Iterative correction for ‘pile-up’ in single-photon lifetime measurement

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Abstract

A novel iterative algorithm is proposed to correct for pulse, or photon, ‘pile-up’ in the measurement of temporal intensity profiles using time-correlated single-photon counting (TCSPC). Despite the existence of correction algorithms since 1968, most TCSPC experiments are operated in a very low photon-count mode to avoid pulse pile-up effects in the data. This is inefficient and time-consuming, but is the preferred mode of operation as the current correction algorithms are only applicable under conditions of constant source pulse energy. The advantage of the proposed algorithm is that it can be operated under conditions of variable pulse energy. The effectiveness of the algorithm on simulated data is demonstrated and compared with that of the existing method. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Time-correlated single-photon counting (TCSPC) is a well-established method for measuring intensity temporal profiles. Its principal application is in fluorescence lifetime measurement [1,2], in which the temporal decay of fluorescence following a short excitation pulse is used to identify and quantify fluorescent materials. It has also been used to measure ‘temporal point-spread functions’ (TPSFs) resulting from the scattering of short-duration illumination by turbid media [3]. The technique consists of repeatedly illuminating a sample with a short excitation pulse and logging the elapsed time for the first photon-event to be detected. In the limit that the probability of detecting a photon-event following any one excitation is very small, it may be shown readily that the histogram of the arrival times is proportional to the temporal intensity profile of the emitted light. Although not well recognised, an important reason for the practical success of the technique is that in the low detection limit, this relationship holds even when the excitation, and hence the emitted, pulse energies fluctuate from pulse to pulse. Operation in this low emission probability limit, however, has the disadvantage that the measurement time to accumulate sufficient photon events can be lengthy, especially if a high signal-to-noise ratio is required say to identify multiple
fluorescence decay times. In practice, systems are operated typically such that one photon event is detected for about every 100 excitation pulses (see [2, p. 111]).

Operation at higher emission levels has the obvious advantage of reducing measurement time (or increasing signal-to-noise ratio), but unfortunately it also has two detrimental effects. Firstly, the measured photon event distribution no longer reflects the intensity profile; at higher levels of detection there is an increased chance that the first detected event will occur at an earlier time, this is referred to as ‘pulse pile-up’. Secondly, as is shown below, the variability of excitation/emission pulse energy begins to affect the photon event distribution.

This problem of lengthy experiment times was recognised as early as 1968 when Coates [4] proposed a simple algorithm to correct for pulse pile-up. Despite the existence of this simple correction method, it is not in widespread use; the preferred method being to avoid pulse pile-up by using the low detection probabilities described above. The reasons for this being the accepted modality are often not made clear (see e.g. [2, p. 111]) but an important reason is that the Coates method is only valid when there is no variability in the excitation pulse energies. Variability of pulsed laser pulse energies can be quite pronounced and hence use of higher detection rates and application of the Coates correction can lead to distortions in the recovered profiles.

This communication describes an alternative iterative pulse pile-up correction algorithm. For the special case of no variability in excitation pulse energy, this iterative method yields results indistinguishable from those given by the Coates method. But the iterative method has the advantage that it can be generalised to correct for pulse pile-up in the presence of excitation energy variability, given a measure of the form of the energy distribution.

The mathematical background to recovery of intensity profiles from single-photon-counting data and the origins of pulse pile-up are presented in the next section. In Section 3, the new algorithm is presented and in Section 4 results demonstrating the performance of the algorithm on computer simulated data are presented.

2. Background to pulse pile-up in the presence of pulse energy variability

In fluorescence measurements, TCSPC operates by illuminating, or exciting, a fluorescent sample with a short pulse of light and then recording the time to detect the first emitted photon event. A photon event is defined as the detection of one, or more, photons during a short time interval. The analysis below shows that if the number of photon events per excitation is very low then the distribution of those times reflects the fluorescent intensity decay of the sample.

Let \( I(t) \) be the fluorescent intensity reaching the photo-detector, normalised such that,

\[
\int I(t) \, dt = 1, \tag{1}
\]

and let \( z \) be the average number of photons that would be detected by the detection system per excitation pulse (if it could register all photoelectrons not just the first one). Let the time after the start of the excitation pulse be divided into a large number of small time intervals of width \( \Delta t \) centred at times \( t_i = (i - 0.5)\Delta t \), and let the intensity during the \( i \)th interval be written as \( I_i \), so that the normalisation may be expressed as

\[
\sum_{i=1}^{\infty} I_i \Delta t = 1. \tag{2}
\]

