Repeatability of Corneal Phase Retardation Measurements by Polarization-Sensitive Optical Coherence Tomography

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PURPOSE. Polarization-sensitive optical coherence tomography (PS-OCT) can evaluate internal tissue structures of the cornea, such as collagen fibers, by phase retardation measurement. In this study, we assessed the repeatability of corneal phase retardation measurements using anterior segment PS-OCT.

METHODS. A total of 173 eyes of 173 patients were measured using PS-OCT. In total, 58 eyes of young subjects with normal corneas, 28 eyes of old subjects with normal corneas, 26 eyes with corneal dystrophy or degeneration, 37 eyes with corneal transplantation, and 24 eyes with keratoconus were evaluated. The 3-mm diameter average of en face phase retardation of the posterior corneal surface was examined using PS-OCT. To evaluate the repeatability, intraclass correlation coefficients (ICCs) were calculated for intraobserver and interobserver repeatability analysis.

RESULTS. Polarization-sensitive OCT showed good repeatability for corneal measurements. Intraobserver correlation coefficients of intraobserver and interobserver repeatability of all the subjects were 0.989 and 0.980, respectively. Intraobserver correlation coefficients of the intraobserver measurements for each group, that is, young and old subjects with normal cornea, cornea dystrophy/degeneration, corneal transplantation, and keratoconus, were 0.961, 0.975, 0.984, 0.978, and 0.996, respectively. Interobserver ICCs for the above-mentioned respective groups were 0.952, 0.964, 0.988, 0.959, and 0.975, respectively.

CONCLUSIONS. Polarization-sensitive OCT showed good repeatability for phase retardation measurements of central corneas not only for normal corneas, but also for various diseased corneas. Polarization-sensitive OCT might be useful for evaluating corneal phase retardation, which is one of the parameters that defines birefringence.

Keywords: repeatability, polarization-sensitive optical coherence tomography, cornea dystrophy, keratoconus, corneal transplantation

Polarization-sensitive optical coherence tomography (PS-OCT) is a functional extension of OCT, which not only gathers morphological information like conventional OCT but also evaluates tissue birefringence.1 Several ocular tissues, such as the cornea, retinal nerve fiber layer, and retinal pigment epithelium, as well as fibrous scars, are known to have birefringence. As the birefringence originates from the fibrous microscopic organization of tissues, PS-OCT is useful for evaluating microscopic structural changes of fibrous tissues.2–6

It is well known that the cornea is optically birefringent owing to the highly organized parallel collagen fibrils aggregated in bundles that give rise to approximately 200 lamellae in the corneal stroma.5 Although several methods, such as polarizing microscopes or polariscopes, have been investigated to measure corneal birefringence, none of those techniques can provide depth resolved information.8,9 Corneal tissue microstructures, such as collagen fibers, have important roles in the pathogenesis of corneal ectatic diseases; in particular, the arrangement of regular fibril lamellae is altered in keratoconus, which results in changes in corneal birefringence. Previous studies reported that the average of en face phase retardation of the posterior surface of the corneas measured by PS-OCT was higher in keratoconus patients.5,10 Moreover, phase retardation was reported to be sensitive to discriminate early keratoconus.5

Low measurement speed increases motion artifacts and affects repeatability and reproducibility. The first-generation anterior segment PS-OCT systems were based on time-domain OCT.10,11 Because the measurement speed of PS-OCT used for the study is based on the swept-source OCT (30,000 A-lines/s) and is 10 times faster than that of the first-generation time-domain PS-OCT (2000 A-lines/s), it has high robustness against sample motion. Good repeatability was obtained in our previous pilot study;5 however, our previous study using a swept-source PS-OCT system was carried out on a limited number of patients and evaluated only normal corneas and keratoconus corneas.5 Thus, in the current study, the repeatability of PS-OCT on cornea phase retardation measurements was investigated in normal and transplanted corneas, as well as in various corneal diseases. We demonstrated that PS-OCT...
shows good repeatability for phase retardation measurements not only in normal corneas, but also in several corneal diseases.

