Myopic Retinal Changes Screening: Comparison of Sensitivity and Specificity among 15 Combinations of Ultrawide Field Scanning Laser Ophthalmoscopy Images

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Keywords

Optimal evaluation indices · Comprehensive method · Efficient screening

Abstract

Aim: The objective of the study was to investigate the evaluation indices (diagnostic test accuracy and agreement) of 15 combinations of ultrawide field scanning laser ophthalmoscopy (UWF SLO) images in myopic retinal changes (MRC) screening to determine the combination of imaging that yields the highest evaluation indices in screening MRC. **Methods:** This is a retrospective study of UWF SLO images obtained from myopes and were analyzed by 2 retinal specialists independently. Five field UWF SLO images that included the posterior (B), superior (S), inferior (I), nasal (N), and temporal (T) regions were obtained for analysis and its results used as a reference standard. The evaluation indices of different combinations comprising 1 to 4 fields of the retina were compared to determine the abilities of each combination screens for MRC. **Results:** UWF SLO images obtained from 823 myopic patients (1,646 eyes) were included for the study. Sensitivities ranged from 50.0 to 98.9% (95% confidence interval (CI), 43.8–99.7%); the combinations of B + S +

I (97.3%; 95% CI, 94.4–98.8%), B + T + S + I (98.5%; 95% CI, 95.9–99.5%), and B + S + N + I (98.9%; 95% CI, 96.4–99.7%) ranked highest. Furthermore, the combinations of B + S + I, B + T + S + I, and B + S + N + I also revealed the highest accuracy (97.7%; 95% CI, 95.1–100.0, 98.6; 95% CI, 96.7–100.0, 98.8; 95% CI, 96.9–100.0%) and agreement (kappa = 0.968, 0.980, and 0.980). For the various combinations, specificities were all higher than 99.5% (95% CI, 99.3–100.0%). **Conclusions:** In our study, screening combinations of B + S + I, B + T + S + I, and B + S + N + I stand out with high-performing optimal evaluation indices. However, when time is limited, B + S + I may be more applicable in primary screening of MRC.

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Introduction

Myopia is an increasingly prevalent condition that is of rising concern around the world, affecting as much as 28.3% of the world's population [1]. It is more common among those of Chinese ethnicity, whose individual prev-

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alence can be up to 46% [2]. Currently, myopia is increasingly affecting people of younger age, commonly between 25- and 29-year-olds (prevalence 47.2%) [1]. Myopia also occurs in children and adolescents during puberty due to unstable ocular development. In Western China, the annual incidence of myopia in children and adolescents was 10.6%, and the annual progression of refraction was -0.43 diopters. The cumulative incidence of myopia (54.9%) here is higher than that in children from other parts of China [3]. In contrast, the prevalence of myopic adolescents in other parts of the world such as Sydney is relatively low, with only 29.6% (14.4%) [4]. As myopia is a common problem among Chinese ethnicity, its complications such as pathological myopia is also a cause of concern. High myopia can lead to pathological changes in the choroid and retina, resulting in uncorrectable vision loss [5]. According to estimates of the current global myopia incidence trend, it is predicted that by 2050, myopia will affect 4.8 billion people worldwide (49.8% of the world), of which high myopia will affect approximately 10% of the world population [6]. In consideration of the prevalence of myopia and its impact on the vision of children and adolescents, it is particularly important to regularly screen for fundus changes in myopia patients to avoid long-term fundus complications which would affect their vision.

Many studies have shown that myopia may lead to retinal anomalous changes due to axial elongation [7–10]. Some of the most common forms of retinal anomalies include white without pressure and lattice degeneration (LD) in the peripheral region of the retina in individuals with myopia [11–14], especially in high myopes, with rates as high as 46.5 and 14.6%, respectively [14]. A comparative study by Kang et al. [15] indicated that refractive surgery might be a causative factor of retinal detachment. In addition, LD can evolve into retinal breaks and is a high-risk factor of rhegmatogenous retinal detachment [16]. However, it can be prevented by prophylactic laser photocoagulation. Prevention of retinal complications in myopes depends on performing myopic screening for retinal changes in patients to achieve early detection of peripheral retinal changes and thus prevent further sight loss.

