The impact of horisontal offset of the cornea during corneal specular microscopy

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ABSTRACT

We are developing automated morphometric analysis of the corneal endothelium. Here, the general impact of horizontal offset of the cornea on morphometry was examined. Errors due to perspective during imaging with a Clinical Specular Microscope (CSM) were analyzed considering semi automated analysis software and fully automated Fourier analysis software. *Methods:* A mathematical model of the cornea was created. Trigonometry was applied to find the relationship between the horizontal offset of the cornea relative to the microscope objective, and the consecutive errors from perspective changes in the image. An experimental setup was created using a cornea made of polymethyl methacrylate (PMMA). The posterior surface of the PMMA cornea was horizontally marked. The PMMA cornea was placed in a holder. Difference in refractive index between real endothelium and aqueous humor was emulated using high refractive index liquid. Images with varying horizontal offset on the PMMA corneal posterior surface, along with their relative offset coordinates were captured, using CSM. *Results:* Experiments using controlled offset of the cornea in relation to its center estimated that analyzable images can be acquired within an interval of 1.26 mm, using central cornea sampling CSM. Because of refractive indices along with light scattering differences between the corneal tissue and PMMA , the 1.26 mm interval should be considered a first estimate for feasible CSM images. The effect of corneal endothelial offset during imaging with CSM or fully automated Fourier analysis should be considered.

Keywords: cornea, endothelium, morphometrics, microscopy, offset, Fourier

1. INTRODUCTION

The innermost layer of the cornea is a monolayer of flat, polygonal cells called the endothelium. A well functioning endothelium is vital for maintenance of the optical properties of the cornea. 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 size variation and frequency distribution for number of cell corners are frequently analyzed variables in corneal endothelium[1].

Presently, the morphology of the corneal endothelium in vivo is most commonly estimated by the use of Clinical Specular Microscopy (CSM)[2]. During development of a fully automated analysis method for use with images acquired by CSM, a large number of corneal endothelium images were examined. It was found that horizontal and vertical cell symmetry among the endothelium images seemed to be varying. This became increasingly interesting, when Fourier transforming the images, as horizontal and vertical frequencies of the images were more readily distinguishable than from observing the original cell structure. The Fourier transform outputs the frequency distribution for the transformed data[3]. The figure below shows an example of how horizontal and vertical cell asymmetry of a corneal endothelium image becomes evident when the original image is Fourier transformed. The bright square pixels (Fig. 1, to the right of the endothelial cells) represent the main cell frequencies of the original image. Even though the resolution is not high, it

is clear that the horizontal cell frequency is higher than the vertical cell frequency. The white circle (Fig. 1, left) highlights the frequency distribution tendency of the cell structure. The horizontal and vertical cell dimensions appear rather equal. The white oval (Fig. 1, right), shows a less symmetric cell structure, hinting of a higher horizontal frequency and thus more narrow cells.

