A novel fit-flexible fluorescence soft imager: Tri-sensing of intensity, fall-time, and life profile

Ali Taimori, Bethany Mills, Erin Gaughan, Aysha Ali, Kevin Dhaliwal, Gareth Williams, Neil Finlayson, and James R. Hopgood, Senior Member, IEEE

Abstract—Time-resolved fluorescence imaging techniques, like confocal fluorescence lifetime imaging microscopy, are powerful photonic instrumentation tools of modern science with diverse applications, including: biology, medicine, and chemistry. However, complexities of the systems, both at specimen and device levels, cause difficulties in quantifying soft biomarkers. To address the problems, we first aim to understand and model the underlying photophysics of fluorescence decay curves. For this purpose, we provide a set of mathematical functions, called “life models”, fittable with the real temporal recordings of histogram of photon counts. For each model, an equivalent electrical circuit, called a “life circuit”, is derived for explaining the whole process. In confocal endomicroscopy, the components of excitation laser, specimen, and fluorescence-emission signal as the histogram of photon counts are modelled by a power source, network of resistor-inductor-capacitor circuitry, and multimetre, respectively. We then design a novel pixel-level temporal classification algorithm, called a “fit-flexible approach”, where qualities of “intensity”, “fall-time”, and “life profile” are identified for each point. A model selection mechanism is executed at each pixel to flexibly choose the best representative life model based on a proposed Misfit-percent metric. A two-dimensional arrangement of the quantified information detects some kind of structural information. This approach showed a potential of separating microbeads from lung tissue, distinguishing the tri-sensing from conventional methods. We alleviated by 7% the error of the Misfit-percent for recovering the histograms on real samples than the best state-of-the-art competitor. Software codes are available online.

Index Terms—Fluorescence lifetime imaging microscopy, lifetime estimation, modelling, model selection, system identification.

I. INTRODUCTION

Fluorescence imaging techniques are photonic-based piece of equipment with numerous applications across biology, chemistry, medicine, materials and environmental sciences [1]. In the life sciences, the time-resolved technique of optical fluorescence lifetime imaging microscopy (FLIM) [2] or spectroscopy [3] are widely employed for microscopy or nanoscopy of biological substances. In FLIM, a specimen is first excited via a light source such as a laser. The reactional response to this excitation leads to photon absorption and emission. The time of the first photon detected is then recorded by a sensitive detector, such as a single-photon avalanche diode (SPAD) sensor and related time-correlated single photon counting (TCSPC) electronic equipment [4]. The cycle continues for a given number of excitations. At the end of process, a temporal histogram is produced by counting photons in different time bins. The mean lifetime, as a biomarker/chemomarker characteristic of the transient response, is estimated and utilised to bring a contrast among diverse locations in the sensed specimen [5–13]. Fig. 1 represents graphically the proposed fit-flexible approach.
B. Background investigation

In the fluorescence techniques literature, the function representing time-resolved measurements from a photon counting process is considered as one of the decaying models of mono-, bi-, tri-, or, more generally, multi-exponentials [14]. For the most general case of an infinite number of exponentials, the fluorescence decay curve is:

\[ v(t) = \sum_{i=1}^{\infty} A_i e^{-\frac{t}{\tau_i}} = A \sum_{i=1}^{\infty} \alpha_i e^{-\frac{t}{\tau_i}}, \]  

where \( A_i \in \mathbb{R}^+ \) and \( \tau_i \in \mathbb{R}^+, \forall i \), denote the amplitude and the lifetime of the \( i^{\text{th}} \) term, respectively. The symbol \( \mathbb{R}^+ \) denotes the set of all positive real numbers. There exists an \( A = \sum_{i=1}^{\infty} A_i, \alpha_i = \frac{1}{\tau_i}, 0 < \alpha_i \leq 1, \forall i, \) and \( \sum_{i=1}^{\infty} \alpha_i = 1 \).

In conventional FLIM, the centre of mass of the histogram of photon counts is determined as the fluorescence lifetime [3]. In (1), this is:

\[ \tau_{\text{mean}} = \frac{\sum_{i=1}^{\infty} \alpha_i \tau_i^2}{\sum_{i=1}^{\infty} \alpha_i \tau_i}. \]  

The derivation of (2) is provided in Section S2 of the supplementary materials (SMs). The histogram of photon counts is usually modelled by a mono-exponential due to its simplicity and applicability [6, 13]. However, a bi- or tri-exponential may be applied for complex materials [9, 15, 16].

