

AUTOMATIC IMAGE PROCESSING APPLIED TO CORNEAL ENDOTHELIUM CELL COUNT AND SHAPE CHARACTERIZATION

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ABSTRACT:

Corneal endothelium cell count, as well as cell hexagonality percentage characterization, are of great importance nowadays to detect anomalies and pathologies of human eye, such as glaucoma. Prevalent technologies used are mainly based in both microscopy and a later image analysis. However, automatic cell count made by microscopes' built-in software is rather inconsistent, therefore many laboratories opt for using manual count as the most reliable alternative. This count is a tedious and time-consuming task that can lead to human error, for this reason, several proposals to automate the process have been made. Present communication shows a procedure for the automatic pre-processing, segmentation and analysis of the images obtained by a confocal microscope, using watershed transform, and the graphics user interface (GUI) created with Matlab® to apply this procedure. In order to quantify the procedure's quality, 30 corneal endothelium images with a number of cells between 90 and 170 were analysed, resulting in a mean error in cell count of 4.3%, which can be considered a reasonably good result. However, results achieved for hexagonality percentage using this method, and with the available image quality, are not as good as expected, which invites to improving image quality, focusing in areas with better cell homogeneity or even considering the application of other algorithms, such as neural networks, for future works.

Keywords: Matlab®, Graphics User Interface (GUI), Hexagonality, Watershed, Opening-Closing by reconstruction (OCBR)

1. - INTRODUCTION

The cornea is a transparent avascular connective tissue composed of five differentiated layers, three cellular (epithelium, stroma and endothelium) and two interface (Bow-man's membrane and Descemet's membrane) [1].

Among them, endothelium is the layer responsible of keeping a constant hydration (and therefore translucency), presenting in its non-degraded form a hexagonal mosaic aspect that resembles a honey-comb [2]. By the natural process of aging, as well as by some pathologies or dysfunctions, both the number of cells as well as their shape change during life, from 3000/4000 cells/cm² in the second decade of life to about 2600 cells/cm² above the eighth. The number of cells with a hexagonal shape is modified in a similar way, from approximately 75% to just 60% of the total number [1,3].

This reduction in the number of cells creates gaps that force the rest of cells to augment their size (polimegatism) and/or lose their hexagonal shape (pleomorfism) to fill them in [1,4], therefore the great importance of cell count and shape characterization to detect some anomalies.

Image processing techniques, as well as the ones used in geometric modelling, are commonly used in ophthalmology for the characterization of the different structures and corneal tissues. All of them aim to detect, diagnose, control and treat different pathologies, such as retinopathies, glaucoma or keratoconus, and characterize their different degrees [5-14].

Currently, there are many techniques for analysing corneal endothelium's cells, like those based in non-contact specular microscopy and confocal microscopy. These ones are the most used techniques mainly due to the low risk of epithelial injuries or infections (there is no contact with the eye) and to the easiness of using their software [15-18].

However, many authors highlight the inconsistency in the automatic cell count made by these microscope's own software [4,16,17,19-21]. For this reason, many laboratories and clinics opt for using manual cell count as the most reliable alternative.

Manual count is a tedious and time-consuming task that can lead to human error. For this reason, several proposals have been developed to automate the process of counting and characterising shape and size of epithelium's cells. All are based in processing the image obtained from the microscope, but each does so with a different approach: unsupervised machine learning [4], genetic algorithms [19] snake and particle swarm optimization [22] or neural networks [21,23], among many others, although it must be noticed that each one has its own flaws.

Present communication shows a procedure for the automatic pre-processing, segmentation and analysis of the images obtained by a confocal microscope. To apply it, it has been used the watershed transform, and a graphics user interface (GUI) created with Matlab®, that conjointly form a more efficient and comfortable way of counting corneal endothelium cells, as well as measuring their hexagonality percentage.

