# Medical diagnosis and remote sensing at fiber-tip: picosecond resolved FRET sensor

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## Abstract

Förster Resonance Energy Transfer (FRET) strategy in popular in fiber-optic sensing. However, the steady state emission quenching of the donor is inadequate to conclude FRET. The resonance type energy transfer from one molecule (donor) to other (acceptor) should meet few key properties including donor to acceptor energy migration in non-radiative way. In the present study, we have coupled the evanescent field of an optical fiber to the covalently attached donor (dansyl) molecules at the fiber tip. By using picosecond resolved time correlated single photon counting (TCSPC) we have demonstrated that dansyl at the fiber tip transfers energy to a well known DNA-intercalating dye ethidium. Our ultrafast detection scheme selectively distinguishes the probe (dansyl) emission from the intrinsic emission of the fiber. We have also used the setup for the remote sensing of the dielectric constant (polarity) of an environment. We have finally implemented the detection mechanism to detect an industrial synthetic dye methylene blue (MB) in water.

**Keywords:** Fiber-optic sensor, Time Correlated Single Photon Counting (TCSPC), Sensitized fiber tip.

## 1. Introduction

FRET based fiber optics sensors are evident since 1990s [1, 2]. FRET is a photo-physical process in which excited state energy from a donor is transferred 'non-radiatively' to an acceptor molecule at close proximity via dipole-dipole coupling. The reports on the FRET based fiber sensors mostly rely on the fluorescence quenching of the donor (probe) molecule in sensitized fiber [3-7]. However, Due to spectral overlap between donor emission and acceptor absorption spectra re-absorption of the donor radiation by the acceptor in the medium may occur which leads to fluorescence quenching of a donor molecule. We have addressed this issue in our recent report on FRET based fiber optic sensing [8]. Upon modification of the earlier reported setup (Figure 1) we were able to address multiple sensing applications. Real time detection of DNA hybridization is one of active area of research during the last decade for better understanding of the cellular and molecular biology, with a significant impact on sensing technology leading to new biosensors and sensing techniques for various applications. The traditional ways to detect DNA hybridization are slow [9], nevertheless, there are several recent works [10-12] regarding the sensitive detection process of the DNA hybridization, starting from rapid detection of fiber optic DNA sensor [13] to label-free electrochemical DNA sensor [10]. In this present work we were able detected ethidium bromide (EtBr) tagged DNA (DNA-EtBr) in-vivo by time resolved fluorescence spectroscopic technique upon utilization of the FRET between dansyl chloride and DNA-EtBr. Using the same detection mechanism it was possible to monitor the dielectric constant of a medium as the excited state lifetime of the probe dansyl heavily depends on the polarity of the immediate host environment. It is important in terms of remotely measuring the polarity of a medium such as the petroleum processing column which is reported to be important for the quality control of the petroleum products [14]. On the other hand synthetic dyes like Methylene Blue (MB) are being extensively used in various industries such as textile, paper and plastics with harmful effect in the environment [15]. The released aromatic amines from MB (benzidine, methylene etc) are found to be potential carcinogen. Extensive research has been devoted to remove MB from waste water (adsorption, filtration or chemical reaction) before dumping. However, monitoring the concentration of MB present in the waste water before or after the treatment is relatively less emphasized. The advantageous aspect of our detection mechanism has been further proven by detecting methyl blue (MB) in water after attachment of the DNA-EtBr in the fiber tip.

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## 2. Materials and Methods

### *2.1. Materials*

Dansyl chloride was received from Moleculer Probes (USA). The calf thymus DNA, (3-Aminopropyl) triethoxysilane (APTES), ethidium bromide (EtBr) and methylene blue were purchased from Sigma-Aldrich (USA). All the optical components used in our studies were received from Thorlabs (USA). The 16 channel TCSPC setup is assembled in our lab with all the components (16-channel PMT module PML-16-1-C, Simple Tau-130EM with SPC-130EM & DCC-100 cards, Express Card 54 and SPCM64 software pre installed in Lenovo ThinkPad laptop-PC) are from Becker & Hickl (Berlin, Germany). A UV pulsed light generated from LDH-P-C-375 picosecond pulsed diode laser heads (PicoQuant) was coupled into the optical fibers in our designed instrumental setup. PDL-80-D PicoQuant laser driver was used to drive the laser with adjustable repetition rates from 31.25 kHz to 80 MHz or CW operation and also with adjustable laser output power option. The overall instrument response of the TCSPC system is found to be 200 ps. For the sensor development we have used multi-mode silica core fiber (FT200UMT) from Thorlabs (USA). The core, clad and overall diameters of the silica fiber are 200 µm, 225 µm and 500 µm respectively.

