Different Effect of Media Opacity on Vessel Density Measured by Different Optical Coherence Tomography Angiography Algorithms

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Purpose: Several studies show that media opacity reduces vessel density (VD) measured by image processing algorithms of optical coherence tomography angiography (OCTA). However, different models of OCTA designed their own algorithms and computational methods, which may have different effects of opacity on VD. This study is aimed to investigate the impact of a simulated model of media opacity on quantitative measurement of two OCTA devices.

Methods: A spectral-domain based OCTA (Cirrus 5000; Carl Zeiss Meditec) and a swept-source based OCTA (Triton DRI-OCT, Topcon Inc.) were used to image retinal microvasculature at the macula using 3 × 3 mm scanning protocol from 22 eyes of 22 healthy subjects. Media opacity was simulated with neutral-density filters (optical density (OD))\(_{\text{D}}\) = 0.10–0.48 in Cirrus; OD\(_{\text{D}}\) = 1050nm, ranges 0.15–0.51 in Triton). The filters were placed in front of each study eye, and signal strength (SS) or signal strength intensity (SSI) was recorded during imaging. The parafoveal VD of superficial capillary plexus was then measured using the built-in software from the two devices. The correlations among OD, SS/SSI, and VD were analyzed.

Results: Increased OD was significantly correlated with decreased SS and SSI (\(r_t = -0.576\) and \(-0.922\), respectively, both \(P < 0.001\)) in Cirrus and Triton, respectively. Although increased OD was significantly correlated with decreased VD in Cirrus (\(r_t = -0.539, P < 0.001\)), there was no significant correlation between OD with VD in Triton (\(r_t = -0.143, P = 0.137\)).

Conclusions: The effect of media opacity on quantitative measurement of VD is different between different Cirrus and Triton OCTA devices.

Translational Relevance: This study demonstrates that the effect of media opacity on VD measurement is different among different OCTA devices, suggesting that caution must be taken when interpreting VD measurement on OCTA, particularly among individuals with media opacity.

摘要:

目的: 通过使用光学 coherence tomography angiography (OCTA) 定量测量视网膜血管密度的结果。不同类型的OCTA 设备使用的算法不同，得出的测量结果可能不同。本课题的目的是研究屈光介质混浊对 Cirrus 5000 OCTA 及DRI Triton OCTA 定量测量结果的影响。

方法: 对22名屈光介质透明的健康成年人用 Cirrus 5000 和 DRI Triton OCTA, 分别在无滤光片 (光密度为 0) 及滤光片 (光密度为 0.10–0.48, Cirrus; 光密度0.15–0.51, DRI Triton) 模拟屈光介质混浊的条件下用 3 × 3mm 血流成像模式扫描右眼黄斑区视网膜。使用OCTA软件测量软件记录测量图像信号强度、中心凹浅层血管密度等数据。比较不同透光率下各参数的变化。

结果: 随着光密度的增加, Cirrus 5000 OCTA 中中心血管密度及信号强度分数减少 (\(r_t = -0.539, P < 0.001\) 以及 \(-0.576, P < 0.001\)); 中心血管密度随信号分数的增加而
Introduction

High-resolution retinal microvasculature can now be imaged noninvasively, safely, and rapidly with optical coherence tomography angiography (OCTA).1,2 Furthermore, quantitative OCTA assessment, such as vessel density (VD) and foveal avascular zone (FAZ) area, can be measured reliably and are being proposed to be a biomarker for macular ischemia in diabetic retinopathy and other retinal vascular diseases.3,4

Media opacity, such as cataract, often presented in the eyes of elderly, can reduce the light entering into the eyes and, therefore, influence the backscattering of light detected by the OCTA devices. For example, studies have shown that media opacity, worsening image quality of OCT, and influences of quantitative measurement of retinal nerve fiber layer thickness,5–8 as well as diagnosis of glaucoma or glaucoma progression.9 OCTA imaging is derived from the OCT signal. Therefore, quantitative measurement on OCTA is expected to be affected by media opacities as a matter of course. A few studies have previously reported that VD measurement is reduced in the presence of media opacity in OCTA devices.10,11

Different OCTA devices have different wavelengths of light source, image processing algorithms, and computational methods.12 Therefore, the effect of media opacity on quantitative OCTA measurement is presumably variable in different OCTA devices.

