In vivo measurement of absorption coefficient (μ_a) in rat brain and statistic analysis

Zhiyu Qian (钱志余)[1] Yueqing Gu (顾月清)[2]

[1] *Depart of Biomedical Engineering, Nanjing University of Aeronautics and Astronautics, Nanjing 210016*

[2] *School of Life Science and Technology, China Pharmaceutical University, Nanjing 210009*

A specific designed steady-state fiber spectrometer system was applied to measure the optical parameters in rat brain tissue. The reduced scattering coefficient (μ_s') spectrum was obtained from the responding empirical formula, while the absorption coefficient spectrum can be fitted by a unique diffusion theoretical model in near infrared range (650–850 nm). 12 rats were performed *in vivo* in real time measurements. The range of absorption coefficient (μ_a) in gray matter and white matter of rat brain tissue were obtained from the systemic statistic analysis by applying the empirical formula and theoretical model, and the results were evaluated by tissue phantom experiment. These data have great value in research and clinic applications.

OCIS codes: 300.6340, 170.6280, 260.1960, 290.5820.

The scattering and absorption characteristics of different kinds of tissue have been reported by Cheong *et al.*[1], with most of the data measured in vitro. Actually the *in vitro* data are much different from that of *in vivo*, due to the variation of the blood drainage, structural alteration, and temperature changes, we cannot use the *in vitro* data to estimate the optical properties of biological tissue.

During the past 20 years, several groups have derived expressions for the steady-state diffuse reflectance that is based on diffusion approximations to radiative transport theory[2−5]. The results from diffusion theory can be matched well with predicting value for long source-detector separation ($d \gg$ MFP (mean free path)). However, Monte-Carlo simulation by Michael *et al.*[6] had proved large errors induced by applying the diffusion theory to a small source-detector separation probe (< MFP). Farrell *et al.*[7] reported a analytic model for the radically resolved diffuse reflectance emitted from the surface of a semi-infinite scattering and absorption medium illuminated by a pencil beam of continuous wave (CW) radiation in 1992. It seems can be used in the small source-detector separation (Farrell *et al.* 1–10 mm) in our research. We modified the Farrell's equation to fit 400-μm separation. Determining the reduced scattering and absorption coefficients in vivo has been reported based on time- and frequency-domain techniques, which estimates the optical path length from analysis of temporal pulse broadening[8,9] or analytic diffusion equation solution first published by Patterson *et al.*[10,11].

In this study, we developed an equation regarding μ_s' on tissue in variance with wavelength by fitting a bunch of tissue phantom data, and further verified it with standard phantom. Meanwhile, a new model for calculation of absorption coefficient on a small source-detector separation was derived by modifying Farrell's theory.

To develop diffusion reflectance model, we assumed biological tissue to be a homogeneous semi-infinite turbid medium with certain reduced scattering and absorption coefficient $\mu_s'(\lambda)$, $\mu_a(\lambda)$ respectively (λ is the wavelength of light). Part of incident light is absorbed in the tissue, whereas a certain fraction of scattering light is collected by probe, the amount of light collected depends on the optical properties $\mu_s'(\lambda)$, $\mu_a(\lambda)$ and the probe size. We employ an expression derived by Farrel *et al.* who calculated the diffuse reflectance from a narrow beam of light incident on the surface of a semi-infinite turbid medium in the diffusion approximation. The reflectance radial density $R(\lambda, r)$ at a distance r from the point of incident is

$$R(\lambda, r) = \frac{I_0}{4\pi}[z_0(\mu_{\text{eff}} + \frac{1}{r_1})\frac{\exp(-\mu_{\text{eff}} r_1)}{r_1^2} + (z_0 + 4AD)(\mu_{\text{eff}} + \frac{1}{r_2})\frac{\exp(-\mu_{\text{eff}} r_2)}{r_2^2}], \quad (1)$$

