Image quality metrics for optical coherence angiography

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Abstract: We characterized image quality in optical coherence angiography (OCA) en face planes of mouse cortical capillary network in terms of signal-to-noise ratio (SNR) and Weber contrast (Wc) through a novel mask-based segmentation method. The method was used to compare two adjacent B-scan processing algorithms, (1) average absolute difference (AAD) and (2) standard deviation (SD), while varying the number of lateral cross-sections acquired (also known as the gate length, N). AAD and SD are identical at N = 2 and exhibited similar image quality for N<10. However, AAD is relatively less susceptible to bulk tissue motion artifact than SD. SNR and Wc were 15% and 35% higher for AAD from N = 25 to 100. In addition data sets were acquired with two objective lenses with different magnifications to quantify the effect of lateral resolution on fine capillary detection. The lower power objective yielded a significant mean broadening of 17% in Full Width Half Maximum (FWHM) diameter. These results may guide study and device designs for OCA capillary and blood flow quantification.

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and diagnosis of neural vascular diseases. Recent studies have shown that OCA can be used to correlate between cerebral blood perfusion and brain metabolism allows for the treatment of diseases and pathological conditions. Ocular diseases such as diabetic retinopathy and age-related macular degeneration impact the morphology of vessels and the formation of capillary networks, and are often accompanied by abnormal blood flow. Additionally, the tight correlation between cerebral blood perfusion and brain metabolism allows for the treatment and diagnosis of neural vascular diseases. Recent studies have shown that OCA can be used to investigate vascular perfusion in ischemic stroke and the hemodynamic response during functional activation [11, 12]. In our recent study we demonstrated that OCA is able to

1. Introduction

Optical coherence tomography (OCT) is a high resolution imaging modality capable of capturing volumetric data sets of highly scattering biological tissue. In the last decade an imaging technique called speckle variance optical coherence angiography (SV-OCA) was developed to detect microvasculature and blood flow in biological tissue. Speckle variance angiography is also known as amplitude decorrelation angiography [1]. SV-OCA has been applied successfully to retinal imaging [2–6] and neural imaging [7–9], using phase-based [4], intensity-based [7] or combined approach [10] contrast detection. SV-OCA is rapid, non-invasive, has a relatively high penetration depth of ~1-2 mm, uses intrinsic contrast rather than dye injection to detect flow, and has high lateral and axial resolution necessary to resolve capillaries (5-15 µm diameter).

Understanding morphological and dynamic changes in capillaries is essential to the study of diseases and pathological conditions. Ocular diseases such as diabetic retinopathy and age-related macular degeneration impact the morphology of vessels and the formation of capillary networks, and are often accompanied by abnormal blood flow. Additionally, the tight correlation between cerebral blood perfusion and brain metabolism allows for the treatment and diagnosis of neural vascular diseases. Recent studies have shown that OCA can be used to investigate vascular perfusion in ischemic stroke and the hemodynamic response during functional activation [11, 12]. In our recent study we demonstrated that OCA is able to
characterize the tissue response from neuroprosthetic electrode insertion in mouse motor cortex [9].

Intensity-based OCA captures speckle signal decorrelation in voxels occupied by moving particles (erythrocytes). Generally, intensity-based decorrelation methods are less susceptible to motion artifact (bulk motion), are less sensitive to beam angle, and have lower computational complexity than phase-based techniques [13, 14]. However, both have similar characteristics and show comparable results for vascular mapping [15].

The experimental and parameter space for intensity-based OCA is extensive and requires informed design decision-making to maximize image quality for specific targeted applications. Trade-offs are necessary for the decorrelation calculation and usually involve the number of acquired A-scans, B-Scan interval, lateral sampling density, and number of B-scans collected at each lateral location (referred to as gate length, N). For high bulk motion applications such as ophthalmology, acquisition speed is paramount. For applications where tissue stabilization is straightforward, as with most neural imaging, longer scan times are acceptable. In all cases where the artifactual motion spectrum overlaps the flow spectrum, image quality will be degraded. In situations where bulk motion is problematic, the field of view or frame rate must be adjusted to keep inter-frame displacements less than the beam waist radius [13]. On the other hand, the inter-frame interval has to be high enough to let moving tissue reach a regime of complete decorrelation in order to detect microvasculature flow.