**PATIENTS AND METHODS**

We examined in total 173 eyes of 173 patients (101 men [58.4%] and 72 women [41.6%]; mean age [±SD], 44.8 ± 23.1 years; age range, 22–89 years) at Tsukuba University Hospital between April 2013 and June 2014. Among these, 58 eyes of healthy young subjects (23.1 ± 1.5 years old), 28 eyes of healthy old patients (66.5 ± 8.1 years old), 26 eyes with corneal dystrophy/ degeneration (55.7 ± 17.1 years old), 37 eyes submitted to corneal transplantation (66.1 ± 10.6 years old), and 24 eyes with keratoconus (36.8 ± 11.3 years old) were evaluated. For healthy subjects, only the right eyes were examined. In the cornea dystrophy/degeneration group, 10 eyes had corneal granular dystrophy type I, six eyes had corneal granular dystrophy type II (Avellino dystrophy), eight eyes had lattice corneal dystrophy type I, and two eyes had band keratopathy. Twenty-eight eyes had undergone penetrating keratoplasty, and nine eyes had undergone Descemet’s stripping automated endothelial keratoplasty (DSAEK). For keratoconus diagnosis, we evaluated the topographic appearance of the corneal map and investigated the presence of one or more of the following clinical findings using a slit-lamp microscope: corneal stromal thinning, corneal protrusion at the apex, apical scar, Fleischer ring, and Vogt striae. The severity of keratoconus was graded according to Amsler-Krumeich classification (9 eyes with grade II, 10 eyes with grade III, and 5 eyes with grade IV). To compare the phase retardation of excessive myopia and high astigmatism corneas, 20 eyes of 20 patients (28.9 ± 5.1 years old, refractive error: 7.9 ± 1.7 diopters [DI]) with excessive myopia and 10 eyes of 10 patients (30.4 ± 5.1 years old, astigmatism: 3.3 ± 0.4 D) were measured by PS-OCT. The research followed the tenets of the Declaration of Helsinki, and written informed consent was obtained from each participant. The study was approved by the institutional review board of the University of Tsukuba.

Measurements were performed by experienced examiners (SE SH, SB, GK) using the three-dimensional (3D) cornea and anterior segment OCT (CAS-OCT; Tomey Corp, Aichi, Japan) and PS-OCT. The subjects were instructed to look at an internal fixation target during scanning with anterior segment OCT and PS-OCT. Each measurement was performed by two different examiners. The first examiner took the images at two different sessions within 3 hours for intraobserver repeatability analysis. The second examiner took images using the same settings as the first examiner, also within an interval of 3 hours. The results between the first and second examiners were compared for interobserver repeatability analysis.

The anterior segment PS-OCT device used in this study has been described in previous articles. In brief, the system was based on swept-source OCT technology and its light source sweeps over 110 nm across a center wavelength of 1.3 μm with a sweeping frequency of 30,000 Hz. The depth resolution of the system was 9.2 μm in tissue by assuming the refractive index of the tissue as 1.38. The PS-OCT system used in this study was based on tomographic Jones matrix measurements and is referred to as Jones matrix OCT. To avoid suture line or transplanted graft border, the 4 × 4 mm² areas of corneas were scanned with a horizontal-fast raster pattern centered around the central corneal reflex. The 3D data of cornea included 512 horizontal × 128 vertical A-lines and then an annular area from 0.4- to 3.0-mm diameter was cropped from the 4 × 4 mm² scan and a 3-mm diameter en face phase retardation map was created. The central 0.4-mm region was excluded because the strong specular reflection from the corneal apex created a flare at this region. These maps were then used for quantitative phase retardation analysis.

En face phase retardation maps were obtained by following processing, which is slightly modified from the one used in our previous study. Anterior and posterior surfaces were extracted from the OCT data using intensity-based analysis. For each B-scan, the matrix product of the Jones matrix of the corneal tissue and the inverse of the Jones matrix of the anterior surface was obtained. This product operation removes the effects of the system birefringence of the PS-OCT device. Phase retardation was finally obtained as the phase difference between two eigenvalues of the product matrix. En face phase retardation maps were thus extracted and pseudo colored over the range of 0 to π using a hue color map shown in Figure 1. This color map was consistently used for all phase retardation maps. Because the phase retardation is cumulative along the full depth of the cornea, this en face phase retardation map at the posterior surface reflects the net polarization property of the entire corneal depth.

The average phase retardation of each cornea is obtained not from the phase retardation map but from the system-birefringence-corrected Jones matrix as follows. First, the Jones...
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RESULTS

Intraobserver and Interobserver Repeatability of Corneal Measurements by PS-OCT

The PS-OCT showed good repeatability for corneal phase retardation measurements. Intraclass correlation coefficients of intraobserver and interobserver repeatability among all subjects were 0.989 and 0.980, respectively. Intraclass correlation coefficients of intraobserver for healthy young subjects, healthy old subjects, cornea dystrophy/degeneration, corneal transplantation, and keratoconus groups were 0.961, 0.975, 0.984, 0.978, and 0.996, respectively. Intraclass correlation coefficients of interobserver for healthy young subjects, healthy old subjects, cornea dystrophy/degeneration, corneal transplantation, and keratoconus groups were 0.952, 0.964, 0.988, 0.959, and 0.975, respectively.

