Published in final edited form as:

Curr Eye Res. 2018 January ; 43(1): 122–127. doi:10.1080/02713683.2017.1373823.

A method for volumetric retinal tissue oxygen tension imaging

Anthony E. Felder^{a,b}, Justin Wanek^b, Pang-yu Teng^b, Norman P. Blair^b, and Mahnaz Shahidi^c

^aDepartment of Bioengineering, University of Illinois at Chicago, Chicago IL, USA

^bDepartment of Ophthalmology and Visual Science, University of Illinois at Chicago, Chicago IL, USA

^cDepartment of Ophthalmology, University of Southern California, Los Angeles CA, USA

Abstract

Purpose—Inadequate retinal oxygenation occurs in many vision-threatening retinal diseases, including diabetic retinopathy, retinal vascular occlusions, and age-related macular degeneration. Therefore, techniques that assess retinal oxygenation are necessary to understand retinal physiology in health and disease. The purpose of the current study is to report a method for the three-dimensional (3D) imaging of retinal tissue oxygen tension (tPO_2) in rats.

Methods—Imaging was performed in Long Evans pigmented rats under systemic normoxia ($N=6$) or hypoxia ($N=3$). A vertical laser line was horizontally scanned on the retina and a series of optical section phase-delayed phosphorescence images were acquired. From these images, phosphorescence volumes at each phase delay were constructed and a 3D retinal tPO_2 volume was generated. Retinal tPO_2 volumes were quantitatively analyzed by generating retinal depth profiles of mean tPO_2 (M_{tPO_2}) and the spatial variation of tPO_2 (SV_{tPO_2}). The effects of systemic condition (normoxia/hypoxia) and retinal depth on M_{tPO_2} and SV_{tPO_2} were determined by mixed linear model.

Results—Each 3D retinal tPO_2 volume was approximately $500 \times 750 \times 200 \mu m$ (horizontal $\times$ vertical $\times$ depth) and consisted of 45 *en face* tPO_2 images through the retinal depth. M_{tPO_2} at the chorioretinal interface was significantly correlated with systemic arterial oxygen tension ($P=0.007$; $N=9$). There were significant effects of both systemic condition and retinal depth on M_{tPO_2} and SV_{tPO_2} , such that both were lower under hypoxia than normoxia and higher in the outer retina than inner retina ($P<0.001$).

Conclusion—For the first time, 3D imaging of retinal tPO_2 was demonstrated, with potential future application for assessment of physiological alterations in animal models of retinal diseases.

CONTACT: Mahnaz Shahidi, mshahidi@usc.edu, Department of Ophthalmology, University of Southern California, 1450 San Pablo St, Los Angeles, CA 90033.

Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/ICEY.

Declaration of interests

None (AEF, JW, PT, NPB); patent (MS).

Mahnaz Shahidi holds a patent for the imaging technology. None of the authors have received financial support or have personal financial interest relevant to the topic of the manuscript.

Keywords

Rat; retina; tissue oxygen tension; three-dimensional imaging; phosphorescence lifetime imaging

Introduction

The retina is one of the most metabolically active tissues in the human body.¹ To maintain visual function, oxygen is continuously delivered to the tissue by the retinal and choroidal circulations.^{2,3} The contributions of these circulations create steady-state gradients of oxygen through the retinal depth.^{4,5} Inadequate retinal oxygenation has been implicated in many retinal diseases, including diabetic retinopathy,^{6,7} central retinal vein occlusions,^{8,9} and age-related macular degeneration.¹⁰ Therefore, techniques that assess retinal tissue oxygen content are necessary to better understand retinal pathophysiology.

Several imaging methods have been developed to measure the oxygen content of the retinal vasculature, including spectrophotometry,^{11–14} phosphorescence lifetime imaging,^{15,16} photoacoustic ophthalmoscopy,¹⁷ and visible optical coherence tomography.¹⁸ However, these techniques can only provide an indirect assessment retinal tissue oxygenation from measurements of vascular oxygen content.¹² In contrast, oxygen-sensitive microelectrodes directly measure retinal tissue oxygen tension (tPO_2) through the retinal depth.^{4,19–21} While the oxygen microelectrode technique is considered to be the gold standard due to high sensitivity and depth discrimination, it is invasive and provides limited spatial assessment of tPO_2 . These limitations were addressed in part by two-dimensional phosphorescence lifetime imaging,^{15,16} which provided non-invasive, depth-resolved measurements of tPO_2 at vertically contiguous retinal locations.²² However, since this method did not measure retinal tPO_2 in three dimensions (3D), identification and assessment of physiological and pathological variations in tPO_2 across regions of the retina were limited. In the current study, we addressed this limitation by volumetric retinal tPO_2 imaging using our previously developed system for the 3D imaging of oxygen tension within the retinal vasculature.²³ This imaging technique overcomes the spatial limitations of existing methods and can provide valuable information about variations in tPO_2 under physiological and pathological conditions.

