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Projection artifact removal improves visualization and quantitation of macular neovascularization imaged by optical coherence tomography angiography

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Abstract

Purpose—To visualize and quantify the size and vessel density of macular neovascularization (MNV) using optical coherence tomography angiography (OCTA) with a projection artifact removal algorithm.

Design—Multicenter, observational study.

Participants—Subjects with MNV in at least one eye.

Methods—Patients were imaged using either a swept-source OCT angiography (SS-OCTA) prototype system or a spectral-domain OCT angiography (SD-OCTA) prototype system. The optical microangiography (OMAG) algorithm was used to generate the OCTA images. Projection artifacts from the overlying retinal circulation were removed from the OMAG OCTA images using a novel algorithm. Following removal of the projection artifacts from the OCTA images, we assessed the size and vascularity of the MNV. Concurrent fluorescein angiography (FA) and

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indocyanine green angiography (ICGA) images were used to validate the artifact-free OMAG images whenever available.

Main Outcome Measures—Size and vascularity of MNV imaged with OCTA before and after the use of a projection-artifact removal algorithm.

Results—A total of 30 subjects (40 eyes) diagnosed with MNV were imaged. Five patients were imaged before and after intravitreal injections of vascular endothelial growth factor (VEGF) inhibitors. Following the use of the projection artifact removal algorithm, we found improved visualization of the MNV. Lesion sizes and vascular densities were more easily measured on all the artifact-free OMAG images. In eyes treated with vascular endothelial growth factor inhibitors, vascular density was reduced in all five eyes after treatment, and in four eyes, the size of the MNV decreased. One of five patients showed a slight increase in lesion size, but a decrease in vascular density.

Conclusions—OCTA imaging of MNV using the OMAG algorithm combined with removal of projection artifacts resulted in improved visualization and measurements of the neovascular lesions. OMAG with projection artifact removal should be useful for assessing the response of MNV to treatment using OCTA imaging.

Keywords

Optical coherence tomography angiography; choroidal neovascularization; projection artifacts

INTRODUCTION

Macular neovascularization (MNV) is associated with several major retinal diseases such as age-related macular degeneration (AMD), high myopia, and central serous chorioretinopathy (CSCR).^[1–3] Without prompt treatment, the exudation, hemorrhage, and fibrosis arising from MNV cause irreversible damage to photoreceptors, which ultimately results in the loss of central vision.^[4–6] To prevent the progression of disease and permanent vision loss, intravitreal injections of drugs that inhibit vascular endothelial growth factor (VEGF) are recommended.

The current imaging strategies for diagnosing and characterizing MNV include fluorescein angiography (FA) and optical coherence tomography (OCT) imaging. While routine OCT provides cross sectional information about macular anatomy and can document the accumulation of macular fluid and hyperreflective signals associated with MNV, FA has been the preferred imaging strategy to visualize the size, location, and diagnostic features of MNV that have been shown to be predictive of disease severity. However, FA is an invasive procedure that requires the intravenous injection of dye, and while the risk of a life-threatening anaphylactic reaction to the dye is small, FA is also time consuming, expensive, and uncomfortable for the patient.

With the advent of OCT angiography (OCTA) ^[7–15], the diagnosis and monitoring of retinal and choroidal vascular diseases within the macula can now be performed noninvasively, safely, rapidly, and more comfortably for the patient. Moreover, OCTA provides invaluable depth-resolved information, which is especially useful for the evaluation of MNV. ^[16–22]

However, it has been reported that OCTA images of structures deep to the retinal vasculature contain projection artifacts from the overlying superficial retinal vessels that create the appearance of “false blood vessels” on OCTA images of these deep macular layers. [23,24] These artifacts are particularly troublesome when the region being imaged includes a highly reflective surface such as the retinal pigment epithelium (RPE). Since MNV is always in close approximation to the RPE, these unwanted projection artifacts complicate the interpretation of MNV. Not only is it difficult to visualize the MNV in the presence of the projection artifacts, but it is also difficult to measure these lesions and determine whether the MNV has changed in response to therapy.

