A New Method for Rapid Mapping of the Retinal Thickness at the Posterior Pole

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Purpose. An objective, quantitative, and sensitive method to map retinal thickness is needed to diagnose more effectively the conditions causing alterations in thickness, such as macular edema and neuroretinal atrophy.

Methods. An instrument, the retinal thickness analyzer, was developed into a rapid scanning instrument, capable of covering macular areas of 2 X 2 mm in 100 or 400 msec and generating a detailed map of the retinal thickness. The performance was assessed in vitro and in five normal subjects who were scanned on three separate visits.

Results. Optimal depth precision was 5 to 10 μm, and the optimal depth resolution was 50 μm. Reproducibility was ±12 μm on the same day, ±15 μm for single maps obtained in multiple visits, and ±10 μm for three averaged maps per visit obtained in multiple visits.

Conclusions. This new method to analyze retinal thickness provides four unique features: multiple optical cross-sectioning of the retina, mapping of retinal thickness, high reproducibility, and short acquisition time. These capabilities promise to improve the diagnosis and management of common diseases such as macular edema and glaucoma. Invest Ophthalmol Vis Sci. 1996;37:1994-2001.

Several ocular diseases are related directly to an alteration in retinal thickness, typically caused by edema or atrophy. Diabetic macular edema, for example, which is considered to be the most frequent cause of vision loss in diabetic retinopathy,1 consists of an accumulation of fluid and the consequent thickening of the retina. The Early Treatment Diabetic Retinopathy Study protocol for managing diabetic macular edema is based on the location and area of regional thickening.2 Macular edema also occurs in retinal vascular occlusion and uveitis and after cataract extraction.3 Accurate mapping of retinal thickness in these diseases would be beneficial for early diagnosis and for explaining visual acuity, as well as for predicting changes in visual acuity. Conversely, retinal atrophy caused by pathologies such as glaucoma and by degenerative diseases are associated with thinning of the retina. An objective, quantitative, and sensitive method to assess these losses would be of clinical value, especially in glaucoma, in the early diagnosis and detection of progressive loss.

The methods used clinically, slit lamp biomicroscopy and stereophotography, provide only a subjective evaluation of retinal thickness. The need for objective evaluation has prompted the development of new methods. Laser scanning ophthalmoscopes have been used to map the retinal surface. These maps reveal local changes in relative surface height but do not assess the retinal thickness and, thus, are of limited clinical use.4 We have developed a method, retinal thickness analysis, that is capable of measuring the thickness of the retina. This method has been shown to detect macular edema more sensitively than does clinical examination or fundus photography.5 Its previous version has been applied to study the onset and early progression of macular thickening and to assess the effect of therapy.6,7 In addition, its principle of
New Method for Retinal Thickness Mapping

RPE-choroid
inner limiting
membrane

FIGURE 1. Schematic diagram illustrating the principle of optical sectioning. The laser beam, coming from the left, intersects with the inner limiting membrane and retinal pigment epithelium-choroid interfaces at the fovea (shown as concentric circles). These intersections are viewed at an angle and seen as two separate lines. The reflection from the inner limiting membrane, concave at the fovea, is seen as a curved line that delineates the foveal depression.

Laser slit illumination has been applied to enhance the visualization of intraretinal and vitreal pathologies. Recently, a method based on low coherence interferometry, optical coherence tomography, was introduced and yielded optical sections with unprecedented details. However, optical coherence tomography and our previous version of retinal thickness analysis yield only individual optical sections. This constitutes a clinical limitation because treatment decisions are based not on the degree of edema alone but also on the size of the affected area and its location relative to the fovea. This prompted us to evolve the instrument into a scanning retinal thickness analyzer capable of rapidly covering a macular area and generating a detailed map of retinal thickness.

MATERIALS AND METHODS

Retinal Thickness Mapping

The principle of retinal thickness analysis is based, as previously described, on projecting a thin laser slit obliquely on the retina and viewing it at an angle in a manner similar to slit lamp biomicroscopy (Fig. 1). The separation between the reflections (and scatter) from the vitreoretinal interface and the chorioretinal interface is a measure of the retinal thickness.

Instrumentation. The new scanning retinal thickness analyzer was developed in collaboration with Talia Technology (Mevasseret Zion, Israel) and was designed to provide three major improvements over the previous instrument: rapid scanning to cover a 2 × 2 mm area in one measurement, noncontact optics, and easy alignment.

