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Direct Regularized Estimation of Retinal Vascular Oxygen Tension Based on an Experimental Model

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Abstract

Phosphorescence lifetime imaging is commonly used to generate oxygen tension maps of retinal blood vessels by classical least squares (LS) estimation method. A spatial regularization method was later proposed and provided improved results. However, both methods obtain oxygen tension values from the estimates of intermediate variables, and do not yield an optimum estimate of oxygen tension values, due to their nonlinear dependence on the ratio of intermediate variables. In this paper, we provide an improved solution by devising a regularized direct least squares (RDLS) method that exploits available knowledge in studies that provide models of oxygen tension in retinal arteries and veins, unlike the earlier regularized LS approach where knowledge about intermediate variables is limited. The performance of the proposed RDLS method is evaluated by investigating and comparing the bias, variance, oxygen tension maps, 1-D profiles of arterial oxygen tension, and mean absolute error with those of earlier methods, and its superior performance both quantitatively and qualitatively is demonstrated.

Keywords

Phosphorescence lifetime imaging; Retinal vasculatures; Oxygen tension estimation; Spatial regularization

INTRODUCTION

Normal function of the retinal tissue depends in part on adequate supply of oxygen. Therefore, accurate estimation of oxygen tension (pO_2) in retinal vessels is needed to understand the role of hypoxia in the development of common eye diseases including glaucoma and diabetic retinopathy.^{1,2} Retinal oxygenation has been investigated using different techniques such as oxygen sensitive microelectrodes, magnetic resonance spectroscopy, or magnetic resonance imaging.^{1,3,10} Phosphorescence lifetime imaging technique has allowed mapping of retinal vascular pO_2 .^{6,7,9} With this technique, oxygen tension nonlinearly depends on the phosphorescence lifetime of an oxygen sensitive molecular probe. Using a frequency domain approach, phosphorescence lifetime is estimated from phase-delayed phosphorescence intensity images using a linear model.⁵ However, the classical least squares (LS) method initially used to estimate intermediate variables produced high variance in pO_2 maps due to noise in phosphorescence intensity images, particularly under high oxygen level condition.^{6,7,12} To overcome these drawbacks, a spatial regularization method with least-squares estimates was proposed and the superior performance of this regularized least squares (RLS) method was shown.¹² However, both LS and RLS methods suffer from certain shortcomings: they do not directly minimize the error for the estimate of parameters of interest but instead optimizes the error for the estimates of intermediate variables. Furthermore, these methods are constrained by the fact that knowledge about intermediate variables is limited. Therefore, there is a need to develop a method that directly targets the error in pO_2 estimates while also taking advantage of the available knowledge in studies that provide models of oxygen tension in retinal arteries and veins.

In this paper, we propose a solution by devising a regularized direct least squares (RDLS) method in which we directly (instead of from intermediate variables as in the RLS method) regularize pO_2 values obtained from LS estimates of the variables. Experimental modeling of pO_2 in retinal arteries and veins is also used as the prior knowledge to be exploited when developing an approximate solution. The proposed RDLS method is compared with the currently available LS and RLS methods using simulated data generated based on *in vivo* pO_2 maps and using real data obtained from rat eyes. Based on evaluation of bias, variance, mean absolute error (MAE), as well as, by evaluation of performances of 2-D pO_2 maps and 1-D pO_2 profiles a clear improvement achieved with RDLS method is demonstrated. A preliminary version of this work has appeared in Yildirim *et al.*¹¹

The rest of the paper is organized as follows. In “Materials and methods” section, we briefly describe the data acquisition, the phosphorescence lifetime imaging technique, and previously available LS and RLS methods. The proposed new RDLS method is also described in “Materials and methods” section and, in “Results” section, its performance is assessed by comparing it with currently available methods using both simulated data and real data obtained from rat retinas. “Discussion” section is devoted to discussions about the work. The paper is concluded in “Conclusion” section.