2. - MATERIALS & METHODS

2.1.- PARTICIPANTS

For this study, a total of 30 eye images from 30 patients (11 men / 19 women, ages ranging from 21 to 52 years) were used. These images were randomly selected from the database of patient's endothelium pictures taken with a Konan NSP-9900 (Konan Medical, Hyogo, Japan) in Vissum Hospital (a centre associated with Universidad Miguel Hernandez in Alicante, Spain). Pictures were taken by an expert technician, and ethical standards of the Declaration of Helsinki (Seventh revision, October 2013, Fortaleza, Brazil) were followed, counting with the approval of the local Clinical Research Ethics Committee, and patient's informed consent.

2.2.- SOFTWARE

The programming environment for image filtering and cell detection algorithms, as well as for creating the GUI, has been the commercial software Matlab® R2017b (MathWorks®, Natick, USA). Matlab® is a powerful tool for image treatment, as it can decompose images and treat them as matrices. The flow diagram of this process can be seen in Fig. 1.

Konan NSP-9900 confocal microscope uses its own software (CellCheck®) for obtaining the endothelial images, allowing their exportation in several formats, having chosen the standard .JPG for the importation to the tailored GUI.

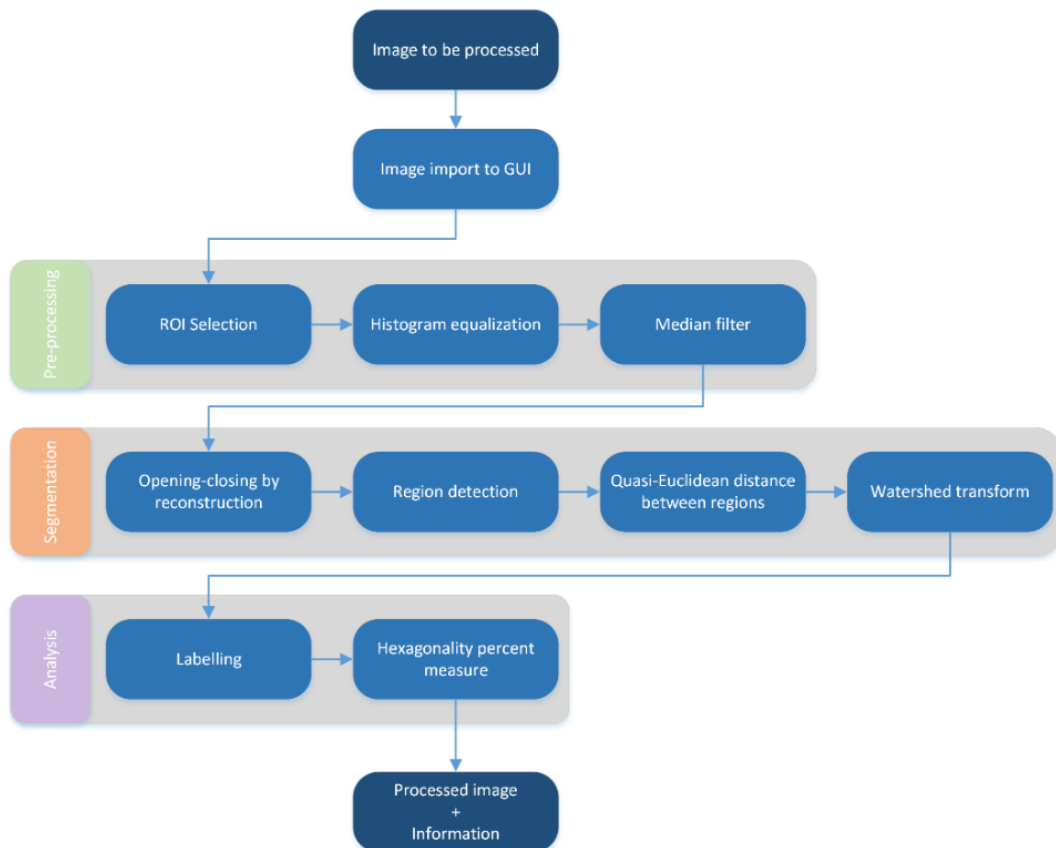



Fig. 1. Flow diagram of the procedure

	<p style="text-align: center;">AUTOMATIC IMAGE PROCESSING APPLIED TO CORNEAL ENDOTHELIUM CELL COUNT AND SHAPE CHARACTERIZATION</p>	<p style="text-align: center;">Computer Sciences</p>
<p>RESEARCH</p>	<p style="text-align: center;">J.S. Velázquez-Blázquez, F. Cavas-Martínez, V.A. Campuzano, J. Alió del Barrio, F.J.F. Cañavate, J. Alió</p>	<p style="text-align: center;">Computer-assisted design</p>

