Objective Measurements of Corneal Light-Backscatter during Corneal Swelling, by Optical Coherence Tomography

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PURPOSE. To demonstrate that corneal light-backscatter can be measured objectively during corneal swelling by optical coherence tomography (OCT).

METHODS. One eye (randomly selected) of 20 non–contact-lens wearers (10 men and 10 women; mean age, 35.6 ± 9.6 years) was patched during 3 hours of soft contact lens (SCL) wear. The contralateral eye acted as the control. Central corneal images were captured before and after SCL wear at 20-minute intervals over 100 minutes using optical coherence tomography (OCT) to obtain corneal thickness and light-backscatter profiles. OCT backscattered light of the epithelial layer (decided by the thickness measurements) and 10 equally divided layers of the remaining cornea were analyzed with a custom software program. Two baseline measurements were taken at different visits before lens wear to test the repeatability of light-backscatter measurements.

RESULTS. From two baseline measurements, repeated measurements showed good repeatability of normalized backscatter results. Immediately after contact lens removal, total central corneal thickness increased significantly by 13.8% (mean ± SD) compared with baseline (P = 0.0001, paired t-test) and then decreased during the deswelling course. Corneal backscattered light changed significantly (repeated-measure ANOVA; F50,950 = 2.22, P = 0.0001) after lens wear, and a significant increase in backscatter was found in the epithelial layer (36.4%) and the most posterior corneal layer (55.6%) immediately after lens removal (post hoc test, P = 0.005). There was a strong correlation (r = 0.9375, P < 0.05) between the change in backscatter and corneal swelling during the deswelling period. The backscatter recovery rate was approximately the same for both epithelial and posterior layers after lens removal.

CONCLUSIONS. Light-backscattering analysis with OCT seems to be a promising and repeatable method of objectively measuring corneal backscatter. This study has demonstrated that corneal backscattered light increased in the anterior and posterior layers of the cornea during corneal swelling induced by contact lens wear and eye closure. (Invest Ophtalmol Vis Sci. 2004;45:3493–3498) DOI:10.1167/iovs.04-0096

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Corneal transparency and visual performance are currently receiving much attention, particularly after contact lens wear and excimer laser surgery. Corneal transparency is dependent on corneal microstructure and normal metabolism. Any disturbance, such as corneal edema and surgery can compromise corneal transparency, resulting in reduced contrast sensitivity and increasing susceptibility to glare.1–5 The loss of corneal transparency induces increased corneal light-backscatter, which is estimated in many clinical conditions by different approaches.4–10 Arbitrary scales have been used as an objective method in most clinics, either by differentiating mild, moderate, or severe haze or by using a scoring scale of 1 to 4.11,12 Kikkawa and Hirayama12 used slit lamp photography to assess light scatter in an attempt to establish the relationship between corneal swelling and light-scattering. Recently, high-frequency ultrasound,13 Scheimpflug photography,14 confocal microscopy through-focusing (CMTF),15,16 and other light-scatter measurements7–9 have been reported; however, none of these is commonly used clinically.

Optical coherence tomography (OCT) has been used to examine the ocular fundus and anterior segment in a noncontact and noninvasive way.16,17 Based on light-backscatter measurements detected with an interferometric technique, OCT has been reported to measure corneal and epithelial thickness across the cornea16–20 and precorneal tear film thickness.21 However, there is no report so far that has attempted to quantify OCT light-backscattering of the cornea and its clinical application for objective estimation of corneal transparency.

This experiment was designed to determine the feasibility of quantifying light-backscatter before and after corneal swelling was induced with soft contact lens wear and eye closure. In addition, a system was designed to segment the cornea into distinct layers from anterior to posterior to determine whether there were regional differences in light-backscattering.

METHODS

Subjects
Twenty subjects (10 men and 10 women; age, 35.6 ± 9.6 years) with no history of contact lens wear or any current ocular or systematic disease were recruited for this study. Informed consent was obtained from each subject after ethics approval was obtained from the Office of Research Ethics, University of Waterloo. All subjects were treated in accordance with the tenets of the Declaration of Helsinki.

