

ON-CHIP FLUORESCENCE LIFETIME EXTRACTION USING SYNCHRONOUS GATING SCHEME

Theoretical Error Analysis and Practical Implementation

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Abstract: A synchronous gating technique was proposed for fluorescent photon collecting. The two- and multi-gate rapid lifetime determination (RLD) technique was applied to implement on-chip fluorescence lifetime extraction. Compared with all available iterative least square method (LSM) or maximum likelihood estimation (MLE) based general purpose FLIM analysis software, we offer a method for the direct calculation of lifetime based on the photon counts stored in on-chip memory and deliver faster analysis to enable real-time applications. Theoretical error analysis of the two-gate RLD technique was derived for comparison. The performance of the algorithms were tested on a single-exponential histogram obtained from a CMOS SPAD detector chip using a 468nm laser diode light source with optimized gate width. Moreover, a multi-exponential pipelined RLD FLIM technique was also proposed and tested on a four-exponential decay DNA sample containing a single adenine analogue 2-aminopurine.

1 INTRODUCTION

Fluorescence lifetime measurements have been used widely to study various scientific and practical applications on optics, chemistry, biology, medicine, medical diagnosis. A large number of different techniques including time-domain and frequency-domain methods have been well developed for measuring fluorescence lifetime (Apanasovich and Novikov, 1992). In time-domain methods, the fluorescence intensity decay is measured through a time-correlated single photon-counting (TCSPC) card after excitation with a short pulse of laser light (Cubeddu et al, 2002), whereas in frequency-domain methods, the fluorescent sample is illuminated with a periodic light source to obtain a measured phase difference between the light source and the fluorescent emission. Irrespective of the method used (Jo et al, 2004), the lifetime extraction is done

using computer software. For general purpose time-domain analysis tools for scientific research demanding high accuracy down to the picosecond timescale or for practical medical/clinical diagnostic applications demanding fast results, a wide range of faint multi-exponential fluorophores must be computed with a lifetime resolution better than 50ps (Becker, 2005). Due to the incapability of the LSM or MLE to resolve a small lifetime with a coarse channel width, the number of bits of resolution of TDCs on photon counting cards is therefore expected to be larger than 11-bit (Becker, 2005). To use LSM or MLE properly, the measurement window is usually set as large as possible otherwise the software would treat the measured data as having a DC offset part and therefore the laser pulse repetition rate is kept low, which further lowers the photon collection speed. Data therefore can be gathered in several days. Moreover, because

fluorescence lifetimes in imaging are determined on a pixel-by-pixel basis, iterative methods can be quite time consuming and make real-time image processing almost impossible. Although one can drop the requirement for short laser pulses by using frequency-domain methods, lifetime extraction still relies on software analysis, which also makes real-time image processing difficult to achieve. As process technology advances, integration of high speed laser drivers and laser diodes on chip is becoming feasible.

2 THEORETICAL ERROR ANALYSIS

The recorded fluorescence intensity $f(t)$ is related to the true decay function $I(t)$ through the integral

$$f(t) = \int_0^t I(t-\tau)IRF(\tau) d\tau \quad (1)$$

where $IRF(t)$ is the instrumental response function, or the convolution of transition spread of the detector and the pulse function of the laser source. The true response $I(t)$ could be obtained through an on-chip digital de-convolution calculation. However, we need to evaluate whether the enhanced precision can justify the cost of the extra chip area for digital de-convolution. Here we assume $I(t) = A\exp(-t/\tau)$, and the ratio of the full width half maximum (FWHM) of $IRF(t)$ over the lifetime is denoted as r . The recorded response $f(t)$ is obtained from (1). As r is larger than 1, it is difficult to obtain a clear response because of the effects of noise and it is inefficient to accumulate enough photon counts for a certain SNR criteria. The smaller the ratio r , the more efficiently and accurately the lifetime can be extracted. Considering the 10ps jitter in the light source, the 80ps transition spread of our SPAD structure, and the 30ps jitter of gate transitions, the overall FWHM is about 100ps. Thus, without on-chip de-convolution function, the smallest lifetime that can be obtained is of the order of 200ps. For first time implementation, we simplify by using longer-lifetime samples as test cases. The assumption of $f(t)$ as a single exponential is quite reasonable. In this paper, we applied the RLD method for simplicity.

