Femtosecond Optical Ranging of Corneal Incision Depth

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Excimer laser ablation has been proposed as a technique for keratorefractive surgery. Clinical acceptance of linear-incision laser keratectomy may depend on the availability of a method for accurately and noninvasively monitoring incision depth during the ablation process. We have developed a femtosecond optical ranging technique for measurement of corneal incision depth. This technique uses nonlinear optical cross-correlation to determine the time-of-flight of an ultrashort laser pulse between the anterior corneal surface and the bottom of the keratectomy incision. Longitudinal and transverse resolution are estimated to be 5 μm and 10 μm, respectively. Invest Ophthalmol Vis Sci 30:99–104, 1989

The ability of argon-fluoride excimer lasers to ablate (remove) corneal tissue in submicron increments has led to great interest in the use of these lasers for keratorefractive surgery, in particular linear-incision keratectomy and large-area surface ablation. Because each laser pulse removes so little tissue, the depth of an incision made with an excimer laser can be controlled much more precisely than the depth of an incision made with a diamond knife. However, the submicron precision possible with the excimer laser is of limited value unless the incision depth can be accurately and noninvasively measured. The number of laser pulses needed to produce an incision of a given depth can be estimated from the experimentally determined values of etch depth per pulse as a function of fluence (incident energy per unit area), but such estimates may be unsatisfactory for clinical use. Although pulse energy can easily be measured with an accuracy of a few percent, accurate measurement of the beam’s spatial intensity profile and cross-sectional area at the corneal surface is difficult. Significant errors in determining the fluence are therefore possible. These errors can result in removal of more or less tissue than predicted, which might lead to corneal perforation or failure to achieve the desired refractive change. Clearly, a method for monitoring the incision depth while the ablation procedure is in progress would be useful.

Ultrasonic pachymetry is an established method for measuring corneal thickness. An ultrasound pulse that travels through the cornea is partially reflected at the cornea-aqueous boundary. The round-trip transit time of the pulse through the cornea is measured; the corneal thickness can then be easily calculated if the speed of sound through the cornea is known. The principal disadvantage of ultrasonic measurement is that it requires direct contact of the probe with the corneal surface. In addition, the transverse spatial resolution of ultrasonic pachymetry is limited by the size of the ultrasound probe tip, which is typically at least 1 mm in diameter. This technique, therefore, is not suitable for the measurement of corneal incisions, where a noncontact measurement is desired and incision width can be less than 100 μm. Several types of optical pachymetry can provide noncontact measurements of corneal thickness, but the application of these techniques to the measurement of narrow, high-aspect-ratio incisions is difficult. A completely different technique, confocal microscopy, is an accurate method of measuring optical thickness that can potentially be used to measure the depths of laser keratectomy incisions.

We report here a technique for measuring corneal incision depth that can be considered an optical analogue of ultrasonic pachymetry. We measure the time required for a femtosecond laser pulse to make a round trip between the corneal surface and the bottom of the incision. This time can be converted to distance by multiplying by the speed of light. By measuring the round-trip transit time of the light pulse between the anterior and posterior corneal sur-
faces, we can also use this technique to determine corneal thickness. We have previously used this method to measure the thickness of the stratum corneum and epidermis of human skin and the corneal thickness of a living rabbit eye.12 This method can detect reflections as small as 10^{-7} of the incident laser pulse energy and has a longitudinal resolution of better than 15 \mu m. Because the laser beam is easily focused to a 10 \mu m diameter spot, with femtosecond optical ranging there is no difficulty in obtaining the high transverse resolution necessary for measurement of slit-like corneal incisions. The longitudinal resolution that we obtain is directly related to the pulse duration. Because the speed of light is so great (0.3 \mu m/fsec), femtosecond pulses are necessary if longitudinal resolution on the order of microns is to be achieved.

Materials and Methods

The apparatus used for femtosecond optical ranging is shown in Figure 1. Because the time resolution of conventional electronic detection techniques is limited to tens of picoseconds and longer, it is necessary to use a cross-correlation technique to make measurements on a femtosecond time scale. This technique performs a correlation of the retro-reflected femtosecond signal from the cornea with a time-delayed reference pulse.