The optics are described schematically in Figure 2. A green (540 nm) HeNe laser is shaped into a narrow slit and directed into the eye by a scanning mirror and a fundus imaging lens. On the fundus, the laser slit is 2 mm in length, and its theoretical width is 10 μm, as shown below. A scanning mirror is placed at the conjugate plane of the pupil; hence, as it rotates, the laser slit is scanned across the macula. The scanner deflects the beam to its new position during the nonrecording interval between consecutive video images. A filter is introduced electronically during alignment to reduce the laser power and is removed during image acquisition.

The image of the intersections of the laser slit with the retina, referred to here as an optical cross-section of the retina, is recorded by a video camera and digitized. The stereobase—namely, the distance at the pupil plane between the incoming laser beam and the outgoing rays—is determined by stops in the optics. Stops yielding separations of 2 to 4 mm, requiring a 4 to 6 mm clearing in the pupil, can be inserted. The results presented here were obtained with a 3 mm separation between the stops (unless otherwise specified), requiring a pupil dilation of 5 mm.

At present, each image is acquired at a video rate of 25 or 50 images per seconds. In one mode, the laser is scanned to generate 10 optical cross-sections 200 μm apart, thus covering a 2 × 2 mm area on the fundus in 200 or 400 msec. In another mode, more detailed coverage is achieved by acquiring optical cross-sections spaced by 100 μm. The images are acquired as 10 fields in 200 msec or 10 frames in 400 msec. The advantage of the higher density of pixels in the frame mode is under evaluation. Technically, there is no obstacle in doubling or tripling the acquisition rate to complete the scan in a fraction of the current time. The optical cross-section occupies only a portion of the charge coupled device sensing element; thus, existing gating techniques can be used to acquire at a rate higher than standard video rate.

To obtain an image adequate for analysis, the power of the 2 mm slit on the retina is maximally 160 μW (540 nm) for a duration of 40 msec. The ANSI 136.1 standards for safe use of lasers do not deal specifically with slit-like configurations. On consultation with ANSI committee members (F. Delori, D. Sliney, personal communication, 1996), we were advised to consider the irradiated area as having a diameter equivalent to the average of the height and width of the slit (or the retinal dimension equivalent to a sub-
tense of 11 mrad). Under these conditions, and considering the fact that the subjects pupil is dilated by mydriatic drugs, we calculated that the maximal permissible corneal power for a 40 msec exposure is 62 mW (or 39 times larger than used). The maximal permissible exposure time for a corneal power of 160 µW is 22 seconds (560 times longer than a single scan duration). The safety of the maximal corneal power of 60 µW used during alignment was evaluated. The maximal permissible exposure time is 66 seconds. This duration is less time than necessary for alignment, and the operator must be aware of this limit. The viewing light irradiates the retina with 60 µW on a field of more than 30°. This yields a retinal power density of 8.5 10^{-5} W/cm², which is permissible for 10,000 seconds.

Infrared diodes illuminate the anterior segment, and the image of the iris is projected on a charge coupled device camera by a lens by a mirror. The image is viewed on a monitor, and, by focusing the image of the pupil and centering it, the operator ensures a constant distance between the instrument and the eye as well as optimal centration of the beams inside the pupil.

An incandescent lamp irradiates a conjugate focal plane of the fundus. At this plane, a screen with multiple fixation targets is placed and is viewed by the subject. This light also provides illumination for the fundus, and the image of the fundus is projected on a charge coupled device camera. The output of the camera is viewed on a screen and recorded after each scan. This allows the operator to verify the fixation, observe the location of pathologies of interest, and document the location of each scan on the fundus. Nine scans are obtained, corresponding to nine different targets, as shown in Figure 3, to cover the central 20° (6 × 6 mm) area around the fovea in the posterior pole.

During operation, the patient sits in front of the instrument and rests his or her chin in a chin rest similar to slit lamp biomicroscopy. The operator uses a joystick to align the pupil while monitoring its image on screen. The patient is asked to fixate on a target, and the image of the optical cross-section, seen on the screen, is optimized by adjusting the focus and the centration on the pupil. The acquisition is activated, lasts 225 or 425 msec, and yields 10 optical cross-sections.

**FIGURE 2.** Schematic layout of the optics. See Materials and Methods section.

**FIGURE 3.** Location of the retinal thickness scans. Nine scans, corresponding to nine fixation targets, cover the central 20° of the posterior pole. Vertical lines in the central box show the location of the 10 optical sections. Graphics have been superimposed on a photograph obtained with a fundus camera.
sections and a fundus image. Immediately after each scan, the 10 cross-sections are displayed on screen (see Fig. 5), inspected, and saved into memory if judged adequate. The patient is then asked to fixate at another target. Scans can proceed quickly because there is no need to refocus the image once the refractive error has been compensated. If the refraction of the patient is beyond the range of focus of the instrument, the procedure can be performed with the patient wearing his or her refractory correction in the form of either spectacles or contact lenses.

