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Fast and robust single-exponential decay recovery from noisy fluorescence lifetime imaging

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Abstract—Fluorescence lifetime imaging microscopy and nanoscopy are valuable techniques for lifetime sensing as an intrinsic characteristic of biological and chemical objects. However, optical photon-count measurements for lifetime estimation are affected by inevitable effects of blurring and noise, leading to degradation of the lifetime accuracy and precision. To tackle the problem, this paper presents a robust and computationally efficient framework for recovering fluorophore sample decay from the histogram of photon-count arrivals modelled as a decaying single-exponential function. In the proposed approach, the temporal histogram data is first decomposed into multiple bins via an adaptive multi-bin signal representation. Then, at each level of the multi-resolution temporal space, decay information including both the amplitude and the lifetime of a single-exponential function is rapidly decoded based on a novel statistical estimator. Ultimately, a game-theoretic model consisting of two players in an “amplitude-lifetime” game is constructed to be able to robustly recover optimal fluorescence decay signal from a set of fused multi-bin estimates. In addition to theoretical demonstrations, the efficiency of the proposed framework is experimentally shown on both synthesised and real data in different imaging circumstances. On a challenging low photon-count regime, our approach achieves about 28% improvement in percent bias than the best competing method.

Index Terms—Adaptive filtering, binning, fluorescence lifetime imaging microscopy, game theory, lifetime estimation, multi-resolution representation, rapid lifetime determination, signal recovery, single-exponential decay.

I. INTRODUCTION

TIME-DOMAIN fluorescence lifetime imaging microscopy (FLIM) and nanoscopy are powerful signal acquisition techniques to characterise biological and chemical objects such as cells, viruses and molecules at micro- and nano-scales. FLIM has diverse applications in fields such as biology, chemistry, physics, medicine, pharmacology, and cancer research [1–4]. In FLIM, a location in a specimen is first excited by a pulsed laser. Absorption of excitation photons can lead to fluorescence, where electrons moved to an excited state return to the ground state resulting in photon emission. The elapsed time from excitation to detection of the

emitted photon associated with the atomic transition from the excited state to the ground state is recorded as a time-stamped event via a sensitive sensor. For a predefined exposure time extending over multiple laser pulses, the measurement cycle is repeated many times. Photon arrival times in a number of quantised time intervals are then counted. Finally, the whole process generates a raw data sequence consisting of a histogram of photon-count arrivals representing a decay function for a pixel of the imaged sample. The data sequence contains noise originating from various sources such as dark counts and shot noise. On-chip histogramming has been reported in a single photon avalanche diode line sensor which permits very high throughput of fluorescence lifetime signals [5]. The histogram decay function can be modelled by a single-, double- or generally multi-exponential, where the fluorescence lifetime, as an intrinsic signature of the imaged object, is defined as the average time that matter stays in the excited state before settling to the ground state. The model of single-exponential decay is widely used across FLIM applications due to its simplicity and utilisability for high-speed imaging. In the literature, different methods have been proposed for estimating decay parameters including the fluorescence lifetime [6–15]. By focusing on both speed and robustness issues for lifetime sensing in a wide range of photon-count regimes, we also make our own contributions to the field, as will be discussed in this section.

A. Review of related arts

We categorise state-of-the-art available lifetime estimation methods into three general families consisting of fitting-based, non-fitting-based, and fit-free approaches. The fitting-based family tries to fit a curve to the histogram of photon counts. Here, the least squares (LS) method is generally employed to estimate parameters of a fluorescence decay model [6]. Maximum likelihood estimation (MLE) is another approach in this category that can account for a more realistic Poisson distribution for the histogram of photon counts, instead of the Gaussian assumption behind the LS fit [7].

In contrast to the direct fitting formulation and solution, non-fitting-based algorithms use specific techniques for finding fluorescence lifetime imaging characteristics. These include rapid lifetime determination (RLD) [8], RLD with overlapping windows (RLD-OW) [9], center of mass method (CMM) [10], and fluorescence lifetime estimation via rotational invariance techniques (FLERIT) [11] are all paradigms of non-fitting-based approaches. The methods available in the RLD class present closed-form solutions for estimating the lifetime parameter [8, 9, 16]. CMM uses the center of mass of the

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single-exponential distribution to derive a simplified lifetime calculation formula suitable for on-board clinical firmware applications. FLERIT models the problem of lifetime estimation into a general class of direction of arrival estimation in array signal processing based on singular value decomposition [17].

Fit-free mechanisms are the third group of lifetime estimation methods. As two well-known approaches in this family, the phasor approach represents the fluorescence lifetime information in a diagram-wise manner [12], and learning-based methods exploit function approximation capability of shallow and deep neural networks for lifetime parameter estimation based on raw decay training samples [13, 14]. In practical FLIM system, lifetime estimation performance and its computation time are two key components. Each of the lifetime calculation families have their own benefits and shortcomings for a compromise between performance and complexity [3].

B. Current challenges

Fitting-based methods are accurate to some extent but are computationally complex. Non-fitting-based algorithms, as addressed in this paper, are fast and provide an apt option for hardware realisation [18]; however, dealing with a range of uncertainties available in the acquired signal simultaneously remains a difficult task. Fit-free phasor and learning-based approaches suffer respectively from a need for intervention of experts for lifetime analysis, and a requirement for a large number of training time series instances. Moreover, non-automatic implementation and the lack of data present two main challenges for medicine-related researchers.

We can fundamentally summarise that the performance of a lifetime estimation approach is affected by three influential dynamics which originate from: 1) the nature of the sample being imaged; 2) the quality of the imaging instrument; and 3) the user experience on tuning acquisition parameters. All the dynamics appear in the captured histogram of photon counts in different forms, making lifetime determination a challenging task [19]. To name a few, low, mid, and high photon counting lead to low-, mid- and high-level signal-to-noise ratio (SNR) acquisitions to be managed [20, 21]. Due to limiting power of light source for preventing damage to sensitive specimens, low photon-count imaging is often observed which leads to amplification of noise components. Blurring effects due to convolving the original decay signal with the instrument response function (IRF) of the imaging device deform the head and tail of a decaying function, which may results in overestimation of the lifetime [15]. Different fluorophores expose varying lifetimes from short to long ranges. User tunable parameters during imaging such as bin width or size of the histogram of photon arrivals for exposure time control also influence the decay curve shape and ultimately the system's accuracy and precision [11]. These factors demand a robust lifetime estimation approach coping with the various regimes, and this is the goal of the method proposed in this paper.

C. Research contributions

To take account of the variability in fluorescence lifetime acquisition, we suggest a corruption-robust lifetime estimation

framework. We first mathematically model the fluorescence decay function in both deterministic and stochastic modes. Afterwards, we propose a fast algorithm to be able to estimate amplitude and lifetime parameters of a single-exponential decay function in the presence of both inevitable blurring and noise corruption. To control the uncertainties existing in a decay signal, we decompose the temporal histogram data as an adaptive multi-bin signal representation. The decomposition is done via a set of adjustable, successive Savitzky-Golay (S-G) low-pass filtering and binning. At each level, decay parameters are estimated from the transformed signals. We formulate the problem of reliable detection of the best representative signal from a set of candidates determined from multiple temporal resolutions using a 2-player game model. Amplitude and lifetime as players act on estimated parameters based on their own payoff functions to be able to recover optimal decay. Our approach falls into the category of non-fitting-based approaches, where the decaying signal can be rapidly and robustly recovered for different regimes. Figure 1 illustrates the suggested method on an exemplar histogram of photon count arrivals. The robustness of our single-exponential decay recovery method at high level of noise is also shown in Fig. 2.

