

# Theoretical Treatment of Scan Time Dependent Optical Bands of Single Molecule Embedded in Polymer or Glass

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A theoretical framework for the description of fluctuating absorption coefficients of a guest molecule embedded in polymer or glass is presented. These fluctuations emerge in experiments with scans of laser frequency. A relation between theoretical expression for the time dependent single molecule absorption coefficient and the absorption coefficient measured in experiments with scanning laser frequency is discussed. The analysis is carried out for a guest molecule whose optical band consists of two well-resolved and poorly resolved optical lines. For well-resolved optical lines, measurement of auto- and cross-correlators is proposed. These correlators enable one to compare the fluctuating absorption coefficient with the time dependent absorption coefficient calculated theoretically with no adjustable parameters. For poorly resolved optical lines, the time dependent absorption coefficient enables one to calculate temporal line broadening of single molecule and to compare it with measured line broadening in time scale of seconds.

## I. Introduction

Measurement of fluorescence of individual guest molecules embedded in a solid matrix and driven by a cw-laser field is a new, powerful method to probe dynamics of individual guest molecules. This single molecule spectroscopy (SMS) method has a significant advantage as compared to spectroscopy of molecular ensembles because SMS enables one to remove inhomogeneous broadening of optical bands.<sup>1–7</sup> Due to this fact, all peculiarities of dynamics of individual guest molecules can be studied in detail.

However, the character of experimental data in SMS differs considerably from that in molecular ensembles because the intensity of single-molecule fluorescence, as a rule, fluctuates. Fluorescence intermittency is a typical phenomenon in SMS. Such blinking fluorescence was discovered in various physical systems, namely, in single light harvesting 2 complex,<sup>8</sup> single polymer molecules,<sup>9–13</sup> single dendrimers,<sup>14–16</sup> proteins,<sup>17,18</sup> and single dye molecules attached to DNA.<sup>19</sup> Single molecule chemical reaction can be monitored by fluctuating fluorescence.<sup>20</sup>

On the one hand, the fluctuating character of experimental data in SMS complicates comparison with the theory based on quantum mechanics because quantum mechanics gives prescriptions for calculation of only probabilities but not fluctuations. However, on the other hand, these very fluctuations of fluorescence are unique sources of information about dynamics of an individual guest molecule. Especially, it is true at room temperature when optical bands are very broad and therefore the shape of the band is noninformative. The fluctuations disappear in molecular ensembles because of ensemble averaging. Development of methods for extracting physical information from fluctuating fluorescence of single molecules with the help of the theory is a problem of great importance.

As a rule, fluctuations of fluorescence are treated statistically.<sup>9–16</sup> Distribution functions found from such averages permit

us to find relaxation constants of processes, which are responsible for fluctuations. However, if we want to build up a microscopic model for the system with fluctuating fluorescence we need to invoke a theory based on quantum mechanics for interpretation of the distribution functions found from statistical treatment of the fluctuations. It was shown in ref 21 how such a theory can help to build a microscopic model for blinking fluorescence of a single polymer molecule measured earlier by the Barbara group.<sup>9</sup>

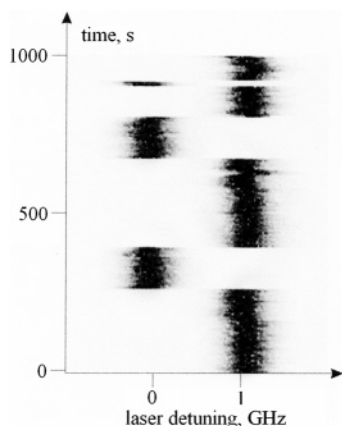
Optical bands of guest molecules at room temperature are broadened by phonons, and absorption bands do not reveal structure. Therefore, fluorescence of single guest molecules measured at room temperature is excited by cw laser light with fixed resonant frequency as it was carried out in refs 8–19. However, there are many works where single molecules are studied at low temperature when optical bands reveal fine structure. In low-temperature experiments fluorescence is excited by cw laser with scanning laser frequency.<sup>2–7,22–24</sup>

Such experiments are carried out in low-temperature polymers and glasses where optical bands of individual guest molecules consist of few narrow lines with fwhm on the order of a few hundred MHz. Scanning of laser frequency is used to facilitate the interpretation of dynamics of such bands. In this case, the view of optical spectra depends on duration of laser scan. Optical spectra became scan-time dependent.

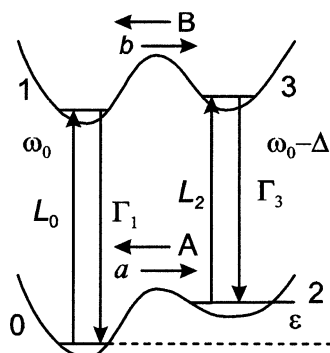
To take into account dependence on laser scan duration, Geva and Skinner proposed a simple model.<sup>25,26</sup> In accordance with this model, TLS can contribute to the optical band only if its relaxation time is shorter than laser scan duration. The longer the scan time, the more TLSs contribute to the optical band. Plakhotnik showed<sup>27</sup> that this model is crude, and he proposed to employ the method of autocorrelation function to treat experimental data obtained with laser scans.

The main goal of this paper is to find how the theory can be used for treating scan-time dependent optical bands of individual guest molecules.

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**Figure 1.** 2D plot of single molecule fluorescence showing jumps of absorption line of single molecule tetra-*tert*-butyl-terylene in polyisobutylene at 7 K.<sup>22</sup> Duration  $t$  of laser scan is 8 s. Rate  $R$  of spectral jumps is less than  $1/t$ .



**Figure 2.** Energy diagram for two-level guest molecule interacting with one TLS.

## II. Two-State-Jump Model

Guest molecules embedded in low-temperature polymer reveal rather narrow optical lines. If frequency of exciting laser is scanned on the time scale of seconds, jumps of absorption optical lines can be observed. Figure 1 shows an example of these jumps. What is the reason to appearance of fluctuating electronic resonance frequency? The appearance of fluctuations in the time scale of seconds can be connected with slow rearrangement of the local environment of individual guest molecule. For last three decades, such rearrangement of atoms or groups of atoms in glasses and polymers is described in the frame of the so-called two-level-system (TLS) model, introduced more than thirty years ago by Anderson et al.<sup>28</sup> and Phillips<sup>29</sup> to explain anomalous properties of low-temperature glasses. An energy diagram describing the TLS model is shown in Figure 2. Here the energy levels 0 and 1 relate to guest molecule with one type of its environment. The energy levels 2 and 3 relate to the same guest molecule after rearrangement of its environment. The diagram shown in Figure 2 is able to describe the appearance of two trails in Figure 1. The right trail corresponds to optical transition 0–1 with frequency  $\omega_0$ . The left trail in Figure 1 corresponds to optical transition 2–3.