Materials and methods

Animals

Nine Long Evans pigmented rats (weight: 300–600 g) were used in this study. The animals were treated in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Anesthesia was induced with intraperitoneal injections of ketamine (100 mg/kg) and xylazine (5 mg/kg), and was maintained during imaging by supplemental doses of ketamine (20 mg/kg) and xylazine (1 mg/kg). An oxygen-sensitive molecular probe, Oxyphor R2 (Oxygen Enterprises, Ltd., Philadelphia, PA) was dissolved in saline and 3 μ L (0.5 mM) was injected intravitreally 1 day prior to imaging, as previously described.²²

Two separate groups of rats were ventilated mechanically (Harvard Apparatus Inc., South Natick, MA) with either 21%/30% fraction of inspired oxygen (FiO_2) to maintain systemic normoxia ($N=6$) or 10% FiO_2 to induce systemic hypoxia ($N=3$), as previously reported.^{24,25} One rat from the normoxia cohort was also ventilated under hypoxia for comparative visualization of tPO_2 within the same retinal region. To verify the systemic condition of rats, arterial oxygen tension (P_aO_2), carbon dioxide tension (P_aCO_2), and blood pH were measured by a blood gas analyzer (Radiometer, Westlake, OH, or Idexx Vetstat, Westbrook, ME) using blood drawn from a catheter inserted into the femoral artery. Body temperature was maintained at 37°C using an animal holder with a closed-loop copper tubing water heater. Prior to imaging, pupils were dilated with 2.5% phenylephrine and 1% tropicamide. One percent hydroxypropyl methylcellulose and a glass cover slip were applied to the cornea to eliminate the cornea's refractive power and to prevent corneal dehydration during imaging.

3D retinal tissue oxygen tension imaging

Our previously described optical section phosphorescence imaging system²³ was used for the 3D imaging of retinal tPO_2 . Briefly, a slitlamp biomicroscope was modified to accommodate a laser (535 nm), an optical chopper, a rotational galvanometer with an attached mirror, a high-pass filter with 650 nm cutoff, and an intensified charge-coupled device (ICCD) camera. The laser was projected at an oblique angle to the retina as a ~ 1 mm vertical line and was modulated by the optical chopper at 1.6 kHz. The laser line was horizontally scanned across the retina, and phosphorescence emission was selectively imaged at each location using the high-pass filter placed in front of the ICCD camera. At each retinal scan location, a series of 6 or 10 phase-delayed, optical section phosphorescence images were acquired by setting incremental delays between the modulated laser and intensifier gain of the ICCD camera. Since the excitation laser and the imaging paths were not coaxial, phosphorescence across the retinal depth appeared laterally displaced in the optical section phosphorescence images.²² Imaging was performed in one eye of each animal, either temporal or nasal to the optic disc. During imaging, room lights were off and rats were light-adapted due to the scanning laser illumination.

A schematic diagram of the methodology for generating 3D retinal tPO_2 volumes is shown in Figure 1. The vertical laser line was horizontally scanned across the retina in $9\ \mu\text{m}$ steps (Figure 1A) and optical section phosphorescence images in the y - z plane of the retina were acquired at locations along the x -axis. The number of scan steps ranged from 14–45, depending on the eye curvature and dilated pupil size. Optical section phosphorescence images acquired at each phase delay were stacked along the x -axis to form phase-delayed phosphorescence volumes (Figure 1B). The volumes were flattened to remove the effect of the eye curvature and then smoothed by a 3D anisotropic averaging filter ($2 \times 6 \times 4$ pixels in the x , y and z -axes, respectively) (Figure 1C). This filter size was selected to smooth each voxel of the phosphorescence volume equally in all dimensions ($18\ \mu\text{m} \times 18\ \mu\text{m} \times 18\ \mu\text{m}$). Using a frequency domain technique,^{15,16} phosphorescence lifetime was determined at each voxel from the phase-delayed phosphorescence volumes and oxygen tension was then calculated using the Stern–Volmer equation to generate a 3D retinal tPO_2 volume (Figure

1D). All image reconstruction and processing was performed using customized algorithms developed in Matlab (MathWorks, Natick, MA).

