
Cell detection in corneal endothelial images using directional filters

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Summary. The article presents an algorithm for the detection of corneal endothelium cells in images obtained with confocal microscopy (KH algorithm). Firstly, pre-processing issues are presented. The proposed methodology is based on image processing algorithms, especially filters. The method outputs images that are prepared for further analysis, e.g. stereological measurements. Each step of the algorithm is discussed in detail and other methods of digital images processing are compared to the research results⁰.

1 Methods for cell detection in digital images of corneal endothelium

Image processing methods are successfully implemented in various branches of science, for example medicine [15]. In computer image analysis (regardless of the analyzed discipline) the main purpose of transformation is to obtain a properly segmented binary image. In order to obtain such images a series of transformations is carried out on the input image. This results in a contrast image, in which objects (e.g. specific objects such as cells) are clearly distinguishable from the background.

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1.1 Input data

A watershed algorithm is used frequently to separate individual cells in images. Unfortunately, this algorithm is sensitive to artifacts in the input images. These artifacts might exist due to image acquisition issues and may be manifested, for example, by the occurrence of stripes or other noise which confound essential information in the image (Figure 1). A detailed description of the structure and performance issues of the proposed system is available in the literature [7]. The other issue is the cell bindings which are visible in pictures, or which cause artifacts (eg nuclei are shown as a "dimples" which are visible during analysis as a cell division which makes one cell looks like two). In order to eliminate such issues it is necessary to perform some transformations that reduce noise and normalize image illumination. This has a crucial significance when analyzing corneal endothelium images as they are characterized by a number of disturbances in the form of numerous horizontal stripes caused by interlacing (Fig. 1(b)), especially in the upper area of the image.

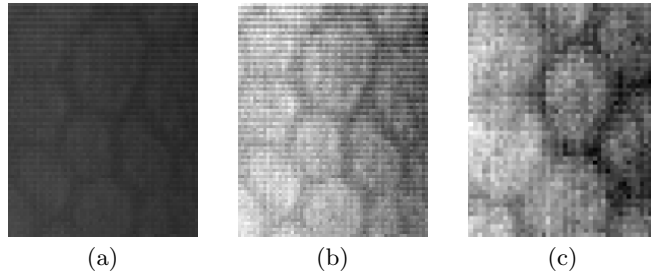


Fig. 1. Fragments of the original (a) and normalized (b) image of corneal endothelium with visible disturbances in the form of horizontal stripes, normalized (c) image of corneal endothelium where noise is visible.

Figure 2 shows disturbances of illumination. The presented graphs (Figure 2) show an average level of pixel brightness in the original image in rows (Figure 2, a) and columns (Figure 2, b).

2 Methods for data preprocessing

Uneven illumination leads to a situation in which the level of brightness tallies in one place with the cell surface, but in another denotes intercellular space. An exceptional irregularity is visible along the columns. Significantly, these issues make it difficult to bring the images to a binary form which is sufficient for further analysis. Consequently, a noise removal methodology has been introduced and compared to commonly used methods (median filtering). These factors make the use of the Watershed algorithms inapplicable [10].

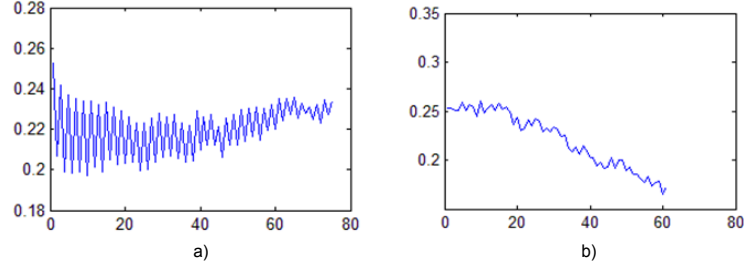


Fig. 2. Values of average levels of brightness in rows (a) and columns (b). The x-axis shows the location of a pixel against the upper left corner of the image – profile, the y-axis shows the illumination value of a given pixel.