It is obvious that Figure 1 demonstrates that the absorption coefficient of the system depends on time and initial conditions. The Silbey group<sup>30,31</sup> undertook an attempt to describe the dynamics of individual molecules by considering a guest molecule interacting with one TLS. This model the authors of ref 31 named the “two-state-jump model” (TSJM). How can the time dependent optical band shown in Figure 1 be considered in the frame of TSJM? To answer this question, Silbey’s group

considered optical Bloch equations with resonant frequency jumping in accordance with a random telegraph process. Fluctuations of the resonant frequency introduced artificially to Bloch equations enabled them to calculate random trails such as those shown in Figure 1. To calculate such trails they used Monte Carlo simulation for temporal behavior of resonant frequency. However, the problem of finding a theoretical expression for the time-dependent absorption coefficient was not solved by this group. This task is solved in our work for the case when only one TLS from the nearest vicinity interacts strongly with a guest molecule, and therefore the optical band of such impurity center consists of two lines. This case is of practical importance because experiment<sup>32</sup> shows that near 40% of individual impurity centers in polymers have such optical bands.

## III. Dynamics of TSJM

Cook and Kimble<sup>33</sup> have shown that quantum jumps in single atoms can be described with the help of rate equations. Since jumps of optical line shown in Figure 1 reflect jumps of the system from left pairs of wells to right pairs of wells, we write rate equations for the diagram shown in Figure 2 as follows:

$$\begin{aligned}\dot{\rho}_1 &= -(\Gamma_1 + b)\rho_1 + L_0\rho_0 + B\rho_3 \\ \dot{\rho}_3 &= b\rho_1 - (\Gamma_3 + B)\rho_3 + L_2\rho_2 \\ \dot{\rho}_0 &= \Gamma_1\rho_1 - (L_0 + a)\rho_0 + A\rho_2 \\ \dot{\rho}_2 &= a\rho_0 + \Gamma_3\rho_3 - (L_2 + A)\rho_2\end{aligned}\quad (1)$$

Here the functions

$$\begin{aligned}L_0(\omega) &= 2\chi^2 \frac{1/T_2}{(\omega - \omega_0)^2 + 1/T_2^2} \\ L_2(\omega) &= 2\chi^2 \frac{1/T_2}{(\omega - \omega_0 + \Delta)^2 + 1/T_2^2}\end{aligned}\quad (2)$$

describe light-induced optical transitions,  $\chi = \mathbf{E}\mathbf{d}/\hbar$  is Rabi frequency, and  $\Gamma_{1,3} = L_{0,2} + 1/T_1$ , where  $T_1$  is the lifetime of the excited electronic level. Here optical dephasing rate is given by

$$\frac{1}{T_2} = \frac{1}{2T_1} + \frac{\gamma(t)}{2}\quad (3)$$

The second term depends on time due to the interaction with a huge number of remote TLSs. These TLSs can be beyond thermal equilibrium. This dependence on time will be discussed in detail in section VIII of the paper.

Constants for fast vertical transitions and slow horizontal transitions shown in Figure 2 satisfy the following inequalities

$$1/T_1 \gg L_{0,2} \gg B, A > b, a\quad (4)$$

In accordance with this hierarchy, jumps between the ground and the excited electronic states of the guest molecule are much faster than jumps between two states of TLS. Therefore, we can use the following approximation:  $\dot{\rho}_1 = \dot{\rho}_3 = 0$ . Then we find from the first and the second lines in eq 1 the following relations

$$\rho_1 = \frac{L_0(\Gamma_3 + B)\rho_0 + L_2B\rho_2}{\Gamma_1\Gamma_3 + b\Gamma_3 + B\Gamma_1} \approx \frac{L_0}{\Gamma_1}\rho_0 + \frac{L_2B}{\Gamma_1\Gamma_3}\rho_2\quad (5)$$

$$\rho_3 = \frac{L_2(\Gamma_1 + b)\rho_2 + L_0b\rho_0}{\Gamma_1\Gamma_3 + b\Gamma_3 + B\Gamma_1} \approx \frac{L_2}{\Gamma_3}\rho_2 + \frac{L_0b}{\Gamma_1\Gamma_3}\rho_0 \quad (6)$$

Inserting these expressions for  $\rho_1$  and  $\rho_3$  into the third and the fourth lines of eq 1, we arrive at the following rate equations for the population of two electronic ground states:

$$\dot{\rho}_0 = -\frac{\rho_0}{\tau_b} + \frac{\rho_2}{\tau_r}, \quad \dot{\rho}_2 = \frac{\rho_0}{\tau_b} - \frac{\rho_2}{\tau_r} \quad (7)$$

These equations are similar those derived by Cook and Kimble<sup>33</sup> for a single atom. Here

$$\frac{1}{\tau_b} = R_b = a + \frac{L_0}{\Gamma_1}b, \quad \frac{1}{\tau_r} = R_r = A + \frac{L_2}{\Gamma_3}B \quad (8)$$

are rate constants depending on laser intensity via the Rabi frequency. Dependence of rate constants on laser intensity yields dependence of line width on laser intensity.<sup>34</sup> Such light-induced broadening in single-molecule optical bands was observed in experiments.<sup>6</sup> These rate equations will be used to find an expression for fluctuating absorption coefficient.

#### IV. Calculation of Quantum Jumps

Vertical bands with interruptions are called trails. Left and right trails in Figure 1 we shall call red and blue trail. It is obvious that each random on-interval in the red trail equals the corresponding off-interval in the blue trail in Figure 1. The degree of blackening in the trails is proportional to the number of the photons of fluorescence. However, this number is also proportional to the probability of the absorption. The probabilities of the absorption are proportional to the probabilities of finding the system in the quantum states 0 and 2 shown in Figure 2.

