Objective Measurement of Backscattered Light from the Anterior and Posterior Cornea In Vivo

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Purpose. To develop an objective and repeatable method of measuring corneal backscattered light from different depths of the cornea in vivo.

Methods. A modified slit lamp (“scatterometer”), with a video camera and synchronous white strobe light, was used to capture images of a 0.1-mm-wide slit beam through the cornea. Image analysis software was developed to measure backscatter from digitized high-magnification images of 82 normal corneas of 41 subjects. Forty eyes of 20 of the same subjects were examined again after 1 month. Mean backscatter from the anterior, middle, and posterior thirds of the cornea was compared between repeated measurements, and expressed in arbitrary scatter units (SU).

Results. Backscatter in the anterior third of the cornea was 451 ± 42 SU (mean ± SD, n = 82), from the middle third was 274 ± 29 SU (n = 82), and from the posterior third was 242 ± 28 SU (n = 82). The difference in backscatter measured a month apart was 5 ± 27 SU (P = 0.54), 2 ± 17 SU (P = 0.42), and 0 ± 15 SU (P = 0.95) in the anterior, middle, and posterior thirds of the cornea, respectively. Minimum detectable differences between measurements were 12, 8, and 7 SU in the anterior, middle and posterior thirds, respectively (α = 0.05, β = 0.20, n = 40).

Conclusions. Backscatter can be measured at different depths of the cornea from high-magnification digitized images of a narrow slit beam through the cornea. The method is objective and repeatable and can be applied in prospective studies of deep and posterior lamellar keratoplasty. (Invest Ophthalmol Vis Sci. 2007;48:166–172) DOI:10.1167/iovs.06-0767

Lamellar keratoplasty has had a resurgence as an optical procedure over the last decade to treat a variety of corneal disorders.1-2 Deep anterior lamellar keratoplasty has been proposed as an alternative to penetrating keratoplasty for keratoconus with the advantage of preserving the host endothelium.3,4 A variety of techniques for posterior lamellar keratoplasty have emerged to treat endothelial dysfunction, with advantages over penetrating keratoplasty of lower postoperative astigmatism, shorter recovery time, and small-incision surgery.5-10

Optical lamellar keratoplasty is a technically challenging procedure in which the final visual outcome may be limited from the irregular lamellar interface,11 possibly by the induction of increased forward light scatter and degradation of the retinal image. During slit lamp examination, haze enables visualization of the interface after lamellar keratoplasty and is graded subjectively. However, an objective method of quantifying haze would enable assessment of small changes in corneal clarity after lamellar surgery. We have designed a noncontact method for objectively measuring backscattered and reflected light—which, if pathologic, collectively constitute haze12—from different depths of the cornea, and we refer to the instrument as a “scatterometer.” We describe our instrument and technique for measuring backscattered and reflected light from normal human corneas in vivo. Similar to most reports of corneal backscatter, we have used the term “backscatter” broadly in this report to include reflected light.

Methods

Instrument

In order to measure backscatter, we used a video pachometer, which has been previously described,13 and modified its image-analysis software. Briefly, a low-level video camera was mounted to the camera arm of a photographic slit lamp (Carl Zeiss Meditec, Inc., Thornwood, NY) and captured images through the left optical element of the binocular biomicroscope. A slit beam 0.1 mm in width entered the cornea at an angle of 55° to the viewing (camera) axis (Fig. 1). The slit beam was aligned with the center of the cornea by using red alignment lights mounted at the vertical center on each side of the objective lens assembly, while the subject viewed a green fixation light. Magnification was changed from ×25 in the previous study13 to ×40 in the present study.

After correct alignment of the microscope with the cornea, images were acquired by stepping on a foot switch, which synchronously triggered a photoflash and captured an image. Images were digitized and transferred to computer memory.

Image Analysis

Each image was composed of an array of pixels (460 × 640), and each pixel (8-bit grayscale or 256 intensity units) represented the brightness at the respective location in the image. An edge-detection algorithm determined the boundaries of the cornea in images as previously described.13 Each pixel extended approximately 3 μm in the horizontal dimension.

After the boundaries of the cornea were detected, the horizontal position of the pixel with maximum grayscale intensity corresponding to the epithelium in every horizontal scan line was fitted to a second-order polynomial. All horizontal scan lines were shifted by an amount determined by the polynomial to straighten the image of the cornea (Fig. 2). The mean intensity of corresponding pixels in all horizontal scan lines was calculated to generate the intensity profile of the image of each cornea (Fig. 2).