Method of adjusting the brightness levels for images of corneal endothelium cells

The last stage of the initial transformation is adjusting the level of image brightness in columns and rows. It is possible to use one of the two proposed methods according to the following formulae. The first formula estimates the average value in each row (2) or column (3) (depending on whether the image is adjusted in rows or columns), compares the calculated value with the average brightness of the image (1). The next step, depending on the obtained result, is to add or subtract a certain value so the average brightness in a row or a column is equal to the average brightness of the image (4, 5) [9]. The second formula differs from the first in the following way: when in a given row or column the average brightness of a pixel is less than the average brightness of the image, a certain number is multiplied instead of added to get a value which is equal to the average brightness of the image (6, 7). This method produces higher contrast (Fig. 4) when the average value in a row or column is lower than the average image brightness. The last method performs illumination adjustment for a round neighborhood of each pixel (9), and is partially similar to first step of the Laws' Texture Energy Measures algorithm [?].

$$S = \frac{\sum_{i=1}^x \sum_{j=1}^y Pin_{ij}}{xy} \quad (1)$$

$$Sw_j = \sum_{i=1}^x Pin_{ij} \quad (2)$$

$$Sk_j = \sum_{j=1}^y Pin_{ij} \quad (3)$$

$$Pout_{ij} = \begin{cases} Pin_{ij} + S - Sw_j & \text{for } Sw_j \neq S \\ Pin_{ij} & \text{for } Sw_j = S \end{cases} \quad (4)$$

$$Pout_{ij} = \begin{cases} Pin_{ij} + S - Sk_i & \text{for } Sk_i \neq S \\ Pin_{ij} & \text{for } Sk_i = S \end{cases} \quad (5)$$

$$Pout_{ij} = \begin{cases} Pin_{ij} + S - Sw_j & \text{for } Sw_j > S \\ Pin_{ij} & \text{for } Sw_j = S \\ \frac{Pin_{ij}S}{Sw_j} & \text{for } Sw_j < S \end{cases} \quad (6)$$

$$Pout_{ij} = \begin{cases} Pin_{ij} + S - Sk_i & \text{for } Sk_i > S \\ Pin_{ij} & \text{for } Sk_i = S \\ \frac{Pin_{ij}S}{Sk_i} & \text{for } Sk_i < S \end{cases} \quad (7)$$

$$Pout_{ij} = \begin{cases} Pin_{ij} + S - Sk_i & \text{for } Sk_i > S \\ Pin_{ij} & \text{for } Sk_i = S \\ \frac{Pin_{ij}S}{Sk_i} & \text{for } Sk_i < S \end{cases} \quad (8)$$

$$Pout_{ij} = Pin_{ij} + (S - Sr_{ij}) \quad (9)$$

where:

Pin - input image

$Pout$ - output image

S - arithmetic mean of brightness levels of all image pixels

Sw_j - arithmetic mean of brightness levels of pixels in j -row in the image

Sk_i - arithmetic mean for the levels of pixels brightness and in i -column in the image.

Sr_{ij} - arithmetic mean of brightness levels for all pixels in the distance equal or less than r from pixel i, j .

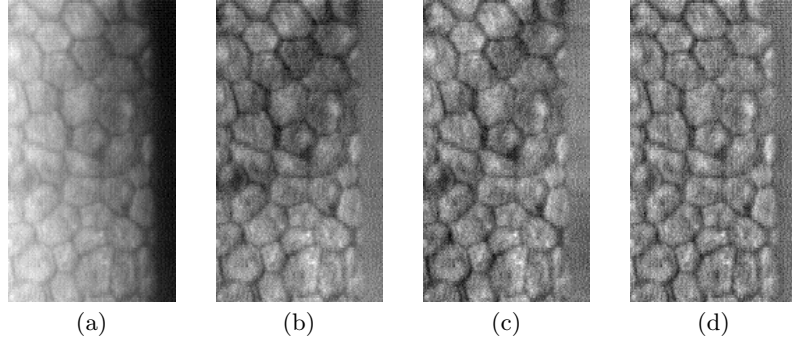


Fig. 3. Normalized (a) image of corneal endothelium with adjusted illumination along the columns (b) and in both directions (c) and in the round neighborhood using $r = 10$ (d).

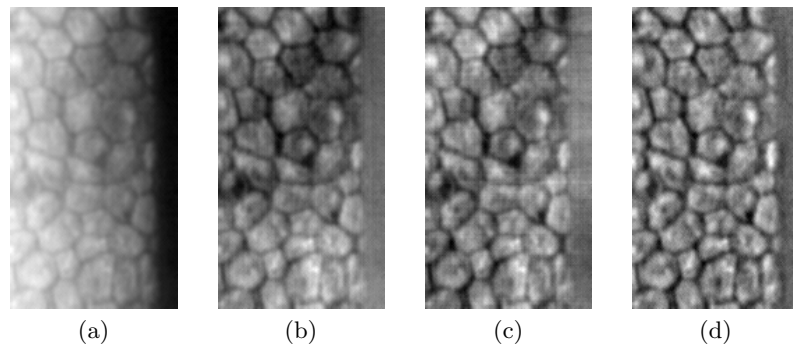


Fig. 4. Normalized and averaged (a) image of corneal endothelium with adjusted illumination along the columns (b) and in both directions (c) and in the round neighborhood using $r = 10$ (d).