Calculation of trails such as those shown in Figure 1 can be carried out if the probabilities  $P_0(t)$  and  $P_2(t)$  to find blue and red on-interval of duration  $t$  are known. How they can be found? It seems, on the first view, that  $P_0(t) \propto \rho_0(t) + \rho_1(t)$  and  $P_2(t) \propto \rho_2(t) + \rho_3(t)$ , where probabilities  $\rho_i(t)$  can be found from eq 1 or from eq 5, eq 6, and eq 7. However, this suggestion is wrong. Indeed the probabilities  $P_0(t)$  and  $P_2(t)$  must approach zero if time increases. The solution of eq 1 satisfies the law of conservation  $\rho_0 + \rho_1 + \rho_2 + \rho_3 = 1$ . The simplified eq 7 was derived under the assumption  $\dot{\rho}_1 = \dot{\rho}_3 = 0$ . Therefore, the probabilities found from eq 7 satisfy another law of conservation  $\rho_0 + \rho_2 = \text{const}$ . Hence neither solution of eq 1 nor solution of eq 7 can describe the distribution function of red and blue on-intervals.

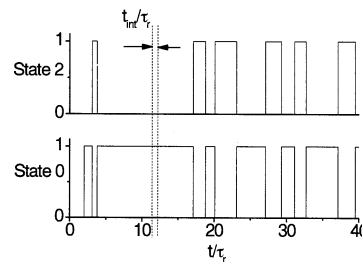
Equations for the distribution functions  $P_0(t)$  and  $P_2(t)$  can be found with the help of eq 7 as follows. If we omit the term  $\rho_2/\tau_r$ , which describes transitions from the red on-states to the blue on-states, we arrive at the following equation:

$$\dot{P}_0(t) = -P_0(t)/\tau_b \quad (9)$$

This is the equation for the probability of finding the system in the blue on-state of the duration  $t$  if the pump from the red on-state is switched off. If we omit the term  $\rho_0/\tau_b$  pumping red on-states, we arrive at the following equation:

$$\dot{P}_2(t) = -P_2(t)/\tau_r \quad (10)$$

The transition from eq 1 to eq 9 and eq 10 is not approximation. This is the way of derivation of equations for the



**Figure 3.** Two quantum trajectories showing fluctuations of the quantum states 0 (low panel) and 2 (upper panel). These fluctuations are simulated with the help of eq 12 and eq 13 for  $\tau_b = 3\tau_r$ . Vertical strip shows data acquisition time  $t_{\text{int}}$  of photon counting.

distribution functions from the set of equations describing the dynamics of the whole system. It has to be noted that eq 9 and eq 10 are *exact equations* for the functions describing distribution of the red and blue intervals. We shall prove this statement in section VII. We shall use these very equations in computer simulation. It can be carried out as follows.

If the system occupies the state 2 at initial moment with the probability equal unit, the equation

$$P_2(t) = \exp\left(-\frac{t}{\tau_r}\right) \quad (11)$$

describes the probability of finding the system in the same state by time moment  $t$ . We may say that  $P_2(t)$  is the probability of finding no jump from the state 2 to the state 0. Therefore, the probability to find a jump from the state 2 to the state 0 is given by

$$W_2(t) = 1 - P_2(t) = 1 - \exp\left(-\frac{t}{\tau_r}\right) \quad (12)$$

Here  $W_2(t)$  is a random value between zero and unit. For instance, it can be realized in Mathcad with the help of the procedure  $\text{rnd}(n)$  in which  $n$  is a step number, i.e., we set  $W_2(t) = \text{rnd}(n)$ . Random time moments of jumps from the state 2 to the state 0 will be given by the solution of eq 12. It looks as follows:

$$t_2 = -\tau_r \ln[1 - W_2(t)] \quad (13)$$

It is obvious that random time moment of the jump from the state 0 to the state 2 will be given by

$$t_0 = -\tau_b \ln[1 - W_0(t)] \quad (14)$$

Here  $W_0(t) = \text{rnd}(n)$  is a random value between zero and unit.

Random values of red and blue on intervals calculated with the help of eq 13 and eq 14 at  $\tau_b = 3\tau_r$  are shown in Figure 3. By blackening the rectangles in Figure 3 and turning this figure by 90° we arrive at the trails that are similar those depicted in Figure 1. Trails of such type have been already calculated in ref 31 by invoking Bloch equations with jumping resonant frequency. Jumps of resonant frequency were characterized by a single intensity-independent rate constant. In our case, the rate constants  $1/\tau_r$  and  $1/\tau_b$  governing the process of jumping are the functions of the microscopic TLS parameters and the laser intensity.

#### V. Time-Dependent Absorption Coefficient

The question is: how can a system with quantum jumps of optical lines be described quantum mechanically? Fluctuations of the absorption coefficient are a clear sign that we deal with

the system, which cannot be described by the density matrix in thermal equilibrium. Indeed, since a photon is emitted, the impurity center (chromophore + TLSs) changes its quantum state and it occupies another quantum state with the probability equal unit. This value of probability differs from the value of the same probability in equilibrium state. Therefore, the value of the probability will evolve with time approaching the equilibrium value. This situation cannot be described within the frame of the system in which TLSs are in thermal equilibrium. Therefore, theoretical expression for the absorption coefficient will include density matrix for TLSs, which depends on time.

The theory for the absorption coefficient of the guest molecule interacting with many TLSs, whose probabilities evolve with time, was developed earlier in ref 35 in connection with spectral diffusion effects found in hole burning experiments. The density matrix in this work depends on time and initial conditions. The absorption coefficient calculated with the help of such density matrix depends on time and initial conditions as well. Later this theory was employed to calculate the autocorrelation function of fluctuating fluorescence of individual chromophores interacting with TLSs.<sup>36–38</sup>

The density matrix approach to the autocorrelation function in SMS was developed also by Kilin et al.<sup>39</sup> for chromophores interacting with one TLS. These authors took into account slow jumps between two TLS states in the ground and excited electronic state. However, the expression for the absorption coefficient was not derived in ref 39.

