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A Spectrometric Setup for Synchronous Total Internal Reflection Fluorescence Measurement at the Solid/Liquid Interface

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Abstract: A spectrometric setup to perform total internal reflection fluorescence (TIRF) and synchronous TIRF measurements at solid/liquid interfaces is presented. The combination of TIRF and synchronous fluorescence was proposed to analyze simultaneously different components at interfaces. The TIRF excitation, emission and synchronous spectra of a water–soluble porphyrin were obtained from water/glass interface using this setup without the existence of a surfactant.

Keywords: Total internal reflection fluorescence, synchronous fluorescence, spectrofluorimeter, meso-tetra(4-sulfonatophenyl)porphyrin.

The interface of two immiscible phases has attracted more and more attention due to its fundamental, biological and industrial importance. However it remains very difficult to probe interfaces using most common analytical methods since the signals from the large amount of solute molecules in the bulk can overwhelm those originating from interfacial molecules. Total internal reflection fluorescence (TIRF) spectroscopy has been proven to be a powerful technique to solve the problem because of its capability of interface-specific[1,2,3]. Nevertheless, The TIRF measurement on a common spectrofluorimeter is still rather limited[2,3]. In this paper, we present a spectrometric setup to perform TIRF measurement at the solid/liquid interface. Moreover, the setup can be also used to acquire synchronous TIRF spectra. Synchronous fluorescence spectroscopy is a useful technique for multi-component analysis[4,5]. The combination of synchronous fluorescence and TIRF would facilitate the investigation of different species at interfaces. To our knowledge, synchronous TIRF spectroscopy has not yet been reported.

The instrument was similar to previous laboratory-made versatile spectrofluorimeter[4] except the TIRF cell as shown in **Figure 1** (1). A prism (quartz or BK7 glass) was attached to the sample cell. When a beam of light struck the interface of the cell wall and the solution in the sample cell at an incidence angle greater than the critical angle, it underwent total internal reflection. A portion of the electromagnetic radiation, typically called the "evanescent wave", penetrated the interface into the less dense solution medium. The evanescent wave induced the fluorescence of molecules at the

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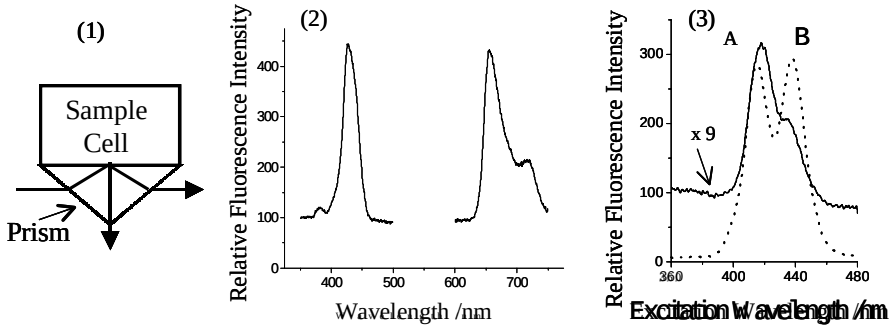
interface region. Thus, interfacial fluorescence spectra could be obtained by TIRF 0technique. For glass interface tests, BK7 glass cell and prism were used. For quartz interface tests, quartz cell and prism were used.

A water-soluble molecule, meso-tetra(4-sulfonatophenyl)porphyrin (TPPS), was tested using the setup. **Figure 1** (2) shows the TIRF excitation and emission spectra of TPPS at glass/water interface at pH 2.5. Interfacial fluorescence signal of TPPS is very weak without the existence of a surfactant[3]. However, using our setup, we can observe clearly the fluorescence spectra of TPPS at the glass/water interface without any surfactant. Synchronous fluorescence spectroscopy can be used to characterize different components simultaneously. We obtained synchronous fluorescence spectra at glass/water interface (**Figure 1** (3)) by combining TIRF technique. Peak A and peak B in the spectra represent unprotonated and diprotonated forms of TPPS, respectively. Peak A was much stronger than peak B in synchronous fluorescence spectra at glass/water interface, whereas the intensities of peak A and peak B were similar in bulk solution. The results indicate that the interface favors the existence of unprotonated form of TPPS. Moreover, a slight red shift of the fluorescence peaks of unprotonated form at the interface was also observed in comparison with those in the bulk solution. The spectrometric setup provides a promising tool to detect the interfacial molecules and probe interfaces.

Figure 1 (1) Optical arrangements for TIRF measurement; (2) Excitation and emission spectra of TPPS at glass/water interface, pH 2.5; (3) Synchronous fluorescence spectra of TPPS at interface (solid) and bulk solution (dot), $\Delta\lambda = 233$ nm, pH 4.6.



Acknowledgments

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