Biomedical Signal Processing and Control 47 (2019) 145–158

Contents lists available at ScienceDirect

Biomedical Signal Processing and Control

journal homepage: www.elsevier.com/locate/bspc

Automatic segmentation of corneal endothelial cells from microscopy images

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ARTICLE INFO

Article history:
Received 30 March 2018
Received in revised form 1 July 2018
Accepted 14 August 2018

Keywords:
Corneal endothelium
Cell segmentation
Iterative cell separation
Morphological processing
Neural network

ABSTRACT

The structure of the corneal endothelial cells can provide important information about the cornea health status. Particularly, parameters describing cell size and shape are important. However, these parameters are not widely used, because it requires segmentation of the cells from corneal endothelium images. Although several dedicated approaches exist, none of them is faultless. Therefore, this paper proposes a new approach to fully automatic segmentation of corneal endothelium images. The proposed approach combines a neural network which is thought to recognize pixels located at the cell boundaries, with postprocessing of the resulting boundary probability map. The postprocessing includes morphological reconstruction followed by coarse cell segmentation using local thresholding. The resulting cells are next separated from each other via iterative morphological opening. Finally, the region between cell bodies is skeletonized. The proposed method was tested on three publicly available corneal endothelium image datasets. The results were assessed against the ground truths and compared with the results provided by selected state-of-the-art methods. The resulting cell boundaries are well aligned with the ground truths. The mean absolute error of the determined cell number equals 6.78%, while the mean absolute error of cell size is at the level of 5.13%. Cell morphometric parameters were determined with the error of 5.69% for the coefficient of variation of cell side length and 11.64% for cell hexagonality.

1. Introduction

The corneal endothelium is a monolayer on the posterior surface of the cornea. It regulates the dehydration of cornea by governing transport of fluid and solutes across the corneal structures that are required to assure the optical transparency. The layer is formed by closely packed, predominantly hexagonal cells of uniform size and the organization which resembles a honeycomb. In the young, healthy adults there are c.a. 3500 endothelial cells per square millimeter [1]. However, the cell density progressively decreases during the lifetime, ranging from about 6000 cells/mm^2 at the birth to about 2300 cells/mm^2 at the age of 85.

The organization of the cells in the corneal endothelium is of great interest for ophthalmologists because it can provide important diagnostic information about the cornea health status or indicate some corneal diseases. Particularly, different pathological conditions may speed up the loss of endothelial cells and thus abnormally decrease cell density. The loss of cells deteriorates the hexagonal pattern. The damaged endothelial tissue does not regenerate. Therefore, neighboring cells expand and migrate to fill void space corresponding to the destroyed cells. This leads to unpredictable cell elongation, thinning and size increase. Cell density is thus one of the most important parameters describing the corneal endothelium health condition. Other morphometric parameters useful for assessment of corneal endothelium aging and health status are cell hexagonality and the coefficient of variation of cell size.

In the everyday clinical routine, the condition of corneal endothelium is usually assessed qualitatively, by visual inspection of corneal endothelium images obtained with a specular microscope. The quantitative assessment using the morphometric parameters and cell geometry analysis is not very common. It is because it requires segmentation of all cells present in the corneal endothelium image. Although several approaches to segmentation of corneal endothelium images have been proposed none of them is faultless. Therefore, to date, obtaining reliable cell contours still requires manual delineation (or at least extensive editing) of cell boundaries. Since there are thousandths of endothelial cells per square millimeter, their manual segmentation is a very

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https://doi.org/10.1016/j.bspc.2018.08.018
1746-8094/

time-consuming activity. Therefore providing a reliable method for automatic segmentation of endothelial cells is still a vital problem.

The automatic segmentation of corneal endothelial cells is not a trivial task. The challenges are caused by a moderate quality of specular microscopy images which exhibits by inhomogeneous illumination. This, in turn, causes lower contrast and varying background intensities. As a result, some of the cell boundaries are difficult to recognize even by a medical expert. Because of these reasons, none of the existing approaches to endothelial cells automatic segmentation is faultless and thus manual editing of the results is required.

To the author's best knowledge, the first approach to automated corneal endothelial cell analysis was proposed about 25 years ago by Nadachi and Nunokawa [2] who suggested using image thresholding followed by scissoring and morphological thinning. Missing boundaries were next edited manually. Similar approaches were also used by Sanchez [3] who preceded thresholding by a scale-space filtering and Ayala et al. [4] in their work on assessment of corneal endothelium health status using granulometric moments. Mahzoun [5] proposed to perform cell segmentation by hexagon detection using directional filters followed by binarization, and thinning. A similar approach was also used in [6], where the authors applied different directional filters and provided a method for background intensity normalization.

