Feasibility Test of a New Method to Measure Retinal Thickness Noninvasively

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There are many devastating ocular diseases that are directly related to an alteration of the retinal or nerve fiber layer thickness, such as glaucoma and macular edema. To diagnose these diseases earlier and to monitor their therapy more sensitively, an accurate measurement of the tissue thickness is needed. Since no clinical method is currently available, we developed and tested a new method capable of measuring noninvasively the retinal thickness. The separation between the images of the anterior and posterior intersections is quantitated by an optoelectronic system. The theoretical performance of the method has been calculated. Tests of the method in a model eye indicated that the measurements were basically diffraction limited, their reproducibility was ±9 μm, and their accuracy was 5.5 μm. Tests performed in vivo indicated that two intersections between the laser and the retina are present and correspond to the anterior and posterior surfaces of the retina. These intersections can be resolved and analyzed to yield quantitative data. These encouraging results indicate that this method is feasible and could yield sensitive measurements of the retinal thickness. Invest Ophthalmol Vis Sci 30:2099-2105, 1989

The evaluation of retinal thickness at the macula is clinically important in several diseases. The retina thickens in macular edema, which is commonly associated with many ocular conditions and is the major cause of visual impairment in a number of them. The observation of retinal thickening has been suggested for identifying the sites of edema accumulation and for following therapy. Moreover, retinal thickening has been used as the sole criterion for early treatment using laser photoagulation. This approach has been found effective in preventing visual loss. In addition, other eye diseases are manifested by a thinning of the retinal tissue.

An objective and accurate measurement of the thickness of the retina, at and around the macula, could thus provide a means for early diagnosis of diseases and a quantitative method by which to evaluate the effectiveness of therapeutic measures. There are currently no quantitative clinical methods to evaluate the thickness of the retina across the macular area. Clinicians obtain an impression of the retinal thickness by one of three methods: slit-lamp biomicroscopy, stereobiomicroscopy and stereophotography. In the first method, a narrow beam of light is directed to the desired retinal location, and its intersection with the retina is viewed under magnification. The separation between the images from the surface of the retina and the pigment epithelium gives the clinician an impression of the retinal thickness. The method is subjective, depends on the angle between the viewer and the illumination, and does not provide a permanent record that can be used for follow-up. The second method involves stereoscopic viewing of the fundus, and the third involves stereophotography and subsequent viewing of the negative. These methods are subjective and insensitive. An attempt at quantitation has been made using a stereoplotter to evaluate the stereophotographs but this requires very expensive equipment, unique operator skills and a great deal of time.

An interesting attempt to measure the retinal thickness noninvasively has been made using scanning ultrasonography. There are a few possible reasons why this method has not been clinically implemented: the location on the fundus from which the echoes are obtained cannot be accurately deter-
mined, very localized areas cannot be probed because the beam size is about 0.5 mm and it is practically impossible to obtain measurement through the crystalline lens.

In response to these needs, we developed an optical method capable of noninvasively measuring the thickness of the retina. In this article we will describe the method, calculate its theoretical performance and present the results of tests, performed in vitro and in vivo, to evaluate the feasibility of the method.

### Methods

#### Description of the Proposed Method

The method is based on exploiting the advantages of slit-lamp biomicroscopy while overcoming its limitations: the resolution is increased, the visual impression is replaced by a quantitative measurement and the results are permanently recorded along with the location of the measurements on the fundus.

The instrument, described schematically in Figure 1, is mounted on a slit lamp biomicroscope. A green helium–neon laser (540 nm) delivers a monochromatic and parallel beam of light controlled by a shutter. The beam diameter is increased by a 5:1 expander and by the magnification optics of the slit lamp microscope. The expanded beam is focused on the fundus by the optics of the slit lamp biomicroscope to which a cylindrical lens has been added. This results in the projection, at an angle, of a narrow slit on the retina. The cornea does not play a role in the focusing because its refraction power is nulled by a planar contact lens. The light is reflected and backscattered by the fundus, picked up by the objective lens, magnified and directed via a beam splitter toward a camera. As illustrated in Figure 2, the image consists of two slits separated by a distance proportional to the retinal thickness.

