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Signal Strength Reduction Effects in OCT Angiography

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Purpose: To elucidate the relationship between vessel density (VD) measurements and signal strength in OCT angiography (OCTA).

Design: Cross-sectional study.

Participants: Healthy volunteers.

Methods: OCT angiography images obtained from healthy volunteers were analyzed to demonstrate the relationship between signal strength index (SSI) and VD. Experiments were performed to determine the effects of signal strength reduction on VD measurements on the Optovue/AngioVue (Optovue, Inc, Fremont, CA) and Cirrus/AngioPlex OCTA (Carl Zeiss Meditec, Inc, Dublin, CA) systems. Signal strength reduction was generated by either neutral density filters (NDFs) or defocus.

Main Outcome Measures: Regression analysis of signal strength effects on VD.

Results: Vessel density decreased linearly with signal strength with high statistical significance on both OCTA systems tested and for all analyzed sources of variation in signal strength. The slope of VD versus SSI was greatest when signal strength was adjusted by NDFs, followed by defocus, interscan difference, interindividual variation, and left–right eye difference. Multivariate analysis revealed that both SSI and age had a significant effect on the interindividual variation in VD.

Conclusions: Vessel density measurements using OCTA were affected significantly by OCT signal strengths on 2 OCTA platforms. Investigators should exercise caution when interpreting VD data from OCTA scans. Quantification algorithms for OCTA should ideally remove the signal strength bias. *Ophthalmology Retina 2019;3:835-842 © 2019 by the American Academy of Ophthalmology*

OCT angiography (OCTA) is a new imaging method[1–7] that can visualize the 3-dimensional pattern of retinal and choroidal circulation down to the capillary level.[8,9] One of the advantages of OCTA is that it allows for quantitative perfusion analyses that are relevant to the investigation of physiology, diagnosis of disease, and assessment of treatment response. For example, the flow index has been used to investigate the retinal vascular autoregulation in response to breathing oxygen-enriched air,[10] the vessel density (VD) has been used to detect diabetic retinopathy[3,11,12] and diagnose glaucoma,[13–15] and the vessel area has been used to assess the response of choroidal neovascularization to anti-angiogenic treatment.[16,17]

OCT angiography perfusion quantification algorithms distinguish vascular from nonvascular pixels on en face OCTA based on the value of the flow signal compared with a threshold value. Some investigators have pointed out that the flow signal is correlated with the amplitude of the reflectance signal and VD measurements could be affected by the OCT signal strength of the image.[18–21] Quantification algorithms that use adaptive thresholding to compensate for signal strength variation have been developed.[22] However, to our knowledge, these advanced algorithms have not been adapted by commercial OCTA platforms. Some clinical investigators are not aware that signal strength could bias VD measurements. This could lead to underestimation or overestimation on the effects of disease or aging on VD. One publication concluded that cataract surgery increases VD because the authors assumed that the increased signal strength after cataract extraction should theoretically have no effect on VD measurement.[23] This misconception is understandable, because the dependence of VD on signal strength has not been described in a clinical journal using a commercial OCTA quantification algorithm, and the mechanisms by which signal strength could affect VD measurement have not been demonstrated and explained convincingly.

In this study, we aimed to demonstrate clearly that VD, as measured by commercial OCTA platforms, is affected by OCT signal strength variation. Two mechanisms of signal strength variation—beam attenuation and defocus—were experimentally investigated to determine how they might affect signal strength and VD measurements differently and whether they might explain the variability of VD measurements in a population with healthy eyes.

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Methods

Participants

This study adhered to the tenets of the Declaration of Helsinki and received institutional review board approval from Oregon Health and Science University. Written informed consent was obtained from all participants. Participants with healthy eyes were recruited. Inclusion criteria were no evidence of retinal pathologic features or glaucoma, intraocular pressure less than 21 mmHg, no chronic or systemic corticosteroid use, and corrected distance visual acuity better than 20/40.