**Analysis.** Analysis is performed by an automated, operator-free, software algorithm. It divides each optical cross-section into 20 segments 100 μm in length, and converts each into a light intensity profile. For each segment, the locations of the vitreoretinal interface and the chorioretinal interface are detected in a manner similar to that previously described. The separation between the two interfaces is calculated and stored as the uncalibrated retinal thickness. An array of 20 X 10 values is thus generated. An automated quality check algorithm is applied to the array to detect occasional points that may deviate significantly from their neighbors. These points are deleted and appear, on the final map, as locations devoid of data. The array is smoothed by a two-dimensional filter (Lorentzian curve with a half-value at the nearest neighbors). The nine arrays, corresponding to the nine targets (Fig. 3), can then be combined into a large array of 60 X 30 points.

The uncalibrated retinal thickness map is sufficient if one is interested in monitoring changes in a given individual or in comparing different areas in the same eye. To convert the values into absolute thickness values in microns, two parameters are needed—the axial length of the eye and the refractive error. The axial length is obtained, once for each patient, by ultrasound in the A-scan mode, and the refractive error is determined by the focus adjustment necessary to acquire the image. Once these measurements are entered, a conversion factor is derived from the light-intensity profile of their image. The array is smoothed by a two-dimensional filter (Lorentzian curve with a half-value at the nearest neighbors). The nine arrays, corresponding to the nine targets (Fig. 3), can then be combined into a large array of 60 X 30 points.

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**Experimental Determination of the In Vitro and In Vivo Beam Width and the Implication on Depth Precision and Resolution.** Two parameters are of interest in our application. The first is depth precision, or the sensitivity with which one can establish the depth of a single interface, e.g., the location of the vitreoretinal interface at one point on the fundus compared to its location at an adjacent point. The second is depth resolution, or the minimal depth separation that can be detected between two surfaces.

In applications such as optical coherence tomography, laser scanning tomography, and retinal thickness analysis, the location of the interfaces are derived from the light-intensity profile of their image. The precision is determined by the width of this light-intensity profile and by its noise. If the noise is negligible, the practical rule of thumb is that the peak can be detected to within 1/5 to 1/10 of the Full Width at Half Maximum (FWHM). Depth precision is then derived by the following equation:

\[
\text{Depth precision} = \frac{\text{FWHM}}{5 \text{ (or 10)} \cdot \text{stereobase angle}} \quad (1)
\]

where the stereobase angle is the angle (in radians) between the incoming laser beam and the imaging path.

Depth resolution also is determined by the FWHM because the light-intensity profiles of the two interfaces must be separated from each other. One common criterion for resolution requires a separation of one FWHM. Depth resolution is thus given by:

\[
\text{Depth resolution} = \frac{\text{FWHM}}{\text{stereobase angle}} \quad (2)
\]

The optimal theoretical FWHM is determined by the divergence of the laser and the diffraction limit of the optics.

For a given wavelength, the beam divergence is inversely proportional to the waist of the laser beam. The laser used has a original beam divergence of 1.2 milliradian; thus, after its beam expansion by a factor of 1.43, the divergence is 1.2 milliradian/1.43 = 0.84 milliradian. The focusing of such a beam by the eye, with an effective focal length of 17 mm, yields a spot size on the retina of 14.3 μm. Because the beam has a Gaussian profile, the FWHM is 0.6 X 14.3 = 8.6 μm.

For the imaging optics, the diffraction limit is determined by the aperture at the pupil and the focal length. The minimum resolved separation of the imaging system (resolution), based on Rayleigh’s criterion, is then given by:

\[
\text{Imaging resolution} = \frac{1.22 \cdot \text{wavelength} \cdot \text{focal length}}{\text{aperture}} \quad (3)
\]

For a 2 mm aperture at the pupil and an effective focal length of 17 mm in air, the diffraction limit is 5.6 μm. The diffraction limits for the laser beam and the imaging then combine to give a theoretical FWHM of \((8.6^2 + 5.6^2)^{1/2} = 10 \, \mu m\).

The optimal in vitro FWHM was assessed by placing a thin plastic slab at the focal length of a 17 mm lens,
informed consent was obtained from each subject, and the pupil was dilated with 1% tropicamide.

Three different reproducibilities were assessed. The first, the intravisit reproducibility, evaluated the reproducibility of multiple measurements obtained in a single session. Three scans (in the frame acquisition mode) were performed at the fovea in each of the five subjects. The instrument was realigned for each scan. The coefficient of variance (SD/mean) of the retinal thickness values in the three scans of each subject was calculated for each of the 20 × 10 points. The average was calculated, and the in vivo intrasession reproducibility was taken as the mean of the values of the five subjects.

