A regionalized automated measurement of ciliary beating frequency

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A REGIONALIZED AUTOMATED MEASUREMENT OF CILIARY BEATING FREQUENCY

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ABSTRACT

Cilia are slender, microscopic, hair-like structures or organelles that extend from the surface of nearly all mammalian cells. Motile cilia, such as those found in the lungs and respiratory tract, present a beating motion that keep the airways clear of mucus and dirt. They are thus of primary importance in many respiratory diseases. The performance of mucous transportation in the nasal cavity can be represented by a ciliary beating frequency. In this paper, we propose a fully automated method that computes the beating frequency from a sequence of images taken with high-speed videomicroscopy. The advantage of our approach is its capacity in computing regionalized frequencies, *i.e.*, various frequencies each associated with one region in the image. Moreover we propose a preprocessing pipeline to alleviate acquistion artefacts due to the camera or to the cell proper motions. We demonstrate the robustness of our approach, and illustrate its performance in comparison to the state-of-the-art.

***Index Terms*—** Cilia, mucociliary clearance, optical flow, registration, segmentation

1. INTRODUCTION

Muco-ciliary clearance is a crucial mechanism of defense against aerial environmental attacks such as micro-organisms or pollution. This clearance is achieved by the coordinated beating of the cilia covering the nasal epithelium. Cilia motility impairment can be either of genetic (primary ciliary dyskinesia) or acquired origin due to environmental attacks and may entailing chronic diseases such as chronic sinusitis and bronchitis. It is of interest for practitioners to evaluate ciliary beating frequency (CBF) easily, robustly and reliably. The estimation of ciliary beating frequency has been a research topic since the middle of the 20th century. One of the first method of reference for measurement of cilary beating frequency was proposed in 1962 and used a photo-sensitive cell [1]. Stroboscopic methods have been replaced by more accurate techniques that use photomultiplier, photodiode and high-speed imaging. Those methods are described and compared in [2]. Analysis via high-speed videomicroscopy is now considered the most accurate method. Hence, the most commonly used technique today for evaluating ciliary function in human being consists of collecting ciliated cells from nasal or tracheobronchial surface mucosa, to observe them under a microscope and to record their motion via high-speed video acquisition. Evaluation, via these records, of ciliary beating frequency and ciliary beating pattern was reported helpful in the diagnosis of primary ciliary dyskinesia [3, 4, 5]. In clinical research, there exist several methods that estimate ciliary beating frequency. Cinematic analyzis [6] counts the number of frames required to complete 10 ciliary beat cycles. It is a time-consuming and user-dependent method, which has to be repeated several times to obtain a reliable result. Kymograph analysis [7] is a linescan-like method where the grey level of a line drawn by the user is analyzed. It is sensible to illumination and vibrations, depends on the location of the line, and is thus also user-dependent.

Some attempts to automate the measurement of CBF have been proposed in the literature. The SAVA System [8] estimates frequencies from small 4×4 pixels windows. Whole frequency spectra can be simultaneously estimated. It is based on grey-level intensity variation, which has shown some limitation if the contrast is not sufficient, rendering the reliability of the technique questionable [9]. CiliaFA [10] provides a frequency histogram of a large number of small regions of interest, assuming low noise and no cell proper motion. The method proposed in [11] uses a sparse optical flow to estimate a single frequency per image. Thus, it is not applicable when several different beating patterns are present in the sequence. Moreover, the method is very sensitive to noise and is easily perturbed by cells proper motion. A linescan-based technique is proposed in [12], coupled with the Fast Fourier Transform, and is evaluated on slices on brain ciliated epithelium. It deals with acquisition problems: the removal of artefacts due to the camera sensor, and frame stabilization. However, the removal needs a blank acquisition sequence and thus access to the camera. More problematic for our applica-

tion, the straight linescan technique needs a straight border of cells, something not always possible with harvested cells.

In this paper, we propose a novel, fully automated tool for the frequency estimation of beating cilia, that overcomes the various limitations of the above methods. In particular, as detailed in section 2.1, our method removes the camera artifacts and stabilizes the inner parts of the cells, leading to greater measurement robustness. More importantly, we propose in section 2.2 to segment the cilia in several zones, each of presenting a consistent area with the same beating frequency that can be estimated using a dense optical flow. We demonstrate in section 3 the validity of our approach, by comparing it to two established methods for cilia beating frequency estimation. Section 4 concludes the paper.

