A novel quantitative methodology for age evaluation of the human corneal endothelium

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ABSTRACT

The human corneal endothelium regulates the cornea transparency. Its cells, that can not regenerate after birth, form a tesselated mosaic with almost perfect hexagonal cells during childhood, becoming progressively bigger and less ordered during aging. This study included 50 patients (in 10 decades groups) and 10 specular microscopy observations per patient. Five different criteria were measured on the manually segmented cells: area and perimeter of the cells as well as reduced Minkowski functionals. All these criteria were used to assess the probability of age group membership. We demonstrated that the age evaluation is near the reality, although a high variability was observed for patients between 30 and 70 years old.

Keywords: quality control; human corneal endothelium; reduced minkowski functionals;

1. INTRODUCTION

1.1 Medical context

The cornea is the transparent tissue in the front of the eye. Its two main roles are to refract the light on the retina, and to protect the eye from the outside. It is constituted of three cell layers; among these layers, the innermost called endothelium is dedicated to a constant deswelling of the cornea, keeping it clear during lifetime. The number of endothelial cells (ECs) per surface unit, called endothelial cell density (ECD in cells/mm^2) is thus crucial to maintain corneal transparency. Below 300 to 500 cells/mm^2, the endothelial function is insufficient and the cornea becomes edematous, causing an irreversible vision loss. The ECD of an healthy adult with a normal cornea varies between 2000 and 3000 cells/mm^2. The corneal endothelium has two particularities: it is a monolayer that can be easily observed with specular microscopes (a routine imaging device first developed in the 60's), and its cells do not regenerate.[1] The latter is of importance because EC loss caused by several ocular severe diseases or traumatism (accidental or iatrogenic) can result in corneal blindness. This explains why ophthalmologists routinely check the endothelial quality using specular microscopes.

Two parameters are universally used: ECD (the most important) and EC morphology. Cell morphology is assessed only by the polymegethism (variations in cell size, given by the coefficient of variation of cell area = standard deviation/mean) and the pleomorphism[2] (variations in cell shape, namely percentage of hexagonal cells). The ECD is an objective and easy to interpret criterion, but the two others are not intuitive and therefore virtually not used by ophthalmologists. The physiological age-related decrease in ECD has been extensively studied in the past and a mean annual cell loss of 0.6% occurs during adulthood.[3] Nevertheless inter-individual variations of ECD are important. In the present article we studied the link between the age and a morphological analysis of ECs. We propose a method for predicting the age from the endothelial parameters with a view of detecting outliers liable to indicate a specific health status.

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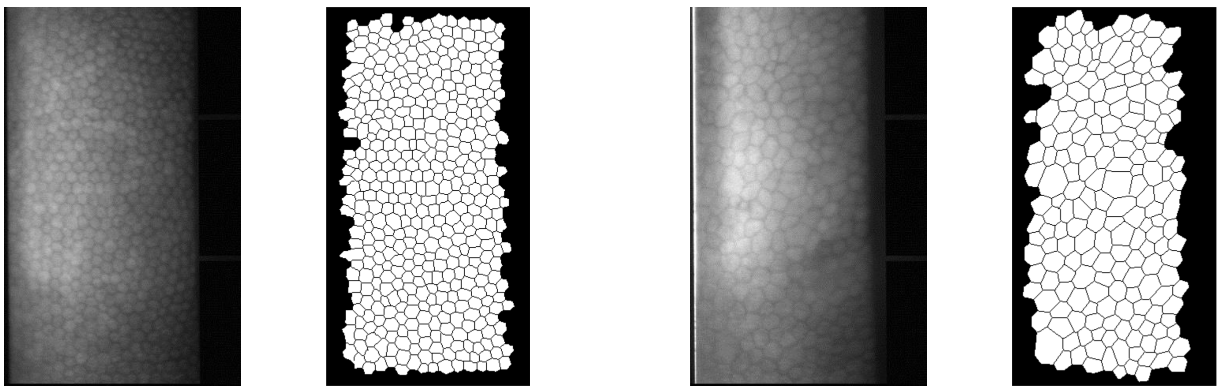
1.2 Corneal endothelium observation and segmentation

The images of the corneal endothelium observations have been taken in vivo using a small field non-contact specular microscope (SP3000, Topcon), see Figs.1. For each decade (0 to 10, 11 to 20, ..., 91 to 100) five patients have been observed. They had no history of corneal disease and benefited from a routine corneal examination. For each patient, five images per eye were taken: one in the central part, and the others at 3 to 4 millimeters from the center in the superior, nasal, inferior and temporal parts of the cornea. A total of 500 images have been manually segmented by an expert ophthalmologist in order to avoid the bias induced by an automatic segmentation methods. The segmentation forms closed cells with thin contours.



