J. Biomed. Eng. Res : 125-130, 2006

Image Analysis Algorithm for the Corneal Endothelium

Young-Yoon Kim, Beop-Min Kim, Hwa-Joon Park, Kang-Bin Im, Jin-Su Lee, Dong-Youn Kim

Department of Biomedical Engineering, College of Health Sciences, Yonsei University, Wonju, Korea
(Received August 12, 2005. Accepted June 7, 2006)

Abstract

The number of the living endothelial cells and the shape of those are very import clinical parameters for the evaluation of the quality of cornea. In this paper, we developed the automated endothelial cell counting and shape analysis algorithm for a confocal microscope. Since, the endothelial images from the confocal microscope has a non-uniform illumination and low contrast between cell boundaries and cell bodies, it is very difficult to segment the cells from the endothelial images. To cope with these difficulties, we proposed the new two stage image processing algorithm. At first stage algorithm, we used a high-pass filter and histogram equalization to compensate the non-uniform brightness pattern and a morphological filter and a watershed method are applied to detect the boundary of cells. From this stage, we could count the number of cells in an endothelial image. At second stage algorithm, we used a Voronoi diagram method to classify the shape of cells. This cell shape analysis and the percent of hexagonal cells are very sensitive in detecting the early endothelium damage. To evaluate the performance of the proposed system, we p가cessed seven endothelial images obtained using a confocal microscope. The proposed system correctly counted 95.5% cells and classified 92.0% of hexagonal cell shapes. This result is better than any others in this research area.

Key words : confocal microscope, endothelium, cell shape, voronoi diagram, watershed, morphological filter

I. INTRODUCTION

The cornea consists of five layers: the corneal epithelium, the Bowman's membrane, the corneal stroma, the Descemet's membrane, and the corneal endothelium as in Fig. 1. Endothelial cell density is the highest at birth, up to 7,500 cells/㎟, and decreases rapidly during early years. Normal density in healthy adult range from 1,600 to 3,200 cells/㎟. Normally, corneal endothelial cells assume the form of uniform hexagonal shape with homogeneous size as in Fig. 2[2]. To keep maintaining the normal transparency of the cornea, various physiological process must be active. If the stroma is allowed to imbibe water, it loses its transparency. Both epithelium and endothelium layers inhibit the leakage of fluid and ions in the stroma. The epithelium consists of 5-6 layers of cells and it is an effective barrier to the diffusion of water into the stroma. The endothelium is a single layer of closely packed hexagonal cells whose permeability to small ions is much smaller than epithelium and its barrier function is weak. However, due to the presence of an active pump mechanism that removes fluid from the stroma, it assumes a critical importance in regulating the optimum level of stromal hydration to maintain corneal transparency. Because of the lack of proliferative capacity of the corneal endothelium, cell damage may be healed by migration and enlargement of remaining cells. This results in a decrease of the cell density, an increase of variation between the cell sizes and a disruption of the normal regular hexagonal pattern[3]. Therefore, the commonly employed parameters in the evaluation of the endothelial pattern are the mean cell density[4] and the percentage of hexagonal cells[5]. It is also important to estimate the health of the endothelial cells before corneal refractive surgery such as LASIK to determined if the subject is eligible for the surgery.

Currently, a non-contact specular microscope, or a confocal microscope are used to acquire the corneal endothelium images. Among these, confocal microscope is becoming the choice of clinicians since it provides the highest images qualities. Clinicians are interested in general cell morphology including density, shape, and size variation, since these features can be used as early indicators of corneal pathologies or in the cell viability test of corneas before transplant [2]. The cell counting and analysis of cell shape are often manually performed by ophthalmologists, which is tedious and time consuming job. Therefore, an automated algorithm to perform these tasks would be significantly helpful. Our study focuses

This study was supported by a grant of the Korea health 21 R&D Project, Ministry & Welfare, Republic of Korea (02-PJ3-PG6-EV07-0002).

