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Surface and Subsurface Fluorometry under Normal Incidence Conditions

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Laser induced fluorescence spectroscopy under normal incidence conditions has been applied to a thick film sample, and identification of surface and subsurface fluorescent components was demonstrated. A model film sample adsorbing different fluorescent compounds on its two sides was prepared. Fluorescence spectra from the surface and subsurface regions were separately obtained for observation angles larger and smaller than the critical angle, and it was shown that the same information as the conventional attenuated total reflection method can be obtained with the normal incidence conditions, which is anticipated by the reciprocal principle. Dependence of fluorescence intensity on observation angle agreed well with theoretical calculation results based on the reciprocal principle, which supports the methodology propriety.

Fluorescence spectroscopy has been applied to spectrochemical surface measurement and characterization of various materials, such as organic solids,[1] surface dynamics of biomolecules,[2] and adsorption behavior of proteins at a solid/liquid interface.[3–6] However, in these works, the attenuated total reflection (ATR) conditions has been used to obtain surface fluorescence, in which an excitation beam is incident on the prism/sample interface at an angle larger than the critical angle and fluorescence is measured from the normal direction to the prism. Recently, we have verified that laser induced fluorescence spectroscopy under normal incidence conditions can be used to determine the monolayer level concentration depth profile of an ultrathin film sample,[7,8] by the method based on the reciprocal theory.[9] From an instrumentational viewpoint, normal incidence excitation can obtain the fluorescence angular distribution without changing the irradiation area, and the excitation beam can be easily scanned. These merits are more suitable than the conventional ATR configuration for the local analyses, future dynamic measurements, and three dimensional analyses.[7–9] Therefore, this method is expected to be developed for various applications.

In this paper, we apply this method to surface and subsurface analysis of a thick film sample. According to the reciprocal theory, this fluorescence method using the normal incidence excitation configuration can obtain the same information as with the conventional ATR configuration. Two kinds of fluorescent dyes are adsorbed on different sides of the model film sample surface, and we demonstrate that fluorescence spectra from a dye distributed in the surface and subsurface regions of the film can be detected separately by changing observation angles. Moreover, the measured dependence of the fluorescence intensity on observation angle is compared with theoretical calculation results which are obtained according to the reciprocal principle.

Theoretical Background

Figure 1 shows a schematic illustration of laser induced fluorescence spectroscopy under normal incidence conditions based on the reciprocal principle. Fluorescence intensity $F(\theta)$ determined at the observation angle θ in the prism side can be expressed as the convolution of the concentration depth profile $C(z)$ of a fluorophore and a transmission function $T(\theta, z)$:[7,10]

$$F(\theta)=k\int_0^t C(z)T(\theta, z)\mathrm{d}z, \tag{1}$$

where t is the film thickness, k is a constant. In the case where the sample is an ultrathin film sample, $T(\theta, z)$ can be obtained by the theoretical analysis of the multiple reflection of fluorescence electromagnetic waves between prism/film and film/air interfaces.[9] On the other hand, in the case when the sample is a bulk or a thick film sample, $T(\theta, z)$ can be obtained by considering the

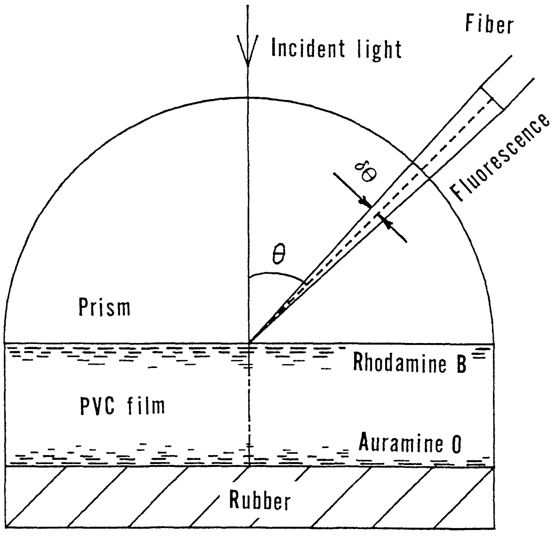

Fig. 1. Schematic illustration of central part of the experimental set up and the model film sample.