### *2.2. Preparation of the sensor*

The sensor was prepares following the earlier reported procedure [8], in short the manually etched [16] fiber tips were thoroughly cleaned in acetone water mixture first. The fiber tips ware then immersed into a hydroxylating agent (3:1 ratio of $H_2SO_4$ and $H_2O_2$, also known as piranha solution) at $80^0$C ant kept for 20 minutes which makes the tip highly hydrophilic. After thoroughly rinsing with milipore water, the fiber tips were immersed in APTES solution at $45^0$C and were kept for 40 minutes. Upon conjugation of the APTES molecules with the surface it is covalently functionalized with a fluorescent dye (dansyl chloride) by exploiting the nucleophilic reactivity of the amine groups. We have used this dansylated fiber tips as a FRET based sensor for DNA-EtBr and for monitoring the complex parameters of micro environments. For the sensing of MB in water the APTS conjugated fiber tips ware immersed into DNA-EtBr solution at $4^0$c and kept overnight. After thoroughly rinsing with water the excess DNA-EtBr was removed from the fiber surface and ware used accordingly.

### *2.3. Experimental setup:*

Upon simplification of the earlier reported TCSPC setup [8] we were able to monitor the change in the excited state lifetime of the probe attached to the fiber tip. The schematic representation of the TCSPC setup is shown in the Figure 1. The PDL-80-D PicoQuant laser driver was used to drive the 375nm UV picosecond pulsed laser (LDH-P-C-375) with a repetition frequency of about 62.5 KHz. The pulsed laser beam passes through a dichroic mirror (which reflects <400 nm and passes wavelengths in the visible range) and a off-axis parabolic mirror (focal length 25 mm), and eventually enters into the proximal end of the sensitized fiber. Finally the fluorescence signal was collected by the fiber bundle (F-100), placed at the focal point of a condenser lens.

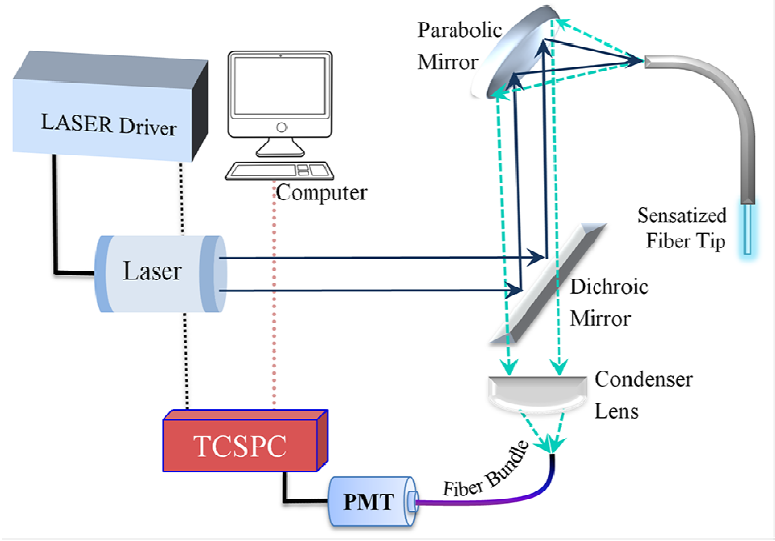


**Figure 1.** Experimental setup: Schematic diagram of the instrumentation based on time resolved based Förster resonance energy transfer (FRET) sensor.

Upon collection of the fluorescence signal by the 16 channel PMT (PML-16-1-C), 16 decays corresponding to 16 different wavelengths ware generated by using the Simple Tau-130EM module consisting of two special purpose data processing cards SPC-130EM and DCC-100. The processed electronic signal is fed to the Lenovo ThinkPad laptop-PC with pre installed SPCM64 software through Express Card 54. The steady state emission spectrum has been generated using the histogram plot, corresponding to maximum intensity of each channel (wavelength) (inset figure 2). All the experiments are performed in dark room to avoid any ambient light exposure.

