# 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 of the corneal endothelium. Pathological conditions and physical trauma may threaten the endothelial cell density to such an extent that the optical property of the cornea and thus clear eyesight is threatened. Diagnosis of the corneal endothelium through morphometry is an important part of several clinical applications. Morphometry of the corneal endothelium is presently carried out 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 and use of fully automated analysis of a very large range 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, normalizing lights and contrasts. The digitally enhanced images of the corneal endothelium were Fourier transformed, using the fast Fourier transform (FFT) and stored as new images. 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 292 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 are 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,

Ophthalmic Technologies XX, edited by Fabrice Manns, Per G. Söderberg, Arthur Ho, Proc. of SPIE Vol. 7550, 75501E · © 2010 SPIE · CCC code: 1605-7422/10/$18 · doi: 10.1117/12.849255

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 sub-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 barrier 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, 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 seldom correct and this is where an operator has to intervene. An example of the semi-automated analysis is shown in the figure below.

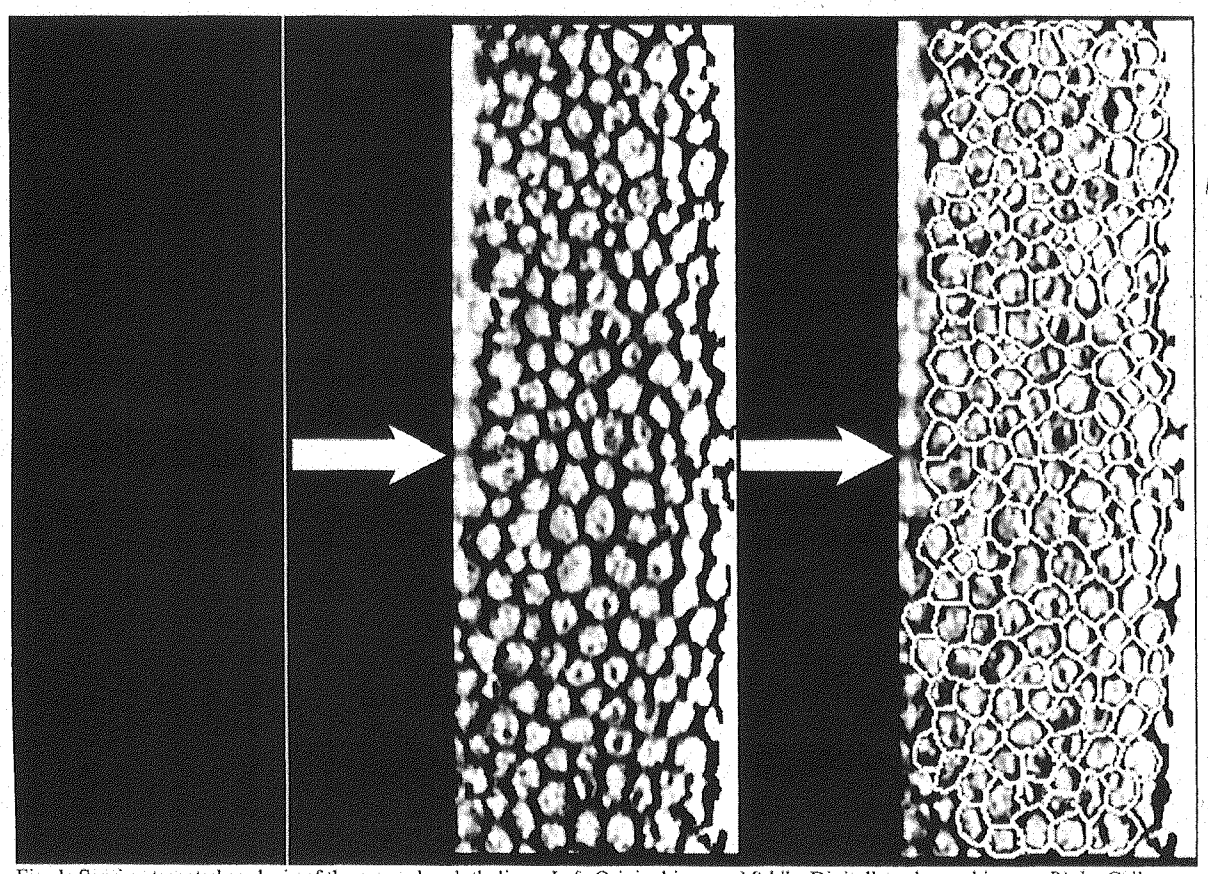

Fig. 1: Semi-automated analysis of the corneal endothelium. *Left*: Original image. *Middle*: Digitally enhanced image. *Right*: Cell barriers identified.

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.

This study is an extension of an earlier study; Bucht et al. (proc. of SPIE 2009)[3].The original study examined the possibilities of analyzing the frequency distribution of CSM image data of the corneal endothelium, using the fast Fourier transform[4] (FFT). A small range of CSM images were used in the original study. This study is an extension of the original study, using a large number of CSM images, as well as a further developed version of the analysis software.

## 2. METHODS

Images of 292 non-pathological corneal endothelia 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.

The original images captured by the CSM are not square, but rectangular (Fig. 1). An algorithm was created for extracting 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.

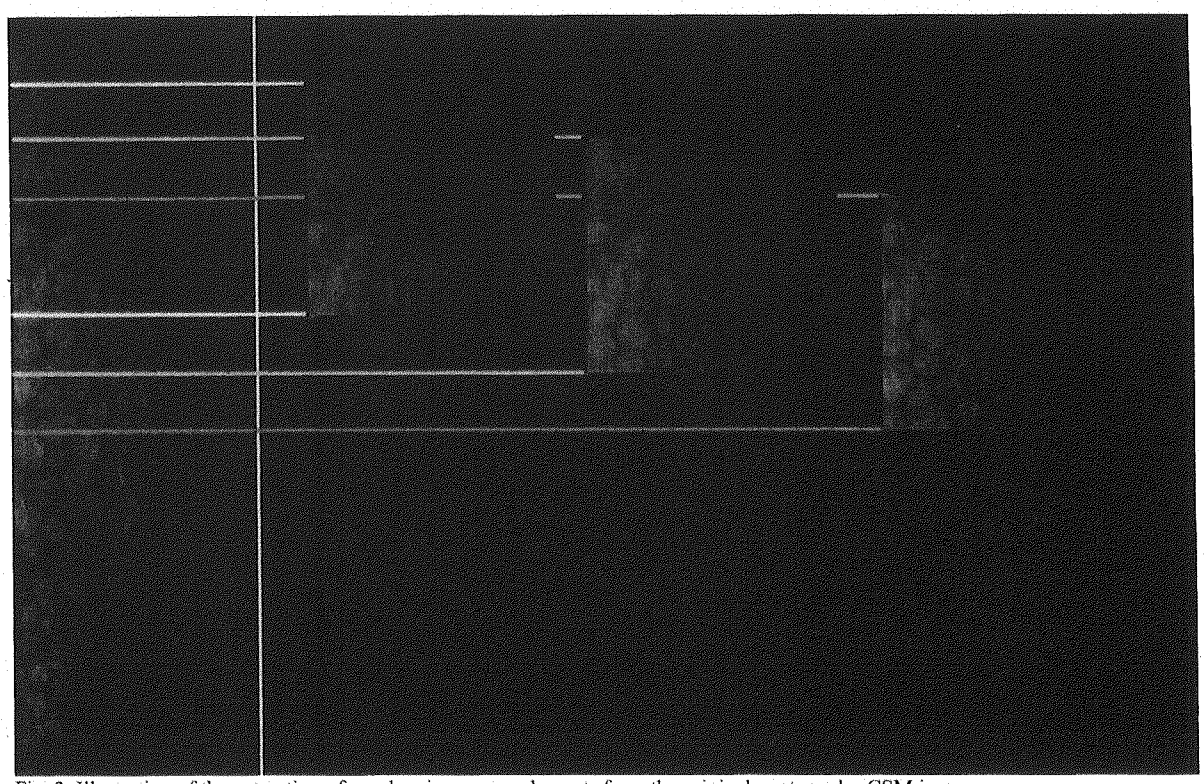

Fig. 2: Illustration of the extraction of overlapping square elements from the original, rectangular CSM image.

