COMMENTARY

Imaging oxygenation of retinal capillaries with depth resolution

Vivek J. Srinivasan[a,b,1] and Ala Moshiri[b]

The inner retina has a three-tiered vascular supply, with capillary beds stratifying in the nerve fiber layer/ganglion cell layer, the inner plexiform layer, and the outer plexiform layer (1). While this trilaminar architecture likely functions to serve the metabolic needs of individual retinal layers, we do not know how metabolism and blood flow regulation differ across layers in the healthy retina. In particular, no noninvasive imaging technique with the potential for human translation has yet successfully distinguished the subtle metabolic signals that arise from these thin layers. For the past decade, visible light optical coherence tomography (OCT) (2, 3) has held the promise of imaging retinal oxygenation based on hemoglobin absorption. However, efforts have been largely limited to quantifying oxygenation of large vessels (4), with rare exceptions (5). In their latest work, Pi et al. (6) take a step toward making visible light OCT a tool for studying depth-resolved oxygenation of retinal capillary beds.

First, they show that their oximetry method is highly repeatable, a must for any measurement to make it to the clinic. Next, they turn their attention toward accuracy. The closest analog of a retinal capillary saturation measurement is a phosphorescence lifetime-based oxygen tension probe (7) or invasive tissue oxygen electrodes (8). How does one validate a technique for which there is no direct and noninvasive gold standard? The authors propose a creative, albeit indirect, method to gauge accuracy. As noted previously by the authors (9), the superficial vascular plexus supply in Brown Norway rats appears to consist predominantly of arterioles and their higher-order two-dimensional capillary branches that supply the surrounding tissue. In this model vascular bed (Fig. 1), Pi et al. (6) show a physiologically plausible decline in oxygen saturation with increasing capillary order and perfusion distance, consistent with delivery to the surrounding nerve fiber layer and ganglion cell layer. Gaining confidence from this sanity check, they next apply their algorithm to investigate the response of the superficial, intermediate, and deep capillary plexuses to hyperoxia and hypoxia. Intriguingly, they find that oxygenation of the capillary beds, where the majority of delivery is purported to occur, is relatively static during systemic modulations, even in the presence of large changes in the macrovascular oxygenation.

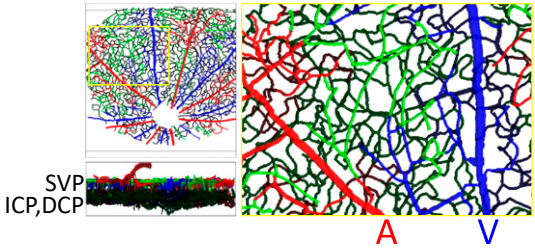

Fig. 1. Arteriole-supplied superficial vascular plexus (SVP) trees in the Brown Norway rat provide Pi et al. (6) with a test bed for their method of retinal capillary oximetry. Comparable SVP trees in a retinal vascular graph of the Sprague-Dawley rat are highlighted in red, with the intermediate capillary plexus (ICP) and deep capillary plexus (DCP) darkened. After showing reasonable oxygen desaturation in this test bed, the authors proceed to investigate the stability of all three capillary plexuses, including the ICP and DCP, in the face of systemic oxygen modulation. A, artery, V, vein.

The ambitious and complex study of Pi et al. (6) is the culmination of numerous advances in the field. First, stable commercial supercontinuum sources (4) and fiber optic couplers (10) have enabled visible light OCT systems to reliably and robustly acquire the high-quality datasets seen here. Second, the split-spectrum amplitude-decorrelation algorithm (11) developed by the authors was necessary to provide high-definition angiograms. Third, the authors propose an OCT angiography-based technique to detect the posterior boundary of individual vessel segments, regardless of size. Fourth, they apply morphological and graph-based analysis methods to OCT angiography data (12), with the aim of optimally

[a]Biomedical Engineering Department, University of California, Davis, CA 95616; and [b]Department of Ophthalmology and Vision Science, University of California Davis School of Medicine, Sacramento, CA 95817

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[1]To whom correspondence may be addressed. Email: vjsriniv@ucdavis.edu.

registering and averaging the subtle capillary signals. Finally, almost in passing, the authors note the utility of visible light OCT en face images to detect connecting vessels between lamina based on their absorption shadows. We feel that this is a very important advantage of visible light OCT, worth highlighting, as such vessels are typically missed in conventional OCT angiography, which is based on red blood cell scattering. These interconnections will ultimately be critical to understand the regulation of blood flow in the trilaminar vasculature.

