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PRECISION AND REPRODUCIBILITY OF MEASUREMENTS OF HUMAN CORNEAL THICKNESS WITH RAPID OPTICAL LOW-COHERENCE REFLECTOMETRY (OLCR)

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ABSTRACT

This study investigates the precision and intraindividual variability of a clinical optical pachometer based on low-coherence reflectometry, which was used to measure the central thickness of a human cornea *in vivo*. The instrument, attached to a slit lamp, is a single mode fiber optic based Michelson interferometer with a high repetition rate as previously described. The same operator performed ten sets of measurements on the same subject, each consisting of 20 consecutive scans, on each day for three consecutive days. By computing the means from every scan series, the thickness of the central cornea with optical pachometry was found to be 519.6 ± 1.2 (range 518–521) μm on day 1, 519.9 ± 0.9 (range 519–521) μm on day 2, and 523.8 ± 0.6 (range 523–525) μm on day 3. The thickness values on day 3, where the subject suffered from a cold without clinical ocular involvement, were different from the two previous days ($p < 0.001$, one way analysis of variance). Optical low-coherence reflectometry measurements of corneal thickness can be performed with high precision of about 1 μm and a high intra- and intersession reproducibility. [S1083-3668(99)01301-5]

Keywords optical low-coherence reflectometry; human corneal thickness; precision and reproducibility of corneal pachometry.

1 INTRODUCTION

The thickness of the human cornea *in vivo* is an important parameter to accurately measure since it may be indicative of corneal endothelial function.[1,2] Damage to either the anterior region (the epithelial layers) or to the single nonregenerative endothelial cell layer on the posterior side of the cornea can result in corneal swelling. Changes in the thickness of the cornea are associated with metabolic abnormalities like the hypoxic situation in contact lens wear. Progressive corneal thinning, on the other hand, may be observed in long term contact lens wear or in degenerative disease like in keratoconus.

There is an increasing demand to accurately measure the thickness of the cornea under various conditions, especially in corneal refractive surgery.[3,4] A precise noncontact, rapid technique with the capability to monitor even minor changes in corneal thickness would be most useful.

There are three types of instruments that can be used to measure the thickness of the cornea at a particular point:

(i) Optical pachometers can be used to measure the thickness of a clear cornea based on the displacement of the slit of light reflected from the anterior and the posterior surfaces of the cornea.[2] Optical techniques require that the average refractive index of the cornea be known.

(ii) A specular microscope can also be used to measure the thickness of a clear cornea by measuring the mechanical displacement of the microscope in order to focus on the anterior surface of the cornea and then on the posterior surface.[5]

(iii) The thickness of the cornea can be also measured with an ultrasound pachometer.[6,7] This technique, which requires contact with the cornea, sends out a sharp pulse of ultrasound and detects the reflections from both the anterior and the posterior surfaces. The time delay for the detection of the reflected signals from both sides of the cornea are used to calculate the corneal thickness. Ultrasound pachometry requires that the speed of sound in the cornea be known.

Currently, ultrasound pachometry is used for clinical routine measurements as it has from the

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available methods the best precision and interobserver reproducibility.[8,9] The major disadvantage of the technique is that a contact between the instrument probe and the cornea is required and that the point of the measurement on the corneal surface cannot be precisely controlled.

Low-coherence reflectometry is an optical technique that was developed in the telecommunications industry to locate faults in fiber optic lines and in integrated optical devices. A book of selected papers and patents on the development of low-coherence reflectometry and its biomedical applications, edited by Masters, is to be published by SPIE as part of the Milestone Series.[10] A recent book on optical time-domain reflectometry is a useful source of information on the physical principles of reflectometry.[11]

To achieve noncontact measurements of corneal thickness with a precision as good as ultrasound, with high repetition rates and with a high level of intrasession and interobserver reproducibility, we have developed an instrument using optical low-coherence reflectometry (OLCR) to measure corneal thickness.[12–14] The instrument has been designed to be mounted on the slit lamp equipment used by ophthalmologists to perform biomicroscopy.[15] With this equipment, we set out to study the precision and reproducibility of repeated measurements of corneal thickness in one subject as well as day to day variations of corneal thickness on three consecutive days.