In the present study, we used natural-density (ND) filters to simulate different degrees of media opacity optically, and compared the impact of media opacity on VD measurement between two different OCTA devices.

Materials and Methods

Subjects

In this cross-sectional study, healthy Chinese subjects between 21 and 28 years of age were recruited. Only the right eye from each subject was included. All measurements were obtained in a very similar environment using the same OCTA devices, which were operated by a trained technician. All participants had normal retinas and did not have any media opacities. All the study eyes had best-corrected visual acuity of at least 20/25 using the Snellen chart, intraocular pressure < 21 mm Hg, and refractive error within ± 6 diopter(D). Subjects were excluded if they had any evidence of systemic or ocular diseases. The research followed the tenets of the Declaration of Helsinki and was approved by the Ethics Review Board of Joint Shantou International Eye Center of Shantou University and the Chinese University of Hong Kong.

The hypotheses are that the mean of each time is equal (H0) and at least two of the means of each time is unequal (H1). The sample size was calculated according to multiple sample 1-way analysis of variance model13:

\[ n = \frac{\psi^2 \left( \sum_{i=1}^{k} S_i^2 / k \right)}{\left( \sum_{i=1}^{k} (\bar{X}_i - \bar{X})^2 / (k - 1) \right) / \left( \sum_{i=1}^{k} S_i^2 / k \right)} \]

where \( S_i \) is the SD of VD each time, \( \bar{X}_i \) is the mean of each time, and \( \bar{X} \) is the mean of \( k \) time means. These parameters were calculated from preliminary examination of five eyes. The \( k \) is the time counts, which is 6 for Cirrus and 5 for Triton, \( \alpha \) is the rate of type 1 error, and \( \beta \) is the rate of type 2 error, and they were set as \( \alpha = 0.05 \) and \( \beta = 10\% \). The \( \psi \) is found from \( \psi \) table according to the degree of freedom \( k - 1 \). The sample size was estimated as 14 for Cirrus and 18 for Triton. In this study, we included 22 subjects.

Light Attenuation Model

There are three types of optical disturbances: light attenuation, refractive aberrations, and light scattering/straylight.7 Previous studies showed that light attenuation in the optical coherence tomography (OCT) and OCTA scanning spot on the retina, expressed as optical density (OD), fully determined loss of OCT and OCTA image quality as provided by the OCT and OCTA systems, irrespective of the nature of the filter, or type of cataract.7,11,14 ND filters have been used to simulate the light attenuation character of media opacity in previous studies.6,10 In the current study, two sets of ND filters (ZAB, PHTODE, CHINA) with known OD were used to simulate media opacity optically for Cirrus OCTA (5 ND filters ranged from 0.10 to 0.48 with wavelength of 840 nm), and...
Table 1. Single Pass Optical Density Values (in Log Units) of the ND Filters

| Filter Nominal Value | Cirrus OCTA | Triton OCTA |
|----------------------|-------------|-------------|
| \( T_{\text{visible light}} \) (%) | \( T_{\lambda=840nm} \) (%) | \( OD_{\lambda=840nm} \) (single pass) | \( T_{\lambda=1050nm} \) (%) | \( OD_{\lambda=1050nm} \) (single pass) |
| 80                   | 80          | 0.10        | 70          | 0.15        |
| 70                   | 60          | 0.22        | 50          | 0.30        |
| 60                   | 53          | 0.28        | 43          | 0.37        |
| 50                   | 42          | 0.38        | 31          | 0.51        |
| 35                   | 33          | 0.48        | –           | –           |

ND, neutral density; T, transmittance; OD, optical density.

Triton OCTA (4 ND filters ranged from 0.15 to 0.51 with wavelength of 1050 nm), respectively.

The ND filters have different transmittance (T) at different wavelengths, and T has a logarithmic relationship with OD: \( OD = \lg \left( \frac{1}{T} \right) \). When the OD is similar, the effect of wavelength is similar. We used OD to represent each filter value as the following: \( OD_{\lambda=840nm} = 0.10, 0.22, 0.28, 0.38, \) and 0.48 on Cirrus OCTA; and \( OD_{\lambda=1050nm} = 0.15, 0.30, 0.37, \) and 0.51 on Triton OCTA (Table 1). This resulted in six scans of Cirrus OCTA and five scans of Triton OCTA from the same region in each eye. Although the ODs were different between the two devices, we did not directly compare the VD between the two devices, but analyzed the correlation of OD and VD in each device.