The chorioretinal and vitreoretinal interfaces were designated from the tPO₂ volume as the location of maximal tPO₂ and 200 μ m anterior to the chorioretinal interface,²⁶ respectively. The retinal tPO₂ volume consisted of 45 *en face* tPO₂ images in the *x-y* plane, extending from the vitreoretinal interface (*en face* tPO₂ image 1 at 0% retinal depth) to the chorioretinal interface (*en face* tPO₂ image 45 at 100% retinal depth) (Figure 1E). The inner and outer retinal volumes were defined as the inner and outer 50% of the total retinal volume^{19,21,26} and extended from *en face* tPO₂ images 1 to 22 and 23 to 45, respectively.

Data analysis

From compiled data in all rats, the relationship between mean tPO₂ of *en face* image 45 and systemic P_aO₂ was assessed by linear regression analysis. From each rat and each *en face* tPO₂ depth, the mean tPO₂ (M_{tPO₂, rat, depth}) and spatial variation of tPO₂ (SV_{tPO₂, rat, depth}) was calculated. Spatial variation represents the lateral variability of tPO₂ in each *en face* image and was calculated as the standard deviation of tPO₂. Combining measurements of M_{tPO₂, rat, depth} and SV_{tPO₂, rat, depth} from all *en face* images in all rats, M_{tPO₂} and SV_{tPO₂} depth profiles under both systemic conditions were generated. Inter-animal variability was assessed by standard deviation of M_{tPO₂} and SV_{tPO₂} measurements at every retinal depth. The effects of systemic condition (normoxia, hypoxia) and retinal depth on M_{tPO₂} and SV_{tPO₂} were determined by mixed linear model. In one retinal region that encompassed both an inferior artery and a superior vein, a mean inner retinal *en face* tPO₂ image (average of *en face* images 1–22) was generated. The relationship between tPO₂ and vertical distance along the image (averaged over 30 μ m vertical contiguous regions) was determined by linear regression analysis. All statistical analyses were performed using SPSS statistical software (version 22, SPSS, Chicago, IL, USA). Statistical significance was accepted at *P* = 0.05.

Results

Qualitative evaluation of tPO₂ volumes

All tPO₂ volumes were inspected for qualitatively recognizable characteristics. Representative tPO₂ volumes generated at the same retinal region (indicated in green; Figure 2A) in a rat under normoxia (Figure 2B) and hypoxia (Figure 2C) are shown. From these volumes, *en face* tPO₂ images at four retinal depths under normoxia and hypoxia are displayed (Figure 2D). As expected, all volumes exhibited greater tPO₂ at the chorioretinal interface compared to the inner retina, and volumes under hypoxia demonstrated lower tPO₂ compared to those under normoxia. Further, tPO₂ changed smoothly across each volume, without abrupt variations in tPO₂. Retinal vessels were not visible on most tPO₂ volumes.

Quantitative analysis of tPO₂ volumes

The P_aO₂, P_aCO₂ and pH levels of rats under systemic normoxia were 95 $\pm$ 15 mmHg, 43 $\pm$ 13 mmHg, and 7.4 $\pm$ 0.15, respectively. For rats under systemic hypoxia, the P_aO₂, P_aCO₂, and pH were 32 $\pm$ 3.5 mmHg, 34 $\pm$ 5.6 mmHg, and 7.4 $\pm$ 0.07, respectively. As shown in

Figure 3, M_{tPO_2} at the chorioretinal interface was linearly correlated with systemic P_aO_2 ($R = 0.82$, $P = 0.007$, $N = 9$).

