

Quantitative In Vivo Retinal Thickness Measurements in Healthy Subjects

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Purpose: The early detection of visual threatening retinal thickness alterations is important for the purpose of offering affected patients proven treatment when indicated. Currently, the clinical methods that are available for obtaining an impression of retinal thickness are subjective and require experience and expertise. The authors present measurements of foveal thickness in healthy individuals obtained by a newly developed instrument that enables a noninvasive, noncontact, safe, and fast measurement of the retinal thickness anywhere in the posterior pole.

Methods: A prototype of the retinal thickness analyzer (RTA) that operates on the principle of laser-slit biomicroscopy was used. Retinal thickness was measured in healthy emmetropic volunteers.

Results: Fifty eyes were measured. The average thickness at the foveola was $178 \pm 44 \mu\text{m}$ (\pm standard deviation; range, 100–322 μm).

Conclusion: The retinal thickness measured by the RTA in healthy subjects correspond well to previous published data (in vivo and histologic) on retinal thickness. This instrument may prove valuable in detecting retinal thickness alteration in macular diseases. *Ophthalmology* 1997;104:639–642

Many ocular diseases cause changes in retinal thickness. Early diagnosis and precise monitoring of these thickness alterations may be possible only if an accurate measurement of the thickness is available. Currently, the clinical methods that are available for obtaining an estimation of

retinal thickness are slit-lamp biomicroscopy and stereo-photography. However, neither method can provide a quantitative measurement of retinal thickness. Several new methods have been evaluated for measuring retinal thickness. Zeimer and his group^{1–5} have suggested laser-biomicroscopy; their method gives a quantitative estimation of the retinal thickness along a line on the retina and required the use of a contact lens. Optical coherence tomography^{6–9} is able to give quantitative measurement of the retinal thickness of a randomly shaped path. At present little data regarding retinal thickness measurements in healthy subjects has been published.^{9,10}

In this article, we describe a prototype of a retinal thickness analyzer based on the laser slit concept and report our in vivo measurements of retinal thickness of the posterior pole in healthy volunteers.

Methods and subjects

Retinal Thickness Analyzer

We used a prototype of the retinal thickness analyzer (RTA; Talia Technology Ltd, Mevaseret Zion, Israel).

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David Landau and Yaacov Rozenman have no proprietary interest in the development or marketing of this or a competing instrument.

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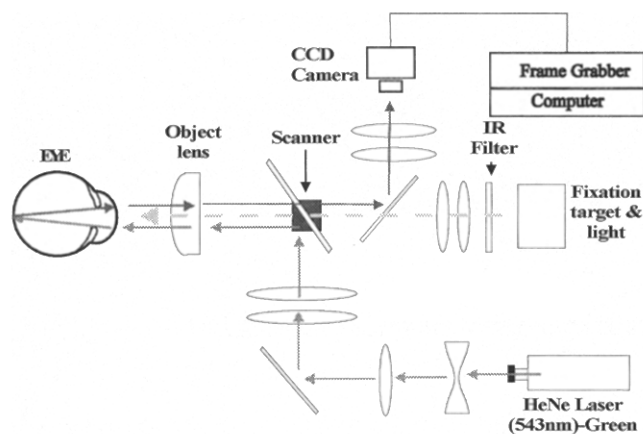


Figure 1. Schematic diagram of the retinal thickness analyzer.