Now the probability, \( P_{1i} \), that a first photon event (i.e. one or more photons detected) occurs during an \( i \)th time interval is given by the product of two probabilities. The first being that zero photons are detected in the intervals 1 to \( i - 1 \) and the second being the probability that one, or more, photons are detected during the \( i \)th interval. Assuming that the intervals are sufficiently small that the intensity is almost constant during each interval, then the product of probabilities may be expressed, using the well-known Poisson photon-counting distribution [5], as

\[
P_{1i} = \exp \left( - \sum_{j=1}^{i-1} S_j \right) (1 - \exp(-S_i)), \tag{3}
\]

where

\[
S_i = z \Delta t I_i. \tag{4}
\]
So the average value of the data $\langle d_i \rangle$ collected during an experiment in which $N$ excitation pulses are used, may be written as

$$\langle d_i \rangle = N P_i$$

$$= N \exp \left( -\sum_{j=1}^{i-1} S_j \right) (1 - \exp(-S_j)). \quad (5)$$

Now consider the low detection limit where $x$ is sufficiently small that the exponentials in the above equations may be approximated as unity plus the exponent, then, the average data are given by

$$\langle d_i \rangle \cong N \left( 1 - \sum_{j=1}^{i-1} S_j \right) S_i, \quad x < 1. \quad (6)$$

On noting the normalisation of Eq. (2) the summation may be rewritten as

$$\sum_{j=1}^{i-1} S_j = x \sum_{j=1}^{i-1} I_j \Delta t \ll x, \quad (7)$$

and so, for the case where $x$ is very much less than unity, the average value of the data is seen to be proportional to the temporal intensity profile, $I(t)$.

$$\langle d_i \rangle \cong N S_i = N x \Delta H, \quad x \ll 1. \quad (8)$$

Fig. 1 illustrates the effect of using different values of $x$. The three solid lines are typical forms of $N S_i$ calculated for $N = 10^6$ and for $x$ values of 0.01, 0.1 and 1. The three sets of symbols are corresponding simulated data $d_i$ calculated using Eq. (5) and corrupted with pseudo-random noise drawn from a Poisson generator. The form of the intensity profile $I(t)$ used is given by the convolution of a double decay curve $G(t)$ of the form given below with $\beta_1 = 0.75, \beta_2 = 0.25, \tau_1 = 1$ ns and $\tau_2 = 5$ ns and a Gaussian instrument response function with a mean of 0.5 ns and a standard deviation of 0.1 ns. The three sets of symbols are corresponding simulated data $d_i$ calculated using Eq. (5) and corrupted with pseudo-random noise drawn from a Poisson generator $[G(t) = \beta_1 \exp(t/\tau_1)/\tau_1 + \beta_2 \exp(t/\tau_2)/\tau_2]$.

Coates [4] proposed a method to invert Eq. (5). The method is very simple and may be expressed as

$$\hat{S}_i = -\ln \left( 1 - d_i / \left( N - \sum_{j=1}^{i-1} d_j \right) \right), \quad (9)$$

where the circumflex denotes an estimator.

Note that implementation of this recovery algorithm requires only the data, $d_i$ and the total number of excitation pulses, $N$, both of which are readily available, and not any knowledge of $x$ which is usually not known accurately. The result of applying this correction algorithm to the data in Fig. 1 is illustrated in Fig. 2. For the low $x$ case where no correction is required the algorithm has a negligible effect on the data, but it is evident for the two larger values of $x$ that the Coates correction algorithm has been successful in correcting the data to reflect more accurately the form of the intensity profile.
Despite the existence of this simple method, it has not been widely applied and the method of choice for dealing with pulse pile-up is to operate at low $\alpha$ values of around 0.01 and to accept the consequence of lengthy measurement times to obtain the required signal-to-noise ratio. One of the main reasons this is done is that there is typically some degree of variability in the excitation pulse energies. Eq. (5) is valid only for the case where the excitation pulses are all of equal energy. Whereas, as is shown below, Eq. (8) is still a good approximation for low $\alpha$ values even when there are variations in the excitation pulse energies.

Assuming that any variation of pulse energies is a random process, a revised form for the expectation of the data values (Eq. (5)) can be obtained by first introducing a unit mean random variable $l$ which represents the variation of the excitation energies about their mean level. Eq. (5) may then be modified by replacing $S_i$ with $lS_i$ multiplying by the probability density function for $l$, $P(l)$, and integrating to yield

$$\langle d_i \rangle' = N \int \exp \left( - \sum_{j=1}^{i-1} \mu S_j \right) \times (1 - \exp(-\mu S_i)) P(\mu) d\mu.$$  \hspace{1cm} (10) 

This may be expressed in a discrete form, using a sampled form of the density function, $P(\mu)$, as

$$\langle d_i \rangle' = N \sum_j \exp \left( - \sum_{j=1}^{i-1} \mu S_j \right) \times (1 - \exp(-\mu S_i)) P(\mu) \Delta \mu.$$  \hspace{1cm} (11) 