Phase Retardation of Cornea

Representative cross-section images and 3-mm diameter average en face phase retardation maps of the posterior corneal surfaces taken with the PS-OCT, as well as photographs, are shown in Figures 1 and 2. Two cases of normal corneas, from a young and an old subject as well as two cases of transplanted corneas are shown in Figure 1. Three cases of corneal dystrophy and a case of keratoconus are shown in Figure 2. The healthy corneas of a young (22-year-old female) and an old (82-year-old female) subject showed low and homogeneous phase retardation, respectively (Figs. 1A, 1B).

The transplanted cornea of a 44-year-old male patient who had undergone penetrating keratoplasty for Acanthamoeba keratitis 2 years earlier and a 57-year-old male patient who had undergone DSAEK for bullous keratopathy 3 years earlier are shown in Figures 1C and 1D. The cornea that underwent penetrating keratoplasty showed some opacity in the anterior photographs. Although the 3-mm diameter average phase retardation did not increase, the en face phase retardation map did not show homogeneous phase retardation. The phase retardation values obtained with the PS-OCT were higher than 0.5 radian in 6 of 28 transplanted corneas that underwent penetrating keratoplasty. Among these patients showing phase retardation values higher than 0.5 radian, five (85%) of six corneas showed apparent opacity in the graft. Similarly to normal cornea, the corneas that underwent DSAEK and showed good transparency by slit-lamp examination had low and homogeneous phase retardation. No increase of phase retardation was found at the interface between host and graft (Fig. 1D).

The case of a 24-year-old female with granular corneal dystrophy type I is shown in Figure 2A. Best-corrected visual acuity was 1.2. Despite the hyaline corneal deposits, no increase of phase retardation was found, and the 3-mm diameter phase retardation was 0.351 radian. On the other hand, the corneal dystrophy type I patient with a decrease in visual acuity showed an increase in phase retardation (Fig. 2B). Strong corneal deposit affects phase retardation, as shown in

FIGURE 2. Four cases of PS-OCT cross-section and en face phase retardation images, and anterior photographs. Three cases of corneal dystrophy; granular corneal dystrophy type I with good visual acuity (A), granular corneal dystrophy type I with decreasing of visual acuity (B), and corneal lipid degeneration associated with lattice corneal dystrophy (C). A case of keratoconus (D). Scale bars: 1 cm.
Figure 3. The graph shows the averages of the 3-mm-diameter phase retardation. The phase retardation is significantly higher in keratoconus eyes than in young and old normal corneas and transplanted cornea. *P < 0.05, ANOVA with Bonferroni correction.

Figure 4. The graph shows the averages of the 3-mm-diameter phase retardation in normal cornea, excessive myopia, and high astigmatism. No significant differences among the three groups are found.

Discussion
The evaluation of measurement repeatability is important for ensuring accuracy when implementing a new method or instrument. As described in the results section, the 3-mm diameter average en face phase retardation maps at the posterior corneal surfaces measured by PS-OCT showed good repeatability. Of note, ICC was higher than 0.9 in all groups.

Previous studies have reported excellent repeatability and reproducibility of conventional swept-source anterior segment OCT. The ICCs of the central corneal thickness and anterior chamber depth measurements with swept-source anterior segment OCT were 0.982 to 0.996. Szalai et al. reported reliability and repeatability of swept-source Fourier-domain OCT in healthy individuals and keratoconus patients. The ICCs of anterior and posterior curvature of healthy individuals and keratoconus patients were 0.982 to 0.986 and 0.995 to 0.997, respectively. The ICCs of corneal thickness and anterior chamber depth of healthy individuals and keratoconus patients were 0.963 to 0.998 and 0.907 to 0.997, respectively.