2.3.- PRE-PROCESSING

Region of Interest (ROI). After selecting the image, first step consists in selecting a ROI inside which the process will take place. For doing it, Matlab®'s library function *roipoly* is used, allowing the creation of a polygonal ROI and obtaining a filtering mask. Then, the complementary of that mask is obtained using the function *imcomplement*, it is transformed to grayscale format (uint8) using *im2uint8*, and finally, the mask transformed polygon in grayscale is added to the original image, pixel by pixel by saturation, with *imadd*.

Histogram Equalization. To better distinguish the inner regions of the cells from their borders, an automatic equalization and expansion of the histogram is the chosen method, and is made by Matlab®'s function *histeq*

Median Filter. To eliminate noise and smoothen the details, several filters such as mean, Gauss or binomial were considered, opting finally for applying a median filter using Matlab®'s function *medfilt2*.

2.4.- SEGMENTATION

To successfully achieve segmentation of images using watershed transform, it is necessary to previously take some steps that allow establishing marks (masses of connected pixels) in the objects.

Opening-Closing by reconstruction. From the great variety of procedures that could be used to "clean" the image, a morphological technique called Opening-Closing by reconstruction (OCBR) was chosen. This operation creates maximum intensity regions inside each object, so it can be detected later.

The procedure followed to perform this technique relies in the following Matlab®'s functions:

1. Image erosion with *imerode* and a disc-shaped kernel previously defined with *strel*.
2. Reconstruction of the eroded image with *imreconstruct*.
3. Dilatation of the image with *imdilate* and a disc-shaped convolution operator like the one in step 1, created with *strel*
4. Reconstruction of the dilated image with *imreconstruct*.
5. Complementation of the result with *imcomplement*.
6. Second median filtering and border erosion to highlight them and split regions that remained joined.

Region Detection. To detect the inner region of the cells, *imregionalmax* function is used, detecting groups of 4 neighbour connected pixels whose intensity is maximum. To remove the remaining small pixel groups, the function *bwareaopen* is then applied. Finally, to resemble the regions of maximum intensity to the inner region of the cells, a morphological closing of the binary image is made with *imclose* function, by means of a disc-shaped convolution operator with a radius of 3 pixels.

Watershed Transform. To obtain the distance between objects it was needed to apply watershed transform, *bwdist* function with Quasi-Euclidean distance is used, thus forcing that the form of the cell tends to a hexagon, instead of tending to a circumference. Then, watershed transform is made with *watershed* function, selecting to show only the highest values of the result (image == 0).

2.5.- ANALYSIS: NUMBER OF CELLS AND HEXAGONALITY

The main objective of this paper is obtaining the number of cells in a corneal endothelium's image, as well as the hexagonality percentage, as these characteristics are used for diagnosing corneal diseases. For that, the following methods have been used:

Labelling. To obtain the value of cellular density, the labelling method is applied using Matlab®'s *bwlabel* function, that gives back the number of labelled objects in the image.

Hexagonality Percentage. To obtain the hexagonality percentage, a mathematic approach has been chosen, defining hexagonality as how close is area/perimeter² ratio to the one of a regular hexagon in each cell. This constant of regular polygons is size-independent and for a regular hexagon takes a value of 0.07216875. To calculate it, *regionprops* function is used to acquire area and perimeter of all objects previously labelled. Once calculated the constant for each cell, the error when it is compared with the constant of the perfect hexagon is obtained with a self-developed code. Thus, this error is expressed as the hexagonality percentage for each cell, and is finally depicted in the GUI as a histogram, along with the mean hexagonality percentage value for all the cells, as can be seen in Supplementary Material section (Fig. 1).

2.6.- GRAPHICS USER INTERFACE

To obtain a readable and intuitive design, the colour palette was homogenized in white, and all GUI was structured placing each object equidistantly and simplifying buttons and/or options, as well as information shown. The designed GUI allows opening the image, showing its histogram, detecting the cells and calculating the number of them and their hexagonality percentage, both individually and as a mean value. Fig. 1 in Supplementary Material section shows the final design of the created GUI.