In summary, our main scientific contributions are:

- a fast algorithm to decode lifetime information from the histogram of photon counts modelled by a single-exponential decaying function;
- an adaptive multi-bin representation of histograms of photon counts to deal with uncertainties of decay variability;
- a game-theoretic model to permit robust recovery of single-exponential decay from a set of signal selection.

D. Paper organisation

The remainder of the paper is organised as follows. In Section II, we present our theoretical analysis behind the lifetime estimation problem, and provide details of the proposed algorithms for single-exponential decay recovery in Section III. In Section IV, a set of coherent experiments for demonstrating the efficiency of our suggested method is described. Section V provides an analysis of the computational complexity of the algorithms. Finally, conclusions are presented in Section VI.

II. THEORY

In this section, we model the decay signal of fluorescence lifetime both deterministically and stochastically. A derivation of FLIM parameter estimation is presented for each case.

A. Deterministic decay model and estimation

In a FLIM system, the fluorescence decay function is ideally modelled by a first-order linear differential equation as:

$$\frac{dx(t)}{dt} = -(k_r + k_n)x(t), \quad (1)$$

where $x(t)$, k_r and k_n denote the quantity at time t , the rate constant of a radiative process, and the cumulative rate constant of an environment-dependent non-radiative process, respectively [7, 22]. Using the separation of variables method

Fig. 1: The proposed multi-resolution fluorescence decay recovery method unfolded and visualised for an exemplar histogram of photon counts. S-G stands for Savitzky-Golay; L_{f_i} denotes window length of the S-G filter at the i^{th} tunable branch; and the parameters A , τ , and M_i represents amplitude and lifetime of the exponential decay, and the number of time bins after binning at the i^{th} level, respectively. The functions $g_i[n]$ and $y_i[m]$ are output signals of S-G filtering and binning at the i^{th} level, respectively. The blue star in the game space denotes the point of Nash equilibrium.

Fig. 2: An illustration of robustness of the proposed single-exponential decay recovery under severe corruption.

on (1) gives the solution $x(t) = Ae^{-\frac{t}{\tau}}$, where $A \triangleq x(0)$ is the initial amplitude and $\tau \triangleq \frac{1}{(k_r + k_n)}$ is the fluorescence lifetime.

In practice, the fluorescence decay is measured at equally-spaced time intervals rather than a given time. In this case, a discrete-time representation of the exponentially decaying continuous-time function with unquantised intensities as

$x[n] \in \mathbb{R}^+$ follows from:

$$x[n] = Ae^{-\frac{\Delta \cdot n}{\tau}}, \forall n = 0, 1, \dots, N-1, \quad (2)$$

where Δ , N and $\mathbb{R}^+$ represent the measurement time interval (in order ranging from picosecond to nanosecond for FLIM devices), the number of measurements, and the set of all positive real numbers, respectively. It is obvious that $A = x[0]$. The function $x[n]$ is a strictly monotonic decreasing function with the descending sort property: $x[0] > x[1] > \dots > x[N-1]$. For a detailed discrete-time signal analysis, we provide standard definitions below to make this paper more accessible to a wider audience.

Definition 1 (Linear difference operator): The linear difference operator $\mathcal{D}\{\cdot\}$ is defined as:

$$d[n] \triangleq \mathcal{D}\{x[n]\} = x[n] - x[n+1], \forall n = 0, 1, \dots, N-1. \quad (3)$$

The descending sort property is held for $d[n]$, where $d[0] > d[1] > \dots > d[N-1]$.

Definition 2 (Maximum fall of signal): We define the point with maximum difference, or maximum fall, of the signal by:

$$n^* = \operatorname{argmax}_{0 \leq n < N} (d[n]). \quad (4)$$

It is obvious that always $n^* = 0$ in the ideal corruption-free case. This means the greatest difference is between the first sample and the second one.

Theorem 1 (Lifetime decoding): Let $x[n] = Ae^{-\frac{\Delta \cdot n}{\tau}}, \forall n = 0, 1, \dots, N-1$ be a histogram of photon-count arrivals. The slope tangent to the point with maximum fall of $x[n]$, i.e. n^* in (4), conveys lifetime information of decay. Geometrically interpreting, for the point n^* , its initial slope just tangent to the exponentially decaying curve intercepts the n -axis at a point near $n \approx \tau$, where the estimated lifetime is $\hat{\tau} = \frac{\Delta}{\ln(\frac{x[0]}{x[1]})}$.

Proof: By using a first-order approximation of the Taylor expansion around the point with maximum difference, the tangent slope can be captured by an appropriate precision, which finally decodes the fluorescence lifetime value. The Taylor expansion of $x[n]$ around the point n^* gives:

$$\hat{x}[n^* + T] = x[n^*] + Tx'[n^*] + \varepsilon, \quad (5)$$

where $x'[n^*] = \frac{x[n^*+T]-x[n^*]}{T}$ represents the first-order difference. Neglecting the higher order terms by setting $\varepsilon \approx 0$, the first-order approximation of $x[n]$ is reached. By substituting $x'[n^*]$ into (5), taking the discrete interval $T = 1$ and reducing to the Maclaurin series for $n^* = 0$ in the ideal model, we have:

$$\hat{x}[1] = x[0]e^{-\frac{\Delta}{\tau}}. \quad (6)$$

If the approximation $\hat{x}[1]$ is the same as the true value $x[1]$, taking the natural logarithm from two sides of (6) will yield:

$$\hat{\tau} = \frac{\Delta}{\ln(\frac{x[0]}{x[1]})}. \quad (7)$$

For the special case $N = 2$, (7) is equal to that of RLD.

B. Stochastic noisy-blurred decay model and estimation

1) *Corrupted measurements modelling:* In real FLIM systems, the signal (2) is a discrete stochastic process, where both A and τ are random variables. Let us model the histogram of photon-count arrivals in the presence of both blur and noise corruption in a pixel as the following quantised measurements:

$$\tilde{x}[n] = \lfloor x[n] * h[n] + \eta[n] \rfloor u[\lfloor x[n] * h[n] + \eta[n] \rfloor], \quad (8)$$

where $\tilde{x}[n] \in \mathbb{Z}^+, \forall n = 0, 1, \dots, N-1$. Operators $\lfloor \cdot \rfloor$, $u[\cdot]$ and $*$ denote the round and step functions and convolution, respectively. The step function in (8) ensures that the photon-count $\tilde{x}[n]$ takes on physical positive counts only. The functions $h[n]$ and $\eta[n] \triangleq \eta_P[n] + \eta_G[n]$ represent the blurring kernel as the IRF of the system, and the noise term, respectively. The noise components $\eta_P[n] \in \mathbb{Z}^+$ and $\eta_G[n] \in \mathbb{R}$ represent, respectively, the signal-dependent shot noise arising from photon sensing, and the signal-independent Gaussian noise due to other background stationary disturbances [23]. The characteristic of shot noise distribution is $\eta_P[n] \sim \mathcal{P}(\lambda)$, where $\mathcal{P}(\cdot)$ denotes Poissonian distribution. Since $\text{SNR} \propto \sqrt{I}$, where I represents the average number of arrived photons, shot noise practically increases for bins with a small number of photons. This means noise effects are higher in bins with lower photon counts such as the tail of a fluorescence

decay. Hence, the average rate of noise dependent on the original signal $x[n]$ is modelled as $\lambda = \left\lceil a \frac{x[0]}{x[n]} \right\rceil \in (0, \infty)$ in simulations. The coefficient a represents a constant and $\lceil \cdot \rceil$ is the ceil function. For the signal-independent noise, it is assumed $\eta_G[n] \sim \mathcal{N}(0, \sigma^2)$, namely a Gaussian distribution with the mean 0 and variance σ^2 .