2. METHODOLOGY

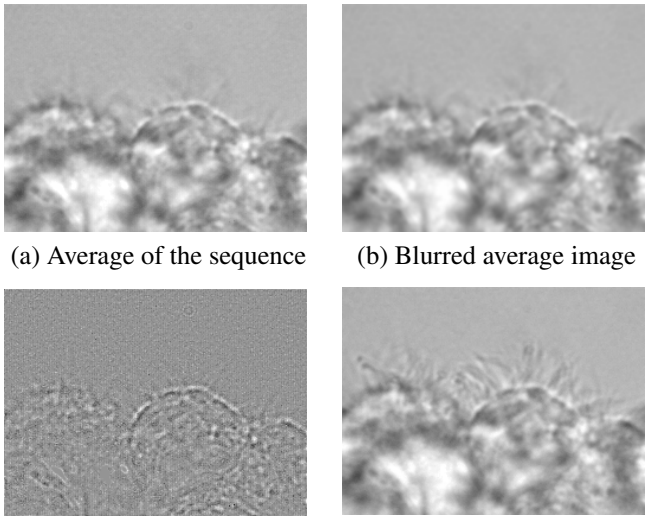
In the field of view, multiple cell groups are often visible, and cilia on a given cell can beat at different frequencies. As a result, many frequencies can be measured in a single field of view. Such frequencies provide information on cilia synchronization, and ultimately on the status of the cells under scrutiny. We seek to segment the field of view into regions that are consistent from the point of view of the beating pattern.

Our method is described as follows: in section 2.1, we remove acquisition artifacts due to the camera and to the experimental protocol. We then segment the images into consistent regions in section 2.2. We first remove the static elements from each image of the sequence, obtaining images where only moving cilia are visible. Inter-images variance allows us to segment each image of the sequence into regions with similar beating pattern. A dense optical flow provides an estimate of the beating frequency on each one of those regions.

2.1. Preprocessing

Two different acquisition artifacts, inherent to the experiment, need to be dealt with: a fixed sensor pattern (a grid-like structure), due to the camera, is visible on the sequence, and harvested cells live in a liquid environment, inducing undesirable cell motion.

Sensor-pattern removal. A sensor pattern is often present on high-speed camera. Since it is associated with the sensor, it never moves, contrary to the content of the acquired sequence, consequently the pixel-wise average $\overline{I} = \frac{1}{n}\sum_j I_j$ of the image sequence integrates the non-moving parts, as well as some residual texture due to the moving parts. A Gaussian filter erases this texture. Subtracting the blurred average frame from the average frame yields a texture image $I_t = \overline{I} - (\overline{I} \star G_{\sigma=1})$. This texture image is then subtracted from each image of the sequence, leading to a pattern-free sequence, as illustrated on Fig. 1.



(a) Average of the sequence (b) Blurred average image

(c) Texture (d) Image without texture

Fig. 1. Sensor-pattern removal (see text).

Sequence stabilization. We developped a robust adaptative rigid registration technique, relying on SIFT keypoints [13]. We first remove SIFT outliers by brute-force matching. We then compute candidate transforms by matching points in pairs, obtaining a similarity transform. We project the transform to the unit circle to estimate a rigid transform. As a final step, we select the most explanatory model between identity, translation-only and rigid transform. The result is a texture-free, stabilized sequence I^s.

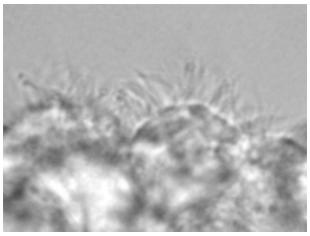
2.2. Region segmentation and frequency estimation

By subtracting from each frame the mean of the stabilized sequence, we obtain a sequence I^{mov} of moving elements on a grey-level background, *i.e.* $\forall j \in \{1 \dots n\}, I_j^{mov} = I_j^s - \frac{1}{n}\sum_k I_k^s$ (Fig. 2.b). We compute the variance $\mathcal{V}$ of S^{diff}, the sequence of difference of frames $(I_i^{mov} - I_{i+1}^{mov})_i$. We then apply a Gaussian filter (G) on $\mathcal{V}$ leading to an image $\mathcal{G} = \mathcal{V} \star G_{\sigma=5}$ in which each zone with a similar beating pattern forms a white blob. We threshold $\mathcal{G}$ at 30% of the maximum intensity, obtaining $\mathcal{G}_s = \mathcal{G}_{\geq 0.3*\max(\mathcal{V})}$. We calculate $\mathcal{M}_1$, the markers of the blobs by morphological erosion and dilation on $\mathcal{G}_s$. We then segment the blobs using a watershed WS [14] with $\| \nabla(\mathcal{G}) \|_M$ the morphological gradient of $\mathcal{G}$

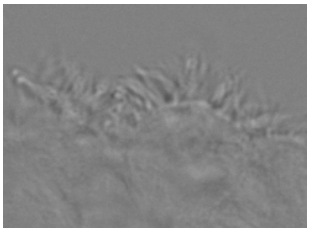
$$\mathcal{R} = \mathrm{WS}(\| \nabla(\mathcal{G}) \|_M, \mathcal{M}_1) \quad (1)$$

We compute a dense optical flow using Färneback's algorithm [15], through which we obtain a dense displacement vector field. In each one of the previously segmented regions, the median of the vectors contained in that region provides the displacement of that region.

