Effect of Angle of Incidence on Macular Thickness and Volume Measurements Obtained by Spectral-Domain Optical Coherence Tomography

Amirhossein Hariri, Sun Young Lee, Humberto Ruiz-Garcia, Muneeswar Gupta Nittala, Florian M. Heussen, and Srinivas R. Sadda

Purpose. Evaluation of the effect of angle of incidence on macular thickness and volume measurements obtained by spectral-domain optical coherence tomography (OCT).

Methods. A total of 30 eyes from 15 healthy young subjects underwent macular cube volume scans (512 × 128 protocol) following dilation using the Cirrus spectral domain OCT. For each eye, scans were obtained by positioning the scanning beam in the center of the dilated pupil, as well as in four eccentric positions (approximately 3 mm from the center), superior, inferior, nasal, and temporal to the pupillary center, to create oblique angles of incidence between the light beam and retina. In all cases, the region scanned by the volume cube was centered on the fovea. Macular thickness and volume measurements were computed for volume scan acquisitions, and differences in values between eccentric scans and the central scan were analyzed.

Results. Retinal thickness and volume values were observed to increase significantly in all subfields for all eccentrically-obtained scans compared to scans obtained through the center of the pupil. The mean increase in thickness for the various scan positions and subfields ranged from 3.76 to 11.38. Scans that were displaced temporally consistently showed the greatest increase in thickness and volume, whereas nasally positioned scans showed the least increase. The increase in retinal thickness for all subfields correlated significantly with angle of inclination or tilting of the retina.

Conclusions. Macular thickness and volume measurement results may be affected significantly by positioning of the scanning beam in the pupil and resultant angle of incidence on the retina. These findings suggest that care should be taken to position the scanning beam consistently in the center of the pupil to achieve reliable measurements. (*Invest Ophthalmol Vis Sci.* 2012;53:5287–5291) DOI:10.1167/iovs.12-9767

From the Doheny Eye Institute and the Department of Ophthalmology, Keck School of Medicine of the University of Southern California, Los Angeles, California.

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Corresponding author: Srinivas R. Sadda, Doheny Eye Institute, 1450 San Pablo Street, Los Angeles, CA 90033; sadda@usc.edu.

Retinal thickness measurements obtained by optical coherence tomography (OCT) have become integral tools in the management of patients in clinical trials and clinical practice.[1–4] The reproducibility and reliability of retinal thickness measurements have been studied extensively by a number of investigators. Previously, we evaluated the accuracy of retinal thickness measurements obtained by Stratus OCT and Cirrus High Definition (HD) OCT.[5,6] We observed that errors in retinal thickness measurements were not uncommon due to incorrect identification of retinal boundaries, particularly in patients with complex retinal diseases that caused significant derangement of these boundaries. A number of investigators have attempted to evaluate factors that affect the reproducibility of measurements obtained with OCT devices. For nerve fiber layer thickness measurements, we observed that signal strength was an important predictor.[7] More recently, investigators have studied the impact of angle of incidence on OCT imaging. In general, when performing OCT scans, OCT operators center the imaging beam in the center of the pupil as they proceed to focus on the retina and obtain the scan. This results in a perpendicular angle between the OCT beam and retina, which in emmetropic patients produces a horizontally oriented B-scan when scans are obtained through the fovea. In many cases, however, due to poor patient cooperation or other operator factors, scans are obtained through eccentric portions of the pupil. In this case, the scanning beam strikes the macula obliquely and the B-scans also may be oriented obliquely (e.g., left side higher than the right side). Generally, this oblique orientation does not hinder the clinical interpretation of the OCT scans and, thus, is not a finding that triggers re-acquisition of scans. Lujan et al., however, demonstrated that angle of incidence could affect visualization of certain structures, such as Henle's fiber layer.[8] They recommended that for adequate evaluation of Henle's layer, OCT scans may need to be obtained from multiple orientations. The impact of angle of incidence on retinal thickness measurements, however, was not evaluated in these prior studies.