3 Methods of binarization of corneal endothelium cells

In order to obtain a particular and logical image of the corneal endothelium it is necessary to apply methods of binarization, as threshold binarization is not sufficient [14], even with proper noise removal and brightness level adjustment.

3.1 Other binarization approaches

There are a few approaches to corneal endothelium image binarization.

In [13] the author describes a binarization process that consists of the following stages: removal of the lowest frequency, gap closing, contrast enhancement, thresholding, skeletonization, and finally improvements to the output image.

An interesting approach which uses shape masks for convolution is presented in [5]. The authors designed three kinds of mask: 'tricorn', orthogonal (vertical and horizontal) and diagonal.

Another method is the use of pyramid methods in image processing [1]. In [4] the authors perform morphological operations to assess the thinned boundaries and next use wavelet pyramidal decomposition to carry out the binarization of corneal endothelial cells.

An interesting method is presented in [6]. The authors propose a scissoring operator which separates cells in the binary image, instead using a type of watershed algorithm.

A very promising approach to binarization is presented in [2]. The authors use the active contour technique to obtain the shape of each cell in the image.

To assess cells contours a supervised classification scheme can be used [12]. The authors propose a multi-scale 2-dimensional matched filter to extract cells components. To obtain the final segmentation, a Support Vector Machine classifier is used.

Adaptive binarization

Adaptive binarization is one of the easiest methods for the binarization of corneal endothelium images, however it is not very effective. This method moves masks with odd sizes (adjusted to the average size of cells in pixels) and determines a value of 0 or 1 for the central element on the basis of the mask context and taking into consideration a particular point. Figure 5 shows logical images which were the result of three different threshold adjustments. This method does not provide satisfying results. The vast majority of cells do not reflect their matrix shape, and in many places the cells either combine or are 'leaky'.

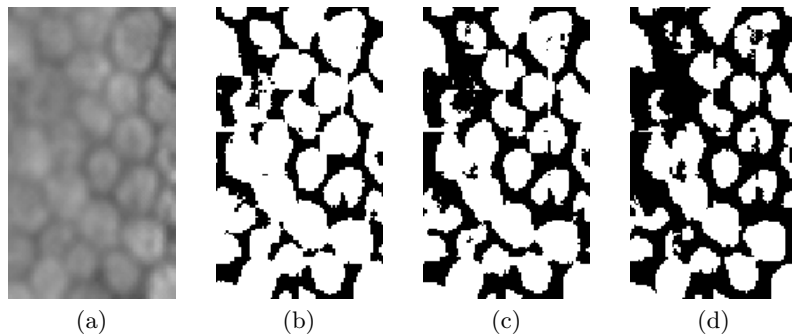


Fig. 5. Part of a normalized and averaged corneal endothelium image (a), binarized with adaptive method with low threshold (b), average threshold (c) and high threshold (d).

Top–Hat binarization algorithm

Top-hat is a process which is performed by subtracting the opening result from the input image. This makes it possible to obtain an image with an adjusted background. The structural element used for the opening process needs to be large enough to successfully remove all corneal cells from the image. However, when it is too large it will cause incorrect background adjustment which further complicates binarization. Unfortunately, even if this method seems to be suitable for such processes as segmentation or binarization, it does not produce good results for corneal endothelium images. Figure 6 shows top-hat binarization results with the use of three different thresholds.

Binarization with the use of a 9×9 mask

One of the suggested methods of binarization of corneal endothelium images uses a 9×9 size mask [8]. It was observed in corneal endothelium images that

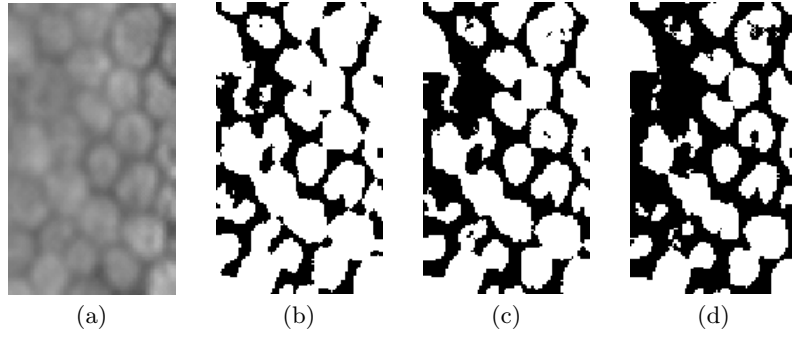


Fig. 6. Part of normalized and averaged image of corneal endothelium (a), binarized with the use of the top-hat algorithm with low threshold (b), average (c) and high (d).