MATERIALS AND METHODS

Data Acquisition

Phosphorescence lifetime intensity images used in this work were acquired from rat retinas by a novel system for optical section phosphorescence imaging developed earlier.^{6,7} Following intravenous injection of an oxygen-sensitive molecular probe which is meso-tetra (4-carboxyphenyl) porphine (Pd-porphyrin) in our study, a diode laser at a wavelength of 532 nm was expanded to a line with a cylindrical lens and focused at an oblique angle on the retina. The relationship between oxygen tension and the oxygen saturation of hemoglobin is determined by the oxygen-hemoglobin dissociation curve and may be altered due to various metabolic conditions. The feasibility of the system for determining oxygen tension changes separately in the retinal and choroidal vasculatures was established by varying the fraction of inspired oxygen in rats. The details and a schematic figure of the imaging system were given in our earlier study.⁶ The imaging optics of a slit-lamp bio-microscope which was fitted with an infrared filter with transmission overlapping the phosphorescence emission of the oxygen probe was utilized to form an image of the retina on a digital intensified charge coupled device (ICCD) camera (Roper Scientific, Trenton, NJ, USA). The incident laser beam was not coaxial with the imaging axis of the instrument, therefore retinal features at different depths were laterally displaced on the phosphorescence optical section retinal image according to their depth location. The laser line was scanned horizontally on the retina by integrating a galvanometer scanner into the system. A series of optical section phosphorescence lifetime intensity images from spatially adjacent locations were acquired. The optical section phosphorescence lifetime intensity images were then combined to generate enface phosphorescence images of the retinal vasculatures in the retinal plane using an image reconstruction technique previously described.⁷ The excitation laser beam was modulated with an optical chopper (Photon Technology, London, ON, Canada) positioned in the beam path and operated at a frequency of 1600 Hz. The digital output from the chopper triggered the timing controller of the ICCD camera. At each location, 10 phase-delayed optical section phosphorescence lifetime intensity images were acquired with a corresponding phase delay increments of 74 μ s.

Animals

Phosphorescence lifetime intensity images of the retinal vasculatures were acquired from 4 Long Evans pigmented rats (450–650 g). All animal experimental procedures were approved by the Animal Care Committee of the University of Illinois at Chicago. The animals were treated in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. An intra-peritoneal infusion of Ketamine (85 mg/kg IP) and Xylazine (3.5 mg/kg IP) at the rate of 0.5 mg/kg/min and 0.02 mg/kg/min, respectively, was used to anesthetize the rats. The percentage of inspired oxygen was controlled by means of a high-flow mask system. Gas mixture containing 21% oxygen (room air, normoxia) was administered to rats for 5 min before and during imaging.

The feasibility of the system was established by demonstrating an increase in the retinal oxygen tension with increased fractions of inspired oxygen. Pupils were dilated with 2.5% phenylephrine and 1% tropicamide to image the retinal vasculatures. A glass cover slip was

placed on the cornea after application of 1% hydroxypropyl to eliminate the cornea refractive power and to prevent dehydration. An oxygen-sensitive molecular probe, Pd-porphine (Frontier Scientific, Logan, Utah), was dissolved (12 mg/mL) in bovine serum albumin solution (60 mg/mL) and physiological saline buffered to a pH of 7, and injected intravenously (20 mg/kg). Imaging was performed at areas within two-disk diameters (600 μm) from the edge of the optic nerve head.

Phosphorescence Lifetime Imaging Method

Since phosphorescence emission from an oxygen sensitive molecular probe is quenched by oxygen, both the intensity and lifetime of the phosphorescence increase with decreased oxygen. Oxygen tension $p\text{O}_2$ and the phosphorescence lifetime, τ , are related by the Stern–Volmer expression:

$$\frac{\tau_0}{\tau} = 1 + (k_\phi)(\tau_0)(p\text{O}_2), \quad (1)$$

where $p\text{O}_2$ (mmHg) is the oxygen tension, $k_\phi(\text{mmHg } \mu\text{s})^{-1}$ is the bimolecular rate constant or quenching constant for the triplet-state phosphorescence probe, and τ_0 is the lifetime in zero oxygen environments. To estimate $p\text{O}_2$ values, we need to determine τ in Eq. (1) given the constants τ_0 and k_ϕ .

