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A Note on Number Fluctuations: Statistics of Fluorescence Correlation Spectroscopy as Applied to Brownian Motion

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Fluorescence Correlation Spectroscopy (hereafter called FCS) is a technique used to determine the diffusion coefficient D by following the number of fluorescent molecules contained in a given spot at various times. We derive an expression, relating the variance of the estimated D to several experimental parameters. The main results give a qualitative description of how the statistical uncertainty of this inherently stochastic technique depends on adjustable variables, such as duration of the experiment, maximal autocorrelation lag, beam size, etc.

KEY WORDS: Fluorescence correlation spectroscopy; Smoluchowski process; diffusion.

The basic idea of fluorescence correlation spectroscopy (FCS) is to compute the autocorrelation function of the fluorescence signal coming off from a small region illuminated by a laser beam. In this note we extended the analysis of statistical accuracy of FCS as performed in a pioneering article by Koppel.[2] If n_i are the number of photon counts in the interval $(i-1)T \leqslant t \leqslant iT$, then the measured function is

$$G_j = \frac{1}{N-j} \sum_{i=1}^{N-j} n_i n_{i+j} \qquad (0 \leqslant j \leqslant M) \tag{1}$$

For a simple diffusing system of noninteracting particles, this permits the

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measurement of the diffusion coefficient D,[1] i.e.,

$$G_j(\hat{D}) = \frac{\beta\bar{n}^2}{1 + 4\hat{D}jT/\omega^2} + \bar{n}^2 \tag{2}$$

We calculated the variance of a measured diffusion coefficient as a function of experimental parameters such as maximal autocorrelation lag M, time window T, beam size ω (the distance between e^{-2} points of an assumed Gaussian intensity profile), number of observations N, and concentration $\bar{c}$ of fluorophores, related to the parameter β by $\beta = (\pi\omega^2/L\bar{c})^{-1}$. We proceed along the same lines as Brenner *et al.*,[3] the important difference being the insertion of the physical process of photon–electron emission in the photomultiplier tube. The Poisson character of this process results in the proportionality between factorial moments of the photon count numbers and the ordinary moments of the photocurrent i:

$$F_1(n) = \left\langle \frac{n!}{(n-1)!} \right\rangle = \langle i \rangle^1 \tag{3}$$

In a second step, the statistics of the photon current are coupled to the movement of the particles in the beam, the latter process being characterized by Smoluchowski statistics.

Adopting the linearization scheme by Koppel,[2] and starting with a linear least-squares procedure, we calculated $\epsilon^2 = \text{var } D/D^2$ under the assumptions of $\ln N \ll N$ and $M \ll N$ and find

$$\epsilon^2 = \sum_{k=1}^{3} (\beta\bar{n})^{k-3}(A_k + B_k/\beta), \qquad A_K, B_K \geqslant 0 \tag{4}$$

A_K and B_K are expressions involving fourth-order autocorrelation functions and were derived in an analogous way as Brenner *et al.*[3] The following comments can be made:

(1) The important factor in determining the accuracy of D is $\beta\bar{n}$, the number of photon counts per molecule per sample time, a result derived earlier by Koppel under the supplementary assumption $T \ll 4D/\omega^2$. This, however, is true only in the limit of $M \ll N$ and $\ln N \ll N$, the expressions for B_2 and B_3 being of this respective order.

(2) The obvious optimal design goes in the direction of increasing $\beta\bar{n}$; however, at a certain saturation value of $\beta\bar{n}$ (determined essentially by photolysis and dead-time errors), we can still improve by decreasing the value of A_3 just by manipulating the other parameters.

(3) In a constrained experimental situation ($NT = \text{const}$), the shape of the function suggests a trade-off between time window and number of observations, eventually resulting in a considerable saving of computing time (Fig. 1).

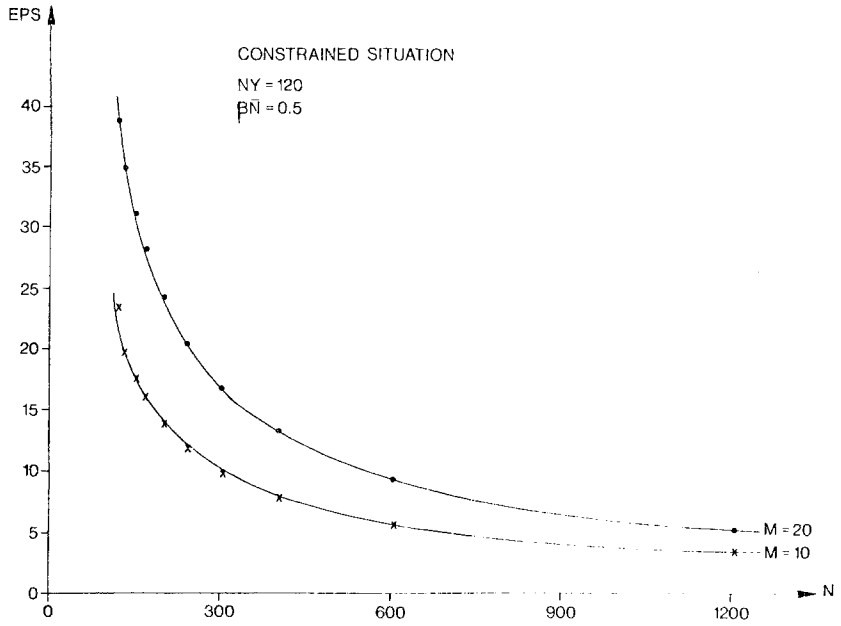

Fig. 1. In the case of constrained systems, ϵ tends to a saturation value, characteristic of continuous sampling.