Several overlapping square elements from the same original CSM image of the corneal endothelium were extracted and saved to disk. Each element underwent a series of automated digital enhancement[3], resulting in a binarized square image element of the corneal endothelium (Fig. 3).

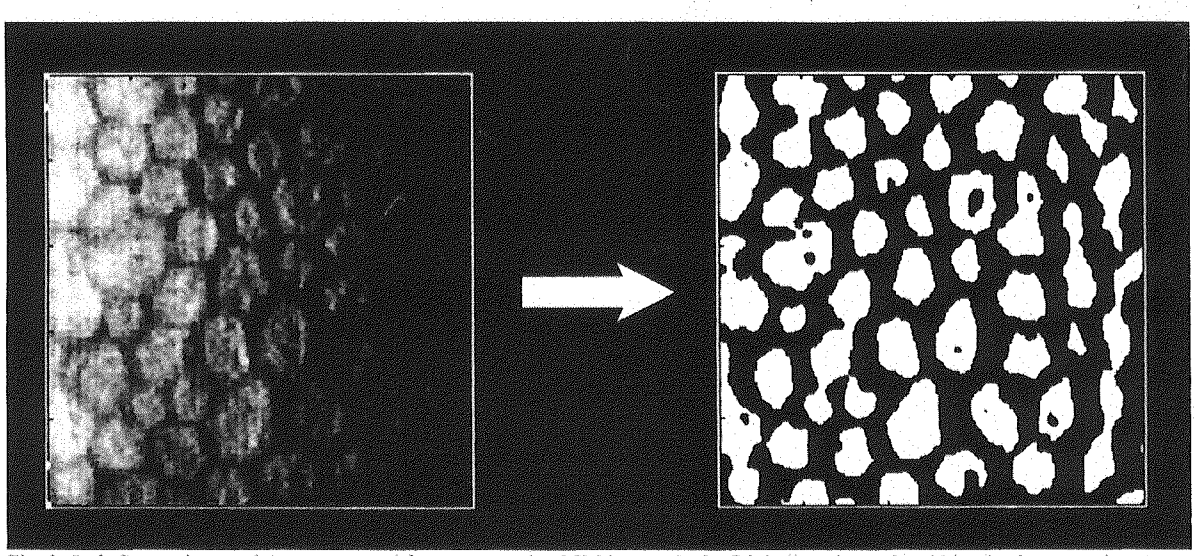

Fig. 3: *Left:* Square image element extracted from rectangular CSM image. *Right:* Digitally enhanced and binarized extracted square image element.

After the automated digital enhancement, each of the extracted square images were Fourier transformed, using FFT2 (Fig. 4).

