Standardization of Corneal Haze Measurement in Confocal Microscopy

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Purpose. Corneal stromal haze cannot be compared in longitudinal studies or across laboratories without standardization. In this study, a method was devised of standardizing image brightness in confocal microscopy of the cornea.

Methods. Thirty-six normal corneas of 18 untreated volunteers and 35 corneas of 18 patients 3 years after LASIK were examined by slit scanning confocal microscopy. The mean image intensity of each frame was adjusted for daily variations in sensitivity of the microscope by using scans through a solution of Amco Clear (GSF Chemicals, Columbus, OH). Adjusted image intensities were expressed in scatter units (SU), representing the concentration of Amco Clear that produced the same intensity as the image. The intensity from the stroma in corneas after LASIK was compared to that in untreated corneas by using generalized estimating equation models.

Results. In the untreated corneas, image brightness was 1079 ± 242 and 758 ± 142 SU in the anterior and mid stroma, respectively. Three years after LASIK, image intensity in the flap was 740 ± 186 SU, approximately 30% lower than in corresponding stroma of the untreated corneas ($P < 0.001$). At mid stroma, brightness was 715 ± 117 SU after LASIK, and was not significantly different from brightness in untreated corneas ($P = 0.26$).

Conclusions. Clinical confocal microscopy provides a high-resolution measurement of corneal haze, and Amco Clear provides a means of standardizing these measurements. This method can detect subtle decreases in haze in the corneal flap 3 years after LASIK and could be used to examine changes in haze after lamellar keratoplasty. (*Invest Ophthalmol Vis Sci.* 2010;51:5610–5616) DOI:10.1167/iovs.10-5614

Eye care professionals have for years assessed the clarity of the cornea by visual inspection with a slit lamp and have used a hazy stroma, epithelium, or endothelium as an indicator of a dystrophic or poorly functioning cornea. This assessment is subjective, and several devices and methods have been described to measure the brightness of light scattered or reflected from the cornea. Photomultiplier and other light-sensing systems have been mounted in the appropriate image plane of slit lamps[1,2] or custom scanning optical systems[3,4] to measure light backscattered from the cornea, and although many of these devices measured light from the full-thickness of the cornea, some allowed investigators to distinguish haze in the anterior, middle, and posterior cornea.[5] Current interest in factors that limit vision after penetrating, anterior, or posterior (endothelial) lamellar keratoplasty have generated a need to examine vision-limiting factors such as corneal haze after these procedures and to resolve the origin of haze in the corneal depth.[6–13] The haze that can develop after refractive surgery and contact lens wear has also stimulated an interest in measurement of scattered light from the corneal stroma.[3,4,14–16]

At best, the spatial resolution of brightness when assessed with a slit lamp and other methods has been limited to approximately a third of the corneal thickness.[3,5] Spatial resolution of the source of haze was greatly improved with the use of confocal microscopes to assess backscatter from the cornea.[9,17–20] With the depth of field of 11 to 26 μm,[21] investigators can identify the layer associated with pathologic scatter, measured as image brightness. Identifying the layer is particularly important when scatter is suspected to increase in surgical interfaces or in the subepithelial region after procedures such as refractive surgery and lamellar keratoplasty.

Most investigators have expressed corneal haze in terms of the specific units of image intensity from the instrument used for measurement. However, image brightness from the cornea or any other tissue can be compared with brightness measured at a different time in longitudinal studies or across laboratories only if the instruments are standardized so that units that express haze are equivalent. Brightness expressed as a digitized video signal is useful only for relative measurements from the same camera and varies depending on the settings of the video camera. Measurements can be standardized in two ways. First, image intensity should be expressed in terms that are meaningful and can be compared across laboratories. Second, measurements of haze must be adjusted for differences or variations in the brightness of the light source and the sensitivity of the light detector—variables that can change over hours as well as months. The standardization should be based on backscatter measured with the same instrument from a substance that does not change from day to day, can be reproduced in any laboratory, and is readily available.