Since the mosaic of corneal endothelial cells in confocal images manifests itself as bright regions separated by visibly darker lines many authors have tried to perform cell segmentation using the watershed algorithm. Attempts have been made to draw the watershed lines along cell boundaries in the inverted corneal endothelium image. Firstly, Vincent et al. [7] proposed an approach based on marker-driven cell segmentation. Their approach, however, may require the manual placing of seeds within each cell, since finding seeds automatically is up to date problematic. To avoid this shortcoming, in [8,9] it was proposed to additionally constrain watershed segmentation by a distance map. The slightly different approach was proposed by Bullet et al. [10] who built watersheds on distance map, but then separated fused cells using Voronoi diagrams. However, as shown in [11] these methods are very sensitive to parameter setting and thus require some experimentation before optimal results are obtained. Recently, Selig et al. [12] have proposed to use stochastic watershed in order to avoid user interaction and empirical setting and adjustment of parameters. Dagher and El Tom [13] used watershed region contours to initialize multiple balloon snakes. Similar, multiple active contour approach was also proposed in [14], where multiple snake active contours are evolving starting from circular regions obtained by thresholding. The latter two approaches are fully automatic and do not require user interaction.

A lot of effort into the problem of corneal endothelial cell segmentation has also been put by the team of Ruggeri. Their approaches include: applying an artificial neural network to classify pixels as cell contour or cell body [15,16], cell shape modeling using a priori knowledge about position, size, shape, and cardinality incorporated into Bayesian framework [17], labeling each pixel as cell vertex, side, or body using support vector machines classifier [18], and evolving population of vertices forming regular hexagons into cell boundaries using genetic algorithm [19]. Each of the Ruggeri's team's solution is more accurate than the previous ones, however in the case of their newest approach the differences between the manual estimation and the contour obtained automatically still cannot be neglected [19].

The most recent approach to corneal endothelium image segmentation proposed by Katafuchi and Yoshimura [20] bases on a convolutional neural network. Although this kind of approaches can be very accurate, they require an extensive training on large datasets which can sometimes take days or weeks. This is also in the case of the Katafuchi and Yoshimura's approach. Additionally, since their approach classifies each pixel separately it still suffers from problems related to pixel-based processing and needs postprocessing and due to filter-like application deteriorates segmentation quality at the image borders.

There also exists commercial software for an automatic analysis of corneal endothelial cell morphometric parameters. These include NAVIS by Nidek Technologies or IMAGEnet by Topcon Medical Systems. However, as reported by some authors (e.g., [21]) the software yields a modest to very substantial overestimation. Therefore, even the most recent studies still involve manual delineation of cell boundaries [22].

Having in mind the above-mentioned limitations, this paper proposes a solution to an automatic segmentation of corneal endothelium images. The proposed approach combines a feed-forward neural network which is thought to recognize pixels located at the cell boundaries, with iterative morphological post-processing of the resulting boundary probability map. In contrary to the existing approaches, instead of concentrating on cell boundaries detection, the method first aims at extracting cell bodies by applying the steps described in the following section.

2. Material and method

2.1. Input data

The approach presented in this paper was developed for a publicly available dataset of 30 images of corneal endothelium obtained from commercially slaughtered pigs [16,23]. The images, each of size 576×768 pixels, were acquired at $200 \times$ magnification from 30 porcine eyes stained with alizarine red using inverse phase contrast microscope (CK 40, Olympus) and analog camera (SSC-DC50AP, Sony) and stored as JPEG (RGB) files. For each image, the corresponding manually created ground truth was provided. On average the area of 0.54 ± 0.07 mm^2 per cornea was assessed, ranging from 0.31 to 0.64 mm^2. This corresponds to the number of cells ranging from 250 to 480 per region of interest. The size of cells in all images was comparable. This dataset is referred in this study as `Dataset 1`.

The proposed method was also tested on two other datasets, referred later in the text as `Dataset 2` and `Dataset 3`.

The `Dataset 2` was described in [24,11] and made available under the CC BY 3.0 License in [11]. It consists of 30 specular microscopy images of corneal endothelium together with the corresponding ground truth segmentation. The size of each image was 152×388 pixels. The images were stored as grayscale PNG files. The images, obtained from both: healthy and diseased patients, were selected to represent low, medium and high cell densities. The images present cells of various sizes, ranging from 27 to 191 endothelial cells within a field of view.

The `Dataset 3` was used by the authors in [25]. It consists of 7 corneal endothelium images of the size ranging from 108×352 pixels to 120×396 pixels and stored as grayscale PNG files. The images were obtained from patients after a cataract surgery. Depending on the image, there were from 59 to 98 cells of similar size within the field of view. Quality of images is low and the contrast at cell boundaries is poor. Images were acquired with a Topcon SP-1000P non-contact specular microscope at the magnification of 150x. Each image was accompanied with manually corrected ground truth boundaries.

The above information about the datasets considered in this study is summarised in Table 1 .