Quantifying spectroscopic concentration ratio by polarization subtraction technique

Bo Lu (吕 博)[1] and Jiwei Wu (吴继伟)[2*]

[1]*Department of Electronics and Information Engineering, Shanghai Normal University Tianhua College, Shanghai 201815, China*

[2]*School of Electronics and Information Engineering, Tongji University, Shanghai 201804, China*

*Corresponding author: wujiwei@tongji.edu.cn

Nonlinear correlation between attenuation and absorption due to the presence of scattering is the main reason for inaccurate spectroscopic quantitative investigations. The polarization subtraction methods are applied to reduce the scattering in order to linearise attenuation to absorption. Monte Carlo simulation shows that the polarized light offers better performance than unpolarized light at giving the most accurate estimate of the concentration ratio of absorbers using the modified Lambert-Beer law. Our results demonstrate that spectrophotometry with polarized technique offers the potential to be a simple and cost-effective system.

OCIS codes: 300.0300, 290.0290.

doi: 10.3788/COL201311.S23001.

Optical spectroscopy of biological tissue is a fast growing field as it provides a noninvasive tool in medical and biological applications, such as the blood volume and oxygenation detection for interrogating tissue tumours. However, the highly scattering properties of tissue cause inaccuracies in spectroscopic measurement and analysis resulting from the nonlinear relationship between attenuation and absorption. This is due to light being heavily scattered along many random paths. Conventional analytical methods for spectrophotometry are based on the Lambert-Beer law, but it is no longer valid in the presence of scattering as the path length is scattered longer than the source-detector spacing. However, if the scattering problems can be overcome then spectroscopic information can be accurately measured.

Mathematical pre-processing techniques have been previously applied to compensate for the effects of scattering[1−3]. Delpy *et al.*[3] have proposed a linear model to approximate the nonlinear relationship between attenuation and absorption by taking into account the effects of scattering, called the modified Lambert-Beer law. It is widely used in quantifying tissue chromophore concentration with the assumptions that the differential path length and scattering loss is constant in a limited range of the absorption changes and that attenuation (A) is linearly dependent on μ_a.

Experimental techniques for reducing the effects of scattering are well known within the field of biomedical optics where the multiple scattering of tissue makes it difficult to perform tissue optical spectroscopy. In this letter, the polarization subtraction method[4−6] is used to reduce the effects of scattering. The polarization subtraction technique has been widely applied in imaging, spectroscopy and the study of layered media by selecting the weakly scattered photons.

Quantifying the concentrations of absorbers in a scattering medium is a common question in the biological science. Absolute concentrations cannot be obtained as the standard Lambert-Beer law is no longer applicable since there are multiple unknown path lengths. However, it is still possible to estimate concentration changes via the modified Lambert-Beer law, as proposed by Delpy *et al.*[3] with the assumption that the scattering loss is constant with wavelength. The validity of this method depends on the constancy of scattering losses and the accuracy of differential path length factor (DPF). Matcher *et al.*[7] have stated that the linear approximation of the modified Lambert-Beer law is only applicable for small changes in attenuation ΔA with respect to absorption $\Delta \mu_a$.

This letter aims to study whether the application range of the modified Lambert-Beer law can be improved by using the polarization subtraction technique. This letter models the estimation of the ratio of concentrations of two absorbers in order to investigate the benefit of the method in practice. The computer simulation is based on the polarized Monte Carlo Model[8].

The Lambert-Beer's law is the basis of quantitative spectroscopic analysis. Based on the Lambert-Beer law, in the absence of scattering, the attenuation A of a sample is linearly related to the total absorption coefficient, μ_a. That is[8]

$$A = \mu_a \cdot d = \sum_{i=1}^{n} \alpha_i c_i \cdot d, \qquad (1)$$

where α is the extinction coefficient in units of mol/L; c is the concentration of absorber in units of L·mol^{-1}·mm^{-1}; and the source detector distance d is in units of mm. In a multi-absorber medium, the overall μ_a is the linear sum of $(\alpha \cdot c)$ for each absorber.