To emphasise potential error resources in the system, we rewrite (8) as $\tilde{x}[n] = x[n] + e[n]$ with the error $e[n] = e_q[n] + e_b[n] + e_P[n] + e_G[n]$, where terms $e_q[n]$, $e_b[n]$, $e_P[n]$ and $e_G[n]$ denote errors introduced by quantisation, blurring, Poissonian and Gaussian noises, respectively. It is notable that the quantisation noise originates from rounding amplitudes, due to the quantised nature of light in photon counting process. Based on the central limit theorem, we can consider the distribution of the error term as i.i.d. $e[n] \sim \mathcal{N}(0, \sigma_e^2)$.

2) *Estimation under corruption:* From the corrupted measurements model, it is known that an efficient minimum variance unbiased estimator (MVUE) [24] does not exist for estimating τ . However, the variance of the estimator based on the Cramér-Rao Lower Bound (CRLB) is given by:

$$\text{var}(\hat{\tau}) \geq \frac{\sigma_e^2 \tau^4}{A^2 \Delta^2 \sum_{n=0}^{N-1} n^2 e^{-\frac{2\Delta \cdot n}{\tau}}}, \quad (9)$$

where the derivation details are provided in the Appendix. However, a MVUE does exist for estimating the amplitude given the true τ , and is given by:

$$\hat{A} = \frac{1 - e^{-\frac{2\Delta}{\tau}}}{1 - e^{-\frac{2\Delta \cdot N}{\tau}}} \sum_{n=0}^{N-1} e^{-\frac{\Delta \cdot n}{\tau}} \tilde{x}[n], \quad (10)$$

with CRLB of:

$$\text{var}(\hat{A}) \geq \frac{\left(1 - e^{-\frac{2\Delta}{\tau}}\right) \sigma_e^2}{1 - e^{-\frac{2\Delta \cdot N}{\tau}}}. \quad (11)$$

We refer the reader to a similar derivation of this in the Appendix. Despite the lack of existence of a MVUE for τ , the theory still inspires the design of a novel estimator for the lifetime and the histogram amplitude, as described below.

It is important to note that the point corresponding to maximum difference does not always occur at zero and its location may generally be a function of a number of parameters such as the IRF, the pulse amplitude and duration of the lighting source, response speed of fluorescence to the excitation pulse, the time bin width, photon detector speed, and any corruption. Therefore, a little displacement to the right may occur. Nevertheless, the descending sort property still holds to some extent after a point m^* , and the lifetime estimation problem can be reformulated by a Taylor expansion around this arbitrary point, $m^* \geq 0$. Consider the M -point signal of $y[m], \forall m, M \leq N$ as a transformed version, including low-pass filtering and dimensionality reduction, of corrupted measurements $\tilde{x}[n]$ (See (18) for a complete definition of the transformation.). By defining the parameters $r_0 \triangleq y[0]$, $r_1 \triangleq y[m^*+1]$, the ratio $r \triangleq \frac{r_0}{r_1}$, and taking natural logarithms from the first-order Taylor expansion equation, the lifetime is estimated as:

$$\hat{\tau} = \frac{\Delta \cdot (m^* + 1)}{\ln(r)}. \quad (12)$$

Even in noisy measurements, points in the vicinity of m^* are somewhat robust to shot noise, because starting points from the falling signal have inherently stronger intensities than other points in the tail of the photon-counts histogram.

However, instead of selecting a specific single point from $y[m]$ for obtaining each of the parameters r_0 and r_1 , then inspired by the CRLB analysis in the Appendix, we propose an estimator to suppress these noise effects. To do this, we employed two left and right weighted average mechanisms of $y[m]$ around the central point m^* , so that:

$$r_0 = \sum_{i=1}^{m^*} \omega_L[i] y[i], \quad (13)$$

in which $\omega_L[i] = \frac{f_L[i]}{\sum_{i=1}^{m^*} f_L[i]}$, $\forall i = 1, \dots, m^*$ is a weighting function. To obey the trend of data which at first exhibits rise and then fall, we utilise the function $f_L[i] \triangleq \frac{1}{2b} e^{-\frac{(m^*-i)}{b}}$, $\forall i$ for the left side of m^* , called the left exponentially growth weighting function. Similarly, for the right side of m^* , let:

$$r_1 = \sum_{k=\mathcal{T}(m^*+1)}^{\mathcal{T}(2m^*)} \omega_R[k] y[k], \quad (14)$$

where $\omega_R[k] = \frac{f_R[k]}{\sum_{k=\mathcal{T}(m^*+1)}^{\mathcal{T}(2m^*)} f_R[k]}$, $\forall k = \mathcal{T}(m^*+1), \dots, \mathcal{T}(2m^*)$ and $f_R[k] \triangleq \frac{1}{2b} e^{-\frac{(k-m^*)}{b}}$, $\forall k$ is called the right exponentially decaying weighting function. The parameter b denotes scale of the exponential functions, which is in relation to τ . In experiments, we set the optimal $b = 3$ by trial and error. On real FLIM measurements, effects such as outlier affect the position of m^* and hence demand a control mechanism on lower and upper bounds of (14). The operator $\mathcal{T}(\cdot)$ as a signal-length truncation function performs that as:

$$\mathcal{T}(m) = \min(m, M-1). \quad (15)$$

The weights in (13) and (14) are normalized, so that $\sum_{i=1}^{m^*} \omega_L[i] = \sum_{k=\mathcal{T}(m^*+1)}^{\mathcal{T}(2m^*)} \omega_R[k] = 1$. Figure 3 illustrates left and right weighting functions. Substituting (13) and (14) into (12) and simplifying by geometric series yields:

$$\hat{\tau} = \frac{\Delta \cdot (m^* + 1)}{\ln \left(\frac{\sum_k e^{-\frac{(k-m^*)}{b}} \sum_i e^{-\frac{(m^*-i)}{b}} y[i]}{\sum_i e^{-\frac{(m^*-i)}{b}} \sum_k e^{-\frac{(k-m^*)}{b}} y[k]} \right)} = \frac{\Delta \cdot (m^* + 1)}{\ln \left(\frac{c_\tau \sum_i e^{\frac{i}{b}} y[i]}{\sum_k e^{-\frac{k}{b}} y[k]} \right)}, \quad (16)$$

in which the constant $c_\tau \triangleq \frac{e^{-\frac{\mathcal{T}(m^*+1)}{b}} - e^{-\frac{\mathcal{T}(2m^*)+1}{b}}}{e^{\frac{m^*}{b}} - 1}$. Also, the approximate amplitude is equal to $\hat{A} = r_0$, i.e.:

$$\hat{A} = \frac{\sum_i e^{-\frac{(m^*-i)}{b}} y[i]}{\sum_i e^{-\frac{(m^*-i)}{b}}} = c_A \sum_i e^{\frac{i}{b}} y[i], \quad (17)$$

where the constant $c_A \triangleq \frac{1 - e^{-\frac{1}{b}}}{e^{\frac{m^*}{b}} - 1}$.

III. ALGORITHMS

The proposed FLIM parameter estimator is summarised in Algorithm 1. Due to applying the difference operator defined

Fig. 3: An illustration of left and right weighting functions.

