Fully automated corneal endothelial morphometry of images captured by clinical specular microscopy

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Abstract

The corneal endothelium serves as the posterior barrier of the cornea. Factors such as clarity and refractive properties of the cornea are in direct relationship to the quality of the endothelium. The endothelial cell density is considered the most important morphological factor. Morphometry of the corneal endothelium is presently done by semi-automated analysis of pictures captured by a Clinical Specular Microscope (CSM). Because of the occasional need of operator involvement, this process can be tedious, having a negative impact on sampling size. This study was dedicated to the development of fully automated analysis of images of the corneal endothelium, captured by CSM, using Fourier analysis. Software was developed in the mathematical programming language Matlab. Pictures of the corneal endothelium, captured by CSM, were read into the analysis software. The software automatically performed digital enhancement of the images. The digitally enhanced images of the corneal endothelium were transformed, using the fast Fourier transform (FFT). Tools were developed and applied for identification and analysis of relevant characteristics of the Fourier transformed images. The data obtained from each Fourier transformed image was used to calculate the mean cell density of its corresponding corneal endothelium. The calculation was based on well known diffraction theory. Results in form of estimated cell density of the corneal endothelium were obtained, using fully automated analysis software on images captured by CSM. The cell density obtained by the fully automated analysis was compared to the cell density obtained from classical, semi-automated analysis and a relatively large correlation was found.

Keywords: cornea, endothelium, Fourier, morphometry, morphology, microscopy

1. INTRODUCTION

The corneal endothelium serves as the posterior barrier of the cornea. Factors such as clarity and refractive properties of the cornea are in direct relationship to the quality of the endothelium. The endothelial cell density is considered the most important morphological factor of the corneal endothelium. Pathological conditions and physical trauma may threaten the endothelial cell density to such an extent that the optical properties of the cornea and thus clear eyesight is threatened. It is therefore important to preoperatively evaluate the corneal endothelium. Average cell size, cell size variation and frequency distribution for number of cell corners are frequently analyzed variables in the corneal endothelium[1]. In this project, focus has been set on cell density, considered the clinically most important morphological variable of the corneal endothelium.

Presently, the morphology of the corneal endothelium in vivo is most commonly estimated by the use of Clinical Specular Microscopy (CSM)[2]. Because of the occasional need of operator involvement this process can be tedious, having a negative impact on sampling size. Depending on the patient and the particular CSM used, the number of cells in one analyzed image is usually in the pro-mille range of the total number of cells of the endothelium. The semi-automated analysis used for comparison on this project is the Image-net retrace algorithm (Topcon). The Image-net method of analysis is based on a computer enhancing the captured image of the corneal endothelium. After the digital enhancement,

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the computer estimates the location of the cell barriers, based on light and contrast variations. The size of each barrier-enclosed area is then calculated, giving the size of each cell. This way, the cell density of the corneal endothelium can be calculated. However, the cell barrier identification is not always correct and this is where an operator has to intervene. An example of the semi-automated analysis is shown in the figure below.