Calibration

The intensity of images was standardized to intensity measured from a reference at every examination, to account for variations in the incident light intensity and the sensitivity of the optical system and camera. The camera and light source were allowed to stabilize for 15 minutes.
before the images were acquired. We used a solid piece of fluorescent glass with a convex anterior surface as the reference. Before examining each subject, at least three images of the fluorescent glass reference were acquired, and the mean intensity ($I_R$) was measured at 450 μm deep to the anterior surface of the glass. $I_w$ was the mean intensity of the same measurement during a reference session. Corneal intensities were multiplied by a factor $C$ to adjust for variation in the light source and sensitivity of the camera with time, where $C = I_w/I_R$. $I_w$ was the same for every subject in this study and for all other studies with our instrumentation. In this situation, the fluorescent glass reference was remeasured and all intensities from the cornea were multiplied by the appropriate calibration factor.

The intensities (grayscale) in the images were converted to arbitrary units of backscatter which we termed “scatter units” (SU). We defined 1 SU as the backscatter produced by a solution with a turbidity equal to 1 NTU (nephelometric turbidity unit), a standard measurement of turbidity. A commercial turbidity standard solution, (AMCO Clear; GFS Chemicals Inc., Powell, OH), supplied at 4000 NTU was diluted to turbidities from 200 to 1500 NTU. The solutions were placed between two contact lenses separated by 0.8 mm by spacers, and backscatter from the solution was measured by the scatterometer. The relationship between image intensity (grayscale) and turbidity was linear from 200 to 1500 NTU, and this included the range of image intensities from normal corneas. Backscatter from the cornea was expressed in SU, meaning the same backscattered light produced by diluted turbidity standard solution of numerically equivalent turbidity (NTU).

FIGURE 1. Representation of the scatterometer. The apparatus is the same as the video pachometer that has been described, with modifications made to the image analysis software. The instrumentation is a photographic slit lamp (Carl Zeiss Meditec, Inc., Thornwood, NY), modified to incorporate a video camera with synchronous flash. Reprinted, with permission, from McLaren JW, Bourne WM. A new video pachometer. Invest Ophthalmol Vis Sci. 1999;40:1593–1598. ©Association for Research in Vision and Ophthalmology.

Corneal thickness was calibrated with images of contact lenses of known thickness, as previously described.

FIGURE 2. (A) Unmodified image of a normal cornea shows peak backscatter from the anterior corneal surface, lower backscatter from the stroma, and slightly higher backscatter (than the stroma) corresponding to the endothelium. The circles adjacent to the image of the cornea are reflections of the alignment lights from the corneal surface. (B) For each horizontal scan line in the image, the anterior and posterior boundaries of the cornea were identified as the intersection of the line fitted to the maximum anterior slope of the epithelial peak or the maximum posterior slope of the endothelial peak, with the respective background intensity. The outer lines (a) represent the pixels in each horizontal scan line that form the boundaries of the cornea; the middle lines (b) represent the pixels that correspond to the maximum anterior or posterior slopes of the epithelial and endothelial peaks, respectively; and the inner lines (c) represent the pixels that correspond to the maximum intensity from the epithelium and endothelium. The pixel with maximum grayscale intensity corresponding to the epithelium in every horizontal scan line was fitted to a second-order polynomial. All horizontal scan lines were shifted by an amount determined by the polynomial to straighten the image of the cornea. The mean grayscale intensity of corresponding pixels in all horizontal scan lines was calculated to generate the grayscale intensity profile of the image of each cornea.

Subjects

Forty-one subjects were recruited from patients attending Mayo Clinic and from staff and their families. Both corneas of each subject were examined, and subjects were excluded if they had any corneal abnormalities or a history of anterior segment disease, ocular trauma or surgery, diabetes mellitus, or use of ocular medications. Systemic medications were permitted unless they were known to affect the cornea or anterior segment. The mean subject age was 31 ± 9 years (mean ± SD, range, 21–54 years). The study was approved by our Institutional Review Board and adhered to the tenets of the Declaration of Helsinki. Informed consent was obtained from all subjects after explanation of the nature and possible consequences of the study.