Algorithm 1 The proposed FLIM parameters estimator

- 1: **Input:** The M -point signal $\mathbf{y} = [y_0, y_1, \dots, y_{M-1}]^T$.
 - 2: Apply the difference operator in (3) on $y[m]$ to find $d[m]$.
 - 3: Obtain the point with maximum fall m^* from (4).
 - 4: Estimate lifetime from (16).
 - 5: Estimate amplitude from (17).
 - 6: **Outputs:** Decoded amplitude $\hat{A}$ and lifetime $\hat{\tau}$.
-

in (3), noise components are naturally amplified. Although decay parameters are usually estimated from the initial samples of the histogram curve, where the SNR is higher due to higher intensities in those time bins, pre-smoothing of the signal $\tilde{x}[n]$ by means of low-pass filtering mechanisms is required in practice to be able to alleviate corruption effects. In this paper, to robustly recover the original fluorescence decay signal, two-step smoothing is used including a sequential S-G filter and temporal binning with an adaptive approach in a multi-resolution representation. Then, based on a game rule, the final decay signal is recovered from decisions made in the multi-resolution temporal space.

A. Multi-bin decay representation

Temporal binning of a histogram as a technique for information representation also concurrently performs dimensionality reduction and intrinsic smoothing tasks [11, 25]. However, finding an optimum bin size in histogram binning is generally a challenging issue in statistics. On the one hand, if the bin size is very small, appearance of a decay curve cannot be sketched well and the shape of the function may be too smooth. On the other hand, if the number of bins is too large, broken combs occur in the histogram of photo counts and a noisy representation of data is made. To address these problems, in this paper a multi-bin representation of the FLIM signal is proposed to be able to gain both smoothness and crispness of the signal without any temporal resolution loss. Naturally, higher-levels of temporal resolution represent crisper signals and lower ones expose smoother signals. To control levels of smoothness/crispness of a signal, we apply a S-G smoothing prior to histogram binning in an adaptive manner according to the degree of the inherent smoothing property in the binning mechanism at different resolution levels of signal.

1) *Adaptive Savitzky-Golay filtering:* S-G filter is a prominent type I finite impulse response signal smoother which uses

a local LS polynomial approximation [26, 27]. Characteristics of interest of the S-G filter are functions of shape and peak preservation. Also, the cut-off frequency of the filter is proportional to the polynomial order, $f_c \propto O_f$, and inversely proportional to the window length of the filter, $f_c \propto \frac{1}{L_f}$. In practice, the polynomial order should be limited to a small number such as $O_f = 2$ to prevent ill-conditioned responses; and, for achieving a filtering process, the window length of the filter should satisfy the condition $L_f > O_f$. For an adaptive filtering, at higher levels of temporal resolution, we use larger L_f to only pass very low frequencies. By moving to lower levels, we exponentially reduce the filter length to gradually widen the pass-band, so they mid- and finally high-frequency components are also passed. At lower levels of resolution, due to strong smoothing by binning itself, initial S-G pre-smoothing is not needed; thus, S-G filter will only mimic the signal and pass all frequencies. The behaviour of the adaptive filtering process is illustrated in Fig. 1, where S-G filter is ineffective in the right branch, and, the same behaviour is seen for binning stage in the left branch.

2) *Adaptive temporal binning*: Consider the S-G filtered N -sample histogram $g[n]$, $\forall n$, where $N = 2^l$ and l is the number of levels in the multi-bin representation of the decay signal. The data points at the i^{th} level of binned space can be determined from the summing formula of:

$$y_i[m] = \sum_{j=1}^{B_i} g[m \cdot B_i + j], \forall m = 0, 1, \dots, M_i - 1, \quad (18)$$

where the function $y_i[m]$, $\forall i = 1, \dots, l$ represents a smoothed, dimensionality reduced version of the signal $g[n]$ and $M_i \triangleq 2^{(l-i+1)} \leq N$ is the number of time bins in the i^{th} reduced dimension from the multi-bin representation. The parameter $B_i \triangleq \frac{N}{M_i}$ refers the number of consecutive time bins that are binned into a new one at the i^{th} level.

3) *Decision fusion*: For each of the temporal resolutions, we can estimate amplitude and lifetime parameters based on Algorithm 1. However, estimated parameters at each level need to be rescaled to the original signal space. To do this for the i^{th} level, we can multiply the estimated amplitude and lifetime values by B_i^{-1} and B_i , respectively. Specifically, rescaling the amplitude with B_i^{-1} assumes a uniform distribution which is not in agreement with a decaying trend. Instead, we use the scale s_i^{-1} for the amplitude, where:

$$s_i = \begin{cases} 1, & i = 1 \\ 2^{i-1-\gamma}, & i > 1 \end{cases}. \quad (19)$$

The bias γ accounts for an exponentially decaying distribution. In experiments, we set $\gamma = 0.25$ as an optimised value.

Initial estimates from diverse levels may encompass optimal parameters, where each level exposes its own properties. For instance, in large binning, uncertainty in estimating amplitude increases, due to combining more intensity values. This also means amplitudes are estimated well at higher levels of temporal binning. Conversely, lifetime estimation at top levels of binning will be generally more sensitive to corruption, where lower levels of temporal resolutions are preferable. This brings a conflict of interest for a final decision making. Hence,

a consensus strategy is suggested to synergically decode the original decay signal based on the game modelled below.

B. Game-theoretic modelling for decay recovery

Suppose the goal is to decide the best parameters for an optimal decay recovery based on the multi-bin histogram representation and corresponding initial estimates. Both of optimal parameters A^* and τ^* are influential on the optimum selection. However, a single objective function is not available to consider both roles. Therefore, we suggest a game in which the problem is modelled by the “game” $\mathcal{G}(v, \mathcal{A}, d)$, where v , $\mathcal{A}$, and d denote the number of players, the set of actions, and payoff function of the game, respectively. We consider the initial estimation behaviour of each of amplitude and lifetime random variables as the players, i.e. there exists $v = 2$ players. The strategy of each player is to choose a set of known actions to be successful. Actions themselves are drawn from multiple temporal binning representations of histogram of photon-count arrivals, where we define the actions set $\mathcal{A} = \{\alpha_1, \alpha_2, \dots, \alpha_k\}$, in which $k = l$ is the number of actions. The game space is shown in the decision fusion stage of Fig. 1 as a grid. The action α_i corresponds to $2^{(k-i+1)}$ -dimensional temporal resolution from the multi-bin decay representation. For the action i^{th} , a single-exponential decay can be recovered from FLIM data of a given pixel, i.e. the pair $(\hat{A}_i, \hat{\tau}_i)$. A Representation of actions taken by each player in the 2-D space $\Omega \in \mathbb{Z}^2$ constitutes a matrix called a reward matrix as $\mathbf{R} = [r_{ij}]$. For one repetition of the game, the matrix's entries takes 1 or 0 values, referring to prize or penalty, respectively.

Theorem 2 (Optimal decay recovery): Let the matrix $\mathbf{R} = [r_{ij}]$ be a reward matrix for the amplitude-lifetime game $\mathcal{G}(v, \mathcal{A}, d)$, where for a repetition of the game, the entry corresponding to an optimal parameter is 1 and other locations are 0. A place in the space Ω of the reward matrix exists by which the underlying optimal decay signal is recoverable.

Proof: Generally, Nash equilibrium proves that an equilibrium point exists for a game, where all players reach their maximum payoff [28]. To show that for the game $\mathcal{G}(v, \mathcal{A}, d)$, consider the equilibrium position as location of optimal estimates. Then, the optimal decay signal recovery is possible by players' payoff function optimisation. Here, optimality is constrained to quality of initial estimates of amplitude and lifetime parameters in the multi-bin decay representation. To detect optimal A^* and τ^* , we first measure distance between each recovered signal from the pair $(\hat{A}_i, \hat{\tau}_j)$, $\forall i, j$ in the game space Ω and received corrupted measurements, and then, find the minimised one (See the blue star in the game space shown in Fig. 1 for illustration.).