The RTA operates on the principle of laser-slit biomicroscopy. There is no contact between the instrument and the examined subject, who fixates on a target in the instrument. A Green helium-neon laser (Melles Griot, San Marco, CA), operating at 543 nm, yields a monochromatic parallel beam. The beam is routed through a number of lenses, resulting in a slanted projection of the beam that forms a 14- μm wide and 2-mm long slit on the retina. The beam is projected onto the retina at a known angle from the visual axis of the eye. The laser power during alignment was 40 μW (safety calculations based on the standards of the American National Safety Institute; the alignment intensity is safe for more than 16 minutes).¹¹ The light reflected back from the retina is viewed by a black-and-white charged coupled device camera (NEMCO-CC-II $\frac{1}{3}$ inch, NEMCO, Inc, Topsfield, MA) at an angle opposite to the incident light (Fig 1). A single scan consists of a sequence of ten slit images, 200 μm apart, projected on the retina within 0.25 second. The length of the slit on the retina is 2 mm; thus, one scan provides thickness information over a field of 2×2 mm on the retina. During a single scan, the power is increased to 80 μW (safe for 50 seconds).¹¹ Green light is strongly absorbed by blood, thus enabling a sharp image of the retinal cross-section to be obtained. In eyes with clear media, only the diffraction characteristics of the optical system limits the sharpness of the image, thus the resolution of the instrument. The image is adversely affected by unclear media. In some cases of media opacities, like those secondary to cataracts (mild to moderate), a change in the stereobase (the angle between the beam incident on the retina and the reflected beam collected by the black-and-white charged coupled device camera) can improve the system performance. The slit images obtained during the scan are digitized using a frame-grabber (Matrox IP-8, Matrox Graphics, Inc, Dorval, Quebec, Canada, 256 gray level = 8 bit). The width of each slit image on the scan (Fig 2) represents the retinal thickness of that spot on the retina. A specialized software, based on the following paradigm analysis algorithm, was developed to analyze the scans. The slit images were first smoothed by a low-pass filter and resampled vertically

along the slit image. The analysis of the thickness was based on the reflections coming from the outer and most inner retinal layers.

Based on the Gaussian nature of the laser beam and reflection characteristics of the above layers and their immediate neighbors,^{12,13} the algorithm considered the extremum of the derivatives (point of maximum steepness) on both sides of the densitometric reading curve as representing the reflections of the outer and most inner layers of the retina (Fig 3). The distance between these two points is proportional to the thickness of the retina at the measured point. Assuming a simple model of retinal light scatter, the relation between retinal thickness and the width of the slit image on the camera is given by the formula: $t = D/(\alpha * M)$, where t = retinal thickness in μm , D = the width of the slit image obtained by the black-and-white charged coupled device camera calculated from the densitometric reading curve in μm (pixel size = 9.4 $\mu\text{m} \times 6.25 \mu\text{m}$), α = stereo base, and M = magnification. The slit images can be processed by various image processing techniques to obtain enhanced detail visualization. As an example, (Fig 4) an indexed color processing technique of an image replaces the gray levels of a monochromatic image by colors based on an arbitrary scale. This image processing technique does not add information to the black-and-white image but allows better detail visualization of different layers of the retina and improved interpretation by a human observer.

Subjects

Retinal thickness measurements were performed in 50 eyes of 31 healthy emmetropic human subjects. The subjects were recruited on voluntary basis. The volunteers were fully informed of the purpose of the examination, and each signed a consent form. We examined 24 female eyes and 26 male eyes. The mean age of our volunteers was 38 years (range, 18–64 years). We examined 25 right eyes and 25 left eyes. Before the retinal thickness measurement, visual acuity and slit-lamp biomicroscopic examinations were performed, the pupils were dilated using one drop each of 2.5% phenylephrine hydrochloride and 0.5% tropicamide. Clear media was assured, and indirect funduscopy was performed to ensure normal-looking retinas. After acquiring the scanned images, retinal thickness was measured at five locations: (1) at the foveola; (2) at a point 500 μm nasal to the foveola, i.e. papillomacular bundle; (3) at a point 500 μm temporal to the foveola; (4) at a point 500 μm superior to the foveola; and (5) at a point 500 μm inferior to the foveola. The reproducibilities were assessed in five of the volunteers (2 women and 3 men).

Two different reproducibilities were assessed. First, the intravisit reproducibility, evaluating nine scans centered on the fovea obtained in a single session in each of the subjects. The instrument was realigned after each scan. One hundred points (10×10) were evaluated from each scan. The coefficient of variance (standard deviation/mean) of the retinal thickness values in all nine scans of each subject was calculated for each of the 100 points.