To mitigate the problem of retinal vessel projection artifacts, Liu et al. [25] proposed a post-image processing approach to minimize the impact of these artifacts. This approach used the pattern of superficial retinal vessels to suppress the projection artifacts on the outer retinal images, and a saliency model was used to recognize the vascular pattern of the MNV. While this approach was able to delineate the MNV and provide measurements for the MNV, the saliency model is dependent on the shape and vascularity of the MNV, which makes automatic assessment difficult. Moreover, the approach did not eliminate the projection artifacts that were present within the MNV, which is not ideal for the evaluation of the vascular complexity associated with MNV. In parallel, Zhang *et al.* [26] proposed another approach in which the overlying retinal microvasculature and the OCT structural information were included in the algorithm for artifact removal, and this algorithm was shown to be effective in removing all the projection artifacts from images of MNV.

To demonstrate the utility of an artifact-removal algorithm when using OCTA imaging for the visualization and measurement of MNV, we report on the use of our artifact-removal algorithm in eyes with MNV and document the quantitative response of MNV to anti-VEGF therapy.

METHODS

Eyes diagnosed with MNV at the Bascom Palmer Eye Institute, Miami, Florida, and the University of Washington Eye Institute, Seattle were enrolled in prospective OCT imaging studies. The Institutional Review Board of each institution approved the study, and patients signed an informed consent in order to participate. The study was performed in accordance with the tenets of the Declaration of Helsinki and compliant with the Health Insurance Portability and Accountability Act of 1996.

Eyes enrolled in this study had the clinical diagnosis of MNV based on history, fundus biomicroscopy, and routine OCT imaging. Color fundus imaging, fundus autofluorescence imaging, FA imaging, and indocyanine green angiography (ICGA) imaging were performed at the discretion of the physician. All eyes treated with anti-VEGF therapy had OCTA imaging on the day of treatment, and subsequent imaging was performed at all follow-up visits. Patients with any macular disease that would confound the interpretation of the OCTA image, such as moderate to severe diabetic retinopathy, or result in a poor quality OCTA image due to a weak signal (signal strength <6), such as a visually significant cataract, were excluded from the study. Scans with a signal strength below six were excluded because this

range was considered the lower boundary of signal strength for reliable imaging, especially in clinical studies, and is a cut-off recommended by manufacturers. All other images were normalized against a signal strength score of 9 in order to minimize the signal strength variation between scans. All eyes were scanned at least once, and patients undergoing anti-VEGF therapy were scanned at follow-up visits.

At the Bascom Palmer Eye Institute, OCTA imaging was performed using a swept-source OCTA (SS-OCTA) prototype system (Carl Zeiss Meditec Inc. Dublin, CA), while at the University of Washington Eye Institute, OCTA imaging was performed using a spectral-domain OCT angiography (SD-OCTA) prototype system (Carl Zeiss Meditec Inc. Dublin, CA).

The SS-OCTA system operated at a central wavelength of 1,050 nm with a scanning speed of 100,000 A-scans per second.^[8,26,27] The swept light source provided a full width at half maximum (FWHM) axial resolution of $\sim 5\ \mu\text{m}$ in tissue and an estimated lateral resolution of $\sim 15\ \mu\text{m}$ at the retinal surface. The OCTA scan was centered on the fovea and measured $3\text{mm} \times 3\text{mm}$ on the retina. In the fast, transverse, x-axis scanning direction, 300 A-lines were used to form one single B-scan. Four consecutive B-scans were performed at each fixed location before proceeding to the next transverse location for further B-scanning. In the slow, vertical, y-axis scanning direction, there were 300 positions over a 3-mm distance.

The SD-OCTA prototype operated at a central wavelength of 840 nm with a speed of 68,000 A-scans per second. The axial and lateral resolutions were approximately the same as those given in SS-OCTA system. In addition, the SD-OCTA prototype was equipped with motion tracking capability, enabling almost motion-free and wide-field imaging through montaging.^[28] In this study, a montage scanning protocol was performed. In each cube scan, a total of 980 B-scans were performed with each B-scan comprised of 245 A-lines and each B-scan repeated four times at each location along slow axis, covering a $2.4\text{ mm} \times 2.4\text{ mm}$ area. With 10% overlap between adjacent cubes, the total area covered by each montaging scan was $6.7\text{ mm} \times 6.7\text{ mm}$.

