Multidisciplinary Ophthalmic Imaging

Reproducibility of Retinal and Choroidal Thickness Measurements in Enhanced Depth Imaging and High-Penetration Optical Coherence Tomography

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PURPOSE. Two optical coherence tomography (OCT) modalities can visualize the choroid: high-penetration OCT (HP-OCT) using a long wavelength, and enhanced depth imaging technique using Heidelberg OCT (EDI-OCT). The purpose of this study was to compare and investigate the agreement among the retinal/choroidal thickness parameters.

METHODS. Twenty-four eyes of 12 healthy volunteers were examined simultaneously using the prototype swept-source HP-OCT and EDI-OCT. Six independent examiners measured the central retinal/choroidal thicknesses on horizontal B-scan images. The reliability was evaluated by intraclass correlation coefficient (ICC). Intervisit reproducibility was assessed by examining 10 of the volunteers 4 months later.

RESULTS. Using HP-OCT, the average of all measurements was 209.1 ± 12.9 μm in the retina and 292.7 ± 77.3 μm in the choroid, and using EDI-OCT, 212.5 ± 13.3 μm in the retina and 283.7 ± 84.1 μm in the choroid. An intersystem comparison showed that the ICCs were 0.661 (95% confidence interval [CI], 0.535–0.754) for the retina and 0.921 (95% CI, 0.875–0.948) for the choroid. Using HP-OCT, the interexaminer ICC reproducibility values were 0.650 (95% CI, 0.447–0.791) for the retinal thickness and 0.912 (95% CI, 0.855–0.958) for the choroidal thickness; using EDI-OCT, the values for the retinal and choroidal thicknesses were 0.788 (95% CI, 0.607–0.898) and 0.970 (95% CI, 0.948–0.985), respectively. The intervisit ICC values for the retinal and choroidal thicknesses were 0.504 (95% CI, 0.376–0.609) and 0.893 (95% CI, 0.864–0.916).

CONCLUSIONS. The retinal and choroidal thicknesses were well-correlated between the instruments. Higher reliability and reproducibility are expected for the choroidal thickness measurements despite with higher morphologic interindividual variations. (Invest Ophthalmol Vis Sci. 2011;52:5556–5540) DOI:10.1167/iovs.10-6811

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The choroid develops many vision-threatening macular pathologies such as choroidal neovascularization (CNV), central serous chorioretinopathy (CSC), polypoidal choroidal vasculopathy (PCV), and high myopia. CNV is neovascularization from the choroid that grows into the subretinal space, causing hemorrhage and/or fibrin formation leading to central visual loss.1 Recent indocyanine green angiographic (ICGA) studies have reported that choroidal vascular hyperpermeability and dilatation of large choroidal vessels cause CSC.2–4 PCV is augmented by abnormal vascular network formation and polypoidal changes at the terminal of the abnormal vessels.5,6 Histologic studies have shown that the myopic choroid is characterized by choroidal vascular occlusion and replacement with fibrous tissue.7,8 Therefore, monitoring the choroidal structures may lead to an understanding of the pathologies and mechanisms of these critical diseases.

ICGA, the most commonly used method to evaluate choroidal vascular changes, is useful and has been applied in many pathologic conditions such as CNV, CSC, and PCV. ICGA provides valuable information about vascular pathologies3–6,9; however, it is still challenging to image the choroidal structural changes in normal and pathologic conditions and to quantify interpretation of the subjective findings from the results. ICGA also is relatively invasive and inconvenient to perform repeatedly during follow-up.

Recent technological advances have facilitated improvements in optical coherence tomography (OCT), one of the most useful tools for diagnosing, making treatment decisions, and monitoring many retinal diseases.10,11 However, imaging the full-thickness choroid is technically challenging because the retinal pigment epithelium (RPE), a high-scattering tissue, attenuates the signals passing through the RPE. Thus, commercial OCTs, with an 800-nm wavelength band light source, are unsuitable for imaging posterior ocular tissue because of this signal attenuation.

Two systems are available to image the deep ocular tissue; high-penetration OCT (HP-OCT) and enhanced depth imaging (EDI) OCT. HP-OCT has an innovative longer light source wavelength12–15 than conventional machines, i.e., a 1-μm band, that provides higher penetration through the RPE, enabling deep choroidal imaging. There are no commercially available HP-OCT machines, and prototypes are used mainly for research. EDI is an innovation of postprocessing using a commercially available OCT. Deep choroidal images are enhanced by taking an inverted image and multiple b-scan averaging (typically 50 to 100 images) to improve the signal-to-noise ratio.16 EDI requires stable fixation for many b-scans and the images must be obtained with an eye tracking system.

The choroidal thickness reportedly is related significantly with specific pathologies. For instance, the choroidal thickness increases in eyes with CSC compared with normal eyes,17,18 and the choroid is thinner with myopic shift or...
axial length elongation. Choroidal thinning also is more prominent in patients with CNV in highly myopic eyes. Thus, the choroidal thickness is being measured increasingly more often and is becoming an accepted procedure clinically and for research. However, very little information is available about the reproducibility of the choroidal thickness measurements. We report the intersystem, interexaminer, and intervisit reproducibility of choroidal thickness measurements using HP-OCT and EDI.

