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High Speed Video System for Tissue Measurement Based on PWM Regulated Dimming and Virtual Instrumentation

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Introduction

Objects investigated by light transmission microscopy usually can't be highlighted using reflection marks or equipped with sensors of cinematic parameters. In this case we often use advantages of image analysis and signal processing. Some methods for frequency measurement (using photodiode and photomultiplier) of biomechanical or microscopic objects can't do the correct analysis of structure pathologies. The most progressive method is high speed digital video method, which brings relatively good results in formation of mathematical and mechanical model of structure movement [1].

On the other side, optimal light conditions in microscope can be achieved using various types of regulators (dimmers). Quality of obtained and analyzed digital images depends on acquisition system and its settings. Tissue measurement in modern medical praxis needs mutual cooperation of medicine, electronics and signal processing.

As example of moving biomechanical system we can consider cilium of respiratory epithelium cell (Fig. 1). Each ciliated cell of respiratory epithelium contains ca. 200 cilias (6 μm long) beating with frequency up to 30 Hz. Cilias are synchronized with metachronal waves propagated in periciliar liquid. From the basic position cilium folds down to the epithelium cell (recovery stroke – 75% of beating cycle) and then rapidly darts up to move mucus with its tip (effective stroke) [2]. Relatively high frequency of cilium movement leads to high requirements for the parts of acquisition system – microscope, camera, acquisition computer and others.

FFT based spectral measurement of cilium movement

Important parameter in measurement process of biomechanical systems or moving structures is object beating frequency (OBF). In the case of respiratory epithelium this parameter has specific name: CBF (ciliary beating frequency). The value of CBF is normally in range 18-30 Hz. Image processing and FFT-based method require high-speed video acquisition system with optimal frame rate up from 400 fps. Older methods [3], [4], [5] were based on photosensitive discrete devices or manual time counting needed for exact number of beating cycles. In this case CBF is:

$$CBF = \frac{N}{T_N}, \tag{1}$$

where N is number of beating cycles (10 e. g.) and T_N represents time needed for these beating cycles.

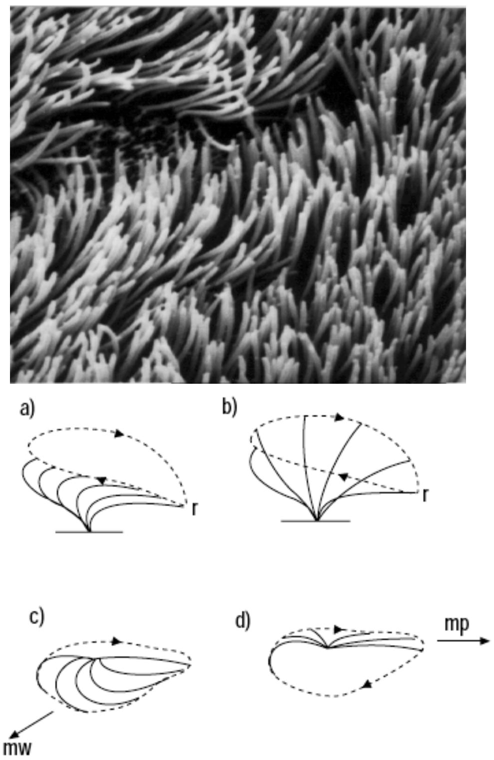


Fig. 1. Ciliated respiratory epithelium in electron microscopy imaging mode and phases of cilia movement

Another way in this analysis uses spectral methods based on FFT analysis of function which represents beating cycles of investigated objects. Measurement method designed by our team is based on frequency analysis of intensity variance curve. This curve is obtained from video sequence by capturing intensity variation in selected region of interest (ROI) (Fig. 2). Intensity variation is discrete function, where each sample

$$I_{avg}(N) = \frac{1}{k.l}\sum_{i=1}^{k}\sum_{j=1}^{l} f(i+T;\, j+L)_N \qquad (2)$$

represents average image intensity (2D mean in ROI area) and local extremes determine beating cycles. N is number of frame from videosequence, k,l represent ROI size, [T;L] are coordinates of left top corner of ROI in actual frame and f(i;j) is pixel value.