In addition, optical parameters (e.g., beam waist and depth of focus) can play a crucial role in determining overall angiogram quality. If the vessel is not sufficiently sampled by the scan beam, width quantification will contain systematic errors. To fulfill the Nyquist criterion, high magnification lenses are preferable in order to achieve a lateral resolution of a few microns. Optics that produce increased lateral resolution also narrow the depth-of-focus (DOF) and shorten the working distance of the objective lens. The former somewhat obviates the depth sectioning advantage of OCT and may necessitate focus tracking. Both may lead to a more complex experimental setup for some applications.

Several quantitative metrics of image quality have been established for conventional OCT, some based upon more traditional imaging modalities [16, 17]. Some techniques examine the histogram of intensity values as a linear composition of background (noise) and foreground (signal) pixels, and the challenge is the delineation and extraction of metrics from overlapping signal and noise components. One approach focused on the number of pixels in certain regions of the histogram from which a quality index was devised [16]. Another approach started from assumptions on the statistical intensity distributions of background and signal pixels [17]. In the case of fluid flow, it is known that the speckle amplitude of voxels is Rayleigh distributed and is Gaussian distributed in static tissue. Pixels partially occupied by flow with small capillary diameter comparable to the lateral B-scan sampling follow a Rician distribution, which is a generalized Rayleigh distribution [18–21].

Previous exploration of SNR specifically related to speckle-variance and angiography is limited. In one study on phantoms, speckle SNR was calculated selecting regions of signal and background over 1000 pixels and SNR showed an ascending trend towards higher N [13]. A similar method was used to quantify the improvements from diverse processing algorithms in en face maximum projections of retinal vessels [1]. Another in vivo study in the chorioallantoic membrane of a chick embryo showed similar results [21]. Although higher gate length are reported to give higher SNR and contrast, high N results in more intense computational processing and longer scan times. Real time processing via graphical processing unit (GPU) and high-speed swept-source OCT have been used to mitigate those limitations [9, 22].

Exploration of SV-OCA parameter space has not been fully investigated. For this reason, the development of standard metrics and methods to assess OCA image quality will allow investigators to make better informed design decisions. The overall goal of this study is to
quantify the parameters that most affect SV-OCA for in vivo applications. We particularly focused on capillary network detection which is challenging because of target dimensions and low flow rate. A novel technique is herein proposed to assess OCA image quality based on the use of a mask to select regions of signal and background in OCA en face images. This method was then used to compare different decorrelation algorithms including approaches based upon standard deviation or variance [6, 9, 13], and averaged absolute difference of adjacent frames [7, 8]. Finally, the same region was imaged using 5 × and 10 × objective lenses to evaluate the effects of system optics on capillary quantification.

2. Materials and methods

2.1 Optical setup

The system and the animal preparation are described elsewhere [9]. Briefly, a custom-built spectrometer-based OCT system was used. The source has a center wavelength of 1315 nm with a bandwidth of 85 nm full-width at half-maximum (FWHM). The spectrometer linear detector has a maximum line rate of 76 kHz. All angiography processing was performed in real-time on the system computer’s GPU.

The optical system was characterized by imaging a nanoparticle-embedded phantom [23] to measure the 3D point spread function (PSF) with both 5 × and 10 × telecentric lenses. The axial PSF was 9.4 µm, which compares well with the theoretical value of 9 µm [24]. For the 5 × scan lens, the lateral PSF width was ~6.5 µm FWHM and the depth of focus (DOF) was ~500 µm. For the 10 × scan lens, the lateral PSF width was ~4.5 µm FWHM and the DOF was ~200 µm. While OCT includes inherent depth sectioning capabilities, the depth range over which the tissue is properly in focus is still governed by optics (i.e., the front objective) for microscopy applications. Invariably this leads to a trade-off between number of volumes acquired in depth and lateral resolution and field of view. For quantitative angiography, the lateral resolution should be better than the diameter of the smallest capillaries (~5 µm). However, slower lenses (i.e., lower N.A.) can be used to maintain focus over a larger depth range (i.e., longer DOF) with a longer working distance for practical considerations (e.g., lens obstructions from electrodes or preparation). Our analysis used a 10 × objective (ThorLabs Inc, LSM02), which allowed resolution of the smallest capillaries (6-8 µm). Similar results are expected for higher power objectives. We also imaged with a 5 × objective (ThorLabs Inc, LSM03), which is often used in angiography for the reasons stated above [7, 9, 12].