2 METHODS

Details of the experimental design of the rapid optical pachometer based on an optical low-coherence reflectometer have been previously presented.[15] The design of the optical reflectometer is based on a Michelson interferometer. A super luminescent diode is the broadband light source with an output power of 7 mW at 850 nm and a spectral width of 18 nm. It is coupled to a fiber optic Michelson interferometer. The light is split between the two arms in a 50/50 coupler. One arm goes to the cornea, and the second arm goes to a rotating cube. An aiming diode laser is integrated into the interferometer with a 95/5 coupler. Longitudinal scanning is achieved with the rotating cube. The detection system is a silicon photodiode, an amplifier, and an oscilloscope to display the detected signal. Large signals are detected at interfaces where there is a large change in the refractive index, i.e., at the air–tear interface, and at the endothelial–aqueous humor interface. The longitudinal scanning system of the instrument, which allows for a scanning speed of more than 20 m per second and a repetition rate of about 400 Hz,[16] is based on a rotating glass cube. The instrument used for the measurements in this study had a longitudinal scanning speed of 500 mm per second and a measurement repetition rate of 15 Hz. The entire instrument is attached to a clinical slit lamp with a chin and head rest to help stabilize the head of the subject. With the help of two converging pilot lasers, the center of the longitudinal scan, which was adjusted to coincide with the focal distance of the slit lamp, was aligned with the cornea at the proper distance (Figure 1). The optical center of the cornea was located by the subject fixating a low power laser, which was aligned coaxially with the 850 nm superluminescent diode (SLD) beam. The diameter of the measuring beam from the superluminescent diode on the surface of the central cornea was about 20 μm.

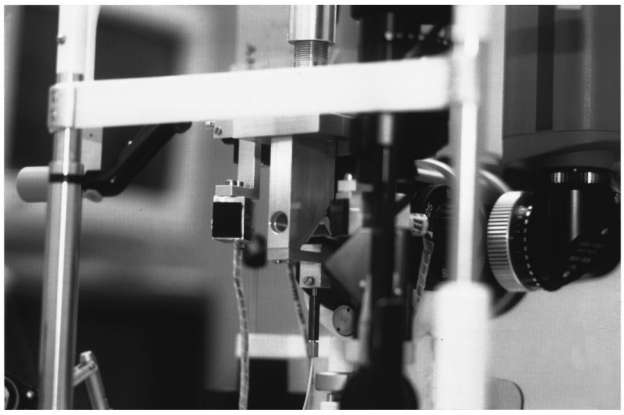

Fig. 1 OLCR pachometer mounted on a clinical slit lamp (Haag Streit, Switzerland). The LCD and the fixation laser signal are placed in front of the front lens of the slit lamp, allowing the observer to direct the instrument with biomicroscopic stereopsis. The pilot lasers are attached horizontally and converge on the focal distance of the slit lamp, facilitating the alignment of the instrument with the *x-y-z* control of the slit lamp.

The instrument was calibrated with a BK7 glass window (Melles Griot, U.S.A.) of 1 mm thickness and a refractive index of $n = 1.5100$ at 850 nm. Measurements were found to be reproducible for a given position on the glass to 1 μm. In order to calculate the thickness of the cornea from the optical distance an average value for the corneal refractive index of 1.376 was used.

To obtain a measurement, a specific strategy for data collection and evaluation was developed. From a series of 20 longitudinal scans, the upper and lower five values were deleted. From the remaining ten scans, the mean was calculated as the current thickness value, and the range and standard deviation were calculated as well.

Measurements were performed between 3 and 5 p.m. on three consecutive days. The subject was seated at a clinical slit lamp with the OLCR instrument. The subject (B.R.M.) was instructed to focus on the fixation light target and the thickness of the central cornea was measured. All of the measurements were obtained on the central cornea of the right eye. Between each measurement set, the instrument was misaligned and the subject moved his head from the measurement position in the chin and head rest.