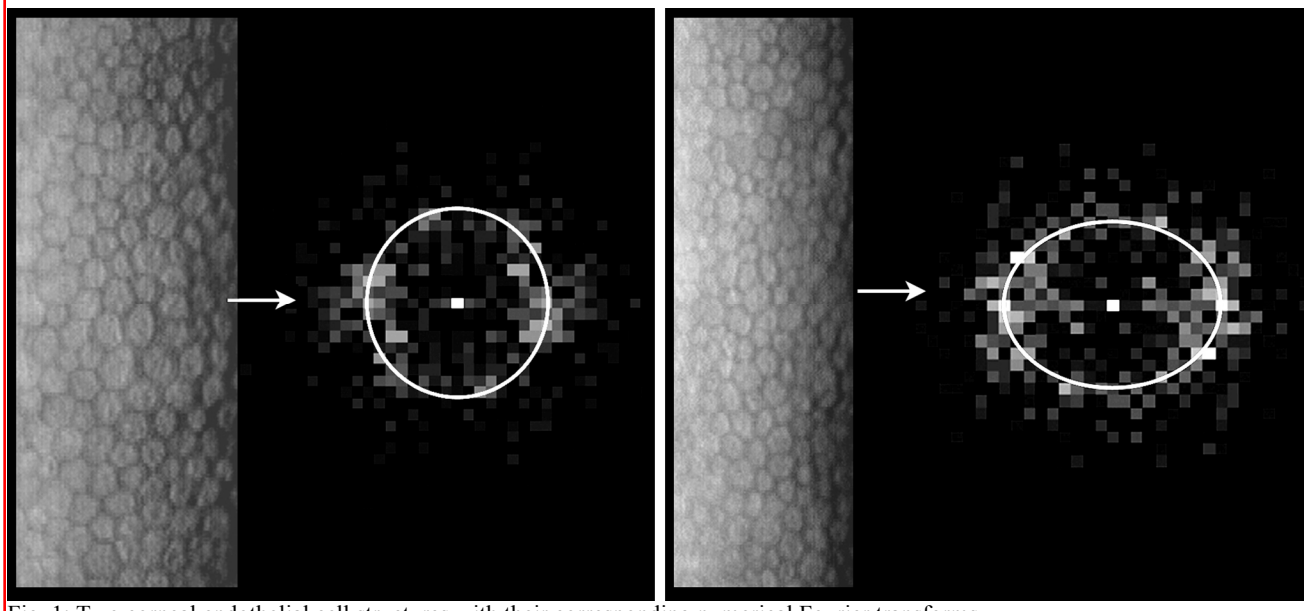

Fig. 1: Two corneal endothelial cell structures with their corresponding numerical Fourier transforms.

This seemingly random asymmetry in the original images of the corneal endothelium was suggested to be effected by a variation in perspective, stemming from an uncontrolled offset from the desired imaging coordinate on the cornea. The perspective is changed because the angle at which the image of the corneal endothelium is captured alters as a function of the offset. It is clear that a small sample of corneal endothelial cells are not always perfectly symmetric. However, the perspective factor seemed plausible enough to warrant a study.

The aim of this study was to examine the theoretical impact on image dimensions of a possible horizontal offset of the cornea relative the desired imaging spot when photographing the central corneal endothelium using clinical specular microscopy. Focus was kept on horizontal offset, as it is harder to spot an eventual offset in the horizontal plane because of the slit nature of the CSM illumination.

2. METHODS

The trigonometric theory explaining the acquired corneal endothelium image asymmetry in relationship to possible offsets from a desired reference point on the cornea was derived. The relational function was implemented in the mathematical programming language Matlab (Mathworks, USA) and a simple graphical user interface was created (Fig. 2, left). Parameters corresponding to normal central corneal dimensions were set. Horizontal offset and default microscope objective viewing angle was interactively controlled though user interface sliders. The relative error due to perspective from horizontal offset was output in terms of percentages. Horizontal offset was chosen in favor of corneal surface offset for the reason of facilitating measurement procedure in the experimental phase.