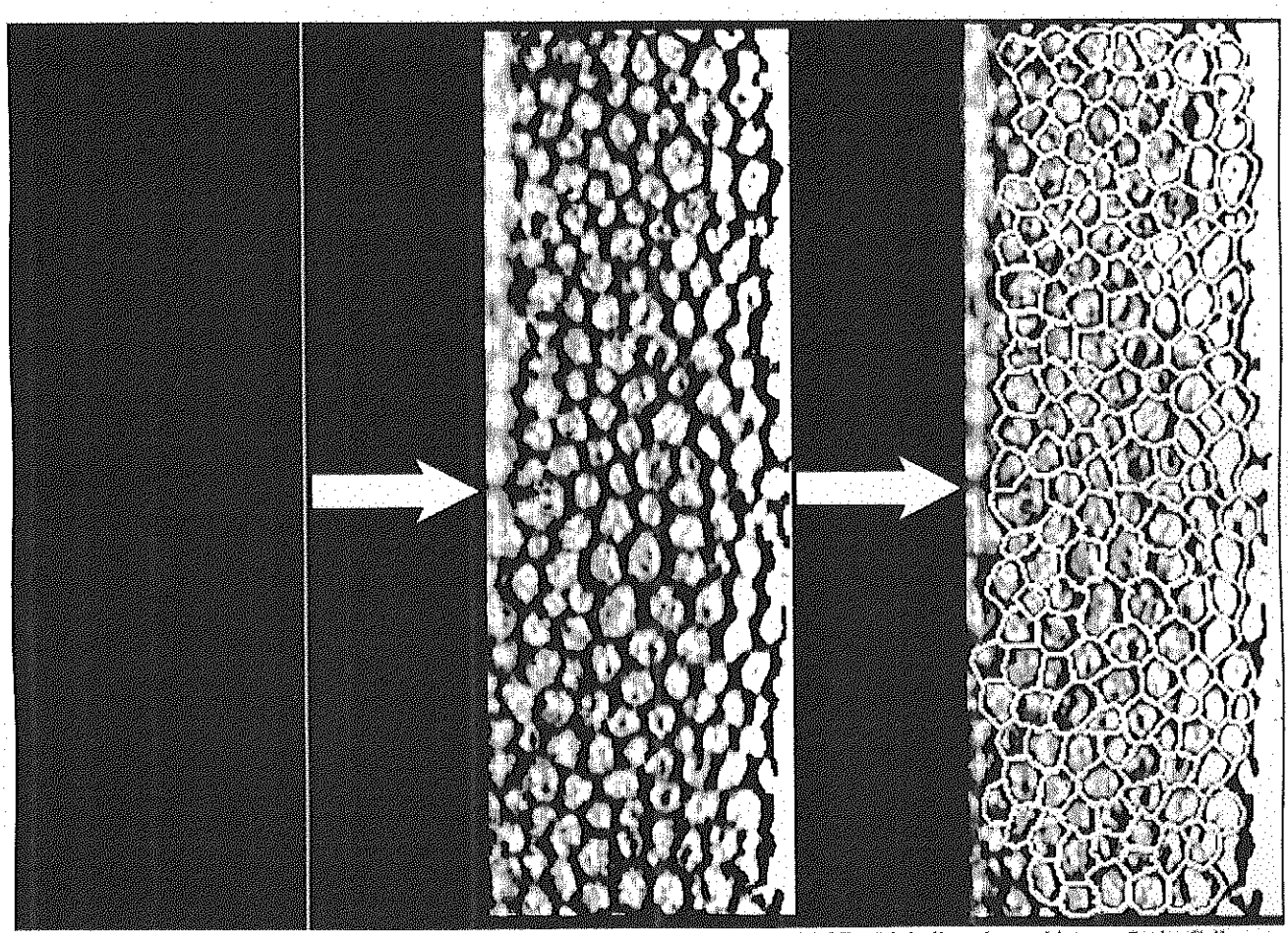

Fig. 1: Semi-automated analysis of the corneal endothelium. *Left*: Original image. *Middle*: Digitally enhanced image. *Right*: Cell barriers identified.

The semi-automated method of analysis has strengths and weaknesses. The strength of the method is that once the cell barriers have been identified, the margin of error in the analysis is relatively small. Because of the direct access to the cell shapes in the time domain, calculations of other interesting morphological variables such as cell size variation and hexagonality are readily available. The weakness of the semi-automated method is that it often requires time consuming operator involvement, resulting in a localized and relatively small sample size.

The aim of this study was to examine the possible benefits from analyzing the image data of the corneal endothelium, using the fast Fourier transform (FFT). The FFT outputs the frequency distribution of the transformed data[3] by mathematically transforming data from the time domain to the frequency domain. In this case, the subjects of transformation were automatically digitally enhanced images of the corneal endothelium, captured by CSM.