The OCTA images were obtained by applying the optical microangiography (OMAG) algorithm to the acquired volumetric datasets in order to extract retinal microvascular flow information.^[7,8,30–32] *En face* angiograms were generated using a maximum intensity projection for each A-scan position. To enhance microvascular visualization, a semi-automated retinal layer segmentation algorithm^[33] was used to separate the retina and choroid into different layers. To identify the MNV, particular attention was paid to a slab with an inner boundary within the outer retinal avascular space (ORAS), the space between the outer nuclear layer and the Bruch's membrane, and an outer boundary of the slab included the inner portion of the choriocapillaris. The slab from the ORAS to the inner portion of the choriocapillaris was designated the outer retina to choriocapillaris (ORCC) slab.

To remove the retinal vessel projection artifacts from the OCTA images of MNV, we developed an artifact-removal algorithm.^[26] The first step in the artifact removal process was to generate an *en face* OMAG OCTA flow image between the outer retina and the

choroid (ORCC slab), which also contained the projection artifacts. A second en face OMAG flow image was then generated from a slab that extended from the inner limiting membrane (ILM) to the boundary between the outer plexiform layer (OPL) and outer nuclear layer. This image was then normalized and inversed. This resulted in a retinal OMAG image that carried the flow information contained within the projection artifacts that were then superimposed onto the image of the MNV. Then, a third en face OCT structural image was generated from the same slab used to visualize the MNV. This image was then normalized and inversed. The final flow image was then obtained by the multiplication of the above three images, resulting in an OCTA image of MNV with minimal projection artifacts. Two masked retinal specialists (CSL, PJR) compared the original and the artifact-free OCTA image and identified the OCTA image with sharper borders and improved visualization of the vascular network, which allowed for quantitative assessment of the MNV. Comparisons with FA and ICGA images were performed when available.

In this study, the quantitative metrics used for assessing MNV and their response in anti-VEGF therapy included changes in the size and vessel density of the MNV. The vessel density was calculated as the ratio of the area occupied by the vessels within the MNV to the overall size of the MNV based on a previously described method.^[34] The contour of the MNV was manually delineated on each image and the size was calculated by the area bounded by the contour line. The observed change in this parameter provided an indication of vessel remodeling within the MNV in response to treatment.

RESULTS

A total of 30 patients were recruited and 40 eyes were diagnosed with MNV at the Bascom Palmer Eye Institute and the University of Washington Eye Institute between September, 2013 and December, 2015. All the neovascular lesions had a prominent type 1 component under the RPE, but careful phenotyping of the neovascular complexes was not performed. Except one, all the 39 eyes of the 30 patients were treatment naïve. Five eyes of five patients received anti-VEGF therapy (with one eye received four injections before the start of this study) and were followed repeatedly with OCTA imaging. Three patients were imaged using SS-OCTA, and two were imaged with SD-OCTA. All five patients were diagnosed with exudative neovascular AMD (Table 1). Follow-up OMAG images were acquired 1 to 9 months after injections. Below, we present the OMAG results in some detail from the 5 eyes that had follow-up visits and were scanned using SS-OCTA and SD-OCTA imaging.

Swept-Source OCT Angiography

Case #1—A 77-year-old man with AMD complained of decreased vision and metamorphopsia in his left eye. Best-corrected visual acuity (BCVA) was 20/30. After examination, FA and ICGA were performed, and MNV was diagnosed (Figure 1 A–D). Early and late transit frames from FA images (Figure 1 A and B) showed hyperfluorescence with late leakage within the central macula. ICGA images (Figure 1 C and D) confirmed the presence of a plaque within the central macula.

The cross-sectional SS-OCT intensity image revealed an irregular detachment of the RPE associated with sub-retinal fluid (Figure 2 A). For easier identification of the MNV, the

OMAG flow signals were overlaid on the intensity B-scan image with colors demonstrating the new vessels (pink) within the RPE detachment. Furthermore, en face OMAG images from the ORCC slab (Figure 2 B, C and D) reveal the MNV clearly within the central macula. In Figure 2B, the retinal projection artifacts surround the MNV. After removal of the projection artifacts^[26] (Figure 2C), the neovascular lesion is well demarcated, which facilitates the measurement of the MNV. An intravitreal injection of bevacizumab was performed on this visit.

Seven weeks later, the patient's BCVA improved to 20/25 and the follow-up OMAG images were acquired. The B-scan OCT image (Figure 2 E) showed a decrease in the amount of sub-retinal fluid; however, the RPE detachment persisted. The *en face* OMAG images (Figure 2 F, G and H) showed a decrease in the size of the MNV and its vascular density demonstrating that the removal of projection artifacts from OMAG images result in easier identification, localization, and quantification of MNV and its response to the treatment.