### *2.4. Formulism:*

The FRET distance between donor and acceptor (R) was calculated following the procedure published elsewhere. Briefly, the Förster distance ($R_0$) is given by,

$$R_0 = 0.211\left[\kappa^2 n^{-4} Q_D J(\lambda)\right]^{\frac{1}{6}} \text{ (in Å)}, \quad (1)$$

where, $\kappa^2$ and $Q_D$ are factor describing the relative orientation in space of the transition dipoles of the donor and acceptor and quantum yield of the donor in absence of acceptor respectively [17]. The degree of spectral overlap between the donor emission and the acceptor absorption is given by,

$$J(\lambda) = \frac{\int_0^\infty F_D(\lambda)\varepsilon(\lambda)\lambda^4 d\lambda}{\int_0^\infty F_D(\lambda) d\lambda} \quad (2)$$

Where, $F_D(\lambda)$ is the fluorescence intensity of the donor in the wavelength range of λ to (λ +dλ) and *ε(λ)* is the extinction coefficient (in $M^{-1}cm^{-1}$) of the acceptor at the wavelength λ. The Donor–acceptor distance (R) has been calculated using the formula,

$$R^6 = \left[R_0{}^6\left(1-E\right)\right]/E, \quad (3)$$

where E is the FRET efficiency, measured by using the lifetime of the donor in the absence ($\tau_D$) and presence ($\tau_{DA}$) of acceptor, defined as,

$$E = 1-(\tau_{DA}/\tau_D) \quad (4)$$

The FRET efficiency (E) is calculated from the amplitude weighted lifetimes $\langle\tau\rangle = \sum_i \alpha_i \tau_i$, where $\alpha_i$ is the relative amplitude contribution to the lifetime $\tau_i$. We have used the amplitude weighted time constants for $\tau_D$ and $\tau_{DA}$ to evaluate E using Equation (4).

## 3. Results and Discussion

### *3.1. Characterizing the fiber sensor length*

The decay transients from the dansylated fiber (1 m) corresponding to 16 different wavelengths are represented in figure 2. It is evident that the decay pattern consists of two decay peaks. The detail investigation of such observation is already reported [8]. The histogram plot of the intensity maximas (inset figure 2) revealed two different emission peaks at 460 nm and 505 nm for peak 1 and peak 2 respectively. At $\lambda_{em}$= 505 nm the decay of peak 1 has an average lifetime of 2.68 ns, where as peak 2 was observed with an average excited state life time of 3.84 ns. Additionally, the path traveled (~ 2m) by light during the time difference (10 ns) between peak 1 and peak 2 is twice the length of the fiber (1 m), which confirms the origin of the second decay (peak 2) is from the sensitized tip.

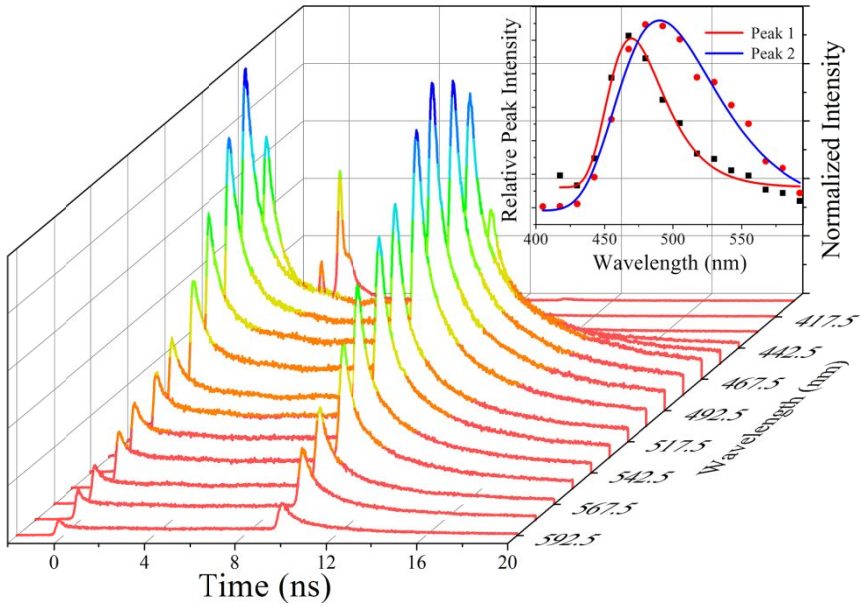


**Figure 2.** Characterization of the fiber sensor: 16 channel decay (400nm -600 nm) from the sensor (Fiber length 1 m) with dansylated fiber tip. (Inset) The histogram plot of the 16 channel decay maxima from both the decays with emission peak at 460 nm and 505 nm respectively.