2. METHODS

Images of non-pathological corneal endothelium of 16 patients were captured by CSM and analyzed by classical semi-automated means. The original, non-enhanced images and their corresponding cell density data were transferred to the computer used for the fully automated analysis.

The FFT is one dimensional and for the transform of two dimensional images, such as the ones captured by CSM, the FFT algorithm has to be applied twice, such as FFT((FFT(image)^T). This is already incorporated in Matlab as FFT2 and this will be the term used from now.

For the sake of later analysis of the frequency distribution, input images for the FFT2 were selected as square. The original images captured by the CSM are not square, but rectangular (Fig. 1), so this had to be accounted for. This was solved by the creation of an algorithm extracting overlapping square parts of the rectangular original CSM image of the corneal endothelium. Extracting several overlapping square fragments of the original rectangular image had double use. Not only does a square image give a frequency distribution which is easier to analyze in this case, but by adding many results from overlapping parts of one image, deviating data due to regions of poor analyzability can be filtered out. Extraction of square elements from the original rectangular CSM image is illustrated below.

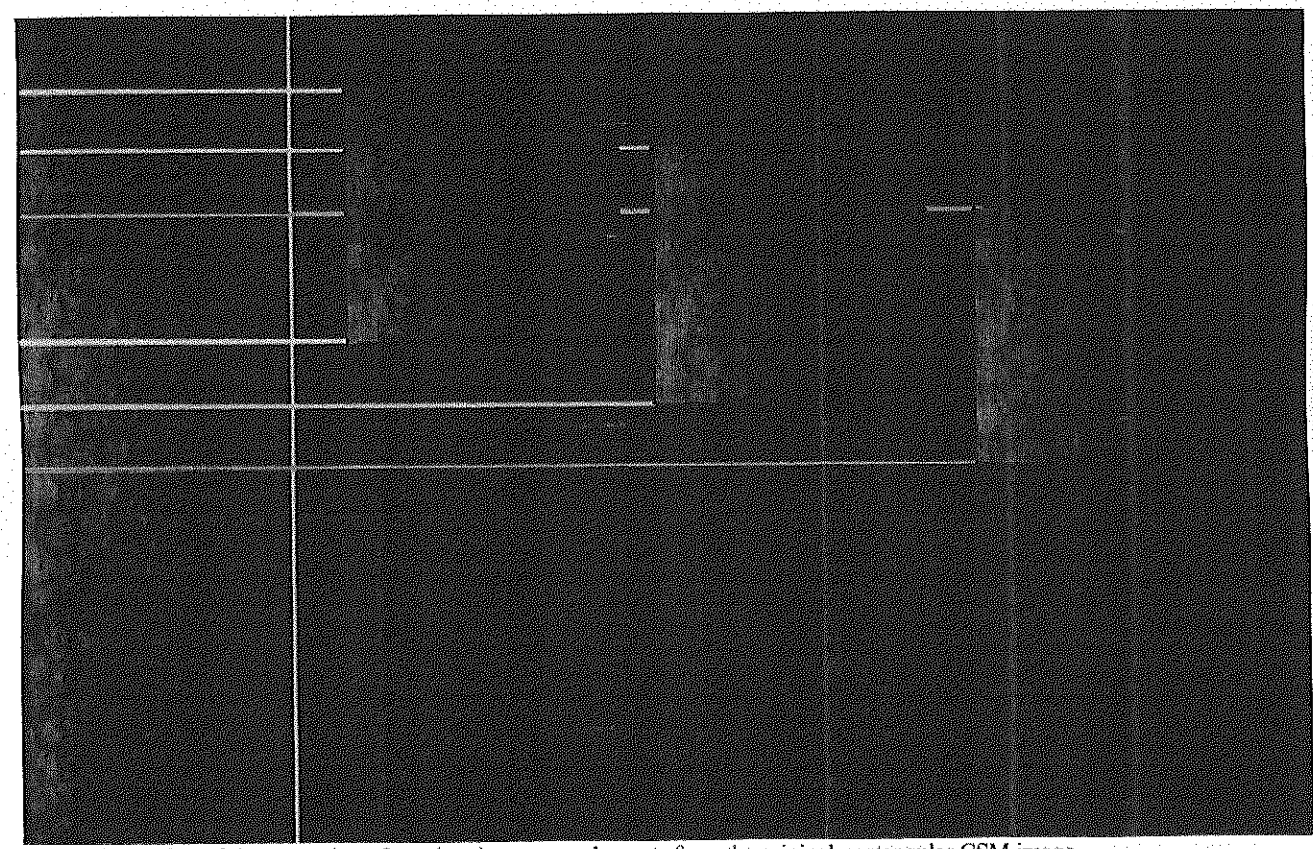

Fig. 2: Illustration of the extraction of overlapping square elements from the original, rectangular CSM image.

Several overlapping square elements from the same original CSM image of the corneal endothelium were extracted and saved. Each element (Fig. 3a) underwent a series of automated digital enhancement. The first algorithm normalizes the light distribution of the image by applying a high bandpass filter. With some imaginative use of absolute values, the same algorithm also filters out some of the high frequency noise from the original image (Fig. 3b).

In the next step of the automated digital image enhancement, a mid/lowpass filter is applied. This is done by a combination of down sampling and nearest neighbor algorithms. This filter is applied for getting rid of the remaining high frequency noise in the image (Fig. 3c).

The last step in the automated digital enhancement was to binarize to image (Fig. 3d). This was done by setting a threshold value of the numeric elements in the matrix composing the image. Anything above threshold would equal a 'one', and anything below would equal a 'zero'. Using a binary image as the FFT2 input was not chosen because of any anticipated improvement of the resulting data of any individual image, but rather for the sake of comparison between images. An illustration of the course of the automated digital image enhancement process is given below.