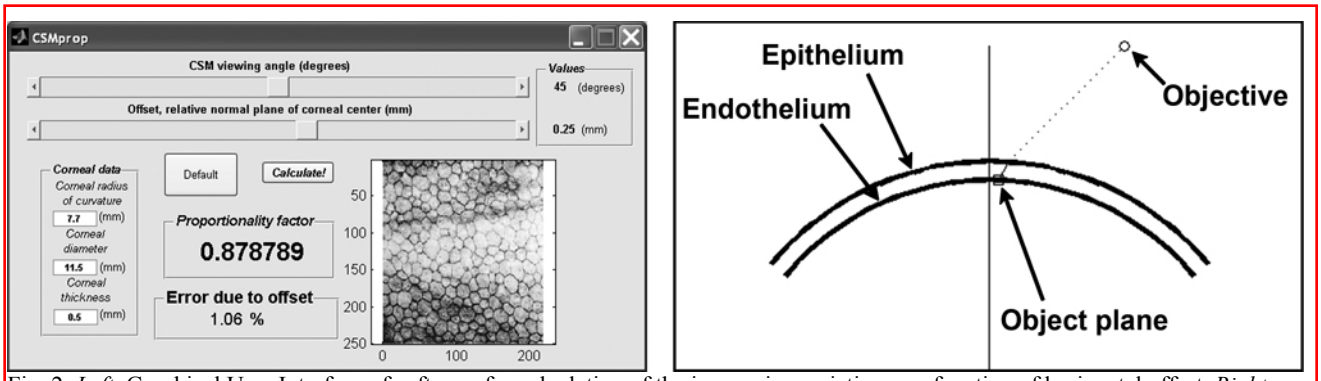

Fig. 2: *Left*: Graphical User Interface of software for calculation of the image size variation as a function of horizontal offset. *Right*: Graphical output of the calculation for given input parameters.

Now a reasonable interval of horizontal offset of the cornea during in vivo CSM photography was to be determined. The idea of a study on human subjects was swiftly discarded. The main reason, that there is no reliable way to know at what offset the images were taken, because of constant eye movement. A substitute for a real eye was created. A cornea was created in polymethyl methacrylate (PMMA). The shape of the PMMA cornea was made to fit normal human central corneal dimensions, with a radius of curvature of 7.5 mm, a diameter of 12.7 mm and a central thickness of 0.5 mm. Focused bursts from a Nd:YAG laser was used to create a horizontal string of reference points with diameters of 30-60 µm along the posterior surface of the PMMA cornea.

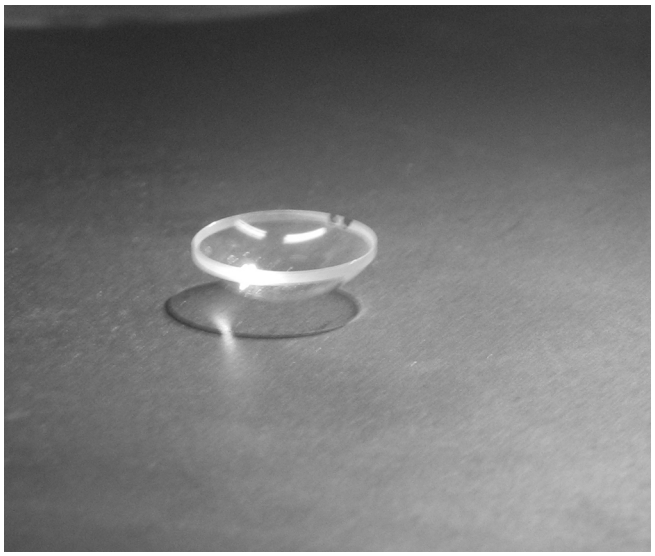

Fig. 3: Polymethyl methacrylate (PMMA) cornea with dimensions of a normal cornea.

Reflective power of an interface between two surfaces is primarily decided by two factors; Angle of incidence of light and difference in refractive indices. The refractive indices of the cornea and the aqueous humor are 1.37 and 1.33 respectively. The cornea and aqueous humor interface difference in refractive index is thus 0.04. The refractive index of the PMMA cornea is 1.49. To emulate a realistic amount of reflection from the posterior interface of the PMMA cornea, a difference in refractive index of 0.04 must be obtained. This was accomplished be putting the PMMA cornea in a specially created holder, with possibility to add a high refractive index liquid to the posterior of the cornea. A water and sugar (61%) solution was used to create the desired refractive index difference at the posterior surface of the PMMA cornea, emulating the reflective power of a real corneal endothelium.

The holder with the attached PMMA cornea and the refractive index balancing liquid was set up for CSM photography and the cornea offset was controlled with an X/Y holder (Fig. 4).

