

Supporting information for “For how long should a system be observed to obtain reliable concentration estimates from the measurement of fluctuations”

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# 1 Supporting information

## 1.1 Computation of the autocorrelation function (ACF)

We consider a system composed of fluorescently tagged and untagged molecules of the same species,  $P_f^t$  and  $P_f^u$ , that diffuse with free diffusion coefficient  $D_f$  and interact with binding sites,  $S$ , forming the bound species,  $P_b^t$  and  $P_b^u$ , respectively. An appropriate assumption is that the binding sites belong to very massive molecules so that  $S$ ,  $P_b^t$  and  $P_b^u$  diffuse with the same coefficient,  $D_s \ll D_f$ . The reaction scheme is given by:



where  $k_{on}$  and  $k_{off}$  are the rates of binding and unbinding respectively and define the dissociation constant,  $K_D = \frac{k_{off}}{k_{on}}$ .

The fluorescence collected by the microscope at time  $t$  is  $F(t) = \Delta t \int d^3\vec{r} I(\vec{r}) q(c_f^t(\vec{r}, t) + c_b^t(\vec{r}, t))$ ,  $\Delta t$  is the sampling time,  $I(\vec{r})$ , is the distribution of the excitation light in the sample,  $q$  is the product of the absorption cross section by the fluorescence quantum yield and the efficiency of the fluorophore, and  $c_f^t$ ,  $c_b^t$  are the number densities of fluorescent molecules in the free and bound forms. We assume that the fluorescent molecules have the same quantum yield in their free and bound forms. The autocorrelation function (ACF) is defined and computed as:

$$G(\tau) \equiv \frac{\langle \delta F(t) \delta F(t + \tau) \rangle}{\langle F(t) \rangle^2} \approx \Delta t \frac{\int \delta F(t) \delta F(t + \tau) dt}{(\int F(t) dt)^2}, \quad (2)$$

where  $\delta F(t) = F(t) - \langle F \rangle$ , is the deviation of the fluorescence from its mean,  $\langle F \rangle$ , which is approximated by the average as shown above. An analytic expression in terms of integrals can be obtained for  $G(\tau)$  in the case of a system as the one considered here. To this end we follow the procedure described in (1). We start with the approximation to the master equation

of the system that is given by the reaction-diffusion equations:

$$\begin{aligned}
\frac{\partial c_f^t}{\partial t} &= D_f \nabla^2 c_f^t - k_{on} c_f^t c_S + k_{off} c_b^t, \\
\frac{\partial c_b^t}{\partial t} &= D_S \nabla^2 c_b^t + k_{on} c_f^t c_S - k_{off} c_b^t, \\
\frac{\partial c_f^u}{\partial t} &= D_f \nabla^2 c_f^u - k_{on} c_f^u c_S + k_{off} c_b^u, \\
\frac{\partial c_b^u}{\partial t} &= D_S \nabla^2 c_b^u + k_{on} c_f^u c_S - k_{off} c_b^u, \\
\frac{\partial c_S}{\partial t} &= D_S \nabla^2 c_S - k_{on} c_f c_S + k_{off} c_b,
\end{aligned} \tag{3}$$

where  $c_S$  is the number density of free binding sites, and  $c_f = c_f^t + c_f^u$  and  $c_b = c_b^t + c_b^u$  are the total concentrations of free and bound particles, respectively and we have used mass action kinetics to describe the reactions. We then linearize Eqs. (3) around the equilibrium solution,  $c_{f,eq}^{u,t} \equiv [P_f^{u,t}]$ ,  $c_{b,eq}^{u,t} \equiv [P_b^{u,t}]$ ,  $c_{S,eq} \equiv [S]$ , that satisfies  $[P_f^t][S] = K_D[P_b^t]$ ,  $[P_f^u][S] = K_D[P_b^u]$ , and write the ACF as:

$$G(\tau) = \frac{\int d^3 \vec{r} d^3 \vec{r}' I(\vec{r}) I(\vec{r}') \sum_{i,j} q_i q_j \langle \delta c_i(\vec{r}, 0) \delta c_j(\vec{r}', \tau) \rangle}{\left( q V_{obs} ([P_f^t] + [P_b^t]) \right)^2}, \tag{4}$$

