Statistics of Molecular Ensemble Blinking Fluorescence

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Fluorescence of M noninteracting molecules, each of which exhibits blinking fluorescence with on- and off-intervals, is considered. Theoretical expressions suitable for statistical analysis of fluorescence of M molecules are derived. The derivation of (i) the distribution functions $P_{on,off}(t)$ for on- and off-interval duration, (ii) the distribution function $w_N(t)$ for the number N of photons emitted at a time interval t, and (iii) the fluorescence autocorrelation function $g^{(2)}(t)$ is carried out with the help of the formulas previously derived for single molecule blinking fluorescence. The statistical treatment of intensity fluctuations in fluorescence of several molecules is carried out to find the three types of the above-mentioned functions. The coincidence of the functions "measured" in blinking fluorescence and those calculated with the help of the theoretical formulas derived proves the validity of the latter.

1. Introduction

Single molecules, embedded into polymer matrices at cryogenic temperatures, exhibit narrow optical lines and therefore, these spectral lines can be used as probes to analyze the parameters of the local environment of the guest molecule.[1−5] Excited by a cw laser field, the guest molecules fluoresce. Fluorescence photons emitted by a single molecule are the source of information about the local environment of individual impurity centers.

However optical spectra of complex organic molecules, embedded into solid matrices at room temperature, are very broad and, therefore, spectral methods used at low temperature become ineffective for studying room temperature single molecule dynamics. Fortunately there are additional possibilities to study room temperature dynamics of single molecules because the integral intensity of single molecule fluorescence fluctuates even at room temperature. For example, it was shown experimentally for a single polymer molecule.[6,7] The thoretical treatment of these experimental data aimed to find the dynamics of a single polymer molecule was realized in refs 8 and 9. The information about single molecule dynamics can be found by statistical analysis of fluorescence fluctuations observed in experiments. The statistical analysis of single molecule fluorescence enables one to find distribution functions of various types.

Such distribution functions are (i) the fluorescence autocorrelation function, $g^{(2)}(t)$, which determines the correlation of the photons emitted by a single molecule and separated by the time interval t; (ii) the distribuion $w_N(t)$ of the number of photons emitted by a single molecule during the time interval t; (iii) the distribuions $P_{on}(t)$ and $P_{off}(t)$ of the time intervals t with the fluorescence light (on-intervals) and without light (off-intervals). The theoretical expressions for all these functions suitable for the statistical treatment of single molecule fluorescence were previously derived by various groups.[8−16]

However, laser spot in single molecule experiments can cover more than one guest molecule. In such a case, theoretical expressions for the distribution functions derived for single molecule fluorescence cannot be used. How can we establish that we measure fluorescence photons emitted by a single molecule? In order to distinguish fluorescence of one and two ions, Itano et al.[17] have measured autocorrelation function $g^{(2)}(t)$ in fluorescence of single Hg^+ ion and two ions. A single molecule cannot emit two photons simultaneously. Therefore the probability of finding two photons at the same time equals zero. This effect is called photon antibunching. It manifests itself in approaching the fluorescence autocorrelation function $g^{(2)}(t)$ to zero at $t \rightarrow 0$. In the fluorescence of two ions the photon antibunching disappears indeed.[17]

However, photon antibunching manifests itself on a nanosecond time scale. How can we distinguish blinking fluorescence of few molecules from single molecule fluorescence if the time resolution of our set up does not permit us to measure photon antibunching on the nanosecond time scale? This is one of the fundamental problems existing in blinking fluorescence of organic molecules at room temperature.

We could solve this problem by measuring all three types of the distribution functions mentioned above in multimolecule fluorescence. However it is impossible to establish the multimolecule character of the emission without a comparison of the measured distribution functions with the calculated ones. Unfortunately, by now there have been no theoretical formulas for the above-mentioned distribution functions acceptable for multimolecule fluorescence. A theory for such distribution functions is built up in the present paper.

Generally, such a theory will be rather complicated because a molecular complex can be of various types. For example, it can consist of a few similar interacting molecules; that is, it can form a dimer,[18] a trimer, etc. It can be a donor−acceptor pair of molecules,[19,20] in which the energy transfer from the donor to the acceptor takes place. Finally, the molecular complex can consist of several similar or different molecules or ions,[17,21] which do not undergo any energy transfer and their resonant interaction can be negligible because of a large distance between the molecules. The latter case is the simplest one, and it appears

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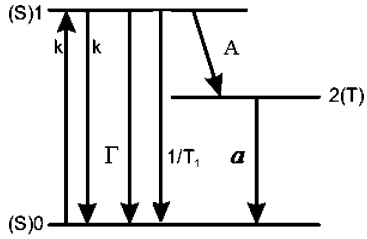

Figure 1. Energy scheme of a molecule with a triplet level producing blinking fluorescence.

natural to begin the research of molecular complex statistics with that very case. Moreover experimental data have already been obtained for such two-atom[17] and two-molecule fluorescence.[21]

The main goal of the present paper is to build up theoretical expressions for three types of the distribution functions which could be used in experiments with blinking fluorescence in which we cannot be sure that we deal with single molecule fluorescence. Comparison of measured distributions and calculated ones with the expressions found here could help us to prove multimolecular character of the blinking fluorescence.

There is also an additional motivation to our work. A single molecule emits pure quantum light. However a large molecular ensemble emits classical light. The second goal of the paper is to monitor how quantum light of a single molecule is converted to classical one with an increase of the number of light emitters.

2. Blinking Fluorescence of Several Molecules

Fluorescence of the majority of organic molecules excited by a cw laser radiation has blinking character, namely, time intervals with fluorescence (on-intervals) are interrupted by pauses without any emission of photons (off-intervals). Such blinking fluorescence emerges due to the existence of a triplet level between the ground and the excited singlet levels in many organic molecules. Figure 1 illustrates the energy scheme of such a molecule.

In Figure 1, k is the rate of light induced transitions between the ground and the excited singlet levels, accompanied by absorption or emission of a laser photon, Γ is the rate of radiation free transitions, and $1/T_1$ is the rate of spontaneous emission from the excited singlet state.