In this article, we propose a method of standardizing image brightness for measurement of scattered and reflected light from the cornea in confocal microscopy. We describe the scatter characteristics from the cornea from two clinical confocal microscopes with different optical designs and characteristics, the ConfoScan 4 (Nidek Technologies, Fremont, CA) and the Tandem Scanning confocal microscope (Tandem Scanning Inc., Reston, VA). This standardization technique was used to measure corneal haze from normal untreated human corneas and from a group of corneas 3 years after laser in situ keratomileusis (LASIK), to demonstrate subtle differences in haze in specific layers of the corneal stroma.

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METHODS

Human Subjects

Thirty-six normal corneas of 18 untreated volunteers (8 men, 10 women) were examined with both confocal microscopes. All participants were examined by slit-lamp biomicroscopy to assure that their corneas and anterior segments were normal. Thirty-five corneas from 18 patients at 3 years after LASIK (4 men, 14 women) were also examined by both confocal microscopes. All control subjects and LASIK patients were participants in other studies in our laboratory.[14,22] At the time of the measurements, the mean age of the control subjects was 43 ± 8 years (± SD, range, 23–55), and the mean age of the LASIK patients was 41 ± 10 years (range, 25–55). Corneas were anesthetized with proparacaine, a drop of viscous gel was applied to the objective of the microscope, and the objective was advanced until the gel contacted the central cornea. The *z*-ring adapter was used with the ConfoScan 4 to stabilize the eye and report the depth of each frame, and corneas were scanned as described previously.[22] In scans from the Tandem Scanning confocal microscope, the depth of the frame was calculated by using a polynomial equation.[23] Each subject gave informed consent to participate after receiving an explanation of the nature and possible consequences of the study. All studies were approved by the Institutional Review Board of Mayo Clinic and conformed to the tenets of the Declaration of Helsinki for research involving human subjects.

Standard Solutions and Confocal Microscopes

Amco Clear (GSF Chemicals, Columbus, OH), a nontoxic, suspended polymer with particle sizes less than 1 μm, was used as a scatter standard. Amco Clear is used to standardize measurements of scatter and turbidity of liquid suspensions in the laboratory. Its stability (shelf life of 1 year) and uniform light-scattering characteristics make it well suited as a brightness standard for confocal microscopy and other measurements of scattered light in the eye. The stock solution of Amco Clear, at a concentration of 4000 NTU (nephelometric turbidity units) was diluted to a series of solutions with concentrations of 0 to 1000 NTU (in steps of 100) and of 1500 NTU.

The Amco Clear solutions were placed in a custom-made spherical glass bulb, with a radius of approximately 8 mm, for scanning by the ConfoScan 4 (Fig. 1). A viscous gel used to examine human corneas (GenTeal Gel; Novartis Ophthalmics, East Hanover, NJ) was placed on the tip of the microscope objective as a contact solution, and the 40× objective lens was aligned and centered on the bright reflex from the posterior surface of the front wall of the bulb. The objective lens and focal plane were advanced approximately 600 μm deep from the surface, the confocal scan was initiated by the operator, and the focal plane was scanned through the solution from posterior to anterior. The step distance was 4 μm and the scan distance was 1000 μm; 350 frames were recorded at 25 frames per second. The illumination control was set to 95% of full brightness in these and all other scans. The image brightness and depth of each frame were exported by the operating program.

The Tandem Scanning microscope scanned the solution in the same glass bulb and in a cylinder with a 5-mm diameter (cut from a 3-mL syringe) to avoid the strong boundary function that was noted after scans in the glass container. The cylinder was filled with the test solution, which was in direct contact with the objective lens of the microscope. The focal plane was withdrawn to just inside the objective lens surface, and a scan was initiated. The step distance was approximately 2.4 μm, the focal plane was advanced in an anterior-to-posterior direction, and the scan was terminated after advancing approximately 1000 μm. Images were recorded at 30 frames per second. The gain and dark level of the video camera (VE-1000 SIT; Dage-MTI Inc., Michigan City, IN) were set manually. The mean brightness of the center of each frame (300 × 300 pixels) was calculated.