Clinically, although traditional methods such as using the Goldmann three-mirror contact lens inspection continue to be important, additional methods such as using digital retinal imaging to document and analyze clinical findings are also necessary. Furthermore, using the Goldmann three-mirror contact lens can be time-consuming, causes discomfort in patients, and might cause corneal abrasions [7, 8]. Recent technological advances in diagnostic imaging of the retina lead to better assessment and

documentation of the posterior and peripheral retina. One such technology available to ophthalmologists is the ultrawide field scanning laser ophthalmoscopy (UWF SLO) by Optos[®] (Daytona, Optos[®] PLC, Scotland, UK) [9]. It uses a bifocal concave elliptical mirror combined with the principle of a confocal scanning laser ophthalmoscope [10] to achieve a super-large retina viewing field with a horizontal direction of 200° and a vertical direction of 170° [17]; it can even achieve a 220° – 240° retinal range under its guided orientation function [18]. Some of the advantages of UWF SLO include obtaining images rapidly even without pupil dilation and being able to acquire a single image with clear pixels and high resolution in just 0.25 s [10, 19]. Furthermore, it can obtain color images of the retina using the red and green dual-color laser imaging principle, which overcomes the lack of color of the previous collected images. It is worth noting that the differing laser wavelengths of individual colors focus on different structures of the fundus. The green laser (532 nm) mainly images the anterior retinal structure and its blood vessels, while the red laser (633 nm) highlights the choroidal structure and its blood vessels [10, 18]. More importantly, UWF SLO has satisfactory consistency in results with Goldmann three-mirror contact lens inspection [7, 8, 20]. Thus, it has been widely used in detecting myopic retinal changes (MRC) since its launch in both domestic and foreign markets.

In clinical practice, in order to perform a complete screening and diagnosis of patients' retina, the UWF SLO images of the fundus are usually collected in 5 fields including the basic posterior pole (B), superior (S), inferior (I), nasal (N), and temporal (T) sides. However, this method often leads to long examination and waiting time. Repeated attempts due to poor cooperation of some patients also cause discomfort in patients. Therefore, this study explores whether there is a screening method with similar sensitivity and specificity as the standard 5-field UWF SLO image even if the number of acquisition field is reduced. Furthermore, the reduced fields of acquisition should also aim to have similar screening effect as the standard 5-field.

Materials and Methods

This study is a retrospective analysis performed according to the tenets of the Declaration of Helsinki. Although there was no informed consent by the subjects as the images were analyzed in retrospect, this study had been approved by the institutional review boards of the Guangzhou Aier Eye Hospital, Guangdong, China (No. GZAIER2018IRB05).

Table 1. Comparison of each combination in different conditions

Combination	Positive rate		False-negative rate		Kappa [#]	
	condition I,* %	condition II, [†] %	condition I,* %	condition II, [†] %	condition I,*	condition II, [†] %
B	8.0	6.4	50.0	59.9	0.627	0.530
B + T	9.2	7.2	42.4	53.0	0.695	0.598
B + S	11.8	10.2	26.1	36.4	0.826	0.746
B + N	9.2	7.7	43.2	52.7	0.686	0.600
B + I	12.3	11.1	24.2	31.8	0.835	0.778
B + T + S	12.6	10.9	21.6	31.8	0.859	0.783
B + T + N	10.4	8.8	35.2	45.5	0.753	0.666
B + T + I	12.9	11.8	20.5	26.9	0.863	0.816
B + S + N	12.8	11.2	20.8	30.7	0.862	0.789
B + S + I	15.7	15.4	2.7	4.6	0.980	0.968
B + I + N	12.9	11.8	20.5	27.3	0.861	0.811
B + T + N + S	13.4	11.8	16.7	26.5	0.891	0.821
B + T + N + I	13.7	12.6	15.9	22.4	0.892	0.847
B + T + S + I	15.9	15.7	1.5	2.7	0.986	0.980
B + S + N + I	16.0	15.9	1.1	2.3	0.986	0.980

B, posterior; T, temporal region; S, superior region; N, nasal region; I, inferior region; MRC, myopic retinal changes. [#] Agreement between the outcomes achieved by each combination and the 5-field analysis. * Condition I: not considering the number of MRC. [†] Condition II: considering the number of MRC.

Subjects

Five-field UWF SLO images of 1,067 eyes were included and analyzed for the study. These images were obtained from adult (18 years and older) myopic patients (diopter range from −1.00 to −6.00 D) who were undergoing screening for MRC prior to refractive surgery at the Refractive Center of Guangzhou Aier Eye Hospital from November 2017 to August 2018.

