Paper title

A model for corneal endothelial morphometry by diffraction

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Abstract

As a part of an ongoing project on corneal endothelium morphometry by diffraction, a model for corneal endothelium simulation has been developed. The model has been developed in the mathematical programming language Matlab™. Images of corneal endothelium were simulated and the diffraction pattern of the image was calculated. The diffraction pattern was calculated for a series of endothelial images while varying important variables in the simulated image. This rendered the theoretical relationships between values of variables in the diffraction pattern and values of morphometric variables in the image. At this stage, the analysis focused on the expression of endothelial mean cell size and coefficient of variation in the diffraction pattern, respectively. As expected from diffraction theory, it was found that there is a direct linear relationship between mean cell size and distance between periodic variations in the diffraction pattern. We further found that the ratio between the intensity in the central maximum and the intensity in the first harmonic of the diffraction pattern was functionally depending on the variation in cell size. The current findings demonstrate that it is possible to theoretically determine average cell size and coefficient of variation of cell size in the diffraction pattern.

Keywords: cornea, endothelium, morphometry, simulation, diffraction

Introduction

The innermost layer of the cornea is a monolayer of flat, polygonal cells called the endothelium. Well functioning endothelium is vital for maintenance of the optical properties of the cornea. The endothelial cells are non-mitotic [Saude T, 1993]. Any surgical trauma may reduce the density of endothelial cells and thereby threaten the clarity of the cornea. It is therefore important to preoperatively evaluate the corneal endothelium. Average cell size cell, cell size variation and frequency distribution for number of cell corners are frequently analyzed variables in corneal endothelium [Kaufman PL, Alm A, 2003].

Presently, morphometric variables of the corneal endothelium are estimated by Clinical Specular Microscopy (CSM). This technique is tedious. It involves several steps as well as a share of human involvement. An image of a small area of the corneal endothelium is analyzed. This image often needs enhancement [Bourne WM, Kaufman HE, 1976]. The goal of the ongoing project is to develop technology for diagnosis of relevant morphometric variables of the corneal endothelium, using the optical phenomenon of diffraction.

The Fraunhofer diffraction (Far field diffraction) pattern theoretically contains all mean information on the periodicities of a two dimensional structure from which it stems [Hecht E, 2002]. The corneal endothelium has the properties of such a structure. A sampled diffraction pattern from a corneal endothelium holds all morphometric information. An optical system, using coherent and monochromatic light impinging on the cornea, is suggested to render an identifiable diffraction pattern.

The aim of this part of the project was to create a model for determination of the relationships between identifiable variables in the diffraction pattern and morphometric variables in the corneal endothelium. Primarily, we focused on the morphometric variables corneal endothelial; cell density, and coefficient of variation of cell size, respectively.

Methods

The mathematical programming language Matlab™ was used to develop the model. The final goal of the software was to be able to simulate a pattern as similar to the corneal endothelium as possible. This pattern should be readily variable with regard to clinically important morphometric variables in the corneal endothelial structure such as cell density and coefficient of variation of cell size. The purpose of the current paper was to define how measurable variables in the diffraction pattern functionally depend on clinically important morphometric variables. Therefore, also, incrementing iterations had to be included in the software. Further, it was decided to include a possibility to analyze the calculated diffraction pattern.

The important criteria for the software were: 1 - Creation of a pattern as similar to the corneal endothelium as possible. 2 - Determinable variation of pertinent morphometrical variables. 3 - Possibility of repetition and analysis of the patterns.

The endothelial cell structure was created, represented as a specular image in a two dimensional matrix. This was developed in a few steps. Cell coordinates were introduced in the matrix, using trigonometrical properties corresponding to a perfect, unperturbed corneal endothelial cell structure. A user adjustable mean cell size variation parameter was added. Once the cell coordinates where distributed, a random perturbation based on variable normal distribution was introduced, to create a more realistic cell structure. The amplitude of this perturbation was made adjustable. To create the simulated cell barriers, the Voronoi geometrical method was used. Now, the software was able to simulate clinically relevant endothelial cell patterns with variable density.

The next step was to introduce the option to determinably adjust the coefficient of variation of cell size of the corneal endothelium. This was solved by creating the option to introduce cells with deviating size in the structure. The amplitude of the deviating size and the number of

deviating cells was introduced as a user parameter in the software. In this way, it was possible to obtain determinable variation of the corneal endothelium coefficient of variation, in the simulated cell structures.