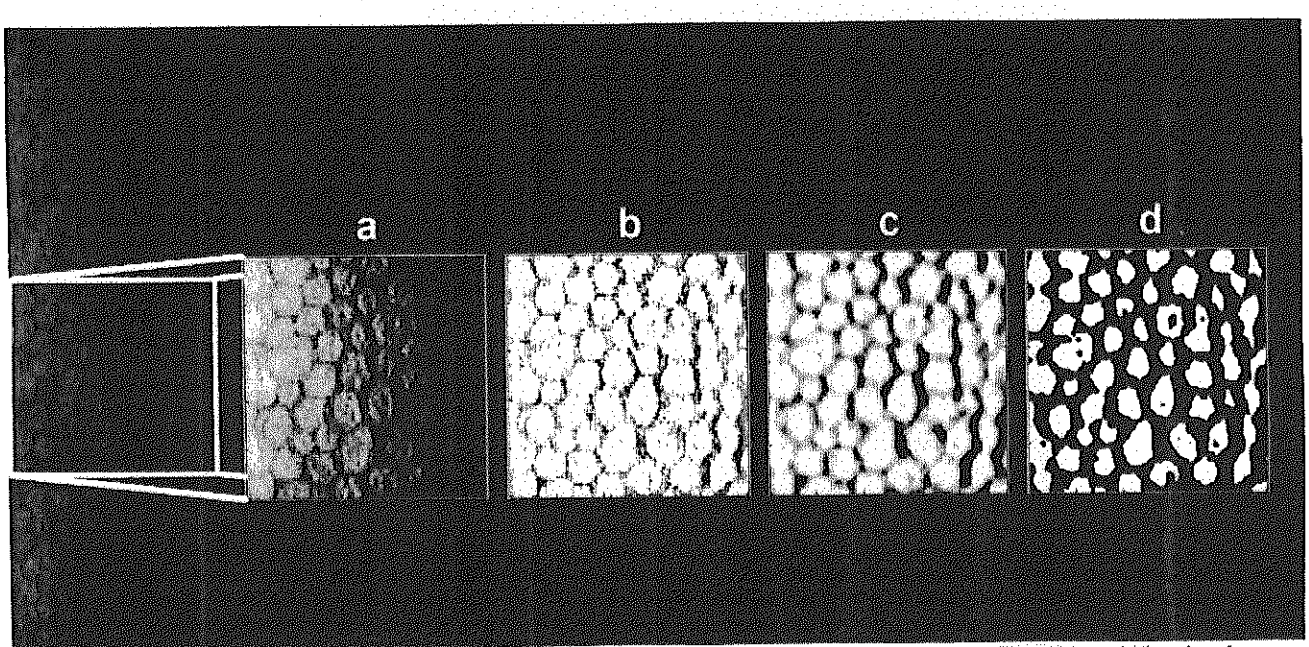

Fig. 3: a) Extracted square image element. b) Normalized and slightly noise filtered element. c) Noise filtered by mid/low bandpass and nearest neighbor algorithms. d) Binary image element.

After the automated digital enhancement, each of the extracted square images were Fourier transformed, using FFT2 (Fig. 4). Each square image element rendered a new image, showing the frequency distribution of the original image element. Analysis tools were developed for identification and interpretation of relevant amplitude characteristics in the frequency distributions. Each frequency distribution was radially sampled from its center, meaning zero frequency, to a frequency corresponding to periodicity in the picture smaller than any realistic cell, thus covering all frequency information generated by the cells in the image. The radial sampling was performed on a 180° arc of each frequency distribution image (Fig. 5). A sampling on 360° would have given the same result, as the frequency distributions are radial symmetric. Figure 4 and 5 below shows the Fourier transform being applied on the enhanced cell image element, as well as the radial sampling of the resulting frequency distribution.

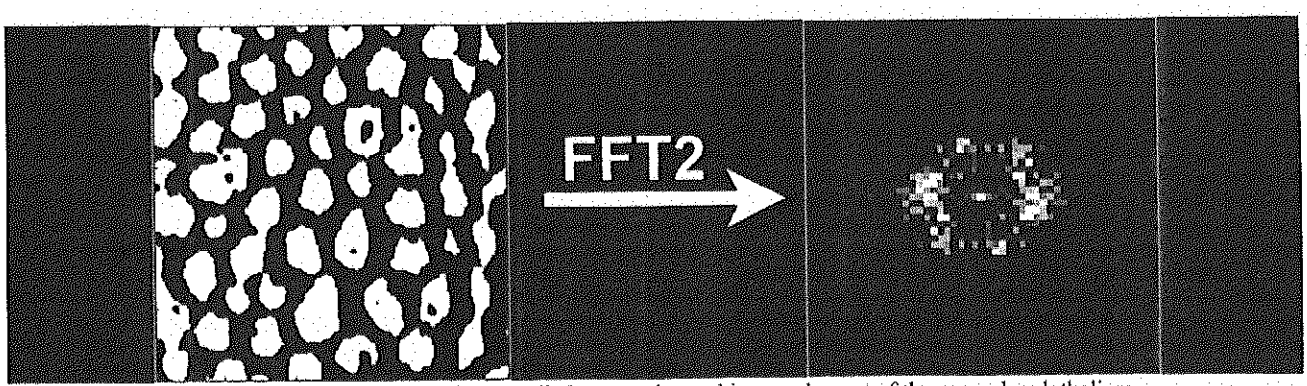

Fig. 4: The two dimensional fast Fourier transform applied to an enhanced image element of the corneal endothelium.