Corneal backscatter was measured from both eyes of all 41 subjects, and both eyes of 20 of these subjects were examined for a second time 1 month later. Subjects were stabilized in the chin rest and fixated the green target light. The slit beam of the microscope was aligned with the center of the left cornea and at least five images were acquired within 2 minutes. The procedure was repeated for the right eye. All
images were acquired by one observer. Central corneal thickness was measured after image acquisition by using an ultrasonic pachometer (DGH 1000; DGH Technologies, Inc., Frazer, PA) as the mean of three measurements.

Data Analysis

The five best images of each cornea were selected by the same observer, ensuring correct alignment, and the mean of the intensity profiles was calculated and used for analysis. Corneal thickness was calculated, and depth in the cornea was expressed as a percentage of corneal thickness. The intensity profile for each cornea was divided into anterior (0%–33%), middle (34%–66%), and posterior (67%–100%) thirds and the mean grayscale intensity in each third of the cornea was calculated.

Repeatability of backscatter (from the anterior, middle, and posterior thirds of the cornea) and corneal thickness among the five images of each cornea was assessed by calculating the coefficient of variation (SD divided by the mean). The means of these coefficients were calculated from six randomly selected eyes of six subjects.

The differences between backscatter and thickness measurements of the same corneas on two different days were compared. Agreement between backscatter from the right and left eyes and between backscatter and corneal thickness on different days was assessed by expressing the difference between measurements as a function of the mean of the measurements, as described by Bland and Altman.15

Correlations between eyes and between repeated measurements were also assessed.

Corneal thickness measured by the scatterometer was compared to corneal thickness measured by the ultrasonic pachometer, and the correlation and agreement were also assessed. Correlations between corneal backscatter and age and between backscatter and corneal thickness were examined.

Differences between eyes were assessed by using two-tailed paired Student’s t-tests for means if the data were normally distributed and Wilcoxon signed-rank tests if they were not. Because we included both eyes of each subject in our analysis, we used general estimating equation (GEE) models to account for correlation between the eyes.16 All reported probabilities are based on the GEE analysis, and P < 0.05 was considered statistically significant. Correlations were also assessed by using GEE models and Pearson’s correlation coefficients. Minimum detectable differences (MDDs) were calculated for nonsignificant differences assuming that there were 40 independent observations (α = 0.05, β = 0.20).

RESULTS

Corneal Backscatter

Peak backscatter originated from the anterior corneal surface of normal corneas, and a smaller peak was usually associated with the posterior surface (Fig. 3). The mean coefficients of variation of repeated estimates of intensity from the five selected images of a cornea were 0.052, 0.047, and 0.045 in the anterior, middle, and posterior thirds of the cornea, respectively.

At the first examination, mean backscatter from the anterior third of the cornea was 451 ± 42 SU (scatter units, mean ± SD), mean backscatter from the middle third was 274 ± 29 SU, and from the posterior third was 242 ± 28 SU. Forty corneas of 20 (of the original 41) patients were re-examined 1 month later, and mean backscatter from the anterior third was 445 ± 43 SU, from the middle third was 270 ± 30 SU, and from the posterior third was 238 ± 31 SU.

Corneal thickness measured by the scatterometer was compared to corneal thickness measured by the ultrasonic pachometer, and the correlation and agreement were also assessed. Correlations between backscatter from the right and left eyes and between backscatter and thickness measurements of the same corneas were 0.95, MDD = 12 SU) in the anterior third, 2 ± 17 SU (P = 0.42, MDD = 8 SU) in the middle third, and 0 ± 15 (P = 0.95, MDD = 7 SU) in the posterior third (Fig. 4B).

Age correlated weakly with backscatter from the anterior third of the cornea (r = -0.29, P = 0.04, n = 82 eyes), but not with backscatter from the middle third (r = 0.10, P = 0.44, n = 82 eyes) or posterior third (r = 0.05, P = 0.70, n = 82 eyes, Fig. 5A).