Corresponding Author : Dong-Youn Kim
Department of Biomedical Engineering, College of Health Sciences and Institute of Medical Engineering, Yonsei University, Wonju, Korea
Tel : 033-760-2434 / Fax : 033-760-2197
E-mail : dongkim77@yonsei.ac.kr

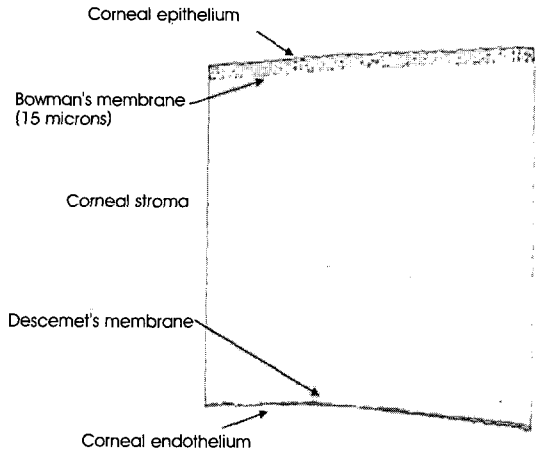

Fig. 1. Typical structure of human cornea composed of five layers [1].

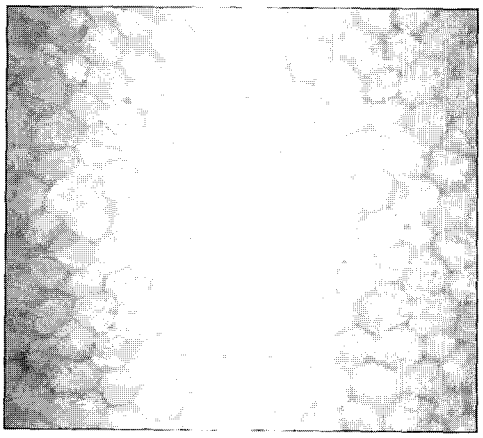

Fig. 2. Endothelial cells in human corneal Image from a confocal microscope [2].

on developing automatic image analysis algorithm for corneal endothelium images from a confocal microscope.

We found that the conventional image processing technique was difficult to implement for this type of problem because of the large difference of the contrast in acquired images which is inherent in the confocal microscope. A human operator can easily extract these features from the corneal images, however it is difficult to perform the same function using computer software. Several researchers have tackled to solve this problem using spatial domain filtering operations[6], Fourier analysis[7],morphological filtering method and watershed[8] and neural networks[9,10]. Apparently, none was able to solve the problem satisfactorily for the whole range of in-vivo images [7].

The objective of our research is to develop an efficient cell count and cell shape classification analysis system which consists of two-stage algorithm. At first stage, we used a high pass filter to correct the low contrast between cell boundaries and cell bodies, then a morphological filter and a watershed method were applied for the segmentation of cells to count the endothelial cells. Second stage, the Voronoi diagram was applied to find the shape of cells. Especially the percentage of the hexagonal cells is very critical to detect the early endothelium damage. Up to now, all the researches related to the analysis of the endothelial images are focused to find the number of cells in an endothelium image. As far as we know, this paper is the first one which performed the classification of the shape of the endothelial cells to count the shape of cells. Since cell shape information is used to detect the early damage of the endothelial cells, this one is considered very important parameter [3]. To evaluate the performance of our algorithm, we used seven corneal endothelium images published in a book under the author's permission [2].

II. MATERIALS AND METHODS

A. Nonuniform Illumination during Confocal Scanning

Fig. 2 shows a non-uniform illumination pattern, which is due to the characteristics of the confocal scanning mechanism. In the central region of Fig. 2, the cells are highly illuminated while the overall brightness decreases near the edge. To illustrate why this phenomenon occurs, we conducted a simple simulation using commercial software ZEMAX[18] and the results are shown in Fig. 3.