OCT Angiography

OCT angiography scans were performed on both eyes of each participant on a 70-kHz spectral-domain OCT system (RTVue-XR Avanti) with AngioVue OCTA software version 2016.2.0.35 (Optovue, Inc, Fremont, CA). The standard definition macular angiography OCTA scan pattern covering a 3 × 3-mm area was used. The split-spectrum amplitude-decorrelation angiography (SSADA) algorithm was applied to detect flow as described previously.[5] The scanning software computed a signal strength index (SSI) value based on the volumetric OCT reflectance signal. The SSI is an integer score that ranges from 0 to 100. Every scan comprised 2 consecutive raster scans performed in orthogonal directions (x- and y-priority) that were registered and merged into a single volume by OptoVue's proprietary Motion Correction Technology.[24,25] In this manner, the prevalence of motion artifacts was reduced. In addition to Motion Correction Technology registration, the tracking system was turned on to reduce motion artifacts caused by blinks and microsaccades.[26]

Vessel density of the superficial vascular complex slab (between the inner limiting membrane and 80% of the ganglion cell complex) was calculated by the AngioVue AngioAnalytics software version 2018.0.0.8. The segmentation of tissue layers was performed automatically by the AngioAnalytics software. No manual corrections of the segmentation were needed. En face projections of the superficial vascular complex were generated by the AngioAnalytics software, and the VD of the entire macula was calculated as the percentage of pixels with suprathreshold flow signal. Two repeat scans were carried out on both eyes of each participant. This allowed us to determine the difference in signal strength and VD between the left and right eyes of the same individual and between repeat scans of the same eyes.

In an additional population of healthy eyes, OCTA scans were obtained from the right eyes with experimental signal strength reduction by 2 mechanisms. In the beam attenuation experiment, serial scans were performed with neutral density filters (NDFs; NEK01, Thorlab, Newton, NJ) placed in front of the eye. Filters with optical densities ranging from 0.1 to 0.6 were used. In the defocus experiment, scans were obtained with varying levels of defocus, ranging from −0.5 to −4.5 diopters (D) relative to the baseline. The baseline measurement for each participant was obtained without NDFs and with optimal focus. The AngioVue has automated software for optimizing focus, along with polarization matching. The software also allowed manual focus setting, which we used to dial in the defocus.

We repeated the NDF experiment in the same participants using the Cirrus/AngioPlex OCT/OCTA system version 9.5 (Carl Zeiss Meditec, Inc, Dublin, CA). Again, the 3 × 3-mm OCTA scan pattern was used. The AngioPlex OCTA uses horizontal-priority raster scanning and active tracking to reduce microsaccadic motion artifacts. The optical microangiography (OMAG) algorithm was used to generate OCTA flow signal.[27] Because VD quantification was not available on the AngioPlex software at the time of the experiment, we calculated VD by applying a fixed threshold on flow signal such that the mean VD of unattenuated scans was the same for healthy participants imaged by both instruments. The AngioPlex metric for signal strength is different from the AngioVue SSI. Signal strength index is an integer value ranging from 0 to 100, whereas the AngioPlex system uses a 10-point scale to measure signal strength. To enable comparison between the 2 OCTA systems, SSI values from the AngioPlex scans were calculated with a custom algorithm to obtain a signal strength scale similar to the AngioVue SSI. The algorithm averaged the logarithmic reflectance (i.e., OCT signal) of the retinal voxels and multiplied the average with a normalization factor. The normalization factor equalized the SSI values of AngioPlex and AngioVue scans obtained in the sets of healthy eyes scanned in the beam attenuation experiments.

Statistical Analysis

Linear and nonlinear regressions were performed using Prism 7 software (GraphPad, San Diego, CA). The slope coefficients from linear regression analysis were compared with the null hypothesis that the slope is 0. The threshold for statistical significance was set at a P value of 0.05. In the NDF and defocus experiments, normalized SSI and VD values were calculated by dividing the SSI or VD values by the baseline measurements for each eye. The baseline measurements were obtained with neither beam attenuation nor defocus.

Results

SSI Partially Explains VD Variation in the Healthy Population

We first determined the relationship between VD and SSI in a population of individuals with healthy eyes on the AngioVue OCTA system (Fig 1A, B). Forty-one participants (23 women and 18 men) were included in this portion of the study. The mean age±standard deviation of the participants was 57.2±20.7 years (range, 26−89 years). Measurements from the 2 scans from the 2 eyes were averaged for this analysis. On univariate regression analyses, VD measurements increased linearly with SSI and decreased linearly with age with a slope of −0.128% per year. Signal strength index also decreased linearly with age with a slope of −0.352 units of SSI per year ($R^2 = 0.638$; $P < 0.0001$, plot not shown). Multivariate regression showed that both SSI ($P = 0.005$) and age ($P = 0.002$) had a significant effect on VD. After accounting for the effect of SSI, the slope of the age effect on VD was reduced to 0.075% per year. The slope of VD versus SSI was 0.152% per unit of SSI in the multivariate analysis.

