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Evaluation of instrument response functions for lifetime imaging detectors using quenched Rose Bengal solutions

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ABSTRACT

Instrument response functions (IRF) in time-domain fluorescence are usually recorded as reflected or scattered excitation light, which is at shorter wavelengths than the observed fluorescence emission. However, its often more appropriate to measure the IRF in the emission spectral region. In this work we show that Rose Bengal water solutions quenched by potassium iodide can be used to measure instrument response functions of single photon detectors in the orange-red wavelength region. We used the quenched RB emission as a reference in time-domain measurements with common detectors and we got practically the same results as with scattering.

1. Introduction

Recording the correct instrument response function (IRF) in order to obtain correct fluorescence lifetimes is an important task in time-resolved fluorescence spectroscopy. IRF is usually recorded as reflected or scattered excitation light, which has a shorter wavelength than the observed fluorescence emission. Sometimes the excitation and emission wavelengths differ significantly and the timing characteristics of photon detectors (especially that of APDs) depend on the wavelength. This problem can be solved by using known standard dyes as a reference, preferably with very short lifetimes. Water solution of erythrosine B was used to obtain the IRF corresponding to the laser pulse convoluted with the detection response in the time-resolved flavin fluorescence experiments [1,2]. Hanley et al. described a multi-point method for calibrating a frequency domain FLIM system. For this purpose they used Rhodamine 6G solutions quenched with iodide, exhibiting single exponential decays [3]. In one of the recent work we showed that erythrosine B can be quenched by KI to about 25 ps and serves as a reference for a green region of spectrum (530–570 nm) [4]. It should be noted that the idea of using a reference dye to avoid color effects is older. Already Harris and Lytle [5], Zuker et al. [6] and Van Den Zegel [7] were well aware of this problem.

The xanthene dyes are very well-known, and because of their photochemical properties often used fluorophores. Rose Bengal (RB) is a one of them. Its chemical structure is similar to that of fluorescein. Both dyes have the same carbon aromatic skeleton but differ in aromatic substitutions. Because of chlorines and iodines on the xanthene ring, RB features a heavy-atom effect which results in a high efficiency of intersystem crossing to the triplet state [8].

RB is a water-soluble photosensitizer with a high absorption coefficient in the red region of the spectrum and a affinity to transfer electrons from its excited triplet state, producing long-lived radicals [9,10]. As a photosensitizer, RB was used to inactivate microorganisms such as viruses [11,12], gram-positive bacterial species [13] and protozoa [14]. The liposomal encapsulation of RB was studied to improve the applicability of the drug in clinical application [15]. Rose Bengal stains damaged conjuctival and corneal cells. It is used as eye drops to identify damage to the surface of the eye [16]. In new technologies dye-sensitized solar cell is fabricated using Rose Bengal dye for sensitization of nanocrystalline TiO_2 and that imparts extension in spectral response towards visible region by modifying the semiconductor surface [17].

In this work we present an ultra-short fluorescence standard in the orange-red region (540–610 nm) which has a lifetime of 16 ps. We demonstrate that such a quenched RB fluorescence can be used as an instrument response function in reconvolution fitting of fluorescence intensity decays. We also evaluated the color dependence of APD responses using a quenched RB reference.

Abbreviations: QY, quantum yield; RB, Rose Bengal; KI, potassium iodide; IRF, instrument response function; RhB, Rhodamin B; Rh6G, Rhodamin 6G; APD, avalanche photo diode; MCP, microchannel plate; PMT, photomultiplier tube; FWHM, full-width at half of maximum.

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2. Materials and methods

All chemicals were analytical reagents or the best grade commercially available. All solutions of chemicals were prepared in deionized water purified by using a Milli-Q Synthesis A10 system produced by Millipore. Rose Bengal, KI, KOH, Rhodamine B and Rhodamine 6G, Ludox (30 wt.% suspension in H_2O) were purchased from Sigma–Aldrich. Rose Bengal solutions for spectroscopic measurements were prepared by adding 100 μl stock solution of RB in water to 2000 μl KI in water. KI solutions were prepared over concentration range from 0 M to saturate (6.03 M). The pH of all solutions was 9.8 and it was achieved by addition 30 μl of stock solution of KOH (0.004 M). Final volume of each sample was 2130 μl. Emission spectra and steady-state fluorescence anisotropies were obtained with 1 cm quartz cuvettes using a Varian Cary Eclipse spectrofluorimeter. For lifetime measurements samples were excited at 470 nm and emission collected at 572 nm. Intensity decays were collected by time-domain technique using a FluoTime 200 lifetime spectrometer (PicoQuant GmbH) equipped with R3809U-50 microchannel plate photomultiplier (MCP-PMT, Hamamatsu) and PicoHarp300 TCSPC module. The excitation source was an LDH470 pulsed laser diode (470 nm, optical pulse duration: 65 ps FWHM) driven by a PDL800-B driver. Polarizers were set to magic angle conditions and the fluorescence was observed through a 100 mm focal length single grating emission monochromator (ScienceTech). Decays were analyzed using the FluoFit software package (version 4.2.1). The analysis involved iterative reconvolution fitting of a sum of exponentials to the experimentally recorded decays:

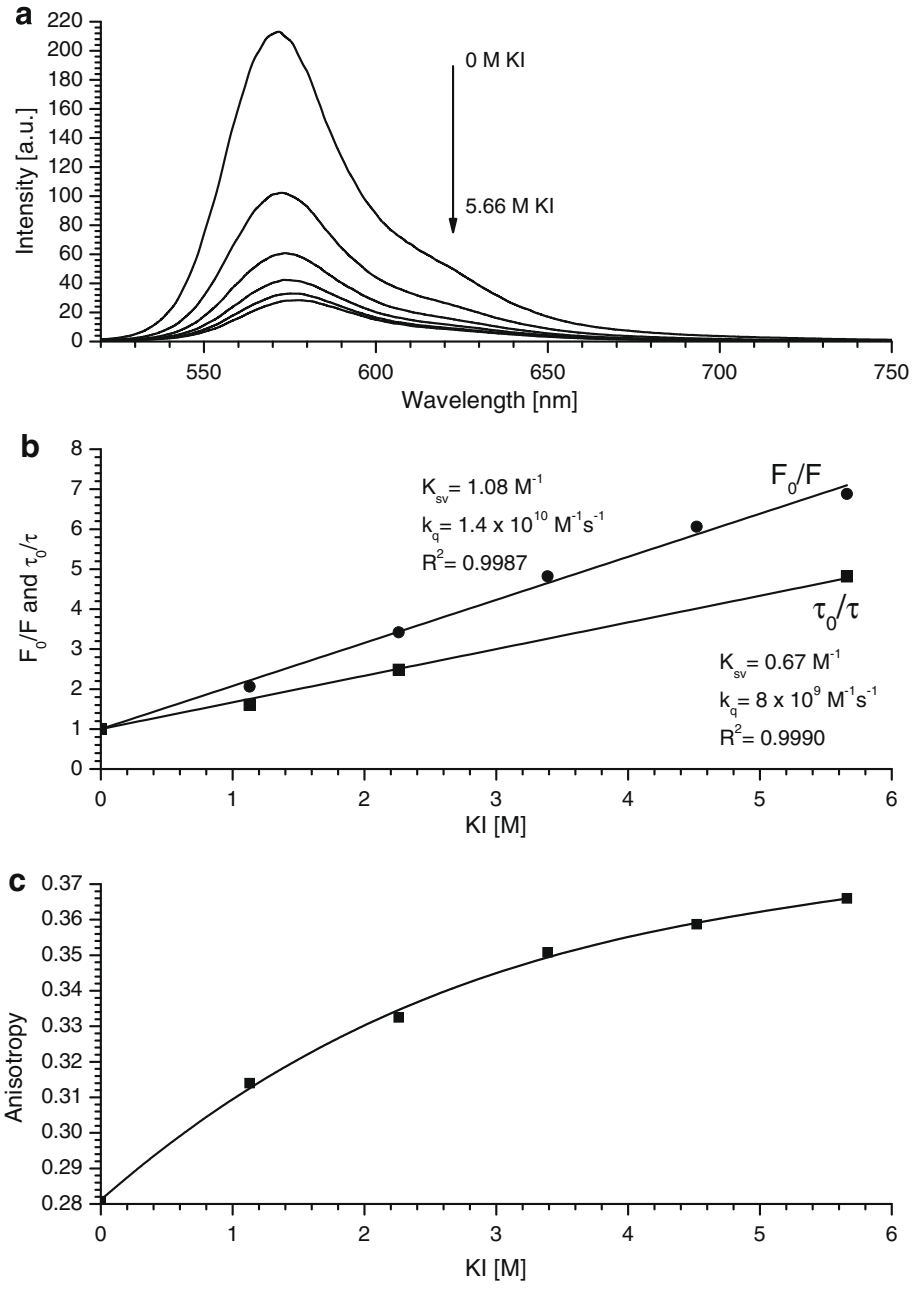

Fig. 1. Fluorescence steady-state measurements for Rose Bengal. (a) Quenching of RB in water by KI. (b) Stern–Volmer dependence in the entire range of quencher concentration. Fluorescence quenching results in seven-fold decrease in the fluorescence intensity in the presence of 5.66 M KI. (c) Fluorescence anisotropy of Rose Bengal in water in presence of KI. The increase in the steady-state anisotropy is due to shorter lifetimes.