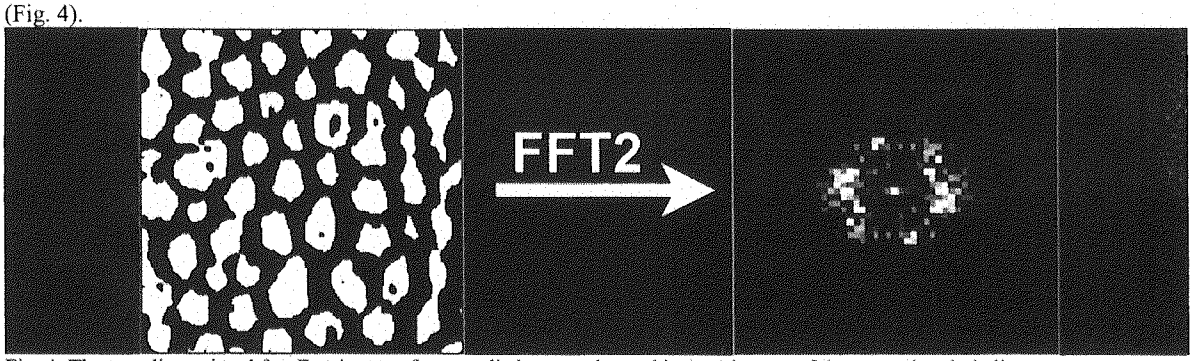


Fig. 4: The two dimensional fast Fourier transform applied to an enhanced image element of the corneal endothelium.

Each square image element rendered a new image, showing the frequency distribution of the original image element. All images of the frequency distribution were added, creating a mean frequency distribution. Analysis tools were developed for identification and interpretation of relevant amplitude characteristics in the frequency distribution. The mean 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 an 180° arc of each frequency distribution image (Fig. 5).

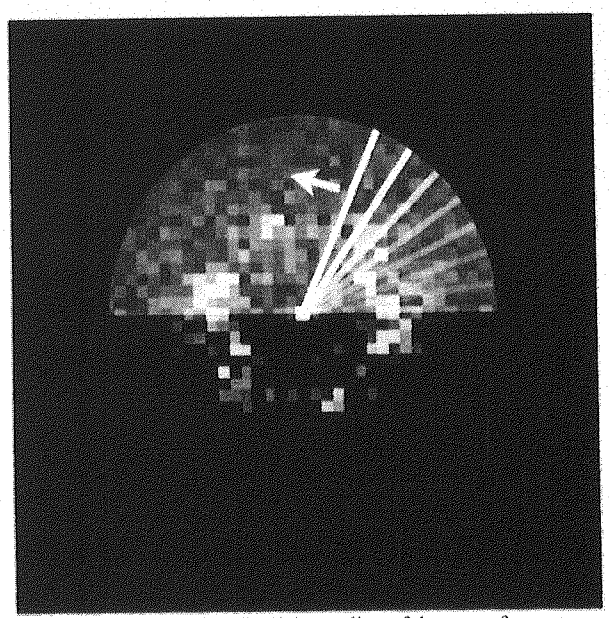

Fig. 5: Schematic of the Radial sampling of the mean frequency distribution.

A sampling on 360° would have given the same result, as the frequency distributions are radial-symmetric.

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 the mean frequency distribution image, giving an estimated value of the cell density of the original image of the corneal endothelium. This value was calculated and squared for all 292 CSM images of a non-pathological corneal endothelium.

The final estimation of cell density of the 292 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 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.

