

Automated Morphometric Analysis of in-vivo Human Corneal Endothelium

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Abstract. In-vivo specular and confocal microscopy provide information on the corneal endothelium health state. The reliable estimation of the clinical parameters requires the accurate detection of cell contours. We propose a method for the automatic segmentation of cell contour. The centers of the cells are detected by convolving the original image with Laplacian of Gaussian kernels, whose scales are set according to the cell size preliminary estimated through a frequency analysis. A structure made by connected vertices is derived from the centers, and it is fine-tuned by combining information about the typical regularity of endothelial cells shape with the pixels intensity of the actual image. Ground truth values for the clinical parameters were obtained from manually drawn cell contours. An accurate automatic estimation is achieved on 30 images: for each clinical parameter, the mean difference between its manual estimation and the automated one is always less than 7%.

Keywords: Corneal endothelium, corneal images, specular microscopy, confocal microscopy, in vivo microscopy, cells contour segmentation.

1 Introduction

Human corneal endothelium is a single layer of uniformly sized cells with a predominantly hexagonal shape covering the posterior corneal surface. It stabilizes the corneal hydration and assures its transparency. Since endothelial cells do not reproduce, the activity and space of a dead cell is replaced by the surrounding cells. Consequently, the total number of cells, their size and regular tessellation are affected by age and pathologies [1]. Thus, the analysis of the main morphometric parameters of corneal endothelium provides clinical information capable to describe the cornea health state. Namely, endothelial cell density (ECD), pleomorphism (or hexagonality coefficient, fraction of hexagonal cells over the total number of cells) and polymegethism (or coefficient of variation, differences in cell size expressed as fractional standard deviation of cell areas) are commonly used as parameters to quantitatively characterize the endothelial cells' condition [2]. In-vivo specular and confocal microscopy allow acquiring noninvasively images of the human corneal endothelial layer, from which density and morphometric parameters can be derived [3].

In order to make this analysis practical in clinical settings, a computerized method that fully automates the accurate recognition of cell boundaries would be needed

[2,4]. The automatic segmentation of images from specular and confocal microscopy is a challenging task because of acquisition noise, illumination drifts and un-focused areas, which all make the contour difficult to be distinguished even by an expert, and the differences among images as regards size and appearance of the cells, due to the different state of health of the cornea. Whereas ECD is often estimated with acceptable accuracy, the quantitative estimation of pleomorphism and polymegethism is significantly affected by errors in contour detection even in few cells, making the reliable estimation of these parameters quite difficult [4].

Several computer programs have been proposed to accomplish this task [2,5,6,7], even if to the best of our knowledge the software used in clinical practice are only semi-automated, or work in a non-clinical context, e.g., with stained cells [8]. In the former case, the cell border detection provided by the computer needs to be edited by the user to correct inaccuracies by manual adjustment. Although manual correction improves the accuracy of this estimation, it usually requires an adjustment of about 50 to 75 per cent of the cell borders, making it tedious, time-consuming and thus impractical in a clinical setting [9]. This often leads the user to reduce the number of outlined cells to just a few tens, greatly affecting the accuracy of estimated parameters and thus the reliability of the clinical outcome. Indeed, it has been reported that at least 75 cells should be evaluated for a reliable estimation of clinical parameters [10], as well as the area covered by the segmented cells should be at least 0.04 mm^2 [11].

We propose here a further development of a recently developed automated algorithm for the segmentation of endothelial cell contours [12,13]. The same reliability of the derived automated morphometric analysis of endothelial cells has been obtained but the execution time of the algorithm we propose now is much lower and more suited for clinical applications.

2 Material

Images acquired with two different instruments were considered in this work: 15 images acquired by the SP-3000P (Topcon, Japan) specular microscope and 15 images acquired by the Confoscan4 (Nidek Technologies, Italy) confocal microscope. Both datasets consisted of images acquired from both healthy and pathological subjects, originally collected at various clinical centers and anonymized for further studies. Images acquired with the specular microscope covered an area of $0.25 \times 0.5 \text{ mm}$ and were saved as 240×480 pixel grayscale images (Fig. 1-A,B,C). Images acquired with the confocal microscope covered an area of $0.46 \times 0.35 \text{ mm}$ and were saved as 768×576 pixel grayscale images (Fig. 1-D,E).