Instrumentation and Lenses
An OCT system (Carl Zeiss Meditec, Dublin, CA) was used in this study. Principles of OCT and measurement of the cornea has been described previously.17,18,20,22,23 In this study, we measured corneal thickness as the distance between the first and last peaks and epithelial thickness as the distance between the first and second peaks, as described in other studies.20,22–24 To ensure that the OCT scanned the central location, a central fixation target within the OCT was provided, and a TV monitor was used to view the scan area. When the observation and scanning axes were coaxial, the scanning axis was orthogonal to the corneal...
surface, and a clear reflection was obtained on the monitor, the OCT image was recorded. Using this method, high repeatability of epithelial and corneal thicknesses was obtained. A single-line scan mode was used to measure corneal thickness, with a scan length of 1.13 mm. There were 100 sagittal scan points in each OCT measurement, which were used to measure corneal thickness, with a scan length of 1.13 mm. Normalized corneal backscatter was introduced and calculated by the following equation

\[
\text{Normalized backscatter} = \frac{\text{mean light reflectivity of each layer}}{\text{mean light reflectivity of the whole cornea}}
\]

Data analysis was conducted on computer (Statistica; StatSoft, Inc., Tulsa, OK). Paired t-tests were used to determine whether there were pair-wise differences \( (P < 0.05) \). Repeated-measurement analysis of variance (Re-ANOVA) and paired t-tests were used to determine whether there was a significant change \( (P < 0.05) \). Repeatability was calculated as the standard deviation of the differences between the test and retest results at each position before lens wear.

**RESULTS**

Normalized corneal backscatter profiles of two baseline visits before lens wear showed good repeatability (standard deviation of the difference of the normalized backscatter between two visits: 0.07) as shown in Figure 1. The magnitudes of backscattered light were different among corneal sections (slices) from the epithelium to the posterior stroma (ANOVA: \( F_{(10,950)} = 30.24, P = 0.000 \)). Immediately after contact lens removal, central corneal thickness increased significantly (15.8\% \pm 2.3\%; mean \pm SD) from baseline \( (P = 0.0001, \text{paired } t\text{-test}) \) then decreased during the deswelling course. The profile of corneal backscattered light changed significantly (Re-ANOVA: \( F_{(50, 950)} = 2.22, P = 0.0001 \)) after lens wear (Fig. 2) and significant increases were found in the epithelium (EP: 36.4\%), anterior (slice \[S1]: 19.4\%), and posterior stromal layers (S10: 35.6\%) immediately after lens removal (post hoc test, \( P = 0.005 \)), compared with baseline, but returned to baseline by 100 minutes. The anterior (S1: \( P = 0.005 \)) and posterior (S10: \( P = 0.003 \)) stromal backscattered light increased significantly compared to the middle layers (S6). Figure 3 shows examples of OCT images of central (1.13 mm) corneal scans before lens insertion and immediately after lens removal. The OCT image shows increased backscattered light depicted in the red and yellow. The quantitative measures of the backscattered light are represented by the reflectivity profiles. The "After" profile shows how the peaks have altered. The graphs of the slice profiles illustrate how the backscattered profile has become bowl shaped, reflecting an increase in light-backscattering in the epithelium, anterior, and posterior stromal layer (lower right graph). There was a strong correlation \( (r = 0.937, P < 0.05) \) between the changes in light-backscattering (the integrated area under each profile curve) and corneal swelling during the deswelling period, as indicated in Figure 4.

Figure 5 shows the light-backscattering recovery of the anterior and posterior stromal layers during the deswelling period. The Pearson correlation \( (r) \) was 0.745 for the anterior layer (S1) and 0.934 for the posterior stromal layer (S10). According to exponential regression, the recovery rate (slope) was \(-1.08\) for the anterior layers and \(-0.906\) for the posterior layers (no significant difference between the two slopes, \( P > 0.05 \)). The recovery of backscattered light in the epithelium and posterior stromal layer correlated positively with corneal deswelling, as shown in Figure 6 (Pearson correlation: \( r = 0.938 \) for the epithelium and \( r = 0.982 \) for the posterior stromal layer).
There was no significant change in light-backscatter in the control eyes (Re-ANOVA: $F_{20, 380} = 0.72, P > 0.05$) compared with baseline. However, total corneal thickness in the control eyes changed significantly during the post-lens-wear period (Re-ANOVA: $F(4,76) = 5.57, P < 0.003$), due to thinning at 60 (0.6%) and 100 (0.5%) minutes compared with baseline (post hoc test: $P < 0.004$).

