·Clinical Research·

Comparison of choroidal thickness measured by two methods

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Abstract

• AIM: To examine the profile of the choroidal thickness (CT) in healthy myopia subjects and emmetropic participants by Heidelberg Eye explore software and Image J software so as to compare the agreement and reproducibility of the two methods.

• METHODS: Thirty-six study participants (36 eyes) were enrolled in this research. The fovea and parafoveal region (the region of 6mm diameter of the fovea as center) of the images were selected by spectral domain optic coherence tomography (SD-OCT). The choroidal thickness was measured manually by the Heidelberg Eye explore software (version 5.3.3.0, Heidelberg Engineering) with a vertical line and the Image J software with a line vertical to the retinal pigment epithelial layer. The agreement and reproducibility of the two methods were described by the Bland-Altmann analysis.

• RESULTS: As compared with Heidelberg Eye explore software (39.9186), the repeatability coefficient is lower calculated by Image J software (27.3525). The Bland-Altmann analysis showed that the limits of 95% CI of agreement analysis is -18.437-63.949μm and the upper limits of the precision of the 95% CI of agreement is between 16.102 and 111.796μm and the lower limits is range from -66.29-21.41μm, which reflected a great variations of the difference.

• CONCLUSION: The repeatability and agreement of measurement implied by Image J software was better than the Heidelberg Eye explore software. The Image J software should be used for measuring the choroidal thickness in future study in China.

• KEYWORDS: choroid; optic coherence tomography; investigative techniques

INTRODUCTION

Choroid which was primarily consists of vascular structures serves many essential functions for the eye [1]. It acts as a heat-sink [2,3] and delivers nutrition for the retina. In depriving of form vision model, the active change in choroidal thickness (ChT) was found in chickens and primates: the changed ChT which move the retina forward and back to bring the photoreceptor layer into the corrective position where the external light will focus on[4-6]. It was also found that choroid was related with many ocular diseases, such as age-related macular degenerative (AMD) [7,8], diabetic retinopathy [9] and glaucoma [10]. In recent studies, Cbakraborty R used a noncontact optic biometer found that not only intraocular pressure, but also choroidal thickness exist diurnal variations[11]. Thus, it is important to investigate this vital structure in border context.

The spectral domain optic coherence tomography (SD-OCT) which use the Fourier domain OCT technology to produce a cross sectional B-Scan image enables to reflect the changes of choroidal thickness reliably. The infrared beam of a super luminescence diode (SLD) of SD-OCT has an average wavelength of 870nm.

The systemic error was inevitable in the process of measuring choroidal thickness because of the manual pattern applied in most of the researches up until now. In this study, we applied two different methods to measuring the choroidal thickness, and the agreement and reliability were compared.

MATERIALS AND METHODS

Subjects From June 2010 to November 2011, thirty-six Chinese emmetropic or myopic subjects (involving 36 eyes) received ophthalmic examination at Tongji Hospital, Wuhan, Hubei, China. The examinations included the best-corrected visual acuity (BCVA), visual field test (Humphrey, 24-2, SITA-standard), intraocular pressure measurement (Goldmann tonometer), anterior segment examination (slit-lamp microscopy), ocular fundus examination (direct and indirect ophthalmoscopes), central corneal thickness

measurement (CCT; A-mode ultrasound) and dioptometry. For emmetropic participants, either of eyes was enrolled randomly. And for ametropic subjects, more severed myopic eye was selected.

The candidates with the following conditions would be excluded from the study if (1) the subject was below 18 years old; (2) BCVA<0.5 (to ensure that the subjects had good central fixation); (3) Candidates had serious cataract and other ocular diseases that make OCT difficult; (4) Candidates had eye diseases such as glaucoma, age-related macular degeneration or received eye operations; (5) Candidate suffered from hypertension, diabetes or had any family history of these conditions.

Written inform consents were obtained from all the study participants before being enrolled in the study and the Declaration of Helsinki was strictly followed throughout the study.