SSI Affects VD Measurement Repeatability

We next examined the effect of SSI on VD differences between repeat scans of the same eyes (Fig 1C). The scans were ordered so that the measurements from the scan with weaker signal strength were subtracted from the scan with the stronger signal strength to obtain SSI difference and VD difference. The slope of VD difference versus change in SSI was 0.378% per unit of SSI. The regression analysis explained 20.4% of the variance ($R^2 = 0.204$).

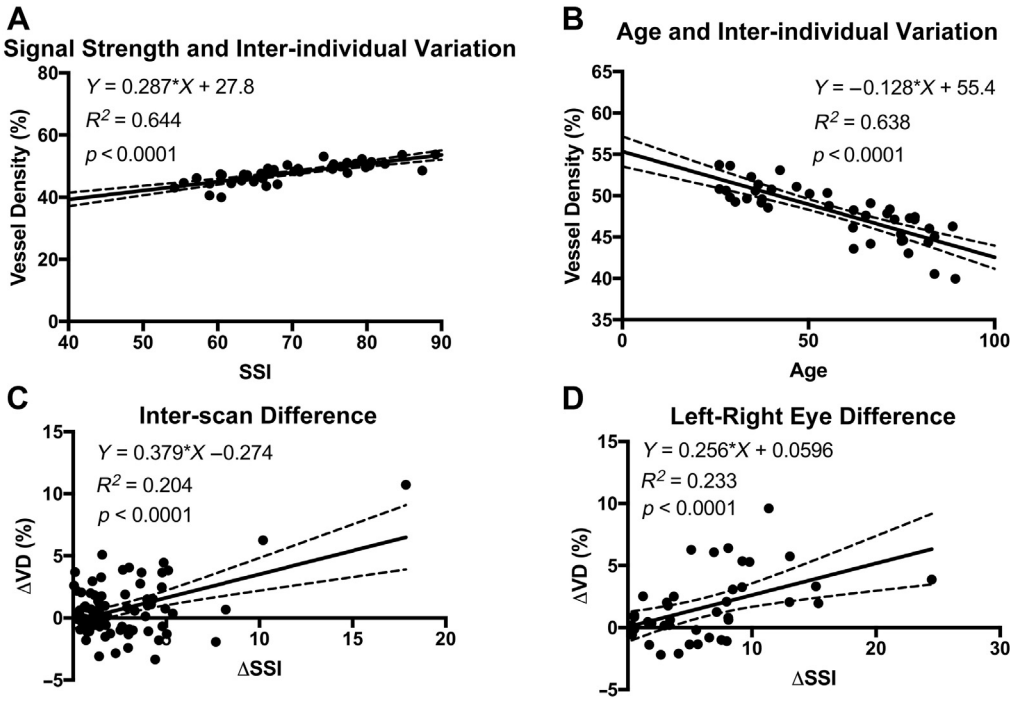

Figure 1. Graphs showing macular retinal vessel density (VD) results obtained using the AngioVue OCT angiography (OCTA) system (Optovue, Inc, Fremont, CA) in persons with healthy eyes. **A**, The VD, averaged between left and right eyes of each individual, is correlated significantly with signal strength index (SSI). **B**, The VD, averaged between left and right eyes of each individual, is correlated significantly with age. **C**, The VD difference (ΔVD) between repeat scans in the same eyes is correlated with the SSI difference (ΔSSI). **D**, Vessel density difference between left and right eyes in the same individual is correlated with ΔSSI.

SSI Partially Explains Intereye Difference

Regression analysis of the difference between left and right eyes of the same individual showed that SSI was a significant factor (Fig 1D). The measurements from repeat scans in each eye were averaged for this analysis. The eyes were ordered so that the measurements from the eye with weaker signal strength were subtracted from the eye with the stronger signal strength to obtain SSI difference and change in VD. The slope of VD difference versus SSI difference was 0.256% per unit of SSI. The regression analysis explained 23.3% of the variance ($R^2 = 0.233$).