2.2 Animal preparation

The FDA White Oak Institutional Animal Care and Use Committee approved a protocol for all animals used in this study. Mice were anesthetized with isoflurane (4% induction, 1.5% maintenance), and their body temperature was maintained with a heating pad during surgery and imaging. A 2 × 2 mm craniotomy, positioned over the mouse primary motor cortex, was made using a high speed dental drill and surgical forceps. A small cover glass window ~120 µm thick was positioned over the craniotomy and secured with dental cement. A metal headbar was secured to the contralateral skull to allow for replicable positioning of the animal’s head during imaging.

2.3 Image acquisition and processing

We acquired 3-D data sets (1.0 × 1.0 × 0.5 mm) from a region over the primary motor cortex of the mice. The focus was set ~350 µm below the cortical surface in the dense capillary network. We implemented real-time GPU-based average absolute difference (AAD) and standard deviation (SD) angiography-processing algorithms. The pixel value (px) was computed using the following equations:
where \( N \) is the gate length, \( A_n \) is the pixel’s intensity at frame \( n \), and \( \bar{A} \) is the pixel’s mean across the set of \( N \) frames.

The former was recently used in a work that aimed at capillary detection and flow quantification [8]. We used the latter in our previous study [9] to quantify vascular remapping due to window and electrode implantation in the mouse primary motor cortex. To visualize the contiguous capillary network, an integrated depth of 40 µm (8 axial slices) around the beam waist was used to create en face images. Single slices were not used because the capillary network appeared discontinuous (compare Figs. 1(c), 1(d)) and Figs. 1(e), 1(f)). All images were obtained through maximum intensity projection (MIP) and in order to minimize any effect of the projection mode, the number of integrated slices was kept as low as possible. Care was taken to integrate the same depth across all the sets. Figure 1 shows the integrated depths around the beam waist and relative en face images for sets acquired using 5 × and 10 × front optics.

The data sets used for SNR analysis were taken in one imaging session, and there was little lateral or axial shift or motion between sets. To confirm this, we identified a vessel in the cross-sectional planes that had the same lateral position in the en face planes (Fig. 1). All en face images show identical morphological features allowing consistent comparisons across sets. To correct any small residual lateral shift or rotation, the en face images were registered using StackReg in Fiji (ImageJ), which does not apply any intensity correction (e.g., brightness or contrast) and thus preserved signal and background content.

SV-OCA is based on the statistical properties of voxel reflectivity. Voxels corresponding to flowing erythrocytes experience decorrelation over time. Algorithms such as SD or AAD have successfully quantified decorrelation of adjacent cross-sections at a given lateral location [8, 9]. While both are able to detect vasculature, noise is embedded in the calculations differently. In addition, the choice of sample size for averaging (gate length \( N \)) involves statistical and non-statistical considerations. Statistical considerations include the level of precision of the estimate, which, for SV-OCA, is the ability to separate signals from moving tissue and static tissue. Non-statistical considerations may include the availability of resources, such as the memory requirements to store a certain number of cross-sections at each lateral position, and the scan time.
Fig. 1. OCT angiograms of mouse cortical tissue. Single cross-sectional images acquired using 5 × (a) and 10 × (b) objective lenses. White lines indicate the integrated depth. En face images of one slice (4 µm) for 5 × (c) and 10 × (d). En face images obtained through MIP of 40 µm for 5 × (e) and 10 × (f) and of 100 µm for 5 × (g) and 10 × (h). Note how capillary networks appear disconnected in single slices. Yellow arrowheads indicate capillaries used as a reference for depth registration.

In order to quantify the influence of the processing algorithm (SD and AAD), gate length, and front optics on the images, these parameters were varied systematically between acquisition sets. Figure 2 compares en face images for the AAD and SD algorithms and a subset of gate lengths. For each region a zoomed image (2 × ) is presented for qualitative comparison.

2.4 Definitions

For traditional reflectance OCT cross-sectional images, separation of the signal and background components is relatively straightforward because the background can be measured in the image region corresponding to the space above the tissue (e.g., aqueous humor for ophthalmic applications and air/media for others). Segmentation techniques are easily applied to separate these regions. However, with en face representation, the background region is difficult to separate from signal. And for angiography, segmentation of the signal (vasculature) is challenging. Moreover, a proper measurement of background is altogether
different compared to applications where signal is comprised simply of the backscattered photons that contribute to the interferometric fringes (AC component) and noise/background is everything else (DC component). The background for angiography includes residual static tissue signal in the case of incomplete suppression of static signal, as well as signal arising from flow in out-of-focus vessels from adjacent depth planes in addition to the background measured in a cross-sectional angiogram. Noise in OCA mainly comes from the moving components (e.g., scanning hardware) and from sample motion that affects inter-frames calculations. These manifest as noisier background and artifactual lines in the en face planes [13]. Thus in this paper for angiography, we define SNR and Weber contrast ($W_c$) as measures of the system ability to detect flow (signal) in a noisy quasi-static biological medium (background):

