

Impact of ocular magnification on retinal and choriocapillaris blood flow quantification in myopia with swept-source optical coherence tomography angiography

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Background: To evaluate the impact of ocular magnification on retinal and choriocapillaris (CC) blood flow quantification in myopic eyes using swept-source optical coherence tomography angiography (SS-OCTA).

Methods: Subjects with myopia were recruited for comprehensive ophthalmic examination and SS-OCTA imaging with 6×6 mm² scanning protocol. Retinal vessel area density (RVAD), retinal vessel skeleton density (RVSD), and percentage of CC flow deficits (CC FD%) were quantified within a 5-mm-diameter circle centered on the fovea before and after magnification correction using the Littman and the modified Bennett formulae.

Results: Images from 28 myopic eyes were qualified for quantitative analyses including 12 eyes with nonhigh myopia (43%) and 16 eyes with high myopia (57%). The mean spherical equivalent (SE) refractive error was -8.18 ± 4.58 diopters (D) and the mean axial length was 27.9 ± 2.5 mm. The mean corrected RVAD was significantly lower than the uncorrected RVAD in all myopic eyes (0.51 ± 0.02 vs. 0.52 ± 0.02 , P<0.001). The mean corrected RVSD was also significantly lower than the uncorrected RVSD in myopic eyes (0.13 ± 0.01 vs. 0.15 ± 0.00 , P<0.001). In highly myopic eyes, the mean corrected CC FD% was significantly higher than the uncorrected CC FD% ($14.9\%\pm4.9\%$ vs. $14.2\%\pm4.5\%$, P=0.009). In non-highly myopic eyes, no statistically significant difference was observed between the corrected and uncorrected CC FD% measurements ($11.7\%\pm5.8\%$ vs. $11.5\%\pm5.8\%$, P=0.133).

Conclusions: Ocular magnification significantly affects the results of retinal and CC blood flow quantification with OCTA in myopic eyes. For accurate determination of the OCTA derived parameters in myopia, magnification correction should be taken into consideration.

Keywords: Optical coherence tomography angiography (OCTA); quantitative analysis; magnification error; myopia; axial length

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Introduction

Myopia is a common cause of vision impairment, projecting to affect approximately 50% of the world's population by 2050 (1). Although the etiology of myopia has not been fully elucidated, lower ocular blood flow has been consistently reported in myopic eyes regardless of the imaging modalities (2). Optical coherence tomography angiography (OCTA) is a novel noninvasive modality that provides images with capillary-level resolution of the retinal vasculature (3,4). Moreover, OCTA has proven clinically useful for the assessment and diagnosis of myopic choroidal neovascularization (5,6).

OCTA is becoming increasingly popular in the investigation of the retinal and choroidal blood flow in myopia (7-12). OCTA parameters such as retinal vessel density (RVD) and choriocapillaris (CC) flow deficits provide information about the perfusion status of the retinal vasculature and CC. In OCTA studies, decreased, unchanged, or increased RVD has been reported in myopic eyes in comparison to emmetropic eves (7-12). However, different from emmetropia, eyes with myopia usually have longer axial length, which may induce significant ocular magnification on OCTA images. According to the Littman $(t = p \times q \times s)$ and the modified Bennett $[q = 0.01306 \times (x - 1.82)]$ formulae, the true size t of a retinal feature increases with axial length when imaging with the same fundus camera optical system (13,14). Currently, commercial optical coherence tomography (OCT) devices do not implement the function to correct image magnification. Therefore, it is essential to manually correct image magnification individually in myopic subjects for accurate measurements of OCT and OCTA parameters. It has been demonstrated that ocular magnification (attributable to axial length) significantly affects the OCT measurements including optic disc size and retinal nerve fiber layer thickness in myopia (15,16). However, there is limited literature has focused on the magnification error of quantitative OCTA measurements in myopic eyes. The purpose of this study is to investigate the potential impact of ocular magnification on retinal and CC blood flow quantification using OCTA in myopic eyes.

Methods

This study was approved by the Capital Medical University (Beijing, China) and the University of Washington (Seattle, USA). The study design followed the tenets of the Declaration of Helsinki. Signed informed consent was obtained from each subject before inclusion in the study.

Study population

Subjects with myopia who manifested spherical equivalent (SE) refractive error of -0.50 diopters (D) or less were prospectively enrolled in this study. Study eligibility criteria included a normal anterior segment with sufficiently clear media and a best-corrected visual acuity (BCVA) of 20/200 or better to allow imaging. Exclusion criteria included any coexisting or previous ocular disease that may confound measurements of the retinal and choroidal blood flow, such as glaucoma, optic nerve disease, chorioretinal pathology other than myopic chorioretinal atrophy. Each subject underwent comprehensive ophthalmic examination including assessment of BCVA, slit-lamp examination, refractive error correction, intraocular pressure measurements with Goldmann applanation tonometry, dilated funduscopy, and ocular biometry (IOLMaster[®] 500; Carl Zeiss Meditec, Jena, Germany). All biometric measurements and imaging scans were performed by trained operators at the Beijing Tongren Eye Center, Capital Medical University, China.