2.1 Theory

The simplest way of calculating fluorescence lifetime is to use the RLD technique with two consecutive gates (Ballew and Demas, 1989) called

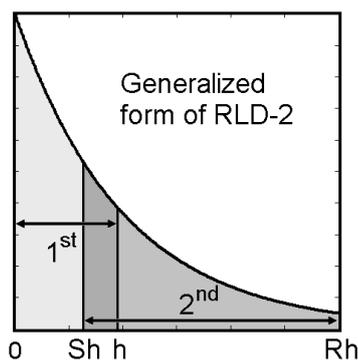


Figure 1: Generalized form of two-gate RLD.

standard RLD. Unlike the LSM or MLE based methods, it is a direct calculation method. The disadvantage of standard RLD is its high sensitivity to the gate width selection. This can be explained by reasoning that when dealing with a short lifetime, the photons are mostly located in the first gate, and the relatively low counts in the second gate becomes the major source of error. To overcome this problem, a gate overlap approach was introduced to the standard RLD (Sharman and Periasamy, 1999) trying to offer greater insensitivity to the Poisson noise in the second gate. This method did offer better resolvability for a range of short lifetimes, but it sacrificed precision for the longer lifetimes. Another approach called SWRLD is proposed (Chan et al, 200) using a square wave driven LED as a light source. SWRLD offers uniform high precision in a much wider range of gate width. However, this method does not easily extract lifetimes shorter than 1ns because the 1ns edge speed of the fastest available LED dictates the minimum lifetime extraction limit, Thus an example of a long lifetime of 2ms has been chosen as an illustration. The second challenge is that SWRLD needs many filters to separate fluorophore emission from scattered laser emission (the IRF). Beyond these limits, SWRLD is indeed a precise method for long lifetime extraction ($\gg 10$ ns). A better approach to achieve better precision for long lifetimes is make the second gate wider than the first and therefore tolerate much higher counts (Moore et al, 2004). This method, however, needs an iteration method to do lifetime extraction. Plus for on-chip implementation, asymmetric gates require the generation of two synchronized clocks with different pulse widths and thus increase the circuit complexity. The best theoretical solution is not necessarily the right one in terms of cost and feasibility. All the amended algorithms mentioned require Monte Carlo to do error analysis. We derive a generalized formula here

for calculating the standard deviation of lifetimes much more conveniently and therefore facilitate location of the optimized lifetime region or measurement window. Figure 1 shows the generalized form of two-gate RLD. The counts N_1 and N_2 in the two gates are related as

$$g(x) = N_2(1-x) - N_1(x^S - x^R) = 0, \quad (2)$$

where $x = \exp(-h/\tau)$ and

$$\begin{aligned} N_1 &= N_c(1-x)/(1-x^R), \sigma N_1 = \sqrt{N_1}, \\ N_2 &= N_c(x^S - x^R)/(1-x^R), \sigma N_2 = \sqrt{N_2}. \end{aligned} \quad (3)$$

with σN_1 and σN_2 being the standard deviations in N_1 and N_2 , respectively for Poisson noise and N_c the total count number. Together with (2), we have

$$\sigma g(x) = \sigma x \cdot |g'(x)|, \quad (4)$$

$$\sigma g = \sqrt{\sigma N_2^2(1-x)^2 + \sigma N_1^2(x^S - x^R)^2}, \quad (5)$$

$$|g'(x)| = N_2 + N_1(Sx^{S-1} - Rx^{R-1}), \quad (6)$$

$$\text{and } \sigma x/x = h\sigma\tau/\tau^2. \quad (7)$$

From (2) to (7), we could obtain

$$\frac{\sigma\tau}{\tau} = \frac{\tau h^{-1} \sqrt{(1-x^R)k(x)} / \sqrt{N_c}}{\left[(S-1)x^{S+1} - (R-1)x^{R+1} - Sx^S + Rx^R \right]} \quad (8)$$