The theory developed in ref 35 is strict but rather complicated. Here we derive expression for absorption coefficient in a more simple way. The absorption coefficient of the system equals the probability to find the system in the initial state multiplied by the probability of optical transition from the initial state to the final state. Applying this concept to the diagram shown in Figure 2 and taking into account that population of the state 0 and the state 2 can be beyond thermal equilibrium, we can write the absorption coefficient as follows:

$$k(t, \omega) = \rho_0(t)L_0(\omega) + \rho_2(t)L_2(\omega) \equiv [1 - \rho(t)]L_0(\omega) + \rho(t)L_2(\omega) \quad (15)$$

Here probabilities  $\rho_0(t)$  and  $\rho_2(t)$  of the initial state in absorption process have to be found from eq 7, i.e.,

$$\rho_2(t) \equiv \rho(t) = \rho(\infty) + [\rho(0) - \rho(\infty)] \exp(-Rt) \quad (16)$$

Here  $R = R_b + R_r$ . The probability depends on time and initial conditions. If we make the substitution

$$\rho(t) \rightarrow \rho(\infty) \quad (17)$$

in eq 15 we consider TLS in equilibrium, as previous theories did. After such substitution we come to a well-known expression for the time-independent absorption coefficient. If we neglect light-induced transitions in TLS described by constants B and b in Figure 2, we find that this probability is function of only temperature:

$$\rho(\infty) = f(T) = [1 + \exp(\epsilon/kT)]^{-1} \quad (18)$$

Here  $\epsilon$  is splitting in TLS. Equations 15 and 16 take into account that TLS is not in thermal equilibrium and therefore the probability  $\rho(t)$  will evolve with time.

Equation 15 for the absorption coefficient depends on time of measurement if this time is short as compared with lifetime  $1/R$  of TLS. The constant  $\rho(\infty) = R_b/(R_b + R_r)$  describes the same probability when the system excited by light is in thermal

equilibrium. The absorption coefficient depends not only on time but it depends on the initial condition  $\rho(0)$  as well. This is a new aspect of the problem concerning the absorption coefficient. This aspect is of great importance if we want to explain the appearance of quantum jumps. Dependence of the absorption coefficient on the initial conditions leads to the existence of two types of the theoretical expressions for the absorption coefficient. Let's consider this statement in detail.

Consider the molecule occupying the state 2 with the probability equal unit at time moment  $t = 0$ . It happens just after the jump to the state 2. At  $\rho(0) = 1$ , eq 15 takes the following form:

$$k_r(t, \omega) = \frac{R_r}{R} [1 - \exp(-Rt)] L_0(\omega) + \left[ \frac{R_b}{R} + \frac{R_r}{R} \exp(-Rt) \right] L_2(\omega) \quad (19)$$

In accordance with this equation, the probability to find the red line decreases with time and the probability to find the blue line will increase. If the molecule jumps to the state 0 we should set  $\rho(0) = 0$ . By inserting this initial condition to eq 15 we arrive at the following expression for the absorption coefficient:

$$k_b(t, \omega) = \left[ \frac{R_r}{R} + \frac{R_b}{R} \exp(-Rt) \right] L_0(\omega) + \frac{R_b}{R} [1 - \exp(-Rt)] L_2(\omega) \quad (20)$$

In accordance with this equation, the probability of finding the blue line will decrease with time and the probability of finding the red line will increase. At  $Rt \gg 1$ , eq 19 and eq 20 are transformed to the following expression:

$$k = \frac{R_r}{R} L_0 + \frac{R_b}{R} L_2 \quad (21)$$

In other words, the system “forgets” about initial conditions if the system is probed at time exceeding relaxation time  $1/R$  of TLS. The ratio of amplitudes in eq 21 depends on the intensity of excitation and on temperature. Our theory predicts the existence of two types of the absorption coefficient only for short times, satisfying the inequality  $Rt < 1$ . These absorption coefficients can be used to describe fluctuating absorption coefficient measured in experiment.

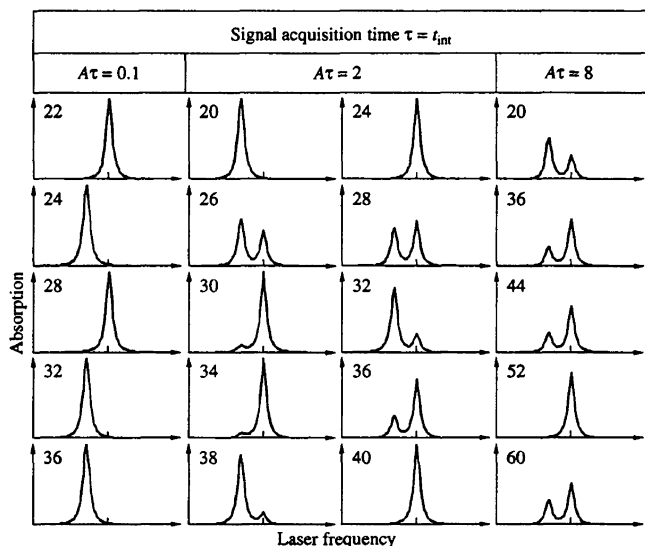
## VI. Simulation of Fluctuating Absorption Lines

The quantum trajectories shown in Figure 3 enable us to calculate fluctuating absorption coefficients and to find how data acquisition time influences the view of fluctuating absorption spectra. The fluctuating absorption coefficient is shown in Figure 4 for various data acquisition times.

The data acquisition time  $t_{\text{int}}$  in which photons are counted is shown in Figure 3 by vertical strip. This time is proportional the duration of a laser scan. Figure 4 shows what type of optical bands we will measure at fast and slow laser scans. In fast laser scans, we will measure only one line (red or blue) in each scan. If  $t_{\text{int}}$  is comparable with  $\tau_r$  we will measure both lines in some scans. If  $t_{\text{int}} > \tau_r$  we will measure the doublet of lines almost in each laser scan.

At  $t_{\text{int}} \gg \tau_r$ , the absorption coefficient does not fluctuate and we will measure the same doublet of lines in each laser scan. This doublet will be time independent and will be described by eq 21.





**Figure 4.** Fluctuations of the absorption coefficient, which will be measured in laser scans if on/off-interval fluctuations are as shown in Figure 3. The data acquisition time  $t_{\text{int}}$  is:  $0.1\tau_r$  for the first column,  $2\tau_r$  for the second and the third columns,  $8\tau_r$  for the fourth column. Numbers staying in each frame correspond to a moment of time in Figure 3.

