Intensification of the emission signals using the reflection mirrors mounted to the sample holder of a fluorimeter

Takao Itoh

Graduate School of Integated Arts and Sciences, Hiroshima University,

1-7-1 Kagamiyama, Higasi-Hiroshima City, 739-8521 Japan

E-mail: titoh@hiroshima-u.ac.jp

Effects of the reflection mirrors mounted to the sample holder of a fluorimeter have been investigated. Analytical expressions are given for the emission intensity measured as a function of the concentration or optical density of the sample, in case one or two reflection mirrors are attached beside the sample holder to intensify the emission signals. The emission intensity calculated as a function of the sample concentration agreed well with the experimental data. By mounting two reflection mirrors, the emission signal was intensified by a factor of near 3.5. However, the degree of intensification depended strongly on the sample concentration and the linearity between the sample concentration and the emission intensity deviated significantly with increasing the sample concentration.

KEY WORDS: Signal intensification; Emission; Fluorimetry; Optical density; Reflection mirrors;

INTRODUCTION

Fluorimetry is known as one of the sensitive tools for detection of the trace amount of chemical species, since unlike UV-VIS optical absorption measurements, it

detects directly the photons emitted by molecules [1]. The reflection mirrors or multiple-pass cells have been utilized to intensify the weak emission signals [2 - 7]. However, detailed analyses on the intensification mechanisms of the emission signals by reflection mirrors are not available.

In the present work, the effects of the reflection mirrors attached beside the sample holder of a fluorimeter have been investigated. Analytical expressions are given for the emission intensity measured as a function of the concentration or optical density of the samples, in case one or two reflection mirrors are mounted to the sample holder of a fluorimeter. Emission intensity calculated based on the analytical expressions are compared with the experimental results. The calculated emission intensity agreed well with the experimental data. The observed emission was intensified by a factor of about 3.3 by using two reflection mirrors for the sample with the optical density near 0.09. However, the linearity between the sample concentration and the emission intensity deviated strongly by using the two reflection mirrors, indicating that the use of the reflection mirrors is effective for measurements of dilute samples such as dilute solutions and low-pressure vapor-phase samples including jet expansion. Thus, one has to consider this in using the systems such as the one shown here for quantitative analyses or obtaining accurate excitation spectra.

EXPERIMENTS

Anthracene obtained from Wako pure chemicals, Japan was recrystallized and dissolved it into hexane to obtain the samples with different concentrations. The fluorescence of anthracene in hexane ranges from 360 to 500 nm, while the first absorption band of anthracene is located at 380 nm in hexane at room temperature. Thus, in order to avoid the effect of re-absorption of the fluorescence emission by the

first absorption band of anthracene, the second fluorescence band at 400 nm was used to measure the emission intensities. Absorption spectra were measured with a Shimadzu UV-2550 spectrophotometer, and fluorescence spectra were measured with a Spex Fluorolog-3 (Model 21-SS) spectrophotometer, equipped with a double-grating excitation monochromator, a high-pressure 450-W Xenon lamp as an excitation-light source, and a photomultiplier tube (Hamamatsu R928-P) in an electric-cooled housing operated in photon-counting mode. For the emission and absorption measurements square 10-mm path length quartz cells were used. Parabolic quartz mirrors coated by silver were used as the reflection mirrors.

We have used a conventional and very simple mirror alignment as shown in Fig. 1(left), where the reflected light is focused by Mirror 1 or 2 to the center of a square 10-mm sample cell and the emission is detected at the position perpendicular to the excitation light pass. The intensity of the light passing through the sample cell and that reflected by Mirror 2 decreases according to Lambert-Beer's low as shown in Fig. 1(right).

RESULTS AND DISCUSSION

Let us start with the equation described by Foerster [8]. In a conventional fluorimeter, the intensity of the emission coming out perpendicular to the exciting light pass is given by, $I = \text{const.} \times kcl \times \exp(kcz)$, where z is the position in the cell from which the emission coming out, c is the concentration of the sample, k is a constant equivalent to the molar extinction coefficient, and l is the cell length (in the present case, l = 10 mm) (see Fig. 1). Since normally we observe the emission coming out from the center of the cell, we put the value of $1/2 \times l$ for z. Then, we obtain,

$$I(0) = \text{const.} \times kcl \times \exp(-kcl/2), \quad (1)$$

where kcl and A are related by $\exp(-kcl) = 10^{-A}$ or $kcl = A \times \ln(10)$, with A denoting the optical density of the sample in a conventional square 10-mm pass-length cell.