3. - RESULTS AND DISCUSSION

Obtained results suggest that in every case it is indispensable to previously acquire a ROI of the image, as there usually have differences in contrast, regions in which cells are not homogenous or in which their borders mix, among other imperfections. The result of the selected ROI and the original image can be seen in Supplementary Material section (Fig. 2).

With the aim of improving the image contrast, in a first stage uniform brightness adjustment filters and linear border enhance filters were tested, with not very satisfactory results as there was a great contrast between dark zones adjacent to very bright ones. To make the program work with the majority of images, it has been necessary to equalize the histogram to enhance contrast between cell's inner area and borders. Results can be seen in Supplementary Material section (Figs. 3 and 4).

Due to the fact that noise present in the images is usually caused by a random occurrence of totally white pixels, applying a median filter allows to reduce the amount of intensity variations between neighbour pixels, and even removing those whose intensity level is very different, achieving a smoothing of the image without deteriorating important details, such as borders, as can be seen in Supplementary Material section (Fig. 5).

The OCBR segmentation method has proven to be critical for obtaining the number of corneal endothelium's cells, as it manages to homogenize almost the whole inner region of them. The result, step by step, of the Opening-Closing by Reconstruction morphological operation can be checked in Figs. 2 and 3.

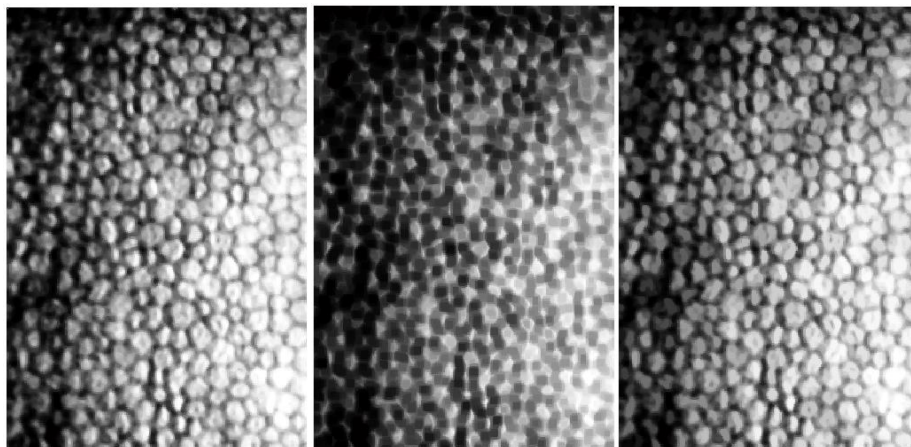


Fig. 2. Image before eroding (left), after eroding (centre) and after reconstruction (right).

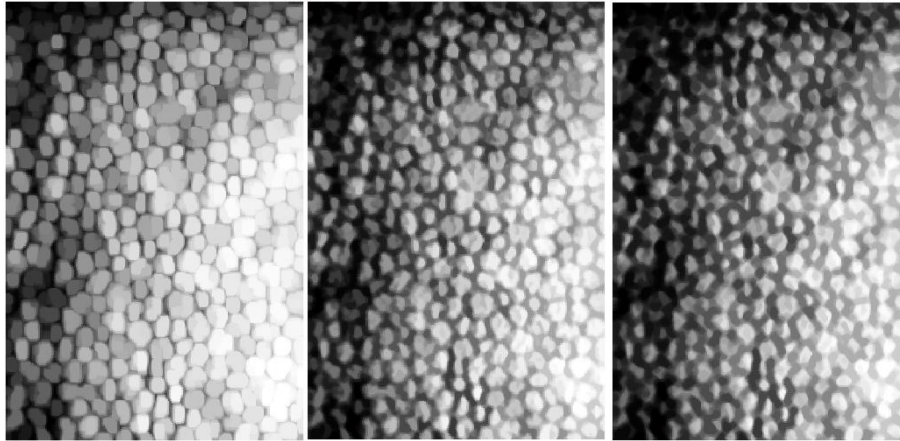


Fig. 3 Image after dilatation (left), after 2nd reconstruction and complementation (centre) and final, after 2nd median filter (right).

