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Primary ciliary dyskinesia assessment by means of optical flow analysis of phase-contrast microscopy images

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ABSTRACT

Primary ciliary dyskinesia implies cilia with defective or total absence of motility, which may result in sinusitis, chronic bronchitis, bronchiectasis and male infertility. Diagnosis can be difficult and is based on an abnormal ciliary beat frequency (CBF) and beat pattern. In this paper, we present a method to determine CBF of isolated cells through the analysis of phase-contrast microscopy images, estimating cilia motion by means of an optical flow algorithm. After having analyzed 28 image sequences (14 with a normal beat pattern and 14 with a dyskinetic pattern), the normal group presented a CBF of 5.2 ± 1.6 Hz, while the dyskinetic patients presented a 1.9 ± 0.9 Hz CBF. The cutoff value to classify a dyskinetic specimen was set to 3.45 Hz (sensitivity 0.86, specificity 0.93). The presented methodology has provided excellent results to objectively diagnose PCD.

1. Introduction

Primary ciliary dyskinesia (PCD) is an autosomal recessive inherited disorder affecting approximately 1:10,000 to 1:30,000 individuals [1–3]. It causes a defect in the action of the cilia lining the respiratory tract (lower and upper, sinuses, Eustachian tube, middle ear), Fallopian tube, cerebrospinal fluid tract and spermatozoid flagella. PCD is characterized by the complete absence of mucociliary clearance, leading to respiratory symptoms and signs typically present since birth and predisposing affected individuals to recurrent respiratory infections [4]. Approximately half of sufferers have situs inversus [5–7].

Motile cilia play a crucial role in clearing mucus and debris from the airways under normal conditions, as can be seen in patients with abnormal airway ciliary beating caused by primary ciliary dyskinesia [8,9]. Motile cilia also play a role in circulating spinal fluid in the ventricles of the brain, where abnormal ciliary beating has recently been linked to hydrocephalus and other developmental cerebral abnormalities [10,11].

Despite persistent symptoms, and often attendance at ear, nose, and throat and respiratory clinics, many patients with PCD are not diagnosed until later in life [12], by which time permanent lung damage has occurred [13]. Early and accurate diagnosis is important, because once made, lung function can be maintained with specialist respiratory care [14–16]. The diagnosis of PCD is traditionally made on the basis of a supportive clinical history and an abnormal ciliary beat frequency (CBF). The most commonly used techniques (the modified photodiode [17] or photomultiplier method [18]) to measure CBF use an indirect method and do not provide information on ciliary beat pattern. New high-resolution digital high speed video (DHSV) imaging has allowed the precise measurement of the beat pattern of cilia [19].

A commonly used method to estimate CBF using a DHSV has been explained in [20,21]. This method computes the Fast Fourier Transform (FFT) of the intensity signals in a window of 3×3 pixels centered on a selected pixel above the cilium. This technique does not consider the global movement of all cilia and local illumination changes can affect the results.

Other novel methods to compute the CBF are based on estimating the movement of pixels or regions in the images by using different techniques, as motion templates [22] or the Lucas–Kanade algorithm [23]. However, these methods are not valid when the cell is moving due to its own ciliary beat.

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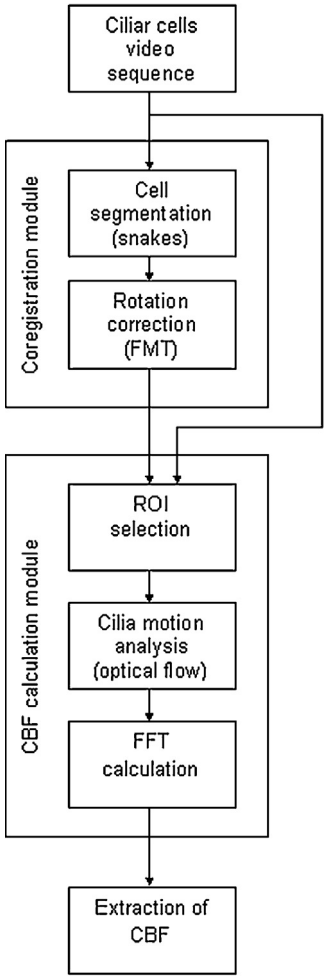

Fig. 1. System flow diagram. FMT: Fourier–Mellin transform. CBF: ciliary beat frequency.

In this paper, we present a method to measure CBF of isolated cells based on the analysis of the cilia global movement by means of optical flow algorithms. A stabilization process is also implemented to eliminate the cell movement effects. The main modules in the system are summarized in Fig. 1. In next section, functionality of each block is described.