When only one reflection mirror (Mirror 1) is attached at the opposite side of the excitation light source, we obtain,

$$I(M1) = \text{const.} \times [b \times \exp(-b/2) + rb \times \exp(-3b/2)], \quad (2)$$

where $b = kcl$ and r is the reflection efficiency of the mirror with $r < 1$. On the other hand, when only one reflection mirror (Mirror 2) is attached at the opposite side of the emission detection system, we obtain,

$$I(M2) = \text{const.} \times (1 + r)b \times \exp(-b/2), \quad (3)$$

Further, when the two reflection mirrors (Mirrors 1 and 2) are attached at the both sides, we have,

$$I(M1 + M2) = \text{const.} \times [(1 + r)b \times \exp(-b/2) + (r + r^2)b \times \exp(-3b/2)], \quad (4)$$

where the values for r of the two mirrors are assumed to be almost exactly the same.

Figure 2 shows the values for I(0), I(M1), I(M2) and I(M1 + M2) plotted against the optical density, A, of the sample. These values have been calculated also using Eqs. (1) to (4) as a function of the optical density, A, with varying the value for r and compared them with the experimental data. Hexane solutions of anthracence with

different concentrations were used tentatively as the model samples. We found that r of near 0.9 agreed well with the experimental results. Solutions of naphthalene were also used as the samples, but almost the same results were obtained. In the present system, the emission signal was intensified by a factor of 3.3 for the sample with the optical density of near 0.09, but the linearity between the sample concentration and the emission intensity deviates strongly by using the two reflection mirrors. As shown in Fig. 3, the linearity is almost retained for samples with the optical densities up to near 0.1, when no reflection was used. On the other hand, when two reflection mirrors were used, the linearity is almost retained for the samples with the optical densities below only about 0.05. Of course, strictly speaking, the data in Fig. 3 shows the nonlinearity between A and I and $I/A < 1.0$ in all over the range of A, but here the linearity is assumed to be almost retained for I/A over 0.9. Figure 4 shows the degrees of the intensification of the emission signal measured as a function of the optical density of the sample. The degree of intensification depends strongly on the concentration of the sample and decreases with increasing the sample concentration. As was mentioned in the experimental section, self-absorption effect is not considered in the present treatment, since the spectral shapes of the absorption and emission spectra differ depending on the molecule, and since the self-absorption effect occurs at somewhat higher concentrations. In the present simple alignment, the maximum of the intensification of about 3.5 has been achieved for the sample with the optical density of 0.02. Thus, one has to take this into consideration in using the systems such as the one shown here for quantitative analyses and obtaining the excitation spectra.

REFERENCES

1. J. R. Lakowicz (1999). *Principels of fluorescence spectroscopy 2nd Ed*., Plenum Press, New York and London.

2. J. U. White (1976). Sample optics for increased sensitivity in fluorescence spectrophotometers *Anal. Chem.* **48** 2089 – 91.

3. T. Itoh and H. Baba (1979) Emission spectra of 1,4-naphthoquinone and its 2-methyl derivative in the vapor phase *Bull. Chem. Soc. Jpn*. **52** 3213 – 16.

4. T. Itoh and H. Baba (1980) Radiative and nonradiative processes in naphthoquinone vapors at low pressure *Chem. Phys*. **51** 179 – 187.

5. T. Itoh (1985) Radiative and nonradiative processes in *p*-benzoquinone vapor at low pressure *Molec. Phys*. **55** 799 – 807.

6. J. El Jabri, S. De Lauzon, N. Cittanova, P. Gervais, J. Mugnier, J. Pouget and B. Valeur (1989) Estrogen fluoroimmunoassay with a fluorimeter designed for low-intensity light detection *Anal. Chim. Acta* **227** 129 - 134.

