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# Probing single molecule kinetics by photon arrival trajectories

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Multitime correlation functions of photon arrival times in single molecule fluorescence resonant energy transfer measurements are computed using a simple model representing slow conformational dynamics described by a collective stochastic Gaussian coordinate. The analogy with time domain nonlinear optical spectroscopy is explored. Various statistical measures of distributions of single photon arrival times and fluorescence lifetimes are employed to analyze non-Poissonian statistics.
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## I. INTRODUCTION

The study of individual molecules in the condense phases is a new and fascinating field which attracts considerable attention of both experimentalists and theorists.[1–4] Single molecule (SM) measurements provide detailed microscopic information about the distributions of various quantities whereas conventional bulk experiments only yield their average. SM signals exhibit stochastic behavior due to coupling with various molecular and environment degrees of freedom.[5–9] Early SM studies obtained fluorescence trajectories binned on a ms time scale when the molecule is subjected to a strong saturating field. Trajectories of intensities or frequencies show evidence for slow kinetics or spectral diffusion.

Two-time correlation functions of fluorescence intensities provide additional information on photon statistics. Brown–Twiss correlations[10] have shown photon bunching, antibunching, and other characteristic signatures of the stochastic nature of the electric field. Such experiments were later performed on single atoms or ions.[11,12] SM measurement in the condensed phase subsequently utilized this technique to provide information about the dynamics of slow coordinates at both cryogenic and room temperature.[1,2,13–18]

Recently, a new experimental technique has been applied for probing SM dynamics by exciting the molecule with a train of pulses and recording a sequence of chronological ($t$) and photon arrival times, i.e., delay ($\tau$) times between an excitation pulse and the emitted photon (Fig. 1).[19,20] The set $\{t,\tau\}$ constitutes a *photon arrival trajectory* (PAT). $t$ is controlled by the incoming pulse train and $\tau$ by the excited state lifetime. It is then possible to simultaneously probe in real time fast (ns to ps) kinetics through $\tau$ and its variation on a much slower (ms, $t$) time scale. Multitime correlation functions are commonly used in the description of nonlinear optical response. Brown–Twiss correlation techniques probe equilibrium stationary fluctuations and are thus the analogs of *frequency domain* nonlinear optical measurements. In contrast, PAT experiments resemble *time domain* multiple pulse nonlinear spectroscopy[21] and have the capacity to provide considerably more detailed and direct information. This includes, e.g., two-time statistics of two measurements separated by $t_1$, and more generally, $N$-time statistics by conducting an $N$-time measurement and obtain $N$ point correlation functions of various moments of $\tau$. Xie and co-workers[20] have demonstrated how this technique may be employed to probe single DNA and tRNA conformational fluctuations on a broad range of time scales through fluorescence resonant energy transfer (FRET).[22]

Models for multitime correlation functions required for the analysis of photon arrival trajectories were developed. Zhao *et al.* computed the four-time correlation function of the third order response functions for a chromophore coupled to a bath consisting either of many two-level systems utilizing the Kubo–Andersen sudden jump stochastic model[23] or harmonic degrees of freedom (the spin-boson model).[24] Because higher order correlation functions provide increasingly more detailed microscopic information on the system and can further be used to compute various measures of correlation of variables of the environment, they serve as a powerful tool in the studies of evolution of slow environments. Barkai *et al.* used the four-time correlation function to study stationary fluorescence fluctuations of a molecule undergoing spectral diffusion.[25]

In this paper we consider FRET dynamics where a chromophore is quenched by an acceptor and their distance is fluctuating due to, e.g., conformational motion. When that motion is faster than the characteristic kinetic time scale, ordinary kinetic rate equations provide an adequate description of dynamics; the dynamics is governed by ensemble averaged rate constants and stochastic trajectories obey Poissonian distributions. However, when the motion is slow, the statistics becomes non-Poissonian.[26–28] We assume that motion of the environment is much slower than the fluorescence lifetime. We compute multitime correlation functions and moments of the distribution of photon arrival times and two-time correlation functions accessible through single and two time measurements. These quantities provide statistical measures for slow dynamics through non-Poissonian arrival time distributions. When data from many stochastic trajectories are collected, the distributions of arrival times are convoluted with static distributions of realizations of the random environment. Moments and correlation functions can thus be viewed as stochastic variables which may be used to deconvolute the two. We relate statistical measures of the dynam-

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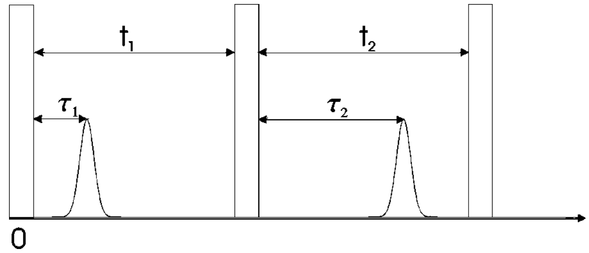


FIG. 1. Macroscopic times ($t$) and arrival times ($\tau$) in a photon arrival time trajectory obtained from a time resolved multiple pulse photon counting experiment.

ics of conformational relaxation to experimentally accessible distributions of photon arrival times.

In Sec. II we compute moments of the distributions of multitime correlation functions of photon arrival times for a stretched exponential distribution of the passage times. We examine several statistical measures which probe the environment dynamics and deviations of the photon arrival time distributions from Poissonian statistics. Distributions and moments of the two-time correlation functions of photon arrival times for single and two time measurements when the fluorescence lifetime depends exponentially on a single slow Gaussian variable are computed in Sec. III. In Sec. IV we establish the one-to-one correspondence between a hierarchy of experimentally accessible quantities expressing probability densities of observing photon arrival times and distributions of fluorescence lifetimes. We model conformational motion as a stochastic Gaussian process. Moments and two-time correlation function of fluorescence lifetime are computed in Sec. V and our results are summarized in Sec. VI.