Case #2—A 69-year-old woman with neovascular AMD had a BCVA of 20/20 while undergoing anti-VEGF therapy in her left eye. The patient received 4 injections of intravitreal bevacizumab previously. At the time of the 5th injection, SS-OCTA imaging was initiated and obtained at each visit thereafter. Over a nine-month period, four injections were performed. Prior to first of her injections, FA imaging showed a diffuse area of early stippled hyperfluorescence with late leakage in superior macula (Figure 3 A, B). ICGA showed a central hyperfluorescent plaque late in the study (Figure 3 C, D). Corresponding OMAG flow images were acquired on the day of the FA and ICGA and at the next visit after the treatment (Figure 4). The routine intensity-based OCT B-scan images showed low-lying, subfoveal detachment of the RPE before and after the injection (Figure 4A, E). OMAG flow images before artifact removal (Figure 4 B, F) identified the MNV before and after the injection, but these images contained projection artifacts from the overlying retinal vessels and residual motion artifacts (horizontal white lines). The MNV was observed in greater detail once the projection artifacts were removed (Figure 4C, D, G, H) and greater vascular detail could be appreciated when compared with the FA and ICGA images (Figure 3).

Case #3—An 81-year-old man presented with blurred vision in the right eye and was diagnosed with MNV. BCVA was 20/100 in the right eye. Early and late transit frames of the FA revealed evidence of stippled hyperfluorescence with staining and late leakage (Figure 5 A and B). ICGA images (Figure 5 C and D) showed mild hyperfluorescence but a central plaque was not identified.

On SS-OCTA imaging, the typical cross-sectional OCT B-scan images showed intraretinal fluid and an irregular elevation of the RPE (Figure 6 A). Flow was detected within the RPE elevation and was presumed to represent neovascularization (Figure 6A). The *en face* OMAG images revealed a multi-lobular neovascular complex (Figure 6 B, C and D) that corresponded to the central areas of hyperfluorescence observed on the FA and ICGA images. After three intravitreal injections of aflibercept, the macular fluid resolved, but a low-lying elevation of the RPE remained (Figure 6E). The *en face* OMAG images demonstrated a decrease in the area of flow with a decrease in the vascular complexity of the remaining neovascularization (Figure 6 F, G, and H).

Spectral-Domain OCT Angiography

Case #4—A 51 year-old woman with neovascular AMD underwent an intravitreal injection of bevacizumab. Prior to injection, her BCVA was 20/70 in the right eye. Widefield SD-OCTA imaging ($6.7 \times 6.7 \text{ mm}^2$) was performed. Prior to injection, the early and late FA images (Figure 7 A, B) revealed a central area of mottled hyperfluorescence with late leakage. The OMAG image of the overlying retina depicts the microvasculature in far greater detail than can be appreciated from the FA images, and the retinal microvasculature appeared normal. (Figure 7 D).

The OCT B-scan before treatment showed an irregular elevation of the RPE associated with sub-retinal fluid and the superimposed color-coded flow signals showed flow within the RPE elevation (Figure 8 A). The OMAG image of the ORCC slab (Figure 8C) demonstrated neovascularization in the central macula, but the borders of MNV were not well demarcated due to the retinal vessel projection artifacts. After the artifact-removal algorithm was applied to the OMAG image, the size of MNV and the vessel caliber were easily observed and could be measured (Figure 8E). Five weeks after the second injection of bevacizumab, OCT B-scans demonstrated a decrease in the subretinal fluid (Figure 8 B) but the repeat widefield SD-OCTA image (Figure 8 D, F) showed a decrease in the vascular complexity of the MNV without an obvious reduction in size.

Case #5—A 74 year-old woman with best BCVA of 20/250 was diagnosed with exudative AMD in her right eye. She declined FA and ICGA due to medical comorbidities. Color fundus imaging showed a large area of subretinal fibrosis with surrounding atrophy (Figure 9A). Widefield *en face* SD-OCTA OMAG imaging did not reveal any obvious abnormalities in retinal vasculature although the motion artifacts due to constant eye movements were apparent (Figure 9B).