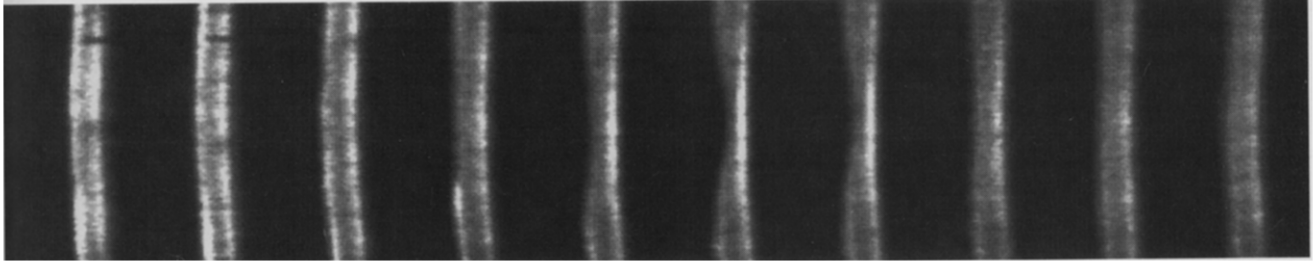


Figure 2. A retinal thickness analyzer scan centered on the foveola consisting of a sequence of 10 slit images 200 μm apart. The sixth slit image from the left was taken through the foveola; the foveolar thinning is well emphasized. Image processing analysis of each slit image can provide information on retinal thickness and retinal layers at this spot.

Second, the intervisit reproducibility, evaluating two scans centered on the fovea obtained from two different sessions (2 days apart) in each of the subjects. One hundred points (10×10) were evaluated from each scan. The coefficient of variance (standard deviation/mean) of the retinal thickness values in all scans of each subject was calculated for each of the 100 points.

Results

Reproducibility

The intravisit reproducibility of scans obtained on the same day was $\pm 5.9\%$, corresponding to $\pm 10.6 \mu\text{m}$. The intravisit reproducibility of scans obtained on sessions 2 days apart was $\pm 6.6\%$, corresponding to $\pm 10.8 \mu\text{m}$.

Retinal Thickness

The average retinal thickness at the five points measured in the 50 eyes examined is summarized in Table 1.

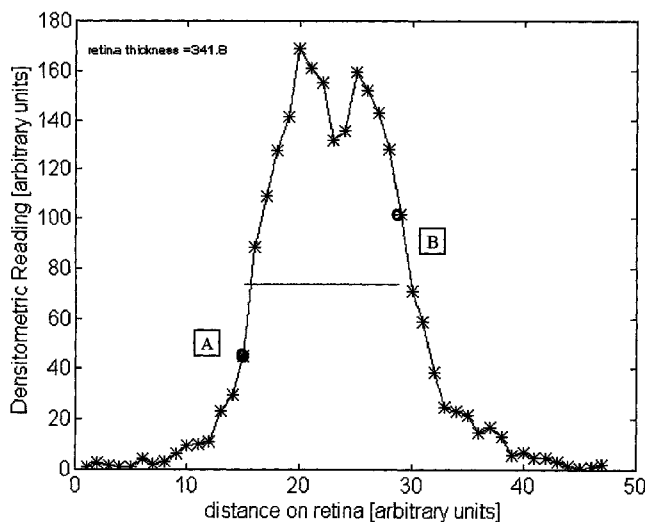


Figure 3. Illustration of a densitometric reading curve of a spot near the fovea. **A**, the point of maximal steepness representing the anterior interface; **B**, the point of maximal steepness representing the posterior interface of the retina. The length of the line between the two points represents the slit image width; it is proportional to the retinal thickness at this spot.

On comparing measurements of approximately the same spot in the two eyes of the same patient (19 patients had both eyes measured), an average difference of $\pm 11.8\%$ was found, corresponding to an intereye difference of $\pm 29 \mu\text{m}$.

The average retinal thickness at the foveola in volunteers younger than 31 years of age was $166 \pm 38 \mu\text{m}$; the average retinal thickness at the foveola in volunteers between the ages of 31 and 50 years was $175 \pm 45 \mu\text{m}$; and the average retinal thickness at the foveola in volunteers older than 50 years of age was $222 \pm 52 \mu\text{m}$ (\pm standard deviation).

Discussion

An accurate and sensitive method for quantifying the retinal thickness is long needed for diagnosing and monitoring a large spectrum of retinal diseases affecting the retinal thickness. In this study, we applied a noninvasive, noncontact device for measuring the retinal thickness.