The dependence of A upon μ_a becomes nonlinear in the presence of scattering. The simulation performed in this letter is based on the modified Lambert-Beer law[3], which is widely used in near-infrared (NIR) spectroscopy analysis and is expressed as

$$A = \alpha \cdot c \cdot \mathrm{DP} + G, \qquad (2)$$

where DP is the differential path length and is proved to be the gradient of A with respect to μ_a[9],

$$DP = v < t > = \Delta A / \Delta \mu_a, \tag{3}$$

where v is the speed of light in the medium and $< t >$ is the mean flight time. In the absence of scattering DP is equal to the slope of the A versus μ_a curve, i.e., DP $= d$. In the presence of scattering the DP is a nonlinear function of both the reduced scattering coefficient ($\mu'_s = \mu_s(1-g)$) and the absorption coefficient (μ_a) which are both wavelength dependent[7]. In Eq. (2) G is the attenuation caused by scattering only.

Figure 1 shows the plots of A versus μ_a and the modified Lambert-Beer law schematically. The standard Lambert-Beer law is valid at $\mu_s = 0$ and the relationship between A and μ_a is linear with the slope of d from Eq. (1). The A versus μ_a plots become more nonlinear with increasing μ_s.

The essence of the modified Lambert-Beer law is to approximate the nonlinear A versus μ_a graph with a straight line. This linear approximation, $A = \mu_a \cdot$ DP $+ G$, is the modified Lambert-Beer law used to approximate the nonlinear A versus μ_a relationship. DP and G are the gradient and the intercept of the straight lines, respectively. It is only a reasonable approximation over a limited range of μ_a and depends on the chosen μ_a range. At high μ_a (such as μ_{a2} in Fig. 1) the gradient DP is close to the slope at $\mu_s = 0$, i.e., $\Delta A_2 / \Delta \mu_{a2} \to d$, but G is much larger than A_s. At low μ_a (such as μ_{a1} in Fig. 1) the intercept G is close to the real scattering losses A_s, i.e., $G_1 \to A_s$, but DP is greater than d. If applying the modified Lambert-Beer law, for example to measure a concentration ratio, the trade-off between G and DP related to the range of μ_a will influence the error.

The polarization method is based on the property of polarization memory of light[4]. Scattering randomizes the polarization state and therefore photons maintaining the original polarization states have travelled shorter path lengths and undertaken less scattering events. Schmitt et al.[5] have discriminated short path length transmitted light by detecting circular polarization maintaining light.
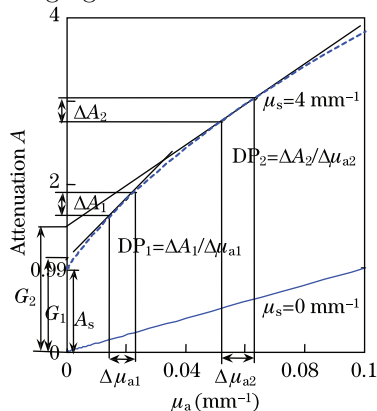

Fig. 1. (Color online) Plots of the A versus μ_a for a slab of 10-mm thickness at different scattering coefficients, i.e., μ_s = 0 (solid line), and 4 mm^{-1} (dashed line). The anisotropy factor is $g = 0.9$. The A versus μ_a curve becomes more non-linear with increasing μ_s. The modified Lambert-Beer law is the straight line with gradient DP and intercept G with the A axis, i.e., $A = \mu_a \cdot$ DP $+ G$.

Polarized light scattering spectroscopy has been used for *in situ* probing of living epithelial cells by using polarized light to discriminate the single backscattered light. This was achieved from a multiply scattered background light from a layered medium via the subtraction of the backscattered co-polarized and cross-polarized light[6].

The polarization subtraction technique is applied in this letter in an attempt to improve the accuracy of quantitative spectroscopy analysis (in model studies). The nonlinear relationship between attenuation and absorption in scattering media can be made less nonlinear through its isolation of short path photons which are more constrained in their path lengths.