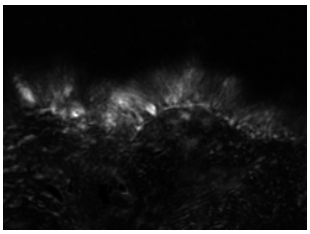
Frequency is then estimated via a Fourier analysis of the



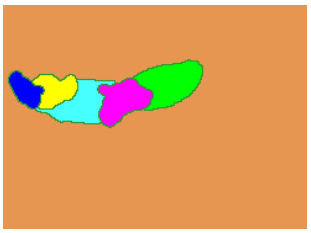
(a) Initial frame



(b) Moving parts



(c) Variance of the sequence



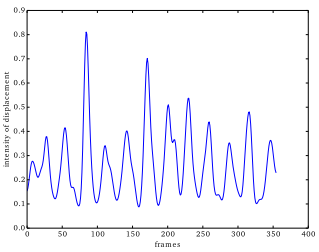
(d) Identification of areas

Fig. 2. The segmentation of the field of view into regions with similar beating pattern relies on the variance of the sequence of the difference between consecutive frames.

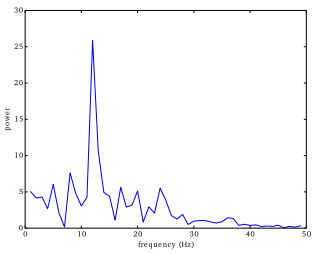
speed variation (*i.e.*, the norm of the displacement vector) over time (Fig. 3).

3. RESULTS AND VALIDATION

Data. We analyzed 10 nasal brushing biopsies from patients of the ENT departement of Henri Mondor Hospital (Créteil, France). Nasal brushing produces significant amounts of cells with beating cilia. The diversity in sequence appearances can be appreciated on Fig. 4.
Brushings were recorded under a microscope minutes after the biopsy, at 358 frames per second with a high speed camera. The spatial resolution was 0.13μm, and the resolution was 256×192 pixels. The record was taken on the border of the groups.



(a) Speed variation



(b) Frequency spectrum

Fig. 3. Fourier analysis of speed variation for one of the sample yields to a frequency of 12.10 Hz.

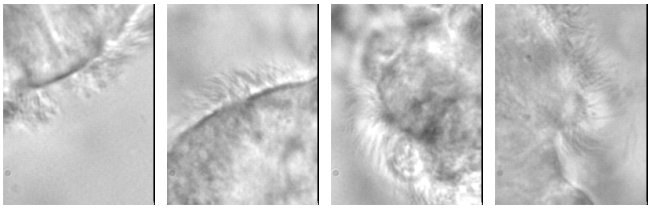
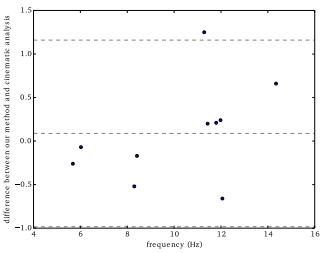


Fig. 4. Four examples of ciliated cells, showing the large variability in our samples.

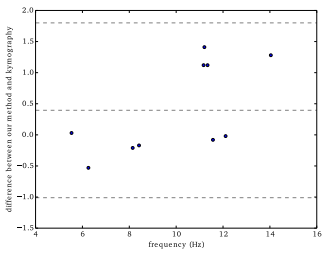
Software. We developped our method using python 2.7.6, Pink [16] for python, numpy, scipy and OpenCV for python. Our application runs in less than 20 seconds on an iMac 3.5GHz Core I7, with 32 GB of memory.

Validation. Bland-Altman diagrams show a repartition of the distance between our method and the two methods of reference (cinematic analysis and kymography). We can observe that our frequency estimations are all contained in the confidence interval of 95% when compared with the kymography method. By comparison with the cinematic anaysis, only one measure is out of the interval, which remains acceptable.

Importance of preprocessing. Preprocessing steps are meaningful in our context. Removing camera artefacts is necessary for the success of image analysis process. The importance of stabilization can be highlighted by some of the image sequences. Indeed, one example shows a dead cell sequence. If the stabilization is not performed, both our algorithm and the classical techniques estimate a beating frequency of 12Hz. A closer look to the video demonstrates that this frequency corresponds to the motion of the cell due to vibrations. After the stabilization, we obtained a frequency of 0 Hz, as expected.



(a) Cinematic analysis



(b) Kymography

Fig. 5. Bland-Altman plots show the consistency between our proposed approach vs. cinematic analyis and kymography.

4. CONCLUSION

In this paper, we propose a regionalized automated measurement of the ciliary beating frequency, capable of coping with several cell groups, each with their own beating pattern. Preprocessing deals with camera artefacts and stabilizes cell proper movement and camera motion to enable a segmentation of the moving parts that remain. Those moving parts are cilia, and are segmented according to their motion. Comparison with semi-automated kymography and cinematic analysis for frequency measurement in the same segmented areas shows that our results are significant.

In future work, we will confirm that our approach is indeed able to process more than one cell group in each field of view. We will also validate our method on a larger database. Furthermore, we believe that we can measure other characteristics beyond frequency from the segmented regions. In particular, an analysis of the qualitative components of beating patterns seems achievable, including a full description of range, rhythm and structure.

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