7. N. L. Vekshin (1989) Multipass cuvettes for spectrofluorimetry *Anal. Chim. Acta* **227** 291-295.

8. Th. Foerster (1951) *Fluoreszenz Organischer Verbindungen*, Vandenhoeck und Ruprecht, Goettingen.

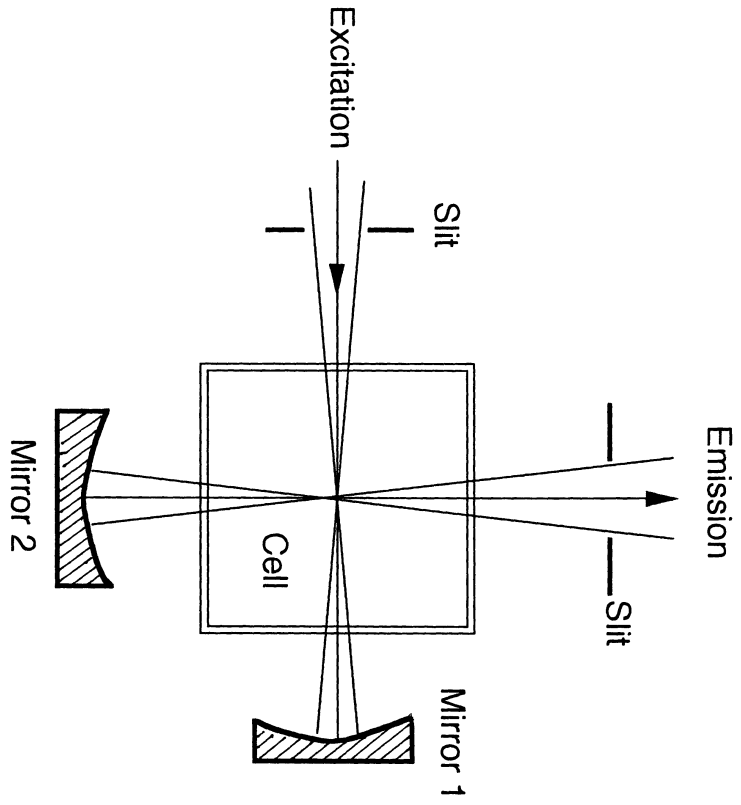
Figure captions

Figure 1. Mirror alignment used in the present system (left), and the consequence of the excition light intensity passing through the sample cell using only Mirror 1 (right).

Figure 2. The values for I(0), I(M1), I(M2) and I(M1 + M2) plotted against the optical density, A, of the sample. Solid curves represent calculated values, while the plots represent experimental data. Anthracene in hexane was used as the model sample, for which optical density at 360 nm and fluorescence intensity at 400 nm were used as A and I, respectively.

Figure 3. The values for I(0)/A, I(M1)/A, I(M2)/A and I(M1 + M2)/A plotted against the optical density, A, of the sample. Solid curves represent calculated values, while the plots represent experimental data: open circles, I(0)/A; closed triangles, I(M1)/A; closed circles, I(M2)/A; and open triangles, I(M1 + M2)/A. All the data are normalized to unity at A = 0. The line I/A = 1 represents the case showing the exact linear relationship between the observed emission intensity and the optical density. The calculated curves for I(0)/A and I(M1)/A are identical to each other, and those for I(M2)/A and I(M1 + M2)/A are also identical to each other.

Figure 4. The values for I(M1)/ I(0), I(M2)/ I(0) and I(M1 + M2)/ I(0) plotted against the optical density, A, of the sample. Solid curves represent calculated values, while the plots represent experimental data.