In order to assess the accuracy of the morphometric parameters estimated by the computerized procedure, ground truth reference values were obtained. For each image, all visible cell contours were manually traced with care by using a public-domain image manipulation program (GIMP v. 2.8, <http://www.gimp.org>), so as to outline the polygonal shape of each cell. On average, 150 cells were manually segmented in both specular and confocal microscopy images, covering an area of about 0.1 mm^2 , which is wide enough to allow a reliable estimation of the morphometric parameters [10,11].

Ground truth parameter values were then estimated from these manually segmented images.

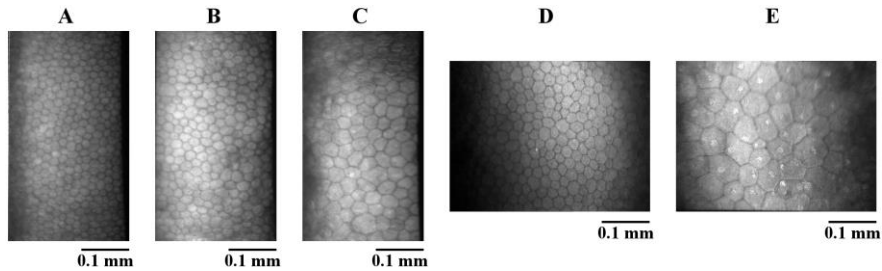


Fig. 1. Example of corneal endothelial images acquired in a normal subject (A), a subject with mild polymegethism (B) and a subject with severe polymegethism (C), with the SP-3000P (Topcon) specular microscope. Example of corneal endothelial images acquired in a normal subject (D) and a subject with severe polymegethism (E), with the Confoscan4 (Nidek Technologies) confocal microscope.

3 Methods

In both specular and confocal microscopy images, cells appear in the image as relatively regular polygons with different sizes, orientations and numbers of sides. Pixel intensity in the contour (dark) is different from the intensity in the inner body (light) of the cell Fig. 2-A. The proposed approach is based on the following steps:

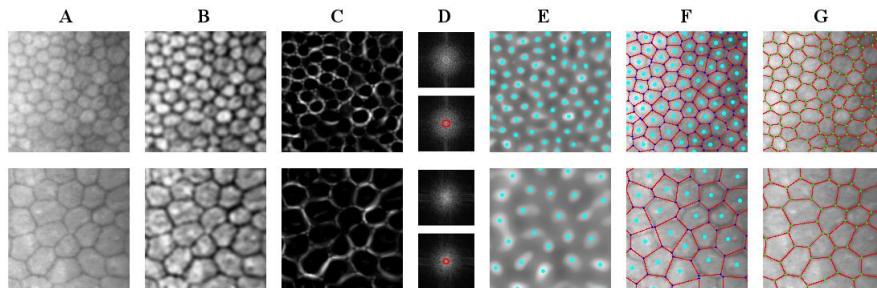


Fig. 2. Successive steps of the proposed algorithm on two representative images.

3.1 Cells size estimation

Illumination correction and contrast enhancement

A homomorphic filter is used to make the illumination of the image uniform [14]. It increases high-frequency components in the log-intensity domain and suppresses very low-frequency components through a Gaussian high pass filter with standard deviation set to 9 and 20 pixels for specular and confocal microscopy images respectively. Contrast enhancement is achieved with the addition of the top-hat transform

and the successive subtraction of the bottom-hat transform to the image [15]. Top-hat and bottom-hat transform are defined as the difference between the input image and its opening (top-hat) and closing (bottom-hat) by a disk with radius equal to 10 and 21 pixels for specular and confocal microscopy images respectively (Fig. 2-B).

The standard deviation of the Gaussian filter and the radius of the disk have been chosen according to the size of the image and its features, independently from the size of the cells.

Cell contours enhancement

The eigenvectors of the Hessian are used to compute the likelihood of an image region to contain lines or other ridges, according to the method described in [16]. The Hessian of the image is computed with a Gaussian 2nd derivative filter at three different values of standard deviation (i.e. 3,5,7 for specular microscopy images and 5,7,9 for confocal microscopy images). For each pixel, eigenvectors and their corresponding eigenvalues are derived for each value of standard deviation, and the maximum response (i.e. combination of the first and second eigenvalue) among the different values of standard deviation is associated to the analyzed pixel (Fig. 2-C). A high response reveals that the analyzed pixel belongs to a line (i.e. a cell contour).