A 2.4mm OCT B-scan image of the central macula showed a thickened hyperreflective detachment of the RPE consistent with fibrovascular scar, and the superimposed OMAG color-coded flow signals depicts flow within this layer (Figure 10A). The *en face* OMAG image of the ORCC slab clearly shows the MNV along with the retinal vessel projection artifacts (Figure 10C). When the projection artifacts are removed, the MNV is more clearly demarcated (Figure 10 E). A red arrowhead marks a prominent vessel communicating with the choroidal circulation. After receiving four injections of aflibercept, widefield SD-OCTA imaging was repeated (Figure 10 B, D, E). The projection artifact-free OMAG images (Figure 10 F) show a remodeled neovascular lesion with a slight reduction in the overall size and complexity of the MNV.

Quantitative Analyses

Quantitative assessments were performed with artifact-free OMAG images from the five patients who had follow-up OCTA scans (Table 2). The size of the MNV and the corresponding vessel densities were calculated. The vessel density decreased in all patients after intravitreal anti-VEGF therapy. A reduction in the size of the MNV was shown in four of five patients.

Discussion

En face OMAG imaging with removal of the retinal vessel projection artifacts resulted in improved visualization and quantitation of the MNV. Previous studies have reported the ability to characterize MNV using OCTA imaging [18,20,21,22,28] and demonstrated the ability to measure these neovascular lesions in treatment-naïve patients [16,18,20,35]. However, the projection artifacts from the retinal vasculature prevented clear visualization of the MNV and quantitative analyses were difficult. The ability to quantitate MNV accurately without apparent noise not only is helpful in diagnosing disease, but also in monitoring of disease progression and the response to treatment.

For the five patients that were imaged after treatment in this report, we chose the change in vessel density within the MNV and the size of the neovascular lesion as surrogate markers of treatment response. Interestingly, all patients showed reduction in the density of vessels and the complexity of vascular networks, but the size of the lesions remained relatively stable in these patients. Even when there was no obvious change in the extent of macular fluid on B-scan OCT imaging, the *en face* images revealed a change within the MNV. The vessel density was reduced in all 5 patients while the decrease in area was only observed in 4 patients. Our results suggest that the decrease in the size of the MNV may not be as sensitive as the change in the vessel density when quantifying a treatment response. However, our sample size was too small to infer a benefit of one parameter over the other. Interestingly, even without a change in overall size, vessel remodeling was seen in some cases. We suspect that vessel remodeling may be due to a decrease in the vascular flow and reorganization of vascular networks while undergoing treatment and also suggests that vessel remodeling may indicate recurrence of MNV. While it is not yet known whether the change in vessel density alone would qualify as a treatment endpoint when there was no evidence of reduction in macular fluid, it does provide a measurement of whether the treatment is having any effect. This change in vessel density may be useful as a new clinical endpoint in assessing an early response to treatment and should be validated in larger trials and additional studies are needed to understand how these possible endpoints relate to clinical outcomes. Of note, our ability to identify and quantify the MNV in the remaining 35 eyes were similar to the results shown with the 5 patients in this report.

The development of OCTA imaging is revolutionizing the way we examine and evaluate retinal and choroidal pathologies. With the introduction of this imaging modality, there are several important considerations in interpreting the OCTA. First, accurate segmentation is critical. Poor segmentation of each slab can lead to erroneous comparisons of lesions at sequential visits. If the comparisons are not made at the same depth, one may conclude that the lesion has either grown or decreased over time. Second, quantitative analyses of the lesion rather than qualitative assessment removes biases and allows for objective measurements, which are critical in monitoring disease progression and treatment decisions. Projection artifact-free OCTA images allow for more accurate detection of MNV, which should result in more reliable quantification of the lesions.