Effects of Beam Attenuation and Beam Defocus

The beam attenuation and defocus experiments were conducted on the right eyes of 8 healthy male participants. The mean age±standard deviation of the participants was 32.8±7.3 years (range, 27−48 years).

The effects of beam attenuation by NDF and beam defocus were qualitatively different (Fig 2). Beam attenuation decreased the flow signal (brightness on en face OCTA images), but the capillaries remained thin and sharply defined. Beam defocus blurred the edges of the blood vessels and made the capillaries appear wider, as well as decreased the brightness of the OCTA images.

Neutral density filters placed in the beam path attenuated signal strength in the expected fashion: the SSI decreased linearly with the optical density of the NDF (Fig 3A). We expected SSI to decrease nonlinearly with defocus; the quadratic fit showed this nonlinearity to be small (Fig 3B). The normalized VD decreased linearly with increasing NDF optical density (Fig 3C). The normalized VD decreased nonlinearly with defocus (Fig 3D); there was almost no VD difference with 1 D of defocus and steep drop in VD beyond 2 D of defocus.

Vessel density measurements decreased linearly with decreasing SSI in both the beam attenuation and defocus experiments (Fig 4). The slope of VD versus SSI was greater for beam attenuation (0.509) compared with beam defocus (0.442), but the difference was not statistically significant ($P = 0.65$). The slopes obtained by experimentally reducing signal strength with NDFs and defocus were significantly steeper than those obtained from interindividual variation (0.15% per unit of SSI after accounting for age-related VD change) and left versus right eye difference (0.26% per unit of SSI), but not for interscan difference (0.38% per unit of SSI; Fig 1).

To ensure that the relationship between signal strength and VD measurements was not specific to a single OCTA system, we also conducted a beam attenuation experiment using the AngioPlex system on random eyes of 3 healthy participants. These participants were separate from the 8 healthy male participants described above. We again found that VD decreased linearly with decreasing SSI (Fig 5). The slope obtained from the AngioPlex data was significantly steeper than that obtained from the AngioVue data ($P < 0.0001$).

Discussion

Understanding the effects of signal strength on VD measurements requires a theoretical framework that considers the various mechanisms behind observed variation in OCT

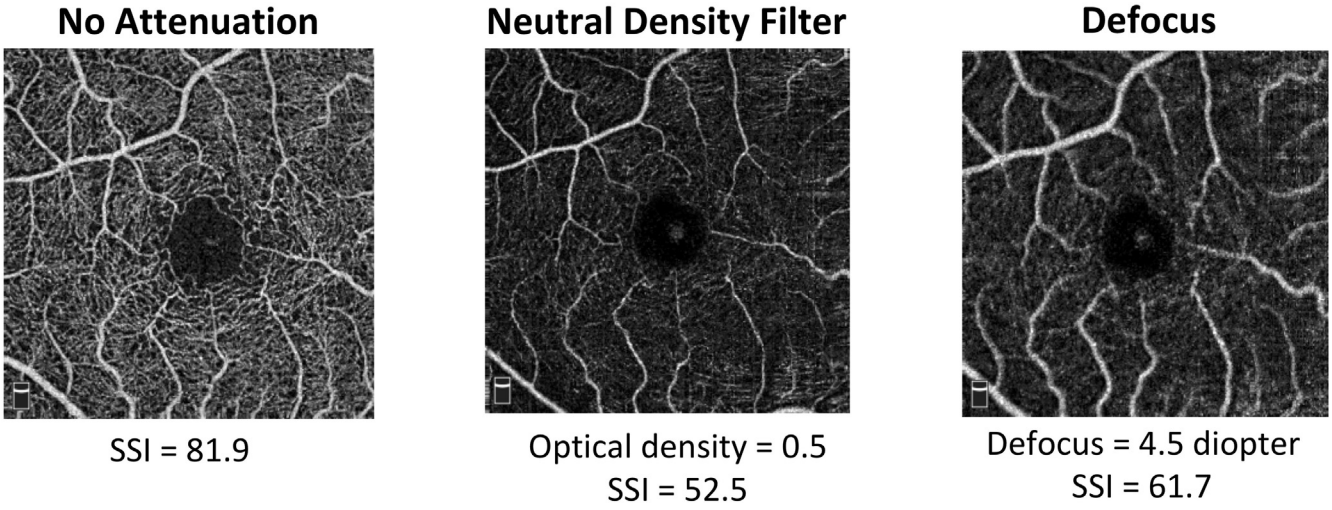

Figure 2. Effects of experimental signal attenuation on macular retinal OCT angiography (OCTA) by the AngioVue OCTA system (Optovue, Inc, Fremont, CA) on a healthy eye. SSI = signal strength index.

signal strength and how they affect the calculation of flow signal in OCTA. These mechanisms fall into 3 categories: beam coupling, beam aberration, and tissue reflectivity. We discuss these in turn.