reflection and refraction effects of fluorescence occurring at the prism/sample interface. Snell's law and basic electromagnetic theory state that light incident from the prism to the prism/sample interface at an angle larger or smaller than the critical angle θ_c will create an evanescent wave or a transmitted wave in the sample, respectively. The transmitted wave propagates through the whole sample and its intensity is homogeneous over the whole sample. On the other hand, the evanescent wave exists only in the surface region of the sample with a depth on the order of the excitation light wavelength from the prism/film interface, and its intensity decreases exponentially with the distance z. According to the reciprocal theory, fluorescence observed in the prism side at $\theta > \theta_c$ and $\theta < \theta_c$ are an evanescent component and a propagating component, respectively.[11-13] Moreover, the reciprocal theory predicts that the evanescent fluorescence component observed must be from a surface region with a depth on the order of the fluorescence wavelength from the prism/film interface, and the propagating fluorescence component observed is from the whole sample including the surface and subsurface regions. The transmission factors $T(\theta, z)$ of the evanescent and propagating fluorescence components are given by the Fresnel transmission factor $T_f(\theta)$ and the attenuation factor $\exp(-2z/dp)$ as Eq. 2a, and the square of the Fresnel transmission factor $T_f(\theta)$ as Eq. 2b, respectively.[10-13]

$$T(\theta, z) = |T_f(\theta)|^2 \exp(-2z/dp), \qquad \theta > \theta_c \tag{2a}$$

$$T(\theta, z) = |T_f(\theta)|^2. \qquad \theta < \theta_c \tag{2b}$$

In Eq. 2a, dp is the penetration depth of the fluorescence which can be obtained from the fluorescence wavelength λ and the refractive indices of prism (n_p) and film (n_f),

$$dp = \lambda / 2\pi n_p[\sin^2\theta - (n_f/n_p)^2]^{1/2}. \tag{3}$$

The Fresnel transmission factor $T_f(\theta)$ and the critical angle θ_c can be obtained from n_p and n_f.[11-13] The fluorescence intensity $F(\theta)$ of the evanescent and propagating components can be expressed as follows by substituting Eqs. 2a and 2b into Eq. 1,

$$F(\theta) = k|T_f(\theta)|^2 \int_0^t C(z) \exp(-2z/dp)\mathrm{d}z, \qquad \theta > \theta_c \tag{4a}$$

$$F(\theta) = k\int_0^t C(z)|T_f(\theta)|^2 \mathrm{d}z = K|T_f(\theta)|^2, \qquad \theta < \theta_c \tag{4b}$$

where the constants K in Eq. 4b can be expressed as Eq. 5,

$$K = k\int_0^t C(z)\mathrm{d}z. \tag{5}$$

Therefore, using the normal incidence excitation configuration shown as Fig. 1, surface and subsurface fluorescence information can be obtained separately at $\theta > \theta_c$ and $\theta < \theta_c$, respectively. This is demonstrated in the following experiement.

Experimental

The experimental set up has been described elsewhere in detail.[7] Figure 1 shows the central part of the set up and the model film sample. An Ar^+ ion laser provided the excitation light (output power, 10 mW), the wavelength of which was selected as 457.9 nm because both the fluorescence compounds Rhodamine B and Auramine O in model film sample can be excited by this laser line. The maximum absorption wavelengths of plasticized poly(vinyl chloride) (PVC) film samples adsorbing Rhodamine B and Auramine O were determined to be about 550 and 440 nm, respectively, by a spectrophotometer and their maximum fluorescence wavelengths were determined to be about 575 and 500 nm, respectively, by a spectrofluorometer. The excitation light was focused on the focal plane of a hemicylindrical prism (n_p, 1.88) which was mounted on the two rotating stages. In order to ensure the model sample made direct contact with the prism, a piece of rubber was used and pressure was applied to the rubber. The side with adsorbed Rhodamine B was in direct contact with the prism. A step index type optical fiber bundle was used to collect the fluorescence. Its diameter was 0.11 cm and the numerical aperture was 0.57. One end of the fiber bundle was fixed at the focal plane of the prism. The other end was coupled with a monochromator. A photomultiplier (Hamamatsu R928) was used as a detector.