The repeatability of corneal measurements using anterior segment PS-OCT has been assessed only in our previous report; the ICCs of 3-mm average phase retardation in keratoconus and normal cornea groups were 0.914 and 0.906, respectively. With regard to repeatability of posterior PS-OCTs, several previously published reports are available. Schütze et al. and Baumann et al. reported good reproducibility of automated lesion size detection in patients with geographic atrophy by using 20,000-Hz PS-OCT. Zotter et al. reported five consecutive measurements of retinal nerve fiber layer birefringence using 70,000-Hz high-speed PS-OCT in 10 eyes of five healthy human volunteers, and five consecutive measurements showed high reproducibility of retinal nerve fiber layer birefringence, retardation, and thickness measurements. Torzicky et al. also reported good repeatability for measuring the choroidal thickness of the human eye using the automatic segmentation performed by a 100,000-Hz PS-OCT with a center wavelength of 1040 nm.

In the current study, 30,000-Hz anterior segment PS-OCT showed good repeatability for corneal measurements. High measurement speed of swept-source PS-OCT is a key source of this high repeatability in the present study. Measurement speed of PS-OCT makes impressive progress. For example, the measurement speed was 2000 A-lines/s in 2004 with time-domain PS-OCT. With swept-source technology, it became 30,000 A-lines/s, 50,000. For posterior eye imaging, 100,000 A-lines/s PS-OCT and multifunctional OCT have been demonstrated. Recent technological development further enables 200,000 A-lines/s PS-OCT and multifunctional OCT. In addition, the subjects were instructed to look at an internal fixation target during scanning. While obtaining the measurements, the scanning position could be visualized on a real-time...
monitor. The inbuilt internal fixation target and high measurement speed may contribute to the high repeatability. The repeatability can be further improved by an automatic eye-tracking system. Szalai et al.\textsuperscript{16} showed that a swept-source anterior segment OCT with built-in eye tracking can provide reproducible measurements.

A previous study reported that phase retardation was sensitive to discriminate keratoconus; therefore, it can be useful to detect very early or even subclinical keratoconus.\textsuperscript{5} The 6-mm and 3- to 6-mm-diameter phase retardation was more sensitive to discriminate early keratoconus than 3-mm. These results indicated that alteration of peripheral phase retardation was more sensitive than central change. However, the direction of fibril orientation with respect to the imaging beam was not orthogonally oriented due to the dome shape of the cornea. Increased phase retardation can be observed at the periphery of the cornea, whereas in the center of the cornea the phase retardation was rather low. Thus, only the central 3-mm-diameter phase retardation was evaluated in the current study.

Recently, lamellar corneal transplant procedures, such as deep anterior lamellar keratoplasty, deep lamellar endothelial keratoplasty, and DSAEK have largely been performed; however, penetrating keratoplasty still remains the important surgical approach for extremely pathologic and deformed corneas in many corneal diseases. Careful postoperative observation is also critical for the prevention of graft failure or other complications. Anterior segment OCT imaging may provide important information for recognizing these changes.\textsuperscript{26–29} For example, anterior segment OCT showed stromal thickening and undulation of the posterior corneal surface in acute graft rejection.\textsuperscript{20} Anterior segment OCT can evaluate the graft-host interface of the incision after penetrating keratoplasty.\textsuperscript{28} Anterior segment OCT is also useful to detect iridocorneal contact as a peripheral anterior synechias in patients who undergo penetrating keratoplasty.\textsuperscript{29} In the current study, approximately 80% of the corneas with higher than 0.5 radian showed apparent opacity in the graft. Polarization-sensitive OCT can provide information regarding the internal tissue properties of the graft, which are difficult to evaluate by anterior segment OCT; therefore, PS-OCT might be useful for postoperative observation after keratoplasty.

In DSAEK, Letko et al.\textsuperscript{30} reported that 90% of eyes regrafted due to unsatisfactory vision had folds or wrinkles in the pupillary area that were apparent on both slit-lamp examination and anterior segment OCT. As folds or wrinkles indicate changes in the orientation of collagen fibers within the graft, PS-OCT might be useful for analyzing these alterations. In the current study, all nine transplanted corneas using DSAEK technique showed good transparency by slit-lamp examination. Moreover, the grafted area showed no increase in phase retardation and anterior segment OCT imaging may be used to obtain phase retardations.\textsuperscript{5}

In conclusion, we confirmed that PS-OCT showed good repeatability for phase retardation measurements not only for normal corneas but also for various other corneal diseases. PS-OCT might be useful for evaluating corneal phase retardation, which is one of the parameters that defines birefringence.

Our study has some limitations. For instance, no other instrument evaluates birefringence with depth resolved information; therefore, it is difficult to compare its accuracy with other instruments. Moreover, the repeatability of PS-OCT depends to a large extent on the measurement algorithm and the speed of the light source.

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