The second and third reproducibilities assessed the variability between visits. Three sessions were performed weeks apart. In each session, three scans were obtained at the fovea. The standard deviation of the retinal thickness values among the three visits of each subject was calculated. The average was then calculated for each subject, and the mean of the values of the five subjects was taken as the in vivo intervisit reproducibility. Two intervisit reproducibilities were assessed. The first, the single scan intervisit reproducibility, was based on one scan per session, and the second, the tri-scan intervisit reproducibility, was based on the average of three scans at each visit. The latter was performed to evaluate the improvement provided by multiple scans.

RESULTS

FWHM and Estimate of Depth Precision and Depth Resolution

In vitro, the FWHM was 10 μm. For a stereobase between the incoming laser beam and the outgoing optical path of 4 mm at the pupil, the stereobase angle was 0.2. The in vitro depth precision, calculated from equation 1, was thus 5 to 10 μm. Similarly, the depth resolution, calculated from equation 2, was 50 μm.

In human subjects, the FWHM was also 10 μm.

FIGURE 5. Multiple optical sections of a normal human fovea obtained in one scan of 400 msec. Note the two interfaces. The right interface, corresponding to the retinal pigment epithelium–choroid (arrowhead), remains flat throughout the 10 optical cross-sections. The left interface (arrow), corresponding to the inner limiting membrane and nerve fiber layer surface, gradually assumes the shape of the foveal depression (shown by the two arrows). The disc is toward the left. Vertical scale = 1000 μm; horizontal scale = 200 μm.

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Quantitative Map of Retinal Thickness
Analysis of the optical cross-sections yielded maps such as the one illustrated in Figure 6. This map, obtained in one of the subjects, clearly shows the depth and the extent of the foveal depression. A thicker retina is seen nasal to the fovea, where the papillomacular bundle is located.

In Vitro Calibration
The results of four measurements performed on four plastic sheets, with thickness in the clinical range, are given in Figure 4. The calibration is linear throughout the range of interest and yields a correlation coefficient $r = 0.998$.

Multiple Optical Sections at the Fovea
The capability of yielding multiple optical sections of the retina is illustrated in Figure 5. The 10 cross-sections of the fovea were obtained in a normal subject in 200 msec. The foveal depression is clearly visible at the central sections. Except at the foveola, the vitreoretinal and chorioretinal interfaces are well separated. The vitreoretinal interface typically is seen as a sharper image than the chorioretinal interfaces because of a number of factors. Among those factors are focus, scatter in the retina, and penetration of light into the retinal pigment epithelium and choriocapillaris.

Intravisit Reproducibility
The mean reproducibility of points on maps obtained on the same day was ±3.6%. The mean thickness measured in this group of subjects was 320 μm. Thus, the point reproducibility was ±11.5 μm.

Single Scan Intervisit Reproducibility
The reproducibility of points in single maps obtained in multiple visits was ±4.1%, corresponding to ±13.1 μm.

Triscan Intervisit Reproducibility
The reproducibility of points in three averaged maps per visit was ±3.2%, corresponding to ±10.2 μm. This value is the average across all the subjects and all the points. To evaluate the local reproducibility, namely, the reproducibility at each point on maps obtained in three different visits, the coefficient of variance was plotted in Figure 7 as a frequency distribution for one patient, selected because the mean reproducibility was 3.3%, which was the closest to the overall average. Ninety percent of the points were found to be within 5% of the coefficient of variance.

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DISCUSSION

The new scanning retinal thickness analyzer provides three major improvements over our previous instrument: rapid scanning to cover a large fundus area in one measurement, noncontact optics, and easy alignment. The scanning mode yields 10 optical sections, 2 mm in height and spaced 200 μm apart, thus covering the 2 × 2 mm fundus area. Clinically, this coverage is sound because it corresponds to a disc diameter, and one such scan is sufficient to cover the fovea. By using a composite of nine scans, arranged in an array of 3 × 3 scans, the central 20° region of the posterior pole can be mapped. This area was chosen to match clinical needs, such as the area to be examined in diabetes to assess clinically significant macular edema, and to provide information on the area most critical to vision. The fact that each scan lasts only 200 or 400 msec is crucial to ensure quality and patient comfort. During fixation, the chance of an eye movement in this time is low because the latency interval for a saccadic eye movement is 200 msec. As mentioned, the scan time could be reduced to well below the time for voluntary eye movement by acquiring the images at a higher rate. Rates above video rate are achieved routinely when the area of interest is less than that of a full frame. This reduction in acquisition time would require a proportionally higher laser power, but this would not affect safety because actual power is at least 38 times lower than permissible exposure.