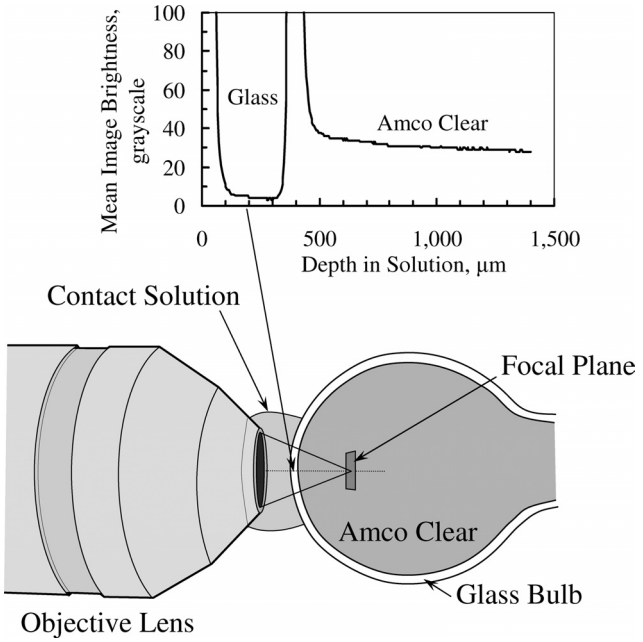

FIGURE 1. Amco Clear was scanned in a glass bulb. A custom glass bulb with a radius of approximately 8 mm was filled with a solution at a known concentration of Amco Clear, and the ConfoScan 4 microscope scanned through the solution. The brightness profile of the scan showed a bright reflex from each surface of the glass wall and low image brightness when the focal plane was within the glass (the direction of the scan was reversed from the native exported format by the ConfoScan 4). The brightness from the solution inside the bulb was dependent on the concentration of Amco Clear.

Standardized Image Brightness

A solution of Amco Clear at 1000 NTU in the glass bulb was scanned immediately after each corneal examination. The mean of image intensity between 100 and 500 μm from the inside surface of the glass bulb was used to compensate for variations in the microscope illumination and sensitivity of the video camera. We assumed that these variations affected the image brightness of the Amco Clear solution by the same proportion as they affected brightness of images of the cornea. The intensity of each frame from a scan through the cornea was adjusted by using:

$$I_c = R \times I \quad (1)$$

where I is the mean intensity calculated from the image, R is the ratio of the intensity from the sample of Amco Clear on a reference day to the intensity of the Amco Clear on the day of the measurement, and I_c is the adjusted intensity.

The adjusted image brightness of the cornea was expressed as scatter units (SU), the concentration of Amco Clear in NTU that produced the same image brightness. The relationship between image brightness and concentration was unique for each microscope (ConfoScan 4 and Tandem Scanning) and was determined from scans of the serial dilutions of Amco Clear. The image intensity from the ConfoScan 4 in scatter units (I_{SU}) was determined by a simple linear relationship:

$$I_{SU} = aI_c + b \quad (2)$$

where, and a and b are constants determined by regression of the mean image brightness and the concentration of Amco Clear.

All scans from the ConfoScan 4 were displayed with the epithelial surface to the left and the endothelial surface to the right, opposite to the direction of the operating software display. This format is consis-