Below are a few illustrations of the potential of the software regarding the simulation and variation of different morphometric states (Fig. 1). The upper left and right pictures show the possibility to simulate clinically relevant corneal endothelial cell structures with different cell densities. The lower pictures are illustrative examples of the ability to vary the coefficient of variation of corneal endothelial cell sizes. The illustrations are small patches of simulated endothelium.

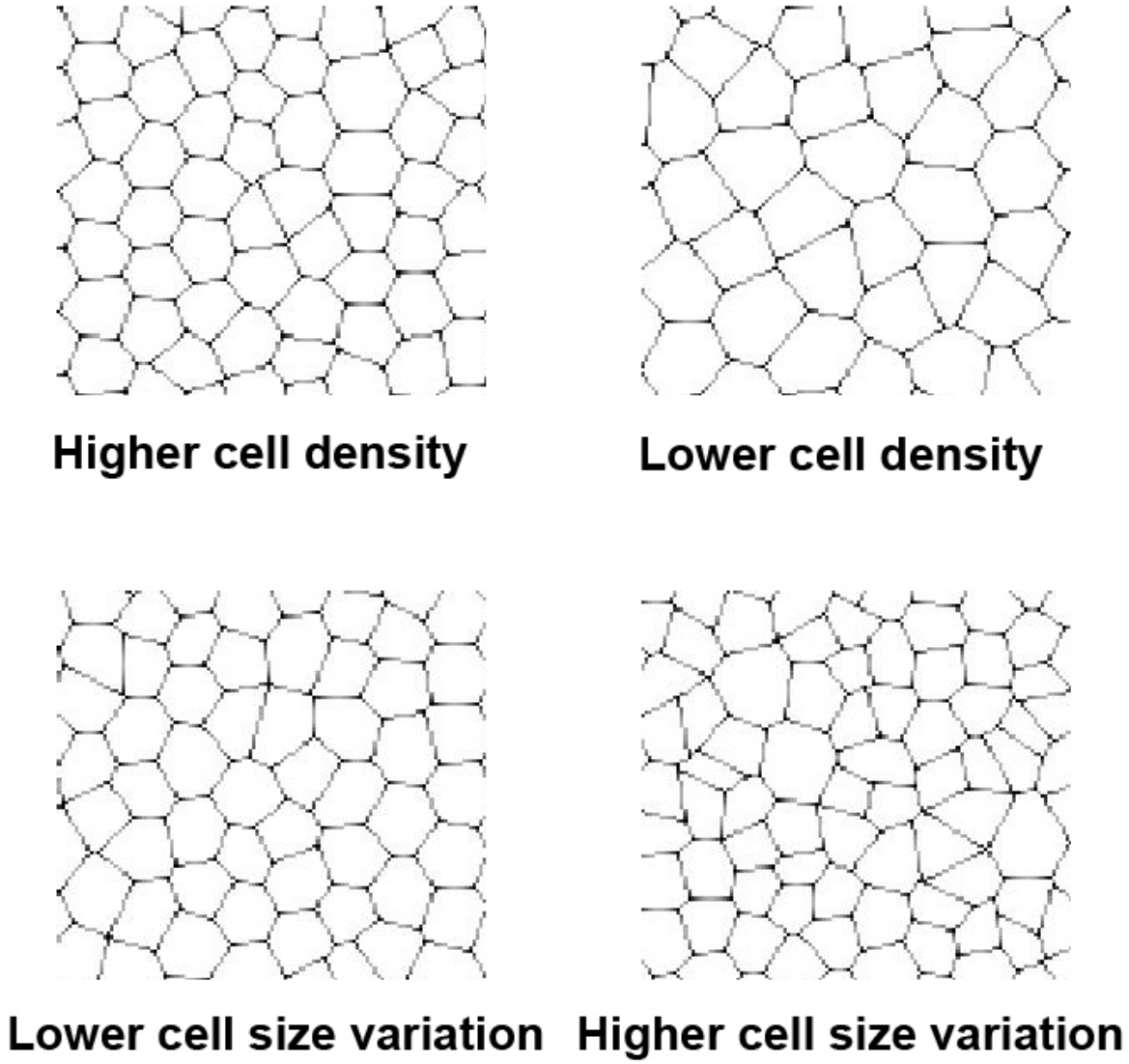

Fig. 1: Examples of patches of simulated corneal endothelium. Upper images are examples of varying cell density. Lower left and right pictures are examples of varying cell size variation/coefficient of variation of cell size.

