OCT Angiography in Healthy Human Subjects

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BACKGROUND AND OBJECTIVE: To noninvasively evaluate the retinal microvasculature in healthy human subjects with optical coherence tomography angiography (OCTA).

PATIENTS AND METHODS: Cross-sectional, observational study of five healthy subjects. OCTA was performed on 3 × 3 mm² sections centered on the fovea, nasal macula, and temporal macula. Retinal vasculature was assessed within three horizontal slabs consisting of the inner, middle, and outer retina. The vasculature within each retinal slab was reconstructed using phase-based and intensity contrast-based algorithms and visualized as separate en face images.

RESULTS: OCTA in healthy subjects demonstrates capillary networks consistent with previous histological studies. No retinal vessels were found in the outer retina. OCT angiography of the inner and middle retinal layers showed region-specific vascular patterns that consistently corroborated qualitative findings from past histological studies.

CONCLUSION: OCTA generates high-resolution, noninvasive angiograms qualitatively similar to conventional fluorescein angiography. OCTA may serve as a bridge to assess some features of the retinal microvasculature between conventionally performed angiograms.


INTRODUCTION

The human retina is one of the most metabolically active tissues in the body and has one of the highest rates of oxygen consumption. The retinal vasculature supports the metabolic demands of the retina and is critical for visual function. Methods that have been used to evaluate retinal vasculature include histology, immunohistochemistry, fluorescein angiography (FA), and indocyanine green angiography (ICGA). The latter two, especially FA, are the gold standard for clinical vascular imaging because they provide excellent resolution of the microvascular anatomy and can help detect vascular abnormalities and neovascularization in cases in which the dye leaks out of incompetent vessels. ICGA is used because its longer wavelength light can penetrate the retinal pigment epithelium (RPE), unlike FA, thus enabling better visualization of the choroidal vasculature. While these two methods provide good imaging of the microcirculation, they are not without drawbacks. The injection of fluorescein causes adverse effects in up to 5% of patients. The most frequently reported adverse effects include nausea (2.9%) and vomiting (1.2%), but rare and severe events such as anaphylaxis (0.083%) can cause death.

The development of optical coherence tomography (OCT) revolutionized ophthalmology by providing a rapid, simple, and noninvasive method to assess retinal structure at the microscopic level. Since its first in vivo use in 1993, OCT has evolved significantly in speed, resolution, and imaging depth, especially with the advent of Fourier-domain OCT. These significant jumps in imaging capability have opened the
potential for OCT to develop new functional capabilities such as Doppler OCT (D-OCT) and OCT angiography (OCTA). D-OCT and its derivatives detect the Doppler shift of moving blood cells to provide information related to vascular structure and blood flow; however, it is complicated by a dependence on the axial flow of blood, poor flow sensitivity, and Doppler angle ambiguity. While techniques using multiple probe beams have been developed to help overcome these challenges, these approaches add to system complexity. More importantly, D-OCT is not applicable at all retinal locations, especially those areas of the tissue capillary networks within the retina.

OCTA overcomes some of these limitations by using variations in the intensity and/or phase properties of the OCT signal that result from movement of blood over multiple B-scans. These decorrelative measurement techniques are independent of flow orientation and have a larger dynamic range of flow velocities, allowing for improved visualization of capillary beds compared to D-OCT. Swept-source OCT (SS-OCT) systems that use longer wavelength ranges (usually around 1,050 nm) and faster scanning speeds offer even more improvements in tissue penetration, signal-to-noise ratio, and spatial resolution. Therefore the SS-OCT system provides an ideal platform for OCTA implementation. The goal of this study is to evaluate the feasibility of noninvasive retinal angiography using a prototype SS-OCT and a combination of phase and intensity-based motion contrast techniques. We compare our results with previous histological reports of normative retinal microvascular anatomy in the literature to assess the feasibility of this method.

**PATIENTS AND METHODS**

Data was acquired using a Cirrus (Carl Zeiss Meditec, Dublin, CA) prototype modified with a swept-source laser system with a central wavelength of 1,060 nm and a scan speed of 100,000 A-scans per second. Five healthy subjects with no prior ophtalmologic or medical history were recruited. A total of nine eyes were imaged. The study was approved by the institutional review board of the University of Southern California, and a signed informed consent was obtained from each subject prior to examination. Study participants were instructed to focus on a fixation target. At least three 3 × 3 mm² scans were taken, centered on the fovea, nasal macula, and far temporal macula. Retinal vasculature was assessed within three horizontal retinal slabs consisting of the inner retina (retinal nerve fiber layer, ganglion cell layer, and superficial inner plexiform layer), middle retina (deep inner plexiform layer, inner nuclear layer, outer plexiform layer, and superficial outer nuclear layer), and outer retina (deep outer nuclear layer to the external limiting membrane). Figure 1 illustrates the scan locations and segmentation scheme. The vasculature within each retinal slab was reconstructed using phase-based and intensity contrast-based algorithms and visualized as separate en face images, as illustrated in Figure 2 (page 512).