## II. MOMENTS OF MULTITIME DISTRIBUTIONS OF PHOTON ARRIVAL TIMES

Consider a single time measurement in which an optical pulse interacts with a SM which emits a photon after the arrival time $\tau$ (see Fig. 1). We assume that $\tau$ depends on a stochastic variable $X$ representing the conformation whose equilibrium distribution is given by $P_{\rm eq}(X)$. When the conformation characteristic time scale $\gamma^{-1}$ is slow compared with fluorescence lifetime $T$ ($T \ll \gamma^{-1}$), the photon arrival times can be described by the passage time distributions $\psi(\tau;X)$ [known also as the waiting time distribution in continuous-time-random-walk (CTRW)].[29–35] For a particular realization $X$, the arrival time probability density $P_1(X,\tau)$ is

$$P_1(X,\tau)=\psi(\tau;X)P_{\rm eq}(X). \tag{1}$$

In a SM photon arrival experiment, data on the number of observed arrival times are collected from many stochastic trajectories; different realizations of $X$ have different $P_1$ profiles and we thus obtain a distribution of survival probability densities. $P_1(X,\tau)$ is a key physical quantity which can be used to construct quantities corresponding to both SM and bulk measurements. For instance, the bulk survival probability density $\langle P(\tau)\rangle$ can be computed as

$$\langle P(\tau)\rangle \equiv \int dX\,\psi(\tau;X)P_{\rm eq}(X), \tag{2}$$

where $\langle \ldots \rangle$ denotes the ensemble average over the equilibrium distribution of $X$. Its $n$th moment is

$$\langle \mu_n\rangle \equiv \int d\tau\,\tau^n \langle P(\tau)\rangle. \tag{3}$$

Different realizations of the random environment give rise to a distribution of $\mu_n(X)$. We define the $n$th moment of $\tau$ for a given $X$,

$$\mu_n(X)\equiv \int d\tau\,\tau^n \psi(\tau;X). \tag{4}$$

We can now look at its $p$th moment,

$$M_p(\mu_n)\equiv\langle(\mu_n)^p\rangle=\int dX(\mu_n(X))^p P_{\rm eq}(X) \quad p=1,2,\ldots. \tag{5}$$

$M_p(\mu_n)$ which is only accessible from individual SM trajectories may be computed using the generating function,[26,32,33] i.e.,

$$M_p(\mu_n)=\left[\frac{d^p}{dy^p}\int dX P_{\rm eq}(X)e^{y\mu_n(X)}\right]_{y=0}, \tag{6}$$

where $y$ is the Laplace variable conjugate to $\mu_n(X)$.

To probe deviations of the distribution of arrival times from exponential (non-Poissonian statistics), we define the difference

$$d_p(\mu_n)\equiv M_p(\mu_n)-[M_1(\mu_n)]^p, \tag{7}$$

and the ratio

$$r_p(\mu_n)\equiv \frac{M_p(\mu_n)}{[M_1(\mu_n)]^p}. \tag{8}$$

For Poissonian statistics, $M_p(\mu_n)=\langle\mu_n\rangle^p$, so that $d_p(\mu_n)=0$ and $r_p(\mu_n)=1$.

In an ensemble of stochastic trajectories, the time evolution of $X$ is convoluted with the distribution of $X$ obtained from the distribution of photon arrival times. A single time measurement reveals equilibrium distributions of quantities of interest, but is not sensitive to slow motion. $d_p$ and $r_p$, provide non-Poissonian signatures of the distribution of arrival times due to static inhomogeneity of conformations. To probe slow environment motions, we need to study the dynamics of correlations of photon arrival times by considering their multitime correlation functions. To that end, we extend a single time experiment to an $N$-time experiment (Fig. 1) in which we send a train of $n$ optical pulses and record a sequence of arrival times $\tau_1,\tau_2,\ldots,\tau_n$ corresponding to a sequence of realizations $X_1,X_2,\ldots,X_n$. This allows to construct a hierarchy of quantities containing all the microscopic information on dynamics of a molecule. In general, we can compute the conditional probability distribution $P_n(X_1,\tau_1,X_2,\tau_2,\ldots,X_n,\tau_n;t_1,\ldots,t_{n-1})$ of detecting $n$ photons corresponding to realizations $X_1,X_2,\ldots,X_n$ and arrival times $\tau_1,\tau_2,\ldots,\tau_n$, with separation intervals $t_1,\ldots,t_{n-1}$ in a series of $n$ consecutive measurements,

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$$P_n(X_1,\tau_1,X_2,\tau_2,\ldots,X_n,\tau_n;t_1,\ldots,t_{n-1})$$
$$\equiv\psi(\tau_n;X_n)G(X_n,t_{n-1}|X_{n-1},t_{n-2})\psi(\tau_{n-1};X_{n-1})$$
$$\times\cdots\times G(X_3,t_2|X_2,t_1)\psi(\tau_2;X_2)$$
$$\times G(X_2,t_1|X_1,0)\psi(\tau_1;X_1), \quad (9)$$

where $G(X_i,t_{i-1}|X_{i-1},t_{i-2})$ is the conditional probability for having $X_i$ at time $t_{i-1}$ given $X_i$ at time $t_{i-1}$, $i=1,2,\ldots$ .

The ensemble-averaged (bulk) joint distribution of observing $n$ arrival times $\tau_1,\ldots,\tau_n$ separated by $t_1,\ldots,t_{n-1}$ is given by

$$\langle P_n(\tau_1,\ldots,\tau_n;t_1,\ldots,t_{n-1})\rangle$$
$$=\int dX_1\cdots\int dX_n$$
$$\times P_n(X_1,\tau_1,\ldots,X_n,\tau_n;t_1,\ldots,t_{n-1})P_{\rm eq}(X_1), \quad (10)$$

and the $n$-time correlation function of arrival times $\tau_1,\ldots,\tau_n$ is

$$\langle\tau^{m_1}(0)\cdots\tau^{m_n}(t_{n-1})\rangle$$
$$=\int d\tau_1\cdots\int d\tau_n\tau_1^{m_1}\cdots\tau_n^{m_n}$$
$$\times\langle P_n(\tau_1\ldots\tau_n;t_1\ldots t_{n-1})\rangle, \quad (11)$$
$$m_1,\ldots,m_n=1,2,\ldots .$$

In an $n$-time SM measurement, $n$-time correlation functions of photon arrival times are evaluated from many SM stochastic trajectories, and are distributed over realizations $X_1,X_2,\ldots,X_n$. We define the multitime extension of Eq. (4),

$$C_{m_1,\ldots,m_n}(X_1,0;\ldots;X_n,t_{n-1})$$
$$\equiv\int d\tau_1\cdots\int d\tau_{n-1}\tau_1^{m_2}\cdots\tau_n^{m_n}$$
$$\times P_n(X_1,\tau_1,\ldots,X_n,\tau_n;t_1,\ldots,t_{n-1}). \quad (12)$$

$C_{m_1,\ldots,m_n}(X_1,0;\ldots;X_n,t_{n-1})$ are stochastic variables, and it is useful to look at their $p$th moment [analogous to Eq. (5)],

$$M_p(C_{m_1,\ldots,m_n},t_1,\ldots,t_{n-1})$$
$$\equiv\int dX_1\int dX_2\cdots\int dX_n$$
$$\times(C_{m_1,\ldots,m_n}(X_1,0;\ldots;X_n,t_{n-1}))^pP_{\rm eq}(X_1). \quad (13)$$

In a previous work, we used a generating function approach to compute multitime correlation functions of relevant physical quantities for SM trajectories in environments with several slow variables with various degrees of correlations among them.[26] The generating function technique [see Eqs. (5) and (6)] may be used to compute $M_p$'s.