A molecule jumps from the ground to the excited level and back absorbing laser photons and spontaneously emitting fluorescence photons. However, if the probability A of the singlet−triplet transition is not too small, the molecule can jump to the triplet state. After this jump the absorption of the laser light stops, and therefore, the fluorescence disappears. After the jump of the molecule to the ground singlet state with the probability a, the light absorption and fluorescence emerges again. Such fluorescence has blinking character, i.e., intervals with light emittion alternate with those without fluorescence light.

The slow kinetics of the molecule connected with the singlet−triplet transitions can be considered without taking into account off-diagonal density matrix elements, in other words, on the basis of rate equations for diagonal matrix elements. These equations, according to the scheme depicted in Figure 1, are represented as follows:

$$\begin{aligned}\dot{\rho}_1 &= -(\Gamma + k + 1/T_1 + A)\rho_1 + k\rho_0\\ \dot{\rho}_0 &= (\Gamma + k + 1/T_1)\rho_1 - k\rho_0 + a\rho_2\\ \dot{\rho}_2 &= A\rho_1 - a\rho_2\end{aligned} \tag{1}$$

Equation 1 describe dynamics of the system. Equations for finding on/off intervals can be derived from eq 1.

2.1. On- and Off-Interval Distribution. A molecule irradiated by cw laser light makes jumps between the singlet states 0 and 1 at random time moments by emitting one photon at a jump from the state 1. The prescription for the derivation of the equations for on- and off-state probabilities with the help of the set of eq 1 was described in ref 22. Briefly it can be described as follows.

If the molecule occupies singlet states 0 and 1 the molecule fluoresces. Since the molecule gets to triplet state 2 the fluorescence stops. Hence eq 1 describes transitions from the bright states 0 and 1 to the dark state 2 and back. If we omit the term $a\rho_2$ in the second line of the set (1), we interrupt jumps from the dark state to the bright state. Hence the equations

$$\begin{aligned}\dot{\rho}_1 &= -(\Gamma + k + 1/T_1 + A)\rho_1 + k\rho_0\\ \dot{\rho}_0 &= (\Gamma + k + 1/T_1)\rho_1 - k\rho_0\end{aligned} \tag{2}$$

will describe evolution of the molecule in on-state. Indeed for the probability

$$\rho_{on} = \rho_1 + \rho_0 \tag{3}$$

of finding the molecule in on-state we find the following equation:

$$\dot{\rho}_{on} = -A\rho_1 \tag{4}$$

It describes decay of on-state and, therefore, it can help us to find lifetime of the on-state. If bin time is considerably more than the average time between two sequentially emitted photons, we will observe fluorescence in on-interval as a continuous radiation. In this case we can make use the quasi-stationary approximation assuming $\dot{\rho}_1 = 0$. Then we find the following relation from the first equation of the set (2)

$$\rho_1 = \frac{kT_1}{1 + (k + A + \Gamma)T_1}\rho_0 \tag{5}$$

Using this formula and eq 3 we can express ρ_1 via ρ_{on}

$$\rho_1 = \frac{kT_1}{1 + (2k + \Gamma + A)T_1}\rho_{on} \tag{6}$$

Substituting this expression in eq 4 we arrive at the following simple formula for the probability of finding the molecule in the on-state

$$\dot{\rho}_{on} = -\rho_{on}/\tau_{on} \tag{7}$$

Here

$$\frac{1}{\tau_{on}} = k\frac{AT_1}{1 + (2k + \Gamma + A)T_1} = kY_{ISC} \tag{8}$$

Y_{ISC} is the intersystem crossing quantum yield. The function

$$\rho_{on}(t) = c_{on} \exp(-t/\tau_{on}) \tag{9}$$

is a solution of eq 7. Here c_{on} is so far an arbitrary coefficient. However, this function can be regarded as the probability density of finding an on-interval of duration t if this arbitrary coefficient is chosen in such a way that the following condition will be satisfied:

$$\int_0^\infty P(t)\, dt = 1 \tag{10}$$

Substituting eq 9 into eq 10, we find that $c_{on} = 1/\tau_{on}$. Hence, the on-interval distribution function is of the following form:

$$P_{on} = \frac{1}{\tau_{on}} \exp\left(-\frac{t}{\tau_{on}}\right) \tag{11}$$

It describes the probability density of finding an on-interval of time duration t. It is evident, τ_{on} is the average on-interval duration.

If the molecule gets to the triplet state, fluorescense stops. Therefore

$$\rho_{off} = \rho_2 \tag{12}$$

is the probability of finding the molecule in off-state. We can derive the equations for this probability if we omit the term $A\rho_1$ in the third line of the set (1), as this term describes jumps of the molecule from on-state to off-state. So the off-state dynamics is described by the equation

$$\dot{\rho}_{off} = -a\rho_{off} \tag{13}$$

Using the same reasoning as we did to arrive at eq 11, we get the following expression for the probability density of finding the molecule in an off-state:

$$P_{off} = a \exp(-at) \tag{14}$$

Here

$$\tau_{off} = 1/a \tag{15}$$

is the average value of off-interval.

2.2. Quantum Trajectory of Fluorescence Intensity for Two Molecules. ***Equations for the Calculation of Fluctuations.*** Transitions from on-states to off-states and back will happen at random time moments. To find these time moments, we will use the equation

$$\mathrm{rnd}(n) = \int_0^{t_n} P(\tau)\, d\tau \tag{16}$$

Here the function rnd(n) determines a random number between 0 and 1 with a constant probability density. Values t found from eq 16 are that very random time moments distributed with the probability density, $P(t)$.

Fluorescence intensity fluctuations of two different molecules calculated in that way are represented in Figure 2a and Figure 2b. Since these molecules do not interact with each other, fluorescence fluctuations of each molecule are independent. Therefore, fluctuations in fluorescence of two molecules are just the sum of individual ones, as Figure 2c shows. Some off-intervals disappear because of this summation. Therefore, all off-intervals will disappear in fluorescence of several molecules. In other words, fluorescence of many molecules will look like continuous classical light.

