



# Single molecule fluorescence in ultrathin capillaries: an electrodynamic study

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## Abstract

The present Letter studies theoretically how the fluorescence lifetime of dye molecules changes when they are confined within glass microcapillaries. The lifetime change is due to the electrodynamic interaction between the emitting molecule and the confining microcavity. The study is done within the framework of the classical theory of spontaneous dipole emission. Numerical results are presented for experimentally relevant conditions. © 1999 Elsevier Science B.V. All rights reserved.

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## 1. Introduction

In recent years, the experimental study of single molecule fluorescence has seen tremendous advances. Of special interest for biological and chemical applications, but also from a pure scientific point of view, are experiments concerning single molecule detection (SMD) and spectroscopy of fluorophores that are solved in solutions (see, e.g., Ref. [1]). The main difficulty in detecting solved single molecules is to discriminate a usually large background (mainly due to Rayleigh/Raman scattering of the exciting laser light) against the single molecule's fluorescence. Because the detected intensity of the scattered light is proportional to the effective volume of the detection region, one tries to minimise this volume. Several experimental techniques were developed for

this purpose: SMD of molecules within a hydrodynamically focused flow [2]; detection of freely diffusing molecules in ultrasmall detection volumes by confocal microscopy [3,4]; and SMD in levitated microdroplets [5]. Recently, new experiments were reported concerning SMD in ultrathin capillaries [6,7].

For both microdroplets and microcapillaries, interesting electrodynamic resonance effects between the molecule's fluorescence and the confining microcavity (microdroplet or microcapillary, respectively) can be expected. Indeed, for microdroplets, both experimental and theoretical results were reported showing changes of fluorescence lifetimes and spectra due to the electrodynamic interaction between the optically excited molecule and the droplet cavity [8–10].

The goal of the present work is to present a theoretical study of the fluorescence emission of solved molecules within a cylindrical microcapillary. The study is done within the framework of classical

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electrodynamics. The fluorescing molecule is considered as an oscillating dipole embedded within a dielectric solvent, which is confined within a cylindrical glass capillary with different dielectric constant. The effect studied here in detail is the change of the fluorescence lifetime. In Section 2, a brief description of the theoretical approach is given. In Section 3, numerical results are presented and discussed for an example with parameter values of practical interest.

## 2. Theoretical background

Starting point of our considerations is the representation of the electromagnetic field of a free oscillating dipole (angular frequency  $\omega$ ) in cylindrical coordinates  $\rho$ ,  $\phi$  and  $z$ . The dipole is supposed to be embedded within a homogeneous medium with dielectric constant  $\epsilon_{\text{in}}$  at position  $(\rho_0, \phi_0, z_0)$ . The time dependence of all fields is proportional to  $\exp(-i\omega t)$ . For  $\rho \geq \rho_0$ , the electric field amplitude of the oscillator is then given by [11]

$$\vec{E}_0(\rho, \phi, z) = \frac{i}{k_0 \epsilon_{\text{in}}} (\text{grad div } \vec{A} + \epsilon_{\text{in}} k_0^2 \vec{A}), \quad (1a)$$

$$\vec{A} = \frac{k_0 \vec{p}}{2} \int_{-\infty}^{\infty} dw \sum_{n=-\infty}^{\infty} \exp[in(\phi - \phi_0) + iw(z - z_0)] J_n(q\rho_0) H_n^{(1)}(q\rho), \quad (1b)$$

where  $\vec{p}$  is the amplitude of the dipole strength,  $k_0 = \omega/c$  is the wave vector of light in vacuum, and  $q = \sqrt{\epsilon_{\text{in}} k_0^2 - w^2}$  with  $\text{Im } q \geq 0$ . The  $J_n$  and  $H_n^{(1)}$  are Bessel functions of the first and third kind, respectively [12].

Next, the dipole is supposed to be situated within a glass capillary with inner radius  $R_1 \geq \rho_0$  and outer radius  $R_2 > R_1$ . The dielectric constant of the glass is denoted by  $\epsilon_{\text{gl}}$ , and the dielectric constant of the medium outside the capillary by  $\epsilon_{\text{out}}$ . The complete electromagnetic field inside the capillary is now composed of two parts: the original field  $\vec{E}_0$  of the free dipole, and a reaction field  $\vec{E}_R$  arising from the interaction with the capillary and its surrounding.