This is a general technique to highlight objects alike lines in an image, because it does not require a priori information about direction, length and thickness of the lines.

Spatial frequency analysis and cells size estimation

A spatial frequency analysis is applied to the images with enhanced cell contours by means of the two dimensional discrete Fourier transform. The magnitude of frequencies is computed (Fig. 2-D) and its maximum value in concentric circular rings, with equally spaced increasing radii, is determined. It has been shown in [17] that the radius of the ring corresponding to the second peak of the computed function represents the average spatial frequency of the cells' repetitive pattern and is strictly related to cell density. Thus, the radius corresponding to the second peak is computed, cell density is derived as in [17] and subsequently cell size d (i.e. mean diameter of cells) is estimated.

3.2 Cell centers recognition

The centers of endothelial cells are automatically detected by convolving the acquired image with a customized two-dimensional Laplacian of Gaussian kernel [18]. Convolution is carried out at three different scales of the filter, with the standard deviation of the original Gaussian prior to the Laplacian operator set to $\sigma=d/(2\cdot\sqrt{2})$ (with cell size d estimated in the previous section), $\sigma-\sigma\cdot 20\%$ and $\sigma+\sigma\cdot 20\%$ respectively. The maximum response among the scales is recorded and used to obtain an image with highlighted cell bodies. Cell centers are then derived by finding the regional maxima, i.e. connected components of pixels with a constant intensity value and whose adjacent pixels all have a lower value (Fig. 2-E).

3.3 Cell contours extraction

The Euclidean skeleton of the recognized cell centers is then derived. It is the set of the connected median lines which are equally distant from nearest centers. From this skeleton, a structure made by connected vertices (Fig. 2-F) is easily derived, since the vertices are the intersection points of the lines and connections between vertices are the lines themselves.

3.4 Cell contours refinement through a genetic algorithm

The derived structure of connected vertices is used as starting population for the genetic algorithm previously developed [12,13]. The location of each vertex is randomly modified, as well as polygons can be modified by adding new vertexes or by splitting, merging, deleting some existing vertices, or modifying some connection between them. Each modification is evaluated considering regularity and pixel intensity, and eventually accepted or not. Indeed, regularity is a well-known anatomical feature of endothelial cells (guaranteed in each image, albeit with different grades), while pixel intensity is relative to the specific image under investigation. The final entire population of vertices forms a set of polygons that fits the underlying cells contours (Fig. 2-G).

The evaluation of regularity is performed for each vertex using ratios between angles and lengths of its connections with other vertices [12,13], thus it is independent from the size of the cell. Pixel intensity is evaluated on three pre-processed images: one with highlighted vertices, one with highlighted sides and one with highlighted body of the cells (see section 3.2). Each pre-processed image is obtained by convolving the original image with customized two-dimensional kernels [18] at three different scales, which are set based on the cell size derived in section 3.1.

The proposed algorithm has been implemented using the Matlab language (The Mathworks Inc., Natick, MA, USA, release 2013b). The procedure analyzes about 150 cells per image and requires less than 1 minute per image.

4 Results

The final contour segmentation in representative images can be seen in Fig. 3.

For each pair of segmented images (automated and manual), only contours positioned in the same region were considered, to avoid that differences in the estimation of the clinical parameters be due to differences in the selected cells. The regions analyzed automatically and manually were wide (0.1 mm^2) and largely overlapped ($\approx 90\%$), thus providing a mean number of overlapped analyzed cells per image equal to 130 in both specular and confocal microscopy images.

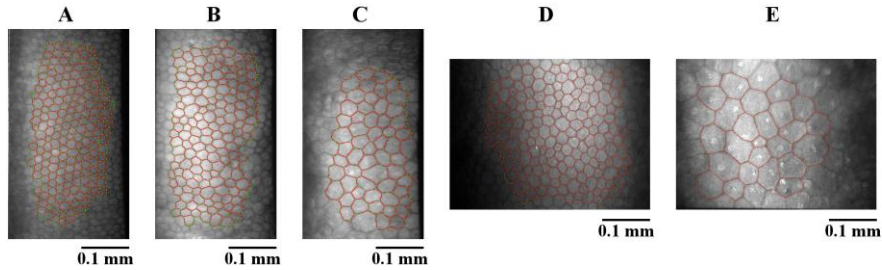


Fig. 3. Automated segmentation of corneal endothelial cell contours in representative images.