The specific inclusion criteria were as follows: (1) the patient's 5-field UWF SLO images (B, S, I, N, and T) were complete; (2) refractive media (e.g., cornea and crystalline lens) were transparent; (3) the image quality and resolution were clear enough to observe the MRC; and (4) images without the eyelid or eyelash artifacts that affected the determination of lesions. Images that were unable to be graded due to operational issues with medical technicians, patients, or equipment were excluded.

Image Acquisition

All UWF SLO images were obtained by a single, experienced medical technician using an Optos 200Tx UWF SLO (Daytona, Optos® PLC, Scotland, UK) with electronic archiving and electronic image transmission. Upper and lower lid retraction were performed by the medical technician wearing disposable gloves after both eyes of the patient were made fully mydriatic with 0.5% tropicamide-phenylephrine eye drops. Images of B and peripheral retina (S, I, N, and T) were obtained. Finally, the medical technician numbered and documented the collected UWF SLO images.

Image Analysis

All of the UWF SLO images were evaluated, and a number of MRC were included but not limited to white without pressure, LD, and retinal breaks. These changes were recorded by 2 retinal specialists independently in chronological order directly on the machine in the refractive department. Both of them had no advanced

knowledge whether the patients' images had MRC. Analysis was conducted with various combinations of 5-field (B + S + I + N + T), 4-field (B + T + N + S, B + T + N + I, B + T + S + I, and B + S + N + I), 3-field (B + T + S, B + T + N, B + T + I, B + S + N, B + S + I, and B + N + I), 2-field (B + T, B + S, B + N, and B + I), and single-field (B) UWF SLO images. When the results obtained by the 2 retinal specialists were inconsistent, a third experienced retinal specialist reviewed those images before making a final decision.

The results of 5-field analysis, including B, S, I, N, and T regions, were used as a reference standard in the study due to its satisfactory consistency in results with Goldmann three-mirror contact lens inspection [7, 8, 20]. Analysis of the UWF SLO images were evaluated based on the reference standard, according to a positive rate, negative rate, false-negative rate, sensitivity, and specificity defined as follows: a positive rate being the percentage of MRC detected in all eyes, while that of MRC that are not detected in all eyes is negative rate; a false-negative rate being the percentage of detecting a negative result in positive eyes based on the reference standard; sensitivity being the percentage of detecting a positive result in positive eyes based on the reference standard; specificity being the percentage of detecting a negative result in normal (negative) eyes based on the reference standard. It is worth to note that only a positive or negative result for MRC were recorded without taking into account the number of MRC found during evaluation (condition I).

However, considering the number of MRC (condition II), the positive rate, negative rate, false-negative rate, and sensitivity were redefined as follows: a positive rate being the percentage of results in which the number of MRC detected was the same as that in the reference standard for all eyes; a negative rate being the percentage of results in which the number of MRC was less than that of the reference standard in all eyes; a false-negative rate refers to the percentage of results in which the number of MRC detected was less than

Table 2. Evaluation indices of each combination

Combination	Sensitivity (95% CI)	Specificity (95% CI)	AUC (95% CI)	Accuracy (95% CI)
B	50.0 (43.8–56.2)	100.0 (99.7–100.0)	0.750 (71.0–79.0)	70.1 (64.2–76.0)
B + T	57.6 (51.4–63.6)	100.0 (99.7–100.0)	0.788 (75.0–82.6)	73.5 (67.5–79.5)
B + S	73.9 (68.0–79.0)	100.0 (99.7–100.0)	0.869 (83.7–90.1)	81.8 (76.0–87.6)
B + N	56.8 (50.6–62.8)	99.9 (99.5–100.0)	0.784 (74.6–82.2)	73.6 (67.6–79.7)
B + I	75.8 (70.0–80.7)	99.9 (99.4–100.0)	0.878 (84.7–90.9)	84.0 (78.4–89.6)
B + T + S	78.4 (72.9–83.1)	100.0 (99.7–100.0)	0.892 (86.2–92.2)	84.1 (78.5–89.7)
B + T + N	64.8 (58.6–70.5)	99.9 (99.5–100.0)	0.824 (78.8–85.9)	77.2 (71.2–83.2)
B + T + I	79.5 (74.1–84.1)	99.9 (99.4–100.0)	0.897 (86.8–92.6)	86.5 (81.1–91.8)
B + S + N	79.2 (73.7–83.8)	99.9 (99.5–100.0)	0.896 (86.6–92.5)	84.6 (79.1–90.2)
B + S + I	97.3 (94.4–98.8)	99.9 (99.4–100.0)	0.986 (97.4–99.7)	97.7 (95.1–100.0)
B + I + N	79.5 (74.1–84.1)	99.8 (99.3–100.0)	0.897 (86.7–86.7)	86.3 (80.9–91.6)
B + T + N + S	83.3 (78.2–87.5)	99.9 (99.5–100.0)	0.916 (89.0–94.3)	86.7 (81.4–92.0)
B + T + N + I	84.1 (79.0–88.2)	99.8 (99.3–99.9)	0.919 (89.3–94.5)	88.7 (83.7–93.7)
B + T + S + I	98.5 (95.9–99.5)	99.9 (99.4–100.0)	0.991 (98.3–100.0)	98.6 (96.7–100.0)
B + S + N + I	98.9 (96.4–99.7)	99.8 (99.3–99.9)	0.993 (98.6–100.0)	98.8 (96.9–100.0)