where we have used the notation,  $\delta c_i$ , for the deviation of the concentration with respect to equilibrium of the  $i$ -th species and  $q_i$  for the corresponding fluorescence efficiency (which is either zero or  $q$ ) and where  $V_{obs} = \frac{\int d^3 \vec{r} I(\vec{r})}{I(0)}$ . In order to find the deviations as functions of space and time we Fourier transform the linearized reaction-diffusion equations. The transformed system can be expressed as  $\dot{C}_\xi = M_\xi C_\xi$ , with  $C_\xi$  the column vector of deviations with respect to equilibrium in Fourier space (the transpose of  $((\delta c_f^t)_\xi, (\delta c_b^t)_\xi, (\delta c_S)_\xi, (\delta c_f^u)_\xi, (\delta c_b^u)_\xi)$ ),  $\vec{\xi}$  the wavenumber (the variable conjugate to the position,  $\mathbf{r}$ , in real space) and  $M_\xi$  given by:

$$M_\xi = \begin{bmatrix} -D_f \xi^2 - k_{on}[S] & k_{off} & -k_{on}[P_f^t] & 0 & 0 \\ k_{on}[S] & -D_s \xi^2 - k_{off} & k_{on}[P_f^t] & 0 & 0 \\ -k_{on}[S] & k_{off} & -D_s \xi^2 - k_{on}[P_f^t] - k_{on}[P_f^u] & -k_{on}[S] & k_{off} \\ 0 & 0 & -k_{on}[P_f^u] & -D_f \xi^2 - k_{on}[S] & k_{off} \\ 0 & 0 & k_{on}[P_f^u] & k_{on}[S] & -D_s \xi^2 - k_{off} \end{bmatrix}, \tag{5}$$

We then seek a solution of the linear system in Fourier space. To this end

we look for eigenvalues and eigenvectors of  $M_\xi$ . The eigenvalues are:

$$\begin{aligned}
\lambda_1 &= -D_s \xi^2, \\
\lambda_2 &= -\frac{1}{2} \left( k_{off} + k_{on}([S] + [P_f]_T) + (D_f + D_s) \xi^2 + \sqrt{\beta_1} \right), \\
\lambda_3 &= -\frac{1}{2} \left( k_{off} + k_{on}([S] + [P_f]_T) + (D_f + D_s) \xi^2 - \sqrt{\beta_1} \right), \\
\lambda_4 &= -\frac{1}{2} \left( k_{off} + k_{on}[S] + (D_f + D_s) \xi^2 + \sqrt{\beta_2} \right), \\
\lambda_5 &= -\frac{1}{2} \left( k_{off} + k_{on}[S] + (D_f + D_s) \xi^2 - \sqrt{\beta_2} \right),
\end{aligned} \tag{6}$$

where  $\beta_1 = (k_{off} + k_{on}([S] + [P_f]_T))^2 + 2(D_f - D_s)\xi^2(k_{on}[S] - k_{on}[P_f]_T - k_{off}) + (D_f - D_s)^2\xi^4$ ,  $\beta_2 = (k_{off} + k_{on}[S])^2 + 2(D_f - D_s)\xi^2(k_{on}[S] - k_{off}) + (D_f - D_s)^2\xi^4$  and  $[P_f]_T = [P_f^t] + [P_f^u]$ . The matrix of eigenvectors is:

$$X = \begin{bmatrix} 0 & \frac{[P_f^t]}{[P_f^u]} \frac{\phi_2}{k_{on}[S]} & \frac{[P_f^t]}{[P_f^u]} \frac{\phi_3}{k_{on}[S]} & -\frac{\phi_4}{k_{on}[S]} & -\frac{\phi_5}{k_{on}[S]} \\ \frac{[P_f^t]}{[P_f^u]} & \frac{[P_f^t]}{[P_f^u]} & \frac{[P_f^t]}{[P_f^u]} & -1 & -1 \\ K_D & -\frac{[P_f]_T}{[P_f^u]} & -\frac{[P_f]_T}{[P_f^u]} & 0 & 0 \\ 0 & \frac{\phi_2}{k_{on}[S]} & \frac{\phi_3}{k_{on}[S]} & \frac{\phi_4}{k_{on}[S]} & \frac{\phi_5}{k_{on}[S]} \\ 1 & 1 & 1 & 1 & 1 \end{bmatrix} \tag{7}$$