The next step in the modeling was to simulate the diffraction pattern corresponding to the simulated corneal endothelial cell structure. This was done by numerical Fourier

transformation of the two dimensional matrix. The Fourier transform yields a diffraction pattern identical in amplitude distribution to the irradiance distribution of the Fraunhofer diffraction.

The Fourier transform was output in another two dimensional matrix in which each coordinate contained a value in the amplitude distribution. An example of an arbitrary simulated corneal endothelial structure and its Fourier transform is depicted below (Fig. 2).

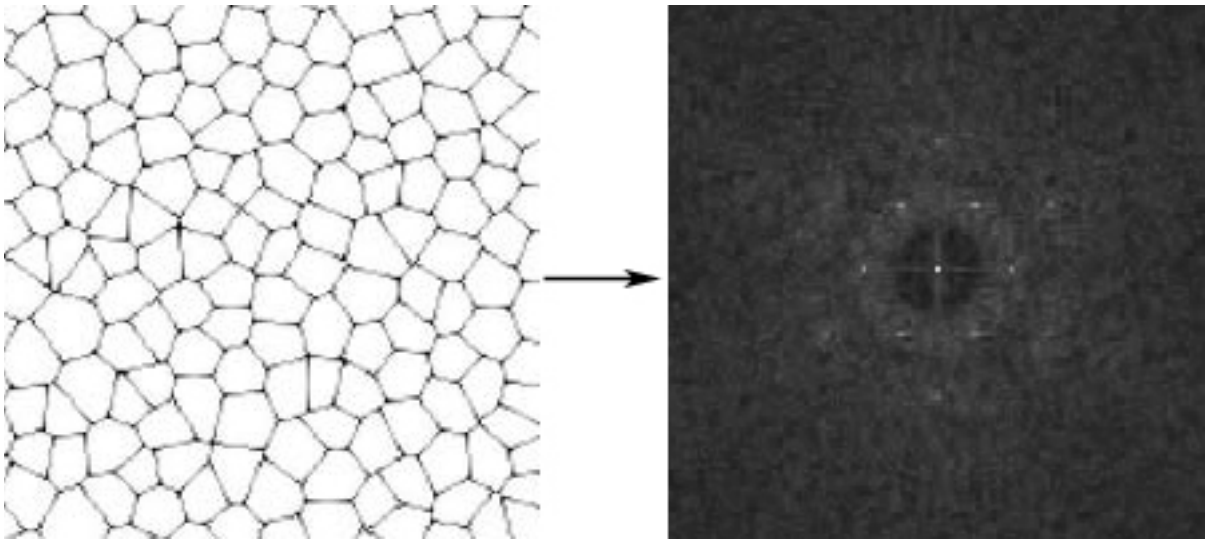

Fig. 2: To the left, a patch of a simulated corneal endothelial structure. To the right, the numerical Fourier transform of the left structure. (Note: The amplitudes in the Fourier transform structure have been slightly edited solely for illustrative purposes)

The subsequent step was to analyze the information contained in the diffraction pattern and to identify relationships between variables in the diffraction pattern and morphometric variables of the simulated corneal endothelial cell structure. Tools were created for the purpose of extraction and analysis of relevant information from the amplitude distribution created with the numerical Fourier transform.

When these tools were available, a large number of simulations were performed and relevant diffraction pattern characteristics were extracted for analysis. The main focus in this part of the project was to find theoretical relationship pertinent to corneal endothelial cell density, and coefficient of variation of cell size, respectively. The simulations were performed, successively varying these quantities.

The diffraction pattern characteristics of greatest interest were the relative amplitudes and positions of the zero'th order peak and the first harmonic of the distribution.

Results

Preliminary simulations showed some theoretically expected results. Only mean cell size effects the distance from the transforms center peak to its first harmonic. A higher coefficient of variation of cell size tends to smear out the subsequent harmonics, lowering their peak amplitudes, making for a greater amplitude ratio between the center peak and the first harmonic.