We further define the corresponding difference and ratio of the $p$th moment. For the two-time correlation function, we have

$$D_p(C_{nm},t_1)\equiv M_p(C_{nm},t_1)-[M_1(C_{nm},t_1)]^p \quad (14)$$

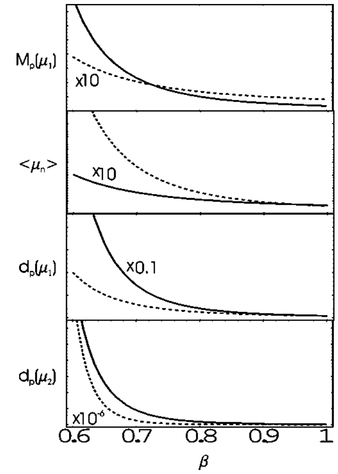


FIG. 2. Top panel: the $p$th moment of the photon arrival times distribution $M_p(\mu_n)$ vs $\beta$ for $n=1$ and $p=1$ (solid line) and $p=2$ (dashed line). Second panel: the ensemble averaged $n$th moment of photon arrival times $\langle\mu_n\rangle$ for $n=2$ (dashed line) and for $n=1$ (solid line). Third panel: the difference $d_p(\mu_n)$ of the $p$th moment of the photon delay times distribution $M_p(\mu_n)$ and the ensemble average of the $n$th moment of photon delay times $\langle\mu_n\rangle$ taken to $p$th power vs $\beta$ for $p=3$, $n=1$ (dashed line) and for $p=2$, $n=1$ (solid line). Bottom panel: the difference $d_p(\mu_n)$ vs $\beta$ for $p=3$, $n=2$ (dashed line) and $M_p(\mu_n)$ for $p=2$, $n=2$ (solid line). For clarity some curves were multiplied by the factors as indicated, $\sigma=a=1$.

and

$$R_p(C_{nm},t_1)\equiv\frac{M_p(C_{nm},t_1)}{[M_1(C_{nm},t_1)]^p}. \quad (15)$$

For Poisson statistics, we have $D_p(C_{nm},t_1)=M_p(\mu_n)M_p(\mu_m)-[M_1(\mu_n)M_1(\mu_m)]^p$ and $R_p(C_{nm},t_1)=r_p(\mu_n)r_p(\mu_m)$. The two-time quantities then carry no new information. Equations (7) and (8) along with Eqs. (14) and (15) probe non-Poissonian signatures in the distributions of photon arrival times which is directly accessible from a single and two-time SM measurement.

Single time measurements probe static fluctuations and may be analyzed using Eqs. (7) and (8). Two time measurements provide information on the dynamics of fluctuations and may be characterized by Eqs. (14) and (15). In the next section we compute the distributions, moments and two-time correlation functions of photon arrival times for a model system.

## III. SINGLE AND TWO TIME MEASUREMENTS OF ARRIVAL TIME DISTRIBUTIONS

We assume a stretched exponential form for the passage time distribution,

$$\psi(X,\tau)=N_0\frac{1}{T}\exp\left[-\left(\frac{\tau}{T}\right)^\beta\right], \quad (16)$$

where $\beta>0$, $N_0=\beta\Gamma(1/\beta)$ is a normalization constant, and $\Gamma(x)$ is the Gamma function. We further assume that the excited state lifetime depends exponentially on $X$,

$$T(X)=T_c\exp[aX], \quad (17)$$

where $T_c$ is the characteristic lifetime and parameter $a$ controls the magnitude of its fluctuation. This corresponds to the

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Dexter mechanism or to quenching by electron transfer. $X$ is taken to be a Gaussian Markovian variable described by the Langevin equation and exponential correlation function,[32]

$$\langle X(t)X(0)\rangle=\sigma^2\exp[-\gamma t], \tag{18}$$

where $\sigma^2=\langle X(0)^2\rangle_{\rm eq}$ stands for the equilibrium magnitude of fluctuations and $\gamma$ is the fluctuation decay rate. Equilibrium distribution of $X$ is

$$P_{\rm eq}(X)=\left(\frac{1}{2\pi\sigma^2}\right)^{1/2}\exp\left[-\frac{X^2}{2\sigma^2}\right]. \tag{19}$$

The conditional probability density $G(X,t;X_0,t_0)$ to find $X$ at time $t$, provided that it was $X_0$ at time $t_0$ is governed by the Smoluchowski equation,

$$\frac{\partial}{\partial t}G(X,t;X_0,t_0)=\frac{\partial}{\partial X}\gamma XG+\frac{\partial}{\partial X}D\frac{\partial}{\partial X}G, \tag{20}$$

where $D$ is the diffusion constant.

Using Eq. (3), we obtain

$$\langle\mu_n\rangle=N_0\frac{1}{\beta}\Gamma\left(\frac{n+1}{\beta}\right)\exp[\tfrac{1}{2}a^2\sigma^2n^2]. \tag{21}$$

$\langle\mu_n\rangle$ for $n=1$ and 2 is plotted in Fig. 2 (upper middle panel) as a function of $\beta$. It monotonically decreases with $\beta$.

For the $p$th moment of $\mu_n$ [Eq. (5)] we obtain

$$M_p(\mu_n)=N_0\left(\frac{1}{\beta}\Gamma\left(\frac{n+1}{\beta}\right)\right)^p\exp\left[\frac{1}{2}a^2\sigma^2p^2n^2\right]. \tag{22}$$

$M_p(\mu_n)$ versus $\beta$ for $p=1$ and 2 and $n=1$ is shown in Fig. 2 (top panel).

The $X$-dependent $n$th moment of arrival times [Eq. (4)], is given by

$$\mu_n(X)=N_0\frac{1}{\beta}\Gamma\left(\frac{n+1}{\beta}\right)\exp[aXn]. \tag{23}$$

Using Eqs. (21) and (22), we have computed $d_p(\mu_n)$ [Eq. (7)] versus $\beta$. The results are displayed in Fig. 2 for $n=1$, $p=2$, and 3 (lower middle panel) and $n=2$, $p=2$, and 3 (bottom panel). All curves decrease monotonically.

