

Chapter 28

Epithelial Cell Detection in Endomicroscopy Images of the Vocal Folds

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Abstract The reasons for a hoarse voice are manifold. Besides structural changes such as additional masses on the vocal folds, changes in the layers of the vocal fold mucus influence the acoustic properties of the voice signal [1]. In our research, we aim to examine this in vivo. One suitable technique for this purpose is the use of micro endoscopes. In contrast to traditional microscopes, the micro endoscopes have a reduced image quality and exhibit strong noise artifacts. Furthermore, images are affected by inhomogeneous illumination. All of the mentioned effects pose a challenge to automatic cell detection and segmentation methods. In this paper, we investigate whether automatic cell detection methods are also suitable for the cells of the epithelium of the vocal folds. Based on band-pass filtering, we could successfully reduce noise and emphasize cell boundaries at the same time. The pass-band was experimentally chosen to emphasize the regular structure of the epithelial cells which can be observed in the frequency domain of the cell image. Subsequently, we applied a watershed segmentation to identify the cell borders. Cell centers were located using a local minima search in the band-pass filtered image. First results indicate that

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the method is able to locate and outline epithelial cells with high accuracy. Future research will focus on the relation between such quantitative measures in cell images to acoustic properties of the voice signal and the mechanical properties of the vocal folds such as the synchrony of their vibration.

28.1 Introduction

Voice hoarseness can be caused by several reasons including laryngitis, cancer of larynx, and structural changes in the vocal folds such as nodules and polyps. Recently, it was shown that changes in the mucus of the vocal folds can be related to acoustic properties of the voice signal [1]. We aim to investigate the vocal fold mucus in vivo using a micro endoscope. An essential step towards this goal is the detection of epithelial cells in the mucus layer which is the topic of this paper.

A plenty of cell detection approaches are available in literature [2–6]. Images of the epithelial cells exhibit two important properties. Firstly, due to physiological reasons, the epithelial cells cover the whole scene. Therefore, the separation between cells and background, which is necessary in several proposed approaches [4, 6, 7], is not required. Secondly, there is a repetitive pattern. The latter was exploited in [8] and [9] for cell density estimation in the corneal endothelium. The purpose of this paper is to investigate whether it is possible to utilize the two aforementioned facts so that basic image processing algorithms can be applied in order to detect epithelial cells in endomicroscopy images of the vocal folds.

28.2 Materials and Methods

28.2.1 Materials

A sample of nine images of the epithelium of the vocal folds were acquired using a micro endoscope of a Cellvizio probe-based confocal laser endomicroscopy (pCLE) system. Figure 28.1 shows two examples.

28.2.2 Detection Pipeline

We apply a band-pass filter on the input image. Cell centers are then found using a minima search procedure. Watershed algorithm is utilized in order to delineate the cell borders. The pipeline is demonstrated in Fig. 28.2. Minima search and watershed in this pipeline are parameterless. On the other hand, the pass-band of the filter must

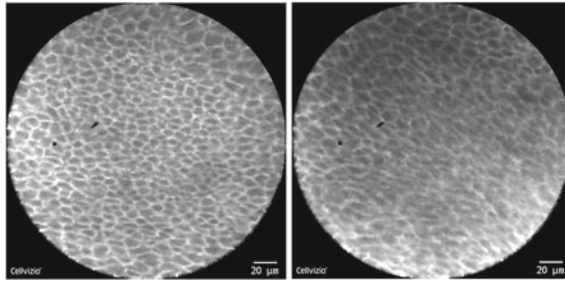


Fig. 28.1 Endomicroscopy images of the vocal fold epithelium

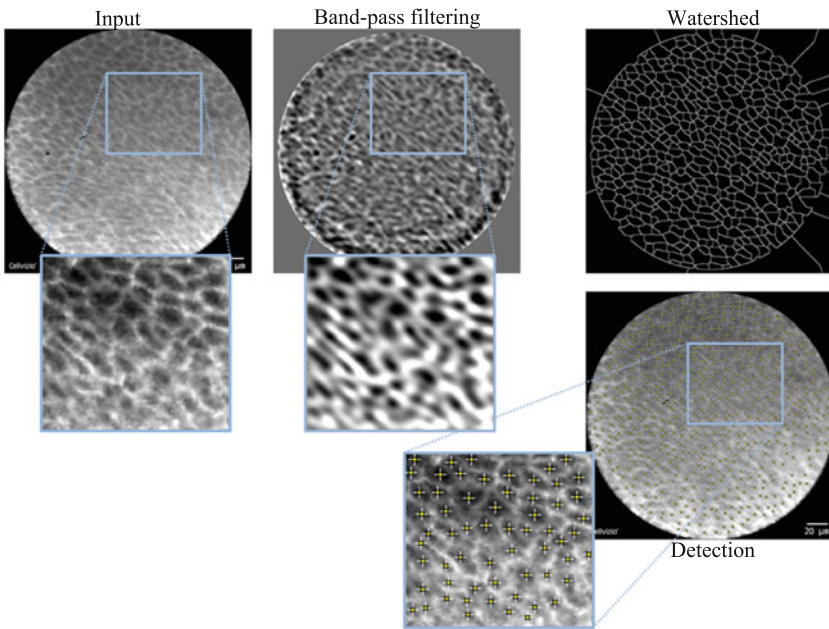


Fig. 28.2 Cell detection pipeline

be tuned. The goal of the tuning is emphasizing the regular pattern of the epithelial cells and at the same time reducing noise and smoothing cellular details.