The relation between the phosphorescence phase angle θ , and θ is given by

$$\tan\theta = \omega_0\tau, \quad (2)$$

where ω_0 is the modulation frequency.

As previously described, using a frequency domain approach, θ can be computed from the observations of image intensity values obtained with different phase delays between the modulated excitation light and imaging, using LS method.^{5–7,9,12} The intensity depends on the phase angle θ , concentration of the probe [Pd], k , and the modulation m , which are unknown. The intensity can be written as a function of phase delay θ_n :

$$I(\theta_n) = k[\text{Pd}] \left(1 + \frac{1}{2}mm_n\cos(\theta - \theta_n) \right) \quad n=1, 2, \dots, L, \quad (3)$$

where m_n is the modulation profile of the image intensifier, θ_n the phase of the gain modulation, and L the number of measurements at each pixel location for different phase values θ_n .

Using the trigonometric identity⁶: $\cos(\theta - \theta_n) = \cos(\theta)\cos(\theta_n) + \sin(\theta)\sin(\theta_n)$;

$$I(\theta_n) = k[\text{Pd}] + \frac{1}{2}k[\text{Pd}]m_n m \cos(\theta)\cos(\theta_n) + \frac{1}{2}k[\text{Pd}]m_n m \sin(\theta)\sin(\theta_n); \quad n=1, 2, \dots, L, \quad (4)$$

Defining $a_0 = k[\text{Pd}]$, $a_1 = \frac{1}{2}k[\text{Pd}]m_n m \cos(\theta)$, and $b_1 = \frac{1}{2}k[\text{Pd}]m_n m \sin(\theta)$, Eq. (4) can be expressed as

$$I(\theta_n) = a_0 + a_1 \cos(\theta_n) + b_1 \sin(\theta_n). \quad (5)$$

Noting that $\frac{b_1}{a_1} = \tan(\theta)$, the phase angle θ in Eq. (3) is obtained as

$$\theta = \tan^{-1} \left(\frac{b_1}{a_1} \right). \quad (6)$$

The pO_2 values at each observation location can therefore be obtained from the LS estimates of a_1 and b_1 using Eqs. (6), (2), and (1).

Regularized Least Squares Method

The intensity values in Eq. (5) are valid in the absence of noise. In the presence of additive noise, the observed intensity vector I^i for the i th pixel can be written in a matrix form as follows:

$$[I_1^i \ I_2^i \ \dots \ I_L^i]^T = \begin{bmatrix} 1 & \cos(\theta_1) & \sin(\theta_1) \\ 1 & \cos(\theta_2) & \sin(\theta_2) \\ \dots & & \\ 1 & \cos(\theta_L) & \sin(\theta_L) \end{bmatrix} \begin{bmatrix} a_0^i \\ a_1^i \\ b_1^i \end{bmatrix} + \begin{bmatrix} N_1 \\ N_2 \\ \dots \\ N_L \end{bmatrix}. \quad (7)$$

The solution for the i th pixel has traditionally been obtained in the least-squares sense:

$$\begin{bmatrix} \hat{a}_0^i & \hat{a}_1^i & \hat{b}_1^i \end{bmatrix}_{LS}^T = Q I^i = [a_0^i \ a_1^i \ b_1^i]^T + Q N, \quad (8)$$

where Q is the pseudo-inverse of the matrix in Eq. (7). The effect of additive noise appears on the LS estimates of parameters as multiplication of the pseudo-inverse of the system matrix Q , and the noise vector N . This effect leads to observation of “spikes” or large deviations in the estimates, causing estimates to lie beyond the physiologically feasible range, which produces an unacceptable local variation in pO_2 maps of retinal vasculatures. The final pO_2 estimate is calculated *via* a nonlinear function based on Eqs. (1), (2), and (6).