Using Eq. (11), we obtain

$$\langle\tau^n(0)\tau^m(t_1)\rangle=N_0^2\frac{1}{\beta^2}\Gamma\left(\frac{n+1}{\beta}\right)\Gamma\left(\frac{m+1}{\beta}\right)\times\exp\left[\frac{a^2\sigma^2}{2}(m^2+2mne^{-\gamma t_1}+n^2)\right]. \tag{24}$$

Figure 3 shows $\langle\tau^n(0)\tau^m(\gamma t_1)\rangle-\langle\mu_n\rangle\langle\mu_m\rangle$ (bottom panel) for $n=m=1$ and $\beta=1$ and 0.8. Both curves decay to zero as $\gamma t_1$ tends to infinity. Correlations of photon arrival times have larger amplitude and last longer for bigger $\beta$.

For the distribution of two-time correlation function of photon arrival times [Eq. (12)] we obtain

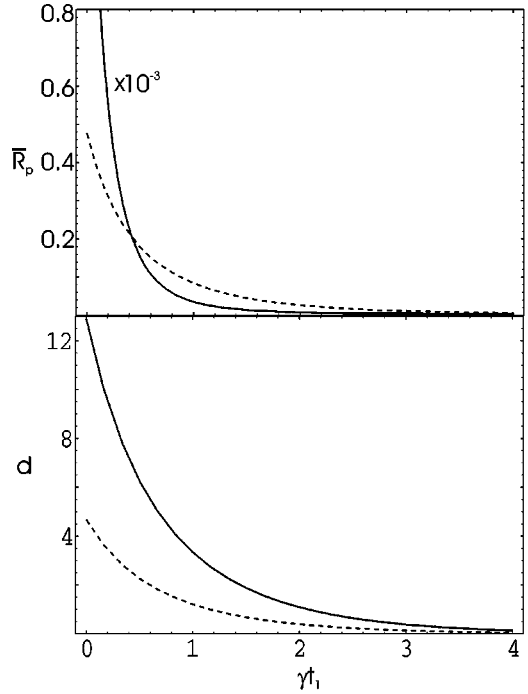


FIG. 3. Top panel: the reduced ratio $\tilde{R}_p(t_1)=R_p(C_{nm},\gamma t_1)-r_p(\mu_n)r_p(\mu_m)$ vs $\gamma t_1$ for $n=m=1$, $\beta=1$; $p=2$ [$\tilde{R}_p(t_1)$, dashed line] and $p=3$ [$10^{-3}\tilde{R}_p(t_1)$, solid line]. Bottom panel: difference $d(t_1)=\langle\tau^n(0)\tau^m(\gamma t_1)\rangle-\langle\mu_n\rangle\langle\mu_m\rangle$ vs $\gamma t_1$ for $n=m=1$; $\beta=1$ (dashed line) and $\beta=0.8$ (solid line); $a=\sigma=1$.

$$C_{nm}(X_1,0;X_2,t_1)=N_0^2\frac{1}{\beta^2}\Gamma\left(\frac{n+1}{\beta}\right)\Gamma\left(\frac{m+1}{\beta}\right)G(X_2,t_1;X_1,0)\times\exp[aX_1n+aX_2m], \tag{25}$$

where the Green's function solution of Eq. (20) is

$$G(X,t;X_0,t_0)=\left(\frac{1}{2\pi\sigma^2(1-e^{-2\gamma(t-t_0)})}\right)^{1/2}\times\exp\left[-\frac{(X-X_0e^{-\gamma(t-t_0)})^2}{2\sigma^2(1-e^{-2\gamma(t-t_0)})}\right]. \tag{26}$$

In Fig. 4 we display the joined distribution $C_{nm}(X_1,0;X_2,t_1)P_{\rm eq}(X_1)$ for $\beta=n=m=1$ and several values of $t_1$. The diagonal ($X_1=X_2$) part of the distribution moves toward lower $X_1$ and $X_2$ and decays whereas the off-diagonal part grows as $t_1$ increases. The diagonal feature representing the $X_1,X_2$ entanglement disappears altogether for long $t_1$ and implies uncorrelated distributions of $X_1$ and $X_2$, i.e., $C_{nm}(X_1,0;X_2,t_1)=\mu_n(X_1)\mu_m(X_2)P_{\rm eq}(X_2)$.

The $p$th moment of the distribution the two-time correlation function of photon arrival times [Eq. (12)] is given by

$$M_p(C_{nm},t_1)=N_0^2\left(\frac{1}{\beta^2}\Gamma\left(\frac{n+1}{\beta}\right)\Gamma\left(\frac{m+1}{\beta}\right)\right)^p\times\exp\left[\frac{a^2\sigma^2p^2}{2}(m^2+2mne^{-\gamma t_1}+n^2)\right]. \tag{27}$$

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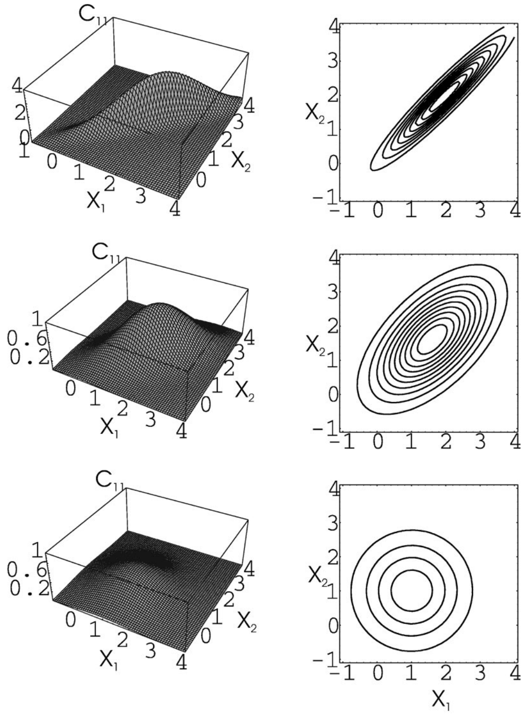


FIG. 4. The joint distribution $C_{nm}(X_1,0;X_2,\gamma t_1)P_{eq}(X_1)$ vs $X_1$ and $X_2$ for $\beta=1$, $n=m=1$ and fixed $\gamma t_1$: $\gamma t_1=0.05$ (top panels), 0.5 (middle panels), and 5.0 (bottom panels); both two-dimensional surfaces (left column) and contour plots (right column) are shown.