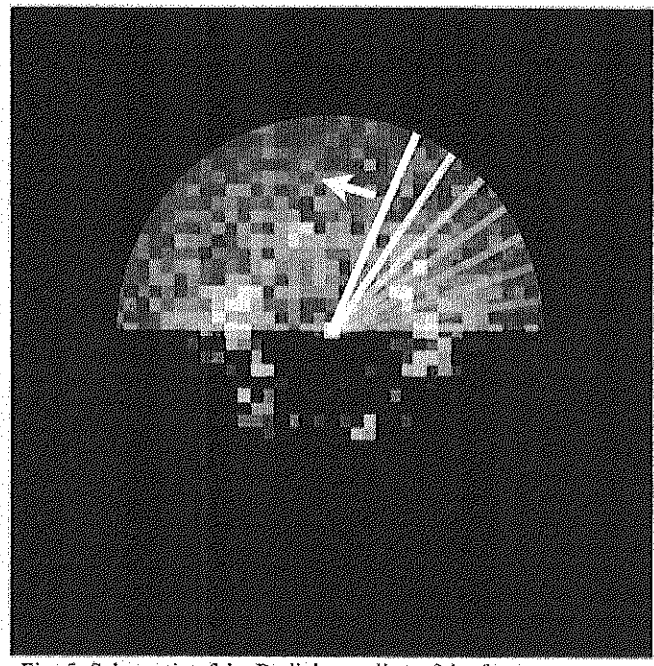

Fig. 5: Schematic of the Radial sampling of the frequency distribution.

To get all possible information out of the radial symmetric frequency distribution, all coordinates of a 180° circumference were used to create sample cuts with the center coordinate (Fig. 5). The cuts were spline interpolated for higher resolution of the extracted data. Knowledge of the structure of the endothelium and the FFT2 coupled with the fact that several unique data sets for each picture were used, allowed for the use of the spline interpolation. Each cut would represent a two dimensional amplitude profile, where the amplitude variations corresponded to frequency prevalence. The absolute mean value (V) of the $(R \cdot \pi)$ radial samples, where R is sampling radius, was calculated for each frequency distribution image.

In the existing literature on using Fourier transform for corneal endothelial morphometry, much larger cell sampling sizes are used. In those cases, simply finding the first order peak of the frequency distribution is enough to get a good estimate on the cell density. In the case of square images extracted from narrow slit CSM images, the number of cells and thus the resulting frequency resolution is too small for such analysis. A dedicated analysis algorithm for finding the mean frequency and thus the square root of the mean cell density of smaller cell number samplings was developed and applied to V. The algorithm used integration over weighed frequency amplitudes. This was done to each square element extracted from the original rectangular CSM image of the corneal endothelium. The mean was taken for every estimated value of the cell density of each square element (Fig. 3a), giving an estimated value of the cell density of the original image of the corneal endothelium. This value was calculated and squared for all 16 CSM images of a non-pathological corneal endothelium.

The final estimation of cell density of the 16 images was normalized and compared to the corresponding value known from the semi-automated analysis. Regressive analysis of the fully automated results as a function of the semi-automated results was performed and plotted, using Matlab.

3. RESULTS

The automated process of digitally enhancing the original images proved to be well functioning. The algorithms proved rugged enough for normalized results from original images of varying quality of light and contrast. The first enhancement algorithm (Fig. 3a to 3b), a high/mid bandpass filter with an added part for filtering some of the high frequency noise, proved particularly suitable for cleaning up the original image.

The individual estimated cell density result from analysis of the FFT2 created frequency distribution did not vary noticeably between using the cleaned up image (Fig. 3c) and the binary image (Fig. 3d). However, comparing estimated corneal endothelium cell density between images was made easier using the binary image. It was also anticipated that tries on cell images with higher deviation in terms of light profiles and contrasts might benefit from using both the cleaned up and the binary image, matching the results as a control criteria.

The use of the two dimensional fast Fourier transform on the binary images resulted in frequency patterns expected from diffraction theory. The extracted radial data cuts over a relevant radius from the frequency distributions and the average of those cuts are shown in the figure below.