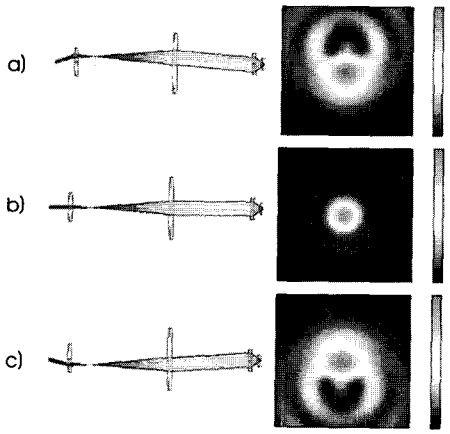

Fig. 3. Light distribution of the focused area

Fig. 3(b) shows the intensity distribution (Airy pattern) in the case that the beam is normally incident to the lens. In this case the intensity distribution is symmetric. However, as the light is obliquely incident into the lens which occurs during confocal scanning (Fig. 3 (a) and (c)), the intensity distribution in the sample become asymmetric and the intensity at the focal point is distributed over larger area. This is one form of the

optical aberrations. Therefore, the confocal images has high illumination in the center area and low illumination near the edges of the images.

Image Processing for Endothelium Images

The objective of our research is to develop a new endothelial cell analysis algorithm. The endothelial cell images from a confocal microscope inherently have a non-uniform illumination and low contrast in dark cell boundaries and in light cell bodies. To solve these problems, we used two-stage algorithm. Fig. 4 is the flowchart of the proposed endothelial cell analysis algorithm.

The first-stage consists of three parts which are the correction of overall illumination, the boundary extraction and the cell count. To correct the non-uniform illuminations, high pass filter and histogram equalization are applied. Then simple thresholding method and morphological operations are used to extract the boundaries of cell. Finally the watershed method is used to find the locations of the center of cell. From these consecutive operations, we can count the number of cell.

The objective of the second-stage is to classify the shape of cells which is very sensitive parameter to detect the early damage in the endothelial cells. The Voronoi diagram method is applied to count the number of cells in a certain shape.

Correction of Overall Illumination

To correct the non-uniform illumination problem, we first used a high-pass filter which are described by the following formulation:

$$\begin{aligned} H(u,v) &= 1 - e^{-D^2(u,v)/2D_0^2} \\ D(u,v) &= \sqrt{(u-M/2)^2 + (v-N/2)^2} \end{aligned} \quad (1)$$

where u and v are spectral coordinates, H is the filter transfer function and D is the distance between DC and the shifted center in the frequency domain. D_0 is the cut-off frequency and $(\frac{M}{2}, \frac{N}{2})$ is the shifted center of the frequency domain. In our research, the adopted cut-off frequency , D_0, is 4 and M and N are 256. Fig. 6. shows the result of the high pass filtered image. Since the high pass filtered image has low contrast, we applied the histogram specification with a Gaussian probability density function to increase the overall contrast [11].

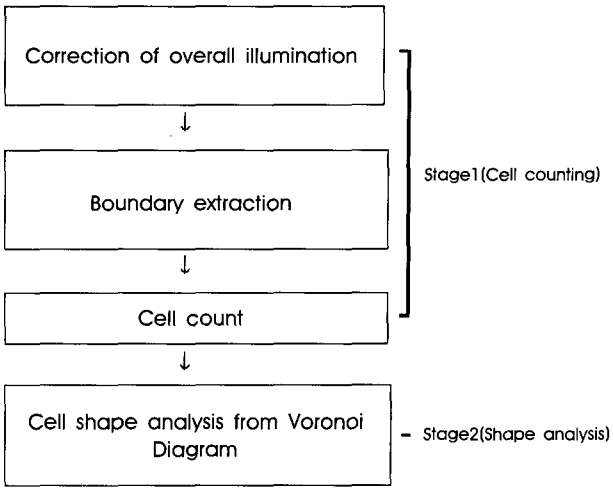

Fig. 4. Flowchart of the proposed algorithm

Boundary Extraction

First a simple thresholding method was applied to generate a binary image. The adopted threshold level was its mean value. Later thinning process was applied to extract the boundaries of cells. Finally, a morphological operations with a 3x3 morphological filter[11] is applied to remove unnecessary boundaries. Fig. 7. shows the results of the morphological filtered cell boundary. During these processes, some cell boundaries are removed. In Fig. 7, those areas are illustrated as shaded region. To recover those boundaries, a watershed method was applied.