The model sample was prepared by an adsorption of fluorescent dyes onto a PVC film (n_f, 1.53) obtained from Okamoto Industry Corporation. The preparation procedure was based on the method proposed by Kaneko et al.[14] in which 1 ml 1×10^{-4} mol dm^{-3} Rhodamine B solution was dropped onto the PVC film sheet (thickness, 0.20 mm). The droplet of Rhodamine B solution remained on the PVC film sheet due to surface tension and the dye molecules diffused into the PVC film, adsorbing in it. After 20 min, the droplet was removed and the PVC film sheet was rinsed with water twice. In the same way, 1 ml 1×10^{-4} mol dm^{-3} Auramine O (with 1×10^{-2} mol dm^{-3} NaOH) solution was dropped onto the other side of PVC film sheet and dye were adsorbed.

Fluorescence microscopic measurements of a cross section of a PVC film sample immersed in 1×10^{-4} mol dm^{-3} Rhodamine B solution for 20 min indicated that the adsorbed fluorescent dye distributed from the surface to a depth of about 40 μm in the PVC film. Similar results were also obtained by the photoacoustic depth profiling method.[15]

Results and Discussion

It has been stated that fluorescence measured at $\theta > \theta_c$ is surface fluorescence which comes from the surface regions with a depth on the order of the fluorescence wavelength from the prism/sample interface, and fluorescence measured at $\theta < \theta_c$ is from the whole sample, including the surface and subsurface regions. The fiber bundle has a finite size, it collects fluorescence from a finite range of observation angles, from $\theta - \delta\theta$ to $\theta + \delta\theta$ as shown in Fig. 1. Therefore the surface fluorescence is obtained practically at $\theta>\theta_c+\delta\theta$. For the prism/PVC film interface, the calculated θ_c from n_p and n_f is 54.5 °C, and $\delta\theta$ is calculated to be 1.5 °C from the fiber diameter (1.1 mm) and the distance (2.14 cm) between the illumination point and fiber bundle. Therefore, in our experiments, surface fluorescence should be obtained at $\theta>56$ °C.

The fluorescence spectra of the model sample was determined at different observation angles under normal incidence conditions, and two kinds of fluorescence spectra were obtained. They are shown in Fig. 2. The fluorescence spectrum obtained at an observation angle=50 °C ($<\theta_c$) is shown by the the solid line, where two peaks appear at about 575 and 500 nm, respectively. They correspond to the fluorescence of Rhodamine B and Auramine O, respectively. The fluorescence spectrum obtained at observation angle=60 °C ($>\theta_c+\delta\theta$) is shown by the dashed line, where only one peak at about 575 nm corresponding to the fluorescence of Rhodamine B is observed. The chain line is the blank fluorescence spectrum, i.e., the fluorescence spectrum of PVC film without adsorption of fluorescent dye.

As shown in Fig. 1, the side of the PVC film surface adsorbing Rhodamine B is in direct contact with the prism, therefore in the prism/film interface region only Rhodamine B is present. On the other hand, apparently Auramine O distributes in the region away from the interface of the prism/film. Therefore, the results of Fig. 2 demonstrate that surface and subsurface fluorescence information can be obtained by changing the observation angle using the normal incidence excitation configuration, which is anticipated in the theoretical section. Although the reciprocity in the angular distribution of fluorescence has been pointed out,[9,11-13] this work is the first spectroscopical demonstration that surface and subsurface information can be separately obtained by the normal incidence excitation configuration.

Figure 3-A shows the theoretical and measured dependence of fluorescence intensity on the observation angle for $\theta<\theta_c$. The theoretical results were obtained from Eq. 4b, and are represented by a solid line. The measured data were obtained at fluorescence wavelength=575 nm, and are shown by circles. The measured fluorescence intensities agree well with the calculated ones. Figure 3-B shows the theoretical and measured dependence of fluorescence intensity on observation angle for $\theta>\theta_c$. The theoretical and measured fluorescence intensities are represented by a solid line and circles, respectively. The measured fluorescence intensities were obtained at fluorescence wavelength=575 nm. The theoretical results were obtained from Eq. 4a assuming $C(z)$ is a constant. As stated above, at $\theta>\theta_c$, the measured fluorescence is from the surface region with a depth on the order of the