where $r_1 = \sqrt{z_0^2 + r^2}$, $r_2 = \sqrt{(z_0^+ 4AD)^2 + r^2}$, $D = \frac{1}{3(\mu_a + \mu_s')}$, $\mu_{\text{eff}} = \sqrt{\mu_a / D}$, $Z_0 = \frac{1}{\mu_a + \mu_s'}$, A is a constant related to the internal reflection of medium ($A = 1$ for $n_{\text{rel}} = 1$, and $A > 1$ for $n_{\text{rel}} > 1$, $n_{\text{rel}} = n_{\text{tissue}}/n_{\text{air}}$), in our research we use $A = 1$ for the index match tissue. I_0 is a coefficient depend on the reduced scattering coefficient and probe size, this is a factor for the equation.

In the small separation situation, take the limit of small $r \to 0$ and obtain[12−14]

$$R(\lambda, 0) = \frac{I_0 \mu_s'^2}{4\pi}\{(z_0\mu_{\text{eff}} + 1)\exp(-z_0\mu_{\text{eff}}) + \frac{1}{(1 + 4A/3)^2}[1 + (1 + 4A/3)z_0\mu_{\text{eff}}] \exp(-z_0\mu_{\text{eff}}(1 + 4A/3))\}. \quad (2)$$

The first term of Eq. (2) is larger 90% than the second term when the source-detector separation is less than 0.5 mm for brain tissue $\mu_s'(\lambda) < 3$ mm^{-1}[13], and the separation of our probes is 0.4 mm, so the second term of Eq. (2) can be ignored, a simplified diffusion model for the steady-state broadband reflectance measured with $r < 0.5$ mm can be obtained approximately as

$$R(\lambda, 0) = \frac{I_0 \mu_s'^2}{4\pi}(z_0\mu_{\text{eff}} + 1)\exp(-z_0\mu_{\text{eff}}). \quad (3)$$

In the brain tissue, the reduced scattering coefficient is larger at least 10 times than absorption coefficient in spectrum range 650–850 nm, but the absorption coefficient is large in the 400–650 nm. To obtain the total light collected by the probe, Eq. (3) must be integrated

over the spatial extent of the light delivery and collection areas. The delivery and collection area radii are $r_{\rm d}$ and $r_{\rm c}$ respectively. Assuming the incident light intensity to be uniform over the entire delivery area, the diffuse reflectance $R(\lambda, \mu_{\rm s}', \mu_{\rm a}, r_{\rm d}, r_{\rm c})$ collected by the probe is given by

$$R(\lambda, \mu_{\rm s}', \mu_{\rm a}, r_{\rm d}, r_{\rm c}) = \frac{1}{r_{\rm d}^2}\int_0^{r_{\rm c}} r{\rm d}r \int_0^{2\pi} {\rm d}\Phi \int_0^{r_{\rm d}} R(\lambda, |r- r'|)r'{\rm d}r', \quad (4)$$

with $|r - r'| = (r^2 + r'^2 - 2rr'\cos\Phi)^{1/2}$, from Eq. (3), for the same reduced scattering and absorption coefficient, R is constant for the result from Eq. (3), so we can got the following equation for the integration

$$R(\lambda, \mu_{\rm s}', \mu_{\rm a}, r_{\rm d}, r_{\rm c}) = R(\lambda, 0)\{\frac{1}{r_d^2}\int_0^{r_{\rm c}} r{\rm d}r \int_0^{2\pi} {\rm d}\Phi \int_0^{r_{\rm d}} r'{\rm d}r'\}. \quad (5)$$