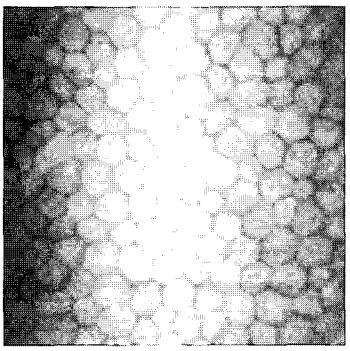

Fig. 5. Original image

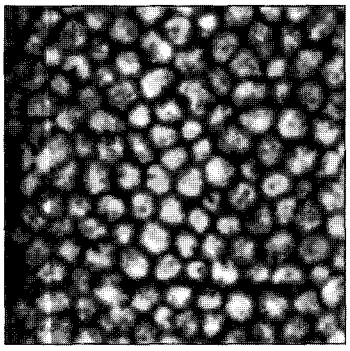

Fig. 6. High pass filtered image

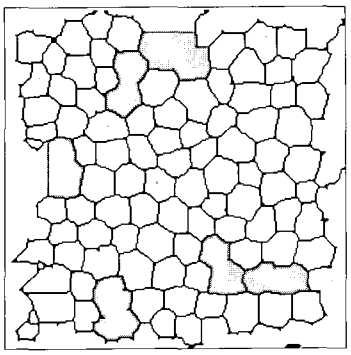

Fig. 7. Result of morphological filtered image

Cell Count

Some researchers are used an average size of cell to identify the cells. They assumed every cell has same size. However, when there is a damage in cornea, the size of cell is different.

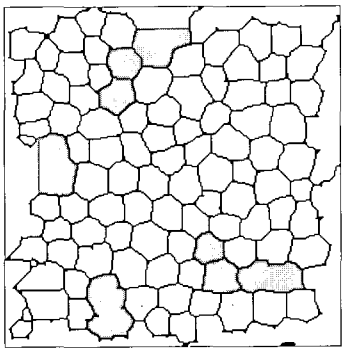

Fig. 8. a) New boundaries from watershed methods

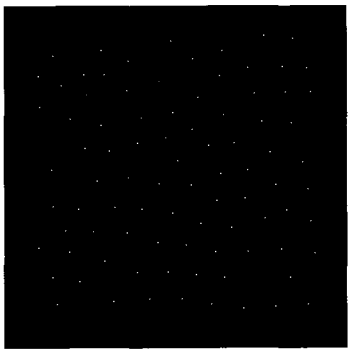

Fig. 8. b) Result of watershed method

So, we applied the watershed method which extracted the boundary of cells from watershed line The following is the watershed method[12].

i) Assign the largest distance value to the boundary of each cell, which are those pixels that are farthest from the center of each pixel.

ii) Compute Euclidean 8-distance value from the boundaries and subtract that distance value from the boundary distance value. Therefore, the center of each cell which is the farthest from the boundary will be assigned the minimum value. Repeat this process to every cell.

iii) By counting minimum values, we can find the number of cells in the topological image.

By applying the watershed method, we could recover all the missing cell boundaries except those of most outer cells which do not have neighbors. Fig. 8.a) shows the new boundaries of endothelium image. Clearly, some boundaries are recovered by the watershed method. Fig. 8.b) displays the final results of watershed method, each cell is represented by the dot.

Cell Shape Analysis from Voronoi Diagram

Up to now most researches are focused to find the number of cells in endothelial images. However, the percentage of the hexagonal shape of cell is very sensitive to detect the early endothelial damage. Voronoi diagrams are used to find the boundaries from the locations of the center of cells, which are already figured out by the watershed method. From those points, the Voronoi diagram partitions space into shape of cells around each center of cell [13, 14].

A Voronoi diagram uses proximity information, what is close to what. Let $p=\{p_1, p_2, \ldots, p_n\}$ be a set of points in the plane. Define $v(p_i)$, the Voronoi cell for p_i, to be the set of points q in the plane that are closer to p_i than to any other points. That is, the Voronoi cell for p_i is defined to be: $V(pi)=\{\| pjq\| < \| piq\|, \forall j \neq i\}$ where $\|pq\|$ denotes the Euclidean distance between points p and q.[19]

We applied Voronoi diagram to the watershed image and Fig. 9 shows the results.