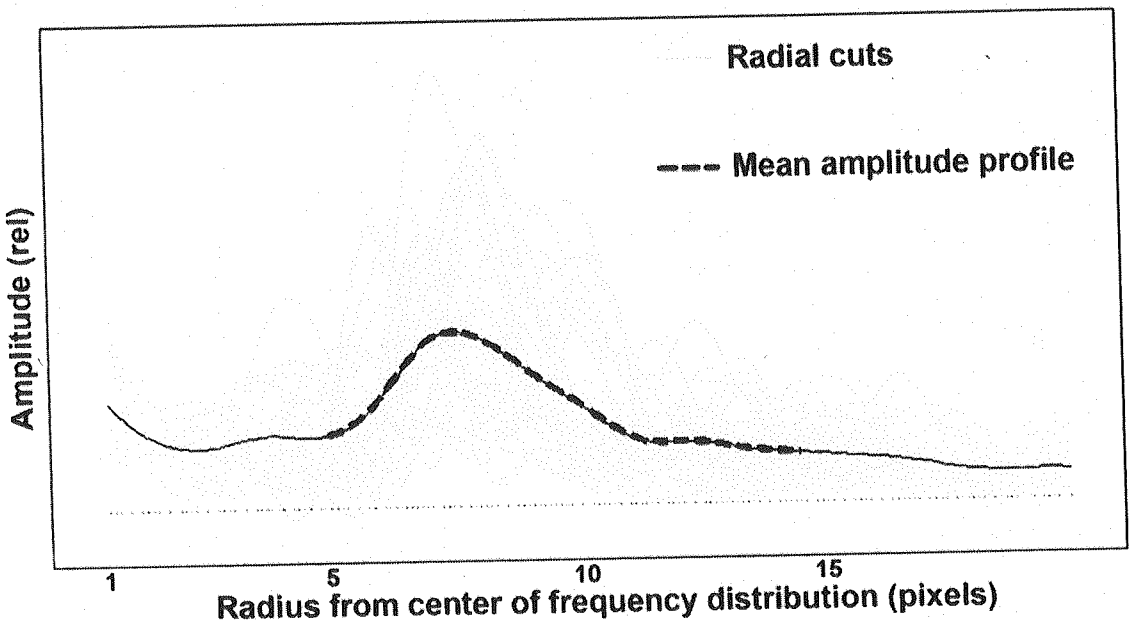


Fig. 6: A set of radial cuts extracted from a frequency distribution and the mean value of those cuts (- -).

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 292 images obtained were compared to the corresponding values obtained by classic, semi-automated analysis. According to the semi-automated analysis, the 292 non pathological CSM images ranged over a corneal endothelium cell density interval with a minimum of 873 cells/mm$^2$ and a maximum of 4750 cells/mm$^2$. The average cell density of the 292 tested images was 2696 cells/mm$^2$ according to the semi-automated analysis. A 95% confidence interval of the residual standard deviation in mean cell size, comparing the fully automated cell density estimate to the semi-automated estimate was found to be [220; 268] cells/mm$^2$. The comparison is plotted in Fig. 7.