(a) Specular image of the corneal endothelium of a 5-year old subject. The cell density is high, and the shapes are regular hexagons.

(b) Manual segmentation of the cells of Fig.1a.

(c) Specular image of the corneal endothelium of a 92-year old subject. The cell density is low and the shapes are irregular.

(d) Manual segmentation of the cells of Fig.1c.

Figure 1: Examples of corneal endotheliums observed by specular microscopy.

2. CHARACTERIZATION OF THE MOSAICS

With some experience, the expert can easily distinguish between normal and pathological endothelial mosaics. In this article, two families of characterizations are presented: the first one will deal with all cells taken separately (called individual cell characterization), and the second one considers the mosaic as a whole (called global mosaic characterization). The measures performed in the first case are the mean ECD and the mean cell perimeter. In the second case, the reduced Minkowski functionals are introduced.

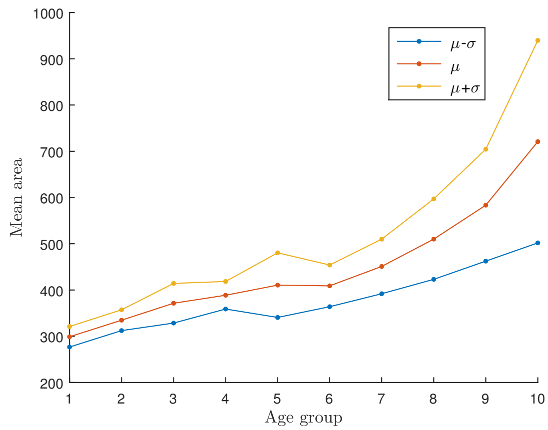
2.1 Individual cell characterization

The statistical distribution of the cells areas and perimeters was previously reported.[4] It logically showed that the mean ECD decreased with age. In addition, the standard deviation was high after 50 years. The perimeter had the same kind of distributions.

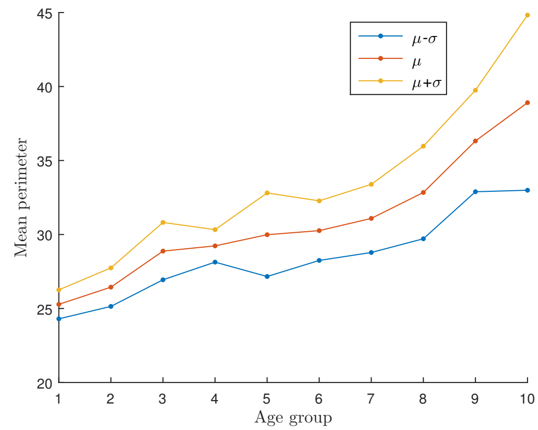
For each age group, the mean and standard deviation is represented in Fig.2. These graphs show that the mean monotonically increases with regard to the age. Roughly speaking, this defines a bijection between the age and the measure, and consequently a specific measurement allows to evaluate an age.

2.2 Global mosaic characterization

The reduced Minkowski functionals[5] are a way to characterize point processes or sets. To perform these measurements in the corneal mosaic, the ECs are simplified to their geometric center, forming a point field. This is mathematically defined as follows.



(a) Area.



(b) Perimeter

Figure 2: Mean (μ) and standard deviation (σ) of the areas and perimeters of the cells per age group.

Let Φ be a finite point field within $W \subset \mathbb{R}^2$ with N points, Φ_r the dilation of Φ by a closed ball b_r of radius $r > 0$. Let $A(r)$, $U(r)$ and $\chi(r)$ be the Minkowski functionals of Φ_r (area, perimeter and Euler number). The reduced Minkowski functionals $a(r)$, $p(r)$ and $e(r)$, are the normalized Minkowski functionals of Φ_r:

$$a(r) = \frac{A(r)}{\pi N r^2} \tag{1}$$

$$u(r) = \frac{U(r)}{2\pi N r} \tag{2}$$

$$e(r) = \frac{\chi(r)}{N}. \tag{3}$$

An illustration of the method is proposed in Fig.3.