The coupling of the OCT beam with sample tissue is affected by media opacity, which attenuates the probe beam by absorption or scattering, and media birefringence, which causes polarization mismatch between the sample and reference beams in the interferometer. These mechanisms attenuate the reflectance signal, making OCT images dimmer. However, they do not affect the beam focal spot size in the retinal plane, thus preserving transverse spatial resolution and the sharpness of the images. In this study, we used absorptive attenuation by NDF to study the effects of beam coupling. The signal strength was measured by the SSI, which is based on the average tissue reflectance amplitude on the OCT image on a logarithmic scale. The measured VD on both the AngioVue and AngioPlex systems were related linearly to SSI; thus, the experiment showed that beam attenuation decreased the flow signal in a log linear fashion. The flow signal in OCTA is based on voxelwise analysis of OCT signal variation between successive B-scans at the same location. The SSADA algorithm[5] measures signal variation using the decorrelation function, which is mathematically equivalent to the variance divided by the square of the average amplitude. The division operation should remove the effect of the average amplitude (signal strength) on flow signal. Thus, some authors have stated that it is mathematically impossible for flow signal to be affected by signal strength.[23] However, the SSADA algorithm also suppresses noise by setting a constant floor value on OCT signal amplitude, thereby reducing the measured flow in low signal voxels. This reduces the flow signal (decorrelation) in voxels with low OCT (reflectance) signal strength. All OCTA algorithms, including SSADA and OMAG, must use a noise-suppression subroutine to avoid artifactual flow in low-signal voxels that are dominated by noise, because noise has random (maximally decorrelated) amplitude and phase. Thus, the dependence of measured VD on SSI is expected, unless a special compensatory algorithm is used to remove this dependence. It should be noted that the slope relating VD and SSI was significantly steeper for the AngioPlex compared with AngioVue. The primary reason is that AngioPlex's OMAG algorithm is based on variance (not normalized to average amplitude) and AngioVue's SSADA algorithm is based on decorrelation (mathematically equivalent to the variance divided by the square of the average amplitude). Thus, an extra normalization step during postprocessing is required by OMAG to quantify vessels in deeper layers such as choroidal neovascularization[28] and choriocapillaris,[29] where signal strength is more variable. The 2 systems may also differ in the proprietary algorithms used to identifying vascular pixels on en face OCTA images, which could affect how much measured VD depends on the signal strength.

Beam aberration is a separate category that includes defocus, astigmatism, and higher-order aberrations. These mechanisms not only reduce the OCT signal but also increase the focal spot size of the OCT beam, making the en face OCT and OCTA images blurry. The blurring effect increases the apparent width of blood vessels. One would expect that this broadening might increase the measured VD, partially counteracting the effect of signal strength reduction. Indeed, we found that the VD versus SSI slope was slightly smaller in the beam defocus experiment compared with the beam attenuation experiment. The effect of beam defocus should be negligible below the Rayleigh range, which is approximately 1 D in the AngioVue system. This was observed in the experiment, which showed drop in VD only beyond 1 D of defocus. The effect of astigmatism on beam spot area is approximately half of defocus on a per-diopter basis. Thus, we would expect less than 2 D of astigmatism to have a negligible effect on SSI and VD for the AngioVue. The effect of defocus and other aberration

