Numerical Simulation of Pile-Up Distorted Time-Correlated Single Photon Counting (TCSPC) Data

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A model, which is based on the binominal distribution, is derived for understanding and investigating the effect of statistical pulse pile-up. The model is applied to constant and exponential decaying sources and is compared with some experimental results.

KEY WORDS: Time-correlated single-photon counting (TCSPC); synthetic data; pulse pile-up; binominal distribution.

INTRODUCTION

The TCSPC method is based on the principle that the deactivation probability of an excited molecule via fluorescence at a time $t_i$ after delta pulse excitation is proportional to the fluorescence intensity at that time. When using a time-to-amplitude converter and a multi-channel analyzer (MCA) for detection, the true probability distribution of the decay is obtained, if the recorded photon is always the only one reaching the photocathode until the next excitation cycle. In any multiphoton event, only the time information of the first arriving photon is recorded and stored in the MCA, which leads to a statistical distortion of the decay histogram. This so-called pulse pile-up effect or type-S pile-up [1] is due to the nature of the method and can be reduced by limiting the start/stop ratio to 1–2% but cannot be eliminated totally. This has, however, the disadvantage of long data collection times, which can cause the problem of introducing systematic errors [2,3].

The precision of kinetic parameters, which are extracted from the raw data, critically depends on the experimental conditions. Of course it is always desirable to obtain a high time resolution and a high number of counts in the peak channel [4,5]. In contrast, the absolute time of data collection should be minimized, because a possible long time drift of the excitation pulse profile could affect the quality of the data, and in reality there will always be a compromise between statistic precision and measuring time. The optimization of decay experiments is especially important if flashlamps with relatively low repetition rates are used and presumes a detailed understanding about the nature of statistical pulse pile-up and the influence on decay curves, which are discussed in the following. Other effects such as detector dead times (type E pile-up) [1] or cross-channel pile-up due to statistical multiplexing [6,7] are of minor importance for standard TCSPC experiments and are not concerned here.

Various methods for the correction of pulse pile-up are known in the literature [8,9]. Coates [10] as well as Davis and King [11] used an equation to correct the raw data. Analytical functions, which are derived from Poisson statistics, are also known for constant, single- and double-exponential functions and can be used to fit the distorted data directly [1,12,13]. These functions have, however, the disadvantage of being time-consuming in
deconvolution procedures. Electronic methods for pile-up discrimination have also been discussed [1,14,15].

The statistical nature of TCSPC data is well defined and therefore it is easy to simulate a desired decay. Such simulation studies are now widely used for testing the possibilities and limitations of decay curve analysis approaches [16-19]. Hence, a simple model for the simulation of pile-up distorted decay curves is derived and applied to constant, single- and double-exponential sources. For the case of a convoluted single-exponential decay, the simulation results are compared with experiments using 9-aminoacridine as fluorophore.

MATERIALS AND METHODS

9-Aminoacridine (9AC) (Sigma Chemical Co.) was used as received. The solvent ethanol (Merck) was of spectroscopic grade, purified by distillation, and dried subsequently to remove traces of water. For deoxygenation, the samples were bubbled for 10 min with high-purity nitrogen and then sealed in 1 x 1-cm cuvettes. The excitation wavelength for all measurements was \( \lambda_{ex} = 381 \) nm. Fluorescence emission was detected at \( \lambda_{em} = 470 \) nm. The optical density of the fluorophore at the excitation wavelength was about 0.3.

Fluorescence decay functions were measured employing the technique of time-correlated single-photon counting as described elsewhere [20]. To take into account a possible long time drift in the excitation pulse profile, lamp and fluorescence data were collected alternately. All measurements were carried out at 293 ± 1 K. The counts were collected into a 512-channel segment of the multichannel analyzer with a time resolution of 3.4 Ch/ns. The total number of counts in the peak channel (CPC) was about 5·10³.

The simulations were carried out on an IBM-compatible personal computer with a 33-MHz 80386 CPU and 80387 coprocessor. The programs are written in GFA-Basic 3.0 (compiler version). The random number generator used was also taken from GFA-Basic 3.0. The randomness was verified by performing different statistical tests (e.g., \( \chi^2 \), runs up/down, serial) [21,22] and the generator was found to be satisfactory for all required applications.

The model functions \( F(t) \) were of an exponential form with \( n = 1,2 \).

\[
F(t) = \sum_{i=1}^{n} A_i \exp \left( -\frac{t}{\tau_i} \right)
\]  

(1)

Due to the fact that the instrument response function \( L(t) \) is not negligibly short compared to the fluorescence function, realistic decay profiles were obtained from a convolution of \( F(t) \) with a synthetic excitation pulse. Here, Eq. (2) was used, giving a pulse shape typical for a hydrogen-filled flashlamp (see Fig. 2).

\[
L(t) = \alpha_1 t^2 \exp( -\beta_1(t + b_1))
+ \alpha_2 [\exp( -\beta_2(t + b_2)) - \exp( -\beta_3(t + b_2))]
\]  

(2)

Since Poisson statistics is generally assumed for TCSPC data, noise was added according to the algorithm of Box-Muller-Marsaglia [21].

SIMULATION OF STATISTICAL PULSE PILE-UP

The pile-up model to be derived here is based on the binomial distribution and calculates the probability function of pile-up distorted data from an arbitrary decay curve using three assumptions.

(i) A number of \( n \) photons is emitted in constant time intervals \( \Delta t \) after an arbitrary excitation pulse. All photons can be distinguished concerning the time of emission \( (N_1 \) is the first, \( N_n \) is the last).

(ii) The photons can move only in discrete paths. The number of those paths is given by the parameter \( b \).

(iii) From all paths \( b \), only a single one is detected. If more than one photon moves in that detection path, only the first one is recorded.

The analogy of items i–iii to the monophoton experiment is as follows: the photocathode area represents a spherical segment around the excited sample. Only a photon which is emitted toward the spacial direction of the photocathode has a chance of being recorded. It is now easy to imagine that a sphere around the sample is covered with photocathodes (= paths \( b \)), but only one of them detects the photons. Moreover, every emitted photon \( N_i \) represents a defined time \( t_i \), which is analogous to a specific channel in the MCA, and thus the counts in path \( i \) are incremented if the event \( N_i \) occurs.

For a source with a constant pulse rate, all photons are emitted with equal probability after delta pulse excitation. Then \( q \) is the probability for a single photon of being emitted via the detection path and is given by \( q = 1/b \). The probability \( p(k) \) to find a number of \( k \) photons in the detection path is defined by the binominal