We have provided quantitative measurements based on projection artifact-free OCTA (OMAG) images, which could be a potential way to effectively monitor the treatment

response. In addition to the vessel density and the size of the MNV, OCTA allows characterization of the vasculature within the neovascular complex. In several cases, we were able to identify the feeder vessel and surrounding vascular networks within the MNV. Most of the vasculature decreased in size, in particular, the peripheral fine vessels within the MNV. In addition, some of these vessels underwent remodeling rather than a decrease in size. The discrepancies in how different types of MNV respond to treatment may be useful in understanding the prognoses associated with different neovascular lesions and why some lesions undergo tachyphylaxis following treatment.^[36] Whether the size of the MNV or the vessel density of the MNV on initial presentation are associated with a certain prognosis or treatment response has not yet been determined.^[37,38]

Currently, routine OCT structural imaging is the standard-of-care for imaging patients with MNV. However, the advantage of OCTA imaging is that both the structural information and the flow information are available from a single volumetric scan. Moreover, with the development of this reliable artifact-removal algorithm and the ORCC slab, which allows for visualization of both type 1 and type 2 MNV, it becomes possible to visualize these lesions without the time-consuming manipulations of the slab boundaries that are currently required for optimal viewing of the MNV. Moreover, OCTA may provide two valuable benefits not available with routine OCT imaging in the management of MNV. First, as we've previously published^[39], swept source OCTA imaging can identify neovascular complexes before any exudation appears. By using the artifact-removal algorithm, it will become possible to accurately follow changes in the size and vascular complexity of these lesions, which may prove useful in predicting the onset of exudation and future vision loss. In addition, we have anecdotal evidence to suggest that once a lesion is treated with anti-VEGF therapy, the change in the size and complexity of the neovascular lesion may precede the onset of recurrent exudation that will require treatment. Thus, the use of the artifact-removal algorithm may offer evidence to suggest that an extension of a treatment interval is not recommended even though the exudation may be controlled. Indubitably, these uses of OCTA and the optimized artifact removal algorithm in the management of MNV need to be studied, and once the algorithm becomes commercially available, clinicians will be empowered to perform these studies.

There are several limitations of our study. Most of our patients were scanned once and two different OCTA modalities were used in this study. Thus, the repeatability and the comparative data between two imaging systems are not available. However, the projection removal algorithm was beneficial in both spectral domain and swept source OCTA imaging. This type of additional post-imaging processing will likely become essential for imaging MNV. However, further studies are clearly needed to confirm the algorithm's reliability, repeatability, and applicability for all types of MNV. Moreover, our follow-up study had a small sample size and short follow-up period. In addition, all quantitative assessments were based on two dimensional *en face* OCTA images rather than three-dimensional images. Since OCTA imaging captures a volumetric dataset, three-dimensional analyses could be applied in the future to more precisely identify the areas of interest. Additional studies with larger sample sizes are needed in which treated patients are followed over their entire treatment course to better understand how improved visualization and quantitation of MNV affects patient care and outcomes.

In summary, both SS-OCTA and SD-OCTA imaging provided accurate and clear identification of MNV in comparison to the current gold standard of FA. The application of projection artifact removal algorithm on the OCTA images of the ORCC slab resulted in improved visualization and quantitation of MNV. Although more studies are needed, these findings suggest that OCTA-based metrics may be useful in future clinical trials for the quantitative assessment of treatment responses to anti-VEGF therapy in patients with MNV. Moreover, the use of the OCTA imaging and artifact removal may improve individualized treatment regimens and improve outcomes in the routine clinical care of patients with MNV. Additional prospective studies with larger sample sizes are needed to better understand and validate the OCTA-based metrics in patients with MNV.

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Figure 1.

Fluorescein and indocyanine green angiographic images of a 77 year-old man diagnosed with macular neovascularization in the left eye. A: An early transit frame from the fluorescein angiography (FA); B: Late transit frame from the FA showing stippled hyperfluorescence and leakage; C: Early transit frame from the indocyanine green angiography (ICGA); D: Late transit frame from the ICGA showing a hyperfluorescent plaque within the central macula.

Figure 2.

Optical microangiography (OMAG) results from a 77 year-old man diagnosed with macular neovascularization (MNV) in the left eye before and after an intravitreal injection of bevacizumab. **A** and **E**: Cross-sectional optical coherence tomography (OCT) B-scan images with color-coded cross-sectional flow signals superimposed. **B** and **F**: *En face* OMAG flow images of the outer retinal to choriocapillaris (ORCC) slab with retinal vessel projection artifacts before (**B**) and after (**F**) intravitreal bevacizumab. **C** and **G**: *En face* OMAG flow images of the ORCC slab with removal of the projection artifacts before (**C**) and after (**G**) intravitreal bevacizumab. **D** and **H**: *En face* color-coded OMAG flow images of the outer retina to choroid (ORC) slab before (**D**) and after (**H**) intravitreal bevacizumab. The colors shown in panels **A**, **D**, **E**, and **H** are coded as follows: red represents superficial retinal layer; green represents deep retinal layer; pink represents the ORCC slab; red represents the remaining choriocapillaris; green represents the remaining choroidal layer. The size of the OMAG images is 3mm × 3mm. The patient was imaged by SS-OCTA.