$M_p(C_{11},t_1)$ for $\beta=1$ and 0.8 is displayed in Fig. 5 for $p=1$ (top panel) and 2 (bottom panel). All curves approach the asymptotic value $M_p(\mu_n)M_p(\mu_m)$ for long $t_1$. $M_p(\mu_n)$ and $M_p(\mu_m)$ become uncorrelated faster for $\beta=1$ compared with $\beta=0.8$. $\overline{R}_p(C_{nm},t_1)=R_p(C_{nm},t_1)-r_p(\mu_n)r_p(\mu_m)$ is displayed in Fig. 3 for $\beta=1$, $n=m=1$ and $p=2$ and 3 (top panel).

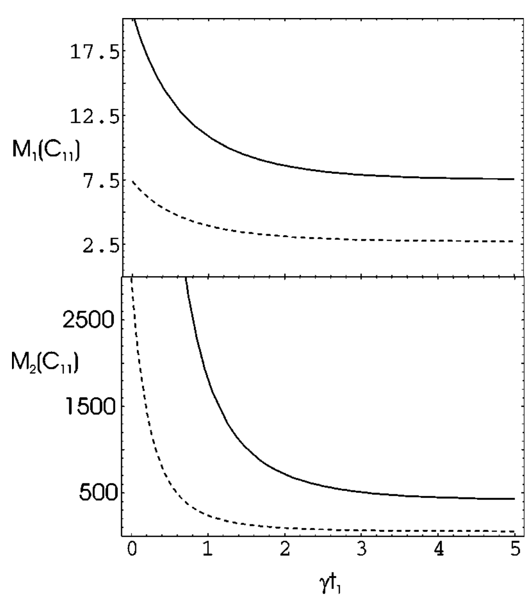


FIG. 5. The $p$th moment of the distribution of the two-time correlation function of photon arrival times $M_p(C_{nm};t_1)$ vs $\gamma t_1$ for $n=m=1$, $\beta=1$ (dashed line) and 0.8 (solid line): $p=1$ (top panel) and $p=2$ (bottom panel); $a=\sigma=1$.

## IV. APPLICATION TO EXPONENTIAL DISTRIBUTION OF PASSAGE TIMES

In this section we analyze the distributions of photon arrival time for the exponential passage time distribution, i.e., setting $\beta=1$ in Eq. (16). In Sec. II we have constructed a set of distributions $P_1,P_2,\ldots,P_n$ containing increasingly more detailed information about correlations among photon arrival times. Similarly, we can construct a hierarchy of quantities containing all the relevant microscopic information about the environment motion. We define the probability distribution of a molecule to have the fluorescence lifetime $T_1$, $F_1(T_1)$, normalized as

$$\int F_1(T_1)dT_1=1, \tag{28}$$

and in general, the joint distribution of a molecule to be in $n$ states with lifetimes $T_1,T_2,\ldots,T_n$ at moments of time separated by $n-1$ intervals $t_1,t_2,\ldots,t_{n-1}$, $F_n(T_1,T_2,\ldots,T_n;t_1,t_2,\ldots,t_{n-1})$ normalized as

$$\int dT_1\cdots\int dT_nF_n(T_1,T_2\ldots T_n;t_1,t_2\ldots t_{n-1})=1. \tag{29}$$

When the conformational fluctuations are much slower than fluorescence lifetime, the distributions of fluorescence lifetimes and photon arrival times are related by the Laplace transforms

$$\begin{aligned} P_1(\tau_1)&=\int_0^\infty dT_1F_1(T_1)\frac{1}{T_1}e^{-\tau_1/T_1},\\ P_n(\tau_1,\tau_2,\ldots,\tau_n;t_1,t_2,\ldots,t_n)&\\ =\int_0^\infty dT_1\int_0^\infty dT_2\cdots\int_0^\infty dT_n&\\ \times F_n(T_1,T_2,\ldots,T_n;t_1,t_2,\ldots,t_{n-1})&\\ \times\frac{1}{T_1T_2\cdots T_n}e^{-\tau_1/T_1-\tau_2/T_2-\ldots-\tau_n/T_n}.& \end{aligned} \tag{30}$$

Equations (30) show the one-to-one correspondence between the distributions of photon arrival times $P_n$'s and distributions of fluorescence lifetimes $F_n$'s. Having evaluated $P_n$'s from experiment, we can compute $F_n$'s by inverse Laplace transforms and thus, gain information on the environment motion.

Hereafter in this section we compute quantities which contain averaged information about the arrival time and fluorescence lifetime statistics as well as environment dynamics. From experimental data one can obtain moments of photon arrival time defined in Eq. (3) and the two-time correlation function of photon arrival times,

$$\begin{aligned}\langle\tau^n(0)\tau^m(t_1)\rangle&\equiv M_{nm}(t_1)\\ &\equiv\int_0^\infty d\tau_1\int_0^\infty d\tau_2P_2(\tau_1,\tau_2;t_1)\tau_1^n\tau_2^m.\end{aligned} \tag{31}$$

These quantities are related to the corresponding moments of fluorescence lifetime,

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$$\langle T^n\rangle \equiv \int_0^\infty dT F_1(T) T^n, \tag{32}$$

and to the two-time correlation functions of fluorescence lifetimes,

$$\langle T^n(0) T^m(t_1)\rangle \equiv K_{nm}(t_1) \equiv \int_0^\infty dT_1 \int_0^\infty dT_2 F_2(T_1,T_2;t_1) T_1^n T_2^m. \tag{33}$$

For our model, using Eqs. (4), (31), and (33), we find

$$\langle T^n\rangle = \Gamma(n+1)^{-1}\langle \tau^n\rangle \tag{34}$$

and

$$K_{nm}(t) = (\Gamma(n+1)\Gamma(m+1))^{-1} M_{nm}(t), \tag{35}$$

where $\Gamma(n+1)=n!$

More detailed information on statistics and dynamics of correlations of the environment can be extracted from higher order correlation functions (see Appendix).

Equations (34) and (35) map the moments and two-time correlation functions and correlation of photon arrival time fluctuations available from experiment onto corresponding moments and $n$-time correlation functions and correlation of fluctuations of fluorescence lifetimes. In the next section we utilize the multitime correlation functions to compute distributions of fluorescence lifetimes and photon arrival times. We also compute moments and the two-time correlation functions of fluorescence lifetime and photon arrival times.