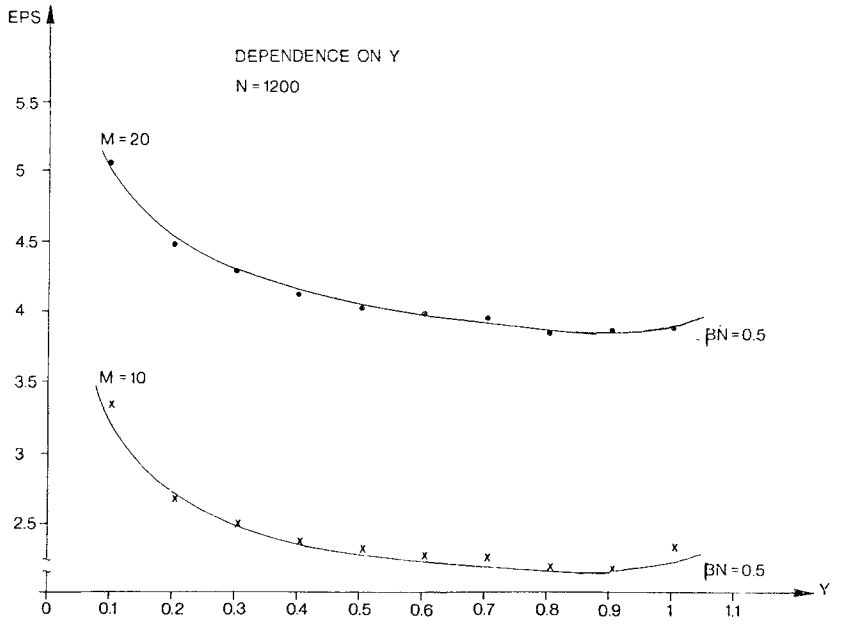

Fig. 2. The ϵ dependence on y shows a broad asymmetry around the minimum. The minimum shifts to smaller y for larger M.

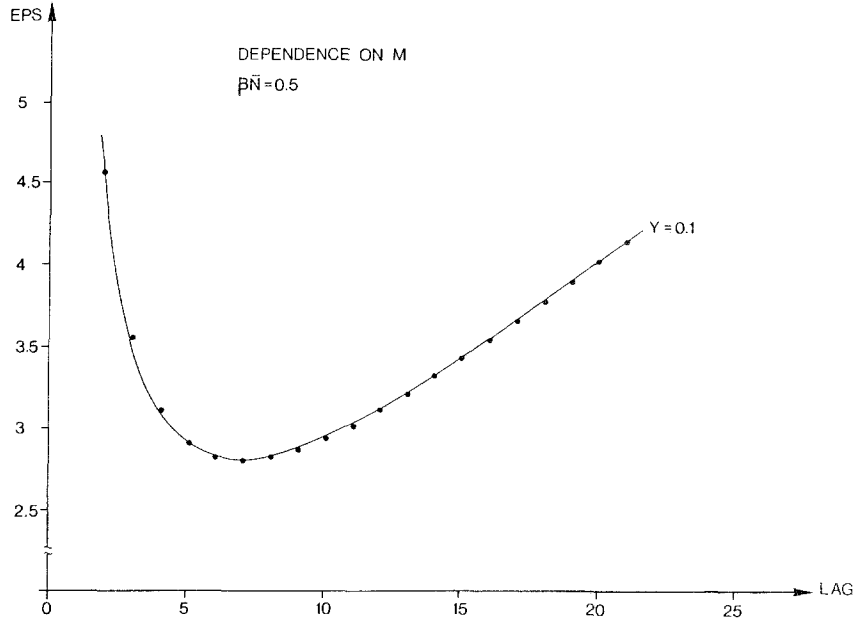

Fig. 3. Plot of ϵ vs M, the maximal autocorrelation lag. Here also the minimum is asymmetric, showing that it is safer to take more channels.

(4) The effect of the sampling window T is coupled to the beam size by $y = 4DT/\omega^2$. Figure 2 shows a rather insensitive minimum for values of $y > 0.1$.

(5) The effect of the maximal autocorrelation lag M is even more dramatically illustrated in Fig. 3. The asymmetry suggests that it is safe to take more channels. One could then cut at a certain point, in order to be as close as possible to the optimal choice of M. Owing to the nature of the linearity assumption, we believe that our results are at least useful in a qualitative fashion. Actually we are performing some experiments on simple diffusing systems.

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