Figure 3.

Fluorescein and indocyanine green angiographic images of a 69 year-old woman diagnosed with macular neovascularization in the left eye before and after treatment with intravitreal aflibercept. A: Late transit frame from the fluorescein angiography (FA) showing stippled hyperfluorescence and late leakage superiorly; B: Late transit frame from the indocyanine green angiography (ICGA) showing a central hyperfluorescent plaque C After treatment, late transit frame from the FA showing stippled hyperfluorescence and decreased leakage superiorly; D: After treatment, late transit frame from the ICGA showing a smaller central hyperfluorescent plaque.

Figure 4.

Optical microangiography (OMAG) results from a 69 year-old woman diagnosed with macular neovascularization (MNV) in the left eye before and after an intravitreal injection of aflibercept. **A** and **E**: Cross-sectional optical coherence tomography (OCT) B-scan images with color-coded cross-sectional flow signals superimposed. **B** and **F**: *En face* OMAG flow images of the outer retina to choriocapillaris (ORCC) slab with retinal vessel projection artifacts before (B) and after (F) intravitreal aflibercept. **C** and **G**: *En face* OMAG flow images of the ORCC slab with removal of the projection artifacts before (C) and after (G) intravitreal aflibercept. **D** and **H**: *En face* color-coded OMAG flow images of the outer retina to choroid (ORC) slab before (D) and after (H) intravitreal aflibercept. The colors shown in panels A, D, E, and H are coded as follows: red represents superficial retinal layer; green represents deep retinal layer; pink represents the ORCC slab; red represents the remaining choriocapillaris; green represents the remaining choroidal layer. The size of the OMAG images is 3mm × 3mm. The patient was imaged by SS-OCTA.

Figure 5. Fluorescein and indocyanine green angiographic images of an 81 year-old man diagnosed with macular neovascularization in the right eye. A: An early transit frame from the fluorescein angiography (FA); B: Late transit frame from the FA showing stippled hyperfluorescence and leakage; C: Early transit frame from the indocyanine green angiography (ICGA); D: Late transit frame from the ICGA showing multi-focal areas of hyperfluorescence in the central macula but no continuous plaque.

Figure 6.

Optical microangiography (OMAG) results from a 81 year-old man diagnosed with macular neovascularization (MNV) in the right eye before and after an intravitreal injection of aflibercept. **A** and **E**: Cross-sectional optical coherence tomography (OCT) B-scan images with color-coded cross-sectional flow signals superimposed. **B** and **F**: *En face* OMAG flow images of the outer retina to choriocapillaris (ORCC) slab with retinal vessel projection artifacts before (B) and after (F) intravitreal aflibercept. **C** and **G**: *En face* OMAG flow images of the ORCC slab with removal of the projection artifacts before (C) and after (G) intravitreal aflibercept. **D** and **H**: *En face* color-coded OMAG flow images of the outer retina to choroid (ORC) slab before (D) and after (H) intravitreal aflibercept. The colors shown in panels A, D, E, and H are coded as follows: red represents superficial retinal layer; green represents deep retinal layer; pink represents the ORCC slab; red represents the remaining choriocapillaris; green represents the remaining choroidal layer. The size of the OMAG images is 3mm × 3mm. The patient was imaged by SS-OCTA.

Figure 7.

Fluorescein angiography (FA) and widefield optical microangiography (OMAG) flow results of a 51 year-old woman diagnosed with macular neovascularization (MNV) in the right eye.

A: An early transit frame from the FA showing mottled hyperfluorescence; B: Late transit frame from the FA showing leakage; C: Magnified early transit frame from the FA representing the same area imaged with OMAG; D: Widefield OMAG image of the total retina covering an area of 6.7mm × 6.7mm. Red represents superficial retinal layer; green represents deep retinal layer. The MNV is not observed in the widefield OMAG image of the retina. The patient was imaged by SD-OCTA.

Figure 8.