The main parameter we are interested in is the change of the lifetime  $\tau$  of the excited state of the fluorescing molecule with respect to its bulk lifetime  $\tau_0$ . Within the classical context presented here, this is equivalent to calculating the emission rate  $S$  which is inverse proportional to the fluorescence lifetime. Following Chance et al. [13], the relative emission rate is given by

$$\frac{S}{S_0} = \frac{\tau_0}{\tau} = 1 + \frac{3\vec{p} \text{Im } \vec{E}_R(\vec{r}_0)}{2n_{\text{in}} k_0^3 p^2}, \quad (2)$$

where  $n_{\text{in}} = \sqrt{\epsilon_{\text{in}}}$  is the refractive index of the solvent (capillary interior), and  $S_0$  denotes the emission rate of the free dipole embedded in a dielectric with refractive index  $n_{\text{in}}$ .

To calculate the reactive field  $\vec{E}_R$  at the dipoles position, we rewrite  $\vec{E}_0$  in terms of the cylinder vector functions  $\vec{M}_{q,n}^{\mathcal{E}}$  and  $\vec{N}_{q,n}^{\mathcal{E}}$  defined by

$$\vec{M}_{q,n}^{\mathcal{E}} = \left[ \frac{in}{\rho} \mathcal{E}_n \hat{e}_\rho - q \mathcal{E}'_n \hat{e}_\phi \right] \exp(in\phi + iwz), \quad (3a)$$

$$\vec{N}_{q,n}^{\mathcal{E}} = \left[ iwq \mathcal{E}'_n \hat{e}_\rho - \frac{nw}{\rho} \mathcal{E}_n \hat{e}_\phi + q^2 \mathcal{E}_n \hat{e}_z \right] \exp(in\phi + iwz), \quad (3b)$$

where  $\mathcal{E}$  denotes any of the Bessel functions. Then, by direct evaluation of Eqs. (1a) and (1b) one obtains

$$\vec{E}_0 = \frac{1}{\epsilon_{\text{in}}} \int_0^\infty dw \sum_{n=-\infty}^{\infty} \left[ A_{q_{\text{in}},n}^0 \vec{M}_{q_{\text{in}},n}^{H^{(1)}} + B_{q_{\text{in}},n}^0 \vec{N}_{q_{\text{in}},n}^{H^{(1)}} \right], \quad (4a)$$

with the abbreviations

$$A_{q,n}^0 = \frac{\sin \beta k^2 \exp(-in\phi_0)}{2q} \times [\exp[i(\alpha - \phi_0)] J_{n+1}(q\rho_0) + \exp[-i(\alpha - \phi_0)] J_{n-1}(q\rho_0)], \quad (4b)$$

$$B_{q,n}^0 = \frac{w \sin \beta \exp(-in\phi_0)}{2q} \times [\exp\{-i(\alpha - \phi_0)\} J_{n-1}(q\rho_0) - \exp\{i(\alpha - \phi_0)\} J_{n+1}(q\rho_0) + i \cos \beta \exp(-in\phi_0) J_n(q\rho_0)], \quad (4c)$$

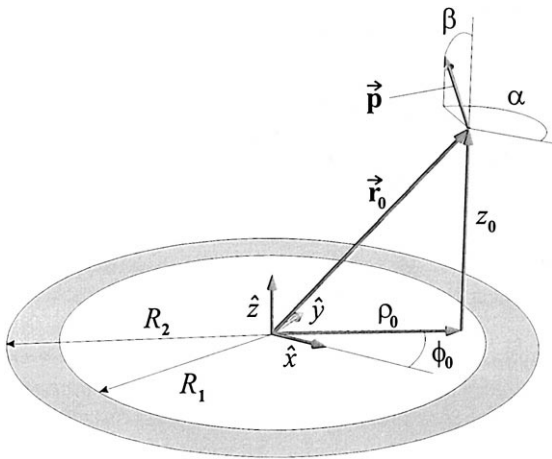


Fig. 1. Geometry of the dipole position and orientation within the capillary (grey ring). Shown are the unit vectors  $\hat{x}$ ,  $\hat{y}$  and  $\hat{z}$  of a Cartesian reference system, the cylindrical coordinates  $\rho_0$ ,  $\phi_0$  and  $z_0$  of the dipole position  $\vec{r}_0$ , and the orientation angles  $\alpha$ ,  $\beta$  fixing the orientation of the dipole  $\vec{p}$ .  $R_1$  and  $R_2$  are the inner and outer capillary radius, respectively.

