Corneal Light Scattering After Excimer Laser Photorefractive Keratectomy: The Objective Measurements of Haze

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ABSTRACT

Background: After photorefractive keratectomy using excimer lasers (193 nm), most corneas show a marginal loss of transparency (haze) and the assessment of its magnitude in clinical studies has been subjective. To address this problem, we have developed a new device for the objective measurement of haze by measuring corneal light scattering.

Methods: A CCD-camera was fixed at 40° to a slit-lamp microscope and connected via frame-grabber to a computer. By incorporating polarizing filters, the system could discriminate between reflected and scattered light. The intensity of light coming from the cornea was measured in gray scale levels using in-house image analysis software. The system was calibrated against three different sizes of microspheres (0.25, 0.50, and 5.00 μm) which corresponded to the size of cellular and extracellular elements known to occur at sites of corneal surgery. Data were obtained from three treated human eyes with measurements before treatment and at five different postoperative intervals with a maximum follow up of 4 months.

Results: All three sizes of microspheres caused disturbances in gray scale levels (36 to 255 units) in the same range of those observed in corneal measurements. Disturbances in corneal light scattering were noted from 1 week postoperatively and persisted throughout the period of observation. We observed an increase in reflected and scattered light until the 2nd postoperative month followed by a subsequent decline.

Conclusions: It appears that this device is very useful to detect and measure objectively disturbances in corneal transparency after excimer laser photorefractive keratectomy. (Refract Corneal Surg 1992;8:114-121.)

A marginal loss of corneal transparency is a phenomenon experienced after photorefractive keratectomy using excimer lasers (193 nm). This loss of corneal transparency, confusingly termed haze, is first apparent from 4 to 6 weeks postoperatively as a diffuse zone of faintly altered light reflex. From clinical observation, haze progressively increases to a maximum at about 6 months and then slowly regresses. Concern has been expressed that even such a marginal loss of transparency may result in some compromise of visual function. To determine whether haze causes any significant problems, investigations have been un-
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![Diagram of the device used for measuring corneal light scattering.](image)

Undertaken to measure visual acuity at various levels of contrast.¹ To equate changes in acuity with different amounts of haze, various groups have attempted to classify changes in transparency. All groups have used slit-lamp microscope examination and a subjective classification of haze on an arbitrary scale of one to four.⁶,⁸ Such methods are difficult to reproduce, being based on the experience and interpretation of individual observers and even when references were made to standard photographs, results were not consistent.⁶

We have developed a digital video system for objectively measuring changes in corneal light scattering. By incorporating polarizing filters, the system can discriminate between reflected and scattered light. It has been used to examine and quantify the loss of corneal transparency associated with photorefractive keratectomy.

This article describes our system, its calibration, and typical results following excimer laser surgery.

**MATERIALS AND METHODS**

The present system was designed to measure scattered light emanating from the cornea and the geometry of the illumination and light collection in the system was not intended to selectively measure specular scattering.

**APPARATUS**

The components of our system are illustrated in Figure 1. The CCD-camera (charge coupled device, Photonic, EEV) was mounted on a bracket on a Haag Streit slit-lamp microscope. The illumination of the slit-lamp microscope was a standard tungsten halogen bulb. The dimensions of the slit limiting the light-source were always kept at 8 mm × 1 mm and the intensity of the illumination was held constant and routinely monitored with a radiometer (United Detector Technology S-370). A switchable linear polarizing filter was mounted in the light-path between the light bulb and the cornea distal to the mirror. This filter was divided into two portions with plane of polarization being at right angles between the upper and lower portion. A second fixed polarizing filter was installed inside the CCD-camera between the lens and the detector with a plane of polarization identical to that in the upper portion of the switchable filter in the light-path. The output of the camera was fed into an Acorn computer (Archimedes 440) fitted with a Wild Vision frame grabber (Hawk V10) which recorded the image at 256 × 256 pixel resolution and 8-bit gray-scale. The images were stored on 3.5-inch floppy disks and could be displayed either on a TV monitor or in graphical form on the plotter.