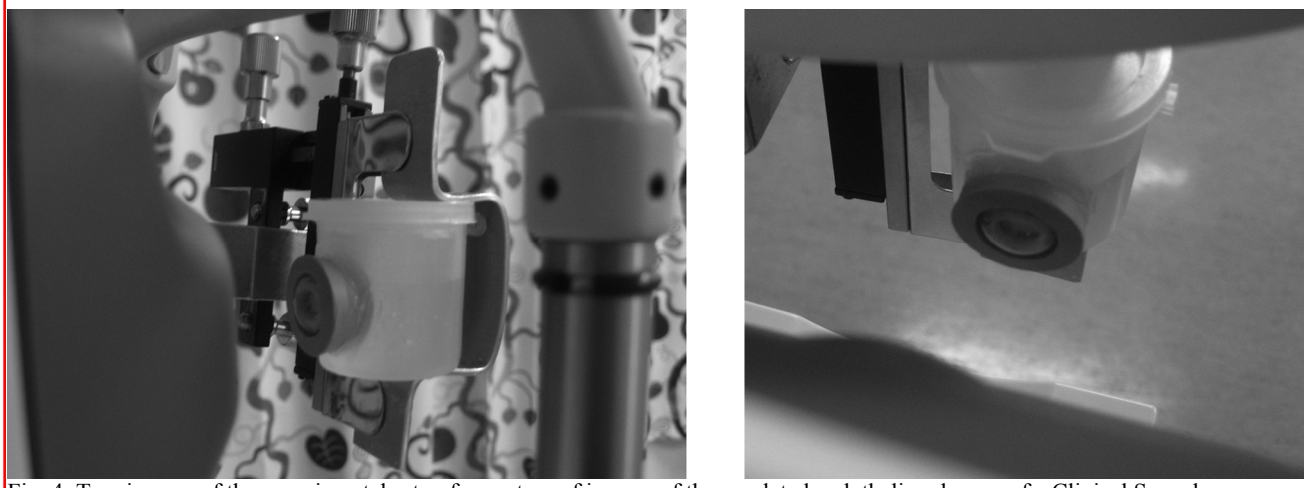

Fig. 4: Two images of the experimental setup for capture of images of the emulated endothelium by use of a Clinical Specular Microscope (CSM).

A total of 24 images were captured of the posterior surface of the PMMA cornea, along the reference horizontal line. When an image was captured, the position on the cornea that had been photographed was recorded. The offset images of the cornea were arbitrarily chosen, but also included identification of the left and right offset limits. These limits were determined as the left and right boundaries where the reflected light was deemed just enough to get an analyzable corneal endothelium image. The left and right offset limits for analyzable images of the posterior of the PMMA cornea were input into the trigonometrics software and the image size effects were calculated.

3. RESULTS

A theoretical relationship between horizontal offset from the center of the cornea and the resulting perspective variation was deduced. Values for the relative image horizontal size error was made available for an interval of -2 mm to +2 mm horizontal offset. This interval was confirmed to be covering estimated relevant intervals for analyzable corneal endothelium images captured in vivo by clinical specular microscope. A user friendly graphical interface provides fast and accurate information on the image effects of horizontal offset during CSM corneal endothelium image capture.

The outer dimensions of the PMMA cornea was considered representative for a real cornea. The noticeable differences were that the PMMA cornea was lacking tissue structures and that its index of refraction was 0.12 units higher than for a real cornea.

The aim of the study was to identify the possible horizontal offset interval when capturing corneal endothelial images using CSM. Because of this, a cell structure on the PMMA cornea was not necessary. The horizontal string of 30-60 µm diameter laser burns was used as a vertical imaging reference on the posterior surface of the PMMA cornea. The PMMA posterior and a magnified part of the Nd:YAG burns are shown below.