In terms of curve-like shape of an exponentially decaying function, we define two critical fixation points for the distance optimisation. The first one is connected to amplitude, which controls the initial position of the decay signal on the y -axis; and, another is related to lifetime, in which the steady-state location of the curve on the x -axis is controlled. It is difficult to seek a single objective function for finding the optimal pair (A^*, τ^*) , in a manner that the metric concurrently copes with the two degrees of freedom well. Instead, we consider a

specific distance measure for each player. Now, the problem is to search apt metrics for satisfying the two fixation points.

The scale of squared error at transition times of a decay curve is usually more than settling times. Hence, for fixing the amplitude, mean squared error (MES) criterion is apt as:

$$d^{\text{MSE}}(\tilde{\mathbf{x}}, \hat{\mathbf{f}}) \triangleq \frac{1}{N} \sum_{i=0}^{N-1} (\tilde{x}_i - \hat{f}_i)^2, \quad (20)$$

where $\hat{\mathbf{f}} = [\hat{f}_0, \hat{f}_1, \dots, \hat{f}_{N-1}]$ represents a single-exponential recovered signal in the space Ω . Conversely, for the tail of the decay curve, the scale is usually higher in terms of Pearson's chi-square test (CHI) metric χ^2 . Therefore, it is appropriate for probing optimum lifetime. The distance χ^2 for the lifetime player is determined by:

$$d^{\text{CHI}}(\tilde{\mathbf{x}}, \hat{\mathbf{f}}) \triangleq \sum_{i=0}^{N-1} \frac{(\tilde{x}_i - \hat{f}_i)^2}{\tilde{x}_i}. \quad (21)$$

Assume vectors $\mathbf{d}^{\text{MSE}}$ and $\mathbf{d}^{\text{CHI}}$ contain MSE and χ^2 distances obtained from the space Ω . Optimal influential parameters are selected from the minimisation of:

$$A^* = \arg \min_{\Omega} (\mathbf{d}^{\text{MSE}}), \quad (22)$$

$$\tau^* = \arg \min_{\Omega} (\mathbf{d}^{\text{CHI}}). \quad (23)$$

Finally, the optimal decay signal is recovered by:

$$f^*[n] = A^* e^{-\frac{\Delta n}{\tau^*}}, n = 0, 1, \dots, N-1. \quad (24)$$

Our robust recovery approach for a pixel is detailed in Algorithm 2. We term our method "Robust RLD".

Example 1: Consider the original decay signal in (2) with $A = 100$, $\tau = 3\text{ns}$, $\Delta = 468.8\text{ps}$, and $N = 32$. The signal was corrupted with the Gaussian blurring kernel $\mathbf{h} = [0.25, 0.5, 0.25]^T$ and noise parameters of $a = 0.05$ and $\sigma^2 = 25$. Figure 1 illustrates the whole process for a random run. For a million random plays of the game, Table I (a) reports accumulated reward matrix normalised on summation of prizes for each player. The Nash equilibrium point is bold in the table. To be able to compare detection performance of our model to that of ground truth decay, we have also repeated the game for the ideal case. For each round of the game, the prize entry in the ground truth reward matrix was determined as the place in which the absolute difference between a ground truth value and corresponding multi-bin estimates is minimum. Outcomes are listed in Table I (b). Again, Nash equilibrium is bold in the table. Comparing bold numbers in Tables I (a) and (b) reveals the congruence of equilibria. Dominant success almost always occurs in upper triangle part of the reward matrices.

IV. EXPERIMENTS

In this section, using synthesised data with known ground truth, we evaluated the robustness of our method under different settings and circumstances through Monte Carlo simulation. We compared our proposed method to CMM, standard LS fit of MATLAB software, Poisson MLE, RLD, RLD-OW and FLERIT approaches. The effectiveness of the proposed method is also shown on real FLIM data.

Algorithm 2 The proposed single-exponential decay recovery

```

1: Input: The  $N$ -point histogram of photo-count arrivals  $\tilde{\mathbf{x}}$ .
2: Initialise  $M = N$ ,  $O_f = 2$ ,  $\mathbf{g} = \tilde{\mathbf{x}}$ ,  $k = 1$ ,  $N_l = \log_2 N$ ,
   and  $\mathbf{n} = [0, 1, \dots, N-1]^T$ .
3: while  $M \geq 2$  do
4:    $L_f = \frac{N}{2^k} + 1$ 
5:   if  $L_f > O_f$  then
6:     Smooth  $\mathbf{g}$  by S-G filter with specs of  $O_f$  and  $L_f$ .
7:   end if
8:   Bin smoothed  $\mathbf{g}$  into  $M$  bins by (18) to make a  $\mathbf{y}$ .
9:   Estimate  $\hat{A}$  and  $\hat{\tau}$  by passing  $\mathbf{y}$  to Algorithm 1.
10:   $\hat{\mathbf{A}}_k = (\frac{1}{\hat{A}_k})\hat{A}$ 
11:   $\hat{\tau}_k = (\frac{N}{\hat{\tau}_k})\hat{\tau}$ 
12:  Update  $\mathbf{g} = \tilde{\mathbf{x}}$ ,  $M \leftarrow \frac{M}{2}$  and  $k \leftarrow k + 1$ .
13: end while
14: Reset counter  $k = 1$ .
15: for  $i \leftarrow 1, N_l$  do
16:   for  $j \leftarrow 1, N_l$  do
17:      $\hat{\mathbf{f}} = \hat{\mathbf{A}}_i e^{-\frac{\Delta}{\hat{\tau}_j} \mathbf{n}}$ 
18:     Measure distance  $d_k^{\text{MSE}}$  between  $\tilde{\mathbf{x}}$  and  $\hat{\mathbf{f}}$  by (20).
19:     Measure distance  $d_k^{\text{CHI}}$  between  $\tilde{\mathbf{x}}$  and  $\hat{\mathbf{f}}$  by (21).
20:      $k \leftarrow k + 1$ 
21:   end for
22: end for
23:  $A^* = \arg \min_{\hat{\mathbf{A}}, \hat{\tau}} (\mathbf{d}^{\text{MSE}})$ 
24:  $\tau^* = \arg \min_{\hat{\mathbf{A}}, \hat{\tau}} (\mathbf{d}^{\text{CHI}})$ 
25:  $\mathbf{f}^* = A^* e^{-\frac{\Delta}{\tau^*} \mathbf{n}}$ 
26: Output: The recovered fluorescence decay signal  $\mathbf{f}^*$ .

```

A. Tests on synthesised data

1) Evaluation on different bin sizes: In this experiment, we changed the bin size of histogram of photon counts from 2^1 to 2^{10} for various decay signals and determined the performance of our method. The amplitude of functions was set $A = 100$. We adjusted the bin width to the formula $\Delta = \frac{5\tau}{N}$, which guarantees settling a decay curve into total measurement cycle for a pixel. All signals were first blurred with a Gaussian kernel of the length 3 and then noise was added with noise characteristics of $a = 0.1$ and $\mathcal{N}(0, 25)$. Figure 4 plots averaged lifetime vs bin size for three fluorescence decay functions with different ground truth lifetimes of 1, 2.5 and 4 in ns. The averaged lifetime reported for each point of the plot was obtained for 100,000 random runs. As seen from the figure, averaged lifetimes are inaccurate at initial points for the bin size of 2, where we have only a line segment for representing a fluorescence decay but not a desired curve. However, the estimations exponentially improve with increasing bin size. Estimations are asymptote to their corresponding ground truth lines for bin sizes more than or equal to 128. Meanwhile, the proposed method delivers appropriate estimations for $N \geq 32$. This is because that size makes a sufficiently continuous decay curve apt for lifetime estimation. Hence, the limit can be considered as a user guide for clinical settings of instruments.