### *3.2. Sensing DNA-EtBr and dielectric constant:*

We have employed time resolved FRET technique to detect DNA (EtBr labeled) with the dansylated fiber sensors (pH ~ 7). Picosecond resolved decay transients of dansylated fiber tip in presence of DNA monitored at 505 nm, shows significant shortening in the fluorescence lifetime. The fluorescence decay of dansylated fiber tip in water (pH~7) revealed multiexponential time constants of 0.50 ns (36%), 2.46 ns (37%) and 10.16 ns (27%) giving an average time constant ($\tau_{avg}$) of 3.84 ns. In presence of DNA (EtBr labeled) time constants obtained as 0.37 ns (57%), 2.1 ns (26%) and 7.19 ns (17%) giving an average time constant ($\tau_{avg}$) of 1.97 ns (Table I). The substantial shortening in the dansyl excited state lifetime upon conjugate formation indicates conclusively that efficient FRET occurs from the dansyl donor to the DNA-EtBr acceptor. Taking the quantum yield of dansylated fiber tips in absence of acceptor as 0.7 [18] and based on the spectral overlap, we have estimated a FRET efficiency of 52% using Eq. (4). The measured Förster distance for the donor-accopter pair is $R_0$ is 2.64 nm and the donor–acceptor distance (r) calculated using Eq. (3) is 2.68 nm.

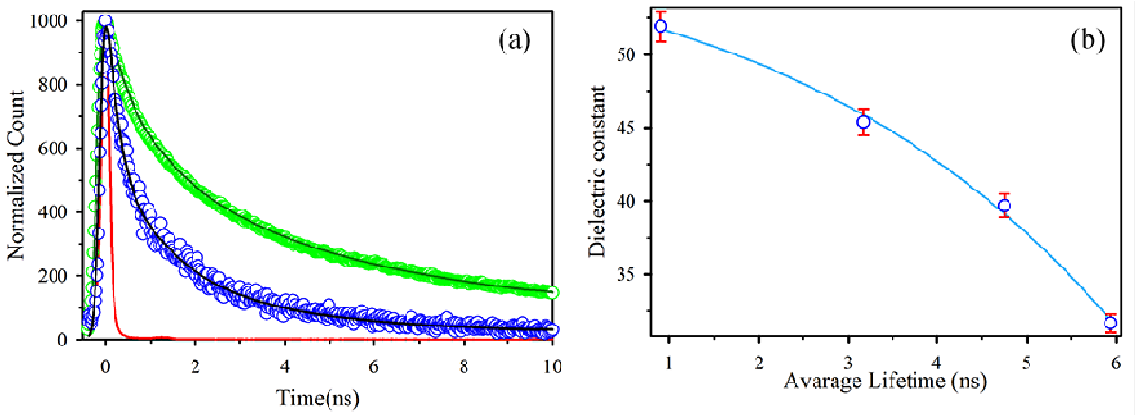


**Figure 3.** Working of the sensor: (a) Picosecond-resolved PL transients of dansylated fiber tip in absence and presence of EtBr-labeled DNA complex monitored at $\lambda_{em}$ = 505 nm. (b) The remote sensing application of the sensor where with increasing water content in the dioxane-water solution the decay becomes much faster.

In order to measure the dielectric constant of an medium [8] micro-environment sensing ability of this sensor with different dielectric mediums were performed. 1, 4-dioxane has a polarity index of about 4.8 which is less, compare to the polarity of water 80. We have used different ratio of dioxane and water (pure Dioxane, 3:1, 1:1 and 1:3) kipping the fixed total volume of 2ml. It is evident from the decay transients of the dansylated fiber tip in different environments the overall decay became faster with increase of water content in the solution from 5.97 ns to 0.89 ns. The dependency between the dielectric constant and the average lifetime of the dansylated fiber tip is represented in the figure 3 (b). Decrease in dielectric constant of the medium results in consequent decrease in average lifetime with an overall exponential dependency.