To suppress the spikes and control the local variation, RLS method was developed in our previous study.¹² In this method spatial regularization was used by defining a cost function as follows:

$$f_{a_1}^i = (a_1^i - A I^i)^2 + \beta (a_1^i - \overline{a_1^i})^2, \quad i=1, 2, \dots, J, \quad (9)$$

where I^i is the observed intensity vector, $\overline{a_1^i}$ the mean value of the parameter to be estimated for the i th pixel, A the second row of the matrix Q in Eq. (7), β the regularization parameter, and J the number of pixels in the image. The first term in Eq. (9) is the LS term called the data fidelity term and the second term is the regularization term controlling the local variation or smoothness of estimates, where the parameter β controls weights of these two terms. The parameter b_1 is also estimated using the same cost function *via* defining A in Eq.

(9) as the third row of the matrix Q in Eq. (8). The final pO_2 maps are obtained from the regularized estimates of a_1 and b_1 using the Eqs. (1), (2), and (6). Due to the limitations of this approach discussed afterward, we re-examine the problem and develop the idea of deriving a regularized direct LS solution by minimizing the error in pO_2 estimates by using knowledge about oxygen tension variations along retinal vessels.

Regularized Direct Least Squares Method

In the RDLS method, we propose a spatial regularization method, which is significantly different from the previous approach.¹² The significant difference is that the regularization is performed using the actual variable of interest, which is pO_2 , rather than regularizing estimates of the intermediate variables to indirectly obtain pO_2 . Moreover, this approach can utilize prior information about retinal vessels oxygen tension distribution which is much more accurately modeled compared with the intermediate variables a_1 and b_1 based on available studies.

Due to the lack of prior information in modeling the distribution of a_1 and b_1 over arteries and veins, we were unable to develop a regularization method that relies on actual measurements from previous studies. The RLS method was applied with a smoothness assumption over the intermediate variables; although in reality they do not need to be smooth since the physical variable that is known to be smooth is pO_2 that depends nonlinearly on the ratio of the intermediate variables.

We propose regularizing the error in estimating the actual variable pO_2 of interest by adapting the cost function (Eq. (9)) for estimates of pO_2 instead of the obtaining pO_2 from the regularized estimates of intermediate variables a_1 and b_1 :

$$f_R^i = (R^i - R_{LS}^i)^2 + \beta (R^i - \bar{R}^i)^2, \quad i=1, 2, \dots, J, \quad (10)$$

where R^i is oxygen tension to be estimated, R_{LS}^i the LS estimate of oxygen tension for i th pixel, β the regularization parameter, J the number of pixels in the image, and $\bar{R}^i$ the mean oxygen tension value calculated for i th pixel adapted to the type of vessel based on prior studies. $\bar{R}^i$ was defined as the sample mean of the pixels in a window with $30 \mu\text{m}$ size in which pO_2 change is sufficiently small, approximately 1 mm-Hg in retinal artery and 0.1 mm-Hg in retinal vein, to capture the local variation.⁷ The cost function in Eq. (10) at each pixel location is dependent on the values of its neighboring pixels due to our definition of the mean value of pO_2 , $\bar{R}^i$. A joint optimization is performed for the total cost. The total cost is equal to the sum of the individual costs of each pixel in Eq. (10), and it is expressed as

$$F_R = \sum_{i=1}^J f_R^i. \quad (11)$$

where, as before, J denoted the total number of pixels.

To find the minimum of this cost function, a gradient-based iterative approach is applied. The iterative algorithm starts with an initial choice of the pO_2 values. Next the gradient of the cost equation is computed as follows:

$$\nabla F_R = \sum_{i=1}^J 2 \left(R^i - R_{LS}^i \right) + 2\beta \left(R^i - \overline{R^i} \right), \quad (12)$$

which is used to determine the search direction for the unknown pO_2 values. The updated value is then obtained as follows:

$$R(k+1) = R(k) - \alpha(k) \nabla F(R(k)), \quad (13)$$

where $\alpha(k)$ is step size at the k th step that is found with a line search, and ∇F is the gradient of the cost function. The iteration is terminated when the stop criterion is achieved, $\|R(k+1) - R(k)\| \leq \varepsilon$. In the proposed study, estimation of oxygen maps did not pose a problem in terms of computation time. Although it is true that gradient based methods sometimes slowly converge, we think that it is not high priority to go into detailed analysis and alternative methods to speed up the convergence, since it did not pose a significant slow convergence in our experiments.

