Evaluation of hypoxic swelling of human cornea with high speed, ultrahigh resolution optical coherence tomography

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ABSTRACT

Hypoxia induced corneal swelling was observed and evaluated in healthy human volunteers by use of high speed, ultrahigh resolution optical coherence tomography (UHROCT). Two dimensional corneal images were acquired at a speed of 47,000 A-scans/s with 3μm x 10μm (axial x lateral) resolution in corneal tissue. The UHROCT tomograms showed clear visualization of all corneal layers, including the Bowman's layer and the Descemet's membrane – Endothelium complex. A segmentation algorithm was developed and used for automatic detection of the boundaries of the different corneal layers and evaluation the individual layer thickness as a function of location. Corneal hypoxia was induced by wear of a soft contact lens (SCL) and an eye patch by 2 healthy volunteers for duration of 3 hours. The thickness of all corneal layers was measured as a function of time, prior to, with and after removal of the SCL. Results from the hypoxia study showed different rates of swelling and de-swelling of the individual corneal layers. About 10% increase in the total cornea thickness was observed, similar to the changes in the stroma, the Bowman's membrane swelled by 20%, while no significant change in the thickness was observed in the Descemet's – Endothelium complex.

INTRODUCTION

The cornea is a transparent, avascular tissue covering the front surface of the eye, which serves multiple purposes: it provides nearly 70% of the eye's refractive power, protects the eye from foreign matter and can act as a minimally invasive pathway for therapeutic drug delivery to the lens, vitreous and retina. Structurally, the cornea consists of a series of laminae with the epithelium being the most anterior structure (~50μm) separated from the structure of greatest bulk, the stroma (~475μm), by an interstitial complex called Bowman's Membrane (~15μm). The inner, most posterior lamina comprises Descemet's membrane and the endothelium (combined thickness of ~10-15μm).

The cornea has active physiological mechanisms to maintain transparency and oxygenation of the tissue[1] and a correlation between the cornea hypoxic state, swelling / de-swelling and a change in the corneal opacity to visible light has been suggested based on previous research. Central corneal thickness measurement plays an important role in gauging a number of aspects of corneal[2] and general ocular health[3]. One important factor directly influencing thickness is hypoxia, the clinical effects of which can include corneal swelling, acidosis, and altered corneal oxygen consumption[4]. Because the cornea is avascular, it is oxygenated from the atmosphere; this is interrupted during eye closure and the cornea and corneal epithelium are thickest when the eye is opened in the morning[5]. Contact lens wear also interrupts the diffusion of oxygen to the corneal tissues and it too affects corneal thickness[6]. The cornea is relatively resistant to short-term hypoxia and the clinical complications of hypoxia with contact lens wear tend to be chronic rather than acute, and so the accumulated degree of hypoxia may be of greater importance than the acute effect in assessing corneal oxygenation[6]. More severe or longer-term hypoxia can result in more serious complications such as microcysts, corneal exhaustion syndrome (CES), corneal vascularization, and decreased resistance to bacterial infection[7]. Clinical studies have shown that corneal swelling, above the non–contact lens wear baseline, can occur, even with high oxygen-permeable lenses[7]. There have been experiments examining the effects of lens wear and eye closure on corneal thickness (e.g., O'Neal & Polse[8,9,10]) and more recently, using a clinical OCT, examining corneal epithelial thickness as well[11,12]. According to the studies of Wang et al performing clinical OCT measurements, corneal epithelial thickness does not

Ophthalmic Technologies XIX, edited by Fabrice Manns, Per G. Söderberg, Arthur Ho
Proc. of SPIE Vol. 7163, 71631G · © 2009 SPIE
CCC code: 1605-7422/09/$18 · doi: 10.1117/12.809431

increase in response to hypoxia from soft contact lens (SCL) wear and eye closure, in contrast to a significant increase in total corneal thickness[11].

Several clinical devices have been used in the past to measure the thickness of some corneal laminae but these devices have limited axial resolution. For example, clinical OCT systems provide axial imaging resolution in the order of 8-18μm, ultrasound biomicroscopy: ~25μm and very high frequency ultrasound: ~21μm. Imaging systems based on the principle of confocal microscopy can achieve significantly better axial resolution in corneal tissue (~3-5μm), however the field of view of this technique is limited to ~0.5mm x 0.5mm in the transverse direction and the imaging procedure is invasive since it requires contact with the corneal surface using anesthesia and sometimes a coupling gel. Recently, high axial resolution (~3μm) images of human corneal were acquired *in-vivo* with UHROCT systems operating in the 800nm wavelength range[14,15] from healthy volunteers and patients with corneal scarring from refractive surgery. Results from these publications show clear visualization of the corneal epithelium and a hint of the interface between the Bowman's membrane and the underlying stroma. However, the imaging probes in these studies were designed for larger field of view at the expense of lower transverse imaging resolution (~20μm - 50μm).