The factor I_0 of Eq. (3), we have discussed, is a factor for different probe and different tissue, which can be fitted from the intralipid and phantom experiment. We can just set a function $\Psi(I_0)$ which contains integration term, just as

$$\Psi(I_0) = \frac{I_0}{r_{\rm d}^2}\int_0^{r_{\rm c}} r{\rm d}r \int_0^{2\pi} {\rm d}\Phi \int_0^{r_{\rm d}} r'{\rm d}r', \quad (6)$$

so the final model for the brain tissue, the reflectance spectrum from the detecting spectrum can be got by Eq. (7), the R should respond to the different wavelength, this is the spectrum of the detecting signal:

$$R(\lambda, \mu_{\rm s}', \mu_{\rm a}, r_{\rm d}, r_{\rm c}) = \frac{\Psi(I_0)\mu_{\rm s}'^2(\lambda)}{4\pi}(z_0(\lambda)\mu_{\rm eff}(\lambda) + 1)\exp(-z_0(\lambda)\mu_{\rm eff}(\lambda)). \quad (7)$$

The equation just can be used in the small source-detector separation, and the reduced scattering coefficient should be less 30 mm^{-1}, actually, the errors from the reduced scattering limitation can be decreased by the fitting function.

The fitting equations of wavelength 750 and 830 nm were got by the power fitting. These equations will be used only for the 100-μm probe.

From Eq. (7), if we want to calculate the absorption coefficient, the function $\Psi(I_0)$ must be fitted out from intralipid or phantom experiment, as shown in Fig. 1. We have got the reduced scattering coefficient fitting equation, the absorption coefficient spectrum can be calculated by Eq. (7). From the fitting profiles, we can see the two wavelength profiles almost the same relation, so we can use one fitting equation for all wavelength (500–1000 nm), and the error is small (error 3%–10%: $0.01 < \mu_{\rm s}' < 3$ cm^{-1}; error 0.01%–3%: $\mu_{\rm s}' > 3$ cm^{-1}). The average data fitting equation for $\Psi(I_0)$ function is

$$\Psi(I_0) = 0.4579(\mu_{\rm s}')^{-1.0531}. \quad (8)$$

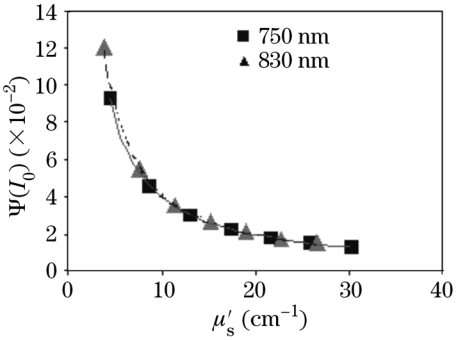

Fig. 1. The reduced scattering coefficient with $\Psi(I_0)$ profile for 100-μm probe from intralipid experiment.

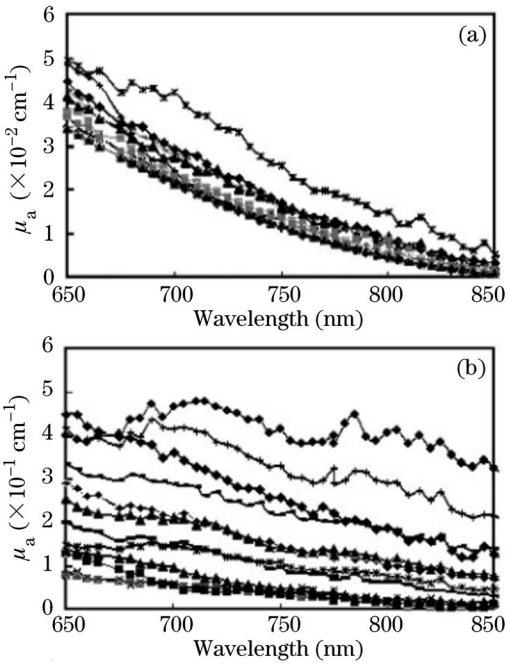

Fig. 2. *In vivo* experiment results for rat brain tissue (100-μm probe). $\mu_{\rm a}$ NIR absorption spectrum of rat brain gray matter (a) and white matter (b).

