Method of Registration of Ciliated Epithelium Cilia Motion

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Abstract. The method of registration of ciliated epithelium cilia motion has been researched. The device for registration and algorithms of registration of the cilia motion have been developed. The example of the laboratory research of the cilia motion has been studied.

1. Introduction

The laboratory examination of patients plays a big role in the right diagnose. Different cells can become the object of the laboratory examination, including airway ciliated epithelium (CE). One of the ways to study cells is to expose them to the aggressive medium and analyze their functional parameters (motion, reproduction) [1-6].

It is known that one of the reasons of bronchial pathology development [7-13] is the disturbance of ciliary activity of airway CE in the system of local protection of lungs. To study the motion activity of CE cilia there have been developed new devices that have modern cameras with in-built high sensitivity digital full frame matrix of high definition. Such cameras allow to send the digital signal into the computer through the memory card or directly [14-16].

The scheme of the device performance is simple (Fig. 1). The activity of CE cilia is observed through the microscope; to the microspore through the fixing scheme (2) a digital camera is connected. It can record with the frequency of 50 shots a second and FullHD (3). The digital camera has an external recording device (4). The recorded information through the readers [19] is sent to the computer [20] where with the help of the special software the parameters of CE cilia motion are calculated.
Modern cameras record big videos; to analyze them it is necessary to develop algorithms of motion fixation and of software [16-18]. To develop an algorithm, PPP MatLab is used; its efficiency for image processing tasks was demonstrated in the work [19]. The result of the laboratory device operation is demonstrated at the example of registration during the motion activity of CE cilia dipped into the solution of the distilled water.

2. Algorithms of registration of CE cilia activity
The algorithm of pixel gradient registration in the image, suggested in the work [19], supposes that each pixel changing its colour in time (with the image change) forms a harmonic signal which is equal in number to the frequency of CE cilia beating. But this rule does not always work. Beating of CE cilia can cause fluctuation of the surrounding medium with lower frequency. If these fluctuations are bigger in number, then the algorithm will identify them as CE cilia beating, and this will be the wrong result. One more disadvantage of the algorithm is the impossibility to measure the amplitude of fluctuations.

Video recording is divided into shots, and shots appear in the form of bidimensional matrix (Fig. 2), in whose sockets there are the values of grey colour (grey). To demonstrate the algorithms, 5-second video recording is used; it consists of 250 shots with the definition of 1920x1080 pixels.
Figure 3. The piece of the shot with ciliated epithelium cilia.

In the whole image in recording, CE cilia take less than 5% and this is the section to analyze. That’s why the specialist chooses this section in video image with CE himself (Fig. 3).

To improve the identification of cilia beating, another algorithm was suggested: the algorithm of registration of the chosen object motion, which works the following the way. The chosen section with cilia is turned the way that the basis with cilia is horizontal and below, then the beating occurs horizontally. In the example the section is turned 60° in clockwise order (Fig. 3).

1. At the chosen section there can been seen the object of observation, namely a part of the cilium removed maximally from the basis. In the given example (Fig.3) the object of observation is highlighted with the square.

2. In the next shot the search of the chosen object is done. The object is looked for only in the horizontal plane with the help of the method of the least squares.

3. Returning the chosen object to the previous state the periodic motion of CE cilia restores and the frequency and amplitude are found for it.

In Fig. 4 there is a result of algorithm operation: in the Y-line the amplitude is measured in pixels; the frequency was 1.56 Hz and the amplitude was 22 pixels. If the scale of observation is known, pixels can be changed into CI units [19].

The suggested algorithm allows to measure the frequency and amplitude of cilia fluctuations. But there is one drawback: there are sections of CE with a large concentration of cilia and the wrong orientation in space, which does not allow to observe a single cilium. In such cases at different stages of motion the cilia are laid upon each other or completely disappear from the visual sector.

Figure 4. The piece of the shot with ciliated epithelium cilia.

A visual analysis of shot-by-shot division of the footage (Fig. 3) allowed to identify at the separate section the amplitude of beating (19 pixels) and the length of the cilium (78 pixels). The results of the manual analysis coincide with the operation of algorithms, which proves their efficiency.
3. Application of the laboratory device

In works [2-13, 20] it was shown that humidity negatively affects the patients with bronchial asthma. One of the reasons for that is the slow-up of the motion activity of CE cilia when they get into the hyposmolar medium.

To prove this fact and assess the laboratory device developed in the Far Eastern Scientific Center of Physiology and Pathology of Respiration, the study of the motion activity of CE in patients with asthma was done. The biopsy sample of the bronchial mucosa obtained with the help of bronchoscopy was dipped into the Hank’s solution. After that the digital camera recording of the CE cilia motion dynamics was done under microspore during 5 minutes. The dynamics of cilia fluctuations is presented in Fig. 5.

![Figure 5. The piece of the shot with ciliated epithelium cilia.](image)

The dynamics of CE cilia fluctuations after dipping into distilled water at the 2nd and 4th minutes is presented in Fig. 6 and 7. A significant decrease of cilia fluctuations frequency has been registered. If before dipping the frequency of cilia beating was 5.46 Hz, then at the 2nd minute the frequency decreased till 3.32 Hz and at the fourth minute till 1.37 Hz, which means that the beating frequency became 3 times lower.

![Figure 6. The piece of the shot with ciliated epithelium cilia.](image)

It is worth mentioning that the registration of the motion was recorded into the memory card and then it was downloaded into the computer. The identification of the motion in the real time demands corresponding expensive equipment. Home and semiprofessional cameras cannot in the real time send video to the computer in FullHD format with 50 shots a second through interface USB2.0/3.0. In the future it is possible to overcome this restriction.
4. Conclusion
The suggested method of registration of the activity of airway CE cilia allows to define the frequency of cilia beating. The laboratory device for registration of CE activity, which consists of a microspore, camera (recording the motion of CE cilia) and a computer (analyzing the motion), has been developed.  

Due to high technological parameters of a modern camera (frequency and definition), the recording of the cilia motion with different frequency was done. The result was compared with the manual analysis to confirm the objectivity of this method of registration.

The suggested laboratory method can be used both to study the influence of negative factors of the environment and to test new methodologies of treatment and effect of the medications.

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Figure 7. The piece of the shot with ciliated epithelium cilia.
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