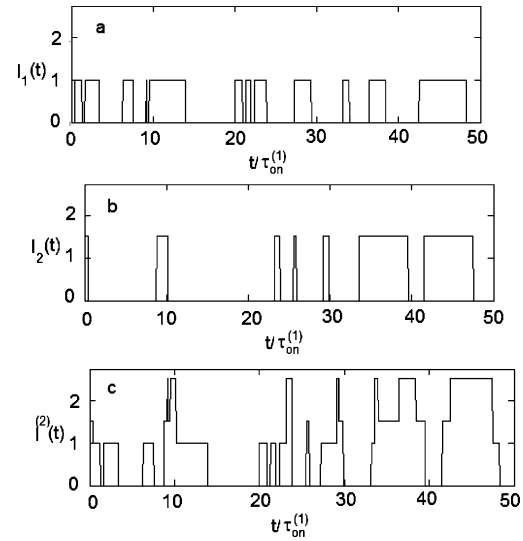

Figure 2. Quantum trajectory of fluorescence intensity of molecule 1 (a) with $\tau_{on}^1 = 3 \times 10^{-3}$ s, $\tau_{off}^1 = 5 \times 10^{-3}$ s and of molecule 2 (b) with $\tau_{on}^2 = 9 \times 10^{-3}$ s, $\tau_{off}^2 = 15 \times 10^{-3}$ s and of total fluorescence of these two molecules (c).

3. On- and Off-Interval Distribution Functions in Two Molecule Fluorescence

Equations 11 and 14 describe the on- and off-interval distributions in single molecule fluorescence. If fluorescence intensity of each molecule is measured independently from each other, we will have two quantum trajectories of the intensity shown in Figure 2, panels a and b. The on- and off-interval distribution functions for molecule 1 and molecule 2 are given by

$$P_{on}^1(t) = \frac{1}{\tau_{on}^1} \exp(-t/\tau_{on}^1), \qquad P_{off}^1(t) = \frac{1}{\tau_{off}^1} \exp(-t/\tau_{off}^1)$$
$$P_{on}^2(t) = \frac{1}{\tau_{on}^2} \exp(-t/\tau_{on}^2), \qquad P_{off}^2(t) = \frac{1}{\tau_{off}^2} \exp(-t/\tau_{off}^2) \tag{17}$$

The parameters for molecule 1 in eqs 8 and 15 were taken as follows:

$$k = 10^5\ \mathrm{s}^{-1}, \qquad \Gamma = 10^7\ \mathrm{s}^{-1}, \qquad 1/T_1 = 10^8\ \mathrm{s}^{-1},$$
$$A = 3.7 \times 10^5\ \mathrm{s}^{-1}, \qquad a = 200\ \mathrm{s}^{-1} \tag{18}$$

Values $\tau_{on}^1 = 3 \times 10^{-3}$ s and $\tau_{off}^1 = 5 \times 10^{-3}$ s relate to parameters (18). Parameters for molecule 2 were chosen in such a way that the average on- and off-intervals for the molecule were three times as long, that is, $\tau_{on}^2 = 9 \times 10^{-3}$ s and $\tau_{off}^2 = 15 \times 10^{-3}$ s. The distribution functions are plotted in Figure 3. Here the histograms show distributions "measured" in blinking fluorescence presented in Figure 2, panels a and b.

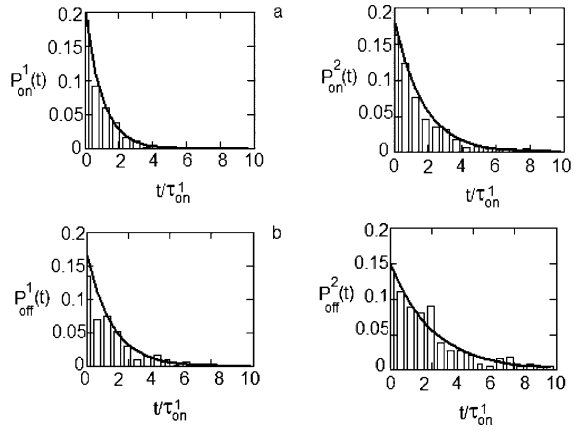

Figure 3. Histogram of on- and off-interval distributions in the quantum trajectory shown in Figure 2, panels a and b. Solid lines shows distributions described by eq 17.

In fluorescence of two molecules plotted in Figure 2c we also see four types of time intervals characterized by four intensity values: 0, I_1, I_2, $I_1 + I_2$. Zero intensity intervals correspond to the off-interval coincidence for the two molecules. Intervals with I_1 intensity correspond to the coincidence of an on-interval of molecule 1 and an off-interval of molecule 2. Vice versa we see for the intervals with I_2 intensity. And, finally, intervals with $I_1 + I_2$ intensity correspond to the coincidence of on-intervals in fluorescence of two molecules.

We should find four distribution functions $P_{off,off}(t)$, $P_{on,off}(t)$, $P_{off,on}(t)$, and $P_{on,on}(t)$ for four types of intervals with intensities 0, I_1, I_2, $I_1 + I_2$. As on- and off-intervals of molecules 1 and 2 are independent, the probabilities for the two molecules are the products of the corresponding probabilities for each of them

$$\begin{aligned} P_{off,off}(t) &= c_{off,off}\exp[-t(1/\tau^1_{off} + 1/\tau^2_{off})], \\ P_{on,off}(t) &= c_{on,off}\exp[-t(1/\tau^1_{on} + 1/\tau^2_{off})] \\ P_{off,on}(t) &= c_{off,on}\exp[-t(1/\tau^1_{off} + 1/\tau^2_{on})], \\ P_{on,on}(t) &= c_{on,on}\exp[-t(1/\tau^1_{on} + 1/\tau^2_{on})] \end{aligned} \tag{19}$$

These functions must satisfy the condition (10) to serve as probability densities. Inserting eq 19 into eq 10 and calculating the integral, we find the explicit expressions for the coefficients c in eq 19. The formulas in 19 take the following form with these expressions

$$P_{off,off}(t) = (1/\tau^1_{off} + 1/\tau^2_{off})\exp[-t(1/\tau^1_{off} + 1/\tau^2_{off})] \tag{20a}$$

$$P_{on,off}(t) = (1/\tau^1_{on} + 1/\tau^2_{off})\exp[-t(1/\tau^1_{on} + 1/\tau^2_{off})] \tag{20b}$$

$$P_{off,on}(t) = (1/\tau^1_{off} + 1/\tau^2_{on})\exp[-t(1/\tau^1_{off} + 1/\tau^2_{on})] \tag{20c}$$

$$P_{on,on}(t) = (1/\tau^1_{on} + 1/\tau^2_{on})\exp[-t(1/\tau^1_{on} + 1/\tau^2_{on})] \tag{20d}$$

Distributions 20a, 20b, 20c, and 20d correspond to the intervals with the intensities 0, I_1, I_2, $I_1 + I_2$, respectively.