The relationship between identifiable diffraction pattern characteristics and the cell density was expected from diffraction theory. The radius of the first harmonic, in the amplitude distribution of the diffraction pattern, is first degree linear inversely related to the mean periodical from which it stems. The mean periodical in this case was the mean cell size of the simulated corneal endothelial structure. This was confirmed in the simulation (Fig. 3).

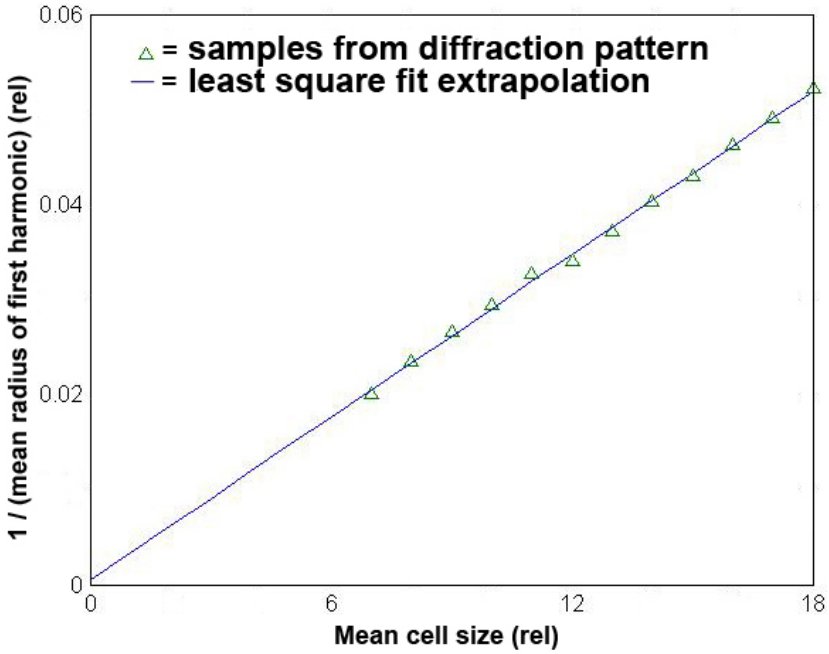

Fig. 3: The first degree linear relationship between corneal endothelial mean cell size and the first harmonic mean radius of the diffraction pattern.

Figure 3 is an illustrated example of samplings from a range of simulated monolayers of corneal endothelial cells with successively decrementing cell density. The horizontal axis shows the mean cell size of the simulated structure. This is the same as the inverse of the cell density. The vertical axis shows the inverse of the mean radius of the first harmonic in the diffraction pattern. The straight line is a least square fit of a first degree polynomial.

Next, the relationship between variation of corneal endothelial cell size and the identifiable characteristics of the diffraction pattern of the cell structures was examined. For this, the endothelial pattern was simulated first with all cells being set to similar size and then successively increasing the fraction of cells with a deviating cell size. The deviation was the same for all changed cell sizes. The mean cell size was kept constant during the whole process. The variable expressing variation of endothelial cell size thus obtained is here called Variation of Endothelial cell Size (VES). In the calculated diffraction pattern, the ratio of the center peak amplitude and the first harmonic peak amplitude of the diffraction pattern, here called *diffraction characteristic* was analyzed.

An example of the functional dependence of the diffraction characteristic, on VES is illustrated to the left in Fig. 4.