where  $\phi_2 = \lambda_2 + k_{off} + k_{on}[P_f]_T$ ,  $\phi_3 = \lambda_3 + k_{off} + k_{on}[P_f]_T$ ,  $\phi_3 - \phi_2 = \sqrt{\beta_1}$ ,  $\phi_4 = \lambda_4 + k_{off}$ ,  $\phi_5 = \lambda_5 + k_{off}$  and  $\phi_5 - \phi_4 = \sqrt{\beta_2}$ . Each component,  $i$ , of the vector,  $C_\xi(t)$ , can be written as  $(C_\xi(t))_i = \sum_{j,\ell} X_{ij}(X^{-1})_{j\ell}(C_\xi(0))_\ell \exp(\lambda_j t)$ . Replacing this expression in Eq. (4) we observe that the ACF depends on the initial correlations between the particle number fluctuations,  $\delta N_i = N_i - \langle N_i \rangle$ , of the various species ( $1 \leq i \leq 5$ ). Here we assume that the correlation distance is short ranged so that  $\langle \delta c_i(\vec{r}, 0) \delta c_j(\vec{r}', 0) \rangle \propto \delta(\vec{r} - \vec{r}')$ . In (1) it is further assumed that  $\langle \delta c_i(\vec{r}, 0) \delta c_j(\vec{r}', 0) \rangle = \langle c_i \rangle \delta_{ij} \delta(\vec{r} - \vec{r}')$ , *i.e.*, that fluctuations in the particle numbers of different species are initially uncorrelated and that each of them obeys Poisson statistics. Here we assume instead that  $\langle \delta c_i(\vec{r}, 0) \delta c_j(\vec{r}', 0) \rangle = \sigma_{ij}^2 \delta(\vec{r} - \vec{r}') / V_{obs}$  with a particle number correlation matrix,  $\sigma_{ij}^2 \equiv \langle \delta N_i \delta N_j \rangle$  that is not necessarily diagonal. The ACF can then be written as:

$$\begin{aligned}
G(\tau) &= \frac{(2\pi)^{-3}}{q^2 V_{obs} ([P_f^t] + [P_b^t])^2} \\
&\times \int d^3 \xi \hat{I}(\vec{\xi}) \sum_{j,l} q_j q_l \sum_s X_{l,s} \exp(\lambda_s \tau) \sum_k (X^{-1})_{s,k} \sigma_{j,k}^2
\end{aligned} \tag{8}$$

where the subscripts  $j, l$ , label the 5 species of the reaction-diffusion system, the index,  $s$ , labels the eigenvalues,  $X^{-1}$  is the inverse of the eigenvectors matrix and  $\hat{I}(\vec{\xi})$  is the Fourier transform of  $I(\vec{r})$ . Given that  $\sigma^2$  is a symmetric matrix, we can define  $Y = X^{-1}\sigma^2$ . Taking into account that only particles in their tagged form are fluorescent and that both the free and bound ones have the same  $q$ , Eq. 8 can be reduced to:

$$G(\tau) = \frac{(2\pi)^{-3}}{q^2 V_{obs} ([P_f^t] + [P_b^t])^2} \int d^3\xi I(\vec{\xi}) \sum_i \alpha_i e^{\lambda_i \tau}, \quad (9)$$

with

$$\begin{aligned} \alpha_1 &= q^2 (X_{11} + X_{21})(Y_{11} + Y_{12}), \\ \alpha_2 &= q^2 (X_{12} + X_{22})(Y_{21} + Y_{22}), \\ \alpha_3 &= q^2 (X_{13} + X_{23})(Y_{31} + Y_{32}), \\ \alpha_4 &= q^2 (X_{14} + X_{24})(Y_{41} + Y_{42}), \\ \alpha_5 &= q^2 (X_{15} + X_{25})(Y_{51} + Y_{52}). \end{aligned} \quad (10)$$