Retinal M_{tPO_2} depth profiles compiled from data obtained in all rats stratified by systemic condition are shown in Figure 4A. At each retinal depth, M_{tPO_2} was lower under hypoxia than under normoxia. Under both systemic conditions, M_{tPO_2} increased with retinal depth, indicating lower tPO_2 in the inner retina and higher tPO_2 toward the chorioretinal interface. Retinal SV_{tPO_2} depth profiles compiled from data obtained in all rats stratified by systemic condition are shown in Figure 4B. Under normoxia, SV_{tPO_2} was approximately 5 and 8 mmHg in inner retinal and outer retinal layers, respectively. Moreover, inter-animal variability in SV_{tPO_2} was around 1 mmHg in the inner retina and 2.5 mmHg in the outer retina. SV_{tPO_2} was lower at each retinal depth under hypoxia as compared to normoxia and highest at the chorioretinal interface under both systemic conditions. There were significant effects of systemic oxygen condition (normoxia, hypoxia) and retinal depth on both M_{tPO_2} and SV_{tPO_2} ($P < 0.001$). In a retinal region that encompassed a horizontally oriented retinal vein superiorly and an artery inferiorly (Figure 2), the fit from the linear regression between inner retinal tPO_2 and the vertical axis was significant under normoxia ($R = 0.88$, $P < 0.001$, $N = 30$).

Discussion

In the current study, we report a technique for imaging of retinal tissue oxygen tension in three dimensions. Volumetric imaging of retinal tPO_2 is essential to identify and assess the lateral variations of retinal oxygenation that occur under normal physiological and pathological conditions. Retinal tPO_2 volumes displayed a lower tPO_2 under hypoxia than normoxia, and all volumes displayed greater tPO_2 toward the choroid, as expected. However, despite retention of the oxyphor only in the extravascular space, retinal vessels, which were devoid of phosphorescence signal, could not be visually identified on most tPO_2 volumes. This was mainly due to the scatter of phosphorescence within the retinal tissue.

In the current study, the M_{tPO_2} depth profiles compiled from all rats under normoxia demonstrated a large oxygen gradient in the outer retina with the greatest M_{tPO_2} occurring at the chorioretinal interface, consistent with previous studies.^{4,19,22} This oxygen gradient is necessary to drive oxygen from the choroid to the photoreceptor inner segments.^{4,27} There was also a significant difference in M_{tPO_2} at each retinal depth between normoxia and hypoxia, consistent with the findings of previous studies.^{5,22,28} However, absolute measurements of tPO_2 through the retinal depth were generally lower than those reported in previous studies. In the current study, inner retinal M_{tPO_2} and maximal M_{tPO_2} under normoxia (18 mmHg, 30 mmHg) were lower than those in light-adapted rats measured by the oxygen microelectrode technique (29 mmHg, 45 mmHg)¹⁹ and phosphorescence lifetime imaging (30 mmHg, 50 mmHg),²² while they were more comparable to other published values measured using the oxygen microelectrode technique (15 mmHg, 50 mmHg),⁴ (13 mmHg, 35 mmHg).²⁹

In the current study, we report SV_{tPO_2} for the first time, revealing physiological variations of tPO_2 across retinal regions. Under normoxia, lateral retinal tPO_2 variability of 5–8 mmHg

was demonstrated and is likely due to the net result of oxygen diffusional gradients within the tissue under steady state. Previously, variations in retinal tPO_2 were suggested based only on differences in pre-retinal oxygen tension between arteries and veins.^{30–32} From compiled data in all rats, SV_{tPO_2} increased with retinal depth and was greatest toward the choroid (8 mmHg), consistent with previous studies that reported similar variations in rat^{19,22,26} and cat^{5,21} under light-adapted conditions. Spatial variation of tPO_2 was also depicted by demonstrating a gradient in inner retinal tPO_2 between an artery and vein pair. This can be attributed to the reduction of oxygen tension in the capillary bed as blood passes from artery to vein, as well as oxygen diffusion to the retinal tissue between vessels.³³ Additionally, from compiled data in all rats, SV_{tPO_2} was lower under hypoxia than normoxia, indicating tPO_2 is less variable during systemic hypoxia. Since the inner retinal tPO_2 range should be related to the difference between the retinal arterial and venous oxygen tension, a decrease in the arteriovenous oxygen tension difference during hypoxia should reduce the SV_{tPO_2} . Indeed, a decrease in the retinal arteriovenous oxygen tension difference was previously reported under hypoxia.²⁴ However, a floor effect on tPO_2 during hypoxia may be present, which can also account for the decreased SV_{tPO_2} . Nevertheless, SV_{tPO_2} can be a valuable metric and may be used to characterize tPO_2 lateral variations across retinal *en face* images for detection of abnormalities, such as retinopathies characterized by multifocal pathologies.