**OCTA Imaging Protocol**

The pupils of all subjects were dilated to at least 6 mm diameter with topical 0.5% tropicamide. Two OCTA devices were used: a spectral-domain OCT device (Cirrus HD-OCT 5000; Carl Zeiss, Dublin, CA) and a swept-source OCT device (DRI-OCT Triton; Topcon, Inc., Tokyo, Japan). The central wavelength of Cirrus is 840 nm and it uses an intensity and phase differentiation-based algorithm for OCTA imaging (OCT microangiography-complex [OMAG]). The central wavelength of Triton is 1050 nm and it uses an amplitude decorrelation ratio-based algorithm for OCTA imaging (OCT angiography ratio analysis [OCTARA]). The macular \( 3 \times 3 \) mm scanning protocol was used for OCTA imaging (Cirrus: 245 B-scans in total, 245 A-scans in each B-scan, and each B-scan is repeated 4 times; Triton: 320 B-scans in total, 320 A-scans in each B-scan, and each B-scan is repeated 4 times).

During OCTA imaging, each ND filter was positioned in front of the eye by using a fixed frame. We carefully checked the positioning of the filters (e.g. potential tilt of the filters) before OCTA imaging. The tilt of filter was estimated to be \(< 10^\circ\). In this manner, a maximum error of 2% was accepted. OCTA imaging was first performed without filter (native) and subsequently with ND filters.

All study subjects were first tested with the Cirrus OCTA, and then with the Triton OCTA. All the subjects were instructed to close their eyes before and after each OCTA scan to avoid the effect of corneal dryness and fatigue. Artificial tears were given if any subjects eyes felt dry or fatigued. Quality control was performed during OCTA scanning. We excluded any OCTA image with poor quality due to significant image artifacts and poor centration, including blink, and doubling of the retinal vessels. Presence of \( > 5 \) artifacts were considered as severe artifacts, and decentration artifact was considered moderate to severe if the central or inner subfields were \(< 10\%\) outside the OCT grid after manual reposition. Images with severe motion or decentration artifacts \( > 10\%\) outside the OCT grid were also excluded. Repeated scanning was performed if the OCTA image deemed poor image quality.

**Quantitative Measurements**

The OCTA images were analyzed using its own built-in software. For Cirrus, AngioPlex software (version 10.0) was used. Signal strength (SS; ranged from 0–10) and parafoveal VD of superficial capillary plexus (SCP) were automatically measured, and there was no error of retinal layer segmentation. For Triton, ImageNet software (version 6.0) was used. The signal strength intensity (SSI; ranged from 0–100) and parafoveal VD of SCP were automatically generated after the error of retina layer segmentation was corrected manually.

Parafoveal VD is divided into subfields (include inner superior, inner nasal, inner inferior, and inner temporal) according to the Early Treatment of Diabetic Retinopathy Study (ETDRS). It is noted that the inner...
diameter of parafovea is 1 mm for both devices, and the outer diameter is 3.0 mm in Cirrus, but 2.5 mm in Triton.

### Statistical Analysis

VD at different regions without filters was taken as baseline measurement. We used percent VD in the analysis, which was calculated as VD divided by its baseline value.

SS, SSI, and percent VD were reported as mean ± SD. Correlation of OD with change in percent VD and SS / SSI by the effect of ND filter was performed using Spearman correlation test. Change in percent parafoveal VD as the result of OD change was evaluated with repeated measurements ANOVA test, adjusted for multiple testing with a Least Significant Difference (LSD) test as the post hoc analysis. Statistics were calculated by SPSS (IBM, SPSS statistics, version 19; SPSS Inc., Chicago, IL), and statistical significance was defined as *P* < 0.05. Bland-Altman plots were used to demonstrate the agreement of VD between without filter and with different filters (5 in Cirrus and 4 in Triton) of VD graphically.

### Results

Twenty-two right eyes of 22 healthy volunteers were included in this study. Table 2 shows the characteristics of the study participants. The mean ± SD of refractive error was −2.02 ± 2.14 D (range: −5.25–3.75 D) in the right eye.