To evaluate this question, we performed OCT volume scans at perpendicular and oblique angles of incidence using the Cirrus OCT, and compared retinal thickness and volume measurements between perpendicular and oblique scans.

Materials and Methods

We recruited 15 healthy young volunteers with known normal retinas (from prior examination) for participation in this study. The study was approved by the institutional review board of the University of Southern California, and written informed consent was obtained from all subjects. The research adhered to the tenets set forth in the Declaration of Helsinki.

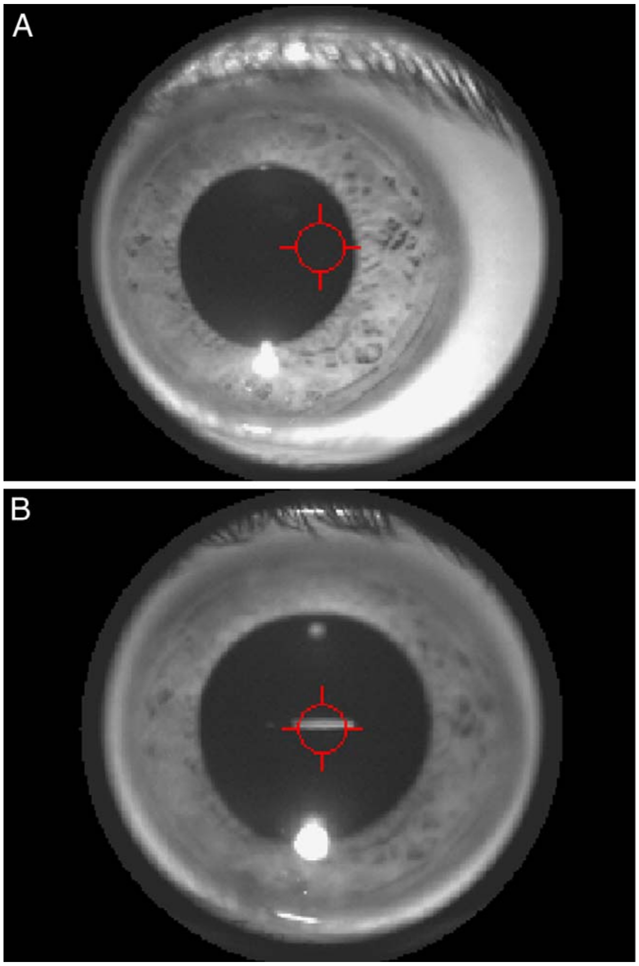

Figure 1. SLO image from Cirrus OCT in which the scanning beam has been displaced (**A**), compared to image in which scanning beam is central (**B**).

Each subject underwent a full ophthalmic examination, including the assessment of visual acuity and refractive error, and a dilated fundus examination with a 90 diopter (D) lens. Both eyes of all subjects were noted to achieve good dilation, with pupils achieving 7–8 mm in size. Subjects then underwent OCT imaging using the Cirrus HD OCT (software version 4.5.1.11; Carl Zeiss Meditec, Dublin, CA) with the macular cube 512 × 128 protocol. Cube scans were obtained with the scanning beam centered in the pupil to achieve a perpendicular angle to the retina and horizontally-oriented B-scans. Cube scans then were obtained after moving the scanning beam to 4 eccentric positions (nasal, temporal, inferior, and superior) approximately 3 mm from the center of the pupil to generate obliquely oriented scans. Figure 1 illustrates the scanning laser ophthalmoscopic (SLO) view of the pupil after temporal displacement of the scanning beam. Note, the area of retina that is scanned still is centered on the fovea despite the eccentric orientation of the light beam through the pupil. All central scans then were repeated to measure reproducibility. If a signal strength of less than 7 was observed for any scan, the scan was repeated and the lower quality scan was not used for further analysis.

Following completion of scan acquisitions, retinal thickness maps were generated for all scans using the Cirrus software. Retinal boundaries were inspected on all B-scans to ensure that segmentation errors were not present. Retinal thickness values for each of the ETDRS subfields as well as the total macular volume were recorded and tabulated for all cases. The differences in measurements for each subfield were computed for each scan position.