In the RDLS method, very specific prior knowledge, such as pO_2 having a decreasing value as we move away from the optic nerve, can be used.⁸ pO_2 was measured at 20 locations along the retinal arteries and veins over a distance spanning 600 μm for four rats.⁸ It was found that pO_2 in retinal arteries decreased linearly with increased distance from the optic nerve head, whereas pO_2 in retinal veins remained relatively unchanged. Therefore, the size of neighborhood used for $\overline{R^i}$ estimate of pixels in retinal vessels was chosen as 30 μm in which pO_2 change is sufficiently small, approximately 1 mmHg in retinal artery and 0.1 mmHg in retinal vein, to capture the local variation.⁸ If the size is chosen larger, local variation in retinal artery oxygenation might be lost. On the other hand if it is chosen too small, the estimate becomes more sensitive to the noise. The window size was decided based on the diameter of retinal vessels⁴ and experimental results conducted to obtain the change in retinal vessels oxygenation.⁸ The size could be decided by changing size of the window in certain range but the method proposed in this study relies on the experimental results. Moreover, as in all regularization approaches, we are introducing a certain amount of bias, and this bias, in theory, may be resulting in loss or some actual non-noise variations. However, this is an inherent property of all regularization techniques and the price paid for significant decrease of variance/noise effects. Therefore, more and less window size would affect bias variance tradeoff in the regularization.

If noise is negligible, the LS estimate of pO_2 map would be smooth but the variation will clearly increase as the level of noise contamination increases. If β is chosen too small, the regularized solution will be very close to the LS estimate and the local variation in the estimated pO_2 map will still remain too high. The parameter β also cannot be chosen arbitrarily large due to the bias-variance trade-off issue in regularization; very large β will cause an unacceptable bias. We are using an experience-based fixed β value for all our

experiments. It is common practice to select such regularization parameters based on experience, and we believe it is an appropriate strategy.

RESULTS

In this section, we perform computer simulations to compare the performance of the RDLS method with the existing methods. We also apply the RDLS method to real data obtained from rat eyes. A simulated pO_2 map containing an artery and a vein was created using a previously reported pO_2 distribution in rat retinal vasculatures.⁸ The variables a_1 and b_1 , and then the intensity images were created based on the phosphorescence lifetime imaging model. An i.i.d. Gaussian noise was added at different SNR values to the intensity images to simulate the noise effect on the real measurements. The MAE performance of RDLS method was compared with the RLS method and LS method. We used MAE to compare performances of the methods since it is less sensitive to the outliers. It is worthwhile to mention that we could not optimize with respect to MAE since the ultimate interest of variable pO_2 cannot be written as a function of observed intensity values. Monte Carlo simulations were used to compare bias and variance performances of the methods at a constant noise level, where the pO_2 maps were computed for 100 repetitive simulations and the bias and variance of these 100 estimates were calculated. The simulated pO_2 map and its estimation by the LS, RLS, and RDLS methods in the presence of noise with 20 dB SNR are shown in Fig. 1. The maps estimated by the LS, RLS, and RDLS methods were chosen since they give the same MAE values as their average MAE values calculated from 100 repetitive estimates. The effect of noise on LS estimates is clearly visible in the form of spikes in the estimated pO_2 map as expected. However, the pO_2 map estimated by the RLS method is piecewise smooth and significantly improved as compared with LS estimate, but inferior as compared to the RDLS method, especially in the artery. Furthermore, the RDLS method provides pO_2 maps that closely resemble to the ground truth, unlike the RLS and LS methods. MAE of the LS method in retinal vessels pO_2 estimation is 11.5 mmHg. The RLS method significantly improved it by generating 1.82 mmHg MAE. The RDLS gave the best result among all methods as its MAE is 0.82 mmHg. The bias and variance performance of the methods obtained from 100 independent estimates were also compared and the results are shown in Figs. 2 and 3, respectively. The RDLS method generated smaller variance value than the RLS method although the RLS method was able to considerably decrease the variance produced by the LS method. The average standard deviation value generated by the LS method is 15.5 mmHg. The average standard deviation value of the RLS method is 2.4 mmHg. The RDLS method generated the smallest average standard deviation value, 1.1 mmHg. The bias performance of the RLS and RDLS methods were close whereas the bias generated by the LS method was significantly higher. The average bias of LS method is 2.54 mmHg. The average bias generated by the RLS method is 0.14 mmHg and by the RDLS method is 0.19 mmHg. In addition to these performance evaluations of 2-D pO_2 maps, we also compared performances of the 1-D profile of oxygen tension maps estimated by RLS, and the RDLS methods in Fig. 4. The 1-D profiles were generated from the estimated pO_2 maps shown in Fig. 1 by extracting pO_2 values along the middle of retinal arteries. Since in the simulation oxygen tension was modeled to decrease along the artery based on a previously published pO_2 distribution in a rat,⁸ we observed that the RDLS method was able