there is regular space between cells (7 pixels in average). With this in mind, a 9×9 size mask was created in order to distinguish intercellular boundaries. This fulfilled its aim relatively well because of its structure. The strongest weight was given to the central point which promoted the darkest pixels. If we invert all elements in the mask, then the mask detects corneal endothelium cells. Elements distribution is presented in (10). The filtering and binarization result from an output image with the use of this mask is presented in Fig. 7.

$$\begin{bmatrix}
 -3 & -3 & -2 & -2 & -2 & -2 & -2 & -3 & -3 \\
 -3 & -2 & -1 & -1 & -1 & -1 & -1 & -2 & -3 \\
 -2 & -1 & 0 & 0 & 0 & 0 & 0 & -1 & -2 \\
 -2 & -1 & 0 & 6 & 12 & 6 & 0 & -1 & -2 \\
 -2 & -1 & 0 & 12 & 32 & 12 & 0 & -1 & -2 \\
 -2 & -1 & 0 & 6 & 12 & 6 & 0 & -1 & -2 \\
 -2 & -1 & 0 & 0 & 0 & 0 & 0 & -1 & -2 \\
 -3 & -2 & -1 & -1 & -1 & -1 & -1 & -2 & -3 \\
 -3 & -3 & -2 & -2 & -2 & -2 & -2 & -3 & -3
 \end{bmatrix} \tag{10}$$

Proposed binarization algorithm with the use of a 9×9 size directional masks (KH algorithm)

The best binarization method so far (among methods presented above) is a 9×9 size filter mask. However, the mask is not perfect as it detects not only intercellular space, but also small objects that look like "holes" in cells, which are probably nuclei. A new method of four 9×9 direction mask emerged whilst taking into consideration filtering with a big mask and analyzing mistakes made during binarization conducted with the previous methods (Tab. 3.1, vertical mask and its transposition to horizontal, and, diagonal masks) [3]. The way these masks work is similar to the previous method in a sense that

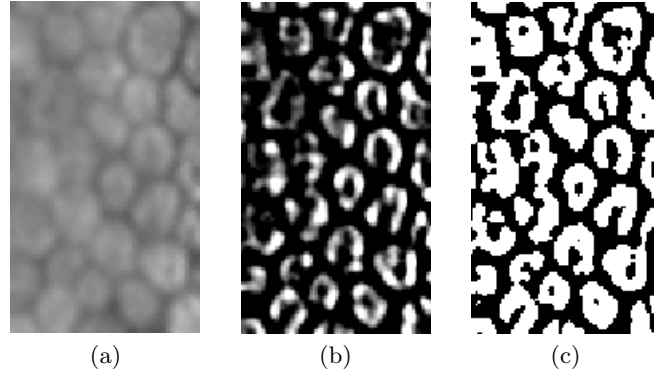


Fig. 7. Normalized and averaged (a) image of corneal endothelium with adjusted illumination along the columns (b) in both directions (c) in the round neighborhood using $r = 10$ (d).

those masks emphasize not only one central point, but also a line in the middle of the mask which is directed at an angle of 0° , 45° , 90° or 135° against the vertical direction. In this way 4 output images were obtained (Fig. 8).