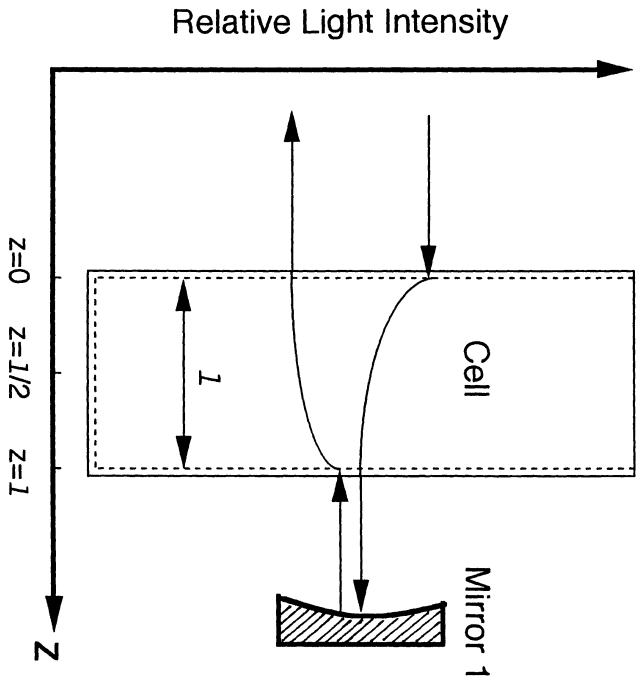


Figure 1., T. Itoh

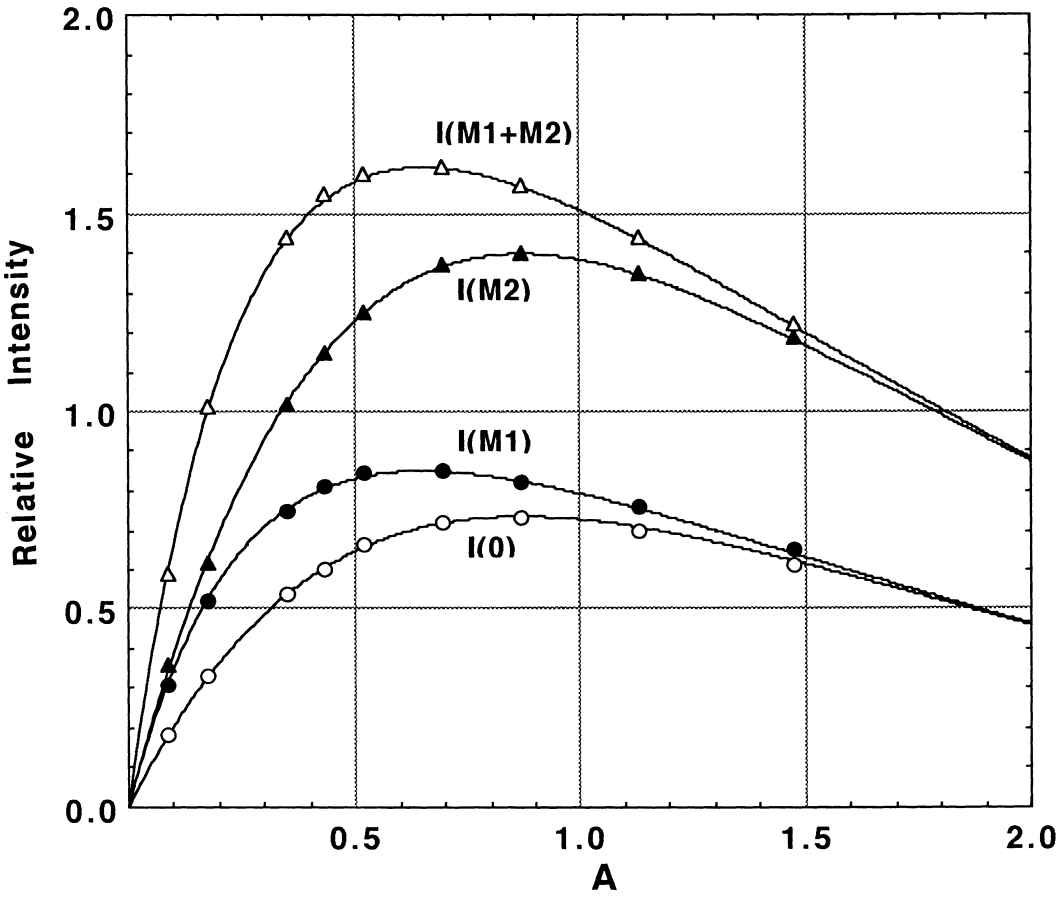


Figure 2., T. Itoh