**IMAGE ANALYSIS**

Images from the camera were digitized using in-house software and analyzed using commercially available software (Foster Findlay Associates). By means of a cursor, areas for analysis could be delineated on the digitized image displayed on a monitor. Analysis could be undertaken using either linear scans made in the horizontal or vertical meridians, or using rectilinear areas of variable but specified dimensions (Fig 2). These results could be plotted graphically and displayed either on the monitor or the plotter. The software allowed for averaging scans, subtracting one from another and determining ratios. We photographed images from the monitor screen using a Canon camera (EOS 620) and Kodak Kodacolor 100 film.

**CALIBRATION**

To determine the relation of gray scale values in digitized images to light intensity, we imaged and digitized a neutral density step-wedge consisting of calibrated Kodak neutral density filters (0.2, 0.4, 0.6, 0.8, and 1.0) mounted on a microscope slide. Each individual Kodak filter had been previously
scanned in a spectrophotometer (Perkin-Elmar, Model 552) between 400 and 800 nm to determine its neutrality, and where necessary allow a more correct calibration than the stated manufacturers value of attenuation. The filter array was located between the slit-lamp microscope light source and the CCD-camera. Images were obtained without using crossed polarizers, scanned, and plotted to relate gray-scale values as a function of neutral density. The step-wedge was imaged as a control prior to every experimental procedure and measurements were expressed relative to this standard.

To determine the relationship of recorded gray scale values to light scattering, the following calibration was undertaken. Various concentrations of latex microspheres (Polysciences) were made up in distilled water, placed in a straight-sided spectrophotometer cuvette, and imaged using the slit-lamp microscope system, both with crossed and uncrossed polarizing filters. Three sizes of microspheres were investigated (0.25, 0.5, and 5.0 μm) at varying dilutions. Numbers of particles per mL were determined with the following equation: Number of particles per mL = \(6W \times 10^{12} \times \frac{\text{px} \times \text{mm}^2}{\text{latex \ g \ for \ 2.5\% \ latex \ \text{g}}^2 \times \text{diameter \ in \ μ \ of \ latex \ particles \ and \ p \ = \ \text{density \ of \ polymer \ in \ grams \ per \ mL \ (1.05 \ for \ polystyrene)}}\). Gray scale levels were plotted as a function of microsphere concentration up to the point of saturation for each size of spheres.

To determine the reproducibility of the method in relation to analysis of the cornea, 20 individual eyes (21 to 66 years) were each imaged twice daily (early AM, late PM) over a 2-week period. Measurements were made both with crossed and uncrossed polarizing filters. The results were plotted for each eye and the range and standard deviation were recorded.

**PROCEDURE**

Patients had sufficient refractive errors prior to surgery that they required correction by either spectacles or contact lenses. As most of the contact lens wearers used soft contact lenses and as such lenses have been reported to affect low contrast acuity, they were asked not to wear these lenses on the day of the measurements. The patients were not dark adapted, but measurements were taken in a darkened room. Patients were seated in front of the slit-lamp microscope and they were asked to gaze directly into the light source. The CCD-camera was focused on the geometric center of the cornea while viewing the image on the monitor. Four separate images were recorded at each sitting for each eye. In summary, images were recorded with crossed and uncrossed polarizers with camera angles either 40° or 90° to the path of the incident light-source. The average time for this whole procedure was approximately 1 minute and no significant differences in time scale were noticed in pre- and postoperative examination.

**SUBJECTIVE AND OBJECTIVE EVALUATION OF PATIENTS**

Patients were recruited from an ongoing clinical trial for refractive surgery evaluating the use of an ExcilMed UV200 excimer laser (Summit Technology). All eyes in this trial had a corneal ablation diameter of 4 mm and attempted corrections varied between -2.00 and -7.00 diopters. The calculated ablation depth over this range was between 12 and 41 μm.

In this pilot study, three eyes were examined preoperatively and at various postoperative periods up to 4 months. Individual details are summarized in Table 1.

The subjective determination of haze was carried out by one experienced clinical observer (D.S.G.), from corneal photographs taken using a standard

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*Ref: Refractive & Corneal Surgery Volume 8 March/April 1992*
protocol. Finally, objective measurements were performed using the apparatus described in this article.