In this study, we used a high speed, UHROCT operating in the 1060 nm wavelength range, a custom designed imaging probe which provides imaging resolution of 3μm x 10μm (axial x lateral), and novel image processing algorithms to visualize and measure the thickness of all corneal structures before and after corneal edema induced by wearing SCL during eye closure. There are a number of issues that arise when developing a new device, perhaps the foremost being that the measurements that you assume are being made are actually occurring. Unfortunately, when developing devices for use with humans, it is not always possible to directly verify that the optically sampled tissue matches the better understood histology. In order, therefore, to demonstrate that the imager is producing valid morphometry, hypothetical tissue manipulation and the resulting image change can be used, where the resulting image reflects the expected appearance produced by the hypothetical tissue manipulation. This is one such experiment: It is as much about construct validity of the UHROCT imager of the cornea as it is about the corneal anatomy and physiology[16].

METHODS

A high speed, UHROCT system developed for ophthalmic applications in the 1060nm spectral region was used for the first time for high resolution imaging of corneal morphology in-vivo in human volunteers. A detailed description of the system design and test performance can be found in a recent publication[17]. Briefly, the imaging system is based on the Fourier Domain OCT design (FD-OCT) and consists of a compact, fiber based Michelson interferometer, interfaced to a custom light source (λc = 1020 nm, $\Delta\lambda$ = 110 nm and Pout = 15 mW). All optical and fiber optic components of the FD-OCT system were selected to support the propagation of broadband light through the systems with minimal spectral and power losses. The interference signal is detected by a high efficiency custom built spectrometer, which utilizes a fast 1024 pixel linear array CCD camera with 47 kHz readout rate. An imaging interface was designed and built for high transverse resolution imaging of the human cornea. The UHROCT system provides 3.2μm axial and ~10μm lateral resolution in corneal tissue.

Imaging of the cornea was conducted on two healthy volunteers in accordance with an approved ethics protocol. In order to induce corneal edema, one eye of two normal, healthy noncontact lens wearer volunteers was taped shut and patched for 3 hours while wearing a hydrogel SCL (<50% water content, 14.0 mm diameter). Two-dimensional corneal images were acquired with a high speed (47,000 A-scan/s), UHROCT (3.2μm x 10μm (axial x lateral) resolution in corneal tissue). Base-line tomograms were acquired before adding the SCL and patching the eye, and subsequent 2D corneal images were acquired immediately after removal of the patch and the SCL, and at intervals of 10-15 minutes thereafter (up to 2 hours 40 minutes). The UHROCT images were processed with a Fuzzy type II wavelet speckle noise reduction algorithm[15] to improve image contrast and with an automatic segmentation algorithm to identify the boundaries of all corneal layers. The thickness of each corneal layer was evaluated from a set of tomograms and the average layer thickness was computed.

RESULTS AND DISCUSSION

A representative corneal tomogram is shown in fig. 1 (left), while image to the right shows the same tomogram with final result from the application of the semi-automatic segmentation algorithm. The images dimensions are 1000 x 512 pixels