From the perspective of system identification [17], the time-resolved fluorescence signal in (1) is analysed as a data-driven, black-box time-series system modelling. In this modelling, inputs are not observed and only the measured outputs, as the histogram of photon counts, are available. This means that, in microscopy of a specimen, an investigator would be only aware of the result of molecular reactions (i.e. the outputs) to be able merely analyse FLIM data. However, there exists a profound gap to the desire of elucidatory understanding physical concepts and detailed origins [14]. This necessitates a white-box modelling beyond the regular demand for analysis of FLIM data [5–7, 9–11, 16, 18, 19]. Such a model, like equivalent electrical circuit consisting of resistor-inductor-capacitor (RLC) elements, characterises objects and their interconnections in a biological system [20], which is not the case in black-box modelling. Consequently, the white-box modelling is particularly informative for two other applications of systems modelling, i.e., “synthesis” and “control”, other than the “analysis” itself [21]. Potential instances include: creating synthetic biomaterials [22] and designing controllers for real tissues [23, 24].

Finding the underlying differential equation [25] which satisfies (1), as a practice of grey-box modelling, reduces the opacity of the model. For mono-exponential models, a photochemistry/photophysics interpretation of fluorescence in terms of Jablonski diagram [26] results in the 1st-order ordinary differential equation (ODE)\(^1\) [13, 27]. However, there is a lack of research on interpretations for 2nd-order and higher-order models [28, 29]. In steady-state fluorescence intensity imaging, but not time-resolved imaging as addressed by this paper, a linear 2nd-order ODE with two distinct real roots for describing the combined effects of photobleaching and photobleaching is proposed in [30]. It models average intensity across video frames as bi-exponential for denoising. Rare studies also exist beyond exponential. For example, the lack of a function other than the exponential for describing environments containing complex materials is identified in [31]. They modelled the decay as a gamma distribution for better experimental data fitting. Lukichev in [32] proposed the stretched exponential Kohlrausch-Williams-Watts (KWW) function, \( f(t) = Ae^{-\left(\frac{t}{\tau}\right)^\gamma}, 0 < \gamma \leq 1 \), with a previous attempt on identifying its origin in relaxation kinetics by Bodunov et al. in [33]. The stretchable parameter \( \gamma \) brings fitting closer to the physical decaying phenomena with time-varying ODEs such as relaxation processes in geophysics, electronics, mechanics, chemistry, biology and medicine [15], and dissipative systems, than the mono-exponential with the integer exponent \( \gamma = 1 \) resulting from an ODE with constant coefficients [13]. That author suggested four circuits including resistor, capacitor, diode, and transistor to obtain some degree of flexibility. It is important to note that the fractional KWW system itself can be expanded via (1), also known as the Prony series expansion in physics [34].

In addition to fluorescence lifetime estimation, the mono-exponential distribution is generally used in a number of other domains such as spectroscopy [15, 35], modelling radioactive decay [36], and probability and statistics for analysing diverse time occurrences such as waiting, arrival, success, and failure times [37]. Statistical models of Rayleigh and Weibull have also employed for modelling the distribution of physical variables from different systems, as a time distribution. Although these functions have not yet been employed in FLIM analysis, the historical evidence for relevant applications in the literature includes: modelling intensity of magnetic resonance imaging (MRI) image pixels in presence of noise [38], controlled release for managing the time distribution of drug delivery in body [24], life sciences for life data analysis purposes [39–41], reliability engineering for analysis of failure time on components [42], materials science for casting processes modelling [43], and wireless communications for combating the multipath fading in received signals [44, 45].