TABLE I: (a) Predicted and (b) ground truth normalised reward matrices for a million rounds of the game in Example 1

(a)							
Player τ	No binning	Binning with $B=2$	Binning with $B=4$	Binning with $B=8$	Binning with $B=16$		
No binning	46.84	0.09	46.37	13.96	25.59	18.94	64.66
Binning with $B=2$	41.01	5.64	14.39	26.07	13.05	33.26	29.78
Binning with $B=4$	8.94	0.03	39.18	2.84	61.36	20.59	76.51
Binning with $B=8$	3.21	11.43	0.06	11.42	0	38.33	37.06
Binning with $B=16$	0	0	0	0.36	0	22.53	74.51

(b)							
Player τ	No binning	Binning with $B=2$	Binning with $B=4$	Binning with $B=8$	Binning with $B=16$		
No binning	0	0	15.94	4.16	19.38	6.15	75.62
Binning with $B=2$	0	0	6.37	0.95	16.7	37.32	8.87
Binning with $B=4$	0	0	77.59	1.43	78.29	21.73	85.33
Binning with $B=8$	0	0	0.1	1.38	0.07	16.22	80
Binning with $B=16$	0	0	0	0	0	0	0

Fig. 4: The performance of our method for a diverse range of bin sizes from three lifetime-varying decay signals.

2) *Effects of blurring and noise:* As mentioned before, fluorescence decay signals are affected by corruption of blurring and noise in practice. However, both the shape and level of signal's corruption may differ from instrument to instrument. This experiment provides two simulation modes for analysing those effects. In the first simulation, for a fixed Gaussian blurring kernel with length 5, we evaluated statistics of estimated lifetimes from a corrupted signal under various shot and background noises. Parameters of the original signal were $A = 100$, $\tau = 1.5\text{ns}$, $\Delta = 58.6\text{ps}$ and $N = 128$. The performance of our method is shown in Fig. 5 for 100,000 random runs. The curves related to mean of lifetime show a stable behaviour of bias (as a measure of accuracy) in our estimator even for severe noise. In this regard, for shot noise with $a = 0.05$ and $a = 0.1$, the initial overestimation of lifetime is due to unrealistic rising of tail of a decay function in the absence of noticeable background noise. This phenomenon is related to our shot noise modelling explained in Section II-B1, and does not occur in real scenarios. As seen for $\sigma^2 \geq 16$, when shot noise is mixed with background noise, estimations are completely controlled around the ground truth line with an acceptable rise in standard deviation (as a measure of precision). Results of the point $\sigma^2 = 0$ in the absence of shot noise mean corruption only by blurring.

In the second simulation, we fixed the parameter of shot noise on $a = 0.05$ and then evaluated mean and standard deviation of estimated lifetimes from a corrupted signal under different blurring kernel shapes and background noise powers. To model the IRF of a FLIM system, different blurring kernels may be utilised. Here, we employed impulse, box, approximate Gaussian and Airy smoothing functions with the same length of 7. It is notable that applying an impulse function means an ideal case without any blurring effect. Therefore, in that case,

Fig. 5: Evaluation of our method under noise variations. Numbers in parenthesis of the legend are average on all σ^2 .

Fig. 6: Evaluation of our method for various blurring kernels.

only the noise effect is present. Parameters of the original signal were the same as the first mode above. Figure 6 plots results for 100,000 random runs. By increasing noise power, a decreasing trend is seen among estimations for all kernels. Responses show small deviations from the ground truth reference. In this regard, the lowest and highest absolute errors between the reference line and mean of lifetime averaged on different noise powers belong to Airy and box kernels, respectively.

3) *Performance comparison under lifetime variations:* The goal of this experiment is to examine the performance of different approaches in short-, mid- and long-lifetime regimes. Figure 7 shows lifetime estimation statistics of various methods on three random signals with short, mid and long lifetimes of 0.5, 3 and 12 nanoseconds, respectively. Common parameters utilised for the signals were $A = 100$, $N = 64$ and $\Delta = \frac{5\tau}{N}$. Corruption characteristics were Gaussian blurring kernel of length 3 and noise parameters of $a = 0.1$, $\mathcal{N}(0, 25)$. Reported statistics were obtained for a million random runs. As is shown in the box plots of Fig. 7, our method outperforms other benchmark approaches. For all lifetime regimes, our

TABLE II: Numerical comparison of the methods in Fig. 8

Method	Region ($\mu \pm \sigma$)		
	A	B	C
FLERIT [11]	9.51 $\pm$ 1.42	6.86 $\pm$ 2.42	2.25 $\pm$ 2.05
Our approach	9.99 $\pm$ 0.19	5.97 $\pm$ 0.17	2.52 $\pm$ 0.58

estimated lifetime is the nearest one to the ground truth value, where the first quartile is aligned with the ground truth line.

The FLERIT approach mainly employs neighbouring pixels information of a central point in an image to construct the observation matrix for estimating the average lifetime. Hence, we separately compare the performance of lifetime estimation between FLERIT and our method on a synthesised image. To do this, we generated an image of dimensions 1024×1024 including three regions with various lifetimes as shown in the intensity map of Fig. 8. Ground truth lifetimes in ns for pixels inside regions of A, B and C are 10, 6 and 2, respectively. Amplitudes were set under a low photon count regime, so that they were $\frac{10}{3}$, 2 and $\frac{2}{3}$ for regions of A, B and C, respectively. Common characteristics of ground truth signals are $N = 32$ and $\Delta = 0.4\text{ns}$. The decay signals were corrupted by Gaussian noise of zero mean and 2.5×10^{-3} variance. In FLERIT, 3×3 neighbours and the number of consecutive merged bins equal to 4 were utilised. In Fig. 8, the left-side intensity map represents summation on all arrived photons, which is the same in FLERIT and our method for a fair comparison. The mid lifetime map visualises lifetime estimation performance. And, the right-side plot depicts lifetimes' histogram. Visual results demonstrate the superiority of our method over FLERIT. For regions A, B and C, Table II tabulates mean $\pm$ standard deviation from estimated lifetimes of FLERIT and our proposed method. The best results are bold in the table. Except for the mean of region C, the evaluation of numerical results demonstrates that the suggested approach brings desired lower bias and variance of lifetime estimates than those of FLERIT.

