ISSN 1726-5749

SENSORS & TRANSDUCERS

vol. 87
1 /08

Sensor Instrumentation, DAQ and Virtual Instruments

International Frequency Sensor Association Publishing

IFSA

Sensors & Transducers

Volume 87
Issue 1
January 2008

www.sensorsportal.com

ISSN 1726-5479

Sensors & Transducers Journal (ISSN 1726-5479) is a peer review international journal published monthly online by International Frequency Sensor Association (IFSA). Available in electronic and CD-ROM.

Sensors & Transducers Journal

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Volume 87
Issue 1
January 2008

www.sensorsportal.com

ISSN 1726-5479

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Sensors & Transducers Journal, Vol. 87, Issue 1, January 2008, pp. 11-18

www.sensorsportal.com

Sensors & Transducers

ISSN 1726-5479

http://www.sensorsportal.com

Kinetics Analysis of Respiratory Epithelium by Virtual Instrumentation

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Received: 20 December 2007 /Accepted: 21 January 2008 /Published: 28 January 2008

Abstract: The article deals with measurement and analysis of kinetic parameters of human respiratory epithelium. The article shows possibilities of data acquisition (videosequences) and postprocessing using virtual instrumentation – LabVIEW. The designed methods enable analysis and measurement of two parameters: cilia beat frequency (CBF) and trajectory of cilia of respiratory epithelium. The problem task is divided into videosequence acquisition of cilia from the microscope, region of interest (ROI) selection for analyzed data reduction, preprocessing of reduced sequences. Frequency analysis is done by intensity method, which records and evaluates intensity variations in ROI. Trajectory analysis uses sophisticated algorithms of object detection in the image called pattern matching. Results of this work are used for diagnostics of some pathology of respiratory apparatus and epithelium. All solution steps are realized in LabVIEW development system. *Copyright © 2008 IFSA.*

Keywords: Cilia, Respiratory epithelium, Virtual instrumentation, LabVIEW, Pattern matching

1. Introduction

Technical means in combination with computers are very useful in modern medical diagnostic methods. Optical tools, such as microscopes and cameras, linked with computer enable not only to examine structural pathologies, but also to record and analyze microscopic motion processes. Image – as the basic element of imaging diagnostic methods – contains a huge amount of information, which cannot be always visible. We can use many software tools to extract this hidden information. One of these tools is LabVIEW with Vision Development module designed for image processing and analysis.

2. Structure and Function of Respiratory Epithelium

The human respiratory apparatus is characterized by large surface in continual interaction with outer environment. Many foreign particles are inhaled to this apparatus in the inbreath phase, but we know some defense mechanisms: surface moisturizing or particles removing by mucociliary transport.

The respiratory mucous membrane is created from glandular epithelium (covering the layer producing mucus) and ciliated epithelium of various types, which moves mucus with foreign particles out of the respiratory apparatus [1].

Each epithelium cell contains around 200 cilia beating with frequency near 1000 min-1 (ca. 17 Hz). Each cilium contains 9 peripheral (double) fibrils, and 2 central fibrils. Cilia movement depends on mutual sliding between fibrils [2]. In Fig. 1 we can see ciliated cell.

Fig. 1. Microscopic image of ciliated cell

One phase of cilia movement is called effective stroke, when cilia pike (end) shifts mucus in oral direction (out of the respiratory apparatus). In the healthy respiratory epithelium, the movements of cilia are synchronized (in one direction) and the mucus is normally transported. Inherited or obtained defects in cilia structure cause immovability or bad synchronization. These structural changes underlie mucus stagnancy and continuous infections of the respiratory apparatus. In case of inherited defects, we can talk about PCD (Primary Ciliary Dyskinesis) syndrome or cystic fibrosis.