The protocol to evaluate the precision of the instrument was on three days to perform 10 sets of 20 measurements on the same eye of the same subject with the same instrument operator. On day 3, the subject (B.R.M.) had clinical symptoms of a cold without obvious eye involvement. Thus, it was decided to perform the series of measurements on day 3 as initially planned.

The results were recorded on-line in a database, which stores the identity of the subject, date, and time of the measurement, and the individual scan values including technical information on their generation from every series. Additionally, the computed mean from every scan series representing the measurement, was recorded manually including range and standard deviation.

A set of ten ultrasound pachometric measurements was performed to confirm the OLCR measurements in magnitude. Data from these measurements were not further processed.

The data were analyzed with Systat for Windows, version 5.01 (Evanston, IL), and graphically presented as Tukey box-and-whiskers plots. The median of the batch values of corneal thickness for each day is marked by the center horizontal line inside the box for days 1 and 2. For the batch data from day 3, the median horizontal line coincides with the lower side of the box. For each box the upper and lower horizontal sides are called the hinges. The median splits the ordered batch of numbers in half, and the hinges split the remaining halves in half again. The vertical whiskers indicate the range of values. When the whisker coincides with the upper or the lower horizontal side of the box it is not shown. The one-way analysis of variance, Systat for Windows, version 5.01 (Evanston, IL), was used to compare the data from the third day with the data from day 1 and day 2.

3 RESULTS

The optical pachometer gave highly reproducible intrasession measurements of corneal thickness. The standard deviations (precision) in every scan series were frequently even below 1 μm (Table 1). When the measurements (consisting of 20 scans each) of one day were computed to form a days total thickness value, we found an average corneal thickness of 519.6 ± 1.2 (range 518–521) μm on day 1, an average corneal thickness of 519.9 ± 0.9 (range 519–521) μm on day 2, and a computed average of 523.8 ± 0.6 (range 523–525) μm on day 3. The standard deviations derived from forming the mean of different measurements from one day can be regarded as the intrasession reproducibility, which is 1.2 and 0.9 μm. The values of day 1 and day 2 were nearly identical, indicating a low intersession variation. The thickness values on day 3, where the subject suffered from a cold, were different from the 2 previous days ($p < 0.001$, oneway analysis of variance) and probably indicate a slight swelling of the

Table 1 The physical thickness of the central cornea of the right eye measured with an optical pachometer based OLCR. Each measurement value in the table was computed from 20 consecutive scans as described in the text and is presented as the average thickness (μm), the standard deviation of the mean thickness, the minimum value, and the maximum value. Measurements were performed on a single subject by a single operator on three consecutive days. After each measurement the instrument was defocused and the subject moved away from the chin and head rest.

Day 1

Av. thick (μm)	Std. dev. (μm)	Min. thick. (μm)	Max. thick. (μm)
519	0.8	518	520
518	0.6	517	519
519	1.0	518	520
521	1.1	519	522
521	0.7	519	522
520	0.8	518	521
518	0.7	517	519
520	0.9	520	522
519	0.6	518	520
521	0.9	519	523

Day 2

Av. thick (μm)	Std. dev. (μm)	Min. thick. (μm)	Max. thick. (μm)
521	0.6	520	522
521	0.8	519	522
521	0.7	520	522
520	1.0	518	521
519	0.8	518	520
520	0.9	518	521
519	0.9	517	520
520	0.8	519	522
519	0.7	518	520
519	1.2	518	522

Day 3

Av. thick (μm)	Std. dev. (μm)	Min. thick. (μm)	Max. thick. (μm)
524	0.8	523	525
523	1.0	521	524
523	1.2	521	525
523	0.6	522	524
524	1.2	522	527
524	0.9	522	525
525	0.7	523	526
524	0.6	525	526
524	0.9	523	526
524	0.9	522	525