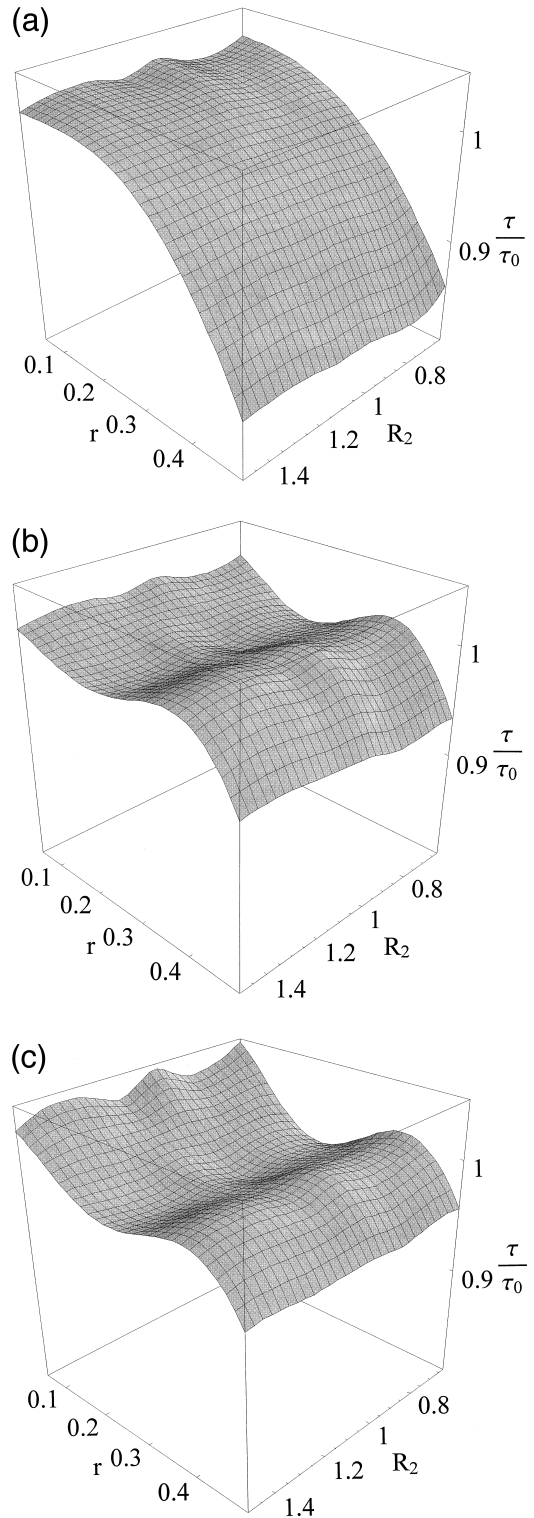
where  $\alpha$  and  $\beta$  are orientation angle of the dipole  $\vec{p}$  defined by the relation

$$\vec{p} = p [\cos(\alpha - \phi_0) \sin \beta \hat{e}_x + \sin(\alpha - \phi_0) \sin \beta \hat{e}_y + \cos \beta \hat{e}_z]. \quad (5)$$

with  $\hat{e}_{x,y,z}$  being unit vectors in a Cartesian coordinate system, see also Fig. 1.

For the unknown reaction field within the capillary, and the unknown field amplitudes within the capillary walls and outside the capillary, an ansatz analogous to Eqs. (4a), (4b) and (4c) is made, but with  $\mathcal{E} \equiv J$  for  $\rho < R_1$ ,  $\mathcal{E} \equiv J, Y$  for  $R_1 < \rho < R_2$ , and  $\mathcal{E} \equiv H^{(1)}$  for  $R_2 < \rho$ , and a corresponding set of unknown constants instead of the  $A_{q,n}^0$ ,  $B_{q,n}^0$  in Eq. (4a). The symbol  $Y$  stands for Bessel functions of the second kind [12].