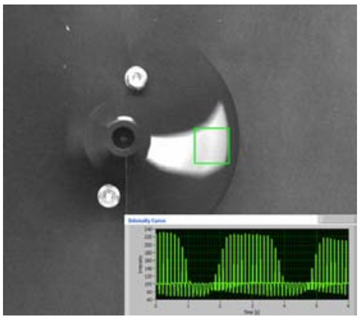


Fig. 2. Capturing of intensity curve from image phantom with frequency of 9 Hz

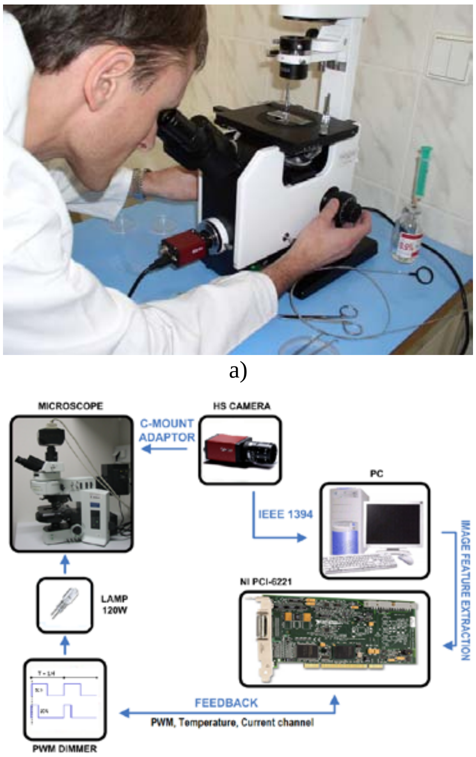
Curve is then analyzed with FFT algorithm, measurement is verified using curve thresholding and envelope analysis. CBF corresponds with harmonic component from FFT or power spectrum (PSD) with maximal value.

For algorithm calibration we used phantoms imitating cilium movement: DSP controlled stepping motor with defined frequency. Measurement is done in graphical development system NI LabVIEW as virtual instrument and results are written as Microsoft Excel XLS file. This component helps to integrate results of investigation to laboratory or clinic information systems. Next advantage of LabVIEW virtual instrument is called Web Publishing Tool. Using this tool we can provide control of whole application through Ethernet or Internet connection.

Design of high speed acquisition system

The first real measurements (in Clinic of pathological physiology, Jessenius Faculty of Medicine, Martin, Slovakia) were taken after algorithm debugging on phantoms (Fig. 3). Because the ciliary beating frequency ”in vitro” goes down from ca. 18 Hz to a half value, primary we used acquisition system with slower camera. AVT Marlin F-046B camera was connected to inverse biological light microscope MODEL IM 1C via C-mount adaptor. Sequences from camera were stored on acquisition computer through IEEE 1394 (FireWire) as uncompressed sequences with parameters: 8 BPP / 640 x 480 pixels / 60 fps.

In the case of usage high speed camera system (Basler), microscope illumination is very important. We have changed microscope condenser light source and made some measurements of intensity by Lutron LX-1102 luxmeter.



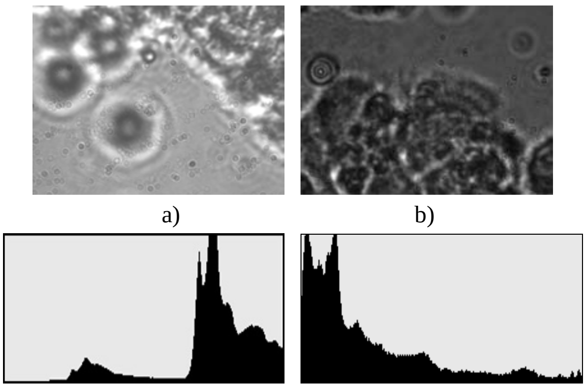
a)

b)

Fig. 3. Experimental workstation – microscope connected to digital camera and block scheme of whole acquisition system

In case of ultra high frame ratio of camera we can meet these essential problems: if the illumination of specimen is too low, frames in video sequence are underexposed and dark; if the illumination of specimen is too high, frames are overexposed and too bright (Fig. 4); high frame ratio causes growth of data for storage, so we must consider optimal connection between camera and acquisition computer. These non-optimal conditions causes same „faults“ in intensity curve [8].