3. Virtual Instrumentation in LabVIEW

Virtual instrument is software analogy of a real meter with all functions. By using universal measuring PC card, we can simulate a large number of expensive meters. User can freely modify the design, controls and indicators of this virtual meter, which is characterized with high degree of flexibility compared to a real one. One of the development systems for virtual instrumentation is the National Instruments LabVIEW.

LabVIEW (Laboratory Virtual Instruments Engineering Workbench) is the graphical development system containing many tools and functions for data and signals measurement, analysis and presentation. Vision Development module contains powerful tools of image processing and analysis

and an independent application Vision Assistant (for building script „skeleton" without programming).

The script for each virtual instrument is called Block Diagram; using icons and color data links makes the script very clear compared to a classical programming language and suitable for fast and easy editing, debugging or repairing.

Graphical user interface (called Front Panel) is automatically generated for each Block Diagram and contains many control items (for inputting constants, strings, arrays; buttons, switches and sliders) and many indicators (windows, graphs, displays, LEDs) [3].

4. Videosequence Acquisition

The issue of kinetic analysis of ciliated cells can be divided into few basic steps: image (video) acquisition, image preprocessing, kinetic parameters analysis (frequency and trajectory).

Hardware acquisition equipment is: inverse biological light microscope and monochrome camera connected to PC through IEEE 1394 (FireWire). LabVIEW application is the software platform for videosequence acquisition. The acquisition system and camera connection with microscope are shown in Fig. 2 and 3.

Fig. 2. Inverse microscope MODEL IM 1C.

Fig. 3. Camera AVT MARLIN F-046B.

The camera supports acquisition in grayscale (8 BPP) mode and two basic formats: Format_0 and Format_7, which differ in resolutions and FPS (from 3,75 to 60 frames per second). Both formats are raw (uncompressed).

Grayscale videosequences are sufficient for further analysis as many LabVIEW tools are applicable only on grayscale image. The given acquisition system can be easily built in medical or laboratory environment. In order to test the applications for sequence preprocessing and kinetic parameters analysis, some phantom sequences were acquired (without microscope or using microscope – microscopic grid movement) or recordings of respiratory epithelium from the internet (Fig. 1).

Fundamental function for image (videosequence) acquisition is LabVIEW express function for communication with the device on IEEE 1394 port and control items enabling to set up the camera shutter, format, FPS, brightness and gain, number of frames (sequence length). Final videosequence is written as raw video file (*.avi) using FOR Loop structure and AVI Create tool. Front Panel of acquisition application is in Fig. 4.

5. Videosequence Preprocessing

Preprocessing has two primary goals: to shorten analysis time and improve kinetic parameters analysis.

The time needed for analysis can be reduced by cutting off of the regions or frames (redundant time intervals) which do not contain relevant information. In this aspect we can talk about time reduction (e.g. frames selection from 7 to 153 of the whole sequence) or spatial reduction (selection of ROI containing isolated cell). Script is very simple and its output is time or spatial (or both) reduced videosequence.

An important phase is improving intensity levels in the whole image. Fig. 5 shows the Front Panel of application for image (sequence) preprocessing. After videosequence opening the following operators and tools can be applied:

Extraction of single color plane
This tool extracts 8-bit gray plane from color image, because many LabVIEW tools and operators work only with grayscale images. The most important is Luminance 8-bit plane (from HSL model, which is RGB derivate). Luminance plane contains information about objects position and minimizes information stored in textures, which is more important for movement detection.

Lookup Table (LuT) operators
Lookup Table is a transfer characteristic (linear, linear in parts, nonlinear) between an original image and a processed image. They enhance and improve intensity relations in the whole image, change brightness and contrast. The kind of LuT operator is **BCG Correction** (B = brightness, C = contrast, G = gamma) [4]. The best known transfer characteristics are shown in Fig. 6. The Lookup exponent is the variable which gives the final curving of the base curve. LuT range is intensity interval on which the LuT curve (characteristic) is applied.