The method for detecting maximum regions works correctly, although its results depend on a great way of previous segmentation and the quality of the original image. The results for *imregionalmax*, *bwareaopen* and *imclose* operations are shown in Fig. 4.

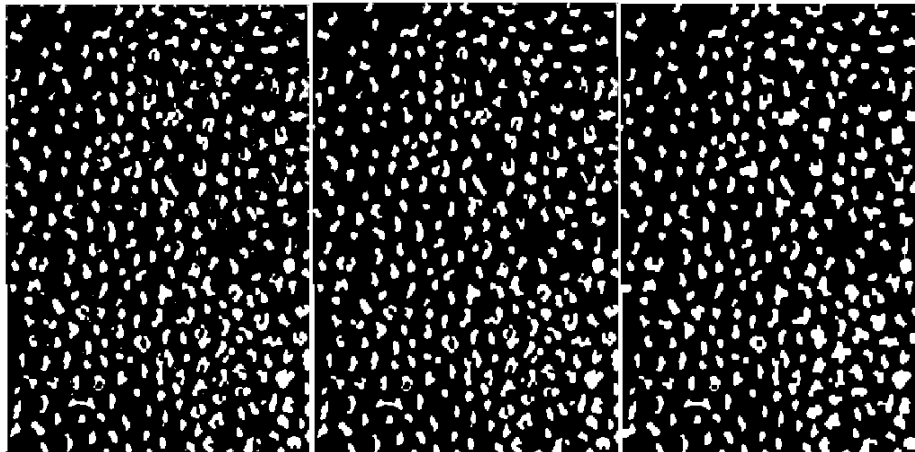


Fig. 4 Image after applying *imregionalmax* (left), *bwareaopen* (centre) and *imclose* (right).

Although many other border detection algorithms were tested, such as “sobel”, “prewitt” or “canny”, we finally opted for using watershed transform with Quasi-Euclidean distance, as it gives the results that most resemble a hexagonal shape, which is the ideal shape of corneal endothelium’s cells.

The labelling method has always accounted the total number of cells previously detected by segmentation, thus error is reduced to the one produced by segmentation on its own. In this case, the image used for cell counting is the complemented of the watershed transform result (Fig. 5), as the function used labels regions of a binary image whose pixel value is equal to 1.

In order to quantify the quality of the segmentation made, 30 images were analysed with the programmed algorithms, with a number of cells in the ROI ranging from 90 to 170. (Fig. 6). To estimate the error made during each cell count, we considered the manual measure as the exact one, and then considered the deviation with respect to it when automatically counting, with the following equation:

$$E_i = \frac{C_a \cdot 100}{C_m} \quad (1)$$

Where E_i is the error in each measure, C_m is the total amount of cells measured manually, and C_a is the total amount of cells measured automatically.

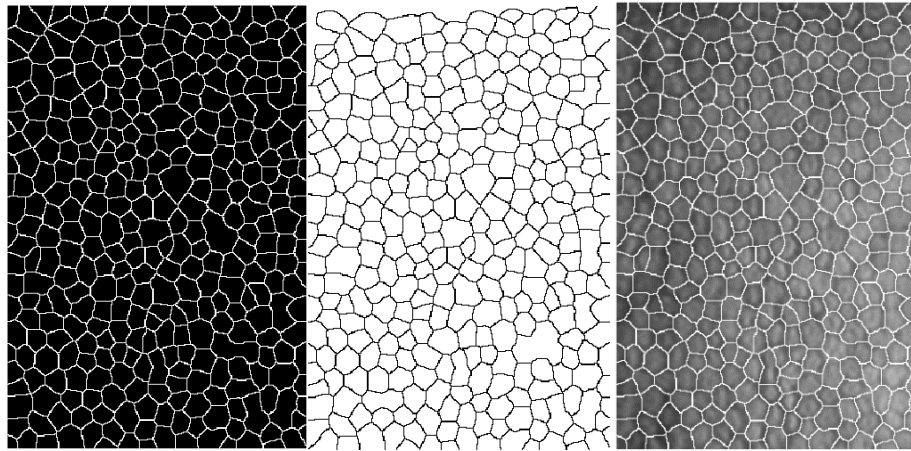


Fig. 5 Watershed transform result (left), its complemented image (centre) and its superposition over the original image (right).