Methods

SD-OCT SD-OCT (Spectralis, Heidelberg, Germany) which has 870nm wavelength and 40 000Hz/second high speed domain frequency was used. The participants who did not use the mydriatic drops fixed head on the holder stably, an inverted representation of the fundus oculi was taken to bring choroidal layer approximate to the zero-delay line. The images were stacked 100 frames for B- scan using the automatic real-time and eye tracking features. The quality bar indicated the signal strength of >25 being used for acquisition of the image. Choroid was defined as the layer extended from RPE to the choroid-sclera hyper-reflective borderline in the profile of the image(Figure 1). Twenty-five intersection points which was 1mm spaces apart in the same direction between eight 45-degree-spaced lines radiating from the fovea and the region of 6mm diameter of the fovea as center (the parafoveal region) were selected (Figure 2). The detailed process of measuring ChT was described below.

Heidelberg eye explore software The Heidelberg eye explore software (intrinsic software, version 5.3.3.0, Heidelberg Engineering, German) of SD-OCT was implied to measuring the mean thickness of choroid (mChT; in the fovea and in the parafoveal region) in emmetropic and myopic participants. The details of measurement method shown in Figure 3, the measuring processes were accomplished depend on the intrinsic vertical line.

Image J software Then the results of the mChT (the mean of the thickness in the fovea and in the parafoveal region) which was perpendicular to RPE layer was calculated manually in the plane by Image J software (version 1.42, National Institutes of Health, USA) (Figure 4). The value of
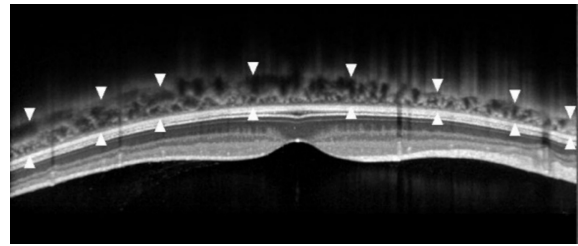

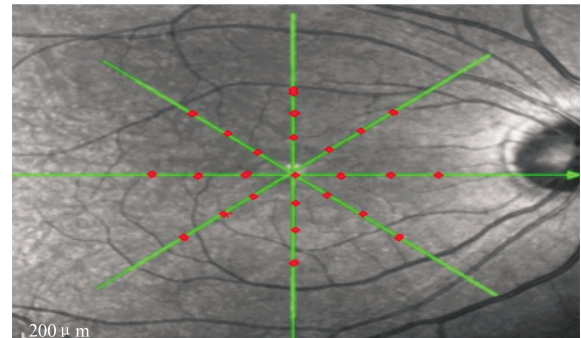**Figure 1 Scope of choroidal thickness obtained by SD-OCT.**

Figure 2 Selection of measurement Points in the profile of retina. 1mm apart from two points in the same direction.

the mChT was conducted twice by one observer separately using the two methods mentioned above. The average value and the difference between the twice repeated measurements for each method were calculated, and the difference of the average value between the two methods was stored for later statistical analysis.

Statistical Analysis

Univariate linear regression analysis between two methods The simple regression analysis was applied and the determinate coefficient (R^2) was used to indicating the association between the two methods.

Measuring repeatability between two sequential measurements for each of the methods The differences and means between two consequence measurement for each method was calculated[12]; we square all the differences, add them up, divide by the number of participants, and take the square root, to get the standard deviation of the differences for each method, the repeatability coefficient (R_c) was twice this[12]. The formulation was shown as below:

$$R_c = 2S_d = 2\sqrt{\frac{x_1^2 + x_2^2 + x_3^2 + \cdots + x_n^2}{n}} \qquad (1)$$

S_d stands for the standard deviation of the differences between two consequence measurement for each method; X_n stands for the individual difference of the subject in two consequence measurement in each method, and n means the sample size of the participants in each method.