Post-processed en face OCT angiograms were analyzed using ImageJ (NIH, Bethesda, MD) to quantify the density of retinal microvasculature. This was per-
formed on each en face slab using the “Auto Local Threshold” plug-in of ImageJ (Landini G., v1.5) to delineate retinal vasculature from background noise. Local thresholding was performed using the “mean method” with a radius of 10 and a C value of –10. Vessel density was calculated as a percentage of the sampled area that was occupied by vessels detected by the thresholding parameters. Statistical analysis was performed using the Student’s t test or the Tukey-Kramer test for multiple comparisons. A P value of less than .05 was accepted as significant. The software JMP Pro (version 11; SAS Inc., Cary, NC) was used for all statistical analysis.

**RESULTS**

Figure 1 (page 511) shows an example of the regions of the retina that underwent OCTA as well as a sample segmentation scheme of the retina. A total of five healthy participants without any history of ocular disorders (other than refractive error) were imaged in this fashion. Figures 2 and 3 show representative OCT angiograms and the results of the ImageJ thresholding algorithm that was applied for quantification of retinal vessel density within each retinal slab. In no cases were retinal vessels visualized in the deep retinal layers, so only results from the inner and middle retinal layers are discussed below. OCTA of the inner and middle retinal layers showed region-specific vascular patterns that consistently corroborated qualitative findings in histological studies and are discussed in detail below.

**Central Macular Region**

Figure 2 shows a representative OCT angiography image set of the macular region. The foveal avascular zone (FAZ) is clearly demarcated with a distinct foveal vascular ring. The fovea has a lower capillary density than parfoveal capillary regions in both the inner and middle retinal slabs. Qualitatively, the inner retina surrounding the macula consists of nine to 10 tertiary vessels (arterioles and venules) that supply the perifoveal vascular network. There are clear areas of perivascular capillary-free zones around most of secondary and tertiary vessels. The vascular network in the inner retina consists of relatively continuous segments of capillaries that mostly travel in the same retinal plane. The middle retinal slab surrounding the fovea contains a more lattice-like pattern of vessels with many small, discontinuous segments. These are consistent with oblique or cross-sectional views of vessels that are travelling between retinal planes in the axial direction. The qualitative findings in both the inner retinal slab and middle retinal slab are illustrated in black-and-white contrast-enhanced ImageJ renderings in Figure 2 for clarity. The vessel density analysis shows a mean total vessel density of 31.68% ± 1.15% in the inner retina and 30.86% ± 1.20% in the middle retina (Figure 5, page 515).

**Nasal Macular Region**

Figure 3 shows a representative OCTA image set of the nasal macular region immediately adjacent to the optic disc. Qualitatively, the inner retina surrounding the optic disc has a distinct pattern of continuous capillary segments that radiate out from the optic disc and appear to run parallel to the axons of the ganglion nerve fibers. The middle retinal slab surrounding the optic disc contains a more lattice-like pattern of
vessels with many small, discontinuous segments similar to those described in the central macular region. The qualitative findings in both the inner retinal slab and middle retinal slab are illustrated in black-and-white contrast-enhanced ImageJ renderings in Figure 3 as well for clarity. Vessel density analysis shows a mean total density of 31.59% ± 1.40% in the inner retina and 31.47% ± 1.62% in the middle retina (Figure 5, page 515).

**Temporal Macula Region**

Figure 4 (page 514) shows a representative OCT angiography image set of the temporal macula. Qualitatively, the temporal macula has a less dense capillary network in the inner retinal and middle retinal slabs. Similar to the above descriptions, the capillaries in the inner retina consist of continuous segments of capillaries that travel in the same retinal plane, whereas the middle retina contains a more lattice-like pattern of vessels with many small, discontinuous segments. The qualitative findings in both the inner retinal slab and middle retinal slab are illustrated in black-and-white contrast-enhanced ImageJ renderings in Figure 4 for clarity. Vessel density analysis shows a mean total density of 22.58% ± 1.66% in the inner retina and 28.21% ± 2.04% in the middle retina (Figure 5).