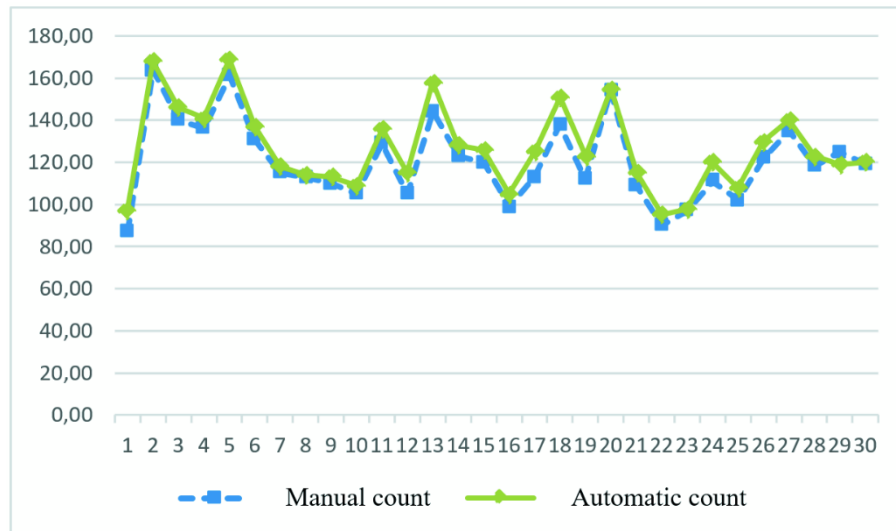


Fig. 6 Number of cells counted in automatic mode vs. manual mode.

After counting endothelium's cells both manually and automatically, the mean error obtained between both procedures, was of about 4.3 % (standard deviation: ± 2.9 %), which can be considered a reasonably good result (Fig. 7). Cases in which error was over 10% were always related with poor image quality and/or high cell size/shape variability.

To determine hexagonality percentage measure error, we proceeded in a similar way, considering manual measure as reference, and compared automatic one with it:

$$H_i = \frac{P_a \cdot 100}{P_m} \quad (2)$$

Where H_i is the hexagonality error in each measure, P_m is the hexagonality percentage measured manually, and P_a is the hexagonality percentage measured automatically. In this case, mean error obtained was over 16 % (standard deviation: ± 8.8 %), so further research will be needed to reduce it (Figure 8):

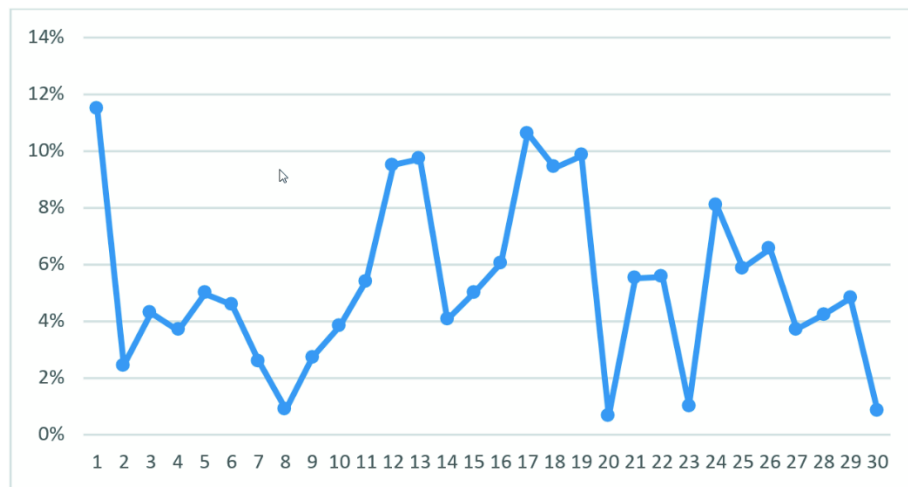


Fig. 7 Cell count errors in automatic mode.