Fig. 2. (a) Relative fluorescence lifetime of a dipole oriented along  $\hat{p} = \hat{x} \cos \phi + \hat{y} \sin \phi$  (perpendicular to the capillary wall) for different dipole positions  $\rho_0$  and outer capillary radius  $R_2$  (both given in units of wavelength). The inner capillary radius is set equal to  $\lambda/2$ . The lifetime is normalised by the bulk lifetime in water/glycol. (b) Same as (a), but for dipole orientation along  $\hat{p} = -\hat{x} \sin \phi + \hat{y} \cos \phi$ . (c) Same as (b), but for dipole orientation along  $\hat{z}$ .



For every value of  $n$ , eight unknown constants have to be determined. These constants can be found by imposing the usual boundary conditions of continuity for the tangential components of the electric and magnetic field amplitudes at the inner and outer capillary walls, yielding an eight by eight linear system of equations (for every value of  $n$ ). Solving this system for the eight constants, evaluating the corresponding reaction field at the dipole's position, and inserting the result into Eq. (2) finally yields the desired information about the change of the emission rate, viz. the fluorescence decay time.

### 3. Numerical results and discussion

In the experimental work of Ref. [7], the capillary was filled with a water/glycerol/Tween 20/dye solution and itself embedded in glycerol to reduce Rayleigh scattering signal. Thus, for the numerical calculations the following values of the dielectric constants were chosen:  $\epsilon_{\text{in}} = 1.4^2$ ,  $\epsilon_{\text{gl}} = 1.51^2$  and  $\epsilon_{\text{out}} = 1.47^2$ . The inner radius of the capillary was set equal to  $R_1 = \lambda/2$ , with  $\lambda$  being the vacuum wavelength. The outer radius  $R_2$  and the radial position  $\rho_0$  of the molecule–dipole were considered as changing parameters. For the three possible orientations of the dipole along  $\hat{\rho} = \hat{x} \cos \phi_0 + \hat{y} \sin \phi_0$ ,  $\hat{\rho} = -\hat{x} \sin \phi_0 + \hat{y} \cos \phi_0$ , and  $\hat{z}$  (a symbol with a hat denoting unit vectors along the corresponding direction), the relative changes of the lifetime in dependence on the outer radius value  $R_2$  and dipole position  $\rho_0$  are shown in Fig. 2a–c. Although the capillary wall thickness is small compared with the wavelength, and the values of the refractive index are very close together, the change in lifetime can reach up to 15%. The largest change occurs for a dipole orientation perpendicular to the capillary wall, similar to the situation of a dipole over a flat surface.

In a real experiment, the position of a fluorophore is not fixed in space. Two different situations may occur: the molecule is freely diffusion within the capillary, or it is adsorbed at the capillary wall. In the first case, the measured fluorescence lifetime will be an average of all lifetimes for all possible dipole positions and orientations in the capillary (it is assumed that the lifetime measurement time is much larger than the characteristic translational and rota-

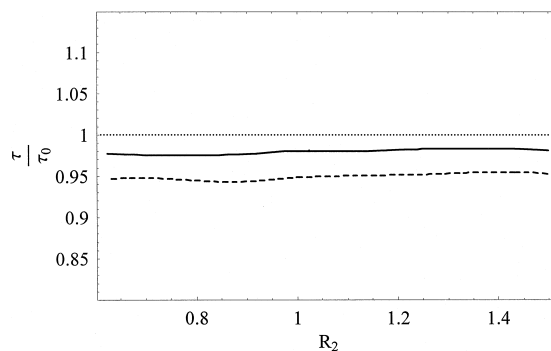


Fig. 3. Measurable lifetime change for freely diffusing dye (solid line), and for dye adsorbed to the capillary wall (dashed line). For comparison, the dotted straight line is the bulk lifetime in water/glycerol (refractive index  $n = 1.4$ ).

tional diffusion times of the fluorophore). In the second case, the dipole position is fixed at  $\rho_0 = R_1$ , and the orientation is mainly an average of all orientations tangential to the capillary wall (usually, adsorbed fluorophores orient their absorption/emission dipole axis parallel to the adsorbing surface). In Fig. 3, a plot for both cases for different values of  $R_2$  is presented. As can be seen, the lifetime change, if averaged over all dipole positions and orientations, is rather small. But under favourable experimental conditions, it should be feasible to measure the lifetime shortening for the adsorbed dye.

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