Convolution (spatial) filtering
Convolution filtering belongs to linear filtering. The filter uses a kernel (an array of odd degree with the defined central pixel). The kernel moves through the image, convolution between the original image and the kernel array is calculated on each position and the result is written on the central pixel position. The result can be divided by normalization factor N, which is the sum of kernel values or 1 [4].

Equalization
Equalization enhances pixel intensities so that they are equally distributed in the defined grayscale range, which is from 0 to 255 in an 8-bit image. The function cumulates equivalent amounts of pixels on a constant grayscale interval. Equalization enables to use all gray intensity values in an image. This

transformation is needed for contrast increasing in images with few intensity values. The equalization range is the value interval on which the transformation is done; this interval will be remapped on 0-255 interval, so we can reveal details from this interval only [4].

Histogram

Histogram shows dependency between the intensity value and the number of pixels of this gray value. It gives us a complex view on the image and facilitates the noise removal or segmentation.

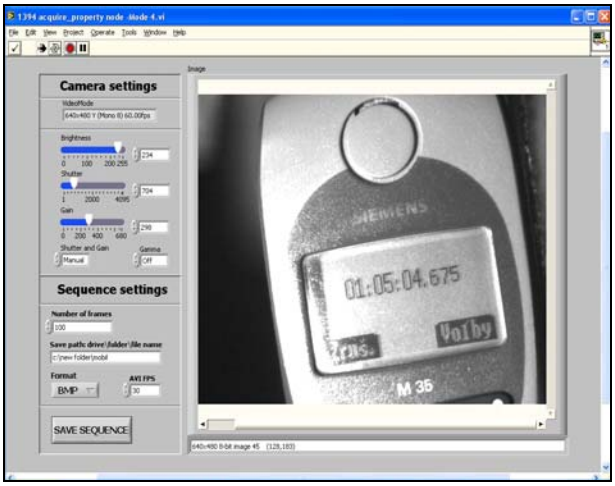

Fig. 4. Acquisition application Front Panel.

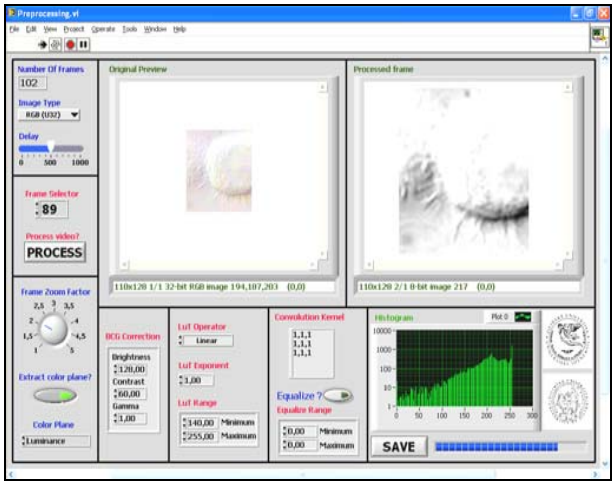

Fig. 5. Preprocessing application Front Panel.

LUT	Transfer Function	Shading Correction
Linear		Increases the intensity dynamic by evenly distributing a given gray-level interval [min, max] over the full gray scale [0, 255]. Min and max default values are 0 and 255 for an 8-bit image.
Logarithmic Power 1/Y Square Root		Increases the brightness and contrast in dark regions. Decreases the contrast in bright regions.
Exponential Power Y Square		Decreases the brightness and contrast in dark regions. Increases the contrast in bright regions.

Fig. 6. LUT Transfer Functions.

6. Measurement of Frequency (CBF)

Healthy cilia make continuous and synchronized moves. The frequency is often called CBF (Cilia Beat Frequency). This parameter can be measured using the intensity method. In this method, we create ROI in near surrounding of beating cilia. Passing of cilia across the ROI causes intensity variations in it. We record average ROI intensity in time and this periodical curve is processed with tools of frequency analysis. By application of FFT (Fast Fourier Transform) we can see the frequency

spectrum of beating. We can also display PSD graph (Power Spectral Density), which is FFT of curve autocorrelation. In Fig. 7 we can see graph of intensity variations and in Fig. 8 PSD of this curve. We can also see (Fig. 8) that measured frequency is in the range of 17 – 20 Hz, which is the physiological value.