to track this change in arterial pO_2 more successfully than the RLS method. MAE of RDLS method is 1.2 mmHg whereas MAE of RLS method is 2.4 mmHg. For different noise variance values between 15 and 30 dB, MAE performance of the methods are compared in Fig. 5. The RDLS method had the highest performance at all SNR values. The difference becomes less notable as SNR gets larger, as expected. MAE of RDLS method at 20 dB is 0.84 mmHg while MAE of RLS method at 20 dB is 1.82 mmHg. We also generated pO_2 maps from real data obtained from rat eyes. The experimental data is obtained using the same set up as before.⁸ We compared the performance of proposed RDLS method with the currently available methods. Oxygen tension maps estimated by LS, RLS, and the RDLS methods are shown in Figs. 6, 7, and 8. The data contains one artery and one vein. The estimates of LS method are spiky and the effect of noise is more predominant in the arterial estimates, as expected because the probe is less sensitive under high oxygen tension conditions and therefore images are more prone to noise. The RLS method considerably suppresses the effect of noise as compared to the LS method, though the image appears piecewise smooth. The RDLS method provides pO_2 maps that are the most acceptable since unpleasant local variations, still exist in pO_2 maps estimated by the RLS method, are observed the least in pO_2 maps estimated by the RDLS method. 1-D profiles of arterial oxygen tension along the retinal artery were also generated. Oxygen tension along the arteries is expected to decrease according to the relative distance from the optic nerve head.⁸ 1-D profiles based on oxygen tension maps generated using the RDLS method displayed previously reported decrease in oxygen tension along arteries⁸ better than the RLS method as shown in Figs. 7 and 9. 1-D profiles of the RLS method did not necessarily decrease according to the relative distance from the optic nerve head. As shown in Figs. 7 and 9, we see steps in the beginning and end of profiles of arterial oxygen tension estimated by the RDLS method though we should see a pure linear decrease along the artery. The proposed RDLS method improved the results of LS and RLS methods but not yet to an extent of meeting the theoretical expectations perfectly. The mean values of pO_2 estimates obtained by the LS, RLS and RDLS methods under 21% oxygen breathing condition are listed in Table 1. The mean pO_2 values estimated in retinal arteries and veins by the LS and RDLS methods do not differ significantly $p>0.8$, which shows that the RDLS method does not produce a noticeable bias, similar to the RLS method. Sample standard deviation of pO_2 values for small regions, whose length was chosen 10 μm as before¹² and width was chosen equal to the vessel width, over the arteries and veins was computed. The average of the sample standard deviation of pO_2 values in retinal arteries and veins calculated for 21% oxygen breathing condition are given in Table 2. Average standard deviation of pO_2 values in retinal arteries and veins generated by the RDLS method was $(0.15 \pm 0.16 \text{ mmHg}; N = 8)$ and was significantly ($p>0.001$) lower than the variance measured by the LS method $(7.6 \pm 3.1 \text{ mmHg}; N = 8)$ and by the RLS method $(1.9 \pm 1 \text{ mmHg}; N = 10)$ under normoxia (21% breathing condition).