$\begin{bmatrix} 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 22 & -2 & -4 & -8 & -16 & -8 & -4 & -2 & 22 \\ 22 & -2 & -4 & -8 & -16 & -8 & -4 & -2 & 22 \\ 22 & -2 & -4 & -8 & -16 & -8 & -4 & -2 & 22 \\ 22 & -2 & -4 & -8 & -16 & -8 & -4 & -2 & 22 \\ 22 & -2 & -4 & -8 & -16 & -8 & -4 & -2 & 22 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{bmatrix}$	$\begin{bmatrix} 0 & 0 & 22 & 22 & 22 & 22 & 22 & 0 & 0 \\ 0 & 0 & -2 & -2 & -2 & -2 & -2 & 0 & 0 \\ 0 & 0 & -4 & -4 & -4 & -4 & -4 & 0 & 0 \\ 0 & 0 & -8 & -8 & -8 & -8 & -8 & 0 & 0 \\ 0 & 0 & -16 & -16 & -16 & -16 & -16 & 0 & 0 \\ 0 & 0 & -8 & -8 & -8 & -8 & -8 & 0 & 0 \\ 0 & 0 & -4 & -4 & -4 & -4 & -4 & 0 & 0 \\ 0 & 0 & -2 & -2 & -2 & -2 & -2 & 0 & 0 \\ 0 & 0 & 22 & 22 & 22 & 22 & 22 & 0 & 0 \end{bmatrix}$
$\begin{bmatrix} 22 & 22 & 22 & 0 & 0 & 0 & 0 & 0 & 0 \\ 22 & -2 & -2 & -2 & 0 & 0 & 0 & 0 & 0 \\ 22 & -2 & -4 & -4 & -8 & -16 & 0 & 0 & 0 \\ 0 & -2 & -4 & -8 & -8 & -16 & -8 & 0 & 0 \\ 0 & 0 & -4 & -8 & -16 & -8 & -4 & 0 & 0 \\ 0 & 0 & -8 & -16 & -8 & -8 & -4 & -2 & 0 \\ 0 & 0 & -16 & -8 & -4 & -4 & -4 & -2 & 22 \\ 0 & 0 & 0 & 0 & 0 & -2 & -2 & -2 & 22 \\ 0 & 0 & 0 & 0 & 0 & 0 & 22 & 22 & 22 \end{bmatrix}$	$\begin{bmatrix} 0 & 0 & 0 & 0 & 0 & 0 & 22 & 22 & 22 \\ 0 & 0 & 0 & 0 & 0 & -2 & -2 & -2 & 22 \\ 0 & 0 & -16 & -8 & -4 & -4 & -4 & -2 & 22 \\ 0 & 0 & -8 & -16 & -8 & -8 & -4 & -2 & 0 \\ 0 & 0 & -4 & -8 & -16 & -8 & -4 & 0 & 0 \\ 0 & -2 & -4 & -8 & -8 & -16 & -8 & 0 & 0 \\ 22 & -2 & -4 & -4 & -4 & -8 & -16 & 0 & 0 \\ 22 & -2 & -2 & -2 & 0 & 0 & 0 & 0 & 0 \\ 22 & 22 & 22 & 0 & 0 & 0 & 0 & 0 & 0 \end{bmatrix}$

Table 1. Four directional masks for cells' boundaries detection

Images obtained with this method were binarized (assuming that during filtering there were some restrictions implemented and each value that exceeded the threshold of 255 was assigned a value equal to this threshold) and

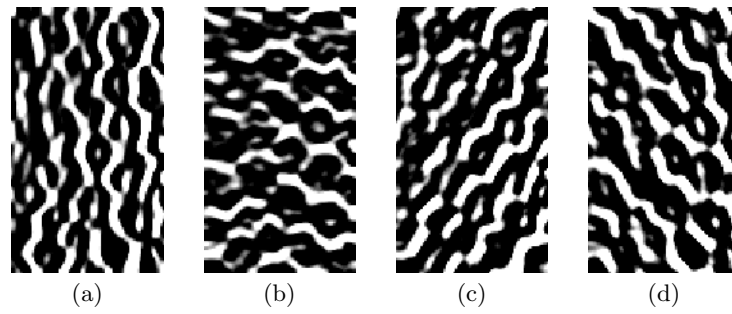


Fig. 8. Output images obtained after filtering with (a) vertical mask, (b) horizontal mask, (c) left diagonal mask, and (d) right diagonal mask.

a logical value 1 was set for all pixels with value 255. Figure 9 shows images that were processed in this way.

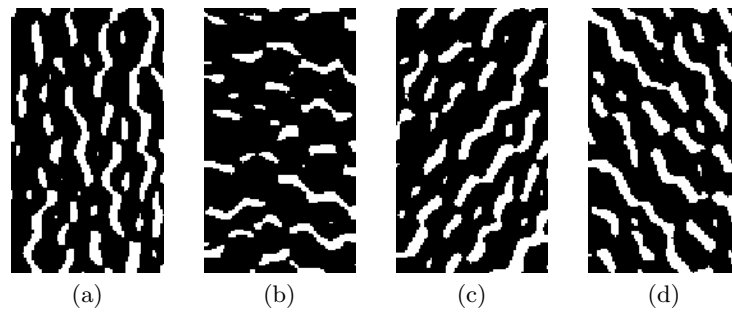


Fig. 9. Image binarization effects.