B, posterior; T, temporal region; S, superior region; N, nasal region; I, inferior region; AUC, area under the curve; 95% CI, 95% confidence interval.

that of the reference standard in positive eyes; sensitivity being the percentage of detecting the same number of MRC as that of the reference standard in positive eyes. (The specific equations were presented in online suppl. Annex 1/Suppl. material; see www.karger.com/doi/10.1159/0005514176 for all online suppl. material.)

Statistical Analysis

The acquired data were analyzed using commercial analytical software program (SPSS21.0; IBM Inc., New York, NY, USA). Diagnostic sensitivity and specificity were calculated for each combination of UWF SLO, with a 95% confidence interval (CI) in condition I; the positive rate and false-negative rate were calculated following the same procedure. The receiver operating characteristic curve and the area under the curve (AUC) were used to evaluate the ability of each combination to select the optimal result based on the findings obtained in the 5-field UWF SLO images analysis. Accuracy and agreement between the outcomes achieved by each form of combination and the 5-field analysis were calculated in condition II, and the level of agreement was measured using kappa statistics. Kappa values were interpreted as follows: 0–0.20, slight agreement; 0.21–0.40, fair agreement; 0.41–0.60, moderate agreement; 0.61–0.80, substantial agreement; and greater than 0.81, highest agreement [21].

Results

Images of both eyes (total 1,646 eyes) from 823 myopic patients were analyzed for this study. According to 5-field analysis of the UWF SLO images, 1,382 of 1,646 eyes (84.0%) were negative for MRC, while 264 eyes (16.0%)

were positive for MRC. All the MRC observed occurred at the peripheral regions of the retina. There were 3 false-positive eyes found in the analysis of 3-field and 4-field combinations which were not counted in the 264 positive results but included in the negative results.

The results and comparisons of each combination are compiled in Table 1. The positive rate of each combination ranges from 8.0 to 16.0% in condition I, while it slightly reduced (6.4–15.9%) in condition II. Of these combinations, the positive rates of B + S + I, B + T + S + I, and B + S + N + I ranked top 3 in both conditions I and II. Although the false-negative rates of B + S + I (2.7 vs. 4.6%), B + T + S + I (1.5 vs. 2.7%), and B + S + N + I (1.1 vs. 2.3%) in condition I were lower than those in condition II, they were still the lowest of all the combinations in both conditions.

In each combination analysis of the UWF SLO images, sensitivity and specificity were calculated and are displayed in Table 2, with 95% CI. The AUCs of each combination suggested the relationship between the sensitivity and specificity, which are presented in Table 2 and Figure 1. Although the specificities of all of the combinations were not much different, ranging from 99.8 to 100.0% with sectionally overlapping CI (99.3–100.0%), the observed trend indicated that the greater the number of imaging fields, the higher the sensitivity, except for the combination of B + S + I, with 97.3% (95% CI, 94.4–98.8%) of sensitivity. With the observed trend, the sensitivities in B

Fig. 1. ROCs and AUCs of various combinations. **a** ROC and AUC of single-field (B) image. **b** ROCs and AUCs of 2-field images. **c** ROCs and AUCs of 3-field images. **d** ROCs and AUCs of 4-field images. ROC, receiver operating characteristic curve; AUC, area under the curve.