Figure 5 (page 515) summarizes the vessel density measurements from the above images and shows that the total vessel density in the temporal macula is significantly less than in the central macula (centered on fovea) or nasal macular retina. It also appears that there is a difference in the vascular density of the middle and inner retinal slabs in the temporal macula that is not observed in the central macula.

**DISCUSSION**

In the current study, we used swept-source OCT to perform noninvasive angiography of healthy participants using a phase- and intensity-contrasting algorithm. Detailed, depth-resolved en face angiograms were obtained in the central macula, temporal macula, and peripapillary retina. These noninvasive angiograms show high-resolution capillary detail throughout the inner and middle retina. As expected, no retinal vasculature was observed in the deep retina. Specifically, the OCT angiograms demonstrate differences in the inner and middle retinal capillary networks that have not been reported previously using fluorescein angiography. Capillary density was quantitated using a local thresholding method to identify and analyze vessels. This method, although simple, shows similar capillary densities across participants at each location and a significant decline in inner retinal capillary density in the temporal macula region compared to all other regions that were studied. It is likely that this method underestimates vessel density in regions of higher vessel density due to limitations in the thresholding method. Nevertheless, these findings suggest that the noninvasive, rapid, and reliable imaging of the retinal microvasculature by OCT angiography can serve a role in the assessment of retinal vascular diseases.

The high resolution of the OCT angiography method described here provides detailed information that
approaches that of post-mortem tissue histology. For example, recent histological analysis of the perifoveal region and peripapillary retina were reported in human cadaver eyes using an elegant micropipette labeling technique and confocal microscopy. These studies corroborate our findings that the peripapillary optic disc has a distinct pattern of continuous capillary segments that radiate outward and appear to run parallel to the axons of the ganglion nerve fibers. In addition, human cadaver studies of the parafoveal and central macular microvasculature also corroborate our qualitative findings of continuous capillary segments (loops) within the superficial retinal sections and more lattice-like discontinuous vessel segments in middle retinal layers. These findings are consistent with the histological observation that retinal capillaries travel some distance from tertiary retinal vessels before diving into the retina. They then travel through the middle retinal layers to reach and nourish the deeper layers such as the outer plexiform layer.

The histological findings from recent human cadaver studies also closely mirror the quantitative findings from our noninvasive angiography method. Unfortunately, a direct comparison of capillary density analysis cannot be made given the difference in methods and the differences in the segmentation of retinal layers. However, a gross approximation of the combined retinal vascular density of the superficial retinal layers from recent histological studies suggests a vascular density between 31% and 55% in the superficial retina, which compares favorably with our estimate of 30% to 32% in the central and peripapillary macular regions (Figures 2 and 3). The same study suggests a vascular density between 27% and 48% for the middle retinal layers, which also compares favorably with our estimates of 30% to 32%.

It is important to note that indiscriminately summing separate optical layers is prone to errors such as the overestimation or underestimation of capillary density due to capillary overlap or inability to quantify vessels traveling perpendicular to the plane of section. The density analysis also does not take into account the decorrelation tail effect in deeper layers from overlying blood vessels. It is also likely that our thresholding method underestimates vessel density in regions of higher vessel density.

OCTA has a number of distinct advantages over conventional FA and may serve a role as an adjunct to conventional FA because the latter cannot be performed regularly due to its invasive nature and adverse effect profile. Clinically, OCTA offers significant work-flow advantages in its ability to generate angiographic images over the course of seconds compared to minutes with FA. OCTA also provides capillary-level resolution of the retinal vasculature in a three-dimensional manner, thereby allowing vascular pathology or anatomical anomalies to be accurately localized. OCTA also has the advantage of eliminating image obscuration by leaking fluorescein.

While OCTA has numerous advantages, our study has certain limitations. In its current state, our OCTA system suffers from motion artifacts and a relatively small field of view, which can be overcome with further development efforts. Although
OCTA eliminates obscuration of fine vascular detail by fluorescein leakage, it also cannot image slow dye leakage, which is often used as a clinical sign of pathologic processes in conventional FA assessment. Lastly, the small sample size and cross-sectional nature of this study limit the generalizability of the qualitative and quantitative findings. Larger and more well-controlled studies will help confirm these findings and identify new clinical applications for this technology.

REFERENCES