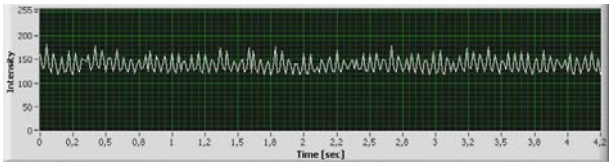

Fig. 7. Average ROI intensity variance graph.

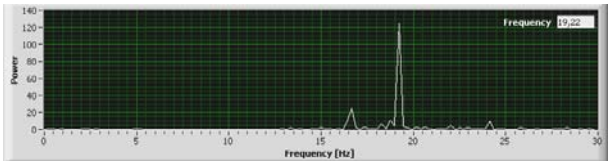

Fig. 8. Power spectral density of variance graph.

LabVIEW contains components working with MS Office, so measurement results can be stored in Excel table. There is Front Panel of application for frequency analysis in Fig. 9.

Second parameter – trajectory (or object position in the frame) is analyzed by algorithms of object detection.

7. Analysis of Cilia Trajectory

The main tool in this phase of work is Pattern Matching. This tool searches a defined template on each frame of videosequence. Pattern Matching is a set of algorithms based on many techniques. The best known algorithm is cross correlation computing between an original image and a template. Computing of correlation consists of many elementary multiplications and it is very time-consuming; cross correlation cannot find rotated objects (rotation against the template max. 5 – 10°) and scaled objects (bigger or smaller than objects in the template) [4]. In practice we often use techniques working in frequency domain, pyramidal matching or other intelligent techniques.

Pattern Matching and trajectory detection were tested on phantom videosequence with circular movement of the object. In Fig. 10, we can see the Front Panel of the application for trajectory detection and Pattern Matching settings and in Fig. 11, 12 and 13 the frame from phantom videosequence, trajectory and angle trajectory, respectively.

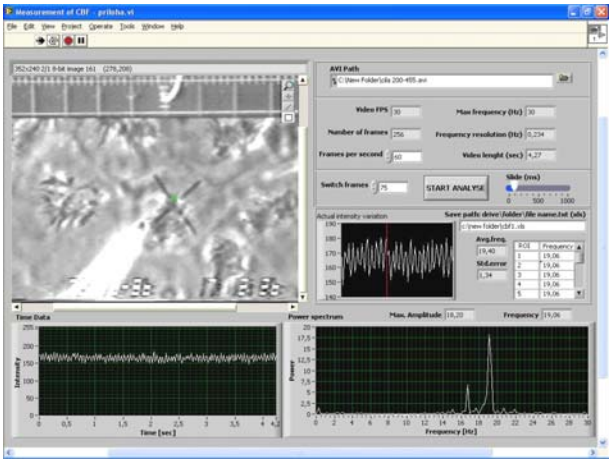

Fig. 9. CBF analysis Front Panel.

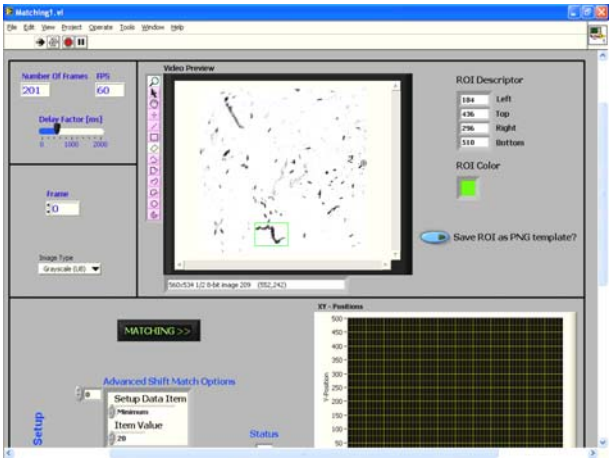

Fig. 10. Pattern Matching detection Front Panel.

