Supporting Information

FCF distortion under the saturation of the fluorescence intensity

The fluorescence intensity of a fluorescent molecule is proportional to the probability of the singlet excited state, $P(\mathrm{S}_1)$ which is given by $P(\mathrm{S}_1) = k_{12}/\{k_{\mathrm{tot}} + (1 + k_{23}/k_{31})k_{12}\}$, as a stationary solution of the Jablonski scheme. The excitation rate k_{12} at position $\boldsymbol{r}$ in 3D Gaussian field is given by $k_{12} = I_0 \exp\{-2(x^2 + y^2)/\omega_0^2 - 2z^2/\omega_z^2\} = I_0\Phi(\boldsymbol{r})$ where $I_0 = \sigma I_{\mathrm{ex}}$. The observed fluorescence intensity of the molecule can be expressed as $i_{\mathrm{em}} = \eta\phi k_{12}/(1 + \alpha k_{12})$ where $\alpha = (1 + k_{23}/k_{31})/k_{\mathrm{tot}}$ with $i_{\mathrm{em}} = \eta k_{21} P(\mathrm{S}_1)$ and $\phi = k_{21}/k_{\mathrm{tot}}$. The average intensity of the molecule can be expanded with the parameter $\xi = \alpha I_0/(1 + \alpha I_0)$ as:

$$i_{\mathrm{em}}(\boldsymbol{r}) = \left\{1 + \sum_{n=1}^{\infty} \xi^n (1-\Phi)^n\right\} \frac{\eta\phi\Phi\xi}{\alpha} = \left\{1 + \sum_{n=1}^{\infty} \xi^n \sum_{m=0}^{\infty} (-)^m \Phi^m \binom{n}{m}\right\} \frac{\eta\phi\Phi\xi}{\alpha} \tag{1}$$

where $\Phi = \Phi(\boldsymbol{r})$. The abbreviation Φ will be used hereafter when the meaning is clear from context. Since ξ and $1-\Phi$ are less than 1, the series will converge when $n \to \infty$. ξ is proportional to the intensity of the molecule at the center of the excitation field. The total intensity at a certain time t is given by totaling the intensity of each molecule at position $\boldsymbol{r}_i(t)$. Therefore, $I(t) = \sum_{i=1}^{N} i_{\mathrm{em}}(\boldsymbol{r}_i(t))$ where N is the total number of molecules in the total volume V. Then, the average intensity is given by $\langle I(t)\rangle = \sum_{i=1}^{N} \int_V d\boldsymbol{r}\, i_{\mathrm{em}}(\boldsymbol{r}_i(t))\nu(\boldsymbol{r}_i(t))$ where the molecule distribution function is $\nu(\boldsymbol{r}) = 1/V$ when the molecules are distributed homogeneously. Here, $\langle\cdot\rangle$ denotes the ensemble average. When the volume V is large enough compared with the excitation-detection field, the finite integration can extend to the infinite one. Consequently, one can obtain

$$\langle I(t)\rangle = (\frac{1}{2})^{3/2} I_0(1-\xi)Cv\eta\phi \left\{1 + \sum_{n=1}^{\infty} \xi^n \sum_{m=0}^{n} (-)^m \left(\frac{1}{m+1}\right)^{3/2} \binom{n}{m}\right\} \tag{2}$$

where $v = \pi^{3/2}\omega_0^2\omega_z$ is the volume element and C is the number density of the molecule N/V, respectively. This series expression does not converge with a saturated value of the emission intensity with $I_0 \to \infty$ because of the infinite space integral. Therefore, this expression cannot describe the experiments under a very high excitation intensity.

When the triplet-singlet conversion is ignored, the fluorescence fluctuation is determined only by the diffusion of molecules in the excitation-detection field. If one considers the non-interacting molecules, the correlation function can be calculated as:

$$\begin{aligned}\langle I(t)I(t+\tau)\rangle &= N\int_V d\boldsymbol{r}\int_V d\boldsymbol{r}'\, i_{\mathrm{em}}(\boldsymbol{r}) i_{\mathrm{em}}(\boldsymbol{r}')\nu(\boldsymbol{r},\boldsymbol{r}';\tau)\nu(\boldsymbol{r}) + N(N-1)\left\{\int_V d\boldsymbol{r} i_{\mathrm{em}}(\boldsymbol{r})\nu(\boldsymbol{r})\right\}^2 \\ &= N/V\int_V d\boldsymbol{r}\int_V d\Delta\boldsymbol{r} i_{\mathrm{em}}(\boldsymbol{r}) i_{\mathrm{em}}(\boldsymbol{r}+\Delta\boldsymbol{r}) P(\boldsymbol{r},\boldsymbol{r}+\Delta\boldsymbol{r};\tau) + \langle I(t)\rangle^2 + O(N/V^{-2})\end{aligned} \tag{3}$$