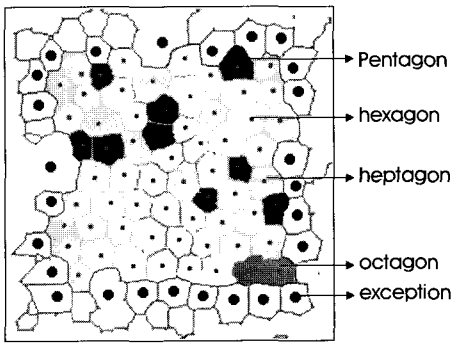

Fig. 9. An endothelial cell image after application of watershed and Voronoi diagram methods.

Since the Voronoi diagram uses the center of neighboring cells, the shape of the cells near the edges cannot be calculated. In Fig. 9. the outer cells which is represented 'exception', thus excluded in the final statistics.

III. RESULTS

The objective of the proposed algorithm is to count the number of cells and to classify the shape of cells to count the number of hexagonal cells. Generally, endothelial images inherently has non-uniform illumination in cell and cell body and has very low contrast between cell body and cell boundary as in Fig. 2. To correct the overall illumination, we first applied a high pass filter and histogram equalization. Fig 6. shows an example image of the results of this processing. Edge detection methods performed poorly on cell images because of the smooth boundaries in cell, so we used the simple thresholding method with its mean and morphological operations. The morphological thinning process is applied to find the shape of the boundary of cells. The morphological opening and closing operation are employed to remove the unwanted lines at this process. Fig. 7 shows the boundary extracted shape of cells. The endothelial cell density counting is tedious and time consuming job to perform. Therefore the automatic counting process is necessary process. We applied the watershed method to count cells automatically. Fig. 8 a) and b) displays the results of the watershed transformed cell image and the location of each cell is represented by dot.

As a measure of our performance, we applied our algorithm to seven different endothelium images. We define the cell discrimination rate by dividing the total number of cells from watershed transform by the total number of cells counted by an ophthalmologist. Table 1 shows the cell discrimination rate for each corneal endothelium image. The obtained average discrimination rate was 95.5% and this one is comparable to or better than others in this research area[6,7,8,9,10].

Table 1. Cell discrimination rate for each cell

corneal endothelium image	cell discrimination rate(%)
1	89.00
2	100
3	92.54
4	100
5	97.67
6	95.35
7	93.97
total average	95.50

From the watershed transformed image, we applied a Voronoi diagram which used Fortune's algorithm[19] which find the shape of cells from the cell boundaries. Fig. 9 shows the result of the Voronoi diagram. Since we used variable frame analysis for our experiment, the outer cells represented by larger dots are not considered. Each cell to be counted is represented by small dots.

In detecting the early endothelium damage, the percentage of hexagonal cells is more sensitive than the cell density that is calculated from average number of cell count in a square millimeter. The percentage of hexagonal cells is defined by dividing the correctly classified number of the hexagonal cells from our algorithm by the number of those by an ophthalmologist. As far as we know, our research is the first one to attempt to calculate the percentage of hexagonal cells.

Table 2. Cell shape classification for seven endothelium images

Expert		The proposed algorithm			
shape	number	pentagon	hexagon	heptagpm	octagon
pentagon	32	32	10	1	0
hexagon	174	0	160	5	0
heptagon	29	0	2	21	0
octagon	0	0	0	1	0
Total	235	32	172	28	0
		232			

From Table 2, it is clear that most endothelial cells take the hexagonal shape, which is typical for normal endothelium. The expert (ophthalmologist) classified the shape of cells from seven corneal endothelium images. The total number of the hexagonal cell is 174, and the pentagonal and the heptagonal one is 32 and 29, respectively. Our algorithm classified all the pentagonal shape cells correctly, but for hexagonal and heptagonal shapes, we failed to classify some cells correctly. The achieved the percentage of hexagonal cell, which is calculated by dividing the number of the hexagonal shape from our algorithm by the that from the ophthalmologist, is 92.0%, which is suitable for clinical applications.

IV. DISCUSSION AND CONCLUSION

We developed the corneal image processing algorithm for the analysis of human corneal endothelial cells. The proposed algorithm calculated the number of the endothelial cells and calculate the shape of those. These parameters are commonly employed parameters in the evaluation of the endothelial pattern to early detect several eye pathologies. In the proposed algorithm, we used two stage algorithm, at the first stage the number of endothelial cells is calculated and second stage the shapes of cells are counted. The classification of cell shape is considered more sensitive parameter for the detection of early endothelium damage. However, most researches are focused to develop the cell count algorithms.