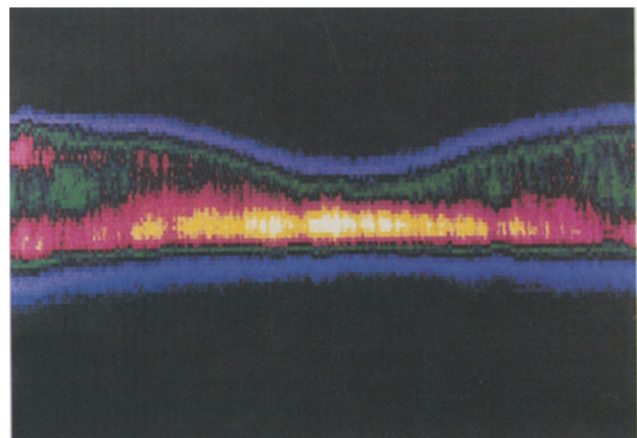


Figure 4. An indexed color image of a slit through the fovea. The foveolar thinning is well emphasized. Different reflection intensities correspond to different retinal layers. This image processing technique demonstrates the ability of the retinal thickness analyzer to distinguish between different layers of the retina. The color coding makes the black-and-white image easier for interpretations and understanding by the observer.

Table 1. The Average Retinal Thickness in Five Different Locations in the Posterior Pole:
Mean \pm SD

Location	Retinal Thickness (μm)	Range (μm)
At the foveola	178 \pm 44	100–322
500 μm nasally to the foveola	314 \pm 62	198–525
500 μm temporally to the foveola	308 \pm 51	192–420
500 μm superior to the foveola	319 \pm 50	251–450
500 μm inferior to the foveola	308 \pm 62	214–574

SD = standard deviation.

With this instrument, we obtained retinal thickness measurements in healthy subjects. At the wavelength used, the anterior reflection probably originates from the internal limiting membrane and the posterior reflection originates from the layer of the retinal pigment epithelium.^{8,12} We found the average retinal thickness at the foveola to be 178 μm , which agrees with the findings of Shahidi et al⁵ and the histologic measurements of 200 μm published by Fine and Yanoff.¹⁴ Recently, a foveolar thickness of 147 μm obtained by in vivo measurement with the optical coherence tomography was published.^{9,10} Although the published retinal thickness measurements in vitro (i.e., 75–120 μm ¹⁵ 130 μm ,¹⁶ 100 μm ¹⁷) are lower than our in vivo findings, it is difficult to compare them to our in vivo findings because the factor of tissue distortion and shrinkage during fixation and preparation is hard to estimate. Interestingly, the foveolar thickness showed a tendency to increase with age, although we could not prove statistical significance. Within 500 μm of the foveola, the retinal thickness is almost the same in all direction, there is a tendency of increased thickness in the nasal and superior parts, although it is not statistically significant. Because the unknown optical power of the eye being examined may induce a slight difference in the calculated magnification power (M) in the equation for retinal thickness, and until further work is completed on the exact origin of the posterior reflection within the retinal pigment epithelium layer, we have some uncertainty regarding our measurements as absolute. Nevertheless, the RTA will give repeatable accurate measurements.

Our work defined the retinal thickness in the foveolar area in healthy subjects, as measured by the RTA (with the current interpretation algorithm), and may be used as a reference number for comparison in measuring retinal thickness in diseased eyes. We have presented a newly developed instrument that is able in a noninvasive, non-contact, safe, and fast way to help measure the retinal thickness anywhere in the posterior pole.

Our measurements in healthy subjects correspond well to previous published data (in vivo and histologic) on retinal thickness. The RTA is able to distinguish between different retinal layers and enables us to measure retinal

thickness. In the current prototype, the image is adversely affected by media opacities, but future technical improvements will hopefully overcome this problem. The exact correlation between the histologic retinal layers and the different reflection layers in the RTA slit image has not been fully established. We believe that this instrument might significantly contribute to early, accurate diagnosis and better monitoring of treatment of clinical significant macular edema and other macular diseases.

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