A general and accurate determination of concentration ratios of two absorbers can be obtained via the principle of similar triangles as shown in Fig. 2,

$$\frac{A_1^* - G}{A_2 - G} = \frac{\mu_{a1}}{\mu_{a2}} = \frac{\alpha_1 \cdot c_1}{\alpha_2 \cdot c_2}, \tag{4}$$

where A_1^*, A_2, and G are the points on the straight line determined by A_2 and A_3 based on the modified Lambert-Beer law, and $\mu_{a1} = \alpha_1 c_1$ and $\mu_{a2} = \alpha_2 c_2$. Therefore, the concentration ratio is

$$\frac{c_1}{c_2} = \frac{A_1^* - G}{A_2 - G} \cdot \frac{\alpha_2}{\alpha_1}. \tag{5}$$

Equation (5) provides an accurate expression of the concentration ratio. Whilst the value of attenuation obtained from the measurement corresponding to absorption μ_{a1} is A_1 rather than A_1^*. Therefore, the accuracy of the concentration ratio depends on the difference between A_1^* and A_1, i.e., $A_1^* - A_1$. In Eq. (5), α_2/α_1 is the ratio of extinction coefficients of two absorbers.

In order to compare the A_1 with A_1^*, another expression for A_1 should be employed. The nonlinear relationship between attenuation and absorption can be mathematically approximated by

$$A_1 = A_s + K_1 \mu_a - K_2 \mu_a^2, \tag{6}$$

where A_s is the real scattering loss, K_1 is equal to $c < t >$ and K_2 is equal to $\sigma^2/2$, where $c < t >$ is the mean path length and σ is the variance of the path length. Crowe et al.[10] have given out detailed description of this. Therefore, A_1 can be expressed by Eq. (6), whilst A_1^* is determined by the modified Lambert-Beer law (Eq. (2)).

$$A_1^* = G + \mu_a \cdot DP. \tag{7}$$

It should be noted that unknown factors G and DP are determined by A_2 and A_3 using the modified Lambert-Beer law. Therefore, the difference between A_1^* and A_1 is

$$A_1^* - A_1 = K_2^*(\mu_{a2}\mu_{a3} - \mu_{a2}\mu_{a1} - \mu_{a1}\mu_{a3} + \mu_{a1}^2). \tag{8}$$

The difference between A_1^* and A_1 is dependent on K_2 and the selection of absorption μ_{a1}, μ_{a2} and μ_{a3}. K_2 has a directly linear effect on the difference between A_1 and A_1^*, that is if $K_2 = 0$, $A_1^* = A_1$. A_s, K_2 is directly related to the variance of path length or time point spread function (TPSF) ($K_2 = \sigma^2/2$), then a narrower TPSF means that A_1 is closer to A_1^* and therefore a more accurate estimate of the concentration ratio is estimated. The effects of the μ_a values are more convoluted, although the effects
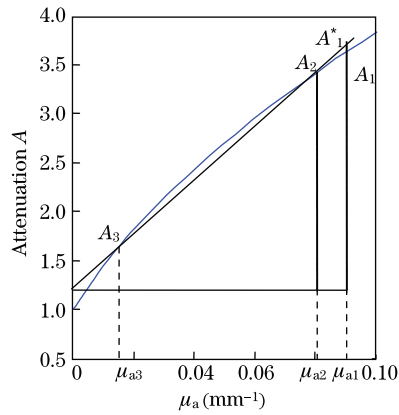
Fig. 2. (Color online) Plots of A versus μ_a. Points A_1, A_2, and A_3 are obtained from simulation corresponding to μ_{a1}, μ_{a2}, and μ_{a3}, respectively. The straight line through A_2 and A_3 is determined from the modified Lambert-Beer law, i.e., $A = \mu_a \cdot$ DP $+ G$. The point A_1^* is on the straight line. Both A_1 and A_1^* correspond to μ_{a1}. The ratio of concentrations of two absorbers is determined through the similar triangle relationship between G, A_1, A_2, μ_{a1} and μ_{a2}. The geometrical triangle relationship is drawn in blue.
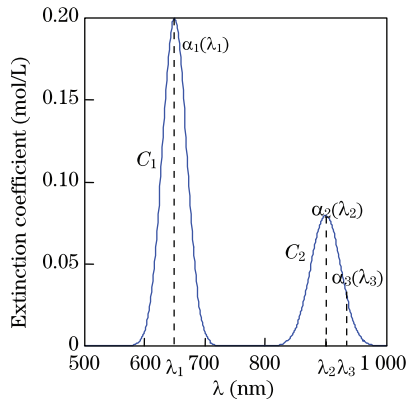

Fig. 3. (Color online) Plots of non-overlapping extinction spectra of two absorbers.

of μ_{a2} and μ_{a3} on the error are symmetrical.