Widefield optical microangiography (OMAG) results from a 51 year-old woman diagnosed with macular neovascularization (MNV) in the right eye before and after an intravitreal injection of bevacizumab. **A and B:** Cross-sectional optical coherence tomography (OCT) B-scan images with color-coded cross-sectional flow signals superimposed before (A) and after (B) bevacizumab. **C and D:** Widefield *en face* OMAG images of the ORCC slabs with the retinal vessels projection artifacts before (C) and after (D) treatment. **E and F:** Widefield *en face* OMAG images of the ORCC slabs with removal of the projection artifacts before (E) and after (F) treatment. The colors shown in panels A and B are coded as follows: red represents superficial retinal layer; green represents deep retinal layer; pink represents the

ORCC slab; red represents the remaining choriocapillaris; green represents the remaining choroidal layer. The size of widefield OMAG image is 6.7×6.7 mm. The patient was imaged by SD-OCTA.

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Figure 9.

Color fundus imaging and widefield optical microangiography (OMAG) flow results of a 74 year-old woman diagnosed with macular neovascularization (MNV) in the right eye. A: A color fundus image showing a central fibrotic scar with surrounding atrophy. The white square represents the area depicted in the widefield OMAG image in B. B: Widefield OMAG image of the total retina covering an area of $6.7\text{mm} \times 6.7\text{mm}$. Red represents superficial retinal layer; green represents deep retinal layer. The MNV is not observed in this widefield OMAG image of the retina. The patient was imaged by SD-OCTA.

Figure 10.

Widefield optical microangiography (OMAG) flow results from a 74 year-old woman diagnosed with macular neovascularization (MNV) in the right eye before and after four intravitreal injections of aflibercept. **A and B:** Cross-sectional optical coherence tomography (OCT) B-scan images with color-coded cross-sectional flow signals superimposed before (A) and after (B) the aflibercept injections. **C and D:** Widefield en face OMAG images of the ORCC slabs with the retinal vessel projection artifacts before (C) and after (D) aflibercept therapy. **E and F:** Widefield en face OMAG images of the ORCC slabs with removal of the projection artifacts before (E) and after (F) treatment.. The colors shown in

panels A and B are coded as follows: red represents superficial retinal layer; green represents deep retinal layer; pink represents the ORCC slab; red represents the remaining choriocapillaris; green represents the remaining choroidal layer. The size of widefield OMAG image is 6.7×6.7 mm. The patient was imaged by SD-OCTA.

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Table 1
Demographics and clinical details of the patients who underwent treatment with vascular endothelial growth factor inhibitors.

Patient No.	Age (yrs)	Gender	Race	Diagnosis	number of injections	Pre-treatment Visual acuity	Post-treatment Visual acuity	Number of follow-up visits	Duration of follow-up [*]
#1	77	Male	Caucasian	Exudative Neovascular AMD	1	20/30	20/25	2	7 weeks
#2 ^{**}	69	Female	Hispanic	Exudative Neovascular AMD	8	20/20	20/20	4	6 months
#3	81	Male	Caucasian	Exudative Neovascular AMD	3	20/40	20/40	3	2 months
#4	51	Female	Caucasian	Exudative Neovascular AMD	2	20/70	20/40	2	5 weeks
#5	74	Female	Caucasian	Exudative Neovascular AMD	3	20/250	20/125	2	3 months
Mean age	70.4±11.7 yrs								

^{*} Duration of follow up is the time from the first to the last OCTA imaging.
^{**} This patient received 4 injections before this study. OCTA imaging was performed at the last 4 injections

Table 2

The measurement between before and after treatment

Patient No.	Quantification	Pre-treatment	Post-treatment	Reduction
#1	Area/Length	1.26 mm ²	0.98 mm ²	22.2 %
	VD [*] (%)	7.4	3.8	48.7 %
#2	Area/Length	2.03 mm ²	1.93 mm ²	4.9 %
	VD [*] (%)	6.6	5.9	10.6 %
#3	Area/Length	1.42 mm ²	1.28 mm ²	9.9 %
	VD [*] (%)	6.6	5.5	16.7 %
#4	Area/Length	2.28 mm ²	2.24 mm ²	1.8 %
	VD [*] (%)	1.5	1.3	13.3%
#5	Area/Length	3.86 mm ²	3.91 mm ²	-1.3 %
	VD [*] (%)	3.5	3.2	8.6 %

^{*} VD: vessel density