## VII. Average of Fluctuating Absorption Coefficient

The fluctuating absorption coefficient should be averaged in order to compare with the theoretical expression for the absorption coefficient. It can be averaged by various ways. The simplest way is to sum up all spectra measured for time  $t$ . If  $t \gg 1/R$ , the averaged optical band will be described by a theoretical formula (eq 21). In this case there is no time dependence. If  $t < 1/R$ , the result of summation will fluctuate. This means that summation of all spectra is unacceptable. We need to find such a procedure of average which could give two average absorption coefficients at  $t < 1/R$ , and these averaged absorption coefficients can be comparable with two absorption coefficients described by eq 19 and eq 20.

Here we offer the way the fluctuating absorption spectra with well resolved lines have to be treated to find the averaged absorption optical band which could be compared with theoretical eq 19 and eq 20. The best way to find such averaged absorption coefficients is to measure with the help of trails auto- and cross-correlators defined below.

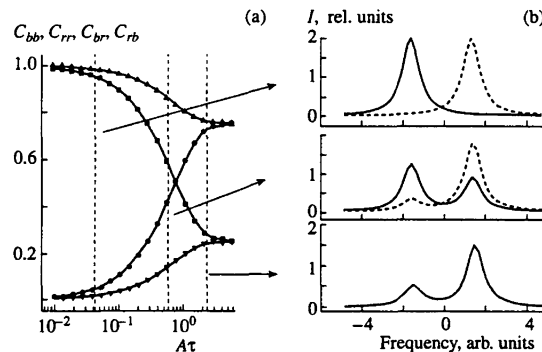
Since fluorescence shown in Figure 1 emerges due to transitions from the levels 1 and 3 shown in Figure 2, we denote the quantum trajectory (QT) shown in the low and upper panel of Figure 3 as  $I_1(t)$  and  $I_3(t)$ , respectively. In fact, these functions describe fluctuations of fluorescence excited by cw-laser light with frequencies  $\omega = \omega_0$  and  $\omega = \omega_0 - \Delta$ , respectively. With the help of two QT, we can find two types of autocorrelation functions (AF):

$$g_1^{(2)}(\tau) = \lim_{t \rightarrow \infty} \frac{\langle I_1(t+\tau)I_1(t) \rangle}{\langle I_1(t) \rangle^2}$$

and

$$g_3^{(2)}(\tau) = \lim_{t \rightarrow \infty} \frac{\langle I_3(t+\tau)I_3(t) \rangle}{\langle I_3(t) \rangle^2} \quad (22)$$

AF are normalized to unit at  $\tau = \infty$ . They can be measured experimentally.<sup>40–42</sup> It is important that these AF also can be calculated theoretically<sup>37,42–44</sup> and they can be related with two



**Figure 5.** (a) Auto- and cross-correlators  $\blacksquare$ ,  $C_{rr}$ ;  $\blacktriangle$ ,  $C_{bb}$ ;  $\bullet$ ,  $C_{br}$ ;  $\blacktriangledown$ ,  $C_{rb}$  calculated with the help of two quantum trajectories shown in Figure 3. The solid lines shown on the same graphic are the result of our calculation of amplitudes in theoretical expressions described by eq 19 and eq 20. (b) The curves are averaged absorption coefficients  $k_r$  (solid lines) and  $k_b$  (dashed lines) calculated by means of eq 27 at three delay times shown by vertical lines in Figure 5a.

absorption coefficients introduced in section VI. To find this relation we introduce a correlator determined as follows:

$$C(\tau) = \frac{\int_{-\infty}^{\infty} I(t)I(t+\tau) dt}{\int_{-\infty}^{\infty} I^2(t) dt} \quad (23)$$

With the help of two QT shown in Figure 3 and denoted by  $I_1(t)$  and  $I_3(t)$ , we can find two autocorrelators:

$$C_{bb}(\tau) = \frac{\int_{-\infty}^{\infty} I_1(t)I_1(t+\tau) dt}{\int_{-\infty}^{\infty} I_1^2(t) dt} \quad C_{rr}(\tau) = \frac{\int_{-\infty}^{\infty} I_3(t)I_3(t+\tau) dt}{\int_{-\infty}^{\infty} I_3^2(t) dt} \quad (24)$$

and two so-called cross-correlators

$$C_{br}(\tau) = \frac{\int_{-\infty}^{\infty} I_1(t)I_3(t+\tau) dt}{\int_{-\infty}^{\infty} I_1^2(t) dt} \quad C_{rb}(\tau) = \frac{\int_{-\infty}^{\infty} I_3(t)I_1(t+\tau) dt}{\int_{-\infty}^{\infty} I_3^2(t) dt} \quad (25)$$

It is obvious that auto- and cross-correlators can be measured experimentally. For convenience, we shall call all photons of fluorescence emitted after excitation via red and blue line as  $r$ - and  $b$ -photons, respectively.

We had no trails measured in real experiment. However, we can consider QT shown in Figure 3 as an experimental result obtained with the help of computer simulation. Substituting intensities  $I_1(t)$  and  $I_3(t)$  in eq 24 and eq 25 by QT shown in Figure 3, we find auto- and cross-correlators, which can be considered as those measured in computer experiment. Symbols in Figure 5a show auto- and cross-correlators calculated with the help of eq 24, eq 25, and random QT shown in Figure 3. The curves in Figure 5a show our calculation of temporal behavior of the amplitudes staying in front of Lorentzians in theoretical expressions (19) and (20) for two absorption coefficients. Coincidence of the symbols and the curves yields the following important equations:

$$\begin{aligned} C_{bb}(\tau) &= \frac{R_r}{R} + \frac{R_b}{R} e^{-R\tau} & C_{br}(\tau) &= \frac{R_b}{R} (1 - e^{-R\tau}) \\ C_{rb}(\tau) &= \frac{R_r}{R} (1 - e^{-R\tau}) & C_{rr}(\tau) &= \frac{R_b}{R} + \frac{R_r}{R} e^{-R\tau} \end{aligned} \quad (26)$$

The auto- and cross-correlators in the left-hand side of eq 26 can be measured experimentally. The probabilities staying in the right-hand side of eq 26 can be calculated theoretically. Therefore, with the help of auto- and cross-correlators measured experimentally, we can build up the following formulas

$$k_r(\tau, \omega) = C_{rb}(\tau)L_0(\omega) + C_{rr}(\tau)L_2(\omega)$$

$$k_b(\tau, \omega) = C_{bb}(\tau)L_0(\omega) + C_{br}(\tau)L_2(\omega) \quad (27)$$

for two absorption coefficients which can be considered as averaged absorption coefficients found in experiment with fluctuating fluorescence by means of measurement of auto- and cross correlators. The averaged optical bands described by eq 27 coincide with two theoretical absorption coefficients  $k_r$  and  $k_b$  described by eq 19 and eq 20. Therefore, eq 27 on one hand and eq 19 and eq 20 on the other hand establish a relation between two averaged absorption coefficients measured experimentally and two absorption coefficients calculated theoretically.