In the future the camera will be replaced by an optoelectronic system that will convert the image into a measurable signal. As illustrated in Figure 2B, the image will be scanned by a slit and the light passing through the slit will be detected by a photodetector. The image will thus be translated into a time-dependent electronic signal, and the time delay measured between the two maxima of the photodetector output will be proportional to the thickness of the tissue. A thickness profile will be obtained by steering a short slit along a preset path and performing numerous thickness measurements.

A measurement is performed as follows. The patient’s pupil is dilated, and the cornea is anesthetized and fitted with a contact lens. The attenuated laser is directed to the region of interest on the fundus. On activation of the system, the powers of the illumina-
Theoretical Feasibility and Performance of the Method

In this section we will evaluate the theoretical performance of the instrument. Overall, we will show that it should be feasible to measure sensitively the retinal thickness with a laser power density that is safe to the eye.

Size of the laser focal spot on the retina: The minimal spot size of a laser beam focused down by a lens is mainly determined by diffraction. The radius, $r_d$, of this spot is given by:

$$ r_d = \frac{1.22\lambda}{Dn} $$

where $\lambda$ is the wavelength of the laser light, $f$ is the focal length of the slit lamp biomicroscope, $n$ is the index of refraction of the medium and $D$ is the diameter of the laser beam at the objective plane. By substituting 100 mm for the focal length, 10 mm for the diameter of the expanded laser beam and 540 nm for its wavelength, one obtains a radius of 6.5 $\mu$m. (This estimate neglects the influence of water in the last 25 mm, taking into account the higher index of refraction of water would reduce the effective focal length of the biomicroscope and actually decrease $r_d$).

Separation between the two spots: As shown in Figure 2, the two laser spots on the fundus are laterally separated from each other due to the angle between illumination and viewing. The separation on the retinal plane ($\Delta x_r$) can easily be derived by

$$ \Delta x_r = \frac{SBn_v}{Z}(RT/n_r) $$

where SB is the stereobase (distance between the center of the objectives) $Z$ is the biomicroscope focal length, $RT$ is the retinal thickness and $n_v$ and $n_r$ are the indices of refraction of the vitreous and retina, respectively. For a slit lamp biomicroscope with objective apertures 23 mm apart and a focal length of 100 mm, the separation at the object plane between the spots varies between 20 and 100 $\mu$m for retinal thicknesses between 100 and 500 $\mu$m.

Thickness resolution: The thickness resolution, namely the minimal thickness that can be measured, can now be estimated. As the thickness decreases, the two spots come close to each other and ultimately overlap. Although "in fact a considerably smaller change could be seen, or at least detected with a sensitive intensity-measuring instrument," we will adapt Rayleigh's criterion of resolving power, namely, a separation equal to the radius of the diffraction limited spot. By substituting 6.5 $\mu$m for $r_d$, in equation (2), one obtains a thickness resolution of 33 $\mu$m. In other words, without relying on sophisticated algorithms such as deconvolution, one could theoretically measure thicknesses that are significantly thinner than those of the normal retina, which varies between 100 and 500 $\mu$m.

Sensitivity: The sensitivity of the measurement will depend on the signal-to-noise ratio. It should be feasible to locate the center of the signal to within one tenth of its width. Thus each spot center could be measured to within 1.3 $\mu$m and the separation to within $\pm(1.3^2 + 1.3^2)^{1/2} = \pm1.8 \mu$m, corresponding to a thickness sensitivity of $\pm9 \mu$m.