However, an experiment can be carried out in such a way that only two types of intervals will be observed in the experiment: without fluorescence at all (off−off) and with any fluorescence intensity (on−off, off−on, and on−on). The on-

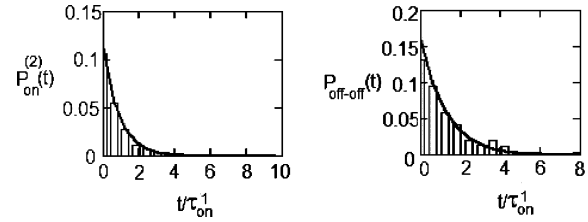

Figure 4. Distributions of on- and off-intervals measured in the quantum trajectory shown in Figure 2c (histograms). Curves show the distribution described by eqs 20a and 21.

interval distribution in fluorescence of both molecules will be given by

$$P^{(2)}_{on}(t) = \frac{\exp([-(b+B)t] + \exp([-(b+A)t] + \exp([-(a+B)t]}{(b+B)^{-1} + (b+A)^{-1} + (a+B)^{-1}} \tag{21}$$

Here $1/\tau^1_{on} = b$, $1/\tau^2_{on} = B$, $1/\tau^1_{off} = a$, and $1/\tau^2_{off} = A$. These distributions are plotted in Figure 4.

The average duration of time intervals with zero intensity in the two-molecule fluorescence is given by

$$\langle t_{off,off}\rangle = \int_0^\infty tP_{off,off}(t)\,dt = \frac{\tau^1_{off}\tau^2_{off}}{\tau^1_{off} + \tau^2_{off}} \tag{22}$$

so it is less than average values τ^1_{off} and τ^2_{off} for off-intervals of individual molecules. As for the intervals with any total fluorescence observed, they are described by the average time

$$\begin{aligned} \langle t^{(2)}_{on}\rangle &= \int_0^\infty tP^{(2)}_{on}(t)\,dt \\ &= \frac{(b+B)^{-2} + (b+A)^{-2} + (a+B)^{-2}}{(b+B)^{-1} + (b+A)^{-1} + (a+B)^{-1}} \end{aligned} \tag{23}$$

It is less than at least one of the two average values of on-intervals τ^1_{on} and τ^2_{on}, or it can be less than both of them. It depends on the average off-intervals.

These decrese of the average intervals in the total fluorescence compared to those in the fluorescence of individual molecules can be seen in Figure 4 as well. Blinking character of single molecule fluorescence will be smoothed in total fluorescence of many molecules. Therefore measurement of on- and off-time distributions in fluorescence of many molecules will be impossible. Such fluorescence is, in fact, classical light.

4. Photon Distribution Functions for Fluorescence of Several Molecules

Let us find expressions for photon distribution functions of several noninteracting molecules. Suppose we know the functions $w^{(1)}_{N_1}(T)$ and $w^{(2)}_{N_2}(T)$ describing the photon distribution in fluorescence of molecule 1 and molecule 2 at time interval T. As the processes of emitting photons by molecules 1 and 2 are independent, the function

$$W(N_1, N_2, T) = w^{(1)}_{N_1}(T)w^{(2)}_{N_2}(T) \tag{24}$$

is the probability of emitting N_1 photons by the first molecule and N_2 photons by the second one during time interval T. If fluorescence photons of the two molecules differ in wavelength, we can always measure both the probability $w_{N_1}^{(1)}(T)$ and the probability $w_{N_2}^{(2)}(T)$ using proper color filters. However, in practice the integral fluorescence intensity of the whole spectrum is measured. In this case all photons emitted by both molecules are counted, and the photon distribution function for two molecules at a time interval T takes now the following form:

$$W_2(N|T) = \sum_{n=0}^{N} w_{N-n}^{(1)}(T) w_n^{(2)}(T) \tag{25}$$

It is easy to generalize this formula for M molecules:

$$W_M(N|T) = \sum_{n_1=0}^{N} w_{N-n_1}^{(1)}(T) W_{M-1}(n_1|T) = \sum_{n_1=0}^{N} w_{N-n_1}^{(1)}(T) \sum_{n_2=0}^{n_1} w_{n_1-n_2}^{(2)}(T) \sum_{n_3=0}^{n_2} w_{n_2-n_3}^{(3)}(T) \ldots \sum_{n_{M-1}=0}^{n_{M-2}} w_{n_{M-2}-n_{M-1}}^{(M-1)}(T) w_{n_{M-1}}^{(M)}(T) \tag{26}$$

With the help of eq 26 we are able to analyze the fluorescence photon statistics of a molecular ensemble. Let us use the Mandel parameter for this purpose. It depends on the first and the second moment of the distribution function and is given by[23]

$$Q = \frac{\langle N(N-1)\rangle - \langle N\rangle^2}{\langle N\rangle} \tag{27}$$

If $Q = 0$, photon distribution is of Poisson type. If $Q < 0$, it is narrower than Poisson distribution; that is, it is of sub-Poisson type. If $Q > 0$, the photon distribution is broader than Poisson distribution, i.e. it is of super-Poisson type. On the basis of eq 25, we can express the Mandel parameter for the fluorescence of two molecules via the Mandel parameters for fluorescence of molecules 1 and 2

$$Q_{1+2} = \frac{\langle N_1(N_1-1)\rangle - \langle N_1\rangle^2 + \langle N_2(N_2-1)\rangle - \langle N_2\rangle^2}{\langle N_1\rangle + \langle N_2\rangle} = Q_1 \frac{\langle N_1\rangle}{\langle N_1\rangle + \langle N_2\rangle} + Q_2 \frac{\langle N_2\rangle}{\langle N_1\rangle + \langle N_2\rangle} \tag{28}$$

Here $N_{1,2}$ are the numbers of photons emitted by the molecules 1 and 2.