There were several limitations of the current study. First, due to the use of laser excitation for phosphorescence lifetime imaging, data can only be obtained in light-adapted rats. Second, intraretinal phosphorescence scattering may reduce depth resolution and alter the tPO_2 depth profiles, though the general trend of tPO_2 retinal depth profiles was similar to that obtained by previous techniques. Third, due to the inverse non-linear relationship between phosphorescence lifetime and tPO_2 , this method is less sensitive to the detection of high tPO_2 , resulting in greater measurement variability. This limitation may, at least in part, account for the observation of higher tPO_2 variability near the choroid. Fourth, eye motion that occurs due to animal respiration during imaging can create shifts between consecutive image locations or blurring of images. To minimize the contributions of these factors, the phosphorescence volumes were smoothed in post-processing, which decreased variations and may have altered calculation of phosphorescence life-time. However, since comparison of tPO_2 was based on data from multiple animals, this image processing step likely minimally affected the results. Last, the calculation of tPO_2 from phosphorescence lifetime relied on oxyphor constants that were derived from *ex vivo* experiments, which may be different from those in the living retinal tissue. This may affect absolute tPO_2 values, the possibility of which cannot be excluded in this study.

In conclusion, we demonstrated a technique for imaging of retinal tissue oxygen tension in three dimensions in rats. This optical imaging technique is a promising tool for detecting lateral spatial variations in retinal tissue oxygen tension in animal models of retinal diseases.

Acknowledgments

The authors would like to acknowledge the contributions of Tara Nguyen and Marek Mori for animal care and data acquisition.

Funding

This study was supported by NIH grants EY017918 and EY001792, Senior Scientific Investigator award (MS) and an unrestricted departmental grant from Research to Prevent Blindness.

References

1. Trick GL, Berkowitz BA. Retinal oxygenation response and retinopathy. *Prog Retin Eye Res.* 2005; 24(2):259–74. DOI: 10.1016/j.preteyeres.2004.08.001 [PubMed: 15610976]
2. Kiel, JW. The ocular circulation Morgan & Claypool Life Sciences; San Rafael, CA, USA: 2010 <http://www.ncbi.nlm.nih.gov/books/NBK53329/>
3. Dartt, DA., Besharse, JC., Dana, R. Encyclopedia of the eye Elsevier, Academic Press; Cambridge, MA, USA: 2010
4. Cringle SJ, Yu DY, Yu PK, Su EN. Intraretinal oxygen consumption in the rat in vivo. *Invest Ophthalmol Vis Sci.* 2002; 43(6):1922–27. [PubMed: 12037000]
5. Linsenmeier RA, Braun RD. Oxygen distribution and consumption in the cat retina during normoxia and hypoxemia. *J Gen Physiol.* 1992; 99(2):177–97. DOI: 10.1085/jgp.99.2.177 [PubMed: 1613482]
6. Gariano RF, Gardner TW. Retinal angiogenesis in development and disease. *Nature.* 2005; 438(7070):960–66. DOI: 10.1038/nature04482 [PubMed: 16355161]
7. Chan-Ling T, Gock B, Stone J. The effect of oxygen on vasoformative cell division. Evidence that ‘physiological hypoxia’ is the stimulus for normal retinal vasculogenesis. *Invest Ophthalmol Vis Sci.* 1995; 36(7):1201–14. [PubMed: 7775098]
8. Traustason S, La Cour M, Larsen M. Retinal vascular oximetry during ranibizumab treatment of central retinal vein occlusion. *Br J Ophthalmol.* 2014; 98(9):1208–11. DOI: 10.1136/bjophthalmol-2013-304580 [PubMed: 24729079]
9. Hardarson SH, Stefansson E. Oxygen saturation in central retinal vein occlusion. *Am J Ophthalmol.* 2010; 150(6):871–75. DOI: 10.1016/j.ajo.2010.06.020 [PubMed: 20875633]
10. Geirsdottir A, Hardarson SH, Olafsdottir OB, Stefansson E. Retinal oxygen metabolism in exudative age-related macular degeneration. *Acta Ophthalmol (Copenh).* 2014; 92(1):27–33. DOI: 10.1111/aos.12294
11. Beach JM, Schwenzer KJ, Srinivas S, Kim D, Tiedeman JS. Oximetry of retinal vessels by dual-wavelength imaging: calibration and influence of pigmentation. *J Appl Physiol (1985).* 1999; 86(2):748–58. [PubMed: 9931217]
12. Felder AE, Wanek J, Blair NP, Shahidi M. Inner retinal oxygen extraction fraction in response to light flicker stimulation in humans. *Invest Ophthalmol Vis Sci.* 2015; 56(11):6633–37. DOI: 10.1167/iovs.15-17321 [PubMed: 26469748]
13. Hammer M, Vilser W, Riemer T, Schweitzer D. Retinal vessel oximetry-calibration, compensation for vessel diameter and fundus pigmentation, and reproducibility. *J Biomed Opt.* 2008; 13(5):054015.doi: 10.1117/1.2976032 [PubMed: 19021395]
14. Hardarson SH, Harris A, Karlsson RA, Halldorsson GH, Kagemann L, Rechtman E, Zoega GM, Eysteinnsson T, Benediktsson JA, Thorsteinsson A, et al. Automatic retinal oximetry. *Invest Ophthalmol Vis Sci.* 2006; 47(11):5011–16. DOI: 10.1167/iovs.06-0039
15. Shahidi M, Shakoor A, Blair NP, Mori M, Shonard RD. A method for chorioretinal oxygen tension measurement. *Curr Eye Res.* 2006; 31(4):357–66. DOI: 10.1080/02713680600599446 [PubMed: 16603469]
16. Shonard RD, Kight AC. Oxygen tension imaging in the mouse retina. *Ann Biomed Eng.* 2003; 31(9):1084–96. DOI: 10.1114/1.1603256 [PubMed: 14582611]
17. Song W, Wei Q, Liu W, Liu T, Yi J, Sheibani N, Fawzi AA, Linsenmeier RA, Jiao S, Zhang HF. A combined method to quantify the retinal metabolic rate of oxygen using photoacoustic ophthalmoscopy and optical coherence tomography. *Sci Rep.* 2014; 4:6525.doi: 10.1038/srep06525 [PubMed: 25283870]
18. Yi J, Wei Q, Liu W, Backman V, Zhang HF. Visible-light optical coherence tomography for retinal oximetry. *Opt Lett.* 2013; 38(11):1796–98. DOI: 10.1364/OL.38.001796 [PubMed: 23722747]