In Vitro Experimental Tests of Feasibility

A bench-top prototype was designed and tested on a simulated eye (Fig. 3). The eye was represented by lens B of 17 mm focal length in air (corresponding to the ocular focal length in water) and a pupil 6 mm in diameter. To simulate the transparent retina, clear plastic sheets were placed at the focal plane. A red helium–neon laser delivered, via a mirror, a parallel beam to the model eye. The scattered and reflected light passed through respective effective pupillary stops of 1 mm in diameter, and separated by 4 mm at the pupil plane. Lens A created a conjugated image onto the plane of a 10 $\mu$m scanning slit. The slit was fixed on a loudspeaker driven by a waveform generator. The light that passed through the slit was detected by a phototransistor. The output was digitized by a waveform analyzer (Data Precision, Danvers, MA), and the delay was measured by fitting cursors on the two peaks.

In Vivo Experimental Test of Feasibility

A prototype of the instrument was developed as described in Figure 1. The delivery system did not include a beam steering mechanism, the laser beam diameter was expanded to 8 mm, and a cylindrical lens, 300 mm in focal length, was placed in front of the left objective. Instead of the optoelectronic detecting system, not yet developed, the back of a 35
A mm camera was connected to the beam splitter by a standard imaging coupler (Zeiss, Germany).

A target was placed in the optical paths of the illuminating system. This target helped the patient fixate and prevented light from reaching the location on the retina where the laser slit was focused. The latter helped reduce the background light and thus enhanced the image of the laser intersection with the retina.

The film was developed and scanned by the prototype scanning densitometer described in Figure 3. The film was illuminated and placed in front of the lens, which was adjusted to create an image on the scanning slit. As mentioned above, the electronic signal from the phototransistor was recorded on the waveform analyzer.

After the protocol was approved by the Institutional Review Board, the instrument was tested on the eye of one of the investigators (RCZ).

Results

In Vitro Tests

Calibration: The slit in Figure 3 was replaced by a reticule, which obstructed the beam periodically as it was moved by the loudspeaker. The times of the troughs in the signal were plotted as a function of the serial number of the trough. At the center of the travel of the speaker, the function was found to be adequately linear, as indicated by an excellent linear correlation of 0.999. The known distance between the marks on the reticule allowed us to convert time units into displacement units along the motion of the slit.

Spot size at the image plane: A flat reflective surface was placed at the location of the artificial retina, and the output of the phototransistor was recorded as a function of time. The width of the laser spot at the image plane was determined by doubling the width at half maximum (measured with the waveform analyzer), and converted in micrometers to yield a value of 69 μm. Transparent targets were used to simulate retinas of different thicknesses. Figure 4 represents the signal obtained from two layers, 127 and 380 μm thick, respectively. The separation between the peaks was excellent and indicated that the two surfaces could be resolved sensitively. The width at half maximum of the anterior peak was 37 μm and that of the posterior peak was 42 μm. The widening of the second peak was due to defocusing at the posterior surface. The width at half maximum of the anterior peak can be compared to the radius of the diffraction limited spot. According to equation (1), a laser beam 0.8 mm in diameter can be focused by a lens 17 mm in focal length to a spot with a radius of 11.3 μm since the laser beam is projected on the slit plane with a magnification of 3 (equal to the ratio between the focal lengths of lens A [50 mm] and lens B [17 mm]). The diffraction limited spot radius at the slit plane of 34 μm. This value is very close to the above experimental value, indicating that the optical system is effectively diffraction-limited.

Thickness measurements: The results of thickness measurements performed on targets with five different thicknesses are shown in Figure 5 (crossed bars), where the separation at the image plane is plotted as a function of the known thickness of the material. The data demonstrate that the separation is proportional to the thickness, with a linear correlation coefficient larger than 0.999.

Reproducibility: The reproducibility was evaluated by computing the standard deviation of five measurements performed after moving the target and refocusing the system. It was less than ±9 μm for thicknesses between 150 and 500 μm.
Fig. 5. Separation on image plane as a function of material thickness. The bars represent the standard deviation of the data used for the linear regression. The open circles represent data obtained subsequently.