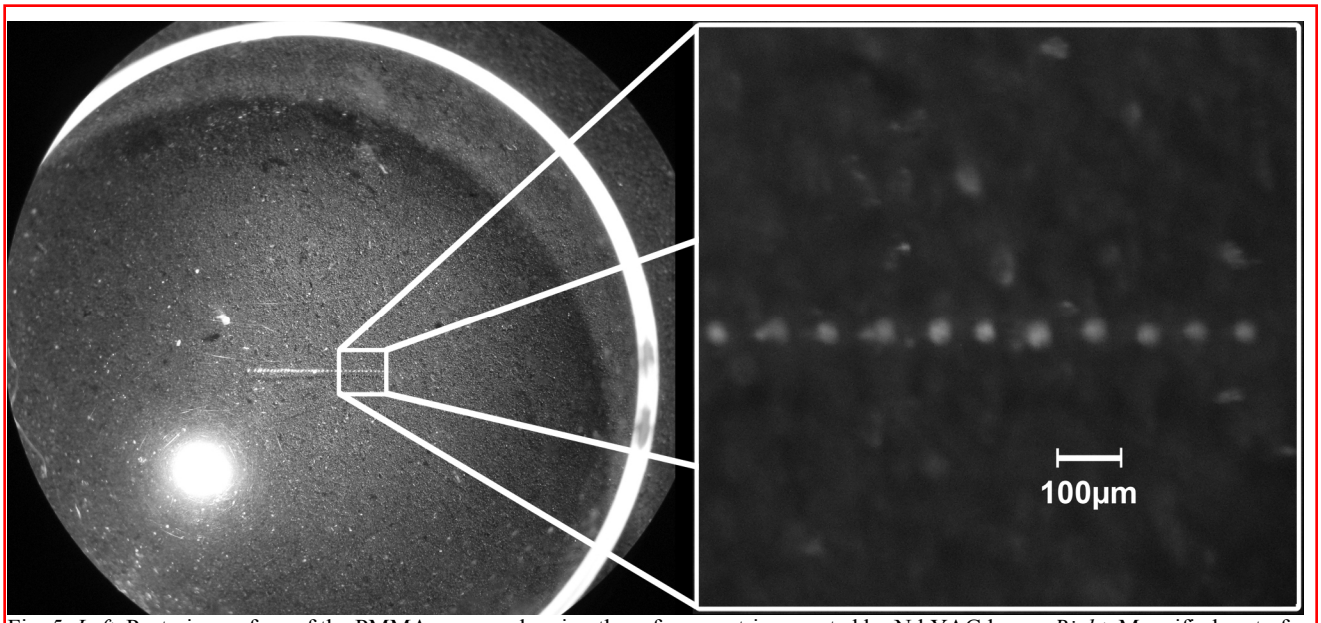

Fig. 5: *Left*: Posterior surface of the PMMA cornea, showing the reference string created by Nd:YAG burns. *Right*: Magnified part of the PMMA reference string.

The texture of the emulated corneal endothelium was of less concern for the study at hand. The reflective power of the PMMA posterior surface was a more important factor. To identify the estimated interval of the cornea at which an analyzable CSM image could be captured, a reflecting power as close a possible to that of the cornea and aqueous humor interface was accomplished. A water and sugar solution (61%) gave the desired 0.04 refraction index difference. Comparing images from the posterior surface of the PMMA with real images of the endothelium (Fig. 7) confirmed that a satisfying reflective power had been reached.

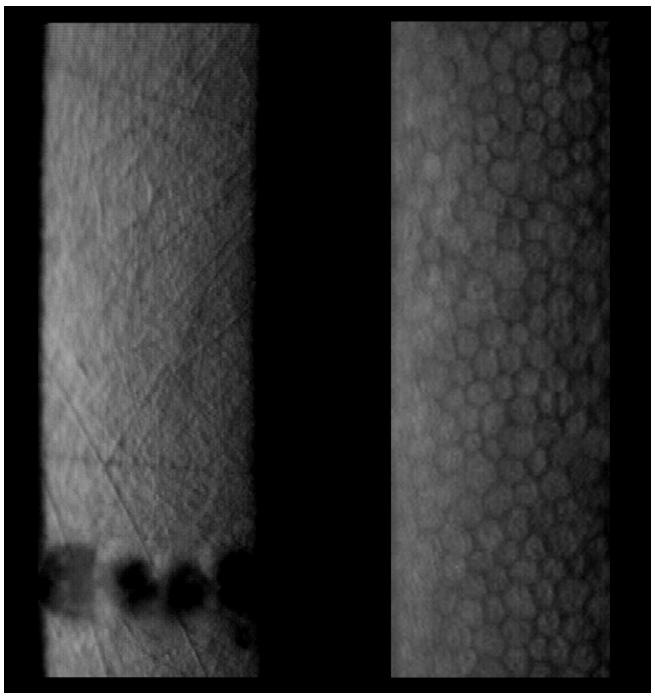

Fig. 6: *Left*: Image of the posterior of the PMMA cornea. *Right*: Image of a normal corneal endothelium.