**RESULTS**

**Calibration**

Throughout the course of this project, radiometric measurements of the slit-lamp microscope light source showed the radiant emission of the source to vary less than 1%. Measured gray scale values were linearly related to the log of light intensity (Fig 3). The only significant deviation from linearity occurred with the 0.8 neutral density filter. Good correlation was also noted between measured neutral density values on the spectrophotometer and those claimed by the manufacturer. Two background values were obtained for the gray scale: 1) the intrinsic dark value of the TV monitor and 2) a bright value obtained by measuring the light passing through the glass holder on which the neutral density step-wedge was mounted but in an area in which no filter was present. All the measured values fell between 36 and 255 gray scale levels.

Good linear relationships were also demonstrated between gray scale measurements and varying concentrations of microspheres (Fig 4). Progressively larger increases in gray scale signals were observed in relation to particle size and concentration for a given size. For the 5.0-micrometer microspheres, relatively low concentrations produced such an elevation in gray scale levels that they rapidly became unmeasurable given the range of our system. Little difference was observed in the measurements obtained with or without the use of crossed polarizing filters. This infers virtually all of the measured signal is generated by scatter and little, if any, by reflection.

The reproducibility of the instrument in obtaining measurements from the human corneas is shown in Figure 5 where the solid symbols show measurements with uncrossed polarizers taken in the morning and afternoon and where the open symbols show complementary measurements made with crossed polarizing filters. No systematic diurnal variations were seen and no systematic variations were observed in relation to sex. Measurements to date have been limited, but a trend was observed that indicated increasing scattering with increasing age. All the gray scale measurements on untreated human corneas fell within the range 90 to 150 units of gray scale. Measurements on this control group showed that optimal signals were obtained with a 40-degree separation angle between CCD-camera and the light source and, as a consequence, we discontinued mak-
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Figure 5: Representative graph showing reproducibility of gray scale measurements from an untreated cornea measured twice daily for 10 days. The filled symbols are measurements made with uncrossed polarizers (see text) while the open symbols are made with crossed polarizers. Morning measurements are depicted by circles while evening measurements are shown as triangles. The square shows the greater gray scale value associated with increasing age and is derived from an 66-year-old man.

This range correlates with that obtained for microspheres between 5.00 and 0.25 μm in diameter.

DISCUSSION

Of the many reports of the ocular properties of the ocular media of the human eye,\textsuperscript{10-27} relatively few describe the system in vivo.\textsuperscript{19-25} Most of the studies have utilized enucleated eyes and concentrate on either the absorption characteristic of the pigment epithelium and choroid\textsuperscript{10,11,13,14} or on the age-related changes in the transparency of the human lens.\textsuperscript{15,16} More recently, several devices have been constructed to obtain data from the living human eye. However, both of the most widely available devices, the Scheimpflug system\textsuperscript{17} and the opacimeters,\textsuperscript{21-23} have again concentrated on lenticular changes in an attempt to screen and classify early stages of developing cataract. Limited attempts have been made to examine corneal transparency in vivo using linear scanning densitometry in conjunction with Scheimpflug photographs.\textsuperscript{24} The results of this technique have demonstrated a differential scatter across the cornea with higher scatter signals being generated in relation to the anterior and posterior surfaces. Using this technique, no correlation was found between increasing scatter and age in a series of measurements of eyes with no known ophthalmic diseases. In contrast, in a second study\textsuperscript{25} using a photometer mounted on a slit-lamp microscope, an increase scatter with increasing age was apparent. The photometer device also demonstrated a correlation between increase scatter and increase corneal thickness resulting from surgical manipulation during cataract extraction. To date, only one study has been undertaken in an attempt to obtain objective measures of corneal transparency after excimer laser photorefractive keratectomy.\textsuperscript{26} In this study, a commercial lensometer was employed, but although some limited correlation was obtained for high degrees of haze, the system was not sufficiently sensitive to obtain useful measurements for patients with more mild haze. This instrument was designed to measure back-scattered light from the lens and, therefore, its limited value for monitoring corneal disturbances is not surprising.