## V. MULTITIME STATISTICS OF ARRIVAL AND KINETIC TIMES

The probability density of a molecule to have fluorescence lifetime $T_1$, is given by

$$F_1(T_1) \equiv \int dX_1 P_{\mathrm{eq}}(X_1)\,\delta(T_1 - T(X_1)), \tag{36}$$

and the joint distribution of a molecule to be in state $X_1$ with lifetime $T_1$ at time $t=0$, and in state $X_2$ with lifetime $T_2$ after time $t=t_1$ is

$$F_2(T_1,T_2;t_1) \equiv \int dX_1 \int dX_2 P_{\mathrm{eq}}(X_1)\,\delta(T_1 - T(X_1)) \times G(X_2,t_1;X_1,0)\,\delta(T_2 - T(X_2)). \tag{37}$$

We can compute the $n$-time distribution of fluorescence lifetimes, i.e.,

$$F_n(T_1,T_2,\ldots,T_n;t_1,\ldots,t_{n-1}) \equiv \int dX_1 \cdots \int dX_n \delta(T_n - T(X_n)) \times G(X_n,t_{n-1};X_{n-1},t_{n-2}) \times \cdots \times G(X_2,t_1;X_1,t_0)\,\delta(T_1 - T(X_1)) P_{\mathrm{eq}}(X_1). \tag{38}$$

Assuming $\beta=1$ and $a=1$ and using Eqs. (36), (37), (30), and (4), we obtain

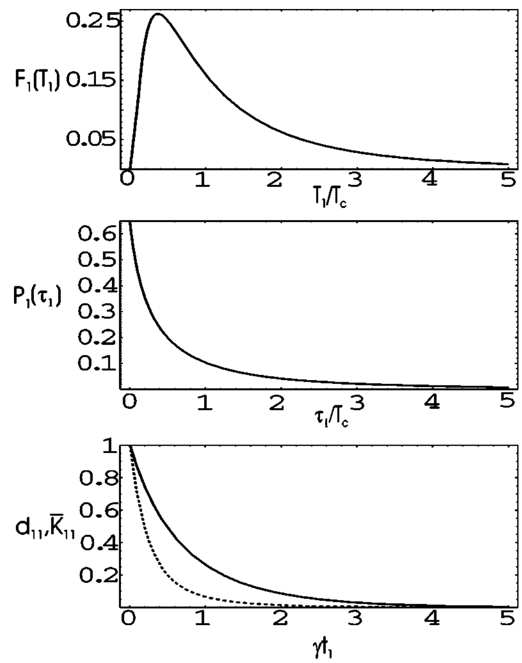


FIG. 6. Probability distributions of fluorescence lifetime $F_1(T_1)$ (top panel) and fluorescence photon arrival time $P_1(\tau_1)$ (middle panel) for $a=1$, $\sigma=1$. Bottom panel: The difference $d_{nm}(t_1)$ (solid line) and $\overline{K}_{11}(t_1)$ (dashed line) as a function of reduced separation time $\gamma t_1$ for $n=m=1$.

$$F_1(T_1) = \left(\frac{1}{2\pi}\right)^{1/2} (T_c e^{x_1})^{-1} P_{\mathrm{eq}}(x_1) \tag{39}$$

and

$$F_2(T_1,T_2;t_1) = \frac{1}{2\pi} (T_c^2 e^{x_1+x_2})^{-1} P_{\mathrm{eq}}(x_1) G(x_2,t_1,x_1,0), \tag{40}$$

where

$$x_{1,2} = \log \frac{T_{1,2}}{T_c}. \tag{41}$$

In Fig. 6 we present $F_1(T_1)$ (top panel) which is peaked around the average value of the fluorescence lifetime, $\langle T\rangle$. In Fig. 7 we display $F_2(T_1,T_2;t_1)$ for several values of $t_1$. Here, the diagonal $(T_1=T_2)$ peak corresponding to the $T_1$, $T_2$-entanglement decays as $t_1$ increases. At longer $t_1$ the off-diagonal part of the density grows relative to the diagonal part, and diagonal feature of the joint distribution is completely washed out. This implies that at longer $t_1$ the $T_1$, $T_2$-correlation decays and $T_1$ and $T_2$ become statistically independent, i.e., $F_2(T_1,T_2;t_1) = F_1(T_1)F_1(T_2)$. To better demonstrate this point, we display in Fig. 8 the difference,

$$d(T_1,T_2;t_1) \equiv F_2(T_1,T_2;t_1) - F_1(T_1)F_1(T_2) \tag{42}$$

for several values of $t_1$. The hump of $d(T_1,T_2;t_1)$ along the diagonal at shorter $t_1$ acquires more off-diagonal features and gradually decays at longer $t_1$. This can be understood in the following way: when separation between any two photons is short and the environment is slow, a molecule retains memory of its previous states, i.e., state $X_2$ with the lifetime $T_2$ remembers state $X_1$ with the lifetime $T_1$ after $t_1$, the fluorescence decays from states $X_1$ and $X_2$ are correlated,

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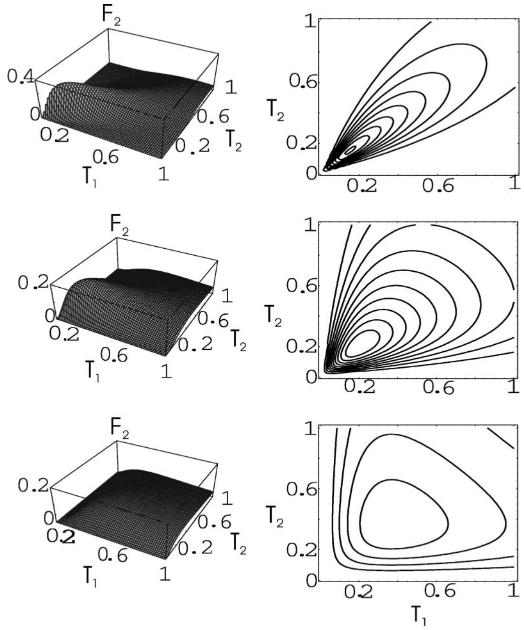


FIG. 7. The joint probability distribution $F_2(T_1,T_2;t_1)$ of observing fluorescence decay lifetimes ($T_1$ and $T_2$ are given in units of $T_c$) in two consecutive PAT measurements for fixed reduced separation time $\gamma t_1=1$ (top panels), 5 (middle panels), and 10 (bottom panels) for $a=1$, $\sigma=1$; both two-dimensional surfaces (left column) and contour plots (right column) are shown.

and the joint distribution of lifetimes $T_1$ and $T_2$ become non-Poissonian. $d(T_1,T_2;t_1)$ probes deviations from Poissonian statistics. However, at longer separation times fluctuations of the environment decay, and a molecule reaches its equilibrium state before being excited again. In this case, $F_2(T_1,T_2)=F_1(T_1)F_1(T_2)$ and the lifetime statistics is Poissonian.