METHODS

Subjects
Twelve healthy volunteers (24 eyes) participated after they provided informed consent. The subjects had no systemic or ophthalmologic symptoms. Both HP-OCT and EDI-OCT (Spectralis; Heidelberg Engineering, Heidelberg, Germany) images were obtained simultaneously at Osaka University Hospital, Osaka, Japan. This study followed the tenets of the Declaration of Helsinki. The institutional review board of Osaka University Hospital approved the usage of prototype HP-OCT and this study. Among the 12 examiners, 10 were examined again 4 months later to assess intervisit reproducibility. All examinations were obtained around noon to avoid diurnal variations.

Prototype 1060 nm HP-OCT
The prototype HP-OCT, used to image the full-thickness choroid and sclera, is based on swept-source OCT technology, with a speed of 50,000 A-scans/second. A 6 × 6-mm retinal region was scanned by fast raster scanning protocol, and the A-scan density was 512 lines (horizontal) × 255 lines (vertical). The center wavelength of the probe beam is 1060 nm. The detailed profile of this instrument was reported previously.

EDI-OCT
The full choroidal thickness was imaged using EDI-OCT with eye tracking and image-averaging systems, as previously described. The OCT device is pushed sufficiently close to the eye to obtain an inverted image. Each section was obtained using eye tracking, and 100 b-scans were averaged to improve the signal-to-noise ratio. A 9-μm horizontal image was obtained that included the fovea. Additional hardware or software is unnecessary with EDI-OCT for the EDI technique. The images were reinverted for display purposes in the present study. Both HP-OCT and EDI-OCT were operated by the same experienced technician.

Retinal and Choroidal Thickness Measurement
The software in the instrument manually measured the foveal retinal and choroidal thicknesses. The retinal thickness was defined as the vertical distance between the internal limiting membrane (ILM) and RPE, and the choroidal thickness between the RPE and the choriocapillary interface. During all examinations, the choriocapillary interface was clearly visualized using both instruments. Six experienced clinicians independently measured the retinal and choroidal thicknesses. A representative image is shown in Figure 1.

Other Examinations
An experienced technician measured the spherical equivalent refractive error using an auto refractometer (Nidek, Gamagohri, Japan) and the axial length (IOLMaster; Carl Zeiss Meditec, Dublin, CA).

Intersystem Reproducibility, Interexaminer, and Intervisit Reproducibility
The 24 eyes were examined using the two instruments. The six independent examiners measured the foveal retinal and choroidal thicknesses. The intersystem and interobserver reproducibility values were evaluated based on the intraclass correlation coefficient (ICC). The same six examiners again evaluated the images from 20 of the 24 eyes 4 months later using both instruments. The intervisit ICC value was also calculated. According to Shrout and Fleiss there are three types of ICC (class 1 to 3). We chose class 2 ICC for the interexaminer reproducibility in each device because we thought more examiners are better than fewer to better simulate the real clinical setting. We also chose class 2 ICC for the intersystem and intervisit reproducibility because these examinations were performed on the same basis. For further details of ICC types, see Reference 24.

Statistical Analysis
Statistical analysis, such as calculation of the ICCs, was performed using statistical software (SPSS version 14.0; SPSS Inc., Chicago, IL).

RESULTS
Demographic Data
The study included 12 subjects (nine men, three women) with a mean age of 37.6 ± 9.3 years (range, 23.9–50.5). The mean spherical equivalent refractive error was −4.0 ± 3.2 diopters (range, −11.25 to 2.25), and the mean axial length was 25.36 ± 1.20 mm (range, 22.71 to 27.34).

Overall Measurement Results
Using HP-OCT, the mean retinal thickness of all measurements was 209.1 ± 12.9 μm (range, 179–253), and the mean choroidal thickness was 292.7 ± 77.3 μm (range, 156–512). Using EDI-OCT, the mean retinal thickness of all measurements was 212.5 ± 13.3 μm (range, 168–260), and the mean choroidal thickness was 283.7 ± 84.1 μm (range, 144–474).

Intersystem Reproducibility
The agreement between the retinal/choroidal thickness using both systems (HP-OCT and EDI-OCT) is shown in Figure 2. The

![Figure 1](image_url)
ICCs were 0.661 (95% CI, 0.535–0.754) for the retina and 0.921 (95% CI, 0.875–0.948) for the choroid.

**Interexaminer Reproducibility**

The interexaminer reproducibility is shown in Figure 3. Using HP-OCT, the ICC for the retinal thickness was 0.630 (95% CI, 0.447–0.791) and 0.912 (95% CI, 0.835–0.958) for the choroidal thickness. Using EDI-OCT, the ICC for the retinal thickness was 0.788 (95% CI, 0.607–0.898) and 0.970 (95% CI, 0.948–0.985) for the choroidal thickness.