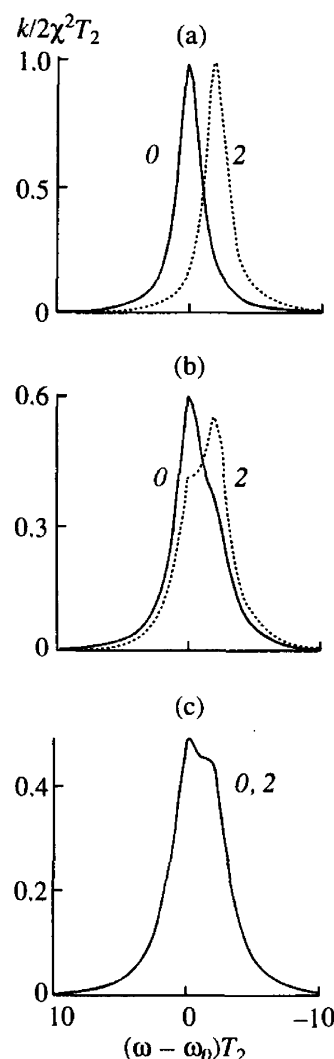
The absorption coefficients calculated with the help of eq 27 are shown in Figure 5b for time delays shown by vertical lines in Figure 5a. At  $R\tau \ll 1$  we arrive at two averaged absorption coefficients, each of which consists of a single line. At longer delay time, when  $R\tau \sim 1$ , each averaged absorption coefficient consists of a doublet of lines. This is scan-time dependent absorption coefficient indeed. At last, at long delay time when  $R\tau \gg 1$ , we arrive at a single absorption coefficient, described by eq 21. Since time delay  $\tau$  in eq 19 and eq 20 and in auto- and cross correlators is the same and rate constants  $R_r$  and  $R_b$  can be easily measured with the help of ratio of amplitudes at  $\tau \gg 1/R$  the theoretical eq 19 and eq 20 have no adjustable parameters.

After deriving eq 9 and eq 10 in section III, we have said that these equations are exact equations for finding probabilities  $P_0(t)$  and  $P_2(t)$ , by virtue of which we can find random QT  $I_1(t)$  and  $I_3(t)$ . Coincidence of symbols and curves in Figure 5a proved this statement. Indeed, expressions in the right-hand side of eq 26 have been calculated with the help of a full set in eq 7. However auto- and cross-correlators in the left-hand side of eq 26 have been found with the help of QT shown in Figure 3. Let us remind that these QT have been simulated with the help of probabilities  $P_0(t)$  and  $P_2(t)$  found from eq 9 and eq 10. If eq 9 and eq 10 were approximate, we had no exact coincidence figures and curves in Figure 5a.

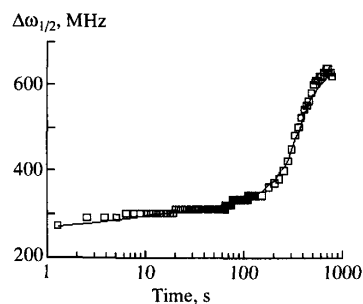
### VIII. Temporal Line Broadening

If two optical lines in the doublet are poorly resolved in frequency scale, we cannot measure cross-correlators and therefore another method has to be developed to compare our theory and experiment. Consider now the case when red and blue lines are poorly resolved in frequency scale. The result of our calculation with the help of eq 19 and eq 20 for this case is shown in Figure 6.

It is difficult to measure independently two absorption coefficients shown in Figure 6. Therefore, Boiron et al.<sup>32</sup> measured, in this case, dependence of the half width of such complex optical band on scan time. Duration of the laser scan was 1 s. Line widths were determined by sliding average.<sup>32</sup> For example, to determine the line width on a time scale  $t = 5$  s, all spectra between 0 and 5 s are averaged, then 1 and 6 s, 2 and 7 s, etc., then these averaged spectra are shifted to the same position of their maximum, then average them all and measure the fwhm. The temporal dependence of half-width of single terrylene



**Figure 6.** Absorption coefficients  $k_b$  (solid line) and  $k_r$  (dashed line) for a molecule interacting with one TLS at various duration of scan time.  $Rt = 3 \times 10^{-3}$  (a),  $Rt = 1$  (b) and  $Rt = 10^2$  (c).  $R_b/R = 0.45$ ,  $T_2\Delta = 2$ . The solid line 0 shows the optical line corresponding 0–1 transition in Figure 2. The dashed line 2 shows the optical line corresponding 2–3 transition in Figure 2.



**Figure 7.** Temporal behavior of measured (squares)<sup>32</sup> and calculated (solid line) fwhm of single terrylene molecule embedded in polyethylene matrix at  $T = 1.8$  K.

molecule measured as was described above is shown in Figure 7. Our calculation of fwhm has been carried out as follows.

To find theoretical expression for fwhm of whole optical band shown in Figure 6, we need to know a theoretical expression for the half width of each line of the doublet. The system described by Figure 2 can be considered as pair of electronic levels 0 and 1 interacting with pair of electronic levels 2 and 3 via relaxation process described by constants  $A$ ,  $a$ ,  $B$ ,  $b$ . Van't Hove and Schmidt<sup>45</sup> considered two sets of Bloch equations

for electronic levels 0,1 and 2,3 at  $A = B$  and  $a = b$ . They derived the following expression for optical dephasing time relating to the 0–1 transition:

$$\frac{1}{T_2(T)} = \frac{a}{A} \frac{\Delta^2 A}{\Delta^2 + A^2} = \frac{\Delta^2 A}{\Delta^2 + A^2} \exp(-\epsilon/k_B T) \quad (28)$$

The model yielding eq 28 is known as the “exchange model”. Here, dephasing time depends solely on temperature, but it does not depend on time of measurement. This means that exchange model takes into account interaction between levels 0,1 and 2,3 within the frame of thermal equilibrium. However, eq 28 can be derived also in the model which describes guest molecules interacting with single TLSs, where  $A$  and  $a$  describe relaxation in TLS and  $\epsilon$  describes energy splitting in TLS.<sup>46</sup> Such approach used<sup>46</sup> is more promising since it enables one to consider a two-level guest molecule interacting with many TLSs. Moreover, it enables one to consider a system with TLSs, which are beyond thermal equilibrium.