where $P(\boldsymbol{r},\boldsymbol{r}';\tau)$ is the probability for the detection of a molecule at position $\boldsymbol{r}'$ at time τ when the molecule is at position $\boldsymbol{r}$ at time 0. The last term of the equation comes from the finite number of molecules in the finite space and will vanish when the volume is large enough. When the molecules are diffusing in free space, the diffusion can be characterized by the probability function $P(\boldsymbol{r},\boldsymbol{r}';\tau) = (4\pi D\tau)^{-3/2}\exp(-|\boldsymbol{r}-\boldsymbol{r}'|^2/4D\tau)$ if the volume V is infinite. Therefore, the task is the calculation of the equation,

$$\langle I(t)I(t+\tau)\rangle = C\int d\boldsymbol{r}\int d\Delta\boldsymbol{r} i_{\mathrm{em}}(\boldsymbol{r}) i_{\mathrm{em}}(\boldsymbol{r}+\Delta\boldsymbol{r}) P(\boldsymbol{r},\boldsymbol{r}+\Delta\boldsymbol{r};\tau) + \langle I(t)\rangle^2. \tag{4}$$

The inside of the integration is the product of the Gaussian functions and the integration can be made analytically. By using eq 1, the inside of the integral can be expanded as a series form of the product of Gaussian functions Φ,

$$i_{\rm em}(\boldsymbol{r})i_{\rm em}(\boldsymbol{r}') = (\frac{\xi}{\alpha})^2 \left[\Phi(\boldsymbol{r})\Phi(\boldsymbol{r}') + \sum_{n=1}^{\infty}\xi^n \sum_{m=0}^{n}(-)^m \left\{\Phi((m+1)\boldsymbol{r})\Phi(\boldsymbol{r}') + \Phi(\boldsymbol{r})\Phi((m+1)\boldsymbol{r}')\right\}\binom{n}{m}\right.$$
$$\left. + \sum_{n=1}^{\infty}\sum_{k=1}^{\infty}\xi^{n+k}\sum_{m=0}^{n}\sum_{l=0}^{k}(-)^{m+l}\Phi((m+1)\boldsymbol{r})\Phi((l+1)\boldsymbol{r}')\binom{n}{m}\binom{k}{l}\right]. \tag{5}$$

The integration of the product of Φ produces the higher order correlation functions as the following schematically written expression,

$$\Phi(n\boldsymbol{r})\Phi(m\boldsymbol{r}') \longrightarrow \frac{v}{8}\left(\frac{2}{n+m}\right)^{3/2}\rho(\frac{2nm}{n+m}\lambda), \tag{6}$$

where $\rho(x) = (1+x)^{-1}(1+x/q^2)^{-\frac{1}{2}}$, $\lambda = 4D\tau/\omega_0^2$ and $q = \omega_z/\omega_0$, respectively. Consequently, the integration of the right hand side of eq 5 is given by

$$\frac{v}{8}\left(\frac{\xi}{\alpha}\right)^2\left\{\rho(\lambda) + 2\sum_{n=1}^{\infty}\xi^n\sum_{m=0}^{n}(-)^m\left(\frac{2}{m+2}\right)^{3/2}\rho(\frac{2(m+1)}{m+2}\lambda)\binom{n}{m}\right.$$
$$\left. + \sum_{n=2}^{\infty}\xi^n\sum_{k=1}^{n-1}\sum_{m=0}^{n-k}\sum_{l=0}^{k}(-)^{m+l}\left(\frac{2}{m+l+2}\right)^{3/2}\rho(\frac{2(m+1)(l+1)}{m+l+2}\lambda)\binom{n-k}{m}\binom{k}{l}\right\}. \tag{7}$$

The first term in the brackets is the correlation function in the absence of saturation and other terms are additional terms due to saturation. The final form can be expressed as:

$$\langle I(t)I(t+\tau)\rangle = \frac{Cv\eta^2\phi^2}{8}I_0^2(1-\xi)^2\left\{\rho(\lambda) + 2\sum_{n=1}^{\infty}\xi^n\sum_{m=0}^{n}(-)^m\left(\frac{2}{m+2}\right)^{3/2}\rho(\frac{2(m+1)}{m+2}\lambda)\binom{n}{m}\right.$$
$$\left. + \sum_{n=2}^{\infty}\xi^n\sum_{k=1}^{n-1}\sum_{m=0}^{n-k}\sum_{l=0}^{k}(-)^{m+l}\left(\frac{2}{m+l+2}\right)^{3/2}\rho(\frac{2(m+1)(l+1)}{m+l+2}\lambda)\binom{n-k}{m}\binom{k}{l}\right\} + \langle I(t)\rangle^2, \tag{8}$$

using a relation $\xi/\alpha = I_0(1-\xi)$. The product, Cv, is the number of molecules (n_0) in the excitation-detection volume(v).