The Minkowski functionals are measured for all patients, and the mean value for each age group is presented in Fig.4. These figures clearly show an evolution of each criterion with the age. To quantify this visual evidence, a specific value is introduced. It is a particular radius value defined for each functional (r_a, r_u and r_e for area, perimeter and Euler number, respectively), such that:

$$\forall r \geq r_a, \quad a(r) \leq 0.3, \tag{4}$$

$$\forall r \geq r_u, \quad u(r) \leq 0.3, \tag{5}$$

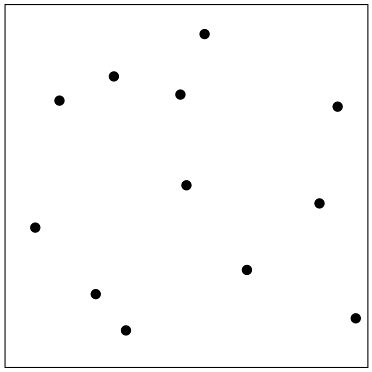
$$\forall r \geq r_e, \quad e(r) = 0. \tag{6}$$

Notice that the value 0.3 is empirically chosen. These values are represented in Fig.5. These graphs show that the mean values are strictly increasing curves. Again, a bijection can be made between the age group and the mean r value, for the three criteria area, perimeter and Euler number. Three other critera are thus defined to evaluate the age of a cornea.

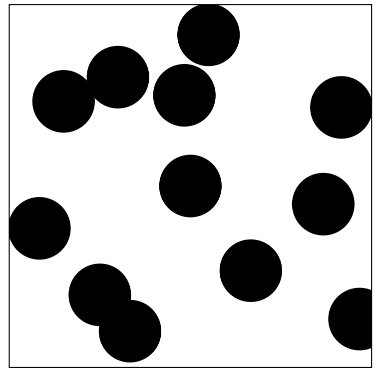
2.3 Evaluation of the corneal age group

So far, five criteria have been presented: mean area and perimeter, and the values r_a, r_u and r_e deduced from the reduced Minkowski functionals. From each criterion, the age group g can be deduced by searching for the closest mean value. Let μ_i and σ_i be the mean and standard deviation of one criterion, for instance the cells density, of the i-th group. Let m be the same criterion measured on one endothelium (the mean value is computed on the 5 images of the same eye). The estimated age group of the endothelium for this criterion is:

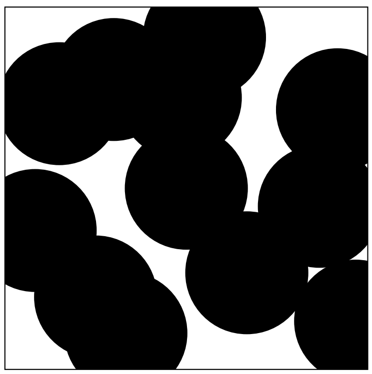
$$g = \underset{i=1,\ldots,10}{\arg\min} |m - \mu_i|. \tag{7}$$



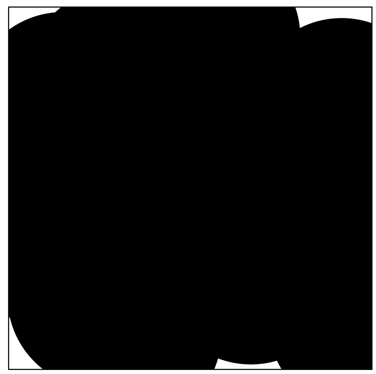
(a) Φ point field.



(b) Φ_r, dilation of Φ with $r = 0.5$.

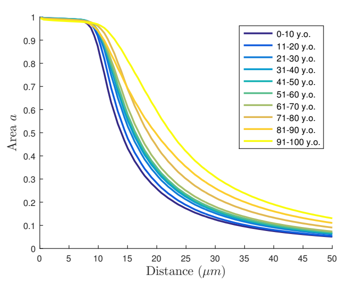


(c) Φ_r, dilation of Φ with $r = 1$.