The laser source used for these measurements is a colliding-pulse-modelocked ring dye laser,13 which produces a train of 65 fsec pulses at 625 nm (repetition rate 100 MHz, average power 20 mW). A beam splitter divides each pulse into probe and reference pulses. A \times5 microscope objective focuses the probe pulse to a 10 \mu m diameter spot at the sample surface. Power at the corneal surface is 5 mW. Beam diameter at the retina is approximately 2 mm, and retinal irradiance is well below the damage threshold. (The laser power required to produce ophthalmoscopically visible lesions in rhesus monkeys is 35 mW at 633 nm for a 10 sec exposure and 460 \mu m retinal image diameter.14) Reflections, which occur at the surface of the sample and at boundaries between layers of the sample that differ in refractive index, are collected by the microscope objective and focused into a nonlinear crystal (potassium dihydrogen phosphate, KDP). The reference pulse is focused into the same crystal after passing through an optical delay line. Because the delay-line mirrors are mounted on a stepping-motor-driven translation stage, the length of the delay line can be easily varied.

When the signal and reference pulses overlap in the crystal spatially and temporally, the crystal generates ultraviolet light at 312 nm, the second harmonic of the laser wavelength (Fig. 2). The amount of ultraviolet light produced is proportional to the cross-correlation function, E(T) = \int_{-\infty}^{\infty} I_s(t) I_r(t - T) dt, where I_s(t) and I_r(t - T) are the time-dependent signal and reference pulse intensities and T is the time delay between the signal and reference pulses. The cross-correlation function is a maximum when T = 0, i.e., when the signal and reference pulses reach the crystal simultaneously. Ranging is performed by measuring second-harmonic intensity as a function of delay-line length. Length can be easily converted to time, since the speed of light is known. Similar methods are used for the measurement of femtosecond and picosecond...
Fig. 3. (A) Histologic section of a deep corneal incision created by an excimer laser. Incision depth determined from this section is 420 \( \mu \text{m} \), compared to 320 \( \mu \text{m} \) determined by examining the unsectioned block of tissue. The infolding of the incision walls is an artifact of tissue processing. Bar = 100 \( \mu \text{m} \). (B) Femtosecond optical ranging of this incision. Cross-correlation traces b and c were taken with the probe beam focused into the incision; a and d were taken with the beam focused onto the unablated corneal surface. Incision depth is 307 \( \mu \text{m} \) (comparing traces a and b) to 280 \( \mu \text{m} \) (comparing traces c and d). The values 270 \( \mu \text{m} \), 15 \( \mu \text{m} \), 220 \( \mu \text{m} \) are the lateral displacements of the eye between successive measurements. Vertical = second-harmonic intensity, horizontal = position of the stepping-motor-driven translation stage.

Results

Femtosecond ranging data for a deep corneal incision are shown in Figure 3. A total of four cross-correlation traces were obtained, one for each of two points on the bottom of the incision and two points on the adjacent, unablated corneal surface. Both ranging and histology show that the base of the incision is not perfectly flat and that one side of the corneal surface adjacent to the incision is higher than the other. These corneal irregularities limit the precision with which the incision depth can be defined. Incision depth determined by femtosecond ranging is 280 \( \mu \text{m} \) (by comparison of one cross-correlation trace obtained with the beam focused onto the unablated...
Fig. 4. (A) Histologic section of a shallower corneal incision created by an excimer laser. Incision depth determined from this section is 310 \( \mu m \), compared to 230 \( \mu m \) determined by examining the unsectioned block of tissue. Bar = 100 \( \mu m \). (B) Femtosecond optical ranging of this incision. For trace b the probe beam was focused into the incision; for traces a and c the probe beam was focused onto the unablated corneal surface. Incision depth is 180 \( \mu m \) (comparing traces a and b) to 208 \( \mu m \) (comparing traces b and c).

Similar data for a shallower incision are shown in Figure 4. Here the incision depth determined by optical ranging is 180–208 \( \mu m \), compared to 310 \( \mu m \) determined by light-microscopic examination of 2 \( \mu m \) sections. Again, agreement is fairly good when the stretching of the tissue in the sectioning process is accounted for. Incision depth on the block of unsectioned tissue is 230 \( \mu m \), in reasonably good agreement with the optical ranging measurements.