Notice from Eq. 9, that the ACF can be expressed as the sum of several components, one for each eigenvalue. Nevertheless, it is not always possible to obtain an analytical expression for it. In (2), (3), it is shown that there exist some limits for which the ACF can be expressed as the sum of diffusive components. One of them is the so called *fast reaction* limit, in which the reactions occur on a much faster time scale than the time it takes for molecules to cross the observation volume. In this limit, the ACF reduces to the sum of three diffusive components which can be expressed as:

$$\begin{aligned} G(\tau) &= \frac{G_{oS}}{\left(1 + \frac{\tau}{\tau_S}\right) \sqrt{1 + w^2 \frac{\tau}{\tau_S}}} + \frac{G_{oColl}}{\left(1 + \frac{\tau}{\tau_{Coll}}\right) \sqrt{1 + w^2 \frac{\tau}{\tau_{Coll}}}} \\ &\quad + \frac{G_{oS_m}}{\left(1 + \frac{\tau}{\tau_{S_m}}\right) \sqrt{1 + w^2 \frac{\tau}{\tau_{S_m}}}}, \end{aligned} \quad (11)$$

where we have considered a gaussian illumination profile,  $I(\vec{r}) = I_0 \exp\left(-\frac{2(x^2+y^2)}{w_r^2} - \frac{2z^2}{w_z^2}\right)$ , with  $w = w_z/w_r$  the ratio of the beam waist along the direction of propagation of light,  $w_z$ , and the perpendicular direction  $w_r$ . The weights and

characteristic times of each component are:

$$\begin{aligned}
G_{os} &= \frac{1}{\langle N^t \rangle^2} \frac{[P_f^t]}{K_D + [P_f]_T} \gamma_1, \\
G_{ocoll} &= \frac{1}{\langle N^t \rangle^2} \frac{[P_f^t]}{K_D + [P_f]_T} \frac{K_D \gamma_2 - [P_f]_T \gamma_3}{[P_f]_T}, \\
G_{osm} &= \frac{1}{\langle N^t \rangle^2} \frac{[P_f^u] \gamma_4 - [P_f^t] \gamma_5}{[P_f]_T}, \quad \tau_S = \frac{w_r^2}{4D_S}, \\
\tau_{coll} &= \frac{w_r^2 (1 + \frac{[S]^2}{K_D [S]_T})}{4(D_f + \frac{[S]^2}{K_D [S]_T} D_S)}, \quad \tau_{sm} = \frac{w_r^2 (1 + \frac{[S]}{K_D})}{4(D_f + \frac{[S]}{K_D} D_S)}, \quad (12)
\end{aligned}$$

where  $\langle N^t \rangle = ([P_f^t] + [P_b^t])V_{obs}$  is the mean total number of fluorescent particles in the observation volume,  $[S]_T$  is the total concentration of binding sites and the various  $\gamma_i$  are correlation factors between the species derived from Eq. 8,

$$\begin{aligned}
\gamma_1 &= \langle \delta N_b^{t2} \rangle + \langle \delta N_f^t \delta N_b^t \rangle + \langle \delta N_f^t \delta N_S \rangle \\
&+ \langle \delta N_f^t \delta N_b^u \rangle + \langle \delta N_b^t \delta N_b^u \rangle + \langle \delta N_b^t \delta N_S \rangle, \\
\gamma_2 &= \langle \delta N_f^{t2} \rangle + \langle \delta N_b^{t2} \rangle + 2\langle \delta N_f^t \delta N_b^t \rangle + \\
&+ \langle \delta N_f^t \delta N_f^u \rangle + \langle \delta N_f^u \delta N_b^t \rangle + \langle \delta N_f^t \delta N_b^u \rangle + \langle \delta N_b^t \delta N_b^u \rangle, \\
\gamma_3 &= \langle \delta N_f^t \delta N_S \rangle + \langle \delta N_b^t \delta N_S \rangle - \langle \delta N_f^{t2} \rangle \\
&- \langle \delta N_f^t \delta N_b^t \rangle - \langle \delta N_f^t \delta N_f^u \rangle - \langle \delta N_f^u \delta N_b^t \rangle, \\
\gamma_4 &= \langle \delta N_f^{t2} \rangle + \langle \delta N_b^{t2} \rangle + 2\langle \delta N_f^t \delta N_b^t \rangle, \\
\gamma_5 &= \langle \delta N_f^t \delta N_f^u \rangle + \langle \delta N_f^u \delta N_b^t \rangle + \langle \delta N_f^t \delta N_b^u \rangle \\
&+ \langle \delta N_b^t \delta N_b^u \rangle, \quad (13)
\end{aligned}$$