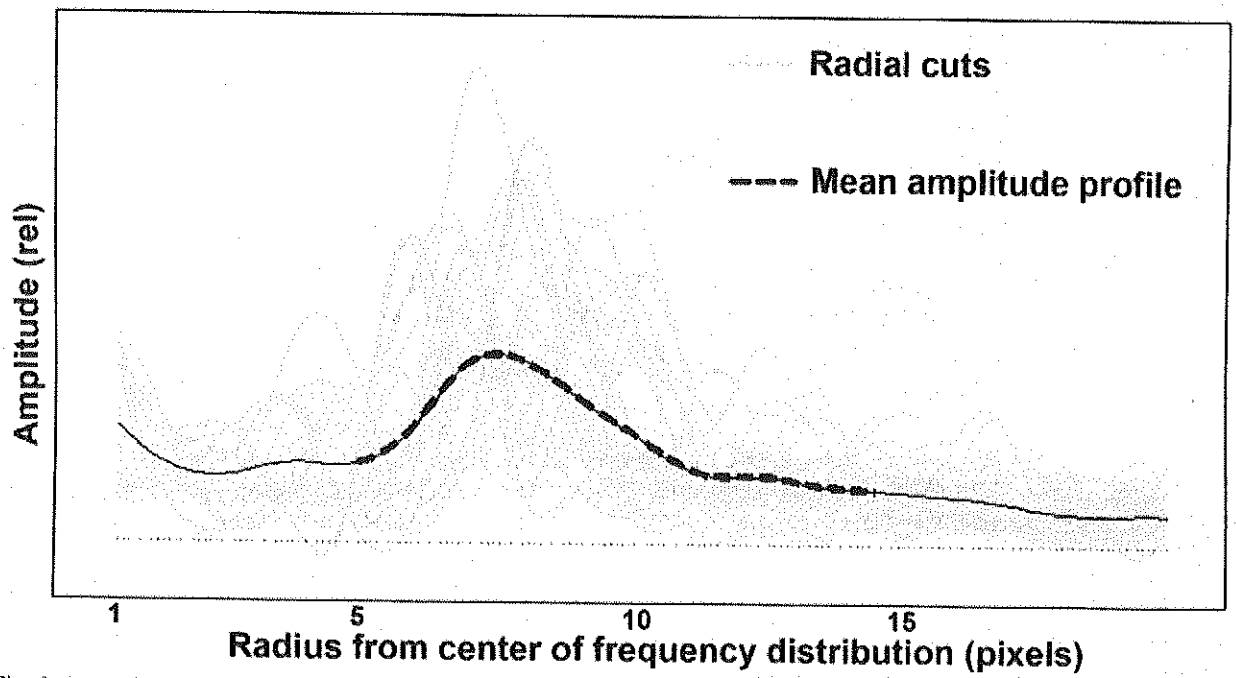

Fig. 6: A set of radial cuts extracted from a frequency distribution and the mean value of those cuts (- -).

The mean dataset (Fig. 6) extracted from the frequency distributions, squared, gave an estimation of the endothelial cell density of the extracted square element (Fig. 2) of the original corneal endothelium CSM image. The estimated values of each overlapping square element from one original image were averaged and a final estimated value of the cell density for that image was found.

The estimated cell densities from the fully automated analysis of the 16 images obtained was compared to the corresponding values obtained by classic, semi-automated analysis. According to the semi-automated analysis, the 16 non pathological CSM images ranged over a corneal endothelium cell density interval from a minimum of 2227 cells/mm^2 and a maximum of 3472 cells/mm^2. The average cell size of the 16 tested images was ~360 μm^2 according to the semi-automated analysis. The residual standard deviation in mean cell size, comparing the fully automated cell density estimate to the semi-automated estimate was found to be ~22.3 μm^2, which corresponds to a dataset mean cell size deviation of ~6.2 %. The comparison is plotted in the figure below.