Original maximal value of illumination (measured between condenser optics and specimen) changed from $8{,}6.10^3$ lx to ca. 80.10^3 lx after replacing 20W halogen lamp for 120W halogen lamp. Heat from condenser must have been removed using active CPU cooler mounted onto microscope or using intelligent dimming tool.



a) b)

c) d)

Fig. 4. Overexposed (left) and underexposed (right) sequence frame and their histograms with grayscale distributions

On the basis of simulations in OrCAD we designed wiring dimmer (Fig. 5). The power supply is made directly from the main supply 230V via input transformer [6]. Dimming feature is provided through one PWM (pulse width modulated) channel, which generates driving impulses of switching transistor [7]. PWM signal is generated in NI PCI-6229 LabVIEW measurement card and is isolated from power circuit through optoelectronic coupler and amplified in transistor driver. PWM has constant frequency of 50 kHz and regulation is done through duty cycle of impulses. This regulation part of acquisition system uses software regulation feedback included in acquisition virtual instrument and will be described later. Power regulation in dependency on duty cycle and its influence on image histogram is shown in Fig. 6.

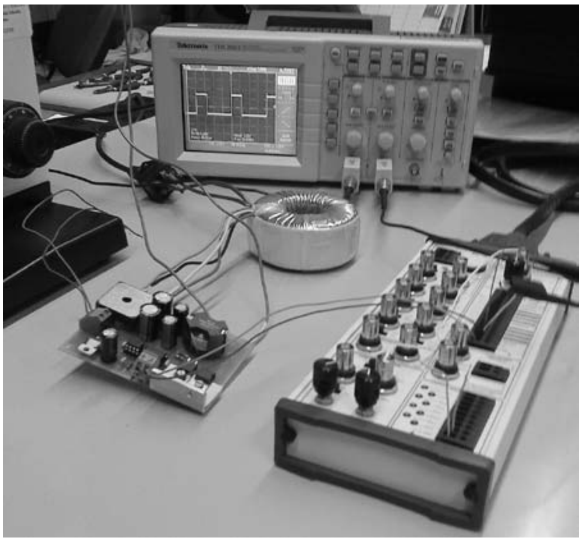
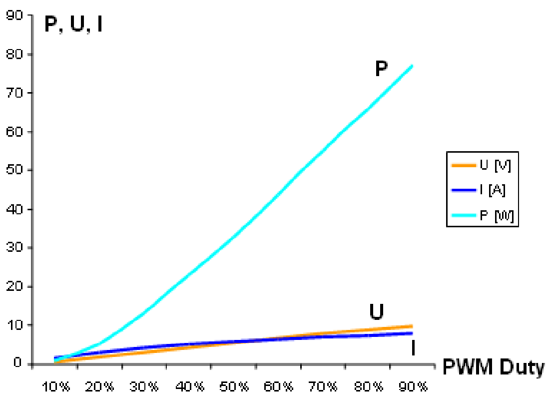
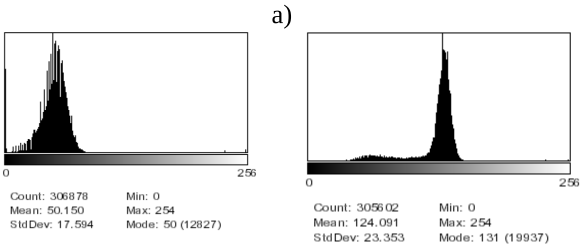


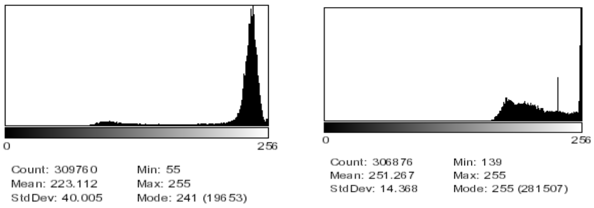
Fig. 5. Single PWM halogen bulb microscope dimmer, testing phase



a)



b) PWM 20% c) PWM 25%



d) PWM 30% e) PWM 40%

Fig. 6. Voltage, current and power regulation in dependency on duty cycle of switching impulses and its influence on image histogram