We arrive at the following result for the Mandel parameter of M molecules

$$Q_M = \frac{\sum_{j=1}^{M} Q_j \langle N_j\rangle}{\sum_{k=1}^{M} \langle N_k\rangle} \tag{29}$$

The consequence of the last two formulas is that the Mandel parameter Q_M for fluorescence of identical M molecules is equal to the Mandel parameter Q for single molecule fluorescence, i.e.

$$Q_M = Q \tag{30}$$

So in this case the type of fluorescence statistics for M molecules coincides with that for single molecule statistics.

In general, it is not true. Equation 30 is broken if fluorescence of molecules gathered in the ensemble have different kinds of statistics. For example, it follows from eq 28 that if the fluorescence of the first molecule is of sub-Poisson type, i.e., $Q_1 < 0$, and the fluorescence of the second one is of super-Poisson type, i.e., $Q_2 > 0$, it is possible that full fluorescence will be of Poisson type because $Q_{1+2} = 0$.

Let us now turn to the expression describing a photon distribution in single molecule fluorescence. The corresponding formula for the photon distribution function was derived in ref 9

$$w_N(T) = \frac{1}{\tau_0} \int_0^T (T-t)\{[s(\lambda)^{N-1}]_t - 2[s(\lambda)^N]_t + [s(\lambda)^{N+1}]_t\}\, \mathrm{d}t, \qquad (N \geq 1) \tag{31}$$

$$w_0(T) = \frac{1}{\tau_0} \int_0^\infty [1 - \int_0^{T+t} s(x)\, \mathrm{d}x]\, \mathrm{d}t \tag{32}$$

The physical meaning of the constant

$$\tau_0 = \int_0^\infty (1 - \int_0^t s(x)\, \mathrm{d}x)\, \mathrm{d}t \tag{33}$$

is simple: it determines the average time interval between emitted photons. In other words, its inverse value equals the average fluorescence intensity $\langle I\rangle$ of a single molecule

$$1/\tau_0 = \langle I\rangle \tag{34}$$

We see that eqs 31−33 are expressed solely via a single function $s(t)$ called the start−stop correlator (or via the N power of its Laplace transform). The physical meaning of the start−stop correlator is determined by the formula

$$\mathrm{d}W_s(t) = s(t)\, \mathrm{d}t \tag{35}$$

Here $\mathrm{d}W_s(t)$ is the probability of emission a photon at the interval $(t, t + \mathrm{d}t)$ if the preceding photon was emitted at the zero time moment. The start−stop correlator describes the correlation of two sequentially emitted photons if a molecule is illuminated by a cw laser light, and that is why it is also called "waiting time distribution".[11] Its specific form is determined by the microscopic model describing the molecule being excited by cw laser field.

Only a small part of emitted photons will be detected. If a molecule has emitted N photons, αN photons with $0 < \alpha < 1$ will be registered. In the simplest case of Poisson distribution $P_N(\langle N\rangle) = \langle N\rangle^N \exp[-\langle N\rangle]/N!$ with $\langle N\rangle = kT$, the decrease of the number N of arriving photons is fully equivalent to less intensive excitation of the molecule (αk instead of k in the

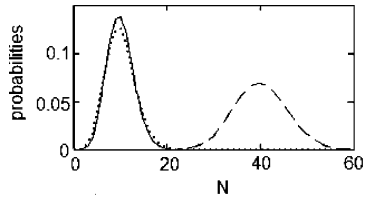

Figure 5. Photon distribution function $w_N(T)$ calculated with the help of eqs 31, 33, and 37 for the time interval $T = 10/k$ (solid line); Poisson distribution with the same maximum position corresponds to a longer time interval (dots); the distribution $W_4(N|T)$ of photons emitted by four identical molecules each of which has the distribution function $w_N(T)$ is shown by dashed line.

distribution formulas). Therefore the decrease of arriving photons can be compensated by the equivalent increase of the time interval T.

In case of non-Poisson distribution, a more complicated rescaling exists. The case of a three-level molecule and its super-Poisson blinking fluorescence was studied in ref 9. As it was shown, we need to substitute αk for k, αA for A, and αa for a in eq 1 and T/α for time interval T. It is equivalent to the statistical treating of αN detected photons instead of N emitted photons. Hence eqs 30−32 can be used for calculation of the distribution of photons arriving at detector.

At the beginning let us consider the simpliest case, which describes a two-level molecule excited by a cw laser. In this case, as it was shown in ref 10, we arrive at the following expression for the start−stop correlator:

$$s(T) = \frac{\lambda_1\lambda_2}{\lambda_2 - \lambda_1}[\exp(-\lambda_1 T) - \exp(-\lambda_2 T)] \qquad (36)$$

Here $\lambda_1 = \gamma - R$, $\lambda_2 = \gamma + R$, $\gamma = k + G/2$, $R = (\gamma^2 - k/T_1)^{1/2}$. G is the rate of all spontaneous transitions from the excited state of the molecule, $1/T_1$ is the rate of spontaneous luminescence, and k is the rate of laser excitation of the molecule. Carrying out the Laplace transform $\int_0^\infty e^{i(\omega+i0)t}e^{-\lambda_j t}\,dt = [\lambda_j - i\omega]^{-1} = [\lambda_j - \lambda]^{-1}$ in eq 36 and raising the result into power N, we arrive at the following expression:

$$s(\lambda)^N = \lambda_1\lambda_2\frac{\lambda_1^{N-1}}{(\lambda_1 - \lambda)^N}\frac{\lambda_2^{N-1}}{(\lambda_2 - \lambda)^N} = \lambda_1\lambda_2 P_{N-1}^{\lambda_1}(\lambda)P_{N-1}^{\lambda_2}(\lambda), \qquad N \geq 1 \qquad (37)$$

Here $P_N^{\lambda_i}(\lambda) = \lambda_i^N/(\lambda_i - \lambda)^{N+1}$ is the Laplace transform of the Poisson function. Carrying out the inverse Laplace transform, we arrive at the following expression:

$$[s(\lambda)^N]_t = \lambda_1\lambda_2\int_0^t dx\, P_{N-1}[\lambda_1(t-x)]P_{N-1}[\lambda_2 x] \qquad (38)$$