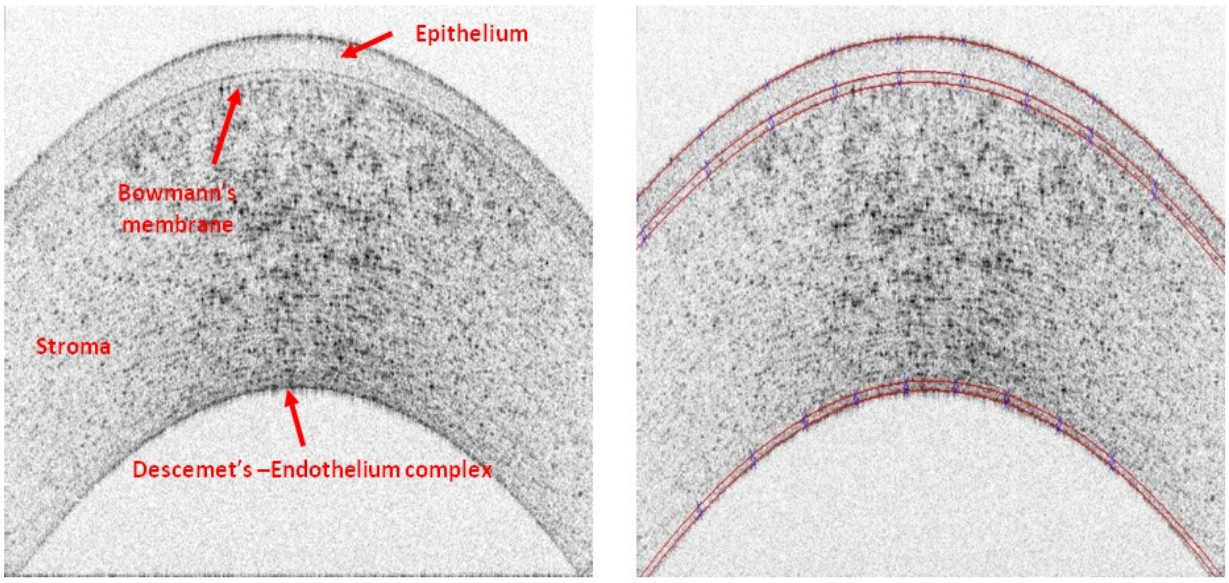

Figure 1. Human cornea tomogram acquired in-vivo with an UHR FD-OCT system with 3µm axial resolution, ~10µm transverse resolution and a Rayleigh range of ~300µm. Acquisition speed was 47,000 A-scan per second.

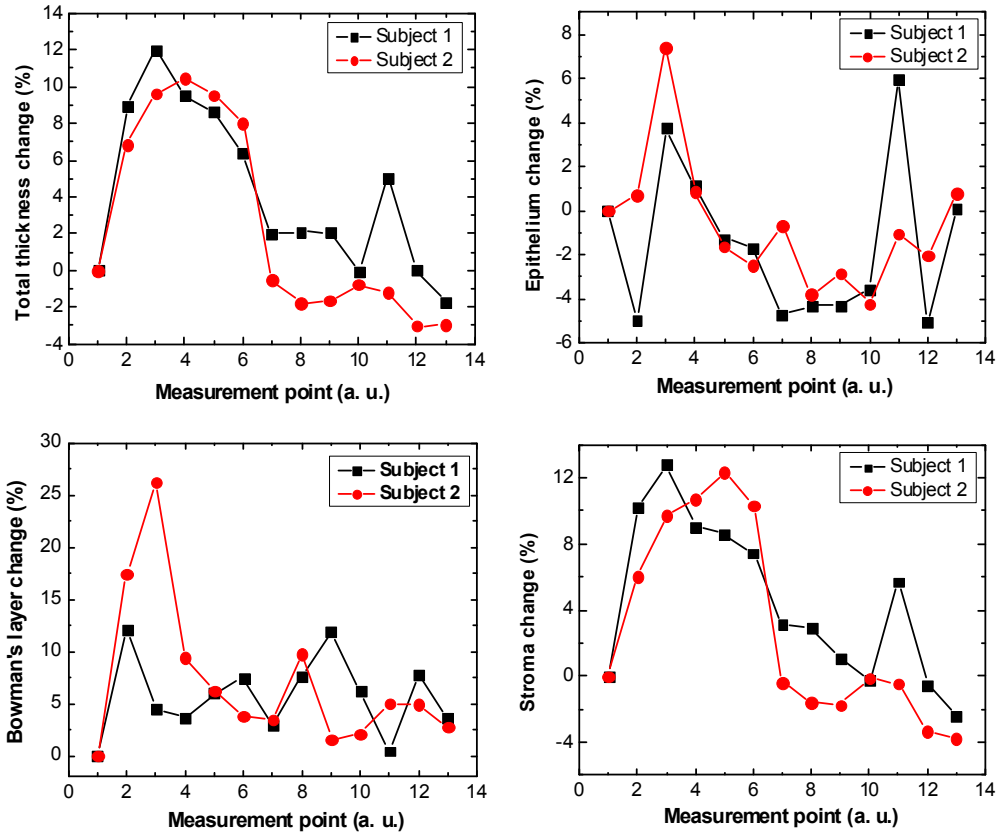

Figure 2 Graphical representation of the thickness of individual corneal laminae as a function of time (number of the measurement). Measurement #1 – control, before placement of the SCL, measurement #2 – with contact lens and after 3 hours of eye patch wear; measurement #3 – immediately after removal of the SCL; measurements #4 - #14 – spaced by time intervals of 10 -15 min.

(lateral x axial), corresponding to 5mm x 1mm in corneal tissue. Note that both images in fig.1 were enlarged by 50% in the vertical direction, for better visualization of the corneal layers. The separate laminae of the cornea are clearly observed, including the very thin layers such as the Bowmann's membrane and the Descemet's – endothelium complex (layers are marked with red arrows and labelled for clarity in fig.1, left). The corneal tomogram also reveals the fibrous structure of the stroma. The highly reflective "black" spots observed within the stroma are most likely knots, or crossings of the collagen fibers, randomly aligned in such a way as to work as "mirrors" and back-reflect the incident optical beam. The epithelial and Bowman's layers appear on the OCT tomogram to have more homogeneous structure as compared to the underlying stroma.