Analysis of the agreement between the two methods The normal distribution of the difference of choroidal thickness measured by two methods was checked by drawing the histogram. The Bland-Altmann analysis [12] was
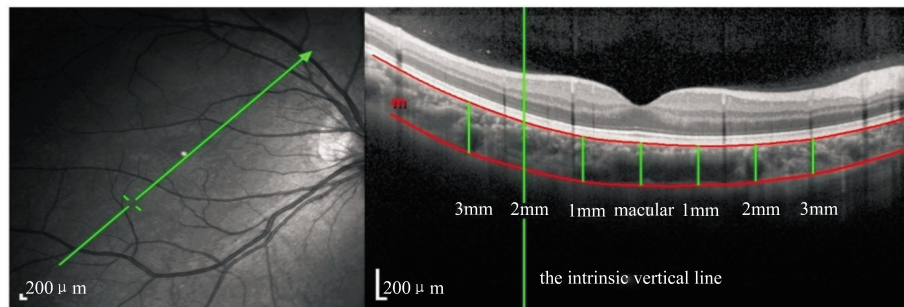
Figure 3 Choroidal thickness implied by Heidelberg Eye explore software.
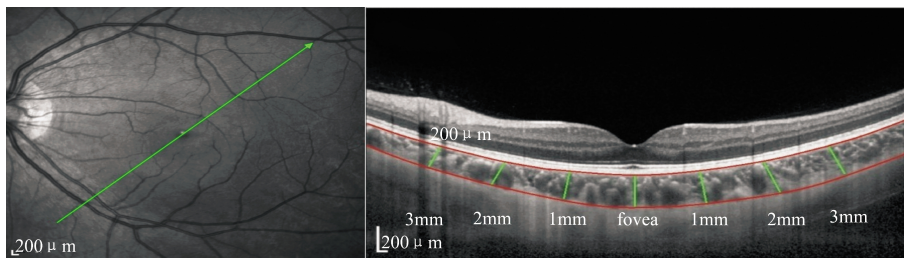

Figure 4 Choroidal thickness implied by Image J software.

used to determine the agreement of the two methods, the 95% confidence interval (95% CI) in the graph can be obtained by calculating the corrected standard deviation. The formulation was shown as below:

$$S=\sqrt{S_d^2+\frac{1}{4}S_1^2+\frac{1}{4}S_2^2} \qquad (2)$$

$$95\%\ CI= (\bar{d}\pm2S) \qquad (3)$$

S is the corrected standard deviation of S_d; S_d stands for the standard deviation of the differences between the means for each method. S_1 and S_2 indicate the standard deviation of differences between repeated measurements for each method separately. $\bar{d}$ stands for the mean difference between the two methods.

Precision of estimated 95% CI of agreement The estimated 95% CI obtained by the agreement analysis was applied to the whole population, different samples will have different limit. So we want to know how precise the values of the estimated 95%CI are. According to the method of the Bland JM and Altmann DG publicated in 1986 in Lancet [12], the standard error (SE) of $\bar{d}\pm2S$ is about $\sqrt{\frac{3S_d^2}{n}}$ (4); the upper limit of the 95%CI is $\bar{d}+2S-(t*SE)$ and $\bar{d}+2S+(t*SE)$ (5); The 95% CI for the lower limit of agreement is $\bar{d}-2S-(t*SE)$ and $\bar{d}-2S+(t*SE)$ (6). The t-value would be calculated if the differences obtained between the two methods follow a distribution which is normal (the t-distribution).

SPSS software package (Version 16.0, SPSS Inc, Chicago, IL, USA) was used for statistical analysis. $P<0.05$ was considered statistically significant.

RESULTS
Statistical Description All the measurements were listed in Table 1. 36 emmetropic or myopic subjects were enrolled in this study.

Univariate Linear Regression Analysis Univariate linear regression analysis showed that the value intrinsic Software measured was well linearly related with the result obtained by the Image J software (Figure 5).

Repeatability Analysis between Two Sequential Measurements for Each Method The results calculated by formulation (1), the repeatability coefficient for intrinsic software was 39.9186; and for Image J, the repeatability coefficient is 27.3525 which were lower than the former (Figure 6).

Agreement Analysis between the Two Methods For analyzing the agreement of the two methods, we drew the histogram of the difference between the two methods first, as Figure 7 displayed, the distribution of the difference is normal, so the Bland-Altmann diagram was drew. From Figure 8 we can see the mean of the difference was 22.756um; the corrected standard deviation (S) is 21.017; thus the 95% CI ($\bar{d}\pm2S$) would be calculated (the upper limit is 63.949μm; the lower limit is -18.437μm) by formulation (2) and (3).