4) *Comparison in various photon-count regimes:* As mentioned earlier, SNR is proportional to square root of the average number of arrived photons I . This parameter is a function of amplitude. Hence, we change the amplitude to simulate low, mid and high photon count regimes, which are interpreted as different levels of SNR. To this intent, we considered three signals with amplitudes of 50, 250 and 1000. We set other common parameters of signals as $\tau = 2\text{ns}$, $\Delta = 312.5\text{ps}$ and $N = 32$. All signals were first blurred with a Gaussian kernel of length 3 and then noise was added with characteristics of $a = 0.1$, $\mathcal{N}(0, 25)$. Those settings result in the values of I approximately equal to 10, 50 and 200 averaged on the measurement cycle for low, mid and high photon counts, respectively. For all of the regimes, Fig. 9 represents mean and standard deviation of estimated lifetimes from different approaches including our proposed approaches in Algorithms 1 and 2. Due to the differentiation operator used in Algorithm 1, we have also evaluated the effect of pre-binning as a simple signal smoother before estimation. To do this, we reduced the number of bins from 32 to reasonably sized 8. Statistics in the bar graph were obtained for a million times random run. For

the challenging low photon-count situation, comparing results of different approaches to the ground truth reference reveals the lowest bias for the proposed Robust RLD with a significant difference from other competing approaches. In the regime, the percent bias of our approach is 7.55%, whereas, for the best competitor (RLD-WO), it is 35.13%, which shows 27.58% improvement. This specifically demonstrates the robustness of our method under severe corruption, as seen in the example of Fig. 2. The accuracy of the estimator in Algorithm 1 is ranked second but in an uncontrolled variance, which is because of noise amplification. However, with an accuracy-precision trade-off, the pre-binning clearly improves the variance of the estimator in Algorithm 1. Thanks to high levels of SNR for the high photon counts regime, most of the lifetime estimators expose close performance. Interestingly, the bias of Algorithm 1 is less than that of Algorithm 2 in this regime. This fact also highlights that for low noise levels, it may not be needed to employ more complex approaches for lifetime estimation. Meanwhile, results of Robust RLD show its appropriateness for utilising in a wide variety of photon-count regimes.

5) *Comparison on decay recovery error:* Although the lifetime is known as the most important parameter in FLIM, some fields like materials science deal with the amplitude information, too. However, all benchmark lifetime estimation approaches do not the capability of amplitude estimation such as CMM. Or, if capable, they may work weakly under difficult situations like RLD and RLD-OW. This fact also means they are not effective for recovering a continuous or discrete version of a decay signal. Among our compared methods, amplitude estimation of LS fit has the best rank. Therefore, to save space, we have reported a performance comparison between our method and that of LS fit in Table III. For this experiment, we considered three signals with small, mid and large amplitudes of 20, 100 and 500, respectively. Other parameters of the three signals were $\tau = 5\text{ns}$, $\Delta = 781.3\text{ps}$ and $N = 32$. Signals were first blurred with a Gaussian kernel of length 3 and then noise was added with characteristics of $a = 0.05$, $\mathcal{N}(0, 64)$. In Table III, bold mean and standard deviation values represent the best performance. For the small amplitude, our method has lower bias than LS fit; and, in mid and large amplitudes, it follows the LS fit with little differences. The LS fit is inaccurate for lifetime estimation in case of the small amplitude, where signal corruption is severe, whereas the proposed method is robust for diverse amplitudes. Regarding recovery error of χ^2 , our method outperforms the LS fit for almost all cases.

B. Tests on real data

1) *Imaging device:* For imaging from real samples, we employed a fibre-bundle microendoscopy system called Kronoscan [29, 30]. The imager facilitates in vivo, in situ optical biopsies of the human lung in real-time at clinical video rates, assisting fast diagnostic and potentially therapeutic interventions. The sensing system incorporates both intensity and lifetime imaging over a range of different optical wavelengths.

2) *Experimental design:* Neutrophil Activation Probe (NAP) is an activatable fluorescent probe used for the detection of Human Neutrophil Elastase (HNE), an enzyme released

Fig. 7: Lifetime estimation statistics of various methods on (a) short-, (b) mid- and (c) long-lifetime regimes.

TABLE III: Decay recovery error of the proposed method than the best competitor among our compared methods

Method	Amplitude estimation ($\mu \pm \sigma$)			Lifetime estimation ($\mu \pm \sigma$)			Decay recovery error of χ^2 ($\mu \pm \sigma$)		
	$A = 20$	$A = 100$	$A = 500$	$A = 20$	$A = 100$	$A = 500$	$A = 20$	$A = 100$	$A = 500$
Least squares fit	15.14 ± 4.66	86.97 ± 5.08	439.29 ± 5.05	15.35 ± 6.67	6.24 ± 0.54	5.77 ± 0.1	682.8 ± 625.9	44.52 ± 36.69	62.17 ± 15.34
Our approach	15.41 ± 5.23	86.55 ± 5.59	433.29 ± 7.3	5.59 ± 3.48	5.21 ± 0.62	5.31 ± 0.21	59.49 ± 114.43	21.36 ± 19.49	42.27 ± 15.98

Fig. 8: Lifetime estimation performance comparison between (a) FLERIT and (b) our method on the same synthesised image. Ground truth lifetimes in ns for regions of A, B and C are 10, 6 and 2, respectively.

by activated, pro-inflammatory neutrophils [31]. Consisting of three internally quenched fluorescein moieties each conjugated to a cleavable peptide sequence specific to HNE, NAP ($\lambda_{ex} = 488\text{nm}$ and $\lambda_{em} = 525\text{nm}$) in its activated form amplifies its fluorescence signal due to the release of fluorescein. By incubating NAP with HNE, we sought to characterise its fluorescence lifetime properties and test the effects of Sivelestat, a specific inhibitor of HNE, as well as Nafamostat mesylate, an antiviral drug currently undergoing clinical trials as a potential treatment for coronavirus (COVID-19) (clinical trial identifier NCT04473053) as a potential inhibitor of HNE.

Another property of the experiment from the lifetime estimation viewpoint, is measuring fluorescence in a homogeneous solution as opposed to those of complex biological

Fig. 9: Mean and standard deviation of estimated lifetimes of different approaches under various photon-count regimes.

objects such as cells. This means that signals from each sample should be consistent, and we expect a uniform, flat lifetime map. Therefore, such partial information about samples' behaviour brings the ability to evaluate the performance of the proposed lifetime estimation method in real-world scenarios.

3) *Samples preparation:* 400nM HNE (HNE-L, Molecular Innovations, USA), diluted in HNE buffer (0.05M Na Acetate, pH 5.0 containing 0.1M NaCl), was incubated with 10 μM Sivelestat (ONO-5046, Tocris, Bristol, UK) or 10 μM Nafamostat mesylate (N0289, Sigma-Aldrich, UK) prior to the addition of 5 μM NAP (diluted in phosphate buffered saline). Solutions were incubated for 15 minutes at 37°C prior to fluorescence intensity/lifetime imaging with Kronoscan. Experimental samples include 5 μM NAP alone, 5 μM NAP+400nM HNE, 5 μM NAP+400nM HNE+10 μM Sivelestat and 5 μM NAP+400nM HNE+10 μM Nafamostat mesylate, labelled respectively A, B, C and D for simplicity.

4) *Sensing and outcomes:* For each sample, microendoscopy was done on green and red spectral bands with wavelength ranges of (498nm $\sim$ 570nm) and (594nm $\sim$ 764nm),

TABLE IV: Results of the proposed method on real samples

Sample	N_f	Spectral band 1 ($\mu \pm \sigma$)		Spectral band 2 ($\mu \pm \sigma$)	
		Intensity (a.u.)	Lifetime (ns)	Intensity (a.u.)	Lifetime (ns)
A	19	13679 $\pm$ 2537	3.23 $\pm$ 0.06	1083 $\pm$ 163	5 $\pm$ 0.71
B	23	44922 $\pm$ 8475	3.57 $\pm$ 0.09	2844 $\pm$ 547	4.19 $\pm$ 0.27
C	31	27060 $\pm$ 5005	3.57 $\pm$ 0.05	1753 $\pm$ 298	4.39 $\pm$ 0.46
D	28	43389 $\pm$ 8822	3.53 $\pm$ 0.07	2658 $\pm$ 566	4.19 $\pm$ 0.3

Fig. 10: Intensity and lifetime maps, and lifetimes' histogram from the band 1, 15th frame, sample C in Table IV.

called bands 1 and 2, respectively. Each sample contains video sequences with N_f frames. Signal parameters are $N = 16$, $\Delta = 800$ ps and the frames dimension of 128×128 . Table IV summarises statistics of samples. Mean and standard deviation values were calculated on pixels inside the probe circle accumulated over all video frames of $f = 0, 1, \dots, N_f - 1$; and, as a pattern of the plain specimens, Fig. 10 shows intensity and lifetime maps as well lifetimes' histogram from the band 1, 15th frame, sample C. The zigzag-wise patterns on maps are mainly due to fibre-bundle and video frame sample artefacts.