**Accuracy:** Three plastic targets with different thicknesses (open circles in Fig. 5) were measured after the correlation curve was obtained. Each target was measured four times and the mean was computed. The regression formula was used to convert the results into thickness for each measurement and the absolute values of the difference between this result and the known thickness were calculated. The accuracy, calculated as the average of the absolute differences, was 5.5 μm.

**Improved retina model:** Additional measurements were performed on a more realistic model that included scattering in the retina and a water-filled cavity to mimic the vitreous. A transparent plastic sheet, which was 270 μm thick, and represented the retina, was glued on a fundus color photograph to somewhat simulate the scattering and reflectance of the fundus.

The results are illustrated in Figure 6. The thickness could be resolved easily despite the appearance of a large peak generated by the color photograph. The width of the peak was again similar to the theoretical diffraction limit. The resolution was even good enough to indicate that the emulsion was not in contact with the plastic but rather was separated by a thin layer of glue.

**In Vivo Test**

Figure 7 shows the image of the intersection of the laser beam and the fundus at a location between the macula and the optic disc. Two lines can be seen clearly, the left one corresponding to a location anterior to the right one. The presence of a blood vessel is seen to cause the disappearance of the posterior intersection but does not influence the anterior intersection. This indicates that the anterior line is due to a reflection from an interface located anterior to retinal blood vessels.

Figure 8 represents a densitometric profile of a segment of the image in Figure 7. It can be seen that two peaks can be resolved clearly and that the distance between them can be quantitated easily.

**Discussion**

We have described a method designed to noninvasively measure the thickness of the retina. The theoretical evaluation has shown that the method is capable of measuring retinal thicknesses as low as 33 μm and that the sensitivity could be ±9n^{-1/2} μm, where n is the number of measurements averaged.

The experimental results obtained using transparent targets and a prototype of the instrument confirmed these estimates. They showed that the measurements were essentially diffraction limited and that the reproducibility was ±9 μm, in exact agreement with the theoretical sensitivity for n = 1. The experimental accuracy of 5.5 μm, determined by four measurements, corresponds with the theoretical error of the mean for n = 4, namely 4.5 μm. These values
for sensitivity and accuracy are encouraging because they are small compared with the retinal thickness in the posterior pole, which varies between 100 and 500 μm. These results were obtained in the absence of scatter, which could occur in the fundus. However, the fact that the addition of a color photograph of the fundus did not degrade the results is also encouraging.

The pilot test performed in vivo clearly indicates that two distinct intersections between the laser and the retina are visible. It is not surprising that the intersection of the laser beam with the posterior interface of the retina is visible since the structures located between the retina and the choroid layer emit most of the light that produces fundus images at 540 nm. Less obvious is the illustration of the anterior intersection. The presence of a well separated anterior intersection is a new observation. The light is most probably due mainly to specular reflections from layers that must be quite superficial, as they were shown to be located anterior to retinal blood vessels. It can be concluded that regardless of the exact physiologic nature of the layers involved in these optical phenomena the distance between them is very close to the anatomical thickness of the retina.

The pilot test in vivo also indicates that the image can be recorded with a safe laser power. The 50 μW laser power delivered to the fundus is equivalent to an intrabeam viewing, with a pupil 0.7 cm in diameter of a beam with a power density at the cornea of 50/π(0.7/2)² = 130 μW/cm². Laser safety guidelines state that under these conditions the maximum permissible exposure is 70 sec, while the photograph can be obtained at ½ sec.

The densitometric profile obtained from film illustrates that in the normal eye adequate quantitative data can be derived from the image since two clear peaks could be resolved and the distance between them could be measured.

In summary, the calculations and the tests performed in this feasibility study indicate that the proposed method to measure retinal thickness noninvasively is promising. More work obviously is needed to improve the instrument, assess the reproducibility in vivo and determine the degree of clarity of the ocular media necessary to obtain valuable results.

Key words: retinal thickness, biomicroscopy, macula, edema, atrophy

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References