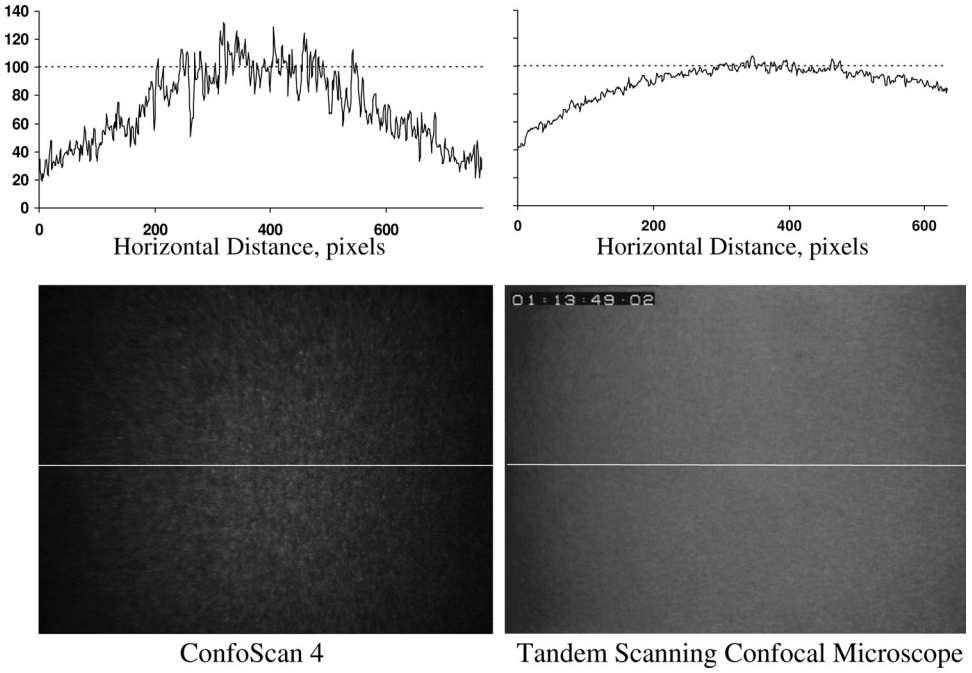

FIGURE 2. Confocal images of Amco Clear, 1000 NTU. Graphs (*top*) show the normalized brightness along the *white horizontal line* in each image. The image was somewhat more granular in the ConfoScan 4, and it manifested as more variation in the brightness trace. Both instruments showed a nonuniform intensity across the image, with the brightest region at the center and a decrease toward the edges of the image.

tent with our earlier work and allowed us to compare profiles of frame brightness directly with those of the Tandem Scanning confocal microscope.

Statistical Analysis

The image intensity and depth of each frame in scans from the ConfoScan 4 were exported from the operating system. The mean image intensity of each frame recorded by the Tandem Scanning confocal microscope was calculated in a central rectangle, 300 × 300 pixels from video images. An investigator reviewed each scan from each microscope and identified the frames that best represented the epithelial surface, the anterior stromal surface (anterior-most keratocytes), the interface in LASIK eyes (bright particles), and the endothelial surface of the cornea.

The depth of each frame in the stroma was scaled from 0% to 100% of stromal thickness so that we could compare stromal regions of similar proportional depths, regardless of corneal thickness. In untreated corneas, image brightness at each percentile of stromal depth was averaged across the corneas. In the corneas that had undergone LASIK, the depths of the frames were scaled in two steps, between the anterior stromal surface and the mean interface depth and between the mean interface depth and the endothelial surface, as a percentage of the full stromal thickness. The mean image intensity in each 5 percentiles of depth was compared between control corneas and LASIK corneas by using generalized estimating equation models to account for possible correlation between fellow eyes of the same subject.[24]

RESULTS

ConfoScan 4: Standard Solutions

Images of Amco Clear were somewhat granular, and the intensity across the video field on a scan line near the center showed considerable random variation (Fig. 2). The central region of the field was brightest, and near the edges of the frame, brightness decreased by as much as 80%. The mean image brightness exported by the operating program was below 5 grayscale units when the concentration of Amco Clear was below 200 NTU, but increased linearly at concentrations higher than this (Fig. 3). Scans through a solvent solution without Amco Clear showed a bright reflex from the surface of the container, but no persistent boundary function beyond 30 μm from this surface. The coefficients of equation 2 were a = 17.1 SU/grayscale and b = 125 SU. The mean ratio of image brightness from the 1000 NTU standard solution on the day of the corneal scan to brightness on a reference day (R in equation 1) was 1.009 ± 0.032 for all control and LASIK corneas (n = 71).

Tandem Scanning Confocal Microscope: Standard Solutions

Images of Amco Clear from the Tandem Scanning microscope were more uniform than those from the ConfoScan 4, and a