To compute the approximate angle of incidence of the light on the retinal surface, the central B-scan (i.e., through the foveal center) from each volume scan, including the machine-generated boundaries, was exported as an image file for further analysis. B-scans were imported and analyzed using the National Institutes of Health image-analysis software (ImageJ 1.42q; developed by Wayne Rasbands, National Institutes of Health, Bethesda, MD). The angle α was defined as the angle between a vertical line/A-scan at the foveal center and the outer retinal pigment epithelium (RPE) boundary line as determined by the Cirrus OCT (Fig. 2A). For the volume scans obtained through the pupillary center, the angle α was close to 90 degrees for all cases in this normal cohort. The "amount of inclination" for each of the eccentrically-oriented scans was calculated as the difference in α angles between the scan obtained through the pupillary center and that obtained at the off-axis pupillary position (Fig. 2B).

To evaluate the differences in retinal thickness measurements between central and eccentric scans, a paired *t* test was performed. The correlation between the angle of inclination, and the macular thickness and macular cube volume changes was evaluated by Pearson's correlation analysis. Significance was set at a $P < 0.05$. All statistical analyses were performed using commercial software (Statistical Package for Social Science, version 19.0; SPSS Inc., Armonk, NY).

Results

We enrolled 15 healthy subjects (30 eyes) with normal ophthalmological examinations in this study, including 7 women and 8 men. The mean age of the subjects was 37.5 years, and mean refractive error (spherical equivalent) of the 30 eyes was −1.74 ± 2.30 (range +0.25 to −8.00) D. The one individual with a refractive error of < −6.00 (i.e., 8.00) had a

Table. Change in Measured Macular Thickness and Cube Volume in Different Eccentric Scans Compared to Primary Position

	Nasal	Inferior	Superior	Temporal
Outer superior subfield	4.68 ± 4.63 (−5, 12)	6.00 ± 4.65 (−5, 14)	8.07 ± 5.33 (−2, 21)	8.44 ± 6.94 (−5, 21)
Outer nasal subfield	4.14 ± 4.55 (−5, 12)	4.97 ± 5.49 (−4, 13)	7.11 ± 4.42 (−4, 14)	7.25 ± 5.89 (−5, 18)
Outer inferior subfield	3.78 ± 4.60 (−1, 16)	6.14 ± 5.31 (−3, 16)	6.90 ± 4.36 (−1, 17)	8.83 ± 5.56 (0, 19)
Outer temporal subfield	6.04 ± 5.61 (−3, 16)	7.38 ± 6.43 (−4, 18)	7.48 ± 4.34 (−2, 15)	9.86 ± 5.68 (−1, 23)
Inner superior subfield	6.21 ± 4.69 (−5, 14)	8.50 ± 5.89 (−5, 18)	9.45 ± 4.63 (−1, 16)	11.38 ± 5.97 (−1, 24)
Inner nasal subfield	6.19 ± 4.26 (−3,16)	8.27 ± 6.24 (−5,19)	8.36 ± 5.17 (−3, 17)	9.26 ± 7.17 (−3, 33)
Inner inferior subfield	5.14 ± 5.29 (−5, 21)	9.11 ± 6.68 (−5, 21)	9.19 ± 4.69 (−5, 16)	10.33 ± 6.82 (−1, 24)
Inner temporal subfield	4.92 ± 5.14 (−5, 15)	9.26 ± 7.10 (−4, 22)	9.34 ± 4.47 (2, 25)	10.62 ± 6.37 (−3, 25)
Central subfield	3.93 ± 3.67 (−4, 12)	7.07 ± 7.30 (−8, 27)	7.30 ± 3.61 (0, 14)	8.17 ± 5.71 (−3, 17)
Cube average thickness	3.76 ± 3.91 (−5, 13)	4.71 ± 5.06 (−4, 13)	7.12 ± 3.47 (0, 13)	7.15 ± 6.16 (−3, 19)
Cube volume	0.13 ± 0.15 (−0.2, 0.5)	0.15 ± 0.20 (−0.3, 0.5)	0.20 ± 0.22 (−0.4, 0.5)	0.23 ± 0.23 (−0.3, 0.7)

Values are mean ± SD (Min, Max). Thicknesses are in micrometer and cube volume is in mm^3. A positive value means the thickness in that scan position is greater than in primary position.