Like any work that pushes the field forward, Pi et al. (6) highlight numerous directions for further validation and confirmation. The potential confounding effects of anesthesia should be addressed with studies in awake subjects. As pointed out by the authors, blood flow measurements would lend credence to biophysical models that predict tissue oxygenation, without requiring an assumed oxygen consumption. The choroidal supply, which has been hypothesized to contribute during hyperoxia (13), must be measured. Finally, while the authors show that capillary segment responses make sense when averaged over branching order and perfusion distance, the accuracy of individual capillary segment oxygenation values is unclear. Accordingly, the authors defer a complete three-dimensional analysis of the oxygen distribution to a future work.

In the absence of these measurements, the authors wisely refrain from speculation. Instead, they succinctly state that their results suggest that healthy microvasculature maintains a stable source of oxygen delivery in the face of oxygenation changes. This simple principle could be a benchmark for future work. For instance, one can imagine testing the stability of microvascular oxygenation in the presence of retinal diseases that may differentially affect the layers served by the various capillary beds. Diseases of the inner retina, such as glaucoma, might alter oxygenation in the nerve fiber layer (14). On the other hand, retinitis pigmentosa and cone dystrophy primarily affect the outer retina. It is natural to expect that the microvascular oxygenation in the outer plexiform layer might be most affected based on anatomical location, remembering though that the outer retina derives substantial oxygen supply from the choroidal circulation.

More broadly, OCT-based oximetry can both test long-held assumptions and reveal insights in diseases where blindness directly results from abnormalities of the ocular vasculature. For example, age-related macular degeneration (AMD) can result in exudation from neovascular tissue, causing damage to the macula. The presence of a cilioretinal artery, suggestive of increased oxygenation of the macula, may protect against this harmful neovascularization (15). The role of macular oxygenation in AMD progression could be tested more directly using OCT-based oximetry. Diabetes causes direct damage to retinal microvasculature through mechanisms related to hyperglycemia. Fluorescein (16), and more recently, OCT (17) angiography have documented the closure of retinal vessels in the context of diabetic retinopathy (18), which presumably leads to retinal hypoperfusion, tissue hypoxia, secretion of factors like vascular endothelial growth factor, and the development of harmful retinal neovascularization. While reduced blood flow can be inferred angiographically, clinical OCT oximetry could more directly assess the degree of hypoxia. As suggested by Pi et al. (6), these observations could be resolved in depth, indicating which layers of the retinal vasculature are first affected and possibly even predicting complicating features such as diabetic macular edema. Similar paradigms can be envisioned for retinal vein and artery occlusions, also important causes of vision loss (19, 20). In applying OCT-based oximetry in disease, we caution that visible light stimulates the retina, inducing metabolic changes and neurovascular coupling, which will undoubtedly change blood oxygenation. This concern could potentially be alleviated by the proper selection of controls when studying retinal pathologies.

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Finally, the potential for OCT retinal oximetry to drive clinical treatment decisions and serve as clinical trials outcomes cannot be overlooked. Currently, retinal exudation in structural OCT is the main clinical tool for treatment decisions in the retina clinic. OCT angiography can be useful in confirming the clinical diagnosis and may be helpful in treatment decision making in select scenarios. OCT retinal oximetry, after it is reliable, accurate, affordable, and proven as an outcome measure in therapeutic clinical trials, may reveal that retinal oxygenation is a prognostic sign or even a primary end point in the treatment of patients with various retinal diseases. With the growing realization that visible light OCT signals from capillaries are informative, we are optimistic that progress toward clinically viable, depth-resolved capillary oximetry will continue.

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