Isolated points of trajectory (red dots) are the centres of ROIs detected as template in the image. Detection errors (white lines heading towards the origin of the coordinate system) is also visible. Detection errors come from some blurred frames, even though higher FPS (30 or 60) was used. These detection errors can be removed by local averaging or by using some interpolation methods.

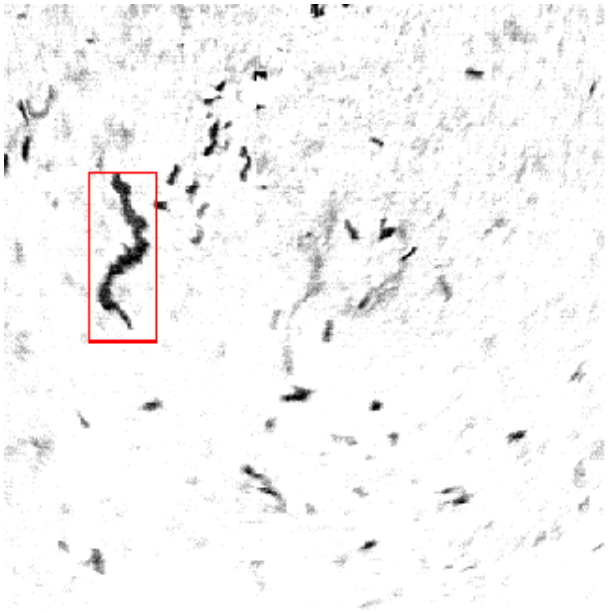

Fig. 11. Phantom video with detection template.

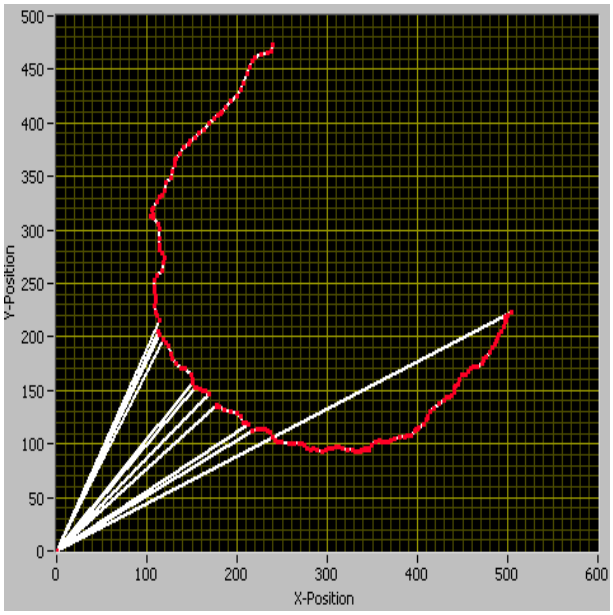

Fig. 12. Circular trajectory graph.

Results of detection on real cilia videosequences are often controversial and chaotic. The reasons are the following: low quality videosequences from internet sources with low resolution (MPEG format with information loss) or a big amount of similar objects on a small surface. The result of the bad detection can be seen in Fig. 14.

