© 2002 OSA/BOSD, AOIMP, TLA 2002

Correction of fluorescence spectra using data from elastic scattering spectroscopy and a modified Beer's law

Ousama M A'Amar and Irving J Bigio
Boston University, Department of Biomedical Engineering, 44 Cummington Street, Boston, MA 02215
Phone: 617-353-0261, Fax: 617-353-1283, email: oaamar@bu.edu

Abstract: We suggest a new method that permits an approximate correction of the spectral distortion of fluorescence due to scattering and absorption. This method is based on the utilization of a modification of Beer's law with data from an elastic scattering spectral measurement.
©2002 Optical Society of America
OCIS codes: (300.2530) Fluorescence, laser-induced

1. Introduction

Light-induced fluorescence spectroscopy (LIFS) is a noninvasive/ minimally-invasive technique that provides clinical information in real time. As various fluorophores may contribute simultaneously to the global fluorescence signal, the real characteristics of each fluorophore cannot be accurately determined. Consequently, the identification of these fluorophores is quite ambiguous. This is mostly due to the superposition of fluorescence emissions and the effect of optical properties of tissue [1,5]. Both effects strongly modify the spectral characteristics of the intrinsic fluorescence. Time-resolved spectroscopy may provide a solution for the superposition effect by decomposing the spectra based on the fluorescence lifetime [1]. However, this technique does not correct the effect of tissue attenuation, which is due to scattering (morphology) and absorption (eg. hemoglobin).
Elastic scattering spectroscopy (ESS), sometimes called diffuse-reflectance spectroscopy, provides spectra that contain information about the morphology of the tissue as well as the chromophore content (e.g. hemoglobin and melanin) [2]. Using the spectral data provided by the ESS, tissue convolution effect can be extracted and used to correct the fluorescence spectra [3-6].
In this work, we use an approximation of Beer's law to compute the total relative attenuation coefficient of light in a turbid medium. Our purpose is to demonstrate that the extracted attenuation coefficient as a function of the wavelength can be used to correct the distortion of fluorescence spectra due to the optical properties of the medium.

2. Beer's law - correction model

Beer's law states that, if light of intensity I_0 travels a distance L through a homogeneous medium with absorption coefficient μ_a, the transmitted light has intensity I_1 given by Eq. (1).

$$I_1 = I_0 \cdot \exp(-\mu_a \cdot L) \qquad (1)$$

In a turbid medium, a modified Beer's law, which takes into account both absorption and scattering events, may be applied to compute the transmitted light intensity [7]. The absorption coefficient μ_a in Beer's law is replaced by the total attenuation coefficient $\mu_t = \mu_a + \mu_s$, where μ_s is the scattering coefficient. Then,

$$\mu_t = -\frac{1}{L_s} \cdot \log\left(a \cdot \frac{I_1}{I_0}\right) \qquad (2)$$

where L_s is the average effective pathlength of the elastically scattered light between source and detector, and a is a proportionality constant to account for the geometry. If the majority of the scattered photons undergo a small number of scattering events before being collected, one can use this modified law in order to estimate the total attenuation coefficient μ_t. The spectral data from ESS represents the ratio of the elastically scattered light intensity I_1 to the incident light intensity I_0. Then if we measure the ratio $R(\lambda_n) = \frac{I_1(\lambda_n)}{I_0(\lambda_n)}$ at any wavelength λ_n, and using the data from ESS, we can relate its value to the full ESS spectrum, so as to calibrate the ESS spectrum.
On the other hand, if we denote the pathlength of the fluorescence light (through the medium to the surface detector) as L_f we can express the measured fluorescence signal f_m at the surface as follows:

$$f_m \cong a_1 \cdot f_i \cdot \exp(-\mu_t \cdot L_f) \qquad (3)$$

© 2002 OSA/BOSD, AOIMP, TLA 2002

where f_i is the intrinsic fluorescence and a_1 is a proportionality constant. By measuring both the fluorescence spectrum and the elastic scattering spectrum $\frac{I_1}{I_0}$ using the same probe at the same location in the medium, we can then approximate the intrinsic fluorescence using Beer's law. Let f_c be the corrected fluorescence, which is an approximation of the actual intrinsic fluorescence f_i. Then, by substituting (2) in (3) we obtain:

$$f_c \cong f_i = a_1 \cdot \frac{f_m}{\exp\left(a_2 \cdot \log\left(a \cdot \frac{I_1}{I_0}\right)\right)} \quad (4)$$

where $a_2 = \frac{L_f}{L_s}$

The backscattered light originates from a directional source at the surface. The fluorescence light is an isotropic source produced by a directional monochromatic light. Since the monochromatic and white lights are directional, their distribution is governed by the optical properties of the medium (scattering coefficient μ_s, absorption coefficient μ_a and the mean cosine of the scattering angle g). If the fluorophore molecules are homogeneously distributed in the medium, the fluorescence distribution will be governed by the excitation light distribution in the medium. Consequently, the fluorescence light source takes the form of the distribution of directional excitation light. The fluorescence re-absorption by the fluorophore itself and the fluorescence emission induced by the broadband lamp (white source) were neglected.

3. Parameters optimization

We consider the non-linear proportionality constants a and a_2 in eq. (4) without paying attention to the pathlengths of light. The proportionality constant a_1 is a linear parameter that does not affect the shape of the corrected spectrum. Let F_i be the normalized fluorescence spectrum measured in pure fluorescent solution (no absorption or scattering in the medium), F_m the normalized fluorescence spectrum measured in the tissue phantom and F_c the normalized corrected spectrum. Using the Least-Square criterion, we need to estimate the parameters a, a_1 and a_2 that minimize the function $[F_i - F_c]^2$. For this purpose we utilized an iterative Broyden-Fletcher-Goldfarb-Shanno (BFGS) formula. BFGS algorithm is available under Matlab software functions (MathWorks).
Then the estimated correction of the fluorescence spectrum F_c is computed using our model as following:

$$F_C(\lambda) = a_1 \cdot \frac{F_m(\lambda)}{\exp[a_2 \cdot \log(a \cdot R(\lambda))]} \quad (5)$$

4. Materials & methods

4.1. Phantoms

Tissue phantoms were 10-ml aqueous suspensions of polystyrene particles to which fluorescein (25µg/ml) and blue dye (Direct Blue 71) were added. The polystyrene spheres had a diameter of 0.426±1% µm. Using Mie theory, the concentration of the polystyrene particles was computed to produce a reduced scattering coefficient μ_s'=20 cm^{-1} (mean cosine of the scattering angle, g=0.82) at 500 nm. The added blue dye produced an absorption coefficient μ_a=11 cm^{-1} at 500 nm.

4.2. Experimental setup

The fluorescence and elastic scattering spectra were measured using a thermoelectrically cooled optical multichannel analyzer. A He-Cd laser at wavelength λ= 442 nm, was used to induce the fluorescence in the medium. The laser source was replaced by a small tungsten lamp in order to record the elastic scattering spectra.
The fiber-optic probe was a single fiber probe (provided with a 50/50 ratio coupler) with 200-µm core diameter and 0.22 NA. The tip of the optical probe was kept vertical and immersed 1 mm into the phantom solutions during the measurements. The elastic scattering and fluorescence spectra were measured five times using the same single-fiber probe and averaged before being used in the correction model. All ESS and fluorescence measurements reported in

this paper were taken in a dark room with background subtraction. ESS measurements were corrected against a spectrally-flat diffuse reflector (Spectralon®).