Swept-source OCTA (SS-OCTA) imaging and processing

SS-OCTA data were acquired using the PLEX[®] Elite 9000 SS-OCT system (Carl Zeiss Meditec, Dublin, CA, USA) with a central wavelength of 1,060 nm and a speed of 100-kHz A-scan rate. The $6\times 6 \text{ mm}^2$ scanning protocol was used for imaging, with 500 A-lines per B-scan and 500 B-scans with two repeated scans in each fixed location. OCTA data were generated using complex optical microangiography (OMAGc) algorithm (17). Per recommendation from the manufacturer, scans with signal strength less than seven or obvious artifacts were excluded from the analyses.

Retinal and CC slabs were segmented using a validated semi-automated segmentation method (18). The retinal vasculature slab was defined as a slab extending from the inner limiting membrane to the outer plexiform layer. Following the guidelines in the previous study (19), the CC slab in myopic eyes was defined as a slab extending from approximately 4 to 14 μ m below the outer boundary of Bruch's membrane (BM). The CC *en face* flow images were generated using the sum projection, followed by removal of the retinal vessel projection artifacts (20) and compensation for the CC signal strength due to heterogeneous optical properties of the retinal pigment epithelium (21) before



Figure 1 Quantitative comparison between the uncorrected (A-C) and corrected (D-F) *en face* OCTA images in an eye with high myopia. First column (A,D): the *en face* retinal blood flow images; second column (B,E): the *en face* CC blood flow images; third column (C,F): the CC flow deficit binary maps for quantification showing the differences before and after magnification correction. Images are from the left eye of a 52-year-old myopic subject with an axial length of 30.59 mm. The top row images are nominal $6\times6 \text{ mm}^2$ fields, and those at the bottom are corresponding corrected $6\times6 \text{ mm}^2$ fields. OCTA, optical coherence tomography angiography; CC, choriocapillaris.

the identification of CC flow deficits (FDs). All images were analyzed with MATLAB (R2017b; MathWorks, Inc., Natick, MA, USA).

The retinal vessel area density (RVAD) and retinal vessel skeleton density (RVSD) were calculated from the *en face* retinal flow images using our previously published algorithm (22). The percentage of CC FDs (CC FD%) was calculated using a previously described global thresholding method, followed by removal of the CC FDs with an equivalent diameter smaller than the normal intercapillary distance of 24 μ m (21,23). All the parameters were quantified within a 5-mm-diameter circle centered on the fovea in the *en face* OCTA images (*Figure 1*). Examples of the *en face* retinal flow image, the *en face* CC flow image, and the quantitative maps are shown in *Figure 2*.

Adjustment for image magnification

For the estimation of corrected or "true" measurements, original OCTA images were adjusted by a scaling factor defined by the Littman and the modified Bennett formulae (13,14). According to the Littman formula, the relationship between the true size *t* of a retinal feature and its measured size s on OCTA image can be expressed as $t = p \times q \times s$, where *p* is the magnification factor related to the OCT system, and *q* is the magnification factor related to the eye (13). The factor *q* can be determined by the Bennett formula: $q = 0.01306 \times (x - 1.82)$, where x is the axial length (14). By using the default axial length 24.46 mm adopted by the PLEX[®] Elite 9000 system for a magnification of 1 (i.e., *t* = *s*), p can be calculated as $1/[0.01306 \times (24.46 - 1.82)]=3.382$. Therefore, the scaling factor of the OCTA for adjustment



Figure 2 Quantitative comparisons between the uncorrected and corrected *en face* OCTA images and quantitative maps from a myopic eye. First column (A,F): the *en face* retinal blood flow images; second column (B,G): the RVAD maps; third column (C,H): the RVSD maps; fourth column (D,I): the en face CC blood flow images; fifth column (EJ): the CC flow deficit binary maps for quantification. Images are from the right eye of a 51-year-old myopic subject with an axial length of 26.47 mm. The top row images are nominal 6x6 mm² fields, and those at the bottom are corresponding corrected 6x6 mm² fields. OCTA, optical coherence tomography angiography; RVAD, retinal vessel area density; RVSD, retinal vessel skeleton density; CC, choriocapillaris.