Non-Poissonian features may also be studied by comparing the two-time correlation function of fluorescence lifetimes $T_1$ and $T_2$ with a product of corresponding moments. It follows from Eq. (35) that correlation function of fluorescence lifetimes can be evaluated from experimentally accessible correlation function of photon arrival times. Using Eqs. (39) and (40) we have computed the $m$th moment and the two-time correlation function of fluorescence lifetime. We obtain

$$\langle T^n\rangle = T_c^n \exp[\tfrac{1}{2}n^2\sigma^2] \tag{43}$$

and

$$K_{nm}(t)=\exp\left[\frac{\sigma^2(1-e^{-2\gamma t})}{2}\left(\frac{\ln T_c}{\sigma^2(1+e^{-\gamma t})}+m\right)^2\right]$$
$$\times\exp[\tfrac{1}{2}(n+me^{-\gamma t}+\ln T_c)^2]$$
$$\times\exp\left[-\left(\frac{1-e^{-\gamma t}}{2\sigma^2(1+e^{-\gamma t})}+\frac{1}{2}\right)(\ln T_c)^2\right]. \tag{44}$$

Note that apart from factors $\Gamma(n+1)$ and $\Gamma(n+1)\Gamma(m+1)$ [see Eqs. (34) and (35) in Sec. IV], $\langle\tau^n\rangle$ and $M_{nm}(t)$ are given by Eqs. (43) and (44), respectively.

In Fig. 6 (bottom panel, solid curve) we display the difference,

$$d_{nm}(t_1)\equiv K_{nm}(t_1)-\langle T^n\rangle\langle T^m\rangle \tag{45}$$

for $n=m=1$ normalized by $d_{nm}(0)$. This quantity directly probes the dynamics of deviations of the distribution of fluorescence lifetimes from Poissonian statistics. At short $t_1$ when $t_1\ll\gamma^{-1}$, distribution of lifetimes is manifestly non-Poissonian. For longer $t_1$ ($t_1=2$ or $3\gamma^{-1}$) we observe small deviations from Poissonian distribution. For long $t_1$ ($t_1=5\gamma^{-1}$) $K_{nm}(t_1)$ tends to $\langle T^n\rangle\langle T^m\rangle$ and the distribution is essentially Poissonian. The same difference function in Eq. (45) probes dynamics of deviations of the distribution of photon arrival times.

To study conformational dynamics we have computed the two time correlation function of fluorescence lifetime fluctuations [Eq. (A4)] for $m=n=1$ and obtained

$$\bar{K}_{11}(t_1)=K_{11}(t_1)-\langle T\rangle(K_{10}(t_1)-K_{01}(t_1))+\langle T\rangle^2, \tag{46}$$

where $\langle T\rangle$ and $\langle T\rangle^2$ are given by Eq. (43) with $n=1$ and $n=2$, respectively. $K_{10}$ and $K_{01}$ are obtained from Eq. (44) by substituting $n=1$, $m=0$ and $n=0$, $m=1$, respectively. $\bar{K}_{11}(t_1)/\bar{K}_{11}(0)$ is displayed in Fig. 6 (bottom panel, dashed curve). This quantity can be evaluated from experimentally accessible correlation function of photon arrival time fluctuations, $\bar{M}_{11}(t)$ (when $n=m=1$, $\bar{K}$ and $\bar{M}$ are same functions, see Appendix). It directly measures decay of fluorescence lifetime fluctuations and thus, can be used to deduce a time scale of motion of the environment.

Finally, we have computed $P_1(\tau_1)$ and $P_2(\tau_1,\tau_2;t_1)$ from $F_1(T_1)$ and $F_2(T_1,T_2,t_1)$ using Eqs. (30). Closed expressions of these quantities are lengthy and are not presented here. In Fig. 6 (middle panel) we plot $P_1(\tau_1)$, and in Fig. 9 we display $P_2(\tau_1,\tau_2;t_1)$ for several values of $t_1$. Similar to $F_2(T_1,T_2,t_1)$, we find decay accompanied by redistribution of the probability density along the $\tau_1$- and $\tau_2$-axes as $t_1$ increases.

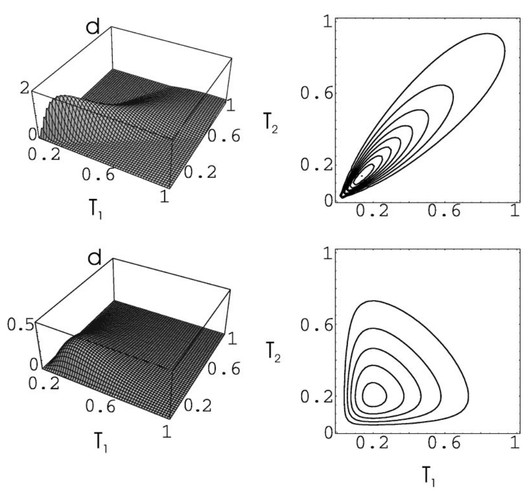


FIG. 8. The difference $d(T_1,T_2;t_1)$ ($T_1$ and $T_2$ are given in units of $T_c$) for fixed reduced separation time $\gamma t_1=1$ (top panels) and 5 (bottom panels) for $a=1$, $\sigma=1$; both two-dimensional surfaces (left column) and contour plots (right column) are shown.