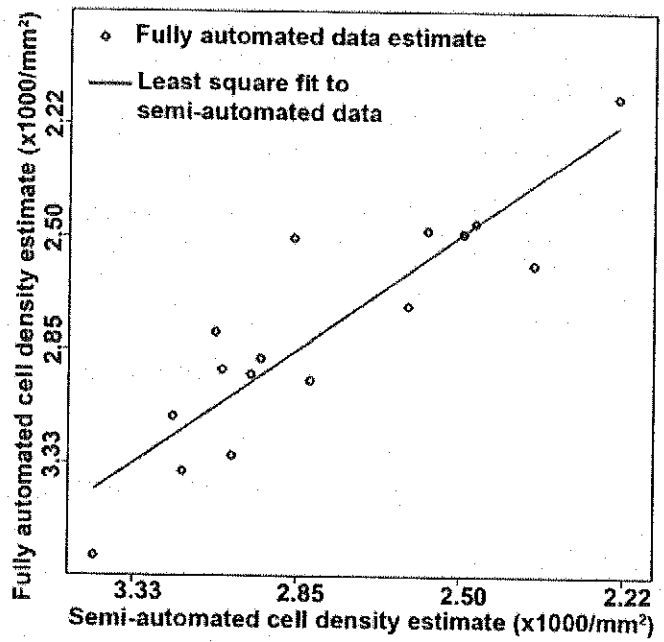

Fig. 7: Fully automated cell density estimate as a function of the semi-automated cell count (cells/mm^2).

4. DISCUSSION

The strengths and weaknesses of the classic semi-automated morphometry of the corneal endothelium are rather clear. It is a reliable and relatively error free way to get accurate estimates of several interesting morphological variables, but at the cost of time.

The use of Fourier analysis for estimating the cell density of the corneal endothelium is in no way new and there are many articles on its possible use in endothelial morphometry[4,5,6]. The use of Fourier analysis as a fast way to find primarily cell density estimates of the corneal endothelium is promising, due to the speed and its inherent robustness. So far, Fourier analysis has predominately been applied to models[7] or larger samples of endothelial cells such as images from corneal donor buttons, resulting in higher quality images and larger sample sizes[8].

A higher number of cells, better quality of cell images and clear models favors the Fourier transform, because a larger number of cells means a higher sampling frequency, which in turn means higher resolution in the frequency domain. This was one of the challenges and incentives in performing this study. Images taken with a narrow slit clinical specular microscope holds rather few cells. Applying the typical way of analysis of the frequency distribution, by simply finding the first order peak, will not work with this few cells. There might be a lot of cells of size A, and the rest of size 2A. The first order peak will be situated at a frequency corresponding to either A or 2A, while the actual mean cell size is somewhere in between. With a large number of cells, e.g. an image of the corneal endothelium of a donor button, the normal or near normal distributed cell sizes makes it so that the frequency domain first order peak will be at, or close to, the actual mean cell size. With a smaller sampling size, e.g. the images from CSM microscopes, it is not longer clear that the cells we sample are normal or near normal distributed in size.

The problem with Fourier analysis on small sampling sizes needed a solution. The solution in this study, was to measure the whole range of frequencies corresponding to plausible cell sizes. This range was then weighted and from integrating over the weights, an estimate of the mean frequency corresponding to the square root of the mean cell size was found. It could be of future interest to see at what number of sampling cells one has to disband the classical way of frequency distribution analysis for the corneal endothelium.

With a relatively small data set of 16 non-pathological images, this study would mainly serve as an indication of the possibilities of using Fourier analysis for corneal endothelial morphometry in images captured by CSM. Yet, seeing that

it is fully automated and operated by the push of one button and that the residual standard deviation was not larger than 6.2 % compared to the semi-automatic analysis of the cell density, the result should be considered satisfying. There are indeed reasons to continue the pursuit of fully automated Fourier transform based morphometry of the corneal endothelium.

One thing to note and to keep in mind when interpreting the result is that in this study, the semi-automated analysis data is considered as error free. This is most likely not the case. Even with the Image-net border finding method and manual operator involvement, the cell density estimate is likely to deviate from the actual state of the examined corneal endothelium.

Future development of this project consist of optimizing the case-specialized frequency distribution analysis algorithm and to extend the test group.

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