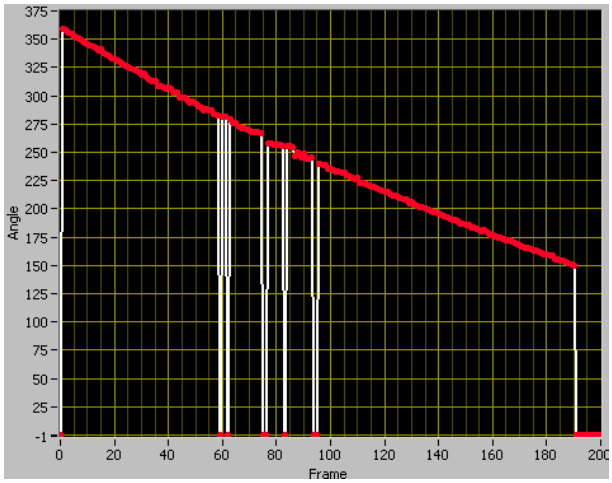

Fig. 13. Angle trajectory.

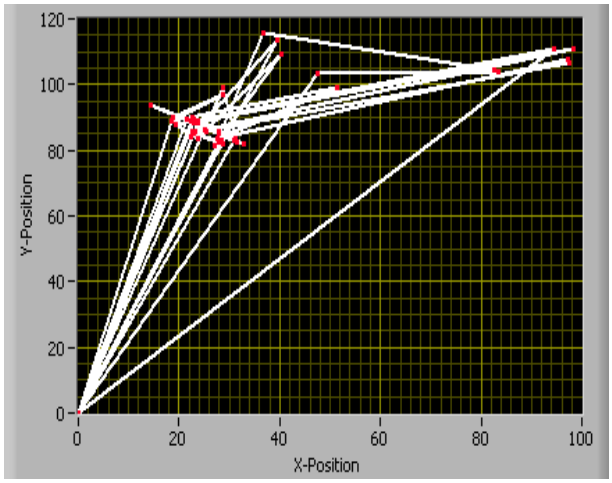

Fig. 14. Bad detection of trajectory.

8. Conclusions

Analysis of the kinetic parameters of the respiratory epithelium is very important in diagnostics of primary ciliary dyskinesis. By using various acquisition methods and image processing methods, we can create bigger videosequence database for determining the reference values for the cilia movement analysis. Pattern Matching techniques seem to be suitable for phantom videosequences. Preliminary experiments were performed on phantoms or videosequences from internet sources, these results are useful in creating feedback for acquisition of own videosequences in the real clinical environment.

Acknowledgements

This work has been supported in part by grant No.1/3107/06 and No.1/3123/06 and No.1/0704/08 from VEGA grant agency and by the grant No.LPP-0237-06.

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Guide for Contributors

Aims and Scope

Sensors & Transducers Journal (ISSN 1726-5479) provides an advanced forum for the science and technology of physical, chemical sensors and biosensors. It publishes state-of-the-art reviews, regular research and application specific papers, short notes, letters to Editor and sensors related books reviews as well as academic, practical and commercial information of interest to its readership. Because it is an open access, peer review international journal, papers rapidly published in *Sensors & Transducers Journal* will receive a very high publicity. The journal is published monthly as twelve issues per annual by International Frequency Association (IFSA). In additional, some special sponsored and conference issues published annually.

Topics Covered

Contributions are invited on all aspects of research, development and application of the science and technology of sensors, transducers and sensor instrumentations. Topics include, but are not restricted to:

- Physical, chemical and biosensors;
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- Theory, principles, effects, design, standardization and modeling;
- Smart sensors and systems;
- Sensor instrumentation;
- Virtual instruments;
- Sensors interfaces, buses and networks;
- Signal processing;
- Frequency (period, duty-cycle)-to-digital converters, ADC;
- Technologies and materials;
- Nanosensors;
- Microsystems;
- Applications.

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Articles should be written in English. Authors are invited to submit by e-mail editor@sensorsportal.com 6-14 pages article (including abstract, illustrations (color or grayscale), photos and references) in both: MS Word (doc) and Acrobat (pdf) formats. Detailed preparation instructions, paper example and template of manuscript are available from the journal's webpage: http://www.sensorsportal.com/HTML/DIGEST/Submition.htm Authors must follow the instructions strictly when submitting their manuscripts.

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