Precision of Estimated 95% CI of Agreement The standard error (SE) of $\bar{d}\pm2S$ is 6.153 and the t-value was 7.78. For the upper limits of 95%CI of the agreement, the estimated scope is 63.949+ (7.78*6.15) to 63.949- (7.78* 6.15), giving 16.102-111.796μm. And for the lower limits of the 95%CI of agreement, the estimated value is -66.29 -21.41μm.
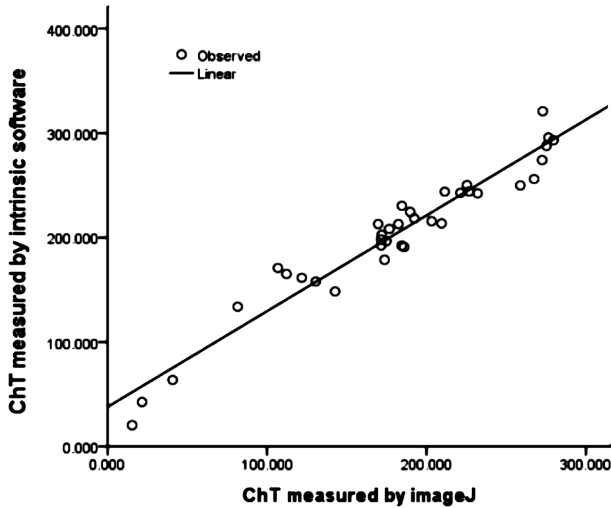
Figure 5 Scatterplot of choroidal thickness of all the subjects measured by the two different methods show a significant correlation $P<0.001$; y=0.916X+37.919; R^2=0.937.

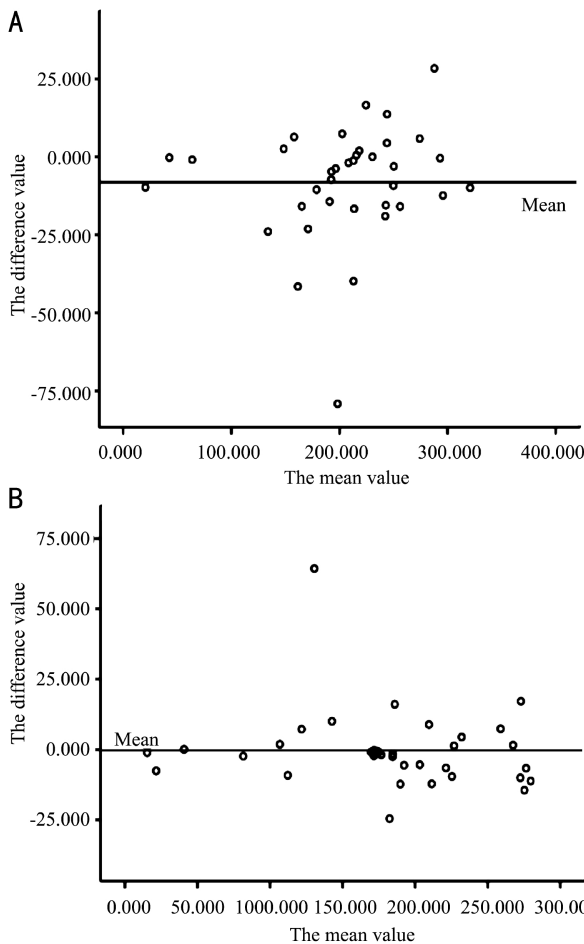Figure 6 Repeatability evaluation of the chorodial thickness measured by (A) Heidelberg Eye explores software and (B) Image J software.

Table 1 Descriptive statistics   mean±SD

Parameters	Emmetropic group	Myopic group
Number	9	27
Sex (male/female)	5/4	15/12
Age(a)	32.22±13.49	26.33±10.64
RE(D)	-0.51±0.23	-7.22±3.31
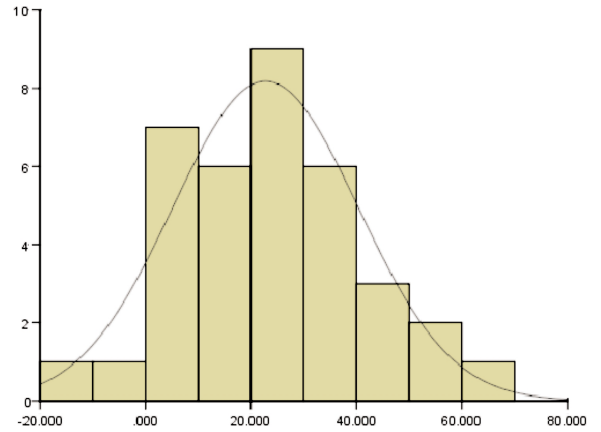
Figure 7 Histograph shows a normal distribution of the difference of choroidal thickness between the two methods.
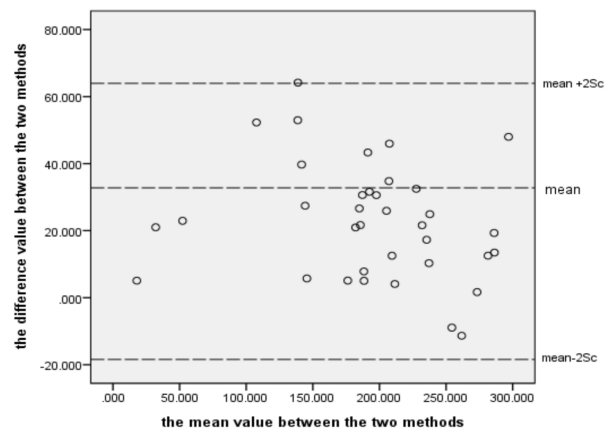