+ T + S + I and B + S + N + I reached the highest, with 98.5% (95% CI, 95.9–99.5%) and 98.9% (95% CI, 96.4–99.7%), respectively.

Accuracy and agreement between the outcomes achieved by each form of combination and the reference

standard are presented in Tables 1 and 2, respectively. These represented evaluations of the screening ability of each combination. With the observed trend, the top 3 that achieve the highest agreement in conditions I and II were B + S + I ($\kappa = 0.980$ vs. 0.968), B + T + S + I

(kappa = 0.986 vs. 0.980), and B + S + N + I (kappa = 0.986 vs. 0.980). Similarly, the greater the number of imaging fields, the higher the accuracy, the highest in the combinations of B + S + I (97.7%; 95% CI, 95.1–100.0%), B + T + S + I (98.6%; 95% CI, 96.7–100.0%), and B + S + N + I (98.8%; 95% CI, 96.9–100.0%), respectively.

Discussion

Peripheral retinal changes are characteristic in the natural progression of myopia, and this pathological complication is more common in patients with high myopia. Meanwhile, refractive surgery may contribute to further complications such as retinal detachment in myopes with peripheral retinal changes [15, 22]. Therefore, it is important to perform screening of peripheral retinal changes in myopic patients. According to 5-field UWF SLO images acquired in this study, we observed obvious retinal changes in the peripheral retina in 16% of myopic eyes. We also found that the greater the number of imaging fields, the greater the number of retinal changes detected. However, the B + S + I combination proved to be the most efficient combination in detecting retinal changes in the myopic eye.

Clinically, UWF SLO allows for better assessment and documentation of the posterior and peripheral retina. It is capable of obtaining images rapidly even without pupil dilation and is also highly efficient, being able to acquire a single ultrawide field image with clear pixels and high resolution in just 0.25 s [10, 19]. In recently published studies by Liu et al. [7] and Peng et al. [8], there is a satisfactory consistency in results between UWF SLO and Goldmann three-mirror contact lens inspection. Our team had also conducted a study with a large sample size ($N = 2,000$) on the consistency of UWF SLO with Goldmann three-mirror contact lens inspection in various myopic degrees and retinopathies [20], results of which (kappa = 0.888–1.000) were consistent with those of Liu et al. [7] and Peng et al. [8]. Up till now, there has been no research which clearly proposed that UWF SLO is able to replace the Goldmann three-mirror contact lens inspection in the clinical role. Thus, results of 5-field UWF SLO images in the study were merely used as a reference standard, rather than a gold standard. Clinically, single-field and 5-field UWF SLO imaging were frequently used. However, single-field imaging might not be enough as lesions located in the peripheral retina would often be missed, while 5-field imaging takes up too much time and resources. Hence, we seek alternative combinations in UWF SLO imaging that could yield high sensitivity, high

accuracy, and greater efficiency to improve the primary screening method.

We investigated the diagnostic sensitivity, specificity, and accuracy of various combinations of UWF SLO photography to search for the optimally balanced combination. We aimed to search for the optimal combination that could yield high sensitivity and specificity with greater efficiency, while not compromising on diagnostic accuracy in reference to the standard method of primary retinal screening. The positive rates, false-negative rates, and agreement of 15 different imaging combinations, excluding the reference standard method, were superior in condition I than condition II (see Table 1). We also observed that the positive rates and false-negative rates in condition I were higher than those in condition II. The reason behind this phenomenon is that the purpose of the discussion under different conditions is different. In condition I, we focused on the presence of MRC without regard to the number of retinal changes in order to explore the positive and false-negative rates of various combinations. In condition II, we focused on exploring whether the various combinations would detect the same number of retinal changes giving better credibility to the results than the reference standard. Therefore, we have formulated more detailed analysis building on condition I.