The experimental setup, using an X/Y holder to adjust the position of the PMMA cornea in front of the CSM worked as anticipated. 24 images were captured along the horizontal markings at the posterior surface of the PMMA cornea. Out of the 24 images, two which were assessed representative for left and right edges of the analyzable interval were identified. Because of the stability of the setup, each capture could be associated with its relative offset position on the corneal horizontal axis. Eight representative images of the posterior surface of the PMMA cornea are depicted below, at their corresponding relative positions (Fig. 7). The left and right edge image captures are highlighted by white rectangles.

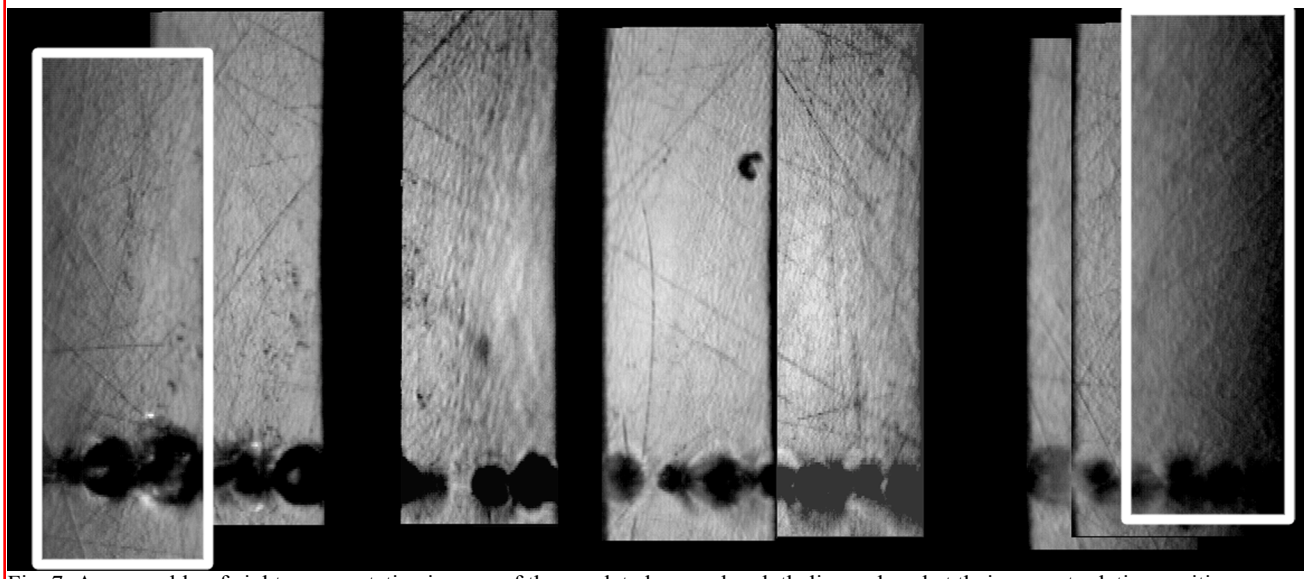

Fig. 7: An assembly of eight representative images of the emulated corneal endothelium, placed at their correct relative positions.

Using images captured from the anterior surface of the PMMA cornea, the interval at which analyzable images of the corneal endothelium could be captured was estimated to 1.26 mm. The edges of the interval were observed to be approximately symmetrically situated around the center of the emulated cornea. An image of the anterior surface of the PMMA cornea can be seen below (Fig. 8, left). The white circles are highlighted markings corresponding to the horizontal position where the posterior images (Fig. 7) were captured. Also, the graphical output cut of the calculated interval can be seen (Fig. 8, right).