Figure 8 The Bland−Altmann analysis, the graph evaluated the agreement of the two different methods.

DISCUSSION

The spectral optic coherence tomography which used in most of studies in recent years attempted to find the variations of choroidal thickness because of the various important functions it belongs, such as thermoregulation, blood supply, refractive compensation. Recently, we found that the choroidal thickness changed accompanied by the alteration of the thickness of photoreceptor layer implied by Image J software, and hypothesized that the choroid and photoreceptor neurons constitute the neurovascular unit [13]. We searched the studies with regard to the choroidal thickness measurement of various ocular diseases in recent three years in PubMed, Medline and about 16 articles using the SD-OCT (Spectralis, Heidelberg, Germany) to capture the posterior image of the profile of retina (81% measured by the Heidelberg Eye explore software and 18% the Image J software was used for measuring). Either the intrinsic software of SD-OCT or Image J, the systemic error inevitably existed because the manual pattern must be implied to calibrate the thickness of choroid.

Ikuno *et al* [14] evaluate the reproducibility of choroidal thickness measurement between the two different machines (SD-OCT and High penetration OCT), the higher reproducibility was found in intersystem comparison. In our study, we compared the reproducibility and agreement of the two different measuring methods.

For evaluating the correlation between the two methods, the univariate regression analysis was implied and the determined coefficient (R^2) was 0.937. The null hypothesis here is that the relationships between the two methods are not linearly related. The possibility is small ($P<0.001$) and we can conclude that the choroidal thickness measurements by the intrinsic software of the SD-OCT and the Image J are related.

From the geometrical theorems of triangle, the choroidal thickness measured by the intrinsic software using the vertical measuring line would cause the measuring error because of the distortional image due to the extremity curvature in high myopia eyes or the tilt image obtained by the process of the image acquisition. And we also know that the high relationship could not mean the two methods agree and the better repeatability of each method was essential to evaluate the agreement of the two methods. As shown in formulation (1), the repeatability coefficient (R_c) calculated by Image J (27.3525) is lower than intrinsic software measured (39.9186), the repeatability coefficient (R_c) is proportional to the standard deviation of the differences for the methods, therefore we could conclude that the repeatability of Image J is better than the intrinsic software of SD-OCT from the result and we could also deduce this result from the Figure 6 which the mean of the difference are 8.137 and 0.25.

It was shown that the distribution of the difference between the two methods are normal (Figure 7), 95% confidence intervals thus can be calculated by finding the appropriate point of the t-distribution with 35 degrees of freedom, and the Bland-Altmann graph was drew. From Figure 8, the limits of 95% CI of agreement analysis is -18.437μm to 63.949μm, thus the result of choroidal thickness measured by Image J may be -18.437μm below or 63.949μm higher than the result obtained by the intrinsic software of the SD-OCT, which might not be accepted for clinical purpose.

From the results of the precision estimated 95% CI of agreement, we can see that the interval is wide, the upper limits is between 16.102μm and 111.796μm and the lower limits is range from -66.29μm to 21.41μm, which reflecting the great variations of the difference. Thus the degree of the agreement between two methods will not be accepted.

In conclusion, the repeatability and agreement of measurement implied by Image J software was better than the intrinsic software of SD-OCT. Instead of the intrinsic software (version 5.3.3.0, Heidelberg Engineering), the Image J software should be used for measuring the choroidal thickness in future study in China.

Our study has some limitations. Not every image shows choroid-sclera borderline clearly, this may fail to include some extravascular choroid. For resolving this problem, implying the enhanced depth imaging OCT which the wavelength is 1060nm may solve this problem.

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