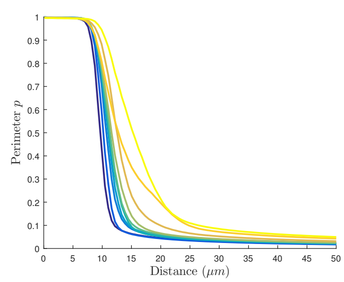


(d) Φ_r, dilation of Φ with $r = 1.5$.

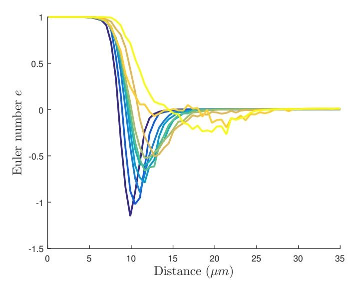
Figure 3: Point field Φ that schematically represents the geometric centers of the cells, and several dilations with different sizes. The reduced Minkowski functionals are performed on the different binary sets.



(a) Reduced area a.



(b) Reduced perimeter u.



(c) Reduced Euler number e.

Figure 4: Mean reduced Minkowski functionals for each age group. The distance in μm is the dilation size.

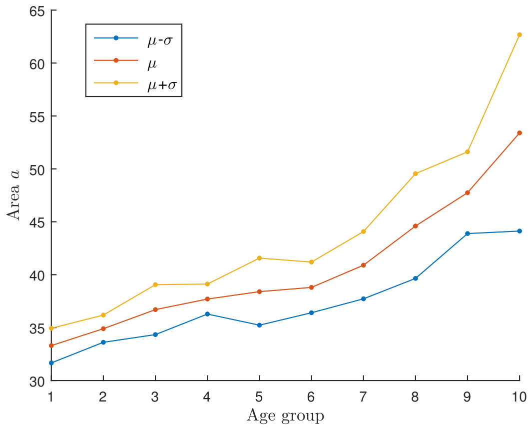
Furthermore, an interval of the age groups to which the endothelium could belong is illustrated in Fig.6 and given by:

$$I = [\min(E), \max(E)] \,, \tag{8}$$

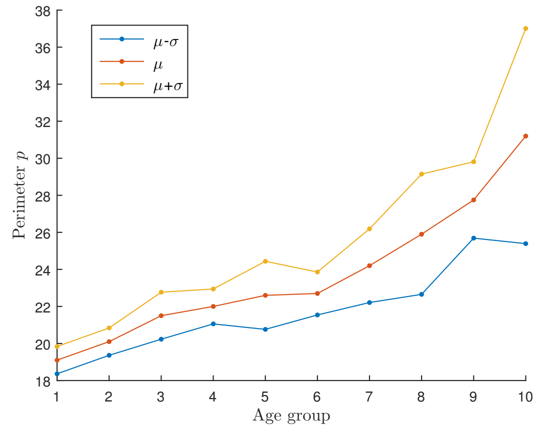
where E is:

$$E = \{i = 1, \dots, 10 \mid m \in [\mu_i - \sigma_i, \mu_i + \sigma_i]\} \,. \tag{9}$$

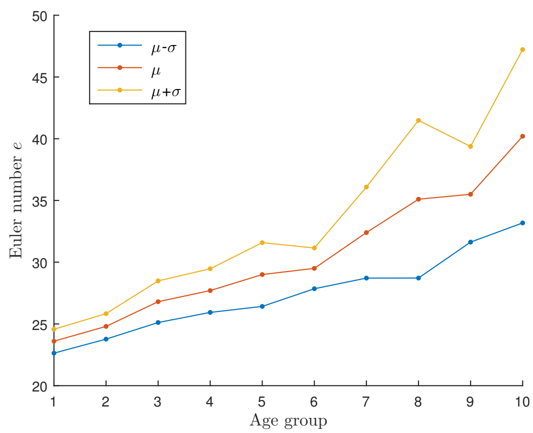
Notice that in Fig.6, the values μ represents a continuous extrapolation of the discrete values μ_i. The representation of these results (the evaluated age g and the age group interval I) is shown as an example (Fig.7) for the reduced Minkowski functionals, and only for the 5th age group. This kind of graph is a bit hard to understand, especially if lots of measures are taken into account.