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Croupo				Potractive error (SE) (D) (range)	Avial longth (mm) (range)
Groups	No. of eyes	Age (years)	BCVA (IOGIVIAR)	Refractive error (SE) (D) (range)	Axiai length (mm) (range)
Myopia	28	33.6±15.0	0.19±0.32	–8.18±4.58 (–0.93 to –16.13)	27.87±2.51 (24.50 to 33.22)
Non-high myopia	12	25.4±12.2	0.06±0.20	-4.00±1.86 (-0.93 to -5.83)	25.49±0.75 (24.50 to 26.47)
High myopia	16	38.5±16.8	0.29±0.37	-11.31±3.29 (-6.93 to -16.13)	29.66±1.72 (26.77 to 33.22)

Table 1 Demographic data of the subjects

Data are no., mean ± standard deviation, or range. BCVA, best-corrected visual acuity; logMAR, logarithm of the minimum angle of resolution; SE, spherical equivalent; D, diopters.

of the ocular magnification can be calculated as: $t/s = 3.382 \times 0.01306 \times (x - 1.82)$. The *en face* OCTA image of the myopic eye was then adjusted by this scaling factor for further quantification (*Figure 2F,G,H,I,f*).

Statistical analysis

Statistical analysis was conducted using the commercial statistical software (IBM SPSS Statistics version 25.0; IBM Corporation, Armonk, NY, USA). Variable normality was inspected using histograms and the Shapiro-Wilk test. The OCTA measurements before and after magnification correction were compared with paired *t*-test or Wilcoxon matched-pairs signed-rank test as appropriate. For all comparisons, a P value of less than 0.05 was considered statistically significant.

Results

Demographics and clinical characteristics

Twenty-eight myopic eyes were analyzed in this study. The myopic eyes were further classified as non-high myopia (axial length <26.5 mm) in 12 eyes (43%) and high myopia (axial length \geq 26.5 mm) in 16 eyes (57%). Baseline characteristics are listed in *Table 1*.

Comparison of OCTA measurements before and after image magnification correction

Based on the Shapiro-Wilk test, the corrected and uncorrected RVSD and CC FD% measurements were compared using the Wilcoxon matched-pairs signed-rank test. The corrected and uncorrected RVAD measurements were compared using the paired *t*-test. Box plots of the RVAD, RVSD, and CC FD% measurements are shown in *Figure 3*, with brackets indicating statistically significant differences between the corrected and uncorrected measurements. The mean and standard deviation values of all OCTA measurements are shown in *Table 2*. The mean corrected RVAD and RVSD were significantly lower than the uncorrected measurements in all groups (all P<0.01; *Figure 3A,B*). In eyes with high myopia, the mean corrected CC FD% was significantly higher than the uncorrected CC FD% (14.9%±4.9% vs. 14.2%±4.5%, P=0.009; *Figure 3C*). However, no statistically significant difference was observed between the corrected and uncorrected CC FD% measurements in eyes with non-high myopia (11.7%±5.8% vs. 11.5%±5.8%, P=0.133; *Figure 3C*).

Discussion

The present study demonstrated that both the retinal vascular measurements and the CC FD measurements evaluated from corrected OCTA images were significantly different from those from uncorrected images in myopic eyes. The corrected RVAD and RVSD measurements were significantly lower than the uncorrected measurements in myopic eyes. Meanwhile, the corrected CC FD% measurements were significantly higher than the uncorrected measurements in highly myopic eyes.

The impact of image magnification on RVAD measurements with OCTA in healthy subjects has been reported by Sampson *et al.* (24). In their study, 51% of the eyes exhibited more than 5% correction of the foveal RVAD measurements, suggesting that ocular magnification may affect foveal RVAD quantification. However, the RVAD was quantified within a 1-mm circle and a 1-mm rim centered on the fovea in their study, which limited a further interpretation of the magnification effect on RVAD measurements in larger regions. Moreover, as a more sensitive parameter for assessing retinal capillary perfusion (22), the magnification effect on RVSD measurement was not assessed.

In the current study, we evaluated both RVAD and RVSD measurements from the $6\times 6~\text{mm}^2$ OCTA scans in myopic



Figure 3 Box plots of the RVAD (A), RVSD (B), and CC FD% (C) measurements in myopia group, non-high myopia group, and high myopia group. Brackets indicate statistically significant differences between the corrected and uncorrected measurements. RVAD, retinal vessel area density; RVSD, retinal vessel skeleton density; CC FD%, percentage of choriocapillaris flow deficits.

eyes. Our results showed that both the corrected RVAD and the corrected RVSD measurements significantly decreased after correcting ocular magnification. In OCTA, the scan area of 6×6 mm² was calculated from the linear distance on the surface of the retina subtended by an angular size of approximately $20^{\circ}\times 20^{\circ}$ using the default axial length adopted by the OCT system. In myopic eyes, a longer axial length would result in a larger retinal area being scanned than the nominal 6×6 mm² area, which makes the retinal vascular network appear artificially denser.