$$k(x) = (1-x)(x^S - x^R)^2 + (1-x)^2(x^S - x^R).$$

2.2 Comparison of RLD-2s and RLD-N

To demonstrate the ability of different RLD schemes, we fix the measurement window (MW). First we compute the standard deviation over the lifetime $\text{SNR} = \sigma\tau/\tau$ in dB. Figure 2 shows the SNR in the range of τ/MW within 0.05 and 1, and gate number N within 2 and 128 under total counts of 2^{17} . It shows the SNR plot converges as $N > 8$ and RLD-2 shows the best resolvability for small lifetime region, but both RLD-2 and RLD-N could not resolve those less than 0.1. It means that with a laser source of repetition rate of 100MHz, they could not resolve those less than 1ns. Moreover, the complexity of implementing N -gate RLD on chip is too large. In terms of implementation, the RLD-2 is much easier than RLD-N. Figure 3 shows a comparison plot of lifetime SNR versus lifetime normalized by measurement window (MW) for theoretical equation (8), equation for the multi-gate scheme not shown here, Monte-Carlo RLD methods and the maximum

likelihood estimator (MLE) (Kollner and Wolfrum, 1992).

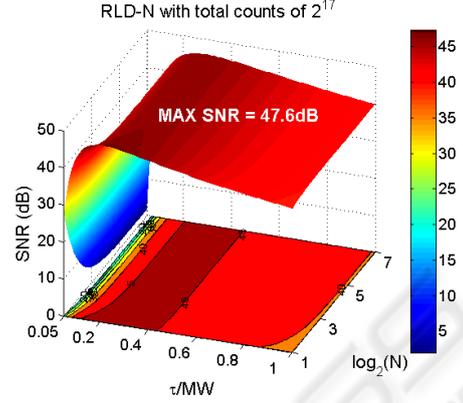


Figure 2: SNR plot for RLD-N under total counts of 2^{17} .

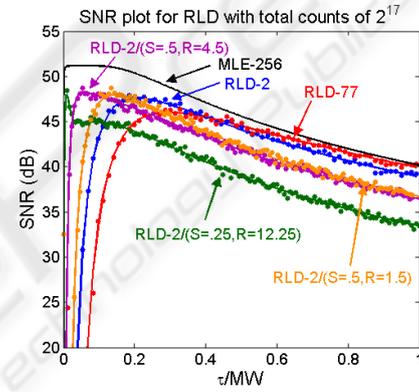


Figure 3: SNR plot for RLD-N with total counts of 2^{17} .

This plot shows the range of lifetime resolvable by each extraction method for a certain laser repetition rate. The results obtained by theoretical equations are marked as solid lines whereas those by Monte-Carlo methods are indicated by dots. They match well. We also compare the MLE results and find that the peak value of the RLD-2 occurs at $\tau/\text{MW} = \tau/(2h) \sim 0.2$ which is coincident with previously reported literature. The plot shows the RLD-77 has best precision and closest to that of MLE only in high lifetime region, while it has the worst resolvability for low lifetime region. Un-equal gate scheme ($S = 0.5$, $R = 4.5$) offers better resolvability than the equal gate-width one ($S = 0.5$, $R = 1.5$). The one with $S = 0.25$ and $R = 12.25$ suggested by (Moore et al, 2004) offers the best resolvability for small lifetime region but sacrifices some precision in the high lifetime region. In terms of implementation complexity, feasibility, and limitations on chip, the equal gate width scheme is the simplest. Of course, if we insist on implementing an un-equal one given

that we have conquered the problem of transition spread of the IRF, we could build an on-chip look-up table to simplify lifetime extraction. For first time on-chip implementation, we simplify by using the equal gate and non-overlap scheme. The overlap scheme will be implemented in the future. Table 1 lists the summary of RLD schemes. Except the RLD- N , the others are all possible candidates.

Table 1: Comparison and summary of RLD schemes.

	Closed Form	$\tau/MW < 0.1$ Resolvability	On-chip Feasibility
Standard RLD-2	Yes	No	Yes/Look-up Table
Standard RLD- N ($N > 2$)	Yes	No	No
Overlap RLD-2 ($R = 1+S$)	Yes	Yes	Yes/Look-up Table
Overlap RLD-2 ($R \neq 1+S$)	No	Yes	Yes/Look-up Table

2.3 Synchronous Gating Scheme

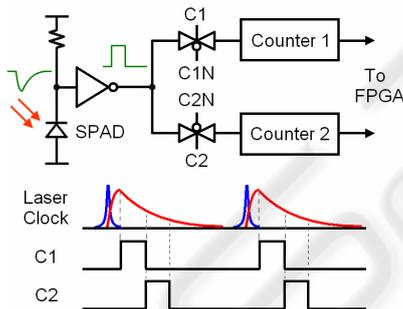


Figure 4: Timing diagram for synchronous gating technique.