**Intervisit Reproducibility**

Using HP-OCT, the intervisit reproducibility is shown in Figure 4. The mean retinal and choroidal thicknesses at baseline were 210 ± 14 μm and 296 ± 74 μm, respectively. Using EDI-OCT, the values were 214 ± 16 μm and 286 ± 81 μm. Four months...
later, the mean retinal and choroidal thicknesses were 217 ± 12 μm and 299 ± 64 μm using HP-OCT and 218 ± 15 μm and 294 ± 74 μm using EDI-OCT. The intervisit ICCs for both systems were 0.504 (95% CI, 0.376–0.609) for the retina and 0.893 (95% CI, 0.864–0.916) for the choroid.

**DISCUSSION**

We determined the agreement of the retinal and choroidal thickness measurements using HP-OCT and EDI-OCT. In choroidal thickness measurement using EDI-OCT system, others found high interobserver correlation (r = 0.93 for right eye and r = 0.97 for left eyes),16 and high repeatability with interobserver coefficients of 32 μm.32 However there has been very limited information available regarding intervisit and interobserver reproducibility of HP-OCT and intersystem consistency. Remarkably, the choroidal thickness measurement had high reproducibility (ICC ≥ 0.90) for intersystem, interexaminer, and intervisit reproducibility. These results indicated that a high level of agreement could be obtained in many situations. For instance, a study with a number of graders can be performed with a large patient population. The choroidal circulation changes in response to various stresses, and the choroidal thickness has diurnal fluctuations.22 In the present study, the intervisit comparison was performed around noon both times to rule this out and the intervisit agreement was good.

Measuring the choroidal thickness is more challenging than measuring the retinal measurement for a few reasons. First, the choroidal thickness varies more than the retinal thickness. For instance, the SD in the present study using HP-OCT was 12.9 μm in the retina but 77.4 μm in the choroid, indicating more variability in choroidal thickness. As long as the measurement is accurate, the deviation does not matter; however, because of the variation, longer time may be required to determine the optimal method for measuring the choroidal thickness. Second, the choriocapillary interface is blurred in the choroidal thickness measurement. Thin, distinct layers are not seen in the choriocapillary interface as in the RPE, and the transitional zone has a blurred border. The internal limiting membrane and RPE in the retina are clearly identifiable and thin, making tracing the lines relatively easier. However, the choriocapillary interface is broader and the traced line may differ among examiners even within the same choriocapillary interface. Third, an automatic detection and segmentation system are still lacking in the choroid, unlike retinal thickness,20 which allows us more accurate and objective analysis, increasing the repeatability. ICCs for retinal thickness did not show the higher correlation than choroidal thickness in the present study. This is probably because the variation of retinal thickness was relatively small in comparison with manual measurement error. One possible reason of higher measurement error was from manual measurement by 6 examiners, as discussed below. ICC is influenced by the SD and measurement error, and smaller deviation also might have lead to the smaller ICC in the retinal thickness measurement. Thus, we must be very careful to compare ICC numbers in different settings, because these parameters are influential.

The retinal thickness has been well studied, and many previous studies have reported more favorable ICCs exceeding 0.90.27–29 The current results showed somewhat lower ICCs with the HP-OCT and EDI (0.6–0.8). The reason for this is unknown; however, a possible explanation is that we measured the thicknesses manually, while most previous OCT studies used automatic segmentation and averaged at least four numbers within the 1-mm circle centered at the fovea. Another possibility is that the present study included six examiners who evaluated the interexaminer variants. Thus, the measuring method might have affected the repeatability of the retinal thickness measurement.

As mentioned previously, the choroidal thickness has varied widely among reports. For instance, Margolis and Spaide30 investigated 54 normal patients (mean age, 50.4 years) using the EDI-OCT and reported a mean choroidal thickness of 287 ± 76 μm subfoveally. We also investigated 86 eyes (mean age, 39.4 years) with HP-OCT and reported a mean subfoveal choroidal thickness of 354 ± 111 μm.31 These observations may raise a question regarding the agreement of the choroidal thicknesses between the two systems. In the present study, the mean choroidal thickness was 293 μm using HP-OCT and 284 using EDI-OCT. The mean numbers were similar and also the intersystem ICC was 0.921 in the choroid, indicating high consistency. Thus, the difference between the two studies is not a result of the system or the wavelength of the light source but presumably something else, such as age, spherical equivalent refractive error, axial length, or perhaps ethnicity.30,31 Also the choroidal thickness varies diurnally.22 Care must be taken to interpret the choroidal thickness data considering these background parameters.

Finally, exploration of the choroidal thickness has just begun, and more information is needed to gain a better understanding of choroidal abnormalities. In addition, it is still somewhat of a controversy, that the hyper-scattering line behind the choroidal vessels actually represents the chorioretinal interface. It might be more accurate to use the polarization-sensitive, 1-μm swept-source OCT to trace the chorioretinal interface.32 The present study included normal eyes, and pathologic
conditions may affect the accuracy of the choroidal thickness measurements. We believe it is important to determine the process of and risk factors for these vision-threatening macular diseases.

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