### *3.3. Sensing MB in Water:*

A strong spectral overall between the emission of donor (DNA-EtBr) with that of the absorption spectrum of the acceptor (MB) is evident from Figure 4a. From the Figure 4a the overlap integral of DNA-EtBr (donor) emission spectrum with that of the MB (acceptor) absorption (equation 2) was estimated to be $5.2 \times 10^{15}$ $M^{-1}cm^{-1}nm^4$. A significant steady state quenching of DNA-EtBr emission at 600 nm at the fiber tip is evident from Figure 4b. A direct evidence of the resonance type energy transfer is obtained from both the steady state quenching (Figure 4b) and time resolved fluorescence transients of the donor DNA-EtBr at the fiber tip as shown in Figure 4c. The faster fluorescence decay of the DNA-EtBr in MB solution is evident from the Figure 4c and table I, which confirms proximity of the synthetic dye at the fiber surface. On taking quantum yield of DNA-EtBr in absence of acceptor MB to be 0.2, we have estimated a FRET efficiency of 44% using Equation (4). The estimated Förster distance, $R_0$, for the FRET pair is found to be 49.16 Å. The donor–acceptor distance (R) using Equation (3) is calculated to be 51.14 Å.

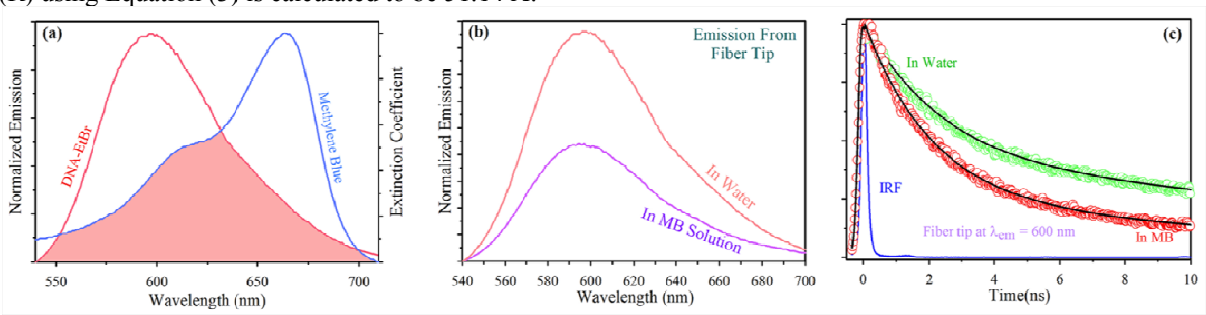


**Figure 4.** Methylene blue (MB) sensing: (a) Spectral overlap between emission spectrum of EtBr-labeled DNA attached fiber tip and the absorption spectrum MB. (b) Steady-state fluorescence quenching of EtBr-labeled DNA attached fiber tip in presence of the acceptor MB (c) Picosecond-resolved PL transients of EtBr-labeled DNA attached fiber tip in presence and absence of MB monitored at $\lambda_{em}$ = 600 nm.

| **Figures** | **Description** | **$\tau_1$ [ns] %** | **$\tau_2$ [ns] %** | **$\tau_3$ [ns] %** | **$\tau_{avg}$ [ns]** |
|---|---|---|---|---|---|
| Figure 2 | 1st Decay ($\lambda_{em}$=500 nm) | 0.27 (45%) | 0.72 (33%) | 10.42 (22%) | 2.68 |
| | 2nd Decay ($\lambda_{em}$=500 nm) | 0.50 (36%) | 2.47 (37%) | 10.16 (27%) | 3.84 |
| Figure 3(a) | Fiber tip in water | 0.50 (36%) | 2.47 (37%) | 10.16 (27%) | 3.84 |
| | Fiber tip in DNA-EtBr | 0.37 (57%) | 2.12 (26%) | 7.19 (17%) | 1.98 |
| Figure 4(c) | Fiber tip with DNA-EtBr | 1.34 (57%) | 11.50 (43%) | - | 5.68 |
| | In MB solution | 1.12 (50%) | 5.14 (50%) | - | 3.13 |

**Table I:** Tri-exponential decay fitting of dansylated fiber tip in different environment corrosponding to Figure 2 and Figure 3(a). Bi-exponential decay filting corrosponding to Figure 4(c).

## 4. Conclusion

In conclusion, we have successfully detected the presence of DNA by resonance type energy transfer scheme in a model FRET based fiber optic sensor. The time domain measurement strategy also eliminates possible

interference from the background emission of the system. FRET mechanism allows us to use dipole-dipole coupling formulism for the estimation of proximity of the acceptor with respect to the sensitized fiber tip (donor) in angstrom resolution. The efficacy of the designed fiber sensor for the detection of various dielectric constants of a liquid medium has also been established. It has also been possible to measure the presence of MB in water by this detection mechanism. In near future it is expected that our FRET based study will find relevance in highly responsive optical sensor development.

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