Starting from cell contours, the estimation of ECD, pleomorphism, and polymegethism was carried out (Table 1 and Table 2). ECD was computed as the total number of cells divided by the sum of individual cell areas. Pleomorphism was computed by counting for each cell the number of neighboring cells (cells along the border of the analyzed area were excluded from this computation) and taking the percentage of cells with hexagonal shape. Polymegethism was computed as the fractional standard deviation of all cell areas.

For all morphometric parameters, a two-sided Wilcoxon rank sum test revealed no statistically significant difference between automated and manual estimate (p -value >0.6 for specular microscopy and p -value >0.3 for confocal microscopy).

Table 1. Automated and manual estimates of the morphometric parameters in specular images, and their percent absolute differences. They are computed on each image and their mean, standard deviation, minimum and maximum are computed across all images.

SP-3000P (Topcon) specular microscope			
ECD (cells/mm²)	<i>Automated</i>	<i>Manual</i>	<i>Absolute Difference</i>
<i>mean</i>	2578	2511	3.32 %
<i>sd</i>	686	636	1.93 %
<i>min</i>	458	493	1.04 %
<i>max</i>	3407	3199	7.64 %
Pleomorphism (%)	<i>Automated</i>	<i>Manual</i>	<i>Absolute Difference</i>
<i>mean</i>	55.59	55.25	4.25 %
<i>sd</i>	11.41	10.90	4.76 %
<i>min</i>	36.90	36.70	0.00 %
<i>max</i>	75.00	71.10	16.05 %
Polymegethism (%)	<i>Automated</i>	<i>Manual</i>	<i>Absolute Difference</i>
<i>mean</i>	37.23	38.03	6.62 %
<i>sd</i>	6.86	6.41	4.44 %
<i>min</i>	22.50	24.90	0.64 %
<i>max</i>	45.70	48.30	16.43 %

Table 2. Automated and manual estimates of the morphometric parameters in confocal images, and their percent absolute differences. They are computed on each image and their mean, standard deviation, minimum and maximum are computed across all images.

Confoscan4 (Nidek Technologies) confocal microscope			
ECD (cells/mm²)	<i>Automated</i>	<i>Manual</i>	<i>Absolute Difference</i>
<i>mean</i>	2096	2083	1.20 %
<i>sd</i>	580	562	1.54 %
<i>min</i>	446	445	0.05 %
<i>max</i>	2879	2780	5.90 %
Pleomorphism (%)	<i>Automated</i>	<i>Manual</i>	<i>Absolute Difference</i>
<i>mean</i>	65.25	64.36	3.21 %
<i>sd</i>	8.16	8.03	2.84 %
<i>min</i>	54.20	51.10	0.00 %
<i>max</i>	85.70	83.30	10.19 %
Polymegethism (%)	<i>Automated</i>	<i>Manual</i>	<i>Absolute Difference</i>
<i>mean</i>	28.47	29.68	5.61 %
<i>sd</i>	4.23	4.28	4.19 %
<i>min</i>	21.80	22.70	0.98 %
<i>max</i>	37.80	39.90	13.76 %

5 Conclusions

We present here a completely automated system for the estimation of cornea endothelium morphometric parameters. The estimates of the clinical parameters provided by the proposed algorithm are in very good agreement with ground truth, obtained with a careful manual analysis. The algorithm is based on the typical endothelial cells regularity and on general image processing techniques, so that it can successfully work with minimal changes in images acquired with specular or confocal microscopes.

The proposed algorithm is based on many steps, the last of which is an adaptation of a previously developed genetic algorithm [12,13]. The pre-processing steps, not present in the original algorithm, provide a preliminary structure made by connected vertices. It is a good starting point for the genetic algorithm, which now reaches the optimal solution very quickly on the whole image, instead of starting from a small structure in the center of the image that is stepwise increased until the whole area of the image is covered. With respect to the original algorithm, the current one allows a significant reduction in the execution time (less than a minute instead of tens of minutes) with the same accuracy in the estimation of morphometric parameters (see results in [12]).

The proposed algorithm appears capable of reliably obtaining the contour of hundreds of cells in regions covering a large area (on average 0.1 mm²), and thus of providing an accurate estimation of ECD and also pleomorphism and polymegethism.

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