(a) r_a.



(b) r_u.



(c) r_e.

Figure 5: Mean (μ) and standard deviation (σ) of r_a, r_u and r_e per age group.

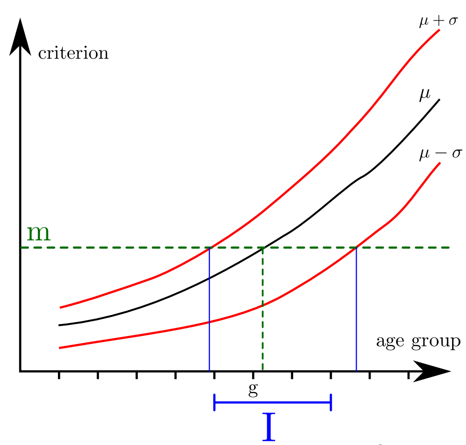


Figure 6: Interval age group definition.

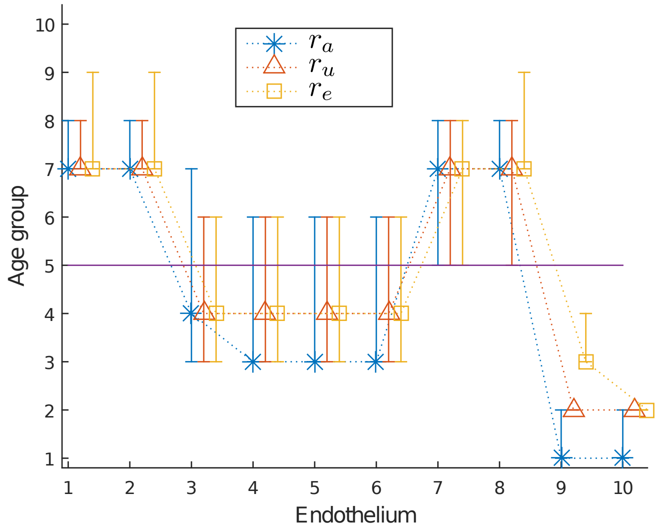


Figure 7: Age group g and interval I of each endothelium the 5th age group according to the criteria r_a, r_u and r_e. There is not specific ordering in the endothelium indices (in abscissa). The age group 5 (in ordinates) should be included in the interval if the measure would have been perfect.

2.4 Probability of age group membership

Let $N = 5$ be the number of evaluation criteria. For each endothelium e and for each age group i, the numbers $n_{g,i}$ and $n_{I,i}$ are defined as follows:

$$n_{g,i} = \sum_{j=1}^{N} \mathbb{1}\{g_j = i\} \tag{10}$$

$$n_{I,i} = \sum_{j=1}^{N} \mathbb{1}\{i \in I_j\} \tag{11}$$

where g_j and I_j are the estimated age group and interval for the j-th criterion ($j \in [1; N]$). For the N criteria (j denotes the j-th criterion), $n_{g,i}$ is the number of times the endothelium e is associated to the age group i, and $n_{I,i}$ is the number of times that the interval I_j contains the age group i.

From these values, a probability of age group membership is constructed. If G_i is the i-th age group ($G_i = [(i-1) \times 10; i \times 10[$ in years), then the probability of the endothelium e to belong to G_i is given by:

$$\mathbb{P}\left[e \in G_i\right] = \frac{1}{(c_g + c_I)} \left(c_a \times \frac{n_{g,i}}{N} + c_I \times \frac{n_{I,i}}{\sum_{j=1}^{N} n_{I,j}} \right), \tag{12}$$

where $c_g = 2$ and $c_I = 1$ are two positive numbers arbitrarily defined such that more weight is attributed to the age evaluation, and less to the interval.

3. RESULTS

The probability of group membership is reproduced in the tables 2, 3, 4 and 5. A color scale is applied to enhance the values (see table 1 for the scale). Only a few groups are represented to simplify the presentation of the results.

Except for the patients between 0-30 years, the variability in ECD and the other measured parameters is high (see standard deviations on Figs. 2 and 5). This simply reflects the reality observed on patients. The

Table 1: Color scale employed in the following tables, in percentage.