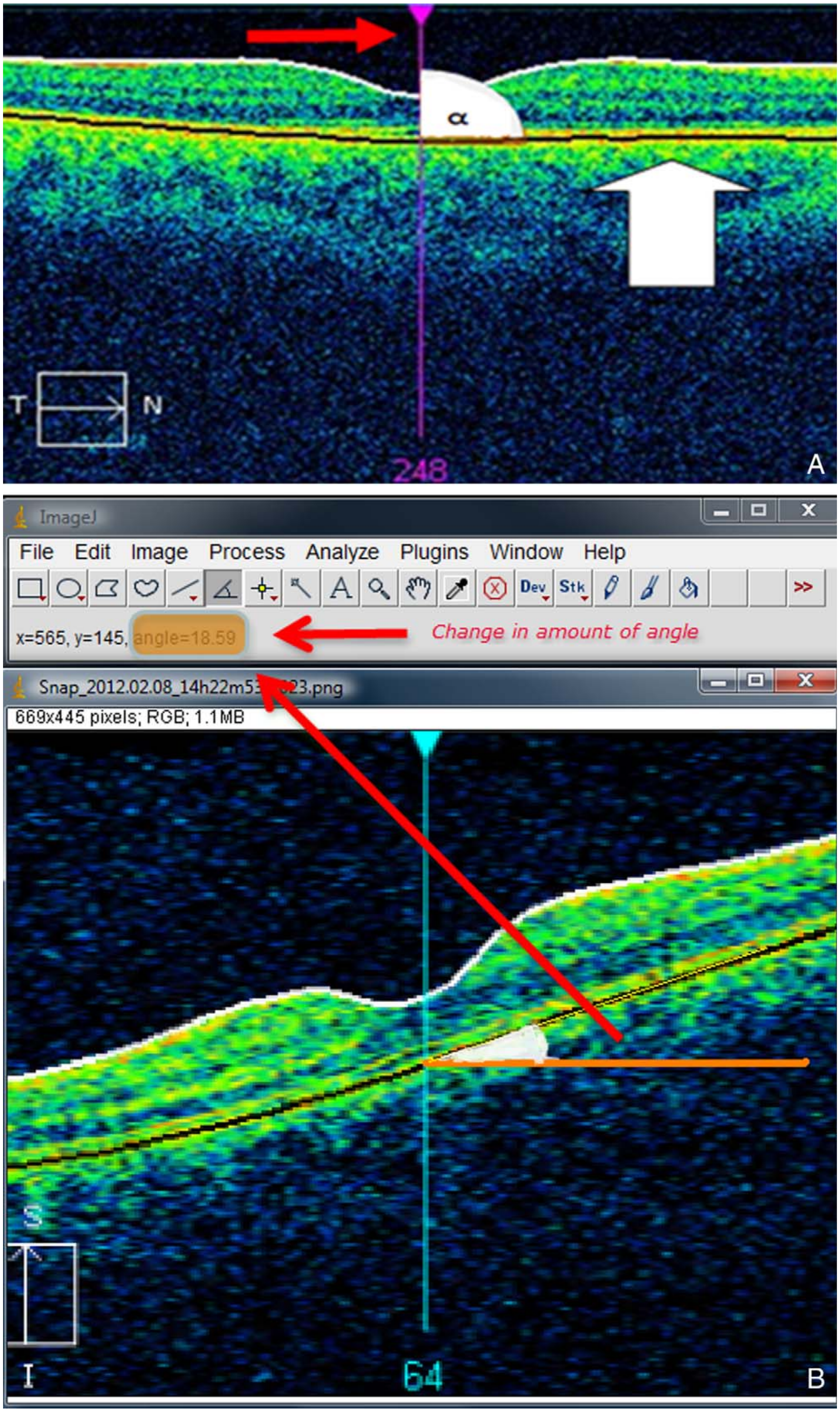

FIGURE 2. Calculation of the angle of incidence (α). B-scan through the foveal center, including the instrument boundary lines, is imported into image J. Angle is calculated between a vertical line through the central A-scan (foveal center) and the RPE boundary line as determined by the OCT instrument. For primary position (**A**) this angle obviously is 90 degrees, but for an off center image due to angulation this angle would be changed. The amount of this change was calculated by Image j as shown in (**B**).

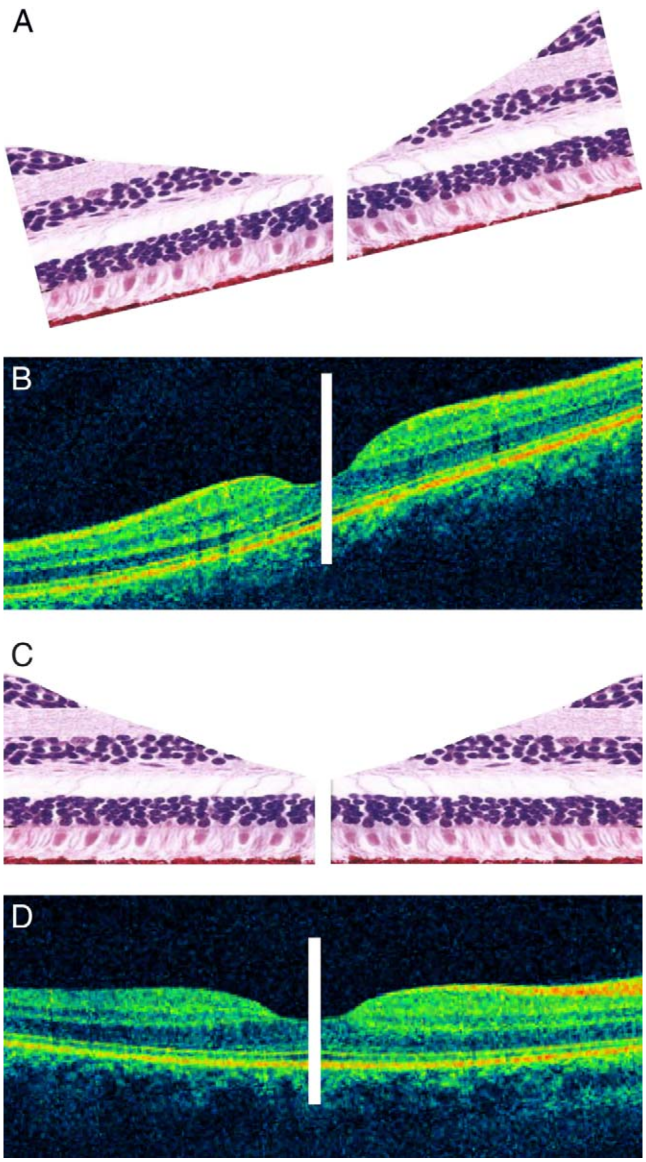

FIGURE 3. A non-perpendicular intersection with retinal surface results in a longer path from the retinal surface to the RPE and, hence, a greater "retinal thickness" in comparison with a perpendicular intersection. Compare images (**A**) and (**B**) with (**C**) and (**D**).

normal-appearing fundus with no fundus features of high myopia or staphylomatous change (i.e., centrally positioned scan in this individual was relatively flat similar to the other emmetropic subjects).

To establish the level of inter-scan reproducibility of the retinal thickness measurements, the central scans were repeated and differences between scans were computed. The mean differences for the various subfields were less than 3 μ and not statistically significant.