So in the case of excitation pulse energy variation, Eqs. (10) or (11) better describe the expected data form than Eq. (5). Now consider the low detection limit where $\alpha$ is sufficiently small that the exponentials in the above equation may be approximated as unity plus the exponent, then, the average data may be approximated by

$$\langle d_i \rangle' \approx N \left\{ \sum_j \mu S_j P(\mu) \Delta \mu \right\} S_i = NS_i,$$  \hspace{1cm} (12) 

Using a similar argument to that used leading to Eq. (8), the summation inside the brackets may be shown to be much less than unity for a small value of $\alpha$ and so the expectation for the data reduces to

$$\langle d_i \rangle' \approx N \left\{ \sum_j \mu P(\mu) \Delta \mu \right\} S_i = N \alpha \Delta I_i, \hspace{0.5cm} \alpha \ll 1.$$  \hspace{1cm} (13) 

The term inside the bracket is simply the average of $\mu$ which is defined above to be unity. So this equation demonstrates that in the limit of a small detection probability, the data are again proportional to the required intensity even in the presence of excitation pulse variation.

Fig. 3 illustrates the effect of using different values of $\alpha$. The three solid lines are as in the previous figures. The three sets of symbols are corresponding simulated data $d_i$ calculated from Eq. (11) corrupted with Poisson noise and for $P(\mu)$ having a Gamma distribution with a mean of unity and a skewness of 1.5.

It is clear for $\alpha = 0.01$, that despite the pulse energy variation the data reflect accurately the form of the intensity profile (as suggested by Eq. (13)), whereas for larger values of $\alpha$ the data (although less noisy) deviate from the intensity profile. The reason for the deviation is as for the previous case but it may be noted that the amount...
Fig. 3. The effect on the expected data of using different values of \( z \) for the case of variable excitation pulse energy is illustrated. The three solid lines are as in Fig. 1. The three sets of symbols are corresponding simulated data \( d_i \) calculated using Eq. (11) and corrupted with pseudo-random noise drawn from a Poisson generator. The variability for this case is described by a Gamma distribution for \( P(\mu) \) with a mean of unity and a skewness of 1.5.

Fig. 4. The effectiveness of applying the Coates correction to the simulated data shown in Fig. 3 i.e. for the case of variable excitation pulse energy is illustrated.

of deviation is slightly greater in this case than for the case of constant excitation energy illustrated in Fig. 1.

Fig. 4 illustrates the results of applying the Coates correction to the simulated data of Fig. 3. As for the correction illustrated in Fig. 2, for the low \( z \) case where no correction is required the algorithm has a negligible effect on the data. However, for the two larger values of \( z \), the Coates correction has yielded a corrected form which is closer to the form of the intensity profile than the uncorrected data but still shows a significant residual deviation. Such deviations can lead to significant errors when fitting to multiple exponential functions, as is commonly required in fluorescent lifetime studies. Fig. 4 thus illustrates the weakness of the Coates method in restoring with sufficient accuracy in the presence of pulse energy variation for \( z \) values above about 0.01. Clearly, the ability of the Coates algorithm to correct will depend on the amount of pulse energy variability. For the case of a Gamma distribution of pulse energies and \( z \) of unity, the Coates method gives a corrected form with a clear deviation from the intensity profile for skewness above about 0.25 (giving a standard deviation of 0.125).

Hence if accurate determination of the intensity profile is required in the presence of pulse energy variability and in short measurement times, there is a need to invert Eq. (11). In the next section an iterative method to achieve this is presented.

3. An iterative correction algorithm

It is instructive to first consider the case where there is no pulse energy variation i.e. the inversion of Eq. (5). The Coates method is an elegant solution to this problem but an alternative method presents itself if Eq. (5) is rewritten in the form

\[
\langle d_i \rangle = NS_i \left[ \exp \left( - \sum_{j=1}^{i-1} S_j \right) (1 - \exp(-S_i))/S_i \right].
\]

(14)

Now for values of \( z \) less than or equal to unity, the terms inside the square bracket will be close to unity. Hence a first-order approximation to \( S_i \) may be written as

\[
S_i^{(1)} = d_i/N.
\]

(15)

Using this first order approximation, higher order approximations may be found based on Eq. (14) by repeated application of
\[ S_i^{(n)} = \frac{d_i}{N} \left[ \exp \left( -\sum_{j=1}^{i-1} S_j^{(n-1)} \right) \right. \]
\[ \times \left. (1 - \exp(-S_i^{(n-1)})/S_i^{(n-1)}) \right], \]
\[ n = 2, 3, 4, \ldots \]  
\[ \text{(16)} \]

Note that like the Coates algorithm, this procedure requires access only to the data and the total number of excitation pulses. Good convergence is generally obtained in about 10 iterations. Application of this iterative procedure to the data of Fig. 1 yields results which are indistinguishable from the recovered profiles yielded by the Coates algorithm and shown in Fig. 2. However, the advantage of this iterative approach is that it may be adapted to provide a means of recovering \( S_i \) from data described by Eq. (11).