Corneal Thickness

The coefficient of variation of corneal thickness measured from the same corneas was 0.010. Corneal thickness was 527 ± 32 μm (n = 82 eyes; range, 446–590 μm) with the scatterometer and 557 ± 31 μm (n = 82 eyes; range, 478–615 μm) with the ultrasonic pachometer (P < 0.001). Although corneal thicknesses measured by the two methods correlated well (r = 0.77, P < 0.001, Fig. 6A), they differed by 30 ± 21 μm (P < 0.001, Fig. 6B). The difference between repeated measurements of corneal thickness with the video pachometer (baseline minus 1 month) was −4 ± 14 μm (n = 40 eyes, P = 0.12, MDD = 6.4 μm, Fig. 7). Corneal thickness did not correlate with backscatter from the anterior (r = -0.05, P = 0.83, n = 82 eyes), middle (r = -0.14, P = 0.82, n = 82 eyes), or posterior (r = -0.06, P = 0.41, n = 82 eyes) thirds of the cornea (Fig. 5B).

DISCUSSION

Corneal backscatter measurement from high-magnification digitized images of a narrow slit beam through the cornea is objective and repeatable. This method can be used to measure backscatter from different depths of the cornea in prospective studies. The instrument that we demonstrated is based on a video pachometer, which has been described, and simultaneously measured corneal thickness.

With our scatterometer, backscatter from fellow corneas correlated highly, and the low mean and SD of the difference between two backscatter measurements of the same corneas on different days indicated high agreement between repeated...
measures with this technique (Fig. 4B). The variation in mean backscatter from the anterior third of the same corneas on different occasions was slightly greater than from the middle and posterior thirds of the cornea (Fig. 4B), and this may have been caused by variations in the anterior corneal surface altering the amount of reflected light. Mean corneal thickness (527 ± 52 μm) was similar to mean thickness measured in an earlier study (514 ± 20 μm), in which the same instrument was used at lower magnification. Corneal thickness measured by the scatterometer was 30 ± 21 μm thinner than measured by ultrasonic pachometry, as has been shown with other optical methods.

The finding that corneal backscatter did not correlate with corneal thickness agrees with the results of Olsen and is because backscatter was calculated as mean intensity in a fixed layer of tissue and not the total backscatter from the entire cornea. Backscatter from the anterior, middle, and posterior thirds of the cornea fell within a narrow range with few outliers, regardless of corneal thickness (Fig. 5B). Backscatter did not correlate with age (Fig. 5A), although we did not have a uniform age distribution of subjects. Smith et al. also found no correlation between backscatter and age measured from Scheimpflug images, whereas Olsen found that backscatter increased with age, although the increase could not be attributed to increasing corneal thickness. Olsen used blue light in his study, and the increased scatter of blue light may account for this difference with our study.

Instruments and methods for objectively quantifying corneal haze have been described and predominantly been used to study haze after excimer photoablation of the cornea. Olsen measured backscatter in vivo from normal corneas and corneas after cataract surgery. He used a modified slit lamp similar to our instrument, with a narrow (0.16 mm) slit and blue light to measure backscatter from the entire thickness of the cornea. McCully et al. measured haze after phototherapeutic keratectomy in rabbits. Their instrument was also a modified slit lamp, but with a wide (2.5 mm) slit and green light. Other methods for measuring corneal backscatter have included Scheimpflug photography and confocal microscopy in vivo.

Lohmann et al. made an important contribution to measuring corneal haze by designing their slit lamp scatterometer with polarizing filters to discriminate between backscatter and reflectance. Incident light on the cornea may be transmitted, absorbed, scattered, or reflected. Corneal haze, as seen by slit lamp biomicroscopy, consists of backscattered and reflected light, and reflected light has little, if any, effect on the retinal image or visual perception. Because reflected light maintains polarization of the source, Lohmann et al. effectively separated reflectance from backscatter by using crossed polarization. With their method, Lohmann et al. correlated corneal backscatter with forward light scatter within the whole eye in five patients after photorefractive keratectomy.

Figure 4. (A) Backscatter correlated between the right and left eyes in the anterior \((r = 0.90, P < 0.001)\), middle \((r = 0.83)\) and posterior \((r = 0.79)\) all third of the cornea in all 41 subjects. (B) Backscatter measured from 40 of the same corneas on different days correlated highly in the anterior \((r = 0.82)\), middle \((r = 0.85)\), and posterior \((r = 0.88; P < 0.001)\) thirds of the cornea. The mean differences between paired measurements were close to 0, as indicated by the data lying very close to the identity line (dashed line).