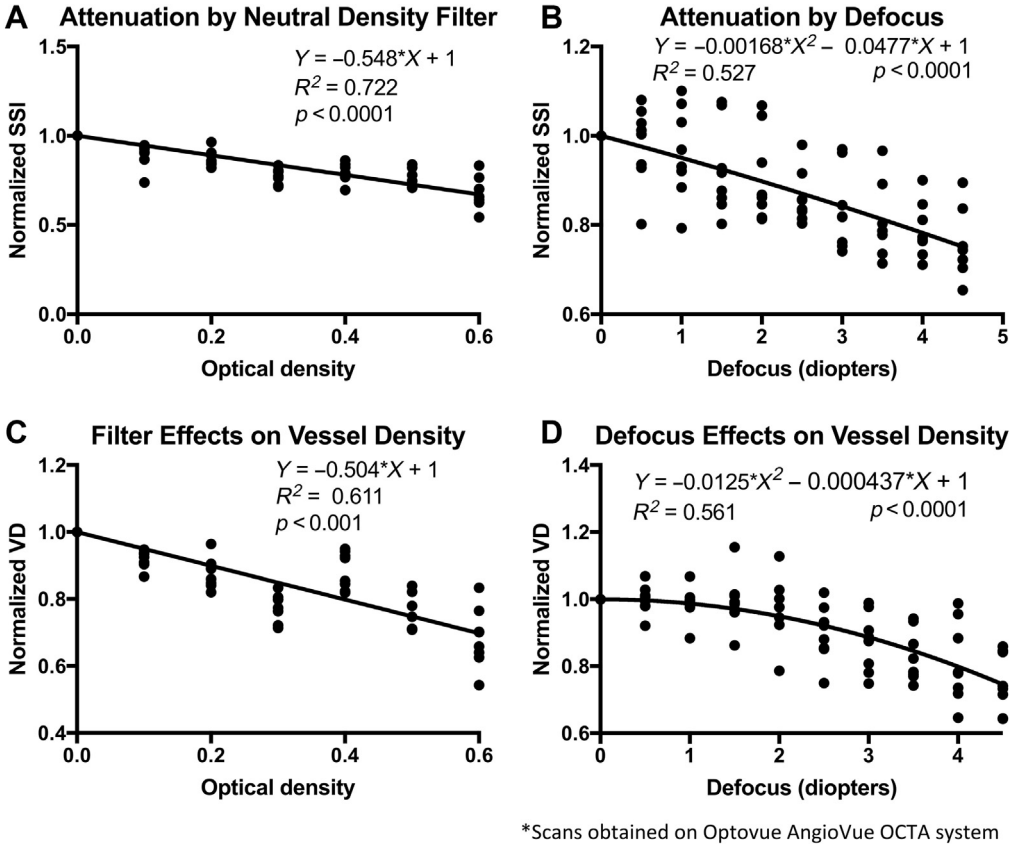

Figure 3. Graphs showing the effects of experimental signal attenuation on macular retinal vessel density (VD) measured by the AngioVue OCT angiography (OCTA) system (Optovue, Inc, Fremont, CA) in persons with healthy eyes. **A**, The signal strength index (SSI), normalized by the unattenuated value, is plotted against the optical density of neutral density filters (NDFs). **B**, Normalized SSI is plotted against diopters of defocus. **C**, Normalized VD plotted against NDF optical density. **D**, Normalized VD plotted against diopters of defocus. Linear regression models were applied to the NDF plots, whereas quadratic models were applied to the defocus plots. The y-intercepts of for the regression models have been set to 1.

would be greater for OCT systems with wider beams (at the corneal plane).

Tissue reflectivity is a third mechanism by which the SSI of an OCT or OCTA image might vary. Some retinal layers, such as the nerve fiber layer (NFL) and retinal pigment epithelium, are more reflective than others. Thus, if the scanned region shows thicker NFL or more highly pigmented retinal pigment epithelium, the SSI is higher, given