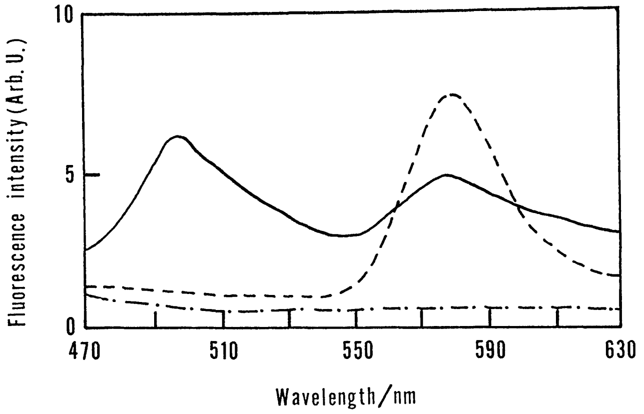

Fig. 2. Fluorescence spectra of the model film sample under normal incidence conditions at different observation angles. The solid and dashed lines represent the fluorescence spectra obtained at 50° and 60°, respectively. The blank fluorescence spectrum is represented by the chain line.

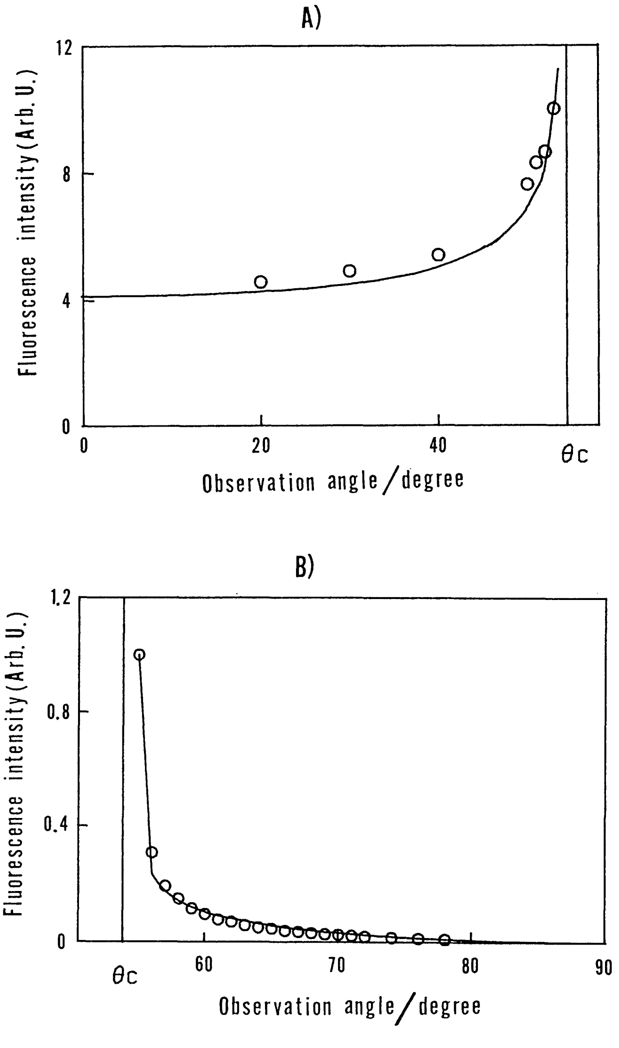

Fig. 3. Theoretical and determined dependence of fluorescence intensity on the observation angles. A: the observation angle is smaller than the critical angle. B: the observation angle is larger than the critical angle.

fluorescence wavelength from the prism/film interface, while adsorbed Rhodamine B is distributed from the surface to a depth of about 40 μm. Therefore the concentration distribution $C(z)$ of Rhodamine B from the surface to a depth on the order of the fluorescence wavelength can be considered as a constant. The results of Fig. 3 indicate that the dependence of fluorescence intensity on observation angle can be quantitatively described by Eqs. 4a and 4b, which confirms propriety of this method, obtained on the basis of the reciprocal principle.[10-13]

In our previous reports, the validity of laser induced fluorescence spectroscopy under normal incidence conditions to an ultrathin film sample was proved.[7,8] In the present work, it was demonstrated that this fluorescence method can also be applied to a bulk or a thick film sample. As a result, the surface fluorescence of these samples can be distinguished from the subsurface fluorescence by this method. The present method provides the possibility to determine the surface fluorescence and subsurface fluorescence simultaneously with two detectors.

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