$$SNR = \frac{\mu_s - \mu_b}{\sqrt{\sigma_s^2 + \sigma_b^2}}$$

(3)

$$W_c = \frac{\mu_s - \mu_b}{\mu_b}$$

(4)

where $\mu_s$ and $\mu_b$ are the mean and $\sigma_s$ and $\sigma_b$ are the standard deviation of the signal and background pixels.

To illustrate the difficulty separating signal and noise in angiography, we implemented a histogram-based approach to quantify image quality via SNR [16, 17]. This method could be applied automatically, without attempt to spatially separate vascular from static tissue with segmentation. Figure 3(a) shows the histogram of pixel intensity values from an en face angiogram (AAD processing, N = 100, 10 x objective, 40 µm MIP). For simplicity, signal and background are assumed to both have Gaussian distributions. The histogram fitting algorithm used an iterative approach, with distribution amplitude, mean, and standard deviation set as free parameters in a nonlinear optimization exercise. From the best fit, pixels are classified as signal or background and signal-to-background calculated. Despite the significant overlap, the algorithm was able to find fits that accurately represented the histograms. Figure 3(b) shows the resultant SNR calculations through Eq. (3). It is clear from comparison of the histogram-based SNR calculation and the qualitative comparison of image quality in Fig. 2, that the histogram technique failed to accurately represent image quality improvement with N. There are a number of reasons for this. First, the assumption of distributions may overly simplify the components contained in the histogram. Second, the uniqueness of the solution is not guaranteed. Last, we know (from the approach below), that vessels occupy a small percentage of en face image area, which does not correspond well with the area under the curve of the signal component found with this technique. Even with a more accurate model for noise (Poisson distribution, Rayleigh) or more complex and constrained fitting, histogram-based techniques may not be able to accurately represent SNR or image quality for angiography.
Fig. 2. Representative capillary network of mouse primary motor cortex using a 10 × objective lens 370 µm below the window, at the center of the focus. Each set was processed using average absolute difference (a-e) and standard deviation algorithm (f-l). En face images were obtained for increasing gate length. For each region a zoomed image (2 × ) is presented for qualitative comparisons.

2.5 Mask-based segmentation method

A mask-based segmentation method was devised to semi-automatically separate signal and background in OCT angiograms. A simple non-automated way to evaluate image SNR is to manually select regions of signal and background in the image. Although this is straightforward, it is not applicable to OCA images of capillaries for several reasons. A capillary generally is a few pixels wide in the en face image unless restricted fields of view
(FOV) are used. However, a wide FOV is usually preferable to visualize a larger extent of the capillary network. In addition, in SV-OCA, speckle noise causes vasculature to appear discontinuous. Thus, manual selection can inaccurately sample the signal component, necessitating an alternate, consistent method. The method we implemented used a mask to split signal areas from background areas in a selected square region. The steps taken to create the mask are shown in Fig. 3(c)-3(h).

![Fig. 3. (a) Histogram of pixel intensity values from an en face angiogram. (b) SNR as a function of N obtained via histogram fit. (c) OCA region of interest (125 × 125 pixels) used to demonstrate segmentation. (d) Four profiles indicated by numbered ROIs were used to obtain a mean full width half maximum (FWHM) profile for the mask. The segmentation steps include: (e) local adaptive thresholding using a Niblack algorithm, (f) particle filtering, (g) skeletonization, and (h) dilation.]

A square region of 125 × 125 pixels was selected in the en face image and converted to binary via a local adaptive threshold using the Niblack algorithm (Fig. 3(e)). The threshold used a 32 × 32 pixel window and 0.1 and 0.2 deviation factors respectively for 10 × and 5 × objective lenses. Noise was removed with particle filtering (Fig. 3(f)) prior to skeletonization (Fig. 3(g)). Finally, the contiguous segments were dilated to the mean diameter of the region, measured by sampling the diameter of several capillaries (Fig. 3(c)-3(d)). As described by Lee et al [25], cerebral capillaries mean diameter is 7 µm and the variation is relatively small so
that the large majority of them fall within 6-8 μm. Considering that the lateral sampling density in this study was 2 μm/pixel, the assumption that all the capillaries have the same diameter does not produce major sampling error. The signal mask was inverted to map the background. Four regions of the same size were analyzed in each en face image. Masks obtained on the sets acquired with N = 100 and the AAD algorithm were applied to those obtained with other parameters. This yielded more consistent and uniform background suppression and more accurate masks. Registration errors, which could result in SNR errors from inaccurate masking, were minimal.