The very transparency of the human cornea renders measurements of small changes in transmission in the system extremely difficult. In essence, changes in transparency could result from attenuation of light by absorption, reflection, or scatter. The anatomical design of the cornea is thought to be optimized to confine absorption within the system to a minimum. Evidence for corneal absorption and spectral changes in relative transmission with age are limited\textsuperscript{18} and, therefore, for the purpose of our analysis, we have chosen to ignore corneal absorption. In contrast, the amount of incident light that is reflected from the surface of the cornea is extensive.
<table>
<thead>
<tr>
<th>Eye</th>
<th>Postoperative Interval</th>
<th>Refraction (D)</th>
<th>Corrected Visual Acuity</th>
<th>Subjective Measurement (Slit-Lamp)</th>
<th>Objective Measurement (Gray-Scale) Polarizers</th>
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*The objective measurements are expressed in relative units (gray scale units) which are proportional to optical density. The visual acuity is the best spectacle corrected visual acuity.

Indeed, so large is the corneal reflex that it is a major constraint in the design of optical instruments whose purpose is to examine the fundus. Scatter may also be a significant attenuator of light entering the eye and both in vitro and in vivo studies have confirmed that scatter increases with any disturbance of the structural array of collagen in the corneal stroma. Such structural alterations and associated scatter are particular noticeable when the array is disturbed by the influx of water.

The image of the cornea that is viewed through a standard slit-lamp microscope consists of components of both reflected and scattered light. Light specularly reflected from a surface does not undergo a change in polarization. In contrast, if polarized light encounters a scatter source, changes in the plane of polarization will occur. In our system, by measuring the total amount of light coming back from subject corneas and by comparing these values with values obtained using crossed polarizers, we can to some extent discriminate between reflected and forward-scattered light as contributory agents to corneal haze.

Any disturbances in corneal transparency must have its origin either in focal changes in refractive indices or in changes in structural elements. Little is known about the refractive indices of discrete components within the cornea, but these are not expected to vary greatly. In relation to structural changes, past studies have indicated three potential causal elements for loss of corneal transparency after excimer laser photorefractive keratectomy. In order of decreasing size these are: migratory or atypical keratocytes (5 μm), microvaculars or intralamellar inclusions (0.25 to 0.5 μm), and newly synthesized atypical collagen (30 to 40 nm). It is somewhat of an oversimplification, but keratocytes are of a size and shape that may result in Mie scatter, whereas microvaculars and collagen could give rise to Rayleigh scatter.

Mie scatter is generated by particles some microns in size. Light scattering by such large particles is much more effective in the forward than in the backward direction. In Rayleigh scatter, submicron particles in the order of less than one tenth the wavelength of light are the causal elements. Such scatter is highly wavelength dependent, in that scatter increases with decreasing wavelength. In Rayleigh scatter, light is equally scattered in the forward and backward direction.

The time course of development of these potential scatter sources has been discussed in relation to excimer postoperative scar formation in a detailed account elsewhere. In summary, if keratocytes are of importance in loss of corneal transparency, then their contribution should be maximal in the first 3 months postoperatively and thereafter should decline. In contrast,
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Figure 6: (A-C) Graphs showing the relationship between objective measurements of corneal transparency in gray scale levels and time after treatment for three different eyes. In these graphs, the corneal light scattering measurements can be related directly to the preoperative value time 0.

newly synthesized collagen and vacuoles or inclusions may be present in the postoperative area for time periods of at least 1 year.1-3,30 The size range of corneal structural disturbances, 0.25 to 5 μm, dictated the size range of microspheres used for mod-elling in our calibration experiments. From Figure 4 it is apparent that, depending on the concentration, all sizes of microspheres between 0.25 and 5 μm could give rise to disturbances in gray scale levels that are comparable to those measured in our patient corneas postoperatively. We cannot discriminate between particles in this size range as to the prime causal elements. However, the long-standing nature of haze seen on slit-lamp microscope observation would suggest that vacuoles, inclusions, and collagen play the predominant role after the 1st 3 months.

The sensitivity of our system seems to be superior to both the lensometer and detailed slit-lamp microscope examination because we have detected minor changes in corneal transparency by measuring corneal light scatter in the 1st postoperative week. This is a time when no haze was noted at the slit-lamp microscope. All groups dependent upon clinically classifying haze have used subjective classifications usually on a scale of one to four, with four being the greatest disturbance. Although these classifications are completely subjective and irreproducible between centers, the finding that the lensometer could not routinely measure haze of “one” is further indication of the superiorly of the current system.

We are now routinely monitoring all our excimer patients with this device. We will shortly report our observations in relation to functional changes, particularly those of low contrast visual acuity.

REFERENCES

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