Figure 1A shows a series of structural B-scans of the two OCTA devices. The boundaries of retinal layers became more indistinct as OD increased in both devices. Figure 1B shows correlations between OD with SS of Cirrus (*r* = −0.576, *P* < 0.001) and SSI of Triton (*r* = −0.922, *P* < 0.001). Table 3 shows the differences found in SS / SSI between scans obtained from different ND filters.

Table 2. Baseline Characteristics

| Characteristics      | Mean ± SD | Range     |
|----------------------|-----------|-----------|
| Age (y)              | 25 ± 2    | 21–28     |
| Male / Female        | 3/19      | –         |
| IOP (mm Hg)          | 15 ± 2    | 12–18     |
| BCVA (log MAR)       | −0.07 ± 0.07 | −0.18 to 0.05 |
| SE (D)               | −2.02 ± 2.14 | −5.25 to 3.75 |

IOP, intraocular pressure; BCVA, best-corrected visual acuity; SE, spherical equivalent; D, diopter.

Figure 2A shows a series of OCTA images with different ND filters. We found that percent parafoveal VD decreased as OD < 840 nm increased in Cirrus (*r* = −0.539, *P* < 0.001), whereas no correlation was found between parafoveal VD and OD > 1050 nm in Triton (*r* = −0.143, *P* = 0.137). In the post hoc analysis, percent parafoveal VD of Cirrus appeared to decrease beyond a threshold OD > 840 nm of 0.22. When the OD > 840 nm was 0.38, percent parafoveal VD was decreased to 11.7 ± 2.5% (*P* < 0.001). Percent parafoveal VD of Triton changed variously in the post hoc analyses. When OD > 1050 nm were 0.15, 0.30, and 0.37, percent parafoveal VD was decreased to 0.9 ± 0.6% (*P* = 0.151), 1.7 ± 0.5% (*P* = 0.003), and 1.8 ± 0.5% (*P* = 0.002), respectively. However, parafoveal VD was increased to 0.5 ± 0.4% (*P* = 0.309) when OD > 1050 nm was 0.51 (see Table 3).

Figure 2B shows the correlations between OD and different regions of parafoveal VD. We found that OD was significantly correlated with VD of subregions (inner superior, inner nasal, inner inferior, and inner temporal) in both Cirrus (*r* = −0.476, −0.438, −0.431, and −0.478, *P* < 0.001, respectively) and Triton (*r* = 0.047, −0.093, −0.100, and −0.072, *P* = 0.627, 0.332, 0.297, and 0.454, respectively).

Bland-Altman plots also showed that as the OD increased, the bias of VD between no filter and with filter increased on Cirrus (Supplementary Fig. S1). However, the bias VD between no filter and with filter did not correlate with OD in Triton (Supplementary Fig. S2).

Although percent parafoveal VD of Cirrus was correlated with SS (*r* = 0.471, *P* < 0.001; Fig. 3A), there was no correlation between percent parafoveal VD and SSI in Triton (*r* = −0.009, *P* = 0.924; Fig. 3B).

### Discussion

In this study, we found a correlation between an increase in OD > 840 nm, and a decrease in percent VD of Cirrus, whereas there was no correlation between OD > 1050 nm and percent VD of Triton. We also found that percent VD decreased as SS decreased in Cirrus OCTA, but percent VD was unchanged as SSI decreased in Triton OCTA. Our study suggested that the impact of media opacity on VD measurement is different between these two OCTA devices.

Previous studies have shown a significant increase of macular VD after cataract surgery tested by Zeiss PLEX Elite 9000 with OMAG algorithm and Optovue RTVue-XR Avanti with SSADA algorithm.19,20 Although these studies suggested that
elimination of media opacity resulted in increase of VD measurement, it did not exclude the factor of retinal vasculature change due to cataract surgery. It has been reported that ND filters as a beam attenuation can decrease the flow signal and VD by using Optovue RTVue-XR Avanti, both SS index and VD decreased linearly with OD of the ND filter, and VD decreased as SS index decreased by using two different OCTA