Generalization of the approach developed in ref 46 for the case where a two-level chromophore interacts with huge number of TLSs, which are beyond thermal equilibrium, was carried out in refs 38 and 47. The contribution to the dephasing rate from the  $j$ th flipping of TLS is given by

$$\frac{\gamma_j}{2} = \frac{\Delta_j^2 A_j}{\Delta_j^2 + A_j^2} \rho_j(t) [1 - \rho_j(t)] < R_j \quad (29)$$

Here

$$\rho_j(t) = \rho_j(\infty) + [\rho_j(0) - \rho_j(\infty)] \exp(-R_j t) \quad (30)$$

is the probability of finding the  $j$ th TLS in an excited state. The contribution is very small if flipping time  $1/R_j$  lies in the time scale of seconds. Nevertheless, we cannot neglect this contribution to the optical dephasing rate because there is great number of TLSs with long relaxation times. Full contribution to dephasing time from all TLS is given by

$$\frac{\gamma(t)}{2} = \sum_{j=1}^{N_0} \frac{A_j \Delta_j^2}{\Delta_j^2 + A_j^2} \rho_j(t) (1 - \rho_j(t)) \quad (31)$$

Here  $N_0$  is the full number of TLS in a sample. Summation can be substituted by integration with functions describing the distribution of rate constants  $R$  and other distributions used in the so-called standard TLS model. For the dipolar interaction between the guest molecule and TLS, the half width of the line depends on time logarithmically:<sup>47</sup>

$$\gamma(t) = cT \log(R_m t) \quad (32)$$

Here  $R_m$  is the largest rate constant of TLSs. By taking into account interaction with phonons and TLSs, we can write for optical dephasing time the following expression:

$$\frac{1}{T_2(t, T)} = \frac{1}{2T_1} + \frac{\gamma_{ph}(T)}{2} + \frac{\gamma(t)}{2} = \frac{1}{T_2(1/R_m, T)} (1 + C \log R_m t) \quad (33)$$

Here the second term results from electron–phonon interaction and the third term results from interaction with a huge number of remote TLS.  $R_m$  is the largest rate constant of TLSs.

Time dependence described by this formula cannot explain experimental data on line broadening obtained by Boiron et al.<sup>32</sup>

over the whole time scale. Equation 33 can explain data only for  $t < 100$  s. However, these data over the whole time scale shown in Figure 7 can be explained if we take into account that both optical lines can contribute to half-width at  $t > 100$  s. Our theory makes it possible to calculate the shape of the optical band consisting of two optical lines at various times.

After making  $N$  laser scans, we will measure the absorption coefficient  $k_b$  and  $k_r$   $N(1-f)$  and  $Nf$  times, respectively. Hence, we can propose the following theoretical expression for finding the theoretical value of fwhm:

$$k(t, \omega) = (1 - f)k_b(t, \omega) + fk_r(t, \omega) \quad (34)$$

The fwhm of this optical band was found with various duration  $t$  of laser scan. This fwhm as a function of the scan time is shown by a solid line in Figure 7. This curve fits experimental data well at  $1/T_2(1/R_m, T) = 90$  MHz,  $C = 0.33$ ,  $R_m = 10^6$  s<sup>-1</sup>. This fact means that our theoretical formula (eq 15) for the absorption coefficient is able to describe scan time dependence of fluctuating absorption band with poor resolved optical lines as well.

## IX. Conclusion

Our paper is focused on the problem of theoretical treatment of single-molecule fluctuating optical bands, which are measured with scanning laser frequency. We consider TLS beyond thermal equilibrium and therefore the probability  $\rho(t)$  to find TLS in excited state 2 in Figure 2 depends on time and initial conditions. Appearance of initial conditions in theoretical expression for the time-dependent absorption coefficient is an important feature, which distinguishes our theory from others. The initial conditions enable us to calculate two types of time-dependent absorption coefficients.

1. For the “two-state-jump model” (TSJM) we derived in section III a theoretical expression (eq 15) for an absorption coefficient that includes transitions in TLS in the ground and excited electronic states. It expresses the rate constants  $1/\tau_r$  and  $1/\tau_b$  via parameters of the microscopic model. Equation 15 is utilized for treating experimental data with a fluctuating optical band measured in single-molecule experiments.

2. We have proposed in section VII the way the trails well resolved in frequency scale have to be averaged. The averaged optical bands obtained after such treatment are described by eq 27. They can be compared with theoretical expressions for two absorption coefficients described by eq 19 and eq 20 with no adjustable parameters. The method of treatment is based on the measurement of two auto-correlators  $C_{rr}(t)$ ,  $C_{bb}(t)$  and two cross-correlators  $C_{rb}(t)$ ,  $C_{br}(t)$  defined by eq 25 and eq 26. The final eq 27 describes two averaged optical bands which can be compared with two theoretical expressions described by eq 19 and eq 20. Figure 5 shows such a comparison.

3. Since we had no trails measured experimentally, we used computer simulation procedure in order to calculate such trails. We have shown in section III that slow modulation of fluorescence can be simulated with the help of simple rate equations.

4. We have shown that eq 19 and eq 20, derived on the basis of eq 15, are able to describe temporal broadening of single molecule optical band with poorly resolved lines.

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## References and Notes

- Moerner, W. E.; Kador, L. *Phys. Rev. Lett.* **1989**, *62*, 2535.
- Orrit, M.; Bernard, J. *Phys. Rev. Lett.* **1990**, *65*, 2716.

