corneal endothelial, cell segmentation, binarization

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## SELECTED ISSUES OF CORNEAL ENDOTHELIAL IMAGE SEGMENTATION

This article concerns the analysis of corneal endothelial image. The basic problems of binarization and segmentation of these images are discussed. Preprocessing methods are proposed, consisting of median and convolution filtration, to remove noise. An algorithm of normalization of the average brightness of the vertical and horizontal is presented. The problem of binarization is discussed. At the end the proposal of segmentation algorithm is reported.

## 1. INTRODUCTION

#### **1.1. CORNEAL ENDOTHELIAL IMAGES**

Corneal endothelium is a monolayer of hexagonal cells covering the posterior corneal surface. Main endothelial function is to maintain stable corneal hydratation level of about 78% by removing water that flows freely into the corneal structure from the anterior chamber. In human (and in primates) endothelial cells have almost no mitotic activity (in contrary to other species); at birth the number of cells is  $6500 \text{ cells/mm}^2$ , and decreases spontaneously during the lifetime, at 80 is  $1700 - 2000 \text{ cells/mm}^2$  [5,6].

Each type of surgery taking place in the anterior chamber (cataract surgery, glaucoma surgery, etc.) results in additional cell decrease, and the decrease rate remains elevated for months after the surgery; many non surgical conditions (trauma, inflammation, elevated intraocular pressure etc.) are reasons of endothelial cells loss as well.

When the cell density decreases below 1000 - 500 cells/mm<sup>2</sup> endothelial insufficiency occurs, leading to irreversible corneal edema, resulting in severe vision loss and requiring corneal transplant; visual function remains permanently impaired [4].

#### **1.2. CORNEAL ENDOTHELIAL IMAGES ACQUISITION**

Corneal assessment in vivo began in 1919, when Vogt described the mirror endothelial reflection using biomicroscope, first specular microscope was presented by Maurice in 1968; in 1976 flesh light was added by Bourne and Kaufman, that improved acquired endothelial images quality [1].

Non contact microscopes are the most popular for in vivo assessment, endothelial image is acquired by black and white camera, and transferred by frame – grabber to the computer, where it may be corrected and analyzed. Cell borders may be manually corrected, or even manually marked, especially when the cell density is extremely low or additional pathological structures are present. Main analyzed and assessed parameters are: CD (cell density – number of cells per square millimeter); H (hexagonality – percentage of hexagonal cells) and CV (coefficient of cell area variation). Corneal endothelial images presented in the study were taken with non – contact specular microscope Topcon SP – 1000.

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# 2. FEATURES OF CORNEAL ENDOTHELIAL IMAGES AND PREPROCESSING

Original corneal endothelial images have dimensions of 160x460. They contain a thermal and device-specific noise, which hinders the correct analysis. The first step is to preprocess images to remove noise.

## 2.1. PREPROCESSING OF IMAGES

The first step in image processing is preprocessing. The main task of preprocessing is to remove noise and distortions.

In the first approach median filter with a mask of 3x3 (Tab. 1a) was tested. The next approach was filtration. We used a convolution filter with mask of 5x5 or 7x7 (presented in tab. 1b and 1c). The best effect is achieved by using convolution mask of 5x5 (Tab. 1b). The alternate method of filtration is FFT 2D filtering [10].

To verify the quality of filtering horizontal profiles can be used. There are examples of profiles on the Fig.3. The original image contains noise, and its profile is jagged (Fig. 3a). Using convolution filtration with the proposed mask makes a profile smooth (Fig. 3c).



Fig.1. Noisy part of an endothelial image - original and normalized brightness.



Table 1. Used masks – a) for median filter, b), c) for convolution filter.



Fig. 2. Using filters: a) original (normalized), normalized view after: b) median, c) mask filter, d) median and mask filter.



Fig. 3. Horizontal profiles in the middle of the images: a) original, b) after median filter, c) after mask filter, d) after median and mask filter. Each red horizontal line means 10 points of brightness.

#### 2.2. VARIABLE AVERAGE BRIGHTNESS OF IMAGES

The way of image acquisition makes non-uniform illumination - a decrease in the average brightness from left to right [7]. The heterogamous illumination appears in the horizontal too. The sample image and average brightness of the vertical and the horizontal are presented on Fig. 4a-4c.



Fig. 4. Original endothelial image a) and: b) average brightness of the vertical c) and the horizontal. Each red horizontal line means 10 points of brightness.