Illumination regulation feedback

In process of sequence acquisition, a ROI placed into image extracts important image feature: average image intensity and histogram distribution. Overexposed image has its histogram concentrated to high intensity values and underexposed image to low values (Fig. 4). Histogram distribution is used as regulation parameter for setting up the PWM for halogen lamp dimmer. Dependency of histogram mean (μ) on duty cycle (d_c) for actual frame ratio (fps) of video system can be described by quasi-linear characteristics (Fig. 7). Regulation algorithm approximates this characteristic with a line using two or more known points A, B (4):

$$y = ax + b\,, \tag{3}$$

$$A = \lfloor \mu_1, d\_c_1 \rfloor;\ B = \lfloor \mu_2, d\_c_2 \rfloor, \tag{4}$$

$$a = \frac{d\_c_2 - d\_c_1}{\mu_2 - \mu_1}\,, \tag{5}$$

$$b = d\_c_1 - \frac{d\_c_2 - d\_c_1}{\mu_2 - \mu_1}\mu_1\,, \tag{6}$$

$$y = \frac{d\_c_2 - d\_c_1}{\mu_2 - \mu_1}x + d\_c_1 - \frac{d\_c_2 - d\_c_1}{\mu_2 - \mu_1}\mu_1 \tag{7}$$

and calculates Δ d_c for setting histogram $\mu_0 \approx 128$, what is half of grayscale range 0–255:

$$\pm \Delta d\_c = d\_c_i - d\_c_0\,. \tag{8}$$

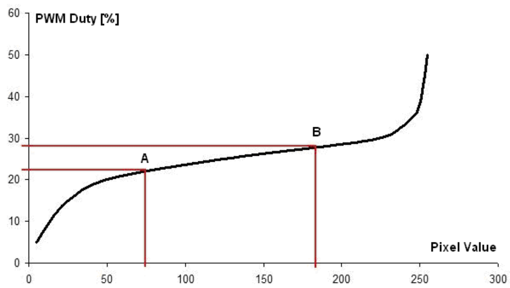


Fig. 7. Illumination characteristics for 60 fps framing. Optimal μ_0 corresponds with d_c_0 = 25%. Acceptable duty cycles lies in range 20-30%, all duty cycles under or over this interval brings underexposed or overexposed image

Delay of the regulation algorithm depends on framing ratio of camera and is 1/fps [s].

Conclusions

In medical praxis – especially in diagnostics of respiratory apparatus – CBF represents very important parameter for describing various pathologies of epithelium. Designed solution for measuring object beating frequency from video sequence using tools of image analysis and spectral analysis simplifies present used methods and reduces usage of hardware devices. Using some development environment (e.g. NI LabVIEW) we can create fully automated application with interactive inputting of some parameters.

In the first approach, algorithms were tested on phantoms with defined frequency. Intensity variance curve analysis can be used in many other applications dedicated to frequency measurement not only in biological environment. Designed hardware acquisition system can be used with or without microscope in applications, where placement of kinematics parameters sensors is not able. Intelligent regulation of condenser illumination through image features extraction and histogram analysis enables fully automated approach to video sequence acquisition.

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Paper focuses on main topics in high speed video acquisition system designing. This video system is used in sophisticated diagnostics of microstructures in human tissue, especially respiratory epithelium cilium. System is linked with optical microscope and uses a phantom generator controlled with DSP processor for calibration and debugging. Algorithms and software are developed as user friendly virtual instruments. Ill. 7, bibl. 8 (in English; abstracts in English and Lithuanian).

D. Koniar, L. Hargaš, S. Štofan, M. Hrianka. Didelės spartos vaizdo sistemos taikymas audiniams matuoti // Elektronika ir elektrotechnika. – Kaunas: Technologija, 2010. – Nr. 10(106). – P. 169–172.

Sudėtingoms mikrostruktūroms tirti (pvz., žmogaus audiniams, kvėpavimo takų epiteliui) taikoma didelės spartos vaizdo sistemos. Tokioje sistemoje svarbus yra optinis mikroskopas ir skaitmeniniam signalų apdorojimui taikomo procesoriaus kalibravimas bei derinimas. Sukurti algoritmai ir programinė įranga. Il. 7, bibl. 8 (anglų kalba; santraukos anglų ir lietuvių k.).