Figure 5. (A) In the 41 subjects, age correlated weakly with backscatter from the anterior third of the cornea \((r = -0.29, P = 0.04)\), but not with backscatter from the middle \((r = 0.10, P = 0.44)\) or posterior \((r = 0.05, P = 0.70)\). (B) In the 82 eyes, corneal thickness did not correlate with backscatter from the anterior \((r = -0.05, P = 0.83)\), middle \((r = -0.14, P = 0.82)\) or posterior \((r = -0.06, P = 0.41)\) thirds of the cornea.
Unfortunately, our scatterometer could not distinguish between backscattered and reflected light. We attempted to incorporate polarizing filters into our design, but images of the cornea in crossed polarization were essentially extinguished because our slit beam was narrow (0.1 mm) and magnification was high (40×). By widening the slit beam to 1 mm, as described by Lohmann et al. and reducing magnification to 10×, we were able to separate backscatter from reflectance, but were no longer able to resolve depth within the cornea. Because our goal was to measure haze from different depths, we designed our scatterometer with a narrow slit beam and without polarizing filters. Although we encountered a narrow, bright reflection from the anterior corneal surface (Fig. 2) with our design, this did not significantly mask light emanating from deeper within the cornea.

The spatial resolution of our scatterometer is a significant advantage over previously described instruments. Smith et al. measured backscatter from the midstroma, but were limited to measuring a 0.26-mm-thick layer in their Scheimpflug images. With our scatterometer, images of corneas with an average thickness were represented by approximately 175 pixels. The spatial resolution of our scatterometer was further enhanced by using a photographic flash of less than 5 μs in duration, which prevented degradation of images by fine ocular movements.

Our slit lamp scatterometer was noninvasive and easily accommodated in the clinical setting. Acquisition of five images of each cornea took less than 2 minutes, and subsequent image analysis was automated, reducing the influence of subjective errors, and took less than 1 minute per cornea. By expressing backscatter in SU, which were numerically equivalent to the turbidity of a commercially available solution, we will be able to compare data between different studies. Measuring backscatter from the interface after optical lamellar
Objective Measurement of Corneal Backscatter

FIGURE 8. Backscatter profiles of an individual cornea at 1 and 6 months after DLEK. The backscatter profile of a normal cornea is shown for comparison. One month after DLEK, increased backscatter in the posterior cornea corresponded to the lamellar interface and endothelium, but increased backscatter was also present in the anterior cornea, corresponding to the subepithelial region. Six months after DLEK, backscatter decreased in the posterior cornea, but remained elevated in the anterior cornea.

keratoplasty may help to explain the limitation in visual acuity that is frequently observed after such procedures.1 Figure 8 shows the backscatter profile of one cornea at 1 and 6 months after deep lamellar endothelial keratoplasty (DLEK). At 1 month, backscatter in the posterior cornea was increased compared to normal, and this corresponded to the location of the lamellar interface and donor endothelium. Of interest, backscatter in the anterior cornea was also increased compared to normal, and this corresponded to changes in the subepithelial region. At six months after DLEK, backscatter from the posterior cornea had decreased, whereas backscatter from the anterior cornea remained elevated. This clearly shows that there are two sources of corneal backscatter that may limit vision after posterior lamellar surgery (Baratz KH, et al. IOVS 2006; 47:ARVO E-Abstract 2372), and being able to separate these may prove important in future studies. Quantifying anterior and posterior backscatter will also be important when comparing newer techniques for posterior lamellar keratoplasty.9,33-34

The main limitation of our method is the inability to separate backscatter and reflectance.12 However, because only 20% of the total light detected may represent reflected light12,35 and because forward light scatter from the whole eye may correlate only with corneal backscatter when the latter is significantly abnormal,27 the combined measure of backscatter and reflectance from corneas after posterior lamellar keratoplasty may indeed be related to visual degradation. Although the relationship between corneal backscatter, forward light scatter and visual degradation may be complex in normal corneas,29,36-38 Lohmann et al.28 reported a correlation between corneal backscatter (without out reflectance) and low-contrast visual acuity. Therefore, the ideal scatterometer would combine high spatial resolution for depth measurements and polarizing filters, to separate backscatter from reflectance and is the subject of future investigation.

In summary, our method of measuring corneal backscatter is objective and repeatable, and allows measurement from different depths of the cornea. The method can be easily applied to prospective and comparative studies of corneas after different types of lamellar surgery.

References