Table 3. Signal Score and Percent Parafoveal Vessel Density Change per Filter of OCTA

| Triton OCTA | OD\(_{\lambda=840nm}\) | SS Mean±SD | Parafoveal VD (%) | \(P\) Value* | \(\triangle\) parafocal VD 95% CI (%) | \(\triangle\) parafocal VD 95% CI (%) |
|-------------|-----------------|-------------|-------------------|--------------|--------------------------------|--------------------------------|
| Triton OCTA | OD\(_{\lambda=1050nm}\) | SS Mean±SD | Parafoveal VD (%) | \(P\) Value* | \(\triangle\) parafocal VD 95% CI (%) | \(\triangle\) parafocal VD 95% CI (%) |
| 0.00        | 9.32 ± 0.84     | 100.00      | –                 | –            | 0.00                           | 63.6 ± 6.8                     |
| 0.10        | 9.23 ± 1.07     | 98.8 ± 12.3 | 0.644             | 1.2 (–4.2 to 6.7) | 0.15                          | 53.2 ± 5.4                     |
| 0.22        | 8.55 ± 1.37     | 93.8 ± 12.1 | 0.026             | 6.2 (8.8 – 11.5) | 0.30                          | 42.9 ± 5.1                     |
| 0.28        | 8.23 ± 1.52     | 92.0 ± 10.8 | 0.002             | 8.0 (3.2 – 12.8) | 0.37                          | 36.2 ± 4.4                     |
| 0.38        | 7.68 ± 1.52     | 88.3 ± 11.9 | < 0.001           | 11.7 (6.4 – 16.9) | 0.51                          | 28.0 ± 4.2                     |
| 0.48        | 6.59 ± 1.53     | 78.2 ± 17.1 | < 0.001           | 21.8 (14.2 – 29.4) | –                             | 18.0 ± 4.2                     |

OD, optical density; SS, signal strength; SSL, signal strength intensity; SD, standard deviation; VD, vessel density; CI, confidence interval.

*LSD, \(P\) value of repeated-measurements ANOVA. \(P\) is significant at 0.05.
Figure 2. SCP and VD illustrating the effect of ND filters on macular OCTA image acquisition. (A) SCP in different OD. The VD of SCP is divided by an ETDRS grid includes parafovea (the annulus area between the two white circles) that contains four regions (inner superior, nasal, inferior, and temporal). The diameter of small white and big white circles is 1.0 mm and 3.0 mm in Cirrus OCTA (2.5 mm in Triton OCTA), respectively. (B) Scatter plots and correlation between OD and each region of parafoveal VD in SCP. Each point from one participant. The $r_s$ and $P$ values were shown ($n = 22$). SCP, superficial capillaries plexus; VD, vessel density; OD, optical density; ETDRS, early treatment diabetic retinopathy study; $r_s$, Spearman correlation coefficient.
Vessel Density Measurement on OCTA in a Media Opacity Model

In our study, the results with Cirrus are similar to previous studies using Cirrus and Optovue RTVue-XR Avanti. However, we found that the effect of media opacity on VD measurement was different in Triton. Specifically, we found that the values of percent VD were similar to OD_{λ=1050nm} increased or SSI decreased in Triton.

Several possibilities may explain the different effect of media opacity on VD measurement between the two devices. First, it may due to the calculation in the OCTA image processing algorithms. The different algorithms of OCTA signal flow between two devices may contribute to different effect of media opacity on VD measurement. The OMAG algorithm in Cirrus is based on the following formula:

\[
Flow(x, z) = \frac{1}{R-1} \sum_{i=0}^{R-1} [C_{i+1}(x, z) - C_i(x, z)],
\]

where \(i\) is the index for the repeated time of B-scans at each \(y\)-scan position, \(x\) is the fast axis scan position, \(z\) is the depth, \(R\) is the number of repeated B-scans in each step, and \(C\) is the motion-corrected complex OCT signal, which incorporates both intensity and phase information from repeated B-scans at the same position. Intensity is an important component of complex. In media opacity, increased OD_{λ=840nm} caused decreased SS, decreased intensity, and decreased \(C\). A new parameter \(a\) is introduced in the formula to represent the change of OCT signal, \(C' = aC\), \(a < 1\) to represent the reduction of OCT signal, and the flow signal in media opacity is:

\[
Flow(x, z)' = \frac{1}{R-1} \sum_{i=0}^{R-1} [C'_{i+1}(x, z) - C'_i(x, z)]
\]

\[
= \frac{1}{R-1} \sum_{i=0}^{R-1} [aC_{i+1}(x, z) - aC_i(x, z)]
\]

\[
= a \frac{1}{R-1} \sum_{i=0}^{R-1} [C_{i+1}(x, z) - C_i(x, z)]
\]

\[
= aFlow(x, z)
\]