Using single-field (B) imaging yielded unsatisfactory positive and false-negative rate resulting in low sensitivity and accuracy. It indicates that B imaging is not enough for the screening of MRC [23] as it would miss retinal changes in the peripheral region and increase the risk of omitted diagnosis. The observed trend indicated that more imaging fields yield higher sensitivity and correspondingly satisfactory high positive and low false-negative rates with greater accuracy (see Tables 1, 2). Of the results of each combination, B + S + I stood out with 97.3% (95% CI; 94.4–98.8%) sensitivity and 97.2% (95% CI; 95.3–99.2%) accuracy, both of which were reflected in the AUC (0.986, 95% CI; 0.974–0.997) (see Table 2; Fig. 1), as well as superior positive and false-negative rate in both conditions I and II (see Table 1). This might be related to the overlap of eyelid or eyelash artifacts [24–26], which resulted in the restricted range in the vertical direction being larger than that of the horizontal direction [27]. More importantly, MRC were mostly located around the vertical meridian, especially at 12 o'clock and 6 o'clock [28–30]. In other words, these results suggest that the deficient range of UWF SLO images would be largely complemented when not obstructed by eyelids and eyelashes.

Although most evaluation indices of B + S + I of 3-field analysis was satisfactory, at least 2.7% of the MRC were

still missed. This might be due to the influence of red-green pseudo-color [31] and that lesions located at the boundary of the images were missed. In addition, as reported by Chen et al. [14] and Kang et al. [15], some of the retinal changes, such as LD in high myopes, were more likely to occur in the temporal regions. Hence, when another field was added in a 3-field analysis, the false-negative rate was reduced and the balance between sensitivity and specificity set improved. Of the different combinations, the sensitivity and specificity of B + T + S + I and B + S + N + I, with the highest agreement, were optimal and not significantly different from the results of the standard method, therefore yielding maximal accuracy results. Thus, B + T + S + I and B + S + N + I, in theory, were the optimal methods in screening for peripheral MRC. However, when nasal or temporal images were added to the B + S + I combination, sensitivity only increased by 1.6%, while the false-negative rate was reduced by 1.6% and no significant change was observed in specificity. In addition, the average time consumption of performing B + S + I was 1.5 min, which is half the time consumption of the standard 5-field acquisition method (2.9 min), while that of B + T + S + I and B + S + N + I was 2.1–2.3 min. It indicated that an additional field added in the B + S + I combination might not be worthwhile with regard to the time spent used in capturing the additional image.

Limitations

This study is a retrospective cross-sectional study that involved patients with a relatively limited age range, type of retinal lesions, and retinal diseases. There were 3 eyes with false-positive results for MRC, and these may have resulted from false-positive artifacts caused by overlapping images. Although it is possible to obtain UWF SLO images without mydriasis using the UWF SLO, obtaining 5-field UWF SLO images without mydriasis requires long intervals of waiting for the pupil to be spontaneously dilated enough to obtain the next image. Hence, although the sensitivity of these screening tests would be overestimated, mydriasis was still used in this study to improve time efficiency in retina screening. Finally, in order to establish the efficiency of 3-field analysis in retina screening, we aimed to utilize similar methods in the screening of retinal changes in other contexts such as screening for diabetic retinal changes. In future clinical studies, more cases need to be analyzed to expand the scope of UWF SLO research and explore for the optimal balanced combination between high sensitivity and specificity and high efficiency.

Conclusion

The UWF SLO is widely used as an auxiliary tool to screen for MRC, a steered imaging method, therefore, is needed to screen these lesions. While 5-field UWF SLO images are able to provide the assistant basis for diagnosis, treatment, and recording of peripheral MRC, B + S + I, B + T + S + I, or B + S + N + I are also efficient and comprehensive methods in screening for peripheral MRC, of which, the B + S + I approach involves a simple clinical operation and has high efficiency, meaning low time and energy consumption while yielding high sensitivity, specificity, accuracy, and agreement and is, therefore, the clinically optimal combination we recommend for efficient screening of peripheral MRC.

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Statement of Ethics

This study is a retrospective and observational analysis performed according to the tenets of the Declaration of Helsinki and approved by the institutional review boards of the Guangzhou Aier Eye Hospital, Guangdong, China (Number: GZAIER2018IRB05).

Conflict of Interest Statement

The authors have not been the recipients of any monetary profit from the device and do not intend to be in the future. The funders had no role in study design, data collection or analysis, decision to publish, or preparation of the manuscript.

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Author Contributions

X.D. conceived and designed the experiments, wrote the paper, and analyzed the data. S.T. wrote the paper. Q.C. and S.L. conceived and designed the experiments. H.L. and Y.H. collected data. J.J. analyzed the data. J.Z. conceived and designed the experiments and contributed the data. All authors revised the manuscript and read and approved the final manuscript.

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