For accurate comparison of the interocular OCTA parameters, RVAD and RVSD were quantified within a 5-mm-diameter circle centered on the fovea in the present study. After correcting magnification in myopic eyes with longer axial length, the foveal avascular zone occupied a relatively larger portion in the 5-mm-diameter circular region, resulting in decreased RVSD measurements from the corrected images compared with the uncorrected ones. Meanwhile, parts of the large-caliber vessels were also excluded from the measuring circle after magnification correction. As a result, the corrected RVAD was lower than the uncorrected RVAD in myopic eyes. Therefore, for more accurate quantification of RVAD and RVSD in myopic eyes, the ocular magnification effect should be taken into consideration.

We also evaluated the magnification effect on the CC blood flow quantification since abnormal CC flow has been reported in myopic eyes (12,25). In the current study, CC *en face* flow images were generated from a 10-µm-thickness slab below BM based on CC thickness measurements in myopic eyes using our previously described algorithm (unpublished data) (26). No statistically significant difference was observed between the corrected and

Table 2 OC IA measurements before and after image magnification corre	and after image magnification correction	Table 2 OCTA measurements before an
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Croups	RVAD		RVSD		CC FD%	
Groups –	Uncorrected	Corrected	Uncorrected	Corrected	Uncorrected	Corrected
Муоріа	0.52±0.02	0.51±0.02	0.15±0.00	0.13±0.01	13.0±5.2	13.5±5.6
Non-high myopia	0.52±0.02	0.51±0.02	0.14±0.00	0.13±0.00	11.5±5.8	11.7±6.1
High myopia	0.52±0.02	0.50±0.02	0.15±0.01	0.12±0.01	14.2±4.5	14.9±4.9

Data are mean ± standard deviation. OCTA, optical coherence tomography angiography; RVAD, retinal vessel area density; RVSD, retinal vessel skeleton density; CC FD%, percentage of choriocapillaris flow deficits.

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uncorrected CC FD% measurements in eyes with non-high myopia. In our preliminary observation, CC FDs in nonhighly myopic eyes were basically uniformly distributed in the central 5-mm-diameter circular region. As a relative value, CC FD% measurements did not change before and after ocular magnification correction in eyes with nonhigh myopia. However, the current study showed that the mean corrected CC FD% was significantly higher than the uncorrected CC FD% in eyes with high myopia. In highly myopic eyes, CC FDs might merge into a large area where CC was completely lost. When these lesions were located in or around the measuring circular region, significant differences were observed between the corrected and uncorrected CC FD% measurements (Figure 1). Consequently, when calculating CC FD% in highly myopic eves, ocular magnification correction is recommended.

While we have demonstrated that magnification correction should be considered for more accurate quantification of OCTA images in myopic eyes, this consideration would be equally applicable to hyperopic eyes. In eyes with hyperopia, a shorter axial length will result in a smaller retinal area being scanned than the nominal defined area in OCT. As a result, the true values of RVAD and RVSD should have been higher than the uncorrected values in hyperopic eyes. Furthermore, magnification correction is also important for accurate assessment of longitudinal changes in retinal and CC blood flow in eyes undergoing axial growth. The decrease of RVAD or RVSD may not be detected if OCTA measurements were not corrected with elongated axial lengths in myopic eyes. When it comes to myopic eves or hyperopic eves with retinal or CC pathologies, changes in RVAD, RVSD, and CC FD measurements before and after magnification correction are even more complicated. Therefore, it is recommended to correct all quantification values derived from OCTA for accurate and reliable assessments of the retinal and CC blood flow in all ametropic eyes.

We acknowledged several limitations to our study. First, due to the poor fixation and low vision status of some myopic subjects, not all OCTA images are optimal for quantitative analysis. Therefore, our study is limited by a relatively small sample size. However, this study still included myopic eyes with a broad axial length ranging from 24.50 to 33.22 mm, which demonstrated the magnification effect on retinal and CC blood flow quantification in low myopia, moderate myopia, high myopia, and extremely high myopia. Second, in the current study, image magnification error was corrected based on Bennett's formula with an abbreviated axial length. For more accurate correction, detailed calculations utilizing the axial length, keratometry, ametropia, anterior chamber depth, as well as the lens thickness might be considered (14). However, measurements of multiple variables might introduce multiple measurement errors in the calculation. Since Bennett's formula has been demonstrated differs little from more detailed calculations (27), we adopted Bennett's method as our magnification correction algorithm.

In conclusion, the current study shows that ocular magnification significantly affects the results of retinal and CC blood flow quantification with OCTA in myopic eyes. For accurate determination of the OCTA derived parameters in myopia as well as other ocular disease investigations, magnification correction should be taken into consideration.

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Footnote

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Ethical Statement: This study was approved by the Capital Medical University (Beijing, China) and the University of Washington (Seattle, USA). The study design followed the tenets of the Declaration of Helsinki. Signed informed consent was obtained from each subject before inclusion in

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the study.

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