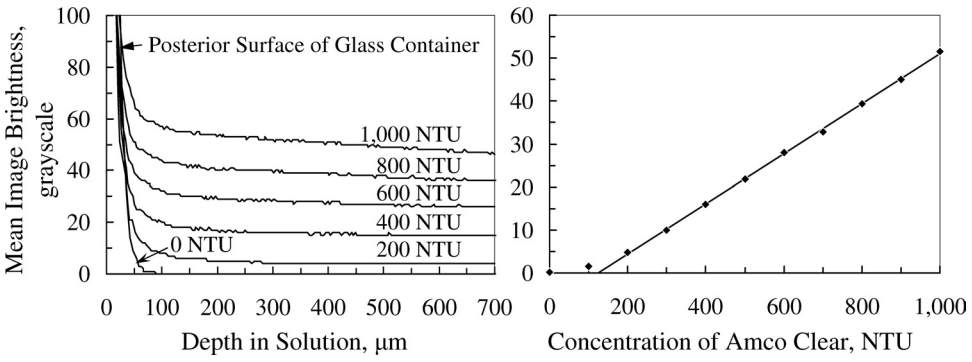

FIGURE 3. *Left*: mean image brightness in grayscale in scans with the ConfoScan 4 through solutions of Amco Clear in a glass bulb. All scans were aligned on the peak from the bright reflection from the inner container wall. Image intensity was uniform or decreased slightly with depth at higher concentrations. *Right*: mean image between 100 and 500 μm from the inner glass surface increased linearly with concentration of Amco Clear at concentrations above 200 NTU.

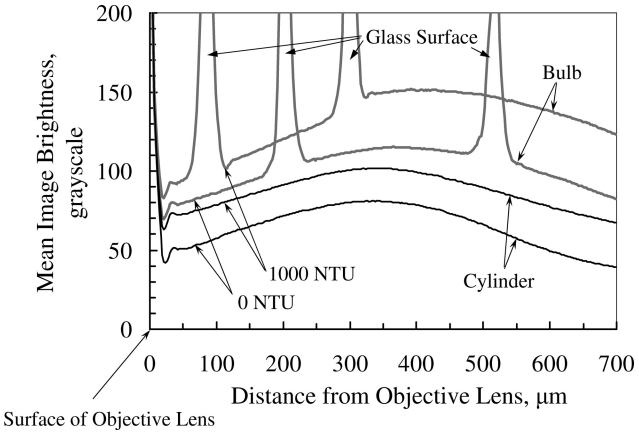

FIGURE 4. Mean image brightness in scans with a Tandem Scanning confocal microscope through two solutions (1000 NTU Amco Clear and solvent without a scattering substance) contained in a glass bulb (Bulb) or a cylinder that placed the solution directly in contact with the objective lens of the microscope (Cylinder). Scans were aligned on the peak reflectance from the surface of the objective lens. In all scans, image brightness increased slightly with depth less than 300 μm, then decrease at depths greater than 400 μm. Scanning through the glass wall increased image intensity throughout the scans of both solutions. The intensity in the scan without scattering substance (0 NTU) in the glass bulb was greater than the intensity of images in the cylinder at 1000 NTU. The effect of scanning through a surface made normalization of scans in the cornea, through the epithelial surface, unreliable.

TABLE 1. Mean Image Brightness of the Stroma in Control Corneas and in Corneas 3 Years after LASIK Measured with the ConfoScan 4 Confocal Microscope

Depth, Percent of Stromal Thickness	Control	LASIK	*P* (GEE-Models)
0–5	1265 ± 250	907 ± 178	<0.001
5–10	1167 ± 251	813 ± 186	<0.001
10–15	1079 ± 242	740 ± 186	<0.001
15–20	1007 ± 225	724 ± 189	<0.001
20–25	950 ± 197	793 ± 192	<0.001
25–30	890 ± 175	821 ± 178	0.12
30–35	839 ± 164	790 ± 161	0.30
35–40	803 ± 151	753 ± 145	0.26
40–45	775 ± 143	730 ± 130	0.28
45–50	761 ± 144	719 ± 124	0.28
50–55	758 ± 142	715 ± 117	0.26
55–60	764 ± 143	725 ± 112	0.32
60–65	782 ± 140	744 ± 116	0.31
65–70	807 ± 146	768 ± 127	0.26
70–75	833 ± 147	798 ± 136	0.27
75–80	860 ± 154	832 ± 145	0.37
80–85	893 ± 170	875 ± 154	0.63
85–90	961 ± 192	982 ± 201	0.90
90–95	1266 ± 318	1403 ± 342	0.13
95–100	2138 ± 399	2204 ± 347	0.53

The image brightness is expressed as the mean ± SD scatter units. The mean interface depth in the LASIK stroma was 18.0% of stromal thickness. GEE, generalized estimating equation.

scan line through the center of a typical image showed considerably less variation than did the scan line from the ConfoScan 4 (Fig. 2). Image brightness decreased by approximately 40% from the center to the edges of the frame. At all concentrations, mean image brightness increased slightly and then decreased with depth in the solution (Fig. 4).