Patient comfort has been enhanced by basing the instrument on noncontact optics. This renders the procedure similar to fundus photography and is practical in the clinical environment. The ease of use for the operator has been improved by the aids during alignment. As in fundus photography, the distance to the eye and the centration in the pupil are important. Compared to conventional cameras, however, these alignments are achieved in a single step by obtaining a sharp, centered image of the pupil on a screen, similar to what is achieved with nonmydriatic fundus cameras. Feedback immediately after acquisition of the optical cross-sections and the fundus image allows the operator to perform a quality assurance check and provides the opportunity to repeat the scan. The fundus image acquired immediately after the scan is advantageous because it provides verification for the location of fixation and eases the registration between the retinal thickness map and fundus disease.

The assessment of scanning retinal thickness mapping concentrated first on depth precision and depth resolution. They are derived from the width of the light-intensity profile of a single interface. Measured in vitro, the FWHM was equal to that measured in normal human eyes and, most interestingly, almost equal to the diffraction limit. This indicates that the optics of the normal human eye and alignment with the instrument do not introduce any degradation, even though the incoming laser beam and the outgoing imaging path are located 2 mm from the center of the pupil. The measurement of the in vivo FWHM allowed the determination of the optimal depth precision, which was found to be 5 to 10 μm. It also indicated that the optimal depth resolution is 50 μm. In clinical practice, this resolution will be degraded by normal and pathologic scatter inside the retina and the optical media. But, because retinal thickness averages 360 μm, even significant degradation can yield a resolution sufficient to separate between the two external interfaces of the retina and to provide delineation of intraretinal pathologies. This is illustrated by the optical cross-sections obtained at the fovea and by our previous images acquired in macular holes with the same principle. Depth resolution and depth precision of scanning laser ophthalmoscopes are 300 and 50 μm, respectively. A comparison with optical coherence tomography is difficult because the in vivo FWHM has not yet been published. A crude evaluation can be obtained from epiretinal membranes because it can assumed that these are thin structures. Depth resolution estimated from the width of the membrane seen in these images is 30 μm. This high depth resolution explains the unprecedented details revealed by the technique. These details are potentially important to the researcher who studies the causes of retinal disease. It remains to be determined, however, whether the tomographic images are advantageous over those obtained by scanning retinal thickness analysis in providing clinical information that affects the management of patients.

The capability of scanning retinal thickness analysis to generate multiple optical cross-sections, illustrated here, is unique to the method. Optical sections clearly show the gradually increasing depression as one approaches the foveola. The other unique property of the method is the capability of the analysis algorithm to generate detailed maps without any operator intervention. The map of the fovea reported here provides a quantitative measure of the thickness and shows variations anticipated from histology. For example, the increased thickness on the nasal side of the fovea is caused by the higher abundance of nerve fibers along the papillomacular bundle.

The in vitro test of models with known thickness showed that the thickness values are linear in the range of clinical interest. Comparisons between in vivo retinal thickness analysis and histology are under way. It is questionable, though, whether they will permit an assessment of accuracy, namely, the difference between the absolute values obtained by retinal thickness analysis and histology because of changes induced by
tissue fixation. These comparisons most likely will assess the reliability of relative measurements.

Reproducibility test results indicated that intravisit reproducibility was $\pm 11.5 \mu m$. The major reason for the high performance of scanning retinal thickness analysis stems from the mapping capability. In one scan, 200 thickness values are obtained, covering a 2 $\times$ 2 mm area. This high, two-dimensional density of points permits smoothing of the data that is equivalent to multiple points averaging, and it yields a low variation. In addition, the fast acquisition time prevents lateral and axial motions.

Surprisingly, the intervisit reproducibility of $\pm 13.1 \mu m$ was close to that of the intravisit reproducibility because once the alignment is performed, there are no variations in parameters that can affect the results. This reproducibility indicates that, on average, one can detect changes of 26 $\mu m$ in retinal thickness with a 95% confidence interval. Results also show that the reproducibility can be improved further by averaging as few as three scans.

In summary, it seems that scanning retinal thickness analysis has four unique properties: multiple optical cross-sectioning of the retina, mapping of the retinal thickness, high reproducibility, and an acquisition time that minimizes artifacts caused by eye motion. The clinical implication of these capabilities must be evaluated.

Key Words
edema, glaucoma, macula, optical sectioning, retinal thickness.

Acknowledgments
The authors thank Salvatore D’Anna for assisting with the recruitment and managing the subjects.

References