28.3 Evaluation

A band-pass filter was manually designed in Fourier domain for each image and the pipeline described above was applied. Figure 28.3 exemplifies the results. The

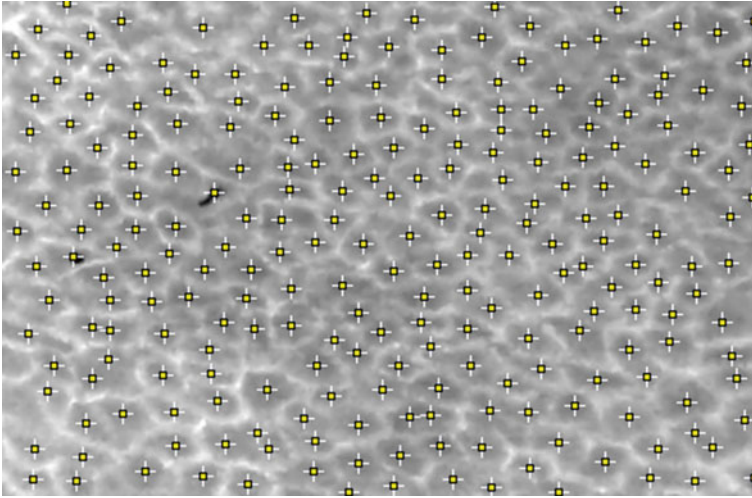


Fig. 28.3 An example of detection results

obtained F-measure of cell detection, averaged over the nine images, was 80.2 ± 4.7 distributed as 94.6 ± 3.7 recall and 70.0 ± 7.3 precision.

28.4 Conclusion and Discussion

It is well known from Fourier analysis that periodicity in space manifests itself in Fourier domain as a peak at the fundamental frequency of the signal. In the case of the 2D Fourier transform, a frequency component along a direction ϕ in space conforms to a peak at the corresponding frequency in the same direction ϕ in the 2D Fourier plane. This fact was exploited in [8] for cell density estimation in the corneal endothelium. Moreover, it was shown that the repetitive pattern information exists inside a ring in the Fourier domain. The radius of the ring is a measure of the endothelial cell density.

We noticed in preliminary experiments (data not shown) that the aforementioned ring is more apparent in the images of the corneal endothelium compared to our images. Therefore, estimating cell density by measuring the ring's radius is a harder task in our case. Nevertheless, the frequency domain is likely to have a distinguishable band. The question which naturally arose was whether there exists a band-pass filter for each image which makes cell detection possible using basic image processing techniques. Our results show that this filter exists. In future work, we plan to design the filter automatically based on the frequency content of the image. In addition, we want to investigate the relation between the quantitative image processing results, the mechanical characteristics of the vocal folds, and acoustic properties of the voice signal.

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References

1. S.A. Klemuk, T. Riede, E.J. Walsh, I.R. Titze, Adapted to roar: functional morphology of tiger and lion vocal folds. *PLoS ONE* **6**(11), e27,029 (2011)
2. G. Becattini, L. Mattos, D. Caldwell, A novel framework for automated targeting of unstained living cells in bright field microscopy. In: *Proceedings of the IEEE International Symposium on Biomedical Imaging: From Nano to Macro*, pp. 195–198 (2011)
3. X. Long, W. Cleveland, Y. Yao, A new preprocessing approach for cell recognition. *IEEE Trans. Inf. Technol. Biomed.* **9**(3), 407–412 (2005)
4. F. Mualla, S. Schöll, B. Sommerfeldt, A. Maier, J. Hornegger, Automatic cell detection in bright-field microscope images using SIFT, random forests, and hierarchical clustering. *IEEE Trans. Med. Imag.* **32**(12), 2274–2286 (2013). doi:[10.1109/TMI.2013.2280380](https://doi.org/10.1109/TMI.2013.2280380)
5. T. Nattkemper, H. Ritter, W. Schubert, Extracting patterns of lymphocyte fluorescence from digital microscope images. *Int. Data Anal. Med. Pharmacol.* **99**, 79–88 (1999)
6. J. Pan, T. Kanade, M. Chen, Heterogeneous conditional random field: realizing joint detection and segmentation of cell regions in microscopic images. In: *CVPR* (2010)
7. F. Mualla, S. Schöll, B. Sommerfeldt, J. Hornegger, Using the monogenic signal for cell-background classification in bright-field microscope images. *Proc. des Workshops Bildverarbeitung für die Med.* **2013**, 170–174 (2013)
8. M. Foracchia, A. Ruggeri, Estimating cell density in corneal endothelium by means of fourier analysis. In: *Engineering in Medicine and Biology, 2002. 24th Annual Conference and the Annual Fall Meeting of the Biomedical Engineering Society EMBS/BMES Conference, 2002. Proceedings of the Second Joint*, vol. 2, pp. 1097–1098. *IEEE* (2002)
9. A. Ruggeri, E. Grisan, J. Jaroszewski, A new system for the automatic estimation of endothelial cell density in donor corneas. *Br. j. Ophthalmol.* **89**(3), 306 (2005)