As \(a < 1\), then \(Flow(x, z)' < Flow(x, z)\) in Cirrus OCTA. The Triton OCTA OCTARA algorithm is based on decorrelation ratio \(r\), between corresponding image pixels as follows:

\[
r(x, y) = 1 - \frac{1}{N} \sum_{i,j} \frac{\min[I(x, y), I_i(x, y)]}{\max[I(x, y), I_i(x, y)]},
\]

where \(I(x, y)\) is the OCT signal intensity, \(N\) is the number of scanned B-scan combinations at a given location, and \(i\) and \(j\) represent two frames within any given combination of frames. In media opacity, OD_{λ=1050nm} increased, caused SSI to decrease, and \(I\) to decrease \((I = aI, a < 1)\).

\[
r(x, y)' = 1 - \frac{1}{N} \sum_{i,j} \frac{\min[I'(x, y), I'_i(x, y)]}{\max[I'(x, y), I'_i(x, y)]}
\]

\[
= 1 - \frac{1}{N} \sum_{i,j} \frac{a \times \min[I(x, y), I_i(x, y)]}{a \times \max[I(x, y), I_i(x, y)]}\]

\[
= 1 - \frac{1}{N} \sum_{i,j} \frac{\min[I(x, y), I_i(x, y)]}{\max[I(x, y), I_i(x, y)]} = r(x, y)
\]
Therefore, the flow signal remains unchanged in Triton OCTA in the presence of media opacity. In brief, the algorithms of OCTA flow signal are different between these two devices. The algorithm of Cirrus OCTA is based on the difference of sequential scans. As OD increases, OCT signal decreases proportionally, OCTA flow signal, which is the difference of two sequential OCT scan decreases, so the vessel density decreases, although the algorithm of Triton OCTA is based on the ratio of sequential scan. As OD increases, OCT signal decreases proportionally, but OCTA flow signal, which is the ratio of the two sequential OCT scan remains unchanged, therefore, the vessel density does not change.

It should be noted that the light source of the two OCTA devices may also affect the VD measurement. A swept-source OCT device has better penetration compared to a spectral-domain OCT device in patients with media opacity.21 In addition, the range of OD differs between the two OCTA devices and the relationship between OD and VD may be nonlinear. Therefore, different wavelengths may explain partially different VD results in the two devices.

VD quantification using OCTA has a wide range of diagnostic and prognostic application in glaucoma and retinal diseases.22,25 Cataract or other media opacity, such as corneal scar and vitreous hemorrhage, are common in clinics. Caution must be taken when interpreting VD measurement on OCTA in individuals with media opacity, in which VD may be reduced or unchanged, depending on the OCTA device used.

Although our hypothesis was supported statistically, some limitations are worth noting. First, the VD measured from the two OCTA devices were measured in different areas (the diameter of parafoveal area in Cirrus was 3 mm, whereas Triton was 2.5 mm), which may cause a difference in the VD measurement. Second, we only reported the VD of the SCP at the macula. The reason for not assessing the deep vessel parameters is that it is difficult to eliminate projection artifacts of vessels from the superficial vascular network in the deep vascular network. Third, some of the SCPs exhibit mild motion and (or) decentration artifacts, which may have affected the VD measurement as well as the correlation of OD. Fourth, the present study only demonstrated the difference of these two devices, but has not developed any algorithms to correct the impact of media opacity on VD measurement, which will be the subject of future studies. Fifth, the current study only used ND filters for mimic light attenuation. Other types of optical disturbances (refractive aberrations and light scattering) were not used. Finally, the performance of algorithms may be different between ischemic retina and normal retina.

The accuracy in healthy subjects is the first step and we intend to investigate the effect of media opacity on VD measurement in diseased eyes in further studies.

In conclusion, the effect of media opacity on quantitative VD measurement is different for Cirrus OCTA and Triton OCTA possibly due to different OCTA flow algorithms used by different models.

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