The characteristic rise and fall of image brightness with scan depth was visible in scans of Amco Clear in the cylinder and in scans though the glass bulb, regardless of the distance between the objective and bulb surfaces (Fig. 4). Scans through the glass wall of the bulb showed a bright reflex from each surface and a strong boundary function that extended from anterior to the glass surface, between the surfaces, and into the solution. In either the bulb or cylinder, images were brighter as the concentration of Amco Clear increased, but the entire profile was shifted upward when measuring through the wall of the bulb. Image brightness of Amco-free solvent in the bulb was higher than image brightness of a solution of Amco Clear at 1000 NTU in the cylinder, which did not require scanning through a container wall.

ConfoScan 4: Corneas

The mean image intensity recorded by the ConfoScan 4 in control corneas had peaks that corresponded to the epithelial surface and the highest density of keratocytes at the anterior stromal surface (Fig. 5). Intensity decreased to a minimum at the mid stroma and then gradually increased toward the endothelium. The highest peak was from the endothelium. The mean intensity at the mid stroma was 758 ± 142 SU (n = 36, Table 1).

In the corneas at 3 years after LASIK, the mean surgical interface depth was 18% of the full stromal thickness measured

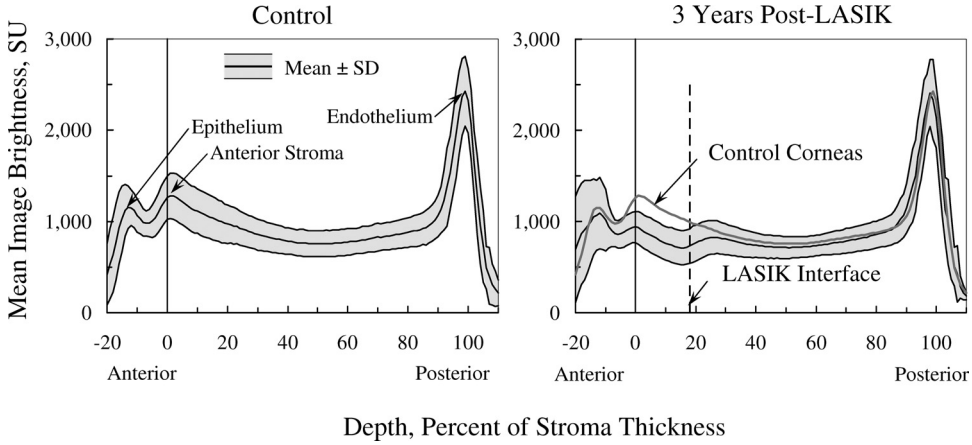

FIGURE 5. *Left*: mean image brightness of 36 normal untreated corneas from 18 subjects examined with the ConfoScan 4. Depth was scaled from 0% to 100% of stromal thickness, from anterior keratocytes to endothelium, and aligned on the anterior boundary of the stroma before averaging. Peaks associated with the epithelial surface, the anteriormost keratocyte nuclei (anterior stroma), and the endothelium were prominent. Mean image brightness is given in SU and represents the concentration of Amco Clear that gave equivalent image brightness. *Right*: mean image brightness (ConfoScan 4) of 35 corneas of 18 patients 3 years after LASIK. Depth was scaled in two steps before averaging. The LASIK flap was scaled from 0 to the mean depth of the LASIK interface as a percentage of stromal thickness, and the stromal bed was scaled from the interface depth to 100% at the endothelium. The mean interface depth was 18% of full stromal thickness. Image brightness after LASIK was lower throughout the flap than it was in analogous regions of the control corneas.

by using the ConfoScan 4. The mean intensity profile showed decreased image brightness between the anterior stroma and the mean interface depth compared with equivalent regions of the untreated corneas (Fig. 5). All mean image intensities in 5-percentile steps between the anterior stromal surface and the interface were significantly lower than the intensity from the equivalent stromal regions in the untreated corneas ($P < 0.001$), whereas all intensities between 25% and 100% of stromal thickness were not significantly different from equivalent regions of the control corneas ($P > 0.1$, Table 1).