5. Results & discussion

Figure 1 shows the normalized fluorescence spectrum F_i of pure fluorescein in an aqueous solution (intrinsic), the normalized distorted fluorescence spectrum for the same fluorophore in tissue phantom F_m and the normalized corrected fluorescence spectrum F_c . The peaks around 590, 670 and 710 nm are artifacts produced by the coupler. Since our model is based on a modified Beer's law, we expected that the optimum correction would be obtained using a single fiber probe because the majority of scattered photons undergo only a small number of scattering events before being collected. Although the correction obtained with the single-fiber probe was satisfactory, we suggest using a dual-fiber probe with a small source-detector separation (< 100 μm). This will permit to avoid the use of an iterative algorithm. We can simply measure the ratio of the scattered light intensity to the incident light intensity at a given wavelength and apply it in our model.

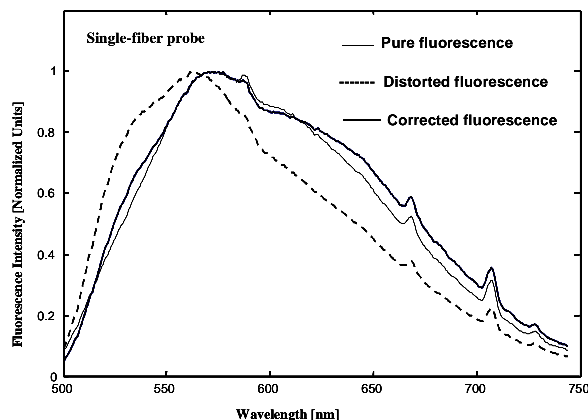

Fig. 1. Fluorescence spectra of fluorescein (2.5%) in aqueous solution (intrinsic) and in a tissue phantom (distorted) composed of fluorescein (2.5%), Direct Blue absorber (μ_a=11 cm^{-1} at 500 nm) and polystyrene particles (μ_s'=20 cm^{-1}, g=0.82 at 500 nm). The corrected spectrum is shown in the same figure. The measurements were made using a single-fiber probe. The squared estimation error between the corrected fluorescence spectrum and the intrinsic fluorescence spectrum was 2.8×10^{-5}.

6. Conclusion

The correction model of fluorescence was developed and experimentally tested. This model permitted to correct the distortion of fluorescence spectra due to the turbidity of the medium. The total attenuation coefficient of light was extracted from the elastic scattering spectra. We have found that the correction was quiet adequate. This could be related to the fact that using the single fiber probe, the majority of photons undergo a very small number of scattering events before being collected by the probe. This fact satisfies the applicability of Beer's law in turbid media.

7. References

1. S. Andersson-Engels and B. C. Wilson, "In vivo fluorescence in clinical oncology : fundamental and practical issues", Journal Cell Pharmacol, **3**, 66-79 (1992).
2. Irving J. Bigio and Judith R. Mourant. "Ultraviolet and visible spectroscopies for tissue diagnostics: fluorescence spectroscopy and elastic-scattering spectroscopy." Phys. Med. Biol. **42**, 803-813 (1997).
3. Qingguo Zhang, Markus G. Mullere, Jun Wu and Michael S. Feld. "Turbidity-free fluorescence spectroscopy of biological tissue." Optics letters, **25** (19), 1451-1453 (2000).
4. Jianan Y. Qu and Jianwen Hua. "Calibrated fluorescence imaging of tissue in vivo." Applied Optics, **78** (25), 4040-4042 (2001).
5. Georges A. Wagnieres, Willem M. Star and Brian C. Wilson. "In vivo fluorescence spectroscopy and imaging for oncological applications." Photochemistry and Photobiology, **68** (5) 603-632 (1998).
6. Zhadin N. N. and Alfano R. R. "Correction of the internal absorption effect in fluorescence emission and excitation spectra from absorbing and highly scattering media: theory and experiment." Journal of biomedical optics, **3** (2), 171-186 (1998).
7. Wilson B. C., Patersson M. S., Flock S. T., "Indirect versus direct techniques for the measurement of the optical properties of tissues", Photochem. Photobiol. **46** (5), 601-608 (1987).