Figure 4 shows the block diagram for the photon counting process. The fluorescence emission is detected by a SPAD detector, and the detected signal is converted into a digital one by a comparator and then sent into two synchronous counters controlled by clocks C1 and C2, respectively. And the photon counts on counters 1 and 2 are sent to a FPGA for post processing.

2.4 Pipelined RLD-2 for Multi-Decays

The above analysis is based on the assumption that the fluorescence emission follows a single-lifetime function. When trying to resolve multi-lifetime

fluorescence decay, we need a simple algorithm. Figure 5 shows an algorithm for lifetime extraction in a two-lifetime fluorescence histogram similar to the concept of pipelined analog-to-digital converters, called pipelined RLD-2 (PL-RLD-2). The lifetime extraction procedure uses RLD-2 to extract the larger lifetime and intensity with the first memory, and subtraction of the extracted extrapolation function from the photon counts stored in the second memory to obtain the second lifetime and intensity. Pipelined algorithms for higher (> 2) decays can follow this procedure until the last lifetime is finally calculated.

3 EXPERIMENTAL RESULTS

3.1 Single-decay

The chip including a 4×16 SPAD array and digital readout circuits was implemented on $0.35 \mu\text{m}$ high voltage CMOS process. The die had the polyimide passivation removed providing around 3-5x increase in photon detection probability in the 500nm range. Each pixel contains a single $15 \mu\text{m}$ -diameter CMOS SPAD (Niclass, 2006).

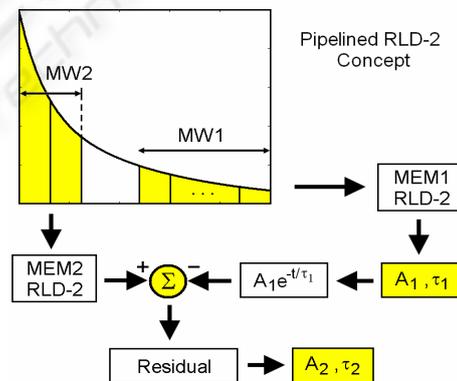


Figure 5: Block diagram of pipelined RLD-2.

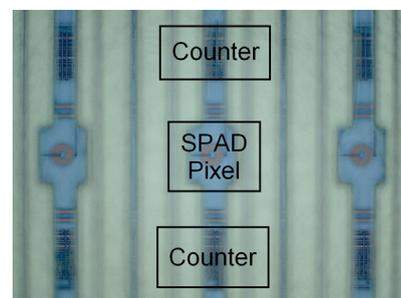


Figure 6: SPAD pixel and two ripple counters.

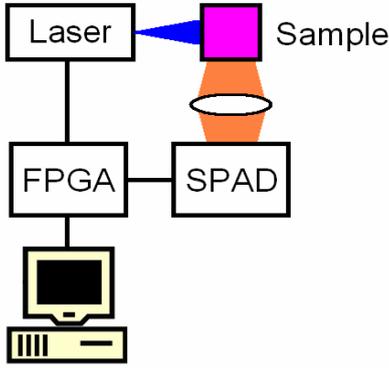


Figure 7: SPAD pixel and two ripple counters.

Figure 6 shows a SPAD pixel with two ripple counters up and down. The gating width could be adjusted over a 48ns range with a 408ps resolution. The imager is controlled by a FPGA and photon count histograms are captured and displayed on a PC. The measurement setup is shown in Figure 7. It consists of a laser diode emitting 88ps pulses at 468nm, 5mW average power, synchronized to the system clock. Without using any photon counting card, the photon emitted is converted into a digital signal and processed by on-chip ripple counters in Figure 4. The fluorophore sample is 1 micro-molar Rhodamine B. Table 2 shows the extracted lifetime using the RLD-2 and the LSM based software. The difference between them is about 7%. Jitter performance of the synchronous gate might contribute some error, because a phase-locked loop PLL has not been integrated to minimize the jitter.

Table 2: Comparison of lifetimes extracted by RLD-2 and software.