Discussion

Significance of Corneal Haze

Corneal haze is routinely assessed by clinicians, to evaluate the diffuse opacity of the cornea. Haze, which we see and measure with various instruments, most likely represents a mixture of light backscattered from small particles and specular reflections from surfaces that divide regions of different refractive index. A change in corneal haze indicates that something in the cornea has changed, such as the number of particles or surfaces that scatter or reflect light, or the reflectance of these structures. Although one often cannot determine the specific nature or origin of the change without additional measurements, a change in haze indicates an improvement or degradation of corneal tissue. The ability to compare haze during an examination with haze at an earlier time or with that in normal corneas is important in assessing progression of corneal disease and detecting differences from normal, in the clinic and in research.

Use of a Scatter Standard for Assessing Corneal Haze

An ideal metric of scatter or reflectance would indicate the brightness of light returning to the observer in absolute units relative to a fixed intensity of light directed toward the cornea. Measurement of reflected light and illumination is complex, and the simpler method of expressing corneal haze in terms of concentration of a standard is well suited for this measurement.

Our samples of Amco Clear were used in two ways to standardize backscatter from the cornea. First, we expressed image brightness in terms of equivalent brightness of the standard (in this case, concentrations of Amco Clear). Standardization of image intensity allows comparisons with haze measured by using similar confocal microscopes that operate with different illumination intensities and camera sensitivities. Second, our measurement of the standard immediately before or after the corneal examination allowed for correction of day-to-day variations in the brightness of the illumination and detection systems of the confocal microscope. Because the standard always produced an image brightness that had a fixed relationship with the overall efficiency of the microscope, variations in illumination and recording sensitivity were compensated for by adjustment for variations in brightness of the standard, according to equation 1. Use of this reference also allows us to extend the range of brightness measurements beyond the dynamic range of the instrument. For example, in a cornea with bright pathologic haze that normally would saturate the detector, the illumination brightness could be reduced to bring the image intensity into a linear working range of the camera. Measurements could then be adjusted for the lower illumination from measurements of the standard at the same illumination.

Amco Clear serves well as a scatter standard for measuring haze with the confocal microscope; it is readily available, stable, provides a relatively homogenous image, and can be diluted to concentrations that produce approximately the same image brightness as the cornea. Amco Clear has been used to standardize image brightness with a slit lamp scatterometer,[5] and although its use in confocal microscopy has been suggested,[21] this is the first demonstration of its use to standardize corneal haze measurements in clinical confocal microscopy. Other standards have been used in a similar way. For example, Formazin is a scatter standard that has been use in nonclinical microscopy[25] and has been suggested as a standard for confocal microscopy (Shaver JH, et al. *IOVS* 2002;43:ARVO E-Abstract 1709). It is a suspension similar to Amco Clear, although the particles are larger and produce a less homogeneous confocal image brightness. In our experience, Formazin also settles out of solution in hours, whereas Amco remains uniformly suspended for days. Solid reflectance standards, such as Spectralon,[1,2] have the advantage of stability. However, most solid standards have a high reflectance and produce an image that is considerably brighter than the cornea. They cannot be diluted as suspensions can, and light reflected by solids may need to be reduced by filters to produce a brightness similar to that of corneal haze. Hillenaar et al. (*IOVS* 2010;51:ARVO E-Abstract 5659) proposed the use of three blocks of polymethylmethacrylate at three opacities as a means of standardizing a wide range of intensities in confocal images.

Differences between the ConfoScan 4 and Tandem Scanning Confocal Microscopes

Scans from the ConfoScan 4 through Amco Clear solution showed a linear response as the concentration of Amco Clear increased. The intensity profile with depth was free of a strong boundary function beyond 25 μm of the bright reflex from the surface of the container. In contrast, scans from the Tandem Scanning confocal microscope showed a variable intensity with depth and a response that was greatly dependent on the bright reflection from the surface (Fig. 4). Image brightness increased in front of, between, and behind the two surfaces of the glass bulb that contained the solution of Amco Clear, regions that scattered very little light. The presence of these surfaces increased the image brightness of the entire scan through solvent without Amco Clear to above that of a solution of Amco Clear at 1000 NTU at all depths. This elevation in image brightness represents a spread of light from the surfaces into frames recorded in front of and behind the reflecting surface and reveals an inefficiency of the confocal principle, which should reject out-of-plane light.