Here $P[ax] = (ax)^N\exp(-ax)/N!$ is the Poisson function. When eq 38 is substituted into eq 31, we can calculate the fluorescence photon distriburion for a two-level molecule. The result is depicted in Figure 5 by a solid line. The photon distribution is close in form to the Poisson one, but it is narrower, i.e., it is of sub-Poisson type. The Poisson distribution with the same maximum position is represented with dots in Figure 5. Substituting the distribution shown in Figure 5 with a solid line into formula 26 we arrive at the distribution for several (in this case four) molecules represented in Figure 5 with a dashed line. The average photon number for M molecule fluorescence is a linear function of M

$$\langle N\rangle_M = M\langle N\rangle \qquad (39)$$

Here $\langle N\rangle$ is the average photon number in single molecule fluorescence. The width of the distribution increases proportionally to $\sqrt{M}$, as

$$\sqrt{\langle N^2\rangle_M - \langle N\rangle_M^2} = \sqrt{M(\langle N^2\rangle - \langle N\rangle^2)} \qquad (40)$$

Let us now consider photon distribution in several molecule fluorescence in which each molecule is characterized by the energy scheme depicted in Figure 1; that is, each molecule has blinking fluorescence. As it was shown in ref 9, the start−stop correlator of such a molecule is the sum of three exponential functions: $s(t) = \sum_{j=0}^2 s_j\exp(-\lambda_j t)$, and the time-dependent component of the Laplace transform in power N has the following form:

$$[s(\lambda)^N]_t = \lambda_1\lambda_2\left(\frac{\lambda_0}{a}\right)^N\left\{\int_0^t dx\, P_{N-1}[\lambda_1(t-x)]P_{N-1}[\lambda_2 x] + \lambda_0\sum_{m=1}^N C_N^m\left(\frac{a-\lambda_0}{\lambda_0}\right)^m\int_0^t dx\, P_{m-1}[\lambda_0(t-x)]\times\int_0^x dy\, P_{N-1}[\lambda_1(x-y)]P_{N-1}[\lambda_2 y]\right\} \qquad (41)$$

Here λ_0, λ_1, and λ_2 are the exponents of the three exponential functions in the start−stop correlator.

When eq 41 is substituted into eq 31, we can calculate the photon distribution in single molecule fluorescence. The result is presented in Figure 6a.

The distribution was calculated for a molecule with parameters (18). The photon distribution for a single molecule consists of a sub-Poisson peak with the maximum at $N = 100$ and a plateau stretching toward small N. The sub-Poisson peak is caused by the fact that we are likely to detect photons from an on-interval coinciding with the interval T. The first term in braces of eq 41 creates this peak. However, as on-interval duration fluctuates, sometimes the interval T will cover two or even three on-intervals. In such cases we will encounter intervals without light, i.e. off-intervals during the observation time. Such situations are described by the sum over m in eq 41. Hence in this case we will detect fewer photons during the interval T than in the case it covers a single on-interval. The plateau manifests itself in the photon distribution due to that very decrease of the detected photon number.

The three-level model discussed here has already been successfully used in ref 9. It theoretically described the distribution of photons measured in ref 7 in fluorescence of a single 1,1′-didodecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI) molecule and a molecule of conjugated polymer dPPV-PPyV. It was shown that eqs 31 and 41 are able to describe measured distributions without any adjustable parameters. All needed parameters have been taken from experimental data of ref 7.

The photon distribution of two identical noninteracting molecules calculated with the help of eq 25 is plotted in Figure 6b. There are already two peaks with the maxima at $N = 100$

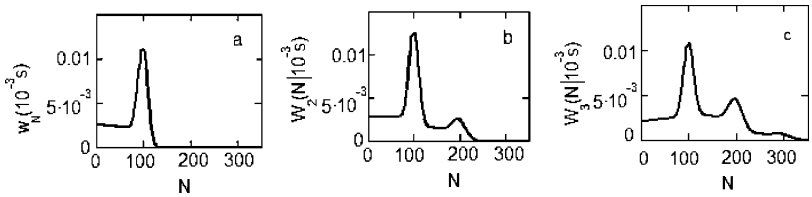

Figure 6. Photon distribution functions in blinking fluorescence of a single molecule (a) of two molecules (b) and three identical molecules (c) calculated with the help of eqs 31, 33, and 41 for set (18) of molecular parameters.

and 200, the second peak being considerably lower than the first one. The same tendency keeps for three noninteracting molecules, the photon distribution of which is shown in Figure 6c. The third peak at $N = 300$ is very small. The forth peak at $N = 400$ in the fluorescence photon distribution for four molecules is practically indiscernible.

5. Fluorescence Autocorrelation Functions

A fluorescence autocorrelation function (AF) determines the correlation between two events separated by value T in time. AF is determined by the formula

$$g^{(2)}(T) = \frac{\langle I(t)I(t+T)\rangle}{\langle I(t)I(t+\infty)\rangle} \tag{42}$$

Here $I(t)$ is the fluctuating fluorescence intensity, and angle parentheses denote the integration of the fluctuating function product over t. At a very large delay T between the events any correlation disappears, and the AF equals unity. Equation 42 enables one to find the AF if the measured function $I(t)$ is at our disposal.

The way to derive theoretically the expression for a single molecule AF was discussed in a number of works,[14,23,24] where the following relation was found:

$$g^{(2)}(T) = \rho_1(T)/\rho_1(\infty) \tag{43}$$

Here $\rho_1(T)$ is the probability of finding a molecule in the excited electronic state at the time moment T, with the initial condition that the molecule was certainly in the ground electronic state at $T = 0$. It means that a photon was emitted at $T = 0$.