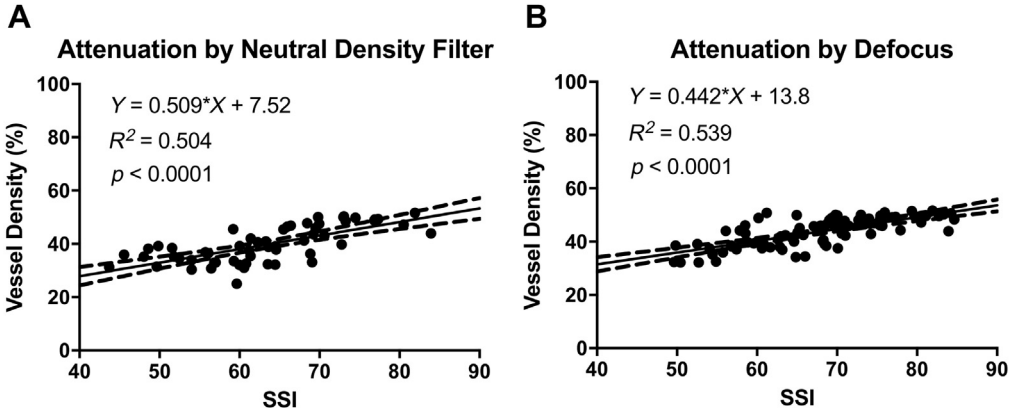

Figure 4. Graphs showing the effects of experimental signal attenuation on macular retinal vessel density (VD) measured by the AngioVue OCT angiography system (Optovue, Inc, Fremont, CA) in persons with healthy eyes. **A**, VD shows significant linear correlation with signal strength index (SSI) in the experiment with neutral density filters. **B**, VD shows significant linear correlation with SSI in the defocus experiment.

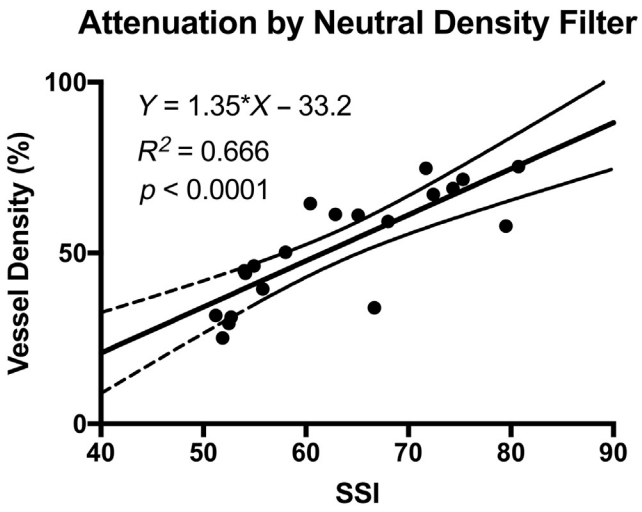

Figure 5. Graph showing the effect of experimental signal attenuation with neutral density filters on macular retinal vessel density measured by the AngioPlex OCT angiography system (Carl Zeiss Meditec, Inc, Dublin, CA) in persons with healthy eyes. SSI = signal strength index.

the same beam coupling and aberration. Because stronger reflectivity of nonvascular tissue could cast stronger shadows and weaken the reflectance from blood cells below, this type of SSI variation should be related inversely with measured VD (e.g., thicker NFL would overshadow and reduce flow signal from deeper retinal vessels), which is opposite of the effect of SSI reduction resulting from beam defocus or attenuation.

The slope dependence of VD was 0.15% per unit of SSI in multivariate regression of interindividual variance, approximately one third of that found in the attenuation and defocus experiments (0.51% and 0.44% per unit of SSI, respectively). The positive slope indicates that SSI variation was mostly the result of beam attenuation and defocus, which overwhelmed the counteracting effect of tissue reflectivity variation. The reduction in SSI with increasing age could be explained by an increase in media opacity (e.g., cataract, vitreous floaters) and increase in media aberrations (e.g., cataract, poor tear film quality), as well as reduced tissue reflectivity (e.g., NFL thinning). The VD versus SSI slope of interindividual VD variance was approximately one third of the slope for experiment beam attenuation and defocus, indicating that most of the interindividual VD variation was real. Aging caused both a real decline in VD as well as an artifactual decline associated with SSI reduction. It is important to note that after adjusting for SSI effect, the rate of VD decline with age dropped from −0.128% to −0.07% per year in our study. Several investigations studied aging changes in VD without accounting for signal strength effects; these may have overestimated the rate of VD decline with aging.[30,31] Our results showed that it is important to take signal strength into account when interpreting physiologic or pathologic changes in VD.

In contrast to interindividual variation, the difference in VD measurements from repeat scans could not be explained by the difference in tissue reflectivity or VD and must be related to the effects of beam coupling or aberration. Indeed, we found the VD versus SSI slope for interscan difference to be statistically equivalent to those resulting from beam defocus or attenuation. This slope is large; thus, studies that monitor VD change over time need to watch closely for artifactual change resulting from signal strength variation. The use of VD measurement algorithms that compensate for signal strength variation could improve the reliability of change analysis.