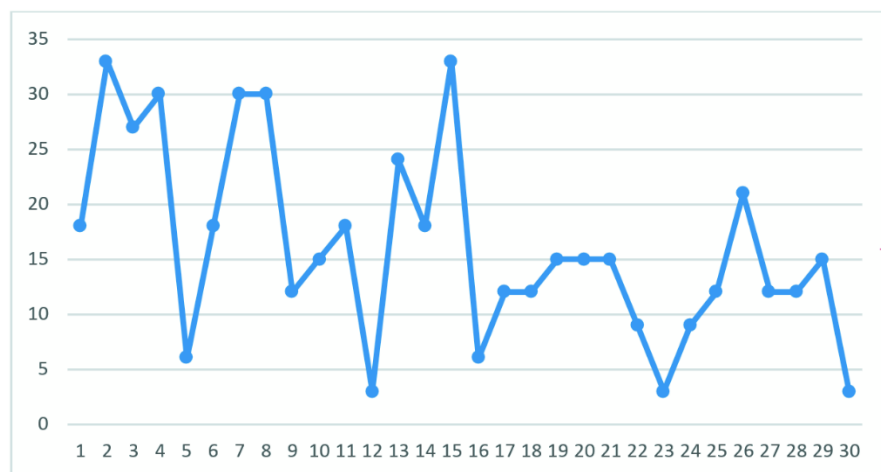


Fig. 8 Hexagonality percent count errors in automatic mode.

4. - CONCLUSIONS

In this work a series of algorithms have been programmed with Matlab® software, looking for obtaining some of the main corneal endothelium's characteristics: cell density and hexagonality percentage. To ease programming, A GUI, also developed in Matlab®, was designed for importing the images and visualizing the results of the analysis.

The objective of designing and programming both a GUI and several cell detection algorithms that allowed reducing the time of analysis and avoiding human mistakes made when using confocal microscope's software, have been accomplished. However, it has been noticed that quality and characteristics of original images considerably affect the level of error.

The results obtained when calculating the number of cells are satisfactory, showing a low mean error, which is in accordance with the results obtained with other approaches: unsupervised machine learning [4], genetic algorithms [19] snake and particle swarm optimization [22] or neural networks [21,23]. Nevertheless, the results achieved for hexagonality percentage using this method, and with the quality of the available images, are not as good as expected, as the mean value of variations between manual and automatic count reaches 16 %, thus it will need future research.

The results obtained with this procedure may be used for analysing corneal features of several corneal diseases, obtaining new representative statistical data from the images and even improving the images obtained of other types of cells.

For future works, quality of the images to be analysed would need to be improved, as well as homogenizing their size, to achieve a total process automation. Also, the same method of segmentation should continue to be evaluated with better quality images, areas with more homogenous shape of cells, and also trying new algorithm programming methods, such as neural networks.

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ADDITIONAL INFORMATION

Conflict of Interest: The authors declare no conflict of interest.

Ethical Approval: All the procedures in this study with human beings followed the ethical rules of the Institution and/or the National Research Committee, and adhered to the tenets of the declaration of Helsinki of 1964 or equivalent ethical rules.