**DISCUSSION**

A novel method was demonstrated in this study to quantify corneal backscattered light in different layers of the cornea by using OCT. After normalization of the OCT data, a repeatable profile was found, as illustrated in Figure 1. In these slice profiles, each data point represents mean light-backscatter in the slice (EP and S1-10) and the changes within the slice were demonstrated quantitatively.

Allemann et al. quantified ultrasonic acoustic backscatter in the anterior 150mu of the stroma in excimer laser-ablated rabbit corneas and found increased acoustic backscatter after surgery. Møller-Pedersen et al. used confocal microscopy through focusing (CMTF), for objective estimation of the haze in the cornea after photorefractive keratectomy (PRK) by digital image analysis based on the light reflectivity of a series of images obtained by confocal microscopy. They found a significant correlation between the objective CMTF haze estimate and clinical haze grading using slit lamp examination. However, the methods used in these studies require direct contact with the cornea. OCT is a noncontact method and has been used to demonstrate an increase in backscattered light at the interface between the corneal flap and the bed after LASIK.

**FIGURE 2.** The changes of normalized backscattered light after lens removal after 3 hours of lens wear and eye closure showed a bowl-shaped pattern due to an increase in epithelial (EP) and posterior stromal layers immediately after lens removal and returning to a relatively flat pattern at 100 minutes.

**FIGURE 3.** Examples of OCT light-backscattering two-dimensional images of the central cornea (1.13 mm) before (top left) and after lens wear (top right). The increase in the red/orange colors is indicative of increased light-backscattering. The magnitude of backscattered light is shown in the reflectivity profiles, higher after lens wear (middle right) in the epithelium, anterior stromal layer (S1), and posterior stromal layer (S10) than that before lens wear (middle left). The graphs illustrate the different normalized backscattered-light profiles before (bottom left) and how light-backscattering increased in the epithelium, anterior (S1) and posterior (S10) stromal layers after lens wear (bottom right).

**FIGURE 4.** Integrated normalized backscatter (percentage of the cornea) versus corneal swelling during the recovery period after 3 hours of lens wear and eye closure. There was a strong correlation between the changes of the light-backscattering (the integrated area under each profile curve) and corneal swelling during the deswelling period.

**FIGURE 5.** Light-backscatter recovery in the anterior and posterior stromal layers.
Using a slit lamp-adapted OCT system, Wirbelauer et al. demonstrated a localized hyper-reflective area, that was found to correlate with a corneal haze after excimer laser phototherapeutic keratectomy (PTK), but the increase was visualized with OCT and not quantified. The authors admitted the limitation of the quantification of increased light-backscatter amplitude because of the variable height and steepness of the slopes of the reflection spikes.

The cornea transmits approximately 90% of the incident light, but is not homogeneous. Normalized backscattered light profiles of the normal cornea (before lens wear) in our study showed a gradual increase in backscattered light from the anterior to posterior stroma as shown in Figures 1, 2, and 3. In the stromal layer, collagen fibrils in the lamellae lie parallel to each other and are nearly uniform in diameter, but increase in diameter from approximately 19 nm in the anterior stroma to 35 nm near the posterior stroma. A stromal hydration gradient was predicted as early as 1979 and measured by others. Komai and Ushiki demonstrated water gradients across the bovine cornea and found that corneal water content was a function of the depth of the cornea from the epithelium. This hydration gradient would be expected to provide a backscattered-light gradient, with the region of greatest hydration (most posterior) inducing the greatest backscatter and the zone of least hydration producing the least. We found a light-backscatter gradient across the cornea.