As far as we know, our research is the first one to calculate the percentage hexagonal cells. When the endothelium cell is in healthy condition, the shape of them is a hexagonal shape. Even though the used watershed transform and a Voronoi diagram are one of the well known image processing techniques, these processing techniques worked very well after several consecutive preprocessing steps which consist of a highpass filtering, histogram equalization. The proposed algorithm counted 95.5% of cells correctly, this result is comparable to and better than others. In cell shape classification, our algorithm counted 92.0% of hexagonal shapes of cells correctly, this result could be suitable for the clinical applications.

REFERENCES

[1] http://medocs.ucdavis.edu/CHA/402/labsyl/01/18/lm09.htm

[2] L. Mastropasqua, M. Nubile, *Confocal Microscopy of the Cornea*, Slack, 2002

[3] M. salerno, F.Sargeni, V.Bonaiuto, P.Amerini, L.Cerulli, and F.Ricci., "A new CNN based tool for an automated morphometry analysis of the corneal endothelium," in *Proc. IEEE Fifth International Workshop*, April 1998, pp.169-174.

[4] Hirst LW, Auer C, Abbey SC et Al., "Quantitative analysis of wide-field endothelial specular microphotographs," *Am. J. Ophthalmol*, vol. 97, pp. 488, 1894.

[5] E.L. Shaw, G.N. Rao, E.J. Arthur et Al., "The functional reserve of the corneal endothelium," *Ophthalmology*, vol. 85, pp. 640, 1978.

[6] R. Nadachi, K. Nunokawa, "Automated corneal endothelial cell analysis," in *Proc. IEEE Fifth Annual Symposium*, June 1992, pp. 450-457.

[7] M. Foracchia, and A. Ruggeri, "Estimating cell density in corneal endothelium by means of Fourier analysis," in *Proc. 2nd Joint IEEE-EMBS/BMSE Conference*, New York, 2002, pp. 1097-98.

[8] L. Ventura, C. A.C. Caetano, S. J.F. Sousa, F. Isaac, C. Chiaradia, R. A. Lotufo, "A corneal endothelium cell analyzer for slit lamps," *IEEE Engineering in Medicine and Biology*, Nov/Dec 2002.

[9] Akira Hasegawa, Kazuyoshi Itoh, and Yoshiki Ichioka. "Generalization of shift invariant neural networks: Image processing of corneal endothelium," *Neural Networks*, vol. 9, no. 2, pp. 345-356, 1996.

[10] Marco Foracchia, and Alfredo Ruggeri, "Cell countour detection in corneal endothelium in-vivo microscopy," in *Proc. 22nd Annual International Conferenceof the IEEE*, Chicago, July 2000.

[11] R.C. Gonzalez, and R.E. Woods, *Digital Image Processing 2nd*, Prentice Hall, 2002.

[12] Jos B.T.M. Roerdink, and A. Meijster, "The watershed transform: Definitions, algorithms and parallelization strategies," *Fundamenta Informaticae*, vol. 41, pp.187-228, 2000.

[13] M. Seul, L. O'Gorman, and M.J. Sammon, *Practical Algorithms for Image Analysis Description, Examples, and Code*, Cambridge, 2000.

[14] M. de Berg, M. van Kreveld, M. Overmars, and O. Schwarzkoph, *Computational Geometry Algorithms and Application*, Springer, 1997.

[15] J.R. Parker, *Practical Computer Vision using C*, Wiley, 1994.

[16] N. Y. Morgan, M. Seul, "Structure of disordered droplet domain patterns in a monomolecular film," *J. Phys. Chem.* vol.99, no.7, 1995.

[17] S. J. Fortune, "A sweepline algorithm for voronoi diagram," *Algorithmica*, pp. 153-174, 1987.

[18] ZEMAX development corporation http://www.zemax.com/zemax /index.html

[19] M.Deberg, M.Vankreveld, M.Overmars, and O.Schwarzkopf, *Computational Geometry: Algorithms and Applications 2nd*, Springer-Verlag, 2000.