Sample	RLD-2	Software
Rhodamine B	2.33ns	2.175ns

3.2 Multiple-decays

The second example is used to test the proposed pipelined RLD-2 algorithm. This data set comes from the fluorophore 2-aminopurine (2AP) inside a singly-labelled 14 base-pair DNA duplex and was measured in an Edinburgh Instruments spectrometer equipped with TCC900 photon counting electronics (Neely et. al, 2005). The excitation source was a Ti-Sapphire femtosecond laser system producing pulses of ~200fs at 76MHz repetition rate. The output of the laser was passed through a pulse picker to reduce the repetition rate to 4.75MHz and then frequency tripled to give an output at 320nm. The emission from the sample was collected orthogonal to the

excitation direction through a polarizer. The fluorescence was passed through a monochromator, and detected by a Hamamatsu PMT (R3809U-50). The instrument response was 50ps FWHM. Fluorescence decay curves were recorded at emission wavelength of 390nm on a timescale of 50ns, resolved into 4096 channels, to a total 10,000 counts in the peak channel. Decay curves were analyzed using the proposed PL-RLD-2 and using the F900 software with standard iterative reconvolution method, assuming a multi-exponential decay function in the following equation.

$$I(t) = \sum_{i=1}^4 A_i \exp\left(-\frac{t}{\tau_i}\right), \quad (9)$$

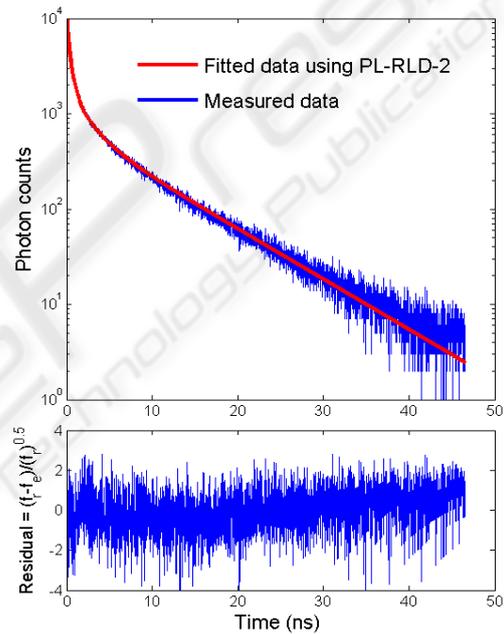


Figure 8: Fitted data and residual using PL-RLD-2.

Table 3: Comparison of lifetimes (ns) and fractional amplitudes (%) extracted by PL-RLD-2 and F900 software.

$\tau_i(\text{ns})/A_i(\%)$	PL-RLD-2	F900 Software
τ_1/A_1	0.136/27	0.14/47
τ_2/A_2	0.481/55	0.47/39
τ_3/A_3	2.179/11	2.19/9
τ_4/A_4	8.225/7	8.15/5

where A_i is the fractional amplitude and τ_i is the fluorescence lifetime of the i -th decay component. Figure 8 shows the logarithmic plot for the measured photon counts starting from the channel with peak counts 10,000 and the fitted data using the proposed PL-RLD-2. The residual plot reveals that the proposed method fits well with the experimental data. The extracted lifetimes and fractional

amplitudes using the PL-RLD-2 and the F900 software are listed in Table 3. The Table shows the extracted lifetimes differ within 4% whereas the amplitudes differ in a significant range. That is why recent literature (Philip, 2003) suggests that fluorescence lifetime measurements offer better precision. These results highlight the potential of RL-RLD-2 for on-chip multiple exponential lifetime extraction, if adaptive gating width technique could also be introduced on-chip.

4 CONCLUSIONS

On-chip fluorescence lifetime extraction including a SPAD array and digital readout circuitry is for the first time implemented on 0.35 μ m CMOS process using the two-gate RLD. Theoretical error equations for several RLD-2/RLD- N schemes were derived and compared to determine a possible implementation strategy. To implement RLD-2, a non-overlap synchronous gating is applied for photon counting. The first on-chip attempt is mainly focused on dealing with single-exponential fluorescence emission, and the extracted result matches with the true value well within 10% including possible contribution from gating jitter. For possible future on-chip implementation for multi-exponential fluorescence lifetime extraction, we proposed a pipelined RLD-2 (PL-RLD-2) and we test this method on a four-exponential experimental data, and the extracted lifetimes match well with those obtained by iteration based software within 4%.

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DISCLAIMER

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