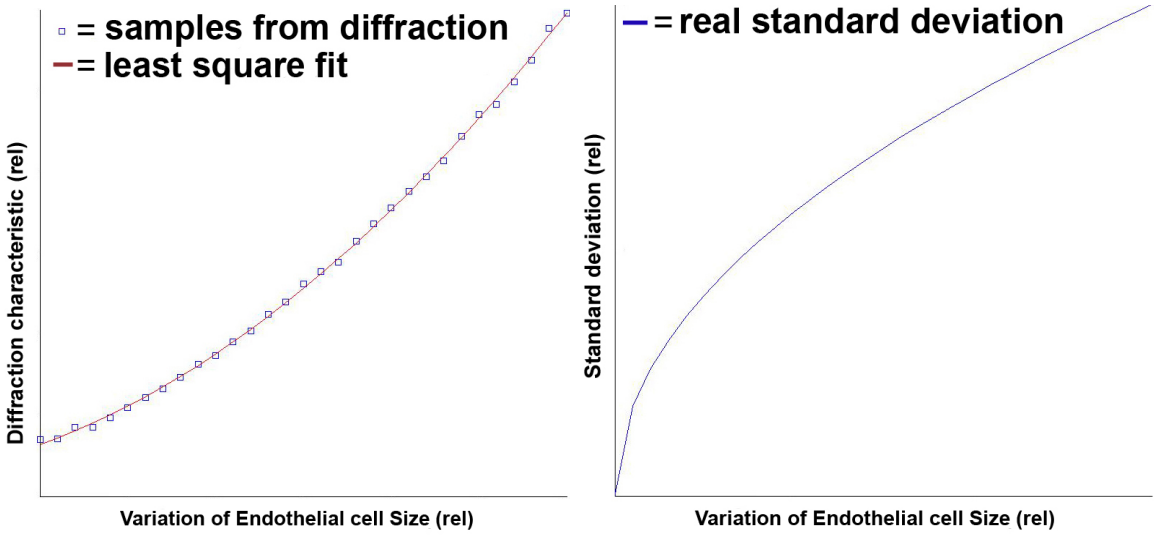

Fig. 4: To the left, the relationship between the diffraction characteristic for variation of endothelial cells (ratio of the center peak amplitude and the first harmonic peak amplitude of the diffraction), and the variation of cell size. To the right, the calculated standard deviation for variation of endothelial cells as a function of variation of endothelial cell size.

It is seen (Fig. 4) that the dependence of the diffraction characteristic on VES can be approximated to a second order polynomial. On the right in Fig. 4 is plotted the standard deviation for corneal endothelial cell size as a function of VES. The standard deviation is in a direct relationship to the coefficient of variation of endothelial cell size.

With a simple modulation of the second order polynomial dependence of the diffraction characteristic on VES, the modulated function (Fig. 5) can be made close to identical to the dependence of the standard deviation for corneal endothelial cell size on VES (Fig. 4, right). This demonstrates that it is possible to use a modulation of the diffraction characteristic to express standard deviation and thus the coefficient of variation of endothelial cell size.

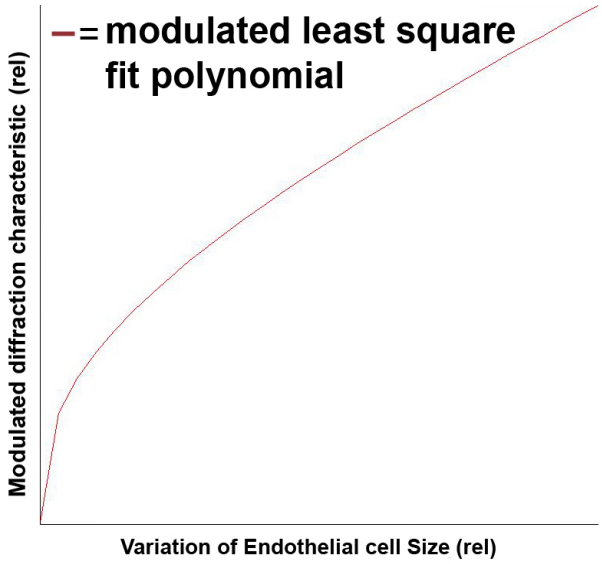

Fig. 5: The modulated polynomial from the left picture in figure 4.

Discussion

The purpose of the current study was to create a model for determination of the theoretical relationship between the diffraction pattern of the corneal endothelium and morphometric quantities of the corneal endothelium. Software capable of simulating clinically relevant corneal endothelial structures was developed. The software was used to determine such theoretical relationships pertaining cell density and the coefficient of variation of the corneal endothelium.

The theoretical relationship between the radius of the first harmonic of the diffraction pattern and the cell density was already known through diffraction theory and was confirmed in the current simulations (Fig. 3). The relationship between the amplitude ratio extracted from the diffraction pattern and VES turned out to be a second order polynomial. A simple mathematical modulation of this relationship rendered a function almost identical to the standard deviation for variability of cell size as a function of VES. Therefore, a modulation of the diffraction characteristic can be used to determine the standard deviation and hence the coefficient of variation for variability of cell size.

The relationships that were established will be used in in vitro calibration processes in a later stage of the project. As the diffraction pattern theoretically contains all information on the structure from which it stems, information on other morphometrical quantities are also waiting to be identified.

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