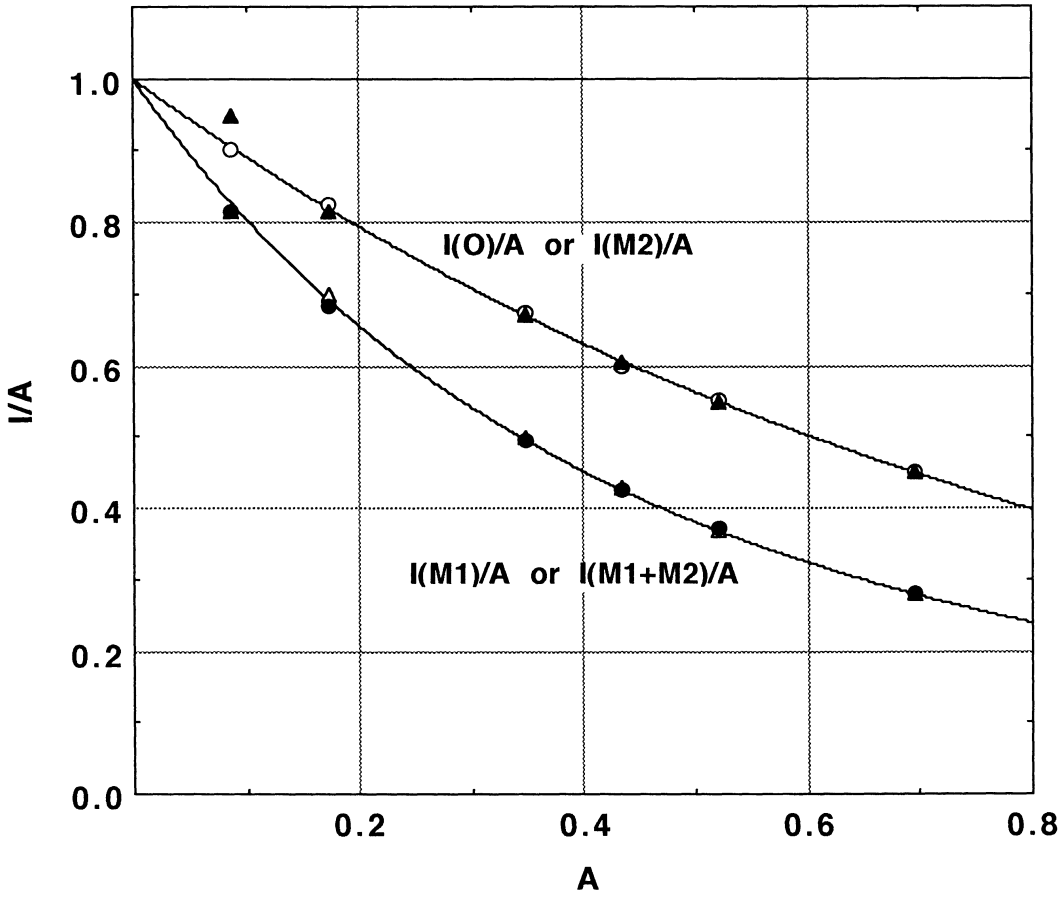


Figure 3., T. Itoh

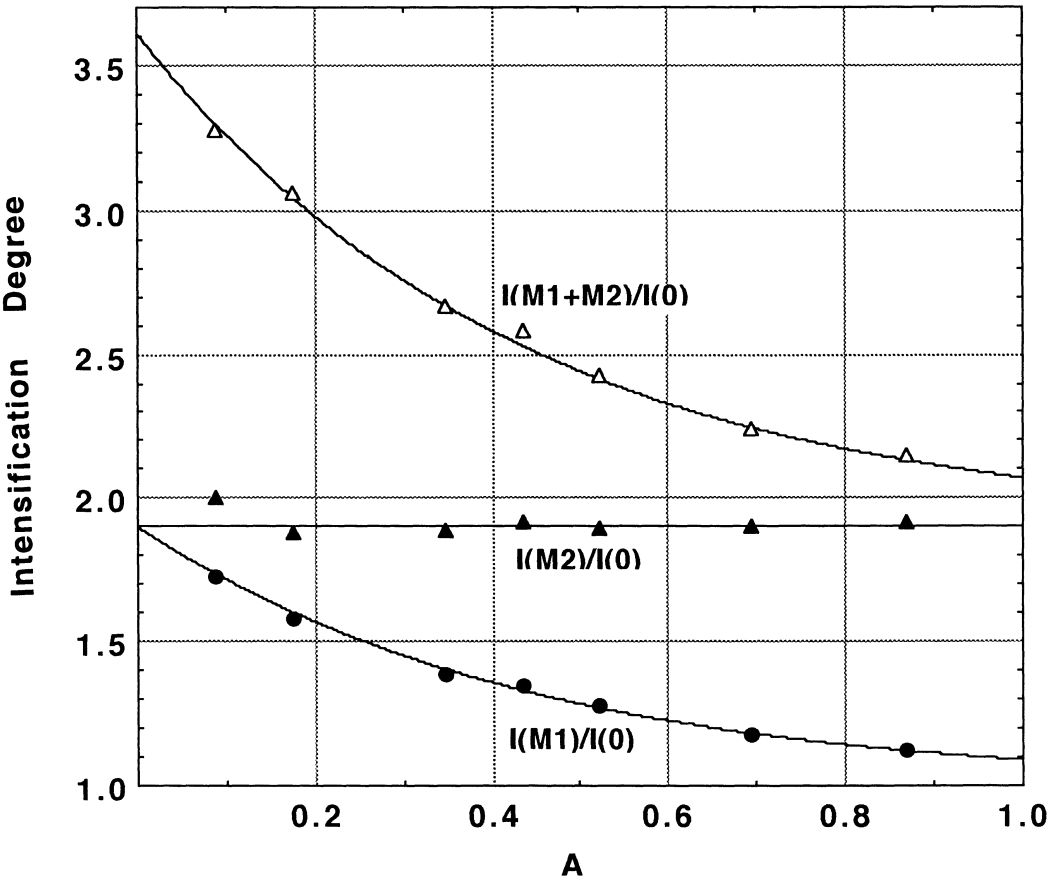


Figure 4., T. Itoh