Obtained results are statistically significant for all samples. HNE activated NAP via the cleavage of peptide sequence to release fluorescein, resulting in increased fluorescence intensity and lifetime. $10\mu\text{M}$ Sivelestat reduced the fluorescence intensity; however, it had no effect on fluorescence lifetime due to the partial inhibition of HNE. No reduction in fluorescence intensity or lifetime was observed with $10\mu\text{M}$ Nafamostat mesylate, indicating that it is not an inhibitor of HNE. Due to the fluorescence emission spectrum properties of NAP, fluorescence was primarily detected in band 1. Thus, standard deviations of band 2 are greater than band 1 counterparts because of noise amplification, where the band 2 red spectrum has lower photon energy than green one and falls largely outside of the NAP emission band. The increased fluorescence lifetime signature of NAP makes it suitable for FLIM.

V. COMPUTATIONAL COMPLEXITY

Except the performance of a lifetime estimator, another important issue in FLIM is computational complexity of the estimator. In this regard, estimators with low complexity enable on-chip lifetime estimation capabilities, which lead to benefits from reducing data transfer rate between a sensor head and a distant computer to portability of a medical device. This section provides approximate computational complexity of our algorithms in terms of the input size of bins number N , yielding an upper bound on their time complexity. We have also done that task for methods presented in Section IV.

TABLE V: Computational complexity of different algorithms

Algorithm(s)	Time complexity
CMM, RLD, RLD-OW, and our estimator in Algorithm 1	$\mathcal{O}(N)$
LS fit, Poisson MLE, and FLERIT [11]	$\mathcal{O}(N^3)$
Our Robust RLD in Algorithm 2	$\mathcal{O}(N \log_2 N)$

As shown in Fig. 1, the proposed Robust RLD algorithm consists of three processing stages at each level of the multi-temporal resolution space and a decision unit at the end. The processing steps include S-G filter, binning, and estimator. The input bin size is variable for each level; and, the complexity of the decider is negligible in comparison to the processing stages. Therefore, the computational complexity is practically bounded to processing applied at the level with the highest resolution. At this level, no binning exists and all photon time-stamps are individually recorded. The proposed estimator in Algorithm 1 has a linear complexity of the order $\mathcal{O}(N)$. Therefore, the dominant term is the complexity of S-G filter [32], i.e. the order of 1-D convolution as $\mathcal{O}(N \log_2 N)$.

The compared algorithms expose two types of complexity, regardless of their implementation details. On the one hand, the complexity of analytic closed-form approaches of CMM, RLD, and RLD-OW grows linearly with increasing N , which is the same as the proposed estimator in Algorithm 1. On the other hand, LS fit, Poisson MLE, and FLERIT have all matrix decomposition-based solutions. Hence, they shows cubic order of complexity. Table V summarises the complexity for different categories. To sum up, our proposed Algorithm 2 is ranked second among them with a linearithmic complexity between the fair order $\mathcal{O}(N)$ and the worst one $\mathcal{O}(N^3)$. The analyses demonstrate that both of our algorithms are suitable options for hardware-level implementations.

VI. CONCLUSION

This paper presented a robust and computationally efficient fluorescence decay signal recovery algorithm in the presence of inevitable blurring and noise occurring during time-resolved single photon sensing. The proposed framework first provides a multi-bin decay representation from the histogram of photon count arrivals using an adaptive signal smoothing approach. Subsequently, each representation is fed to our lifetime decoding algorithm. Finally, estimated parameters from different temporal resolutions are fused to each other based on game-theoretic modelling. We theoretically proved that our method is capable of recovering optimal fluorescence decay robustly under a wide variety of imaging situations.

In addition to being robust, due to the non-fitting-based nature of our method, it can be considered as a rapid approach for hardware-level realisation. This capability is of great importance in real-time fluorescence lifetime sensing systems such as in vivo, in situ microendoscopy and nanoendoscopy.

APPENDIX

DERIVATION OF CRAMÉR-RAO LOWER BOUND AND MVUE

Starting from the corrupted observations model $\tilde{x}[n] = Ae^{-\frac{\Delta \cdot n}{\tau}} + e[n]$, likelihood function for the given τ is:

$$f_{\tilde{X}}(\tilde{x}|\tau) = \prod_{n=0}^{N-1} \frac{1}{\sqrt{2\pi\sigma_e^2}} e^{-\frac{1}{2\sigma_e^2} \left[\tilde{x}[n] - A e^{-\frac{\Delta \cdot n}{\tau}} \right]^2}. \quad (25)$$

The first-order partial derivative of the log-likelihood gives:

$$\frac{\partial \ln f_{\tilde{X}}(\tilde{x}|\tau)}{\partial \tau} = \frac{A\Delta}{\tau^2 \sigma_e^2} \sum_{n=0}^{N-1} \left[n e^{-\frac{\Delta \cdot n}{\tau}} \tilde{x}[n] - A n e^{-\frac{2\Delta \cdot n}{\tau}} \right]. \quad (26)$$

Repeating the differentiation yields:

$$\frac{\partial^2 \ln f_{\tilde{X}}(\tilde{x}|\tau)}{\partial \tau^2} = \frac{A\Delta^2}{\sigma_e^2 \tau^4} \sum_{n=0}^{N-1} \left[n^2 e^{-\frac{\Delta \cdot n}{\tau}} \tilde{x}[n] - 2A n^2 e^{-\frac{2\Delta \cdot n}{\tau}} \right]. \quad (27)$$

The estimation variance satisfies $\text{var}(\hat{\tau}) \geq \text{CRLB}$, where:

$$\text{CRLB} \triangleq \frac{1}{-\mathbb{E} \left[\frac{\partial^2 \ln f_{\tilde{X}}(\tilde{x}|\tau)}{\partial \tau^2} \right]} = \frac{\sigma_e^2 \tau^4}{A^2 \Delta^2 \sum_{n=0}^{N-1} n^2 e^{-\frac{2\Delta \cdot n}{\tau}}}. \quad (28)$$

Now, the condition of existence of a MVUE is to check $I(\tau)(\hat{\tau} - \tau) = \frac{\partial \ln f_{\tilde{X}}(\tilde{x}|\tau)}{\partial \tau}$ for a function $I(\tau)$ as:

$$I(\tau)(\hat{\tau} - \tau) = \frac{A^2 \Delta}{\sigma_e^2 \tau^3} \sum_{n=0}^{N-1} n e^{-\frac{2\Delta \cdot n}{\tau}} \left\{ \frac{\tau \sum_{n=0}^{N-1} n e^{-\frac{\Delta \cdot n}{\tau}} \tilde{x}[n]}{A \sum_{n=0}^{N-1} n e^{-\frac{2\Delta \cdot n}{\tau}}} - \tau \right\}, \quad (29)$$

which $\hat{\tau} \triangleq \frac{\tau \sum_{n=0}^{N-1} n e^{-\frac{\Delta \cdot n}{\tau}} \tilde{x}[n]}{A \sum_{n=0}^{N-1} n e^{-\frac{2\Delta \cdot n}{\tau}}}$ shows the MVUE does not exist due to being a function of the true parameter τ . ■

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