The advantage of the linearised system is that the optical path length is localized, resulting in a narrower TPSF and so smaller K_2 (variance of TPSF). Therefore, it is expected that for a linearised system the concentration ratio is more accurate than a nonlinearised system and the accuracy is independent of the selection of μ_a.

The simulation is run under the assumption that the extinction spectra of the two absorbers presented in the sample follow Gaussian distributions that do not overlap. The two Gaussian distributions have variances of 20 and 25 corresponding to c_1 and c_2, respectively. The concentration ratio of two absorbers is set to be $c_1/c_2 = 10/5 = 2$. The extinction spectra ($\alpha(\lambda)$) are shown in Fig. 3 and the absorption spectra ($\mu_a(\lambda)$) are shown in Fig. 4(c). The attenuation of transmitted light through a 10-mm-thick semi-infinite slab at $\mu_s' = 0.5$ mm^{-1} is calculated for the cases of the unpolarized, circularly polarized, and linearly polarized light, respectively. The attenuation spectra are distorted and do not follow the original Gaussian distributions due to the presence of scattering (Fig. 4(a)) and nonlinear dependence of A upon μ_a. The A versus μ_a plots are made more linear by

linearly and circularly polarized light (Fig. 4(b)). Hence the modified Lambert-Beer law is a closer estimate to reality.

The concentration ratio is estimated using Eq. (5). Figure 5 shows the simulation results of the concentration ratio (c_1/c_2) versus μ_a for linearly polarized, circularly polarized and unpolarized light. Changing μ_a is for the purpose of changing the accuracy of linear approximation obtained from the modified Lambert-Beer law.

From Eq. (8), the error size of the estimation linearly depends on the variance of path lengths or TPSF and in addition the accuracy of estimation shows weak dependence on μ_a for small variance system. As expected, linearly polarized light gives the most accurate estimate of c_1/c_2 at each μ_a. Circularly polarized light presents the intermediate performances and followed by unpolarized light. This is because linearly polarized light gives the narrowest TPSF and followed by circularly polarized light and unpolarized light. Whilst for a system with big TPSF variance (unpolarized light) the accuracy of the
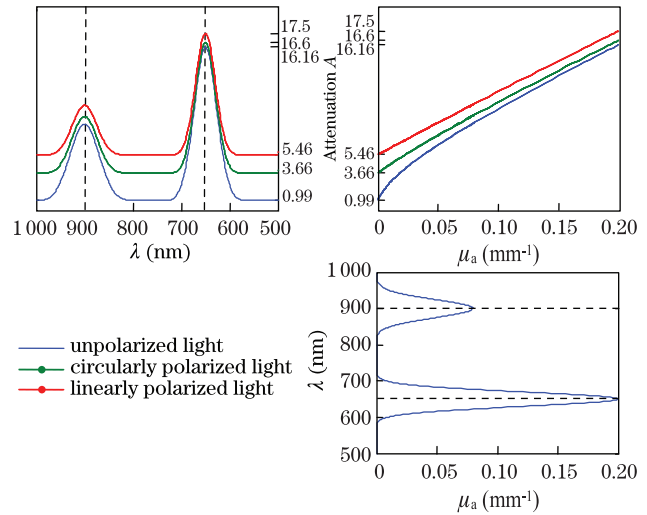