Once the masks were applied to the raw images, SNR and Wc were computed. The SNR defined above was previously used on phantoms, but has never been applied to SV-OCA angiograms [13]. It was chosen for its ability to distinguish two overlapping statistical populations. In addition, since the capillary coverage estimated with our mask-method was ~25% across all analyzed regions, Weber contrast was preferred for OCA over other metrics because of its accuracy when applied to images with small features on a uniform background.

3. Results

Figure 4 shows the SNR and Weber contrast for both objective lenses and both algorithms for gate lengths from N = 2-100. Each data point is the mean (± SD) of four different regions of the same en face image.

SNR and Weber contrast show similar trends in Fig. 4, in part from the similarity of the measures evident in Eq. (3) and Eq. (4). Significant differences were found between AAD and SD processing algorithms. For AAD (black lines) a pronounced increase takes place for 5 × and 10 × lenses up to N = 10 (16% SNR increase and 35% contrast increase for N = 10 with respect to N = 2). Thereafter the values ascend with a lower slope to N = 100. Conversely, the
values for SD (red lines) peak at N = 6 for SNR and at N = 4 for contrast for both lenses (12% SNR and 8% contrast increase with respect to N = 2). Then, they drop to a minimum at N = 25 and plateau afterward. The decrease in SD for medium N values is attributed to animal respiration that increases background noise.

The data acquired with a 5 × objective generally had a lower variance than the 10 × objective data. The overall brightness of the 5 × images was higher, owing to increased collection aperture of this objective, and also possibly due to its longer depth of focus. Window reflection artifacts superimposed on the angiograms could also have increased the brightness of both signal and background. The increased brightness resulted in saturation of some of the capillaries, which slightly compressed the signal range.

In order to evaluate the effect of the beam waist on fine capillary detection, the diameter (FWHM) of the same 25 capillaries acquired at 5 × and 10 × and processed through AAD (N = 100) was measured. Beam waist influences the accuracy of spatial sampling. If the beam waist has a Gaussian profile, and a capillary is approximated by a step reflectivity profile, the appearance of the vessel will be dictated by the convolution of the two functions as the beam scans over the vessel. If the beam waist is significantly smaller than the vessel, the imaged diameter will be accurate. However, if the beam waist is equal to or larger than the vessel, significant broadening can occur. The difference in measured capillary diameter between the 5 × and 10 × objectives was evaluated with a two-tailed Student’s t-test with p < 0.05 (α = 5%). Although both lenses resolved fine capillaries, the measured diameter was 17% higher (p = 0.0018) when imaged with the 5 × objective. Figure 5 shows representative images for 5 × (Fig. 5(a)) and 10 × (Fig. 5(b)) lenses. Figure 5(c) shows a capillary profile from those ROIs. In addition the capillaries were separated into two groups by the system lateral resolution for the 5 × objective. The measured diameter of capillaries narrower than 6.5 µm was 24% higher (p = 0.0001) for the 5 × objective. The measured diameter of capillaries wider than 6.5 µm was 14% higher (p = 0.0399) for the 5 × objective.

![Fig. 5. Profiles of the same capillary in data sets acquired using different objective lenses.](image)

(a) Example ROI for 5 × (a) and 10 × (b) data sets. (c) Profile of the capillary indicated in (a) and (b), continuous line (5 × ) and dashed line (10 × ). Symbols indicate FWHM values. (d) Bar chart showing mean diameter (error bars = ± SD) in capillaries grouped by diameter size. Two groups were separated by the lateral resolution using 5 × objective (6.5 µm). Statistical significance is shown for each group.
4. Discussion

In this paper we assessed OCA image quality with respect to processing algorithm, gate length, and system optics for angiograms collected from mouse primary motor cortex. Evaluation included the possible sources of experimental noise in the setup. The comparison used signal-to-background or signal-to-noise (SNR) and Weber contrast image quality metrics and may guide study designs for OCA applications.