DISCUSSION

Availability of oxygen is important for normal metabolic function of the retinal tissue. An abnormality in the level of retinal oxygenation is potentially an important cue to the onset or presence of some common retinal diseases. However, current knowledge of oxygen's

function in many retinal diseases is incomplete. Accurate assessment of oxygen tension in the retinal vasculature is therefore important to establish the role of oxygen in the development of retinal disease and to aid in the onset or presence of hypoxia. In past work oxygen tension was obtained using the classical least squares (LS) estimate of intermediate variables whose noise-contaminated linear combinations were observed as phosphorescence intensity images. The LS method produces high variance estimates of oxygen tension that are outside of the physiological range, impeding a reliable analysis of retinal oxygenation. An improved estimation method was proposed later for assessing oxygen tension in retinal blood vessels by regularizing the LS estimates of the intermediate variables. A regularized LS (RLS) method, implemented with an iterative algorithm to minimize a regularized LS cost function, was shown to yield estimates whose variance is lower than that obtained with the classical LS method and which are more robust to the presence of noise. The classical and the regularized LS (RLS) estimates of intermediate variables do not, however, yield an optimum estimate of oxygen tension due to its nonlinear dependence on the ratio of intermediate variables. In this work, for the problem of estimating oxygen tension values in retinal blood vessels, we proposed the method of spatially regularized direct LS (RDLS) estimation in which the cost function is defined directly in terms of oxygen tension instead of intermediate variables and where results of studies that provide models of oxygen tension in retinal arteries and veins are exploited. The RDLS method produced more reliable oxygen tension estimates so that an increased fraction of the estimates fall within the previously reported range¹³ and with a significantly reduced variance compared with that of the classical LS and RLS estimates. This performance improvement is achieved without introducing a significant bias so that the mean oxygen tension values do not differ significantly from those obtained with the LS estimates of large retinal vessels. We illustrated the robustness of the proposed procedure to different levels of noise by means of using a simulated data set and its efficiency in decreasing the variance of the LS and RLS estimates without altering the mean significantly for the experimental data set. The method was motivated by the need to estimate oxygen tension values directly using the priors and measurements unlike the previous approaches that obtained oxygen tension values indirectly by estimating the model parameters. We tested the performance of the proposed method comprehensively by comparing it with the currently available methods. We have shown that the method improved the results of the previous approaches, which obtained oxygen tension values indirectly from the estimates of the model parameters. Abnormalities in retinal oxygen delivery and consumption are thought to play a significant role in retinal diseases, such as diabetic retinopathy and vascular occlusions. Application of the method presented in this study for improved oxygen tension measurements in the retinal vasculatures will provide information on fundamental mechanisms that implicate hypoxia in the development of retinal pathologies. This information is greatly needed to broaden knowledge of disease pathophysiology, and thereby advance diagnostic and therapeutic procedures.

CONCLUSION

In this paper, we developed a method to regularize the LS estimates of oxygen tension values by applying regularization directly to the physical parameter of pO_2 maps. The proposed method was developed based on prior knowledge about oxygen tension

distribution along retinal veins and arteries. We have shown that the proposed RDLS method provides improved and more reliable results compared to the existing approaches which obtain oxygen tension values from the estimates of intermediate parameters. In this work, we considered estimation of oxygen tension values in retinal veins and arteries. We used results of studies, when developing the proposed method, performed earlier for the analysis of oxygen tension distribution in retinal veins and arteries. We plan to apply the ideas proposed in this paper to the estimation of oxygen tension gradients along retinal blood vessels, thus providing information on retinal tissue oxygen extraction.

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FIGURE 1.