19. Lau JC, Linsenmeier RA. Oxygen consumption and distribution in the Long-Evans rat retina. *Exp Eye Res.* 2012; 102:50–58. DOI: 10.1016/j.exer.2012.07.004 [PubMed: 22828049]
20. Pournaras CJ, Riva CE, Tsacopoulos M, Strommer K. Diffusion of O₂ in the retina of anesthetized miniature pigs in normoxia and hyperoxia. *Exp Eye Res.* 1989; 49(3):347–60. DOI: 10.1016/0014-4835(89)90045-6 [PubMed: 2792232]
21. Braun RD, Linsenmeier RA, Goldstick TK. Oxygen consumption in the inner and outer retina of the cat. *Invest Ophthalmol Vis Sci.* 1995; 36(3):542–54. [PubMed: 7890485]
22. Shahidi M, Wanek J, Blair NP, Little DM, Wu T. Retinal tissue oxygen tension imaging in the rat. *Invest Ophthalmol Vis Sci.* 2010; 51:4766–70. DOI: 10.1167/iovs.09-4710 [PubMed: 20375336]
23. Shahidi M, Wanek J, Blair NP, Mori M. Three-dimensional mapping of chorioretinal vascular oxygen tension in the rat. *Invest Ophthalmol Vis Sci.* 2009; 50(2):820–25. DOI: 10.1167/iovs.08-2343 [PubMed: 18824736]
24. Wanek J, Teng PY, Blair NP, Shahidi M. Inner retinal oxygen delivery and metabolism under normoxia and hypoxia in rat. *Invest Ophthalmol Vis Sci.* 2013; 54(7):5012–19. DOI: 10.1167/iovs.13-11887 [PubMed: 23821203]
25. Teng PY, Wanek J, Blair NP, Shahidi M. Response of inner retinal oxygen extraction fraction to light flicker under normoxia and hypoxia in rat. *Invest Ophthalmol Vis Sci.* 2014; 55(9):6055–58. DOI: 10.1167/iovs.13-13811 [PubMed: 25183761]
26. Wanek J, Blair NP, Shahidi M. Outer retinal oxygen consumption of rat by phosphorescence lifetime imaging. *Curr Eye Res.* 2012; 37(2):132–37. DOI: 10.3109/02713683.2011.629071 [PubMed: 22070458]
27. Delaey C, Van De Voorde J. Regulatory mechanisms in the retinal and choroidal circulation. *Ophthalmic Res.* 2000; 32(6):249–56. DOI: 10.1159/000055622 [PubMed: 11015035]
28. Linsenmeier RA, Braun RD, McRipley MA, Padnick LB, Ahmed J, Hatchell DL, McLeod DS, Luty GA. Retinal hypoxia in long-term diabetic cats. *Invest Ophthalmol Vis Sci.* 1998; 39(9):1647–57. [PubMed: 9699554]
29. Yu DY, Cringle SJ, Alder VA, Su EN. Intraretinal oxygen distribution in rats as a function of systemic blood pressure. *Am J Physiol.* 1994; 267(6 Pt 2):H2498–507. [PubMed: 7810746]
30. Petropoulos IK, Pournaras JA, Stangos AN, Pournaras CJ. Preretinal partial pressure of oxygen gradients before and after experimental pars plana vitrectomy. *Retina.* 2013; 33(1):170–78. DOI: 10.1097/IAE.0b013e318261a6b5 [PubMed: 22972446]
31. Yu DY, Cringle SJ, Alder VA. The response of rat vitreal oxygen tension to stepwise increases in inspired percentage oxygen. *Invest Ophthalmol Vis Sci.* 1990; 31(12):2493–99. [PubMed: 2265989]
32. Alder VA, Yu DY, Cringle SJ, Su EN. Changes in vitreal oxygen tension distribution in the streptozotocin diabetic rat. *Diabetologia.* 1991; 34(7):469–76. DOI: 10.1007/BF00403282 [PubMed: 1916051]
33. Teng PY, Blair NP, Wanek J, Shahidi M. Oxygen tension and gradient measurements in the retinal microvasculature of rats. *Graefes Arch Clin Exp Ophthalmol.* 2012; 250(3):361–67. DOI: 10.1007/s00417-011-1859-6 [PubMed: 22101835]