Measurements of the cornea with the Tandem Scanning confocal microscope would include only one brightly reflecting surface, the epithelium. However, we cannot predict how much the boundary function from this surface affects measurements of scatter in deeper regions, such as the stroma. The boundary function from the epithelial surface would most likely increase scatter from the stroma, although the amount and its depth dependence is unknown. For this reason, we did not attempt to standardize images from the Tandem Scanning confocal microscope further. Images from the stroma are likely to be consistent with each other and could be stabilized on a day-to-day basis, but because of the uncertainty of the boundary function from the anterior corneal surface, they cannot be expressed in terms of an equivalent of intensity from images of the standard. Others have reported stromal scatter with this instrument, although none have attempted to express these measurements in a standardized form.[9,18,19,26] It is possible that our Tandem Scanning confocal microscope had unique response characteristics, because of optical alignment and adjustments, although the status of other instruments would not be known without examining their response to a boundary, as illustrated in Figure 4.

Other Measurements of Corneal Haze

Backscattered light and reflectance have been measured in human corneas by other instruments, and some investigators have standardized their measurements. McCally et al.[1,2] and Taboada et al.[3] measured backscatter from the whole cornea by using a custom device based on a slitlamp and standardized by using a solid reflectance standard. Patel et al.[5] demonstrated the profile of haze through the depth of the cornea by using a slit lamp scatterometer that was standardized with Amco Clear.[5] The optical characteristics of this slit lamp, however, allowed resolution of only the anterior, middle, and posterior stroma, and peaks from the epithelium, endothelium, and surgical interfaces in some corneas.

Confocal microscopes have an advantage of a much higher spatial resolution than measurements based on the slitlamp. The depth of field of our ConfoScan 4 was approximately 26 μm,[27] and this was apparent in the width of the peak in scans through a brightly reflecting surface. This resolution allowed us to distinguish the epithelial peak from the anterior stroma and to examine specific regions of the stroma, such as the flap and residual stromal bed 3 years after LASIK.

The Clinical Importance of Corneal Haze Measurement

During slit lamp examination, normal corneas scatter and reflect light back toward the observer, and this effect provides a means of identifying structures, such as the epithelium, keratocytes, stroma, and surgical interfaces. Corneal haze is an elevation of this background scatter and is usually considered an indicator of disease. The use of a properly standardized confocal microscope or other device for measuring this haze will allow clinicians to compare the brightness of the haze with that of normal corneas, to identify sources of the elevated haze within the cornea, and to follow progression or regression of haze in patients. However, elevated corneal haze may or may not affect vision, depending on whether it is associated with elevated forward-scattered light.

The relationship between corneal haze, regardless of how it is assessed, and the forward-scattered light that degrades vision is complex.[28,29] Haze has been weakly correlated with forward scatter after keratoplasty.[6–8] Therefore, measurement of corneal haze may help explain visual function, in addition to being a valuable method of assessing general corneal health. In this study, scans from the ConfoScan 4 demonstrated a subtle decrease in brightness of the flap after LASIK compared with untreated control corneas (Fig. 5). Decreased backscatter and reflectance from this region has not been reported, although it has occasionally been noted by clinicians anecdotally (WMB) and may be related to the decrease in keratocytes in this region.[30] Vision after lamellar keratoplasty may be affected by forward scatter associated with haze, and indeed, after endothelial keratoplasty for Fuchs' dystrophy, Patel et al.[3] showed residual haze in the anterior and middle thirds of the cornea. The slit lamp scatterometer that was used to identify this elevated haze could resolve only the anterior, middle, and posterior thirds of the cornea. Use of the confocal microscope to measure haze and identify structures visually will allow association of specific structures, such as the posterior epithelium or anterior stromal surface, with haze. Measuring the recovery of optical properties in these structures over time after surgery will provide clues to how restoring endothelial cell function can revive optical clarity in the anterior cornea. These longitudinal studies are possible with proper standardization of scatter from confocal images.

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