In the binary images that emerged, it is visible that besides intercellular spaces, the mask has also detected small objects which look like "holes" in cells. With the use of indexation it was possible to get rid of elements smaller than 40 pixels. This is shown in Figure 10. Binary images obtained in this way were bounded (binary addition), and then negations were created and the final result was obtained. Figure 11 presents the proposed algorithm schema.

4 Results and comparison of selected binarization methods

The algorithms presented in the research paper were compared according to the quality of binarization results. In figure 12 binarization results are presented with the use of all 4 algorithms. Based on the obtained results it is

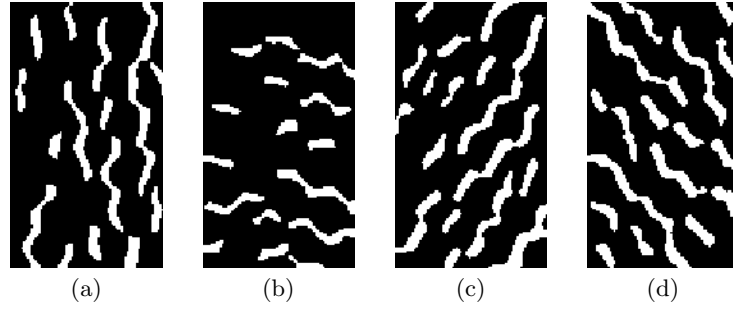


Fig. 10. Images excluding objects with an area less than 40 pixels.

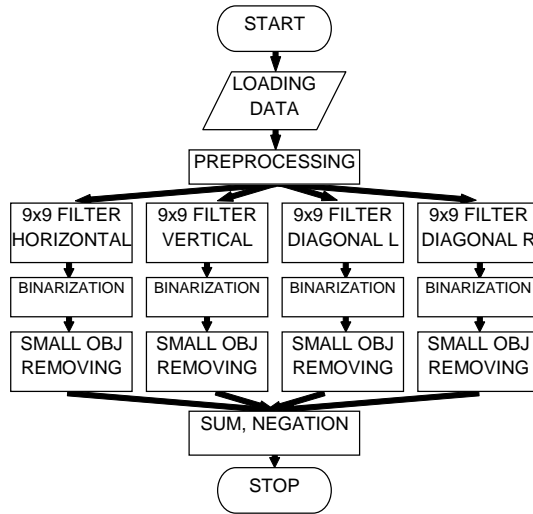


Fig. 11. The proposed algorithm schema.

possible to subjectively evaluate the quality of output images. The proposed algorithm had the best performance in distinguishing cells by giving them a regular edge shape.

5 Summary

The described research presents an adaptable algorithm for the detection of objects that represent corneal endothelium cells. A detailed description of the methodology of clarifying blurry images and binarizing such images has been presented and its use has been compared to general and popular methods of image processing. The proposed methodology seems to be satisfactory from the point of view of the medical specialist. It is unfortunately sensitive to image resizing, which is the basis for the Authors to conduct further research.

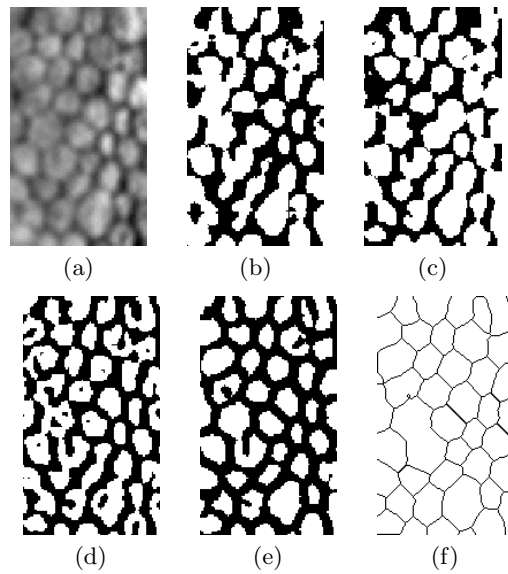


Fig. 12. Results of binarization of non-normalized image with adaptive method (b), with the use of top-hat (c), with the use of mask size 9x9(d), proposed algorithm (using 4 direction masks size 9x9) (e) and final segmentation (f). Input image (a) - normalized.

Further work involves a research on methods of segmentation (eg. precise segmentation [11]), a construction of a grid based on 'triple points'. An interesting issue will be an adaptation of an algorithm for neighborhood map [10] for binarization of corneal endothelial microscopy images.

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