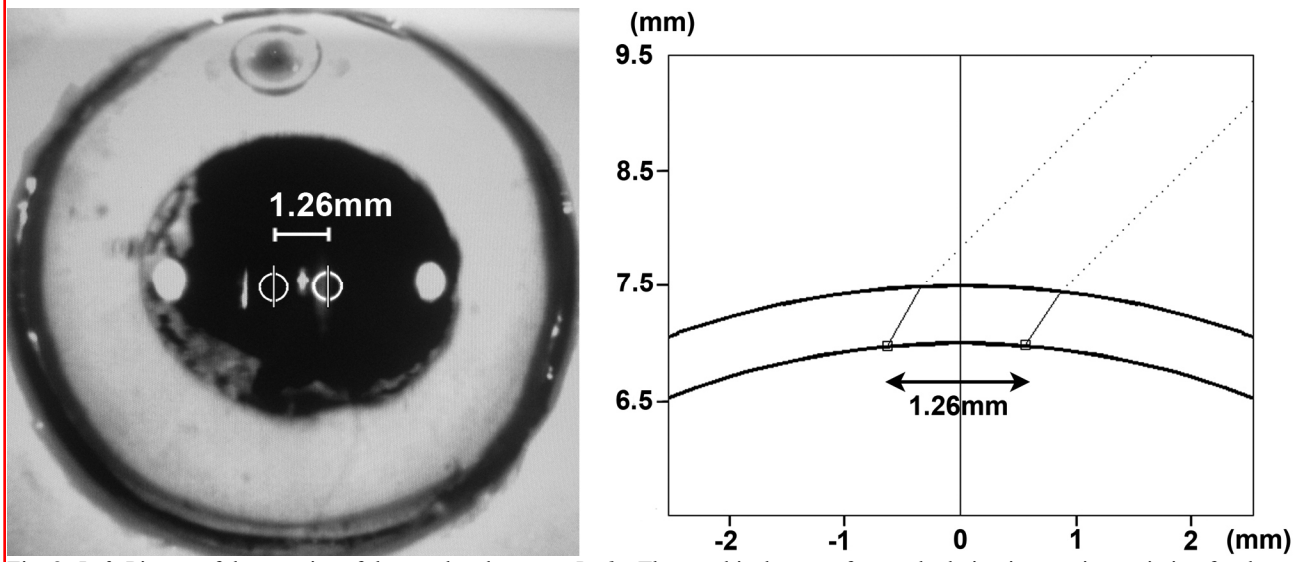

Fig. 8: *Left*: Picture of the anterior of the emulated cornea. *Right*: The graphical output from calculating image size variation for the allowed offset interval.

The trigonometry software calculated the corneal endothelium size error from the approximated offset interval edge values -0.63 mm and +0.63 mm. The two situations were plotted (Fig. 8). The negative offset of -0.63 mm gave a size error of -2.98 % and the positive offset of +0.63 mm gave an error of +2.66 %. The maximum relative image size error is thus close to 3 %, and the relative image size range can be over 5.6 %. The relative image size change as a function of offset from the center was plotted for the offset range of -2 mm to +2 mm and the estimated +/- 0.63 mm offsets were marked in the graph (Fig. 9).

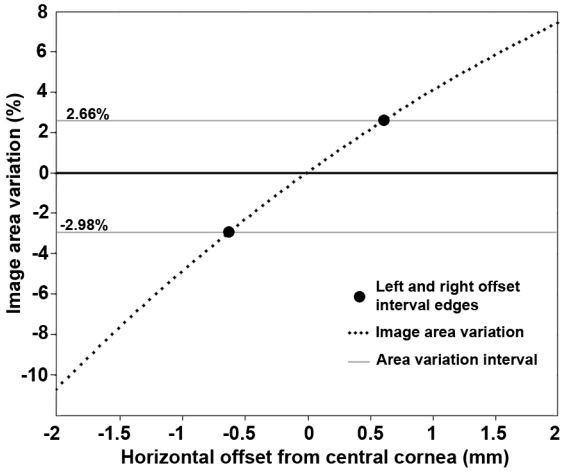

Fig. 9: Relative corneal endothelium image size variation (%) as a function of horizontal offset (mm) from the corneal center.