- (3) Ambrose, W. P.; Basche, Th.; Moerner, W. E. *J. Chem. Phys.* **1991**, 95, 7150.
- (4) Basche, Th.; Moerner, W. E.; Orrit, M.; Talon, H. *Phys. Rev. Lett.* **1992**, 69, 1516.
- (5) Moerner, W. E.; Basche, Th. *Angew. Chem. Int. Ed. Engl.* **1993**, 32, 457.
- (6) Moerner, W. E.; Plakhotnik, T.; Irgartinger, Th.; Croci, M.; Palm, V.; Wild, U. *J. Phys. Chem.* **1994**, 98, 7382.
- (7) Basche, Th.; Moerner, W. E.; Orrit, M.; Wild, U., Eds.; *Single-Molecule Optical Detection, Imaging, and Spectroscopy*; VCH: Weinheim, 1996.
- (8) van Oijen, A. M.; Ketelaars, M.; Kohler, J.; Aartsma, T. J.; Schmidt, J. *J. Phys. Chem. B* **1998**, 102, 9363–9366.
- (9) Vanden Bout, D.; Yip, W.-T.; Hu, D.; Fu, D.-K.; Swager, T.; Barbara, P. *Science* **1997**, 277, 1074–1077.
- (10) Yu, J.; Hu, D.; Barbara, P. *J. Am. Chem. Soc.* **1999**, 121, 6936–6937.
- (11) Yu, J.; Hu, D.; Barbara, P. *Science* **2000**, 289, 1327–1330.
- (12) White, J.; Hsu, J.; Yang, Shu-Chun; Fann, W.; Pern, G.; Chen, S. *J. Chem. Phys.* **2001**, 114, 3848–3852.
- (13) Mirzov, O.; Cichos, F.; von Borczyskowski, C.; Scheblykin, I. G. *Chem. Phys. Lett.* **2004**, 386, 286–290.
- (14) Gensch, T.; Hofkens, J.; Heirmann, A.; Tsuda, K.; Verheijen, W.; Vosch, T.; Christ, Th.; Basche, Th.; Mullen, K.; de Schryver, F. *Angew. Chem. Int. Ed.* **1999**, 38, 3752–3756.
- (15) Hofken, J.; Maus, M.; Gensch, Th.; Vosch, T.; Cotlet, M.; Kohn, F.; Herrmann, A.; Mullen, K.; de Schryver, F. *J. Am. Chem. Soc.* **2000**, 122, 9278–9288.
- (16) Vosch, T.; Hofkens, J.; Cotlet, M.; Kohn, F.; Fujiwara, H.; Gronheid, R.; van der Bierst, K.; Weil, T.; Herrmann, A.; Mullen, K.; Mukamel, S.; van der Auwer, M.; de Schryver, F. *Angew. Chem. Int. Ed.* **2001**, 40, 4643–4648.
- (17) Vale, R.; Funatsu, T.; Pierce, D.; Romberg, L.; Harada, Y.; Yanagida T. *Nature* **1996**, 380, 451–453.
- (18) Dickson, R.; Cubitt, A.; Tsien R.; Moerner, W. E. *Nature* **1997**, 388, 355–358.
- (19) Ha, T.; Enderle, Th.; Chemla, D. S.; Selvin, P. R.; Weiss, S. *Chem. Phys. Lett.* **1997**, 271, 1.
- (20) Lu, H.; Xun, L.; Xie X. *Science* **1998**, 282, 1877–1882.
- (21) Osad'ko, I. S. *JETP* **2003**, 96, 617.
- (22) Naumov, A. V.; Vainer, Yu. G.; Bauer, M.; Zilker, S.; Kador, L. *Phys. Rev. B* **2001**, 63, 212302.
- (23) Naumov, A. V.; Vainer, Yu. G.; Bauer, M.; Kador, L. *J. Chem. Phys.* **2002**, 116, 8132.
- (24) Naumov, A. V.; Vainer, Yu. G.; Bauer, M.; Kador, L. *Opt. Spectrosc.* **2003**, 94, 864.
- (25) Geva, E.; Skinner, J. L. *J. Phys. Chem. B* **1997**, 101, 8920.
- (26) Geva, E.; Skinner, J. L. *J. Chem. Phys.* **1998**, 109, 4920.
- (27) Plakhotnik, T. *J. Lumin.* **1999**, 83–84, 221.
- (28) Anderson, P. W.; Halperin, B. I.; Varma, C. M. *Philos. Mag.* **1972**, 25, 1.
- (29) Phillips, W. A. *J. Low Temp. Phys.* **1971**, 7, 351.
- (30) Barkai, E.; Jung, Y. J.; Silbey, R. *Phys. Rev. Lett.* **2001**, 87, 207403.
- (31) Jung, Y. J.; Barkai, E.; Silbey, R. *Adv. Chem. Phys.* **2002**, 123, 199.
- (32) Boiron, A. M.; Tamarat, Ph.; Lounis, B.; Brown, R.; Orrit, M. *Chem. Phys.* **1999**, 247, 119.
- (33) Cook, R. J.; Kimble, H. J. *Phys. Rev. Lett.* **1985**, 54, 1023.
- (34) Osad'ko, I. S. *JETP* **2001**, 93, 865.
- (35) Osad'ko, I. S. *JETP* **1996**, 82, 434.
- (36) Osad'ko, I. S. *JETP* **1999**, 89, 513.
- (37) Osad'ko, I. S.; Yershova, L. B. *J. Chem. Phys.* **2000**, 112, 9645.
- (38) Osad'ko, I. S. "Selective spectroscopy of single molecules" **2002**, Springer series in Chemical Physics, v. 69.
- (39) Kilin, S. Ya.; Maevskaya, N. M.; Nizovtsev, A. P.; Shatokhin, V. N.; Berman, P. R.; von Borczyskowski, C.; Wrachtrup, J.; Fleury, L. *Phys. Rev.* **1998**, A57, 1400.
- (40) Fleury, L.; Zumbusch, A.; Orrit, M.; Brown, R.; Bernard, J. *J. Lumin.* **1993**, 56, 15.
- (41) Kettner, R.; Tittel, J.; Basche, Th.; Brauchle, C. *J. Chem. Phys.* **1994**, 98, 6671.
- (42) Plenio, M. B.; Knight, P. L. *Rev. Mod. Phys.* **1998**, 70, 101.
- (43) Osad'ko, I. S.; Yershova, L. B. *J. Lumin.* **2000**, 86, 211.
- (44) Osad'ko, I. S.; Yershova, L. B. *J. Lumin.* **2000**, 87–89, 184.
- (45) van t'Hove, C. A.; Schmidt, J. *Chem. Phys. Lett.* **1975**, 36, 460.
- (46) Osad'ko, I. S. *Phys. Rep.* **1991**, 206, 43.
- (47) Osad'ko, I. S.; Yershova, L. B. *J. Chem. Phys.* **1999**, 111, 7652.