Feature of non-uniform illumination makes binarization very difficult – an example is shown on Fig. 5. To solve this problem, a special kind of normalization is needed.



Fig. 5. Threshold binarization of an original image.

# 2.2.1. NORMALIZATION OF THE AVERAGE BRIGHTNESS OF THE VERTICAL AND HORIZONTAL

The normalization of the average brightness can overcome disadvantages of different illumination. The algorithms are proposed:

Normalization of the average brightness of the vertical (NAV):

- for each pixel – add a difference between average image brightness (or fixed value) and average brightness of the pixel's column due to the formula (1)

$$p'(x, y) = p(x, y) + L - \sum_{j=1}^{N_y} p(x, j) * \frac{1}{N_y}$$
(1)

where:

- p(x,y) original brightness level of pixel (x,y),
- p'(x,y) new brightness level of pixel (x,y),
- *Ny* number of horizontal lines (in pixel, height of the image),
- L average brightness of image or constant value, for example 128.

Normalization of the average brightness of the horizontal algorithm (NAH): The algorithm is analogous – it uses formula (2):

$$p'(x, y) = p(x, y) + L - \sum_{i=1}^{N_x} p(i, y) * \frac{1}{N_x}$$
(2)

where:

- Nx – numer of vertical lines (in pixel, width of the image).

The effect of proposed algorithms is shown on Fig. 6. For the input image (Fig. 6a) next images show normalization NAV and normalization NAV and NAH.



Fig. 6. Sample image: a) original, normalized, b) normalized NAV, normalized brightness, c) normalized NAV and NAH, normalized brightness.

The sample horizontal profiles in the middle of the image before and after normalization of the average brightness are presented on Fig 7. These profiles (Fig. 7b and 7c) show that proposed algorithms makes possibility to determine cells and cell boundaries (binarization).



Fig. 7. Horizontal profiles for images: a) original, b) after AV normalization, c) after NAV and NAH normalization. Each red horizontal line means 10 points of brightness.

## 3. ANALYSIS OF CORNEAL ENDOTHELIAL IMAGES

The main objective of the present article is to perform segmentation of corneal endothelial images. To do this first binarization should be performed.

#### **3.1. BINARIZATION**

The specificity of contrast and brightness of images endothelium does not allow the use of classical binarization algorithm. In [10] a binarization algorithm is proposed which adapts its threshold levels to the current context. In this paper, its variety is used. The principle of operation is to determine the context (9x9) for each point and verify that the value of this point is closer to the local minimum or maximum (assumed threshold of 50%). This approach allows to adapt to non-uniform contrast and brightness.

#### 3.2. ANALYZE OF AVERAGE CELL BOUNDARIES PROFILES

Automatic binarization quality assessment can be based on analysis of average profiles of cell boundaries. To do this, for all vertical and horizontal lines sections between cells are tested. All of pixels on the section between cells, marked in the binarization as '0', create an average profile (vertical or horizontal). The values of adequate successive points of original image are added to the profile tables for the profiles of a given size. The most frequent profile size for the cell boundaries is a characteristic of the patient, in most authors' studies, it oscillates around a value of 7 pixels. Fig.8 presents different profiles - for improper binarization (Fig. 8a), for using original images (Fig. 8b, c) and for images with normalized the average brightness of the vertical (Fig. 8d). Automatic binarization quality determination can be made by analyzing the coefficients of interpolating polynomial.



Fig. 8. Average cell boundaries profiles: green - horizontal, red - vertical, black - both (added).

#### 3.3. SEGMENTATION

The last step is segmentation. In [2,8,11] authors proposed to use the watershed algorithm. In current work the proposed algorithm is simpler.



Fig. 9. An idea of segmentation.

### Simple segmentation algorithm:

The algorithm consists of determining the center of the cell boundaries profile (Fig. 9).

For each vertical and horizontal cell boundaries profile the center point is marked on the original image. After processing all cell boundaries profiles output image is obtained.

An example of the effect is shown in Figure 10. The algorithm selects the cells division quite well for binarization carried out properly.



Fig. 10. An example of segmentation using proposed algorithm -a) original image, b) binarized image, c) segmented image.

## 4. SUMMARY

The article shows an attempt to automatic segmentation of corneal endothelial cells. There are proposed numerous algorithms for preprocessing and analyzing. The proposed algorithms are not yet the end of the study, they are promising. The aim is to achieve results comparable with other solutions and perform other medical tests and analyzes than those proposed in the known literature [3].

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