Fig. 4. (Color online) Plots of transmitted light spectra through a 10-mm thick semi-infinite slab for $\mu_s' = 0.5$ mm^{-1} (a) A versus λ, (b) A versus μ_a, (c) μ_a versus. λ. The spectra of unpolarized, circularly polarized, and linearly polarized light are shown as a solid line, dashed line, and dotted line, respectively.
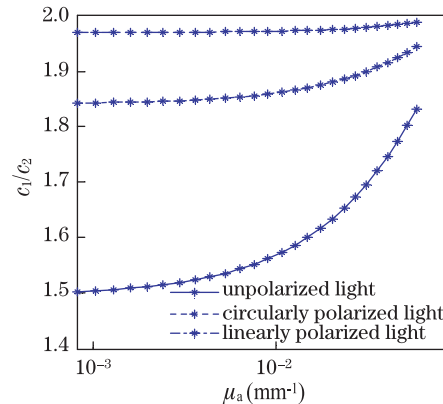

Fig. 5. (Color online) The concentration ratio of two absorbers at different μ_a for unpolarized (solid line), circularly polarized (dashed line), and linearly polarized (dotted line), respectively.

estimate is strongly dependent on the selection of μ_{a}. Figure 5 shows that for unpolarized light more accurate estimates are obtained at higher μ_{a}. This is because a closer value of A_1 to A_1^* is obtained at higher μ_{a}.

It should be noted that the concentration ratio is determined by the selection of absorption ($\mu_{\mathrm{a}1}$, $\mu_{\mathrm{a}2}$, and $\mu_{\mathrm{a}3}$) corresponding to three wavelengths (λ_1, λ_2, and λ_3). In this letter, only one absorption ($\mu_{\mathrm{a}3}$) is changed to show the effects of absorption. This is because the effects of $\mu_{\mathrm{a}2}$ and $\mu_{\mathrm{a}3}$ to the estimate are symmetrical as discussed upward. It is obvious that the closer of $\mu_{\mathrm{a}1}$ to $\mu_{\mathrm{a}2}$ the more accurate estimate can be obtained when the straight line of the modified Lambert-Beer law is fixed.

In conclusion, simulation results show that polarized light gives a more accurate estimation of concentration ratios than unpolarized light and the accuracy of the estimation is independent of the selection of absorption. A simple case is considered where two absorbers have non-overlapping extinction spectra. Factors affecting the accuracy of the estimation of concentration ratios of two absorbers are analyzed analytically. The estimate of concentration ratios depends on the variances of optical path length or TPSF and the selection of absorption. The accuracy of the estimate has a linear dependence on the variance. The polarization subtraction method improves the localization of the optical path lengths resulting in a small variance of the TPSF. As a result,

polarized light offers a better performance over unpolarized light. For unpolarized light (with wider variance), the accuracy of the estimate of concentration ratios more depends on the selection of absorption.

References

1. L. Stordrange, F. O. Libnau, D. Malthe-Sørenssen, and O. M. Kvalheim, J. Chemometrics **16,** 529 (2002).
2. P. Geladi, D. MacDougall, and H. Martens, Appl. Spectrosc. **39,** 491 (1985).
3. D. T. Delpy, M. Cope, P. van der Zee, S. Arridge, S. Wray, and J. Wyatt, Phys. Med. Biol. **33,** 1433 (1988).
4. F. C. MacKintosh, J. X. Zhu, D. J. Pine, and D. A. Weitz, Phys. Rev. B. **40,** 9342 (1989).
5. J. M. Schmitt, H. Gandjbakhche, and R. F. Bonner, Appl. Opt. **31,** 6535 (1992).
6. V. Backman, R. Gurjar, K. Badizadegan, I. Itzkan, R. R. Dasari, L. T. Perelman, and M. S. Feld, IEEE J. Quantum Electron. **5,** 1019 (1999).
7. S. J. Matcher, M. Cope, and D. T. Delpy, Phys. Med. Biol. **39,** 177 (1994).
8. B. Lu, S. P. Morgan, J. A. Crowe, and I. M. Stockford, Appl. Spectrosc. **60,** 1157 (2006).
9. S. R. Arridge, M. Cope, and D. T. Delpy, Phys. Med. Biol. **37,** 1531 (1992).
10. J. Crowe, D. Morris, M. Woolfson, P. Rodmell, and J. Walker, J. Opt. Soc. Am. A **24,** 1969 (2007).