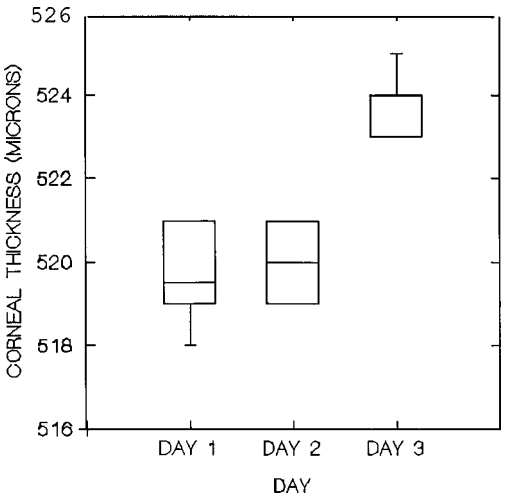

Fig. 2 Graphic presentation (Tukey box and whisker plots) of the distribution of ten measurements, each consisting of 20 consecutive scans, on three consecutive days. See Sec. 2 for explanation of Systat box-and-whisker plots.

cornea from this condition. The distribution of results is shown in a box-and-whisker plot in Figure 2.

4 DISCUSSION

The thickness of the cornea is an important physiological parameter, which has been used to study corneal hydration control under a variety of physiological and pathological conditions. The classical optical methods like slit-lamp pachometry[2,17] or the use of the specular microscope[5] today are not considered precise enough due to their rather high intrasession variability of up to 20 μm. Corneal ultrasound pachometry, on the other hand, today has become the routine method for corneal pachometry, offering a precision of 3.5–13.7 μm.[6,9] However, as this method requires contact with the cornea and shows rather high intrasession variation, a faster and more precise method is needed.

The method of optical low-coherence reflectometry has attained increasing attention in the past years, as it enables for a measurement of layer thickness which differ in refractive index. Experimental setups have been described to measure anterior eye structures with a precision of 1 to 2 μm.[18,19] Consequently, this method has been applied for imaging of the anterior (and the posterior) segment by combining lateral and z scanning in a method called optical coherence tomography (OCT).[20] Partial coherence interferometry has been used for precision biometry of the anterior segment of the human eye.[21] Optical coherence tomography has a great potential for future applications in precision biometry and tomography.[22] There are other noninvasive optical techniques such as confocal microscopy and multiphoton excitation microscopy which are being developed for the imaging of thick, highly scattering tissues in the human body *in vivo*.[23–25] For example, multiphoton excitation microscopy based on NAD(P)H fluorescence can provide functional imaging of cells and tissues.

With the newly designed instrument and setup reported here, a quasicontinuous measurement of a defined spot in the optical center of the cornea has become practicable. The scanning principle with a rotating glass cube allows for a high repetition rate of near to 400 Hz.[16] This instrument is capable of measuring corneal thickness even under conditions of low surface reflectivity and under surgical corneal wounds.[13,14] In a study comparing ultrasound and OLCR, we found the speed and precision of the OLCR instrument to be more suitable to obtain pachometric data in clear corneas.[15]

The value of the corneal thickness derived from the technique of optical low coherence reflectometry is dependent on the refractive index of the cornea. In the normal cornea there are variations of refractive index as a function of depth in the cornea. For cases of corneal swelling due to surgery or injury the normal values of refractive index will be different. Optical reflectometry techniques usually specify an average value of the refractive index for the cornea. The role of the thickness of the tear film on measured values of corneal thickness is being studied, e.g., during excimer surgery. Since optical reflectometry techniques measure the optical thickness of the cornea it cannot readily separate changes in corneal thickness from changes in the average refractive index of the cornea.

The study presented here investigates for the first time the intrasession and intersession variation of pachometric readings, which were found to be very low and better than any other technique. By incidence of an obviously somewhat more hydrated cornea of our subject on day 3, we have demonstrated that even a thickness change of less than 4 μm in a cornea of more than 500 μm thickness can be measured with a high level of significance. It is not clear if the increase of corneal thickness measured on the third day was a thickness change of the cornea, the tear film, or both. The role of these factors in the interpretation of corneal thickness measurements with optical low-coherence reflectometry is under investigation.

5 CONCLUSIONS

The precision of the instrument will most probably enable us to measure minor changes in corneal thickness, indicating a response to a wide range of stimuli, which we have not been able to measure before.

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