0-9%	10-19%	20-29%	30-39%	40-49%	50-59%
	60-69%	70-79%	80-89%	90-99%	100%

Table 2: Probability of age group membership for the 1st age group.

	6 y.o.	6 y.o.	5 y.o.	5 y.o.	4 y.o.	4 y.o.	4 y.o.	4 y.o.	10 y.o.	10 y.o.
0-10	33.3	37.8	100.0	100.0	70.0	52.5	74.2	75.6	100.0	94.4
11-20	43.3	58.5			30.0	47.5	25.8	24.4		5.6
21-30	16.7	3.7								
31-40	3.3									
41-50	3.3									
51-60										
61-70										
71-80										
81-90										
91-100										

characterization method is a good way to reflect the evolution of the corneal endothelium, compared to an average evolution. The prediction of the age was more efficient for extreme ages (younger, 0-20 years old, and older people, 80-100 years old.) than for the rest of the population. Nevertheless, some old patients may appear much younger from a “corneal endothelium point of view”. For example, in the 5-th age group, some patients appear to be classified either in the younger age groups or in the older age groups, denoting a high disparity of evolution of the corneal endothelium.

4. CONCLUSION AND PERSPECTIVES

As it has been previously stated, this article presents a novel methodology for age evaluation of the corneal endothelium. The results are quite good for these five criteria. Some other criteria are under development and evaluation, and it is planned to published these new results, with an analysis between all these criteria. Furthuer analysis will determine whether the other criteria will efficiently help determining the corneal age.

Using the methodology presented in this article, a second series of hundred patients with one central observation in each cornea will be analyzed. It is also planned to perform a long term study (at least for ten years) with a high number of patients (to be determined) that could link the corneal age with other clinically determined ages.

ACKNOWLEDGMENTS

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Table 3: Probability of age group membership for the 5th age group.

	41 y.o.	41 y.o.	49 y.o.	49 y.o.	42 y.o.	42 y.o.	42 y.o.	42 y.o.	43 y.o.	43 y.o.
0-10									24.4	39.2
11-20									54.8	60.8
21-30			7.2	48.8	47.9	34.6			17.0	
31-40			73.9	35.4	34.6	47.9			3.7	
41-50			7.2	8.8	7.9	7.9	7.9	5.6		
51-60			7.2	7.0	7.9	7.9	7.9	5.6		
61-70	51.9	50.3	4.3		1.6	1.6	61.3	62.6		
71-80	38.6	39.5					21.3	22.6		
81-90	7.1	7.7					1.6	3.7		
91-100	2.4	2.6								

Table 4: Probability of age group membership for the 6th age group.

	52 y.o.	**52 y.o.**	**54 y.o.**	**54 y.o.**	**56 y.o.**	**56 y.o.**	**57 y.o.**	**57 y.o.**	**56 y.o.**	**56 y.o.**
0-10										
11-20			47.1	47.1						
21-30		1.5	38.6	38.6	5.6	2.9			19.1	1.6
31-40		3.0	7.1	7.1	46.9	4.3			47.2	1.6
41-50	7.4	20.9	7.1	7.1	33.6	20.6	5.9	7.9	20.6	21.3
51-60	7.4	34.2			6.9	47.2	5.9	21.3	7.2	47.9
61-70	62.6	34.2			5.6	20.6	76.5	47.9	4.3	21.3
71-80	22.6	6.1			1.4	4.3	9.8	21.3	1.4	6.3
81-90							2.0	1.6		
91-100										

Table 5: Probability of age group membership for the 10th age group.

	92 y.o.	**92 y.o.**	**95 y.o.**	**95 y.o.**	**93 y.o.**	**93 y.o.**	**93 y.o.**	**93 y.o.**	**92 y.o.**	**92 y.o.**
0-10										
11-20										
21-30					19.4					
31-40					46.1					
41-50				7.0	7.6	3.9				
51-60				20.4	7.6	3.9				
61-70			2.6	35.4	17.9	36.5				
71-80			18.5	35.4	1.5	49.8				
81-90		5.6	52.8	1.8		3.9				
91-100	100.0	94.4	26.2			2.0	100.0	100.0	100.0	100.0

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