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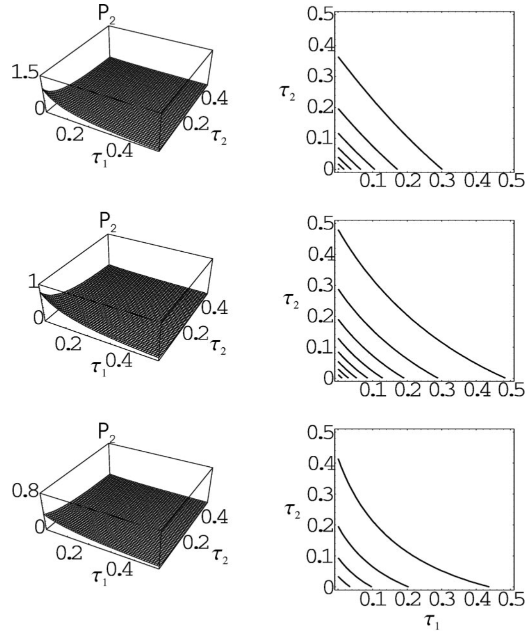


FIG. 9. The joint probability distribution $P_2(\tau_1,\tau_2;t_1)$ of arrival times $\tau_1$ and $\tau_2$ ($\tau_1$ and $\tau_2$ are given in units of $T_c$) in a two time PAT measurements for fixed reduced separation time $\gamma t_1 = 1$ (top panels), 5 (middle panels), and 10 (bottom panels) for $a=1$, $\sigma=1$; both two-dimensional surfaces (left column) and contour plots (right column) are displayed.

## VI. CONCLUSIONS

In this paper we computed moments of multitime distributions and the correlation functions of photon arrival times, quantities accessible through PAT. These quantities may be used to study the evolution of distributions caused by a slow environment. Non-Poissonian statistics provides signatures of correlation and dynamics of the environment. Both static (due to various realizations of the random environment) and dynamic (time evolution) properties of the environment surrounding the molecule may be studied.

Our analysis assumed that the fluorescence lifetime is much faster than the environment (otherwise SMS recovers bulk results) and we modeled the conformational dynamics as a stochastic process with stretched exponential passage time distribution. Statistical measures which probe deviations of distributions of photon arrival times from Poissonian statistics and carry information on the dynamics of correlations were computed. Moments and two-time correlation functions of photon arrival times accessible through single and two-time measurement when the passage time distribution is exponential were analyzed. We explored the one-to-one correspondence between a hierarchy of probability densities of photon arrival times, quantities available experimentally and a hierarchy of distributions of fluorescence lifetime, quantities directly probing dynamics of the environment surrounding a molecule. Moments and the two-time correlation function of fluorescence lifetime were computed and related to the corresponding moments and the two-time correlation function of photon arrival times, respectively.

We have also studied the two-time correlation functions for the fluorescence lifetimes and lifetime fluctuations, and related them to the corresponding correlation functions of photon arrival times and arrival time fluctuations. These quantities directly probe slow motion the environment and can be used to evaluate its relaxation time scale.

In general, when the condition $T \ll \gamma^{-1}$ is not met, the distributions of photon arrival times accessible through single, two- and $n$-time measurement are given by

$$P_1(\tau_1) = \left\langle \exp\left[ -\int_0^{\tau_1} dt \frac{1}{T_1(t)} \right] \right\rangle, \tag{47}$$

$$P_2(\tau_1,\tau_2;t_1) = \left\langle \exp\left[ -\int_0^{\tau_1} dt \frac{1}{T_1(t)} \right] \times \exp\left[ -\int_{t_1}^{t_1+\tau_2} dt \frac{1}{T_2(t)} \right] \right\rangle \cdots,$$

$$P_n(\tau_1,\tau_2,\ldots,\tau_n;t_1,\ldots,t_{n-1}) = \left\langle \exp\left[ -\int_0^{\tau_1} dt \frac{1}{T_1(t)} \right] \exp\left[ -\int_{t_1}^{t_1+\tau_2} dt \frac{1}{T_2(t)} \right] \times \ldots \times \exp\left[ -\int_{t_{n-1}}^{t_{n-1}+\tau_n} dt \frac{1}{T_n(t)} \right] \right\rangle,$$

respectively. These quantities can be evaluated by path integrals and are reminiscent of correlation functions appearing in nonlinear spectroscopy.[21,24] Discrete (multistate) $n$-state jump models of the slow environment[23–25] can be computed as well utilizing the present approach.

## ACKNOWLEDGMENTS

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## APPENDIX: THE *N*-TIME CORRELATION FUNCTIONS OF PHOTON ARRIVAL TIMES AND FLUORESCENCE LIFETIME

The $n$-time correlation functions of $\tau$'s and $T$'s, $M_{m_1,m_2,\ldots,m_n}(t_1,t_2,\ldots,t_{n-1})$ and $K_{m_1,m_2,\ldots,m_n}(t_1,t_2,\ldots,t_{n-1})$ are related by

$$K_{m_1,m_2,\ldots,m_n}(t_1,t_2,\ldots,t_{n-1}) = \prod_{i=1}^{n} \Gamma(m_i+1)^{-1} M_{m_1,m_2,\ldots,m_n}(t_1,t_2,\ldots,t_{n-1}), \tag{A1}$$

where

$$K_{m_1,m_2,\ldots,m_n}(t_1,t_2,\ldots,t_{n-1}) = \int_0^\infty dT_1 \cdots \int_0^\infty dT_n \times F_n(T_1,T_2,\ldots,T_n;t_1,t_2,\ldots,t_{n-1}) T_1^{m_1} \cdots T_n^{m_n} \tag{A2}$$

and $M_{m_1,m_2,\ldots,m_n}(t_1,t_2,\ldots,t_{n-1})$ is defined in Eq. (11).

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Another experimentally accessible useful measure of correlations of the environment is the correlation functions of fluctuations in the photon arrival time, $\delta\tau \equiv \tau - \langle\tau\rangle$, defined as

$$\overline{M}_{m_1,m_2,\ldots,m_n}(t_1,t_2,\ldots,t_{n-1}) \equiv \int_0^\infty d\tau_1 \cdots \int_0^\infty d\tau_n \times P_n(\tau_1,\tau_2,\ldots,\tau_n;t_1,t_2,\ldots,t_{n-1})\,\delta\tau_1^{m_1}\cdots\delta\tau_n^{m_n}, \tag{A3}$$

this quantity is directly related to the $n$-time correlation function of fluctuations in the fluorescence lifetime, $\delta T = T - \langle T\rangle$,

$$\overline{K}_{m_1,m_2,\ldots,m_n}(t_1,t_2,\ldots,t_{n-1}) \equiv \int_0^\infty dT_1 \cdots \int_0^\infty dT_n \times F_n(T_1,T_2,\ldots,T_n;t_1,t_2,\ldots,t_{n-1})\,\delta T_1^{m_1}\cdots\delta T_n^{m_n}. \tag{A4}$$

For example, two-time correlation functions of $\delta\tau$ and $\delta T$ are related by $\overline{K}_{11}(t) = \overline{M}_{11}(t)$.


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