2. Materials and methods

Fresh sample of nasal mucosa were obtained from 28 subjects, and a 5 s duration digital video of its movement was acquired. From these 28 videos, 14 belonged to healthy volunteers, with a normal ciliary movement (normal group), while 14 had a ciliary dyskinetic movement (dyskinetic group). The observations were performed by two investigators, members of the Primary Ciliary Dyskinesia Unit, who used homogeneous criteria and who previously were validated by a *K*-test (>85%) [24].

All image processing was carried out using MATLAB R2010a (The MathWorks, Inc., Natick, MA) on an AMD 2.8 GHz computer with 4 GB of RAM, running Windows XP.

2.1. Image acquisition

Ciliary cells were imaged with a digital high speed video (DHSV) imaging technique using an Eclipse TS100 microscope (Nikon Corporation, Tokyo, Japan) with a 40× Nikon phase-contrast objective and a 10× ocular lense, providing a final optical gain of 400×.

The microscope was connected to a CCD camera (Digital Quad High Speed Progressive Scan Camera, JAI CV-A33 CL, Jai UK Ltd., Uxbridge, United Kingdom) that records the images with a matrix size of 649 × 494 pixels, and a rate of 120 frames per second.

Images were acquired into a HP Workstation xw6200 Xeon 3.4 GHz with 2 Gb of RAM system (Hewlett-Packard Company, Palo Alto, CA, USA) by means of an image acquisition board (NI PCIe-1429, Full Configuration Camera Link Image Acquisition, National Instruments, Austin, TX, USA).

2.2. Stabilization

Ciliated samples are placed in a liquid solution. Therefore, ciliary beat can cause a movement of the cells to analyze. In general, there are two groups: isolated cells that have a rotation movement, and cells that are sticked on the bottom of the slide and keep still. In the first case, it is necessary to eliminate this rotation movement to estimate CBF [25].

2.2.1. Cell segmentation

For initiating the stabilization process, it is necessary to localize the cell to analyze. We propose a semi-automatic segmentation method based on gradient vector flow snakes to perform this task. A snake is an energy-minimizing spline guided by external constraint forces and influenced by image forces that pull it toward features such as lines and edges [26]. Instead of exploiting only image information as low-level edge-detection techniques do, snakes also use information about the boundaries as part of an optimization procedure. Snakes are active contour models: they lock onto nearby edges, localizing them accurately.

The active-contour model involves vertices connected by edge segments with, in general, two associated force terms. The internal force is computed based on the local shape of the contour. The external force (or image force) that drives the active contour to the boundary can be based on any conventional edge-detection technique. The internal and external forces may be weighted differently.

Mathematically, a snake can be defined in discrete form as a curve $x(s) = [x(s), y(s)]$, $s \in [0, 1]$ that moves through the spatial domain of an image to minimize the energy function

$$E = \int_0^1 \frac{1}{2}(\alpha|x\prime(s)|^2 + \beta|x''(s)|^2) + E_{\text{ext}}(x(s))ds \quad (1)$$

where α and β are weighting parameters that control the active contour's tension and rigidity respectively, and govern the effect of the derivatives of $x(s)$. The external energy function E_{ext} is derived from the image so that it takes on its smaller values at the features of interest such as boundaries.

A snake that minimizes (1) must satisfy the Euler equation

$$\alpha x''(s) - \beta x''\prime(s) - \nabla E_{\text{ext}} = 0 \quad (2)$$

where $F_{\text{int}} = \alpha x''(s) - \beta x'''(s)$ and $F_{\text{ext}} = -\nabla E_{\text{ext}}$ comprise the components of a force balance equation such that $F_{\text{int}} + F_{\text{ext}} = 0$.

Xu and Prince [27] proposed Gradient Vector Flow (GVF) to improve the capture range of the image force. GVF involves a vector field derived by solving a vector diffusion equation which diffuses the gradient vectors of a gray-level image. The solution for the GVF vector field involves a combination of Laplacian and gradient terms, and a blending factor is used for governing the trade-off between them. GVF snakes replace the potential force $-\nabla E_{\text{ext}}$ by the gradient vector flow field. The GVF field can be defined as the vector field $v(x, y) = (u(x, y), v(x, y))$ that minimizes the energy function

$$\varepsilon = \iint \mu(u_x^2 + u_y^2 + v_x^2 + v_y^2) + |\nabla f|^2|v - \nabla f|^2 dxdy \quad (3)$$