OCT-detected backscatter (as shown in Fig. 3), which includes light that is specularly reflected from keratocyte nuclei, is similar to that detected by a confocal microscope with similar spikes. Many studies indicate the keratocyte density is greatest in the anterior stroma in the human cornea. Although keratocyte nuclei cause reflectivity, which dominates backscatter when detected at specular angles, the backscatter obtained with the OCT does not appear to depend solely on keratocyte density. If the backscatter is associated solely with keratocyte density, we would have found a decrease (instead of an increase) in backscattering in the posterior stroma during corneal swelling, because the cell density decreased.

The refractive index of the anterior stroma was found to be greater than the posterior stroma in the bovine and human cornea. Patel et al. suggested that the corneal refractive index varies along corneal depth and is most likely a function of local hydration. Results from an in vitro study of bovine eyes showed the refractive index decreased from 1.379 to 1.376 in the anterior stroma and from 1.373 to 1.367 in the posterior stroma when corneal swelling was induced (Dennis S, et al. IOVS 2003;44:ARVO E-Abstract 886). Backscattered light has been found to be closely related to corneal swelling, although Kikkawa and Hirayama have paradoxically shown that the relationship was dependent on layers of the cornea. They found little anterior corneal swelling with intense light-scattering and the opposite effect in the posterior corneal layers. We found a strong correlation between recovery of backscattered light in the epithelium and posterior stroma, and corneal deswelling. This suggests that corneal backscattered light in the epithelium may relate to the distortion of the microstructure (not necessarily thickness changes) and the backscattered light in the stroma may be related to local hydration and refractive index. Further studies are needed to establish a method to predict localized corneal swelling from the changes in backscattered light after contact lens wear.

In our study, backscattered light was analyzed in each slice of the cornea after 3 hours of contact lens wear and eye closure. A significant increase of backscattered light was found in the epithelium and posterior stromal layer, in agreement with previous observations. The changes of corneal transparency from corneal swelling has been well documented, but reduction of vision was only found if significant swelling was induced. Lambert and Klyce found increased light-scattering in the epithelium during corneal swelling induced by oxygen deprivation and demonstrated how this caused subjective halos. They concluded that the visual halos were due to the change in transparency of the epithelium, although, paradoxically, epithelial thickness did not increase. The absence of epithelial increase from hypoxia has been found by others. Corneal striae and folds (localized light scatter) appear in the posterior stroma accompanying corneal swelling after contact lens wear. Korb and Exford described the phenomenon of central corneal clouding due to PMMA lens wear. Farris et al. and Korb and Exford reported that this phenomenon is due to a localized epithelial response to PMMA lens wear, resulting in a focal increase of backscattered light.

The light-backscattering changes that are demonstrated in Figure 2 support the theory that the stroma has regions with distinctive swelling properties. The anterior and posterior corneal layers prevent excessive inhibition of fluid, and removal of either layer results in corneal swelling. Cristol et al. reported that stromal swelling occurred more rapidly through the posterior corneal surface than the anterior surface in human and rabbit eyes. This is probably due to the anterior stroma having narrower and more interwoven lamellae. The anterior and posterior corneal layers are more vulnerable to swelling than the central stroma because of the proximity of the aqueous and precorneal tear film. Our results support this, because backscattered light increased in the anterior (S1) and posterior (S10) layers compared and not in the midstroma.

Our results showed that light-backscattering recovery in the epithelium and posterior stroma occurred simultaneously after lens removal, which supports the theory that active mechanisms are present to maintain corneal thickness and thus corneal transparency. The normalization used to analyze the OCT data caused an apparent decrease in light-backscatter in the midstroma because of the increase in the epithelium and posterior stroma, which caused a net increase in light-scattering by the cornea. Therefore, the layer without light-scatter changes (or small changes) would be shown as a relative decrease (such as the slice shown in Fig. 2) due to the normalization process. The increase of backscattered light of the anterior and posterior layers does not seem to be a random effect, as evidenced by the systematic course of recovery after lens removal.
In summary, light-backscatter analysis with OCT seems to be a promising method for the objective estimation of corneal light-backscattering. Corneal light-backscattering increases in the epithelium and posterior stromal layer of the cornea during corneal swelling induced by contact lens wear and eye closure.

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**References**