### 1.1.1 Uncorrelated Species

The general expression given by Eqs. (12)–(13) embraces several cases. Let us consider first the usual case in which the species are not correlated and obey Poisson statistics, *i.e.*  $\langle \delta N_i \delta N_j \rangle = \langle N_i \rangle \delta_{ij}$ . The weights of the compo-

nents then become (2):

$$\begin{aligned}
 G_{os} &= \frac{[P_b^t]^2}{V([P_f^t] + [P_b^t])^2[S]_T}, \\
 G_{ocoll} &= \frac{[P_f^t]}{V([P_f^t] + [P_b^t])^2} f_t \left( 1 + \frac{[S]^2}{K_D[S]_T} \right), \\
 G_{osm} &= \frac{1 - f_t}{V([P_f^t] + [P_b^t])},
 \end{aligned} \tag{14}$$

where  $f_t \equiv [P_f^t]/([P_f^t] + [P_b^t]) = [P_b^t]/([P_b^t] + [P_b^u])$  is the fraction of tagged molecules at equilibrium.

### 1.1.2 Immobile binding sites

We now consider the same case as before but with the constraint that the binding sites do not diffuse, *i.e.*  $D_S = 0$ . The number of binding sites inside the observation volume is then fixed,  $N_S + N_b^t + N_b^u = N_{S_T}$ , thus  $\delta N_b^t + \delta N_b^u + \delta N_S = 0$ . Therefore  $N_b^t$ ,  $N_b^u$  and  $N_S$  are correlated, following a multinomial distribution such that  $\langle \delta N_S \delta N_b^t \rangle + \langle \delta N_S \delta N_b^u \rangle = -\langle \delta N_S^2 \rangle$ . There are no other correlations in the system. From Eq. 13 it can be seen that  $\gamma_1 = 0$ , causing the weight associated to  $D_S$  to become exactly zero, *i.e.*,  $G_{oS} = 0$ . The other  $\gamma_i$ s remain the same as in the previous case, so that the other two weights do not change.

### 1.1.3 Confined Volume

If the total volume where the particles are allowed to move,  $V_T$ , is not much larger than the observation volume,  $V_T \gtrsim V_{obs}$ , the number of particles inside  $V_{obs}$  is correlated with the number of particles outside. The distribution of particles of each type inside and outside the volume follows a Binomial distribution with parameter  $p = \frac{V_{obs}}{V_T}$ . Then the variance of the number of particles of each species is  $\langle \delta N_i^2 \rangle = \langle N_i \rangle (1 - \frac{V_{obs}}{V_T})$ . Replacing this expression in Eqs. (12)–(13) we observe that the resulting ACF is the same as in the  $V_T \gg V_{obs}$  but multiplied by  $1 - p$ .

## Supporting References

1. Krichevsky, O., and G. Bonnet, 2002. Fluorescence correlation spectroscopy: the technique and its applications. *Reports on Progress in Physics* 65:251.

2. Sigaut, L., M. L. Ponce, A. Colman-Lerner, and S. P. Dawson, 2010. Optical techniques provide information on various effective diffusion coefficients in the presence of traps. *Phys. Rev. E* 82:051912.
3. Ipiña, E. P., and S. P. Dawson, 2013. From free to effective diffusion coefficients in fluorescence correlation spectroscopy experiments. *Phys. Rev. E* 87:022706.