Our investigation showed that OCT signal strength could have a significant impact on retinal VD measurements with OCTA. This is of growing clinical significance because the use of OCTA is increasing. Retinal VD quantification in OCTA has a wide range of diagnostic and prognostic applications in glaucoma and retinal diseases.[3,11,13,14,16] Thus, it is important to find effective methods to reduce the biases on OCTA measurements caused by signal strength variation.

There are several approaches to reduce the effects of signal strength variation: quality improvement, quality control, and algorithmic compensation. Operators of OCTA systems should be trained to optimize scan quality by minimizing defocus, polarization mismatch, tear film disturbance, and pupil vignetting. But because media opacity, astigmatism, and other ocular aberrations are always present in the human population, methods to control and compensate for signal strength variation are also important.

The quality control solution involves excluding OCTA scans with signal strength of less than a certain threshold. A wide range of signal strength cutoff values has been used as quality control measures in OCTA studies.[32–34] The manufacturer's guidelines for the AngioVue system specify a minimum SSI of 45 (of 100) for macular OCTA. The AngioPlex system uses a 10-point scale to measure signal strength and recommends an inclusion criterion of 6 or more. A limitation of the quality control solution is that the acceptable scans still range widely in signal strength. Between the SSI scores of 45 and 80, there is a 5% artifactual variation of VD based on our multivariate population analysis of AngioVue results (0.15% per unit of SSI) and approximately 15% VD variation if the SSI change was caused by beam attenuation or defocus. Lim et al[18] reported a significant relationship between VD and signal strength up to a signal strength value of 9 on the AngioPlex system by directly comparing the differences in VD quantification between different signal strength values. It is not practical to reduce variation further through stricter quality control, because this would exclude too many patients who need OCTA evaluation. Thus, although it is important to have quality control criteria, methods to compensate for signal strength variation are needed in OCTA quantification algorithms.

Gao et al[22] developed a retinal VD quantification algorithm that compensates for signal strength variation by slab-based local reflectance analysis. The inner retinal slab was used, but the highly variable NFL was excluded to minimize the effect of tissue reflectivity variation. The algorithm was effective in minimizing the VD dependence on SSI, improving repeatability, and narrowing the reference range from a normal population.[22] This was a significant improvement for the purpose of glaucoma diagnosis and monitoring.[13] However, there remains a concern that this

algorithm could be biased by retinal tissue reflectivity variation when applied to patients with retinal diseases. The ideal compensation algorithm would need to separate beam coupling and aberration effects completely from tissue reflectivity effects. Camino et al[35,36] developed a regression-based bulk-motion subtraction algorithm that iteratively optimized the separation of vascular flow and nonvascular voxels and analyzed the reflectance in these 2 types of voxels separately. A side benefit of this algorithm is that VD measurement is adjusted based on the reflectance of vascular flow voxels. Because the reflectance of blood cells should not vary much between participants, this approach could theoretically better separate the effects of beam coupling and aberration from tissue reflectivity variation. This algorithm was effective in reducing the VD dependence on SSI in 24 scans from a population of 8 healthy participants.

Given the results of our study, we urge investigators to exercise caution when interpreting OCTA vessel density measurements using current commercial OCTA platforms. Operators should be trained to minimize defocus and optimize signal strength. A minimum signal strength threshold should be included as a quality-control measure. An SSI should be used as a covariate in statistical analysis. Measurements made in eyes with dense cataract, high astigmatism, or high aberration should be interpreted cautiously. We urge manufacturers of OCTA systems to implement quantification algorithms that compensate for signal strength variation and provide quality assessment output that should include an SSI. A defocus index would also be useful as an additional covariate for analysis because we have shown that signal strength variation resulting from beam aberration (e.g., defocus), beam decoupling (e.g., media opacity), and tissue reflectivity changes may have different effects on OCTA measurements.

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Abbreviations and Acronyms:
D = diopter; **NDF** = neutral density filter; **NFL** = nerve fiber layer; **OCTA** = OCT angiography; **OMAG** = optical microangiography; **SSADA** = split-spectrum amplitude-decorrelation angiography; **SSI** = signal strength index; **VD** = vessel density.

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