4. DISCUSSION

When working on developing new methods of morphometric analysis of images of the corneal endothelium, captured by in vivo Clinical Specular Microscopy, lateral asymmetries were detected amongst some of the images. There are several factors that can effect the symmetry of the images because the corneal endothelial cell structure is constantly changing, but the possibility of yet one factor became interesting. That factor was the risk that an image of the corneal endothelium is captured when the object plane is laterally offset from the desired spot. Because of two primary factors, refraction at the posterior surface of the cornea and the radius of curvature of the cornea, horizontal variation of the object plane gives rise to perspective variations and thus a size variation of the captured image. The captured image size is directly related to an important morphological variable such as corneal endothelial cell density. The magnitude and relevancy of the possible size variation of in vivo captured corneal endothelium images were investigated.

The development and use of a trigonometric tool for finding the relationships between cornea offset and the resulting corneal endothelium image size showed that at large enough offsets, the image size variation could render a noticeable error. E.g. at 45 degrees viewing angle and -1 mm horizontal offset from the center, an intrinsic cell density error of -4.9 % is produced. Considering that the analysis of corneal endothelial cell density with CSM is not error free and based on the dimension of the original image, the effects from a potential perspective error from horizontal offset of the object plane could be relevant.

There are other factors that limit the offset perspective error. Foremost, the very mechanics of in vivo CSM. The cornea is illuminated by a slit lamp and the light is in theory specularly reflected from the epithelium and the endothelium. The light source and the objective of the CSM are at opposite angles and if the light was indeed totally specularly reflected from the endothelium, it would be impossible to actually record any light with the slightest offset on a curved surface as the cornea. However, the corneal endothelium is not a totally specularly reflecting surface. The light is scattered, not only in its way through the epithelium and stroma, but also in the reflection at the endothelium and aqueous humor interface. This makes it possible to capture images even when not observing the corneal endothelium at the correct angle.

Finding the range at where enough light was reflected back from the corneal endothelium for an analyzable image was a main task in this study.

A human anterior eye was emulated, using a polymethyl methacrylate (PMMA) cornea, with the aqueous humor represented by a water and sugar (61 %) solution. This emulation was necessary for controlled measurements of offset limits. There were limiting factors in this setup. First, the refractive index of the PMMA cornea is higher than in a real cornea. This leads to a slightly steeper refraction at the anterior of the PMMA cornea, than in a real cornea. The effect of this higher refraction is a slightly higher possible offset limit for analyzable images. On the other hand, more light is reflected and therefore lost at the anterior surface of the PMMA cornea, which would lead to a smaller possible offset limit. The amount of light reflected back at the objective from the posterior PMMA cornea at the different offsets have been used to approximate the effect on a real cornea. Regardless, the use of a plastic anterior eye with the correct corneal dimensions and posterior corneal surface reflective power, showed of good potential for use in future applications.

The resulting estimate of the interval at which enough light was reflected from the posterior surface of the PMMA cornea to form an analyzable image was interesting. At a horizontal interval of 1.26 mm of possible endothelium images, the possible image size variation from edge to edge, was approximated to be over 5.6 %. This is an intrinsic variation in the original image and analytic errors from operators or software are applied after this error. Of course, it should be clear that this is in case of maximum variation and in most cases, the perspective error is anticipated to be smaller.

Another point worth of discussion is the development of new methods for analysis of the corneal endothelium. In particular the use of numerical Fourier transform of corneal endothelial images for morphometric purposes[4,5,6]. The use of the Fourier transform as a way to find primarily the cell density of the corneal endothelium is promising. So far, this method has been applied to models[7] or larger samples of endothelial cells, such as images of corneal donor buttons[8]. In the cases where the field of view of the corneal endothelium is wide and in focus, there are no real perspective issues. Also, the very nature of the Fourier transform makes it particularly applicable when the cell count is large.

When adapting the Fourier transform method for analysis on in vivo images of the corneal endothelium, captured by narrow slit lamp CSM, circumstances changes. Because of the fact that specially tailored algorithms has to be used to extract reliable data from the Fourier transform in a small cell count case, perspective errors may impact the outcome of the analysis. Perspective errors therefore should be considered when developing Fourier transform analysis morphometry of the corneal endothelium.

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