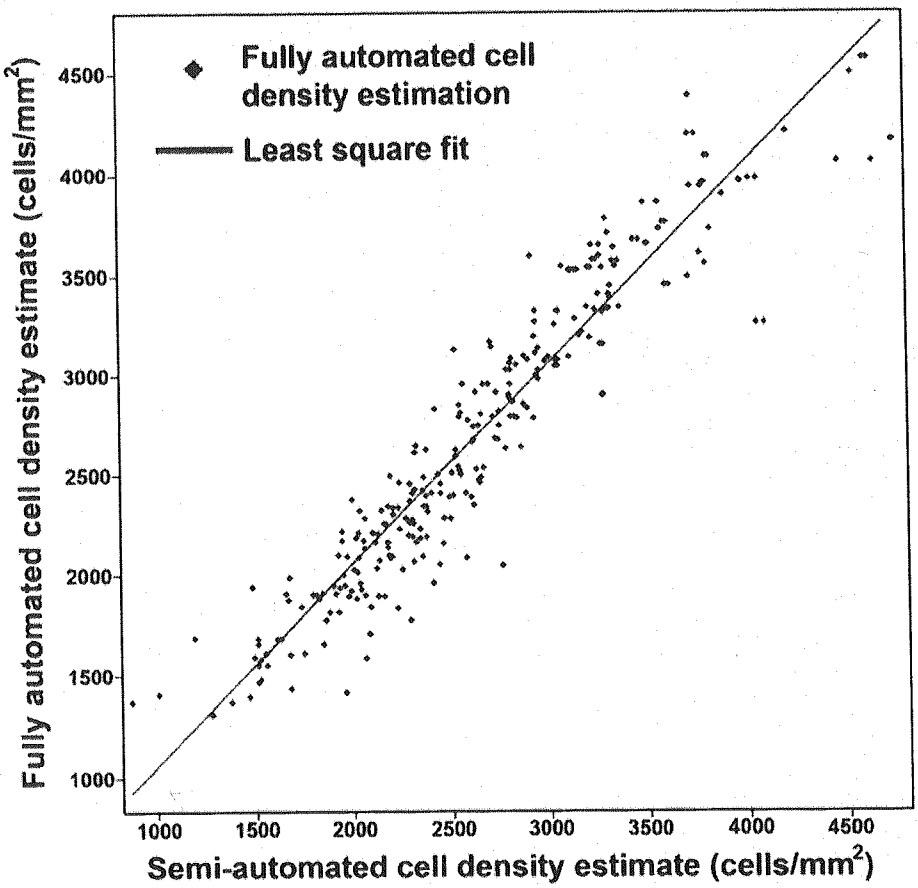


Fig. 7: Fully automated cell density estimate as a function of the semi-automated cell count (cells/mm$^2$).

## 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 and sample size.

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[5,6,7]. 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[8] or larger samples of endothelial cells such as images from corneal donor buttons, resulting in higher quality images and larger sample sizes[9].

A higher number of cells, better quality of cell images and clear models favors the Fourier transform, because a larger number of cells mean 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 of an arbitrary radial frequency cut, 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 and then find the mean of this range. A weighted mean was performed and analyzed as well, although the results presented here stem from the non-weighted algorithm. 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. For an even more robust, but not necessarily of higher resolution analysis for less conditioned images, a combination of mean, weighted mean and integration over boundaries should be considered.

With a relatively large dataset of non-pathological images, this study serves as an indication of the possibilities of using Fourier analysis for corneal endothelial morphometry in small cell sample images captured by CSM. Seeing that the method is fully automated and operated by the push of a button along with the well correlated resulting cell density estimate compared to the far more tedious semi-automated process, the results are encouraging. There are indeed reasons to continue the pursuit of fully automated Fourier transform based morphometry of the corneal endothelium, even for the small sample case of CSM acquired images.

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. In reality, this is 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.

## ACKNOWLEDGMENTS

This study was supported by ELFA Research foundation, the Swedish research council, project K2006-74X-15035-03-2, Karolinska Institute PhD funding, Karin Sandqvists foundation and Gun and Bertil Stohnes foundation.

## REFERENCES

1 Kaufman. P and Alm. A, *Adler's Physiology of the eye - clinical application*, 10, pp. 59-66. Mosby, St. Louis, MO, 2003.

2 Bourne. W. M, McCarey. B. E and Kaufman. H. E, "Specular microscopy of human corneal endothelium in vivo", Am J Ophthalmol, **81**, 319-323, 1976

3 Bucht. C, Söderberg. P. G and Manneberg. G, "Fully automated corneal endothelial morphometry of images captured by clinical specular microscopy", *Ophthalmic technologies XIX*, Manns. F, Söderberg. P and Ho. Vol. 7163. SPIE, SPO, 2009.

4 Hecht. E, "Optics - International Edition", *Optics - International Edition*, IV, pp. 452-471 519-529. Addison Wesley, San Francisco, 2002.

5 Ruggeri. A, Grisan. E and Jaroszewski. J, "A new system for the automatic estimation of endothelial cell density in donor corneas", British Journal of Ophthalmology, **89**, 306-311, 2005

6 Doughty. M. J, Spiteri. M and Dilts. D. M, "Determination of the unit size of the corneal endothelial cell mosaic from Fourier component image analysis", Tissue Cell, **29**, 229-238, 1997

7 Fitzke. F. W, Masters. B. R, Buckley. R. J and Speedwell. L, "Fourier transform analysis of human corneal endothelial specular photomicrographs", Exp Eye Res, **65**, 205-214, 1997

8 Bucht. C, Söderberg. P. G and Manneberg. G, "A model for corneal endothelial morphometry by diffraction", *Ophthalmic technologies XVI*, Manns. F, Söderberg. P and Ho. A, 7, pp. 6138O1-6138O8. SPIE, Bellingham, WA, 2006.

9 Foracchia. M and Ruggeri. A, "Automatic estimation of endothelium cell density in donor corneas by means of Fourier analysis", Med Biol Eng Comput, **42**, 725-731, 2004