SUPPLEMENTARY MATERIAL

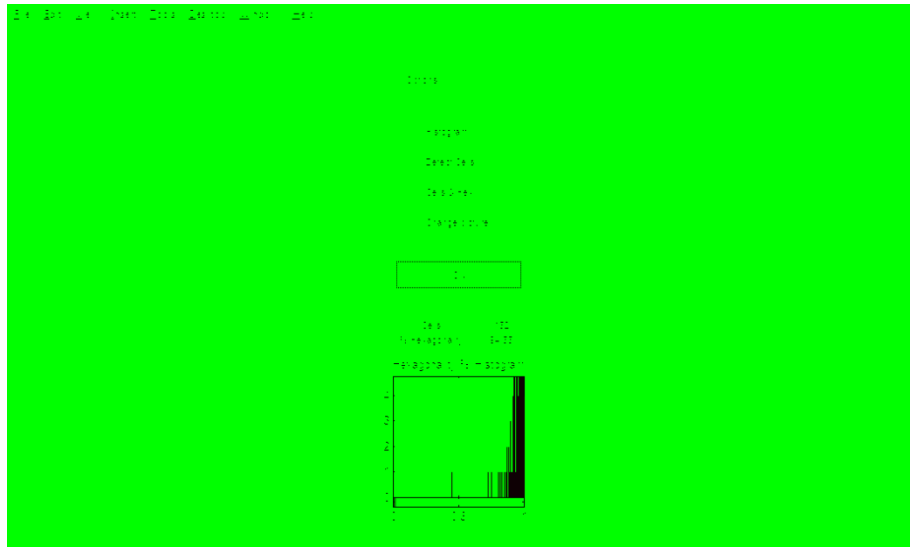


Fig. 1. Graphics User Interface.

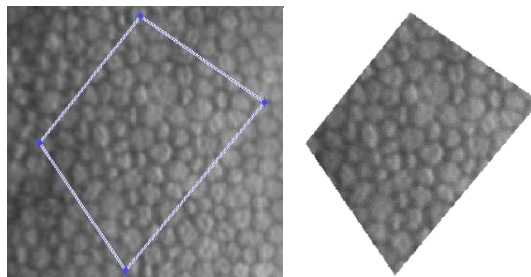


Fig. 2. ROI selection example.

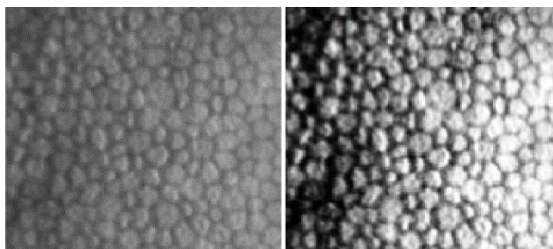


Fig. 3. Image without equalization (left) vs. equalized image (right).

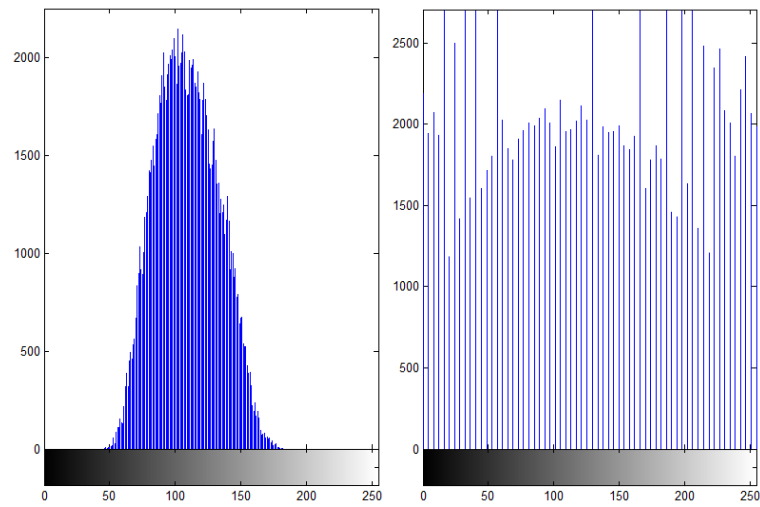


Fig. 4. Non-equalized histogram (left) vs. equalized histogram (right).

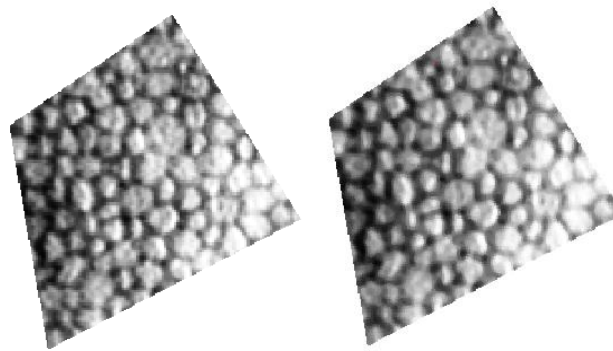


Fig. 5. ROI before median filtering (left), ROI after median filtering (right).