The original simulated oxygen tension map (38×80 pixels, equivalent to $76 \times 160 \mu\text{m}$) (left-top) and the estimated maps in the presence of noise with 20 dB SNR using the LS (right-top), RLS (left-bottom), and RDLS (right-bottom) methods, respectively. V and A denote retinal vein and artery, respectively. The color bars represent oxygen tension values in mmHg.

FIGURE 2.

Bias values of the simulated oxygen tension map (38 × 80 pixels, equivalent to 76 × 160 μm), computed for 100 repetitive Monte-Carlo simulations, of the LS (top), RLS (middle), and RDLS methods. V and A denote retinal vein and artery, respectively. The color bar represents relative bias values in mmHg.

FIGURE 3.

Standard deviation values of the simulated oxygen tension map (38×80 pixels, equivalent to $76 \times 160 \mu\text{m}$), computed for 100 repetitive Monte-Carlo simulations, of the LS (top), RLS (middle), and RDLS methods. V and A denote retinal vein and artery, respectively. The color bar represents standard deviation values in mmHg.

FIGURE 4.

1-D profile, generated from the estimated oxygen tension maps shown in Fig. 1 by extracting oxygen tension values along the middle of retinal arteries of the arterial oxygen tension values, of the original simulated oxygen tension map (dotted), RLS (dashed) and RDLS (solid) methods.

FIGURE 5.

The mean absolute error (MAE) of the oxygen tension maps estimated by using the LS (dotted), RLS (dashed), and RDLS (solid) methods for different SNR values between 15 and 30 dB.

FIGURE 6.

Oxygen tension maps (61×36 pixels, equivalent to $305 \times 180 \mu\text{m}$) of a rat generated using the LS (left), RLS (middle) and RDLS (right) methods. V and A denote retinal vein and artery, respectively. The color bar represents estimated oxygen tension values in mmHg.

FIGURE 7.

1-D profile, generated from the estimated oxygen tension maps of the rat shown in Fig. 6 by extracting oxygen tension values along the middle of retinal arteries of the arterial oxygen tension values, of the RLS (dashed) and RDLS (solid) methods.

FIGURE 8.

Oxygen tension maps (210×140 pixels, equivalent to $420 \times 280 \mu\text{m}$) of a rat generated using the LS (left), RLS (middle) and RDLS (right) methods. V and A denote retinal vein and artery, respectively. The color bar represents estimated oxygen tension values in mmHg.

FIGURE 9.

1-D profile, generated from the estimated oxygen tension maps of the rat shown in Fig. 8 by extracting oxygen tension values along the middle of retinal arteries of the arterial oxygen tension values, of the RLS (dashed) and RDLS (solid) methods.

TABLE 1

The mean values of oxygen tension estimates in retinal arteries and veins obtained by the LS, RLS, and RDLS methods under 21% oxygen breathing condition.

Animal	Retinal blood vessel	Mean pO_2 by the LS in mmHg	Mean pO_2 by the RLS in mmHg	Mean pO_2 by the RDLS in mmHg
Rat1	Artery	62.5	61.8	60
	Vein	27.9	26.9	27.5
Rat2	Artery	33.2	33.2	32.1
	Vein	26.9	26.4	25.4
Rat3	Artery	35.2	34.3	33
	Vein	28.5	27.8	26.9
Rat4	Artery	44.3	45	43.3
	Vein	35	34.6	34.3

TABLE 2

The average of the sample standard deviation of oxygen tension estimates in retinal arteries and veins calculated for 21% oxygen breathing condition.

Animal	Retinal blood vessel	SD pO_2 by the LS in mmHg	SD pO_2 by the RLS in mmHg	SD pO_2 by the RDLS in mmHg
Rat1	Artery	14.6	1.6	0.4
	Vein	6.5	0.7	0.03
Rat2	Artery	7.7	3	0.13
	Vein	6.8	2.8	0.4
Rat3	Artery	8.1	3.2	0.06
	Vein	6.1	2.4	0.05
Rat4	Artery	6.7	1.1	0.14
	Vein	4.3	0.7	0.02