We observed differences in SNR and contrast when processing the same data sets with two different algorithms [6, 8, 9, 13]. The difference was attributed to added noise from mouse respiration motion. When the total acquisition time for N B-scans at a given lateral position is comparable or higher than the animal respiration rate (100-120 breaths/min), axial and transverse shifts between the acquired B-scans in the set can occur, even for very stable setups. Pixel-to-pixel shifts in the cross-sectional B-scans cause additional noise that leads to artifactual bright lines across the MIP en face image.

Figure 6 shows the acquisition time as function of gate length. The total acquisition time is split into scan time (dark gray) and GPU processing time (light gray). The range of respiration typical of an anesthetized mouse (100-120 breaths per minute, or 0.5-0.6 pulses per second) is shown in blue dashed lines. When the respiration rate is close to the acquisition time, SNR and contrast for the SD algorithm decreases while AAD appears unaffected. As an example, Figs. 2(g) and 2(h) show artifactual horizontal lines for the sets acquired with N = 6 and N = 10 and processed with the SD algorithm. As N increases above 25, the number of frames unaffected by motion exceeds those affected by motion and the horizontal lines become less visible (Figs. 2(i)-2(l)). However, the noise is still present. The AAD algorithm is superior in terms of SNR and contrast because the angiogram is calculated using adjacent B-scans with a short interval (~7.5 ms in this study), whereas the SD algorithm angiogram is calculated using a mean value over a longer interval, which includes contributions from additional noise sources (Eq. (1) and Eq. (2)) such as respiration. As expected, no differences were observed in SNR and contrast when comparing data acquired using 5 × and 10 × lenses.

It is not unexpected that SNR and contrast asymptotically increase with N. Mariampillai et al. compared speckle variance SNR to structural SNR for several gate lengths in intralipid and silicone gel phantoms and found similar results [13]. The degree to which SNR and contrast plateau, however, is unexpected in that it occurs to a significantly greater extent than would be expected for simple averaging. In the case of simple frame averaging, a $\sqrt{N}$ dependency is expected and the results in Fig. 4 are several times below that for high N. The lower SNR and contrast for higher N is most likely due to additional noise sources like respiration as described in the previous paragraphs. The choice of gate length depends on the application, and involves consideration of scan time, FOV, lateral pixel density, memory and disk allocation, and real-time processing and display requirements. In particular, for applications in which bulk tissue motion is high (e.g., ophthalmology), implementation of longer gate lengths is impossible without high precision eye tracking [14]. If flow velocimetry via the autocorrelation function is necessary [8], then longer gate lengths are required. Our results indicate that for structural angiography, N = 8 produces acceptable results: significantly better than N = 2 and not appreciably worse than N = 100. For flow velocimetry, longer gate lengths than N = 8 are required, to capture the flow profile, but produce only minor SNR gain in the angiograms.
Fig. 6. Acquisition time as function of the gate length. The acquisition time is split into scan time (dark grey) and GPU processing time (light grey). They account for the total time needed to obtain a processed B-scan. Two dashed blue lines delineate the approximate range of mouse respiration (100-120 breaths per minute).

Recently, the finest capillaries have been resolved with OCT. Accurate quantification of vessel diameter depends on the degree of lateral resolution that the imaging system can achieve. We evaluated the effect of beam waist on fine capillary quantitation. In this study, we tested two objective lenses with magnification of 5 × and 10 × [7]. They were both able to visualize the fine capillary network (Fig. 5(a)-5(b)). However, the measured vessel diameter when the 5 × objective lens was used was significantly higher than when the 10 × objective lens was used, even for vessels slightly larger than the measured lateral resolution of the 5 × objective. This fact should be heeded when designing studies to quantify capillaries. An additional consideration is the DOF. When the target depth range is wider than the DOF, some trade-offs between the lateral resolution and the total acquisition time (to acquire multiple focal planes) are necessary.

In conclusion, we devised a novel mask-based segmentation method to selectively analyze signal and background in OCA en face planes. Through this method, we demonstrated that SNR and Wc are valuable metrics to evaluate image quality in OCA and that AAD is superior to SD at any given N. In particular, the AAD algorithm achieves higher suppression of bulk tissue motion noise. Moreover, the use of very high N may not be justified in terms of SNR and Wc if only morphological qualitative angiograms are needed. Standard techniques to assess image quality will serve as a guide in the correct choice of parameters for other angiography imaging methods as well, such as adaptive optics scanning laser ophthalmoscopy or wide-field laser speckle imaging.

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