The probability of observing a photon at the interval $(t,t + dt)$ under the condition that the first photon has been emitted at the zero time moment is given by

$$dW_p(t) = p(t)\,dt \tag{44}$$

The density probability $p(t)$ is called full two-photon correlator.[15,22] It is given by

$$p(t) = \rho_1(t)/T_1 \tag{45}$$

The full two-photon correlator determines the correlation of two photons separated by the time interval t with any number of intermediate photons emitted at this interval. The fluorescence AF can evidently be expressed via the full two-photon correlator as follows:[25]

$$g^{(2)}(T) = p(T)/p(\infty) \tag{46}$$

Solving eq 1 for a three-level molecule we find the following expression for the full correlator:[15,22]

$$p(t) = \frac{k}{T_1}\left[\frac{a}{\gamma^2 - R^2} + \left(1 - \frac{a}{\gamma - R}\right)\frac{e^{-(\gamma-R)t}}{2R} - \left(1 - \frac{a}{\gamma + R}\right)\frac{e^{-(\gamma+R)t}}{2R}\right] \tag{47}$$

Here

$$\gamma = \frac{G + 2k + a}{2}, \qquad R = \sqrt{\left(\frac{G + 2k - a}{2}\right)^2 - Ak} \tag{48}$$

and $G = \Gamma + A + 1/T_1$ is the sum of rates of all spontaneous transitions from the excited electronic state. The full two photon correlator calculated with the help of eq 47 and set of parameters (18) is shown in Figure 7. The decrease of the full correlator at a delay shorter than 10^{-8} s demonstrates the effect of photon antibunching. It is caused by the fact that the probability of emitting the second photon after the first one approaches zero when the time interval t separating the two photons tends to zero. This fact leads to the sub-Poisson fluorescence photon statistics of the two-level nanoparticle discussed above.

The AF decrease at time of the order of 10^{-3} s coincides with the time τ connected with the average on- and off-intervals t_{on} and t_{off} by the following simple expression:

$$1/\tau = 1/t_{on} + 1/t_{off} \tag{49}$$

There is a plateau at times longer than τ in the full correlator. The value of the correlator at a long delay is equal to the average fluorescence intensity

$$p(\infty) = \langle I\rangle \tag{50}$$

Let us now consider fluorescence of two molecules. We have a succession of photons emitted by both molecules in this case. If the fluorescence wavelengths of the first and the second

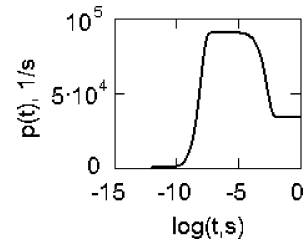

Figure 7. Full two-photon correlator calculated with the help of eq 47 for set of parameters (18). Approach to zero at short times shows photon antibunching. Decrease at 10^{-3} s shows photon bunching due to off-intervals.

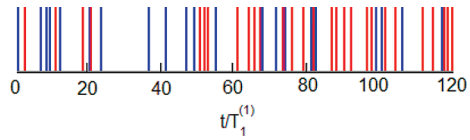

Figure 8. Instances of photon emission by molecule 1 (red lines) and by molecule 2 (blue lines).

molecule differ, we can detect photons emitted by molecule 1 and molecule 2 separately using color filters. Such a succession of photons emitted by the two molecules is shown in Figure 8. Photons emitted by different molecules are depicted with vertical segments of different colors. Apparently, we can measure four types of the probabilities in this case

$$dW_{nm}(t) = p_{nm}(t)\, dt \tag{51}$$

Here the first index $n = 1, 2$ indicates a photon emitted by n-th molecule at zero time moment, and the second index $m = 1, 2$ indicates a photon emitted by mth molecule at a time interval $(t,t + dt)$. We will call the probabilities $p_{11}(t) = p_1(t)$ and $p_{22}(t) = p_2(t)$, which are measured for both photons emitted by molecule 1 or molecule 2, respectively, the full correlators of molecules 1 and 2. The probabilities $p_{12}(t)$ and $p_{21}(t)$, which are connected with the initial and the final photon emitted by different molecules will be called cross-correlators.

As the molecules emit photons independenly of each other, the following relations are evident:

$$p_{11}(\infty) = p_{21}(\infty) = p_1(\infty), \qquad p_{22}(\infty) = p_{12}(\infty) = p_2(\infty) \tag{52}$$

Let us note once again that the first index denotes the molecule which emits the first photon of the pair under consideration, and the second index denotes the molecule which emits the second one. Equation 52 reveals the fact that there is no correlation between any two photons at long delays.

According to antibunching of two photons emitted by one and the same molecule we find

$$p_{11}(0) = p_{22}(0) = 0 \tag{53}$$

However for cross-correlators we can write

$$p_{21}(0) = p_1(\infty) \quad \text{and} \quad p_{12}(0) = p_2(\infty) \tag{54}$$

Equations 52 and 54 result in obvious relations

$$p_{21}(0) = p_{21}(\infty) = p_{21}(\tau) = p_1(\infty),$$
$$p_{12}(0) = p_{12}(\infty) = p_{12}(\tau) = p_2(\infty) \tag{55}$$

In other words, there is no correlation between any pair of photons emitted by molecules 1 and 2. The cross-correlator is determined by the count rate of photons closing the interval τ.

It is evident that

$$P_1 = p_1(\infty)/[p_1(\infty) + p_2(\infty)],$$
$$P_2 = p_2(\infty)/[p_1(\infty) + p_2(\infty)] \tag{56}$$

are the probabilities of finding a photon of one type and a photon of another type in the whole succession of the photons presented in Figure 8. That is why

$$p_{(2)}(\tau) = P_1[p_{11}(\tau) + p_{12}(\tau)] + P_2[p_{22}(\tau) + p_{21}(\tau)]$$
$$= \frac{p_1}{p_1 + p_2}[p_1(\tau) + p_2] + \frac{p_2}{p_1 + p_2}[p_2(\tau) + p_1] \tag{57}$$

is the photon count rate for any pairs separated by the time interval τ. Here abbrivated notations are used: $p_1(\infty) = p_1$ and $p_2(\infty) = p_2$. Therefore, the fluorescence AF for two molecules, which equals unity at infinity, is given by

$$g^{(2)}_{(2)}(\tau) = p_{(2)}(\tau)/p_{(2)}(\infty) = [p_1(\tau)p_1 + p_2(\tau)p_2 + 2p_1p_2](p_1 + p_2)^{-2}$$
$$= [p_1^2 g_1^{(2)}(\tau) + p_2^2 g_2^{(2)}(\tau) + 2p_1p_2](p_1 + p_2)^{-2} \tag{58}$$

This formula expresses the fluorescence AF for two molecules via the AFs for each molecule.