Following the approach used to write Eq. (14), Eq. (11) may be re-written in the form
\[ \langle d_i \rangle' = NS_i \sum_l \exp \left( -\sum_{j=1}^{i-1} \mu_j S_j \right) \]
\[ \times \left\{ (1 - \exp(-\mu_i S_i))/\mu_i S_i \right\} \mu_i P(\mu_i) \Delta \mu. \]
\[ \text{(17)} \]

For values of \( \alpha \) less than or equal to unity the exponential term and the term inside the curly brackets will both be close to unity and hence the summation will also be close to unity. Hence the first order \( S_i \) may be approximated by
\[ S_i^{(1)} = \frac{d_i}{N} \]
\[ \text{(18)} \]

and the higher order by
\[ S_i^{(n)} = \frac{d_i}{N} \sum_l \exp \left( -\sum_{j=1}^{i-1} \mu_j S_j^{(n-1)} \right) \]
\[ \times \left\{ (1 - \exp(-\mu_i S_i^{(n-1)}))/\mu_i S_i^{(n-1)} \right\} \]
\[ \times \mu_i P(\mu_i) \Delta \mu, \quad n = 2, 3, 4, \ldots \]
\[ \text{(19)} \]

It should be noted that the implementation of this iteration requires only the data \( d_i \), the number of excitation pulses \( N \), plus the form of \( P(\mu_i) \) which is the distribution of pulse energies (scaled to have a mean of unity) which could be measured with a high light-level detection system taking some fraction of the excitation beam. Importantly, knowledge of \( \alpha \) is not required.

The results of applying the iteration of Eqs. (18) and (19) to the data of Fig. 3 are shown in Fig. 5. Once again for \( \alpha = 0.01 \), where little correction is required, the algorithm has no discernible effect on the data, but for higher \( \alpha \) values the algorithm has clearly produced corrected data which are a much better representation of the intensity profile than that produced by the Coates method.

A more quantitative assessment of the improvement in the validity of the corrected data may be obtained by attempting to recover the decay times and decay amplitudes from the uncorrected and corrected data. Table 1 shows the results of using a simple least-squares fitting procedure to recover these values from the uncorrected data shown in Fig. 3 and the corrected data shown in Figs. (4) and (5). For the uncorrected data the fitting procedure failed for \( \alpha = 1 \). The errors quoted are the standard deviation of results from 10 runs with independent Poissonian noise applied to each simulated data set and hence reflect the statistical variation in the results.

As expected, the statistical variation decreases as \( \alpha \) increases for all the three recovery methods. It may be observed for \( \alpha = 0.01 \), where both correction methods have a negligible effect, that the decay times recovered by the three approaches are all within the statistical uncertainties. But for...
\[ a = 0.1 \text{ and more markedly for } a = 1, \text{ it may be seen that the timescale values recovered via use of the iterative correction values are within the statistical uncertainty of the true values whereas the values recovered from the uncorrected data and using the Coates formula are not. It should be noted that the above results have been obtained using a somewhat idealised Poissonian noise model. Real data may deviate from this ideal model due to detector artefacts such as dead-time.}

### Table 1

<table>
<thead>
<tr>
<th>Decay curve parameters</th>
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<td>( \beta_1 )</td>
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<td>Original values</td>
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<tr>
<td>( a = 0.1 )</td>
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<tr>
<td>( a = 1 )</td>
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<td>( a = 1 )</td>
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Values recovered by fitting to the data corrected using Coates method (as in Fig. 4)

| \( a = 0.1 \) | 0.777 ± 0.02 | 988 ± 0.28 | 0.254 ± 0.016 | 5.05 ± 0.47 |
| \( a = 1 \) | 0.774 ± 0.011 | 997 ± 0.09 | 0.260 ± 0.009 | 4.84 ± 0.26 |
| \( a = 1 \) | 0.776 ± 0.006 | 999 ± 0.08 | 0.260 ± 0.004 | 4.93 ± 0.18 |

Values recovered by fitting to the data corrected using the iterative method of Eqs. (18) and (19) (as in Fig. 5)

4. Conclusion

The failure of current pulse pile-up correction methods to accurately correct data recorded in the presence of pulse energy variability has been described and its effectiveness demonstrated by application to simulated data corrupted with idealised Poissonian noise.

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**References**