Figure 1.

A schematic diagram illustrating the steps for the generation of a retinal tissue oxygen tension (tPO₂) volume. (A) A vertical laser line is scanned laterally across the rat retina, as indicated by green lines, to acquire a series of phase-delayed optical section phosphorescence images. (B) The series of zero-phase delay optical section phosphorescence images are stacked along the *x*-axis. Vertical red lines on the rightmost optical section phosphorescence image indicate vitreoretinal (left) and chorioretinal (right) interfaces. (C) The zero-phase delay phosphorescence volume is shown. (D) Using the set of phase-delayed phosphorescence volumes, the phosphorescence lifetime was calculated at each voxel to generate a retinal tPO₂ volume, shown in pseudo color. Color bar indicates tPO₂ between 0 and 50 mmHg. (E) The tPO₂ volume contains 45 *en face* tPO₂ images in *x*-*y* planes across the retinal depth. Arrows (from left to right) point to *en face* tPO₂ images at 0%, 33%, 66% and 100% retinal depth.

Figure 2.

(A) Representative retinal region imaged in a rat, indicated by green lines superimposed on the red-free image. Retinal tissue oxygen tension (tPO₂) volumes generated in the same retinal region under normoxia (B) and hypoxia (C). (D) From the tPO₂ volumes, *en face* tPO₂ images at four retinal depths under normoxia and hypoxia are displayed. Color bar indicates tPO₂ in mmHg.

Figure 3.

Mean retinal tissue oxygen tension (M_{tPO_2}) at the chorioretinal interface plotted as a function of systemic arterial oxygen tension (P_aO_2). Compiled data from nine rats.

Figure 4.

(A) Mean retinal tissue oxygen tension (M_{tPO_2}) depth profile under normoxia (red, $N = 6$) and hypoxia (blue, $N = 3$). (B) Retinal tissue oxygen tension spatial variation (SV_{tPO_2}) depth profile under normoxia (red) and hypoxia (blue). Error bars indicate standard error of the means.