Figure 1 (right) shows the output of the semi-automated model based segmentation algorithm with red lines marking the boundaries of the individual corneal laminae. The algorithm uses enhanced intelligent scissor[18] (EIS), a semi-automatic segmentation method to identify epithelium (upper) and the endothelium (lower) layer of the cornea using minimal user input. Using these extracted boundaries, a correspondence model is established between the upper and lower layer of the cornea using medial axis transform (MAT) theorem. A discrete optimization method then uses parameter estimation to find the five corneal layer boundaries using Parzen-windows within the search space provided by the correspondence model. The optimization results can then be used to produce a mathematical model, in the form of a curve or a spline, for each boundary. The model based approach allows the method to be robust to speckle noise and regions with low signal to noise ratios. The output of the algorithm provides the layer thickness at each spatial location in the cornea, as well as the average thickness and standard deviation for each layer.

Results from the hypoxia experiment are summarized in fig.2, where total corneal thickness (top left), epithelial (top right), Bowman's membrane (bottom left) and stromal thickness (bottom right) are graphed as a function of the measurement number. Measurement number 1 corresponds to OCT images acquired prior to patching the eye, number 2 – with the lens, number 3 – immediately after the removal of the contact lens, numbers 4 to 14 correspond to subsequent measurements performed every 10-15min after the contact lens removal.

The total corneal thickness graph (fig. 2, top left) shows 7 – 9% swelling after 3 hours wear of soft contact lens and the eye patch. The swelling appears to increase by a few percent within the 15 – 30min after removal of the eye patch and the SCL. The rate of de-swelling of the total cornea thickness is fast (~30min delay between max and min thickness), and within 2.5 – 3h after removal of the SCL and eye patch, the total corneal thickness returns to normal. Similar behaviour is observed in the stroma (fig. 2, bottom right), however the swelling and de-swelling of the epithelial and Bowman's layer show very different patterns. We have observed an increase (~8%) of the epithelial thickness within 10 min after the removal of the SCL and the eye patch. Within the next hour, the corneal epithelium de-swells to a thickness ~4% below the control measurement, before the placement of the SCL. By the end of the experiment, ~2.5 hours after removal of the SCL, the corneal epithelial thickness returns to baseline values. The Bowman's membrane shows a sharp and significant change in thickness (~25%) in Subject 2, and ~10% in Subject immediately after the removal of the SCL. For the next 2.5 hours the thickness of the Bowman's layer appears to oscillate by a few percent about a steady value, ~5% larger than the thickness of this layer determined form the control measurement at the beginning of the experiment.

	Epithelium [μm]	**Bowman's Membrane [μm]**	**Stroma [μm]**	**Descemet's – Endothelium complex [μm]**	**Total corneal Thickness [μm]**
Before lens	**55**	**16**	**488**	**15**	**574**
Immediately after lens removal (0:00h)	**59**	**20**	**536**	**14**	**629**
Last measurement (2:41h)	**55**	**16**	**470**	**15**	**556**

Table 1 Thickness values of the individual corneal laminae as a function of time, measured in Subject 2.

Physical thickness values for the different corneal laminae, calculated from the UHROCT tomograms are shown in Table 1 for Subject 2. An average refractive index of 1.39 was used for all corneal layers to convert the optical distance measured by the UHROCT system, to physical thickness. The values of the total corneal and the epithelial thickness agree very well with previous measurements conducted in healthy subjects by use of commercial, low axial resolution OCT systems.

CONCLUSION

A high speed, UHROCT system was used to image the structure and boundaries of all corneal laminae in two healthy volunteers. A novel segmentation algorithm was designed and implemented for automatic determination of the thickness of the individual corneal layers. Results from the preliminary hypoxia study show distinctly different rates of swelling and de-swelling of the individual corneal layers, while greater similarity in the rates in observed for the stromal and total corneal thickness. In contrast to the total corneal changes provided by hypoxia, the changes in epithelium thickness, is much less. The reason might be due to an auto-regulation mechanism effecting corneal function in response to lens wear, postulated by previous studies[11]. This investigation is being continued using more volunteers to systematically examine the effects of hypoxia-induced edema on the corneal laminae. There are other factors that have to be taken into consideration while studying the effect that hypoxia may have on the corneal structures, such as diurnal variation, altitude[19] and different types of contact lenses.

ACKNOWLEDGEMENTS

The authors wish to thank Y. Feng for assistance with the corneal measurements and H. Van der Heide and J. Szubra of the University of Waterloo Science shop for their help with electronic and mechanical work on the imaging system. We would like to acknowledge financial support provided by NSERC and the University of Waterloo.

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