Equations 57 and 58 can easily be generalized for the case of M various noninteracting molecules

$$p_{(M)}(\tau) = \frac{1}{\sum_{j=1}^{M} p_j} \sum_{j=1}^{M} \left[p_j p_j(\tau) + \sum_{k=1}^{M} (1 - \delta_{jk}) p_j p_k \right] \tag{57a}$$

$$g^{(2)}_{(M)}(\tau) = \sum_{j=1}^{M} \left[p_j^2 g_j^{(2)}(\tau) + \sum_{k=1}^{M} (1 - \delta_{jk}) p_j p_k \right] \left(\sum_{j=1}^{M} p_j \right)^{-2} \tag{58a}$$

Fluorescence AF for two identical Hg^+ ions and for two identical pentacene molecule have already been measured in refs 17 and 21. Figure 9 shows that eq 58 fits well experimental data for two identical Hg^+ ions.

If time resolution of our set up is low and it does not allow to observe photon antibunching in single molecule fluorescence, we have $p_1(0) \neq 0$ and $p_2(0) \neq 0$. These values depend on time

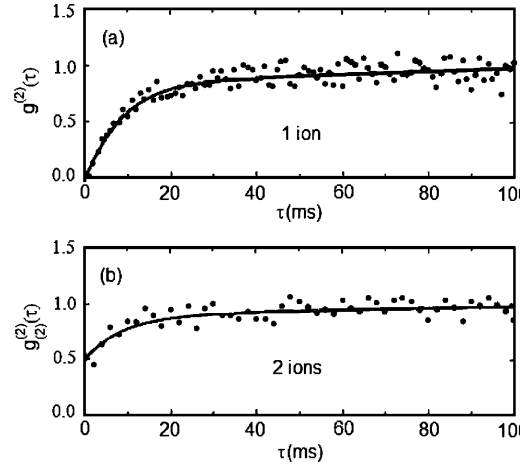

Fig.9

Figure 9. Fluorescence AF for one (a) and two (b) identical Hg^+ ions measured in ref 17 Curves were calculated with the help of eqs 46 and 58.

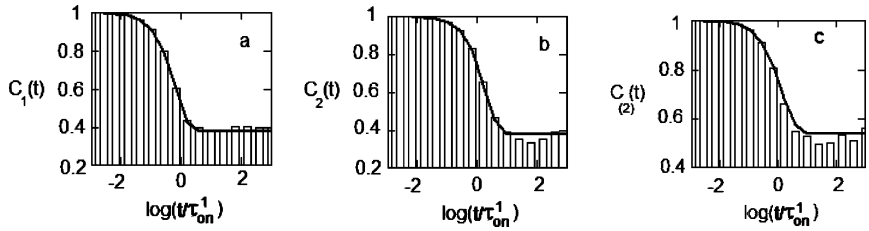

Figure 10. Histograms for correlation functions "measured" in the quantum trajectories shown in Figure 2a−c (computer experiment). Solid lines are result of the theoretical calculation with the help of eqs 59 and 60 (theory).

resolution of our set up. Then we can introduce new correlation functions

$$C_1(\tau) = p_1(\tau)/p_1(0), \qquad C_2(\tau) = p_2(\tau)/p_2(0) \tag{59}$$

for the fluorescence of each molecule and the corresponding correlation function for two molecules as a whole

$$C_{(2)}(\tau) = p_{(2)}(\tau)/p_{(2)}(0) = \frac{p_1p_1(\tau) + p_2p_2(\tau) + 2p_1p_2}{p_1p_1(0) + p_2p_2(0) + 2p_1p_2} \tag{60}$$

The correlation function for M molecules is given by

$$C_{(M)}(\tau) = \frac{\sum_{j=1}^{M}[p_jp_j(0)C_j(\tau) + \sum_{k=1}^{M}(1 - \delta_{jk})p_jp_k]}{\sum_{j=1}^{M}[p_jp_j(0) + \sum_{k=1}^{M}(1 - \delta_{jk})p_jp_k]} \tag{61}$$

Equations 58a and 61 show how the fluctuation smoothing, which inevitably occurs in fluorescence of several molecules, manifests itself in AF and in the full correlator measured in experiments. The difference in values, called "contrast", at small τ and at $\tau \rightarrow \infty$ for these functions can be calculated if we insert eqs 58a and 61 in $g^{(2)}_{(M)}(\tau) - 1$ and $C_{(M)}(\tau) - C_{(M)}(\infty)$. The formulas of the contrast for identical molecules are simplier and can be expressed in terms of the single molecule contrast

$$g^{(2)}_{(M)}(\tau) - 1 = [g^{(2)}(\tau) - 1]/M,$$
$$C_{(M)}(\tau) - C_{(M)}(\infty) = \frac{p(0)}{p(0) + (M-1)p}[C(\tau) - C(\infty)] \tag{62}$$

The contrast of these functions approaches to zero when the number M of molecules increases. Besides, photon antibunching also disappears with the increase of M. The correlators $C(t)$ for two molecules are plotted in Figure 10. Solid lines shows the correlators calculated with the help of eq 60. The histograms represent the result of statistical treating the quantum trajectories of intensity shown in Figure 2. The sufficient coincidence of the curves calculated with the help of eq 60 and the histograms obtained with the help of computer simulation proves the validity of eqs 60 and 61, which we have derived. It is seen from Figure 10c that the contrast, i.e., the difference in values $C_{(2)}(t)$ is less for two molecules than for one molecule. The contrast disappears at all (becomes zero) with the increase of the number of molecules as fluorescence becomes continuous.

6. Conclusion

If each molecule has blinking fluorescence, fluctuations of the fluorescence of several molecules do not disappear at all but become smaller. It is possible to treat such fluorescence with smoothed blinks statistically with the aim of finding such functions as (i) the on- and off-interval distribution, (ii) the photon number distribution at a time interval t, and (iii) fluorescence autocorrelation function.

In the present paper, we have derived the relevant expressions for all the three types of the distribution functions. Moreover we have proved their validity by comparing them to the functions found by the statistical analysis of the fluctuating fluorescence of several molecules simulated with computer and by comparing with the experimentally measured autocorrelation functions shown in Figure 9.

The second goal of the paper was to monitor the conversion of the quantum light of a single molecule to classical light emitted by an ensemble of such molecules. This conversion manifests itself in smoothing fluctuations and in disappearance of on- and off-intervals. It was shown that the best way to observe the conversion of quantum light to classical one is the measurement of the fluorescence autocorrelation function. The disappearance of the "contrast" and photon antibunching in the fluorescence autocorrelation function is sure a sign of such a conversion.

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