Table 1. Absorption Coefficient Statistic Results of 12 Rats

	Wavelength (nm)	Average (cm^{-1})	Errors (cm^{-1})	Max (cm^{-1})	Min (cm^{-1})
Gray	650	0.039	0.006	0.049	0.034
Matter	750	0.014	0.004	0.025	0.011
	830	0.003	0.002	0.009	0.001
White	650	0.243	0.133	0.447	0.078
Matter	750	0.155	0.125	0.415	0.032
	830	0.097	0.105	0.353	0.002

So Eq. (7) can be used to calculate for the absorption coefficient after we got the reduced scattering coefficient and reflectance.

The purpose of this research was to assess the performance of spatially resolved reflectance by using short source-detector separations (< 0.5 mm), the reduced scattering coefficient of brain tissue in vivo equation was fitting from the intralipid and phantom experiments, the μ_s' spectrum can be calculated from the reflectance spectrum by the fitting equation. The absorption coefficient spectrum of brain tissue *in vivo* was calculated by the short separation model developed from the diffusion theory, which was calibrated by the intralipid experiment.

The absorption coefficients of 12 rats were measured *in vivo*, and Fig. 2 is the near infrared (NIR) spectrum of rat's gray matter and white matter from experiments. Table 1 is the absorption coefficient statistic results. The absorption results are matched well with that of the Ref. [1], so we can use the steady-state spectrum to measure the scattering coefficient and absorption coefficient, this is a useful method for *in vivo* experiments, the results were verified from the phantom and intralipid experiments, and match well with the animal results.

In conclusion, one new method for measuring absorption coefficient with steady-state spectrometer is introduced, and the fitting equation of 100 micron probe (specific probe of our lab) was given from diffusion theory model. This is a series research *in vivo* of rat brain tissue optical parameters.

References

1. W. F. Cheong, S. A. Prahl, and A. J. Welch, IEEE J. Quantum Electron. **26,** 2166 (1990).
2. L. Reynolds, C. Johnson, and A. Ishimaru, Appl. Opt. **15,** 2059 (1976).
3. R. A. J. Groenhuis, H. A. Ferwerda, and J. J. T. Bosch, Appl. Opt. **22,** 2456 (1983).
4. J. M. Steinake and A. P. Shepherd, J. Opt. Soc. Am. A **5,** 813 (1998).
5. J. J. M. Schmitt, G. X. Zhou, and E. C. Walker, J. Opt. Soc. Am. A **7,** 2141 (1990).
6. M. G. Nichols, E. L. Hull, and T. H. Foster, Appl. Opt. **36,** 93 (1997).
7. T. J. Farrell and M. S. Patterson, Med. Phys. **19,** 880 (1992).
8. B. Chance, J. S. Leigh, H. Miyake, D. S. Smith, S. Nioka, R. Greenfeld, M. Finander, K. Kaufmann, W. Levy, M. Young, P. Cohen, H. Yoshika, and R. Boretsky, Proc. Natl. Acad. Sci. USA **85,** 4971 (1988).
9. D. T. Delpy, M. Cope, P. van der Zee, S. Arridge, S. Wray, and J. Wyatt, Phys. Med. Biol. **33,** 1433 (1988).
10. M. S. Patterson, B. Chance, and B. C. Wilson, Appl. Opt. **28,** 2331 (1989).
11. M. S. Patterson, J. D. Moulton, B. C. Wilson, K. W. Berndt, and J. R. Lakowicz, Appl. Opt. **30,** 4474 (1991).
12. M. Johns, C. A. Giller, and H. Liu, Appl. Spec. **55,** 1686 (2001).
13. M. Johns, C. Giller, and H. Liu, J. Biomed. Opt. **4,** 437 (1998).
14. H. Liu, D. A. Boas, Y. Zhang, A. G. Yadh, and B. Chance, Phys. Med. Biol. **40,** 1983 (1995).