentals of High-Speed Imaging** – online seminar, Cambridge, 2011.

[6] Chilvers, M. A., O'Callaghan, C., 2000. "**Analysis of ciliary beat pattern and beat frequency using digital high speed imaging: comparison with the photomultiplier and photodiode methods**", in BMJ Publishing Group Ltd & British Thoracic Society [online].

[7] C Zhang, L., Sanderson, M. J., 2003. "Oscillations in ciliary beat frequency and intracellular calcium concentration in rabbit tracheal epithelial cells induced by ATP", in **The Journal of Physiology** (J Physiol) [online], Volume 546, Number 3.

[8] R. T. Collins, R. Gross, and S. Jianbo, "**Silhouette-based human identification from body shape and gait**," in Proceedings of 5th IEEE International Conference on Automatic Face Gesture Recognition, 2002.

[9] A. Gmiterko, M. Kelemen, I. Virgala, R. Surovec, and M. Vacková, „**Modeling of a snake-like robot rectilinear motion and requirements for its actuators**, "in Proceedings of 15th International Conference on Intelligent Engineering Systems, 2011.

[10] W. Burger, M. J. Burge, **Digital Image Processing**. 1st ed. New York: Springer, 2008. ISBN 978-1-84628-968-2.