Eccentric scanning resulted in an expected inclination or angulation of the B-scans relative to the relatively flat orientation of the B-scan obtained in primary position. However, despite attempts to position the scanning beam in the pupil at the same eccentricity or distance from the visual axis, the angle of inclination appeared to vary significantly depending on the direction of displacement relative to the visual axis. The greatest angle was observed with temporal positioning and the smallest with nasal positioning (14.52 ± 2.63 and 8.69 ± 1.91, respectively).

The difference in measurements between the various eccentric positions compared to primary position is shown in the Table. Thickness and volume measurements for all subfields were significantly greater in any eccentric position compared to primary ($P < 0.01$ for all comparisons with primary), with the greatest difference observed with temporal displacement and the smallest with nasal positioning. The maximum observed difference between eccentric and primary position for any case was 33 μ, or a percent difference of 10.6%. The differences in thickness and volume measurements in each subfield and the entire macula were correlated with the angle of inclination of the central B-scan (relative to primary position). A statistically significant relationship ($P < 0.05$) was observed for every subfield with Pearson correlation coefficients.

Discussion

In our study, eccentric positioning of the OCT scanning beam within the pupil resulted in a significant increase in retinal thickness and volume measurements generated by the Cirrus OCT. In some cases and some subfields, differences greater than 30 μ or more than 10% of the retinal thickness could be observed. The amount of the increase in the retinal thickness appeared to correlate strongly with the degree of tilting or inclination of the B-scan caused by the eccentrically positioned beam. Importantly, this increase in thickness was significantly greater than the levels of intersession reproducibility observed in this study, as well as what has been described in previous reports.[9,10] The cause of this observed increase in retinal thickness is not certain. However, one likely and important explanation is that eccentric positioning, with resultant non-perpendicular intersection between the scanning beam and retina, results in a more tangential or angled section through the retina. This non-perpendicular intersection with retinal surface results in a longer path from the retinal surface to the RPE and, hence, a greater "retinal thickness." This is illustrated in Figure 3. This explanation also would be consistent with the observation that greater angles of inclination were associated with greater retinal thickness measurements.

Despite efforts to displace the scanning beam to the same extent (3 mm from the pupillary center) in all directions, temporal displacement consistently yielded a greater inclination and greater retinal thickness measurements in all cases. Although the reason for this difference is not immediately apparent, we suspect it is related to the normal curvature of the posterior pole of the eye and normal temporal position of the foveal center relative to the optic nerve.

The findings from our research may be of importance in longitudinal studies monitoring retinal thickness changes over time. For most studies, thickness changes of 10% or more are considered clinically meaningful. However, our study suggests that angle of incidence-related effects can result in changes in thickness of 10% or more in some cases. Thus, when designing OCT scanning protocols, maintaining a central and consistent position of the scanning beam would appear to be an important parameter to specify. Our findings also may have import when scanning non-foveal or peripheral areas of the retina when perpendicular scanning is not possible. Retinal thickness measurements performed in these areas may be increased artifactitiously as a result of this effect. A similar phenomenon may occur in patients with significant staphylomatous change or abnormal curvature of the posterior pole (e.g., eyes with pathologic myopia). Because of the close relationship between the angle of inclination and the observed thickness measurement, it may be possible to develop corrective algorithms to compensate for the non-perpendicular angles of incidence.

Our study is not without limitations. First, our series is relatively small. However, it is worth noting that the findings were highly consistent across all eyes and subfields in this analysis. Second, though efforts were made to position eccentric scans at a consistent distance from the visual axis, this still was an approximation and small displacements could not be excluded. Nonetheless, our findings would appear to highlight the importance of consistent scanning position and angle of incidence in quantitative OCT studies.

In summary, we observed that eccentric positioning of the OCT scanning beam in the pupil relative to the visual axis can result in inclination of the B-scan and an increase in measured retinal thickness. These increases can be significant, exceeding 10% of the actual thickness in some cases. The increased thickness appears to be predictable and related directly to the degree of inclination. Accounting for the effect of angle of incidence may be important when interpreting the results of longitudinal quantitative OCT analyses.

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