For the plastic block (Fig. 5), the incision depth determined by femtosecond ranging is 173–185 \( \mu m \), compared to 195 \( \mu m \) determined by scanning electron microscopy. Slight spatial nonuniformities in the excimer-laser intensity (which can cause depth variations along the length of the incision), along with the surface roughness of the plastic block, probably account for this small discrepancy.

**Discussion**

The use of ultrashort light pulses for ranging and imaging in biological systems was first proposed by Duguay and Mattick in 1971. A picosecond ranging technique that uses optical heterodyning (interference between probe and reference beams that differ slightly in frequency) as the method for cross-correlation has been described, and the possibility of applying this technique to tissue studies has been suggested. Optical time domain reflectometry using femtosecond pulses has been demonstrated for the characterization of defects and scattering in optical fibers. However, because of the need for high spatial resolution and sensitivity, very few investigations of biological systems have been performed. The recent development of femtosecond laser sources and high-sensitivity detection techniques has made possible the application of short-pulsed optical ranging techniques that have depth resolution on the micron scale.

We have demonstrated the feasibility of using femtosecond optical ranging as a noninvasive, noncontact probe of corneal incision depth. Ranging measurements can be made quickly and easily once the sample is appropriately positioned with respect to the probe beam. Each of the cross-correlation traces shown in Figures 3 and 4 was obtained with a single scan of the stepping-motor-driven translation stage at a scan rate of about 30 \( \mu m/sec \). Signal-to-noise ratio for these scans is very good, and it should be possible to scan faster without losing any essential information. Alternatively, signal-to-noise ratio could be improved still further by scanning more slowly.

Any discrepancies between our ranging measurements and histological results are probably due to changes in corneal dimensions during tissue processing. The close agreement between ranging and scan-
Fig. 5. (A) Scanning electron micrograph of an incision made by an excimer laser in a plastic block. Incision depth is 195 \( \mu m \). The surface roughness is due to the mold in which the block was formed. Bar = 100 \( \mu m \). (B) Femtosecond optical ranging of this incision. Incision depth is 185 \( \mu m \) (comparing traces a and b) to 173 \( \mu m \) (comparing traces b and c).

The depth resolution of femtosecond optical ranging is limited by the precision with which the peak positions in the cross-correlation traces can be determined. Using 65 fsec pulses, the depth resolution is about 5 \( \mu m \). In more complex systems which have multiple internal boundaries or are highly scattering, the resolution is limited by the details of the laser pulse shape and the dynamic range of the cross-correlation measurement. Resolution is reduced if it is necessary to measure weakly reflected echoes in the presence of closely delayed strong ones.

The construction of a slit-lamp delivery system that can be used simultaneously for femtosecond optical ranging and excimer laser linear-incision keratectomy is probably feasible. A substantial amount of optical and mechanical design work is required, but there are no fundamental scientific obstacles. However, several potential problems with the clinical use of femtosecond ranging should be mentioned. First, femtosecond ranging of incision depth must be performed on a relatively dry eye. If the corneal surface is moist, the incision gradually fills with saline. In this situation, ranging detects the reflection from the surface of the saline rather than the reflection from the bottom of the incision, leading to significant underestimation of incision depth. Second, the eye must be carefully positioned so that the probe beam is perpendicular to the corneal surface. The effective aperture of the detection system is relatively small (approximately f/8); reflections from the cornea which occur outside the collection aperture are not detected and the signal is correspondingly reduced. Finally, the eye must be stationary during the course of the measurement if accurate results are to be obtained.

In principle, femtosecond ranging could be used not only to monitor corneal incision depth during linear-incision keratectomy, but also to monitor corneal curvature during large-area surface ablation. In the case of large-area ablation, however, the high transverse resolution possible with femtosecond ranging is not required, and measurement techniques such as photokeratoscopy are probably more useful.

**Key words:** femtosecond optical ranging, keratorefractive surgery, excimer laser, corneal incisions

**References**

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