To estimate \( \tau_{\text{mean}} \), the unknown parameters of (2) should be estimated. Lifetime estimation methods can be categorised into three main groups: fitting-based, non-fitting-based, and fit-free approaches. In fitting-based procedures, a decaying function is first hypothesised for modelling the distribution of the temporal signal. Then, its unknown parameters are estimated by approaches such as least squares (LS) curve fitting [46], maximum likelihood estimation (MLE) [5], or Laguerre expansion [18]. Non-fitting-based approaches usually suggest an explicit closed-form formulation for obtaining the fluorescence lifetime [6–9]. For example, rapid lifetime determination (RLD) [6], RLD with overlapping windows (RLD-OW) [7], Robust RLD [13], Center of Mass Method (CMM) [8], and fluorescence lifetime estimation via rotational invariance techniques (FLERIT) [9] belong to this family. Fit-free methodologies rely on information visualisation [10] and learning [11, 16]. For instance, Digman et al. in [10]
However, it should be noted that the roots can be generally articulated a decay function by linearly combining $n$ roots of $r_i$. The solution $f(t)$ is valid for a homogeneous ODE with $q$ distinct real poles at $s_i = \frac{-1}{r_i}$. Proposed a 2D graphical representation of mono- or bi-exponential lifetime distribution from FLIM pixels. This works on a Fourier-domain-connected calculations called the “phasor approach”. The method requires observer’s interpretation. Additionally, fit-free machine learning-based techniques [11, 16] employ the inherent function approximation capability of neural networks to estimate parameters of a decay model by pre-training from massive amounts of synthetic data.

C. Problem statement

Let $f(t) = A \sum_{i=1}^{n} a_i e^{-\frac{t}{r_i}}$ be a truncated representation of (1). Here, we aim to highlight the function $f(t)$ is the homogeneous solution of an $n^{th}$-order linear non-homogeneous ODE with constant coefficients [47]. Assume the input-output functions $e(t)$ and $f(t)$ of this ODE represent the processes of excitation and fluorescence-emission, respectively. An equiv- alency between the radiation source and the rate of changes of the fluorescence emission exists, which describes a balanced input-output energy with the ODE of:

$$\frac{d^n f(t)}{dt^n} + a_1 \frac{d^{n-1} f(t)}{dt^{n-1}} + \cdots + a_{n-1} \frac{df(t)}{dt} + a_n f(t) = e(t), \quad (3)$$

in which $a_i, \forall i = 1, \ldots, n$, denotes a constant coefficient. The solution $f(t)$ is valid for a homogeneous ODE with $e(t) = 0$, where its corresponding characteristic function as $r^n + a_1 r^{n-1} + \cdots + a_{n-1} r + a_n = 0$ contains $n$ distinct real roots of $r_1, r_2, \ldots, r_n$. So, the $n$-exponential would be able to articulate a decay function by linearly combining $n$ segments. However, it should be noted that the roots can be generally of distinct real, repeated real and complex conjugate forms [31, 32, 34], resulting in different homogeneous solutions that should be taken into account. A best practice in (3) would be to convert the ODE-based grey box to a transparent white box with fully identifiable components like an electrical circuit (See Fig. 2.). Hence, the first question addressed in this research is: “Q1: How can we represent the fluorescence phenomenon using white-box modelling?”

Secondly, another problem with the methods developed in the literature is that they act based on only a specified presumed model; e.g., a fixed single model chosen from a small set, such as mono- and bi-exponential, is considered for describing the fluorescence phenomenon throughout all the pixels within a specimen [19]. However, different locations from a sample may not obey a given parametric model due to diversity of type, dynamics, and environment of biological substances present in the sample. This lack of flexibility introduces modelling error. Therefore, the second question is: “Q2: What model minimises curve fitting error on all the real time-resolved measurements?”

Thirdly, it is assumed that the intensity is maximum at time $t = 0$ in both mono- and bi-exponential decay. This means an impulsive rise-time, i.e., a zero growth time in the curve of Fig. 1 (b). However, because of natural lag in the physical systems, the response may not completely follow a strictly monotonically decreasing trend. Although very small, it takes a short time before the response reaches its maximum strength, as shown in the growth phase of the curve in Fig. 1 (b). In the literature, this behaviour is justified by convolution of the decay with an instrument response function (IRF) [5, 11, 13]:

$$v(t) \doteq IRF(t) * f(t), \quad (4)$$

where $f(t)$ generally denotes the multi-exponential function. For the ideal case of an IRF $(t) = \delta(t)$ with $\delta(\cdot)$ as the Dirac delta [13], (4) is simplified $v(t) = f(t) \doteq h(t)$, which means the decaying multi-exponential function is the impulse response of a FLIM system, i.e., the function $h(t)$. Fig. 2 electrically models the system, where the input energy is relaxed via different parallel pathways. An IRF can be estimated by either blind deconvolution techniques [48], or experimentally quenching processes of fluorescent components [49]. Physically, the temporal response of fluorescence first follows a rise (called a “growth phase”) and then a fall (called a “decay phase”) trend similar to any charging and discharging events, as shown in the curve in Fig. 1 (b). We define a fluorescence “life cycle” as the sum of growth and decay phases. Nevertheless, if the tunable parameter that is the time bin width is selected sufficiently large, a strictly monotonically decreasing curve may be observed due to combining photons of neighbouring bins [4], preserving the importance of the models. The problem is in connection with the technological limitation on temporal resolution of sensing electronic devices (about few picoseconds in TCSPC-based technique [50]). It prevents high resolution details of the rise-time or natural fluctuations of the time series. Fig. 3 shows samples of the time series. We define a fluorescence “life profile” as the shape or envelope of a time series regardless of any growth or decay local fluctuations. Our third research question is: “Q3:
What error metric should one use for selecting the optimal descriptor among a set of fluorescence life models?"

D. Our approach and contributions

To tackle the problems of limited and rigid life model [19, 31, 32], we introduce a novel, fourth family of estimators termed a fit-flexible approach. This process is similar to model selection techniques used in statistical modelling and parameter estimation [51], but is extended to consider further physical constraints. To help motivate the models, we first build on the work in [32] and scale down the whole complex photonic process of time-resolved fluorescence imaging as an electric circuit by leveraging their analogy as will be discussed in Section II. Specimen’s microorganisms are modelled as a network of parallel RLC circuits as shown in Fig. 1 (a). To detect matched profiles in connection to circuits’ responses, we design a fluorescence “life model-set”. We have considered 1st- and 2nd-order dynamical systems [52]. The benefits of these models are the low-order simplicity and the appropriate coverage of systems dynamics. We specifically derive life circuits where their responses lead to a few well-behaved statistical distributions that can fit different shapes of histogram of photo counts in practice. In a search mechanism, we select the optimal life model order and model type describing the shape or envelope of a time series regardless of any growth or decay local fluctuations. In the paper is concluded in Section VI.

Our experiments on the lung demonstrate that quantifying both the fall-time as a stacked histogram in terms of models’ distribution and the life pattern map expose informative contrast among points. These act as complementary information about behaviour of a sample. It may be useful in discovering molecular and cellular structural information towards diseases treatment. Our contributions and novelties are:

- analogically modelling FLIM system with explainable electrical circuits; and,
- separating microbeads from lung tissue by our imager.

In the remainder of this paper, Section II electrically models the process of FLIM. Section III designs an algorithm for the proposed imager. In Section IV, we prepare both synthesised and actual experiments to validate our approach. Section V discusses cost-benefit analysis of the fit-flexible approach, and the paper is concluded in Section VI.

II. Electrical modelling

A. Excitation-emission modelling

Based on the Jablonski diagram, when a specimen is excited, electrons of its excited molecules move from a ground state to an excited state and may or may not release photons of visible light and then come back to the base state [26]. Similarly, in an RLC circuit, after flowing periodic current, electrons in the circuit move to establish the fast events of charging and discharging. With this analogy, we desire to model the whole process electrically to give a physical interpretation for the theoretical models of photon counting, as shown in Fig. 1 (a). We use the pair of the current \(i(t)\) and voltage \(v(t)\) functions as representatives of the excitation \(e(t)\) and fluorescence-emission \(f(t)\) functions, respectively. It is also possible to equivalently describe the whole process as a mechanical system containing mass-spring-damper components or by bond graph theory [52]. However, electrical circuits have been chosen simply to reflect both the nature of electron movement and convenient means for physical interpretation of relaxation phenomena by inspiration from [32].

B. Specimen modelling

Modelling biological systems [21] tries to understand real biochemical processes for goals such as synthesising artificial biological systems with similar functions. To model a specimen such as lung tissue, we discretise the surface of the continuous sample into infinite extremely small units, each modelled by an RLC circuit excitable by an external laser. The light flow passes through the sample, introducing light reaction as photon emission, and heat and gas propagation as negligible absorption events. To electrically translate this, a spot of the specimen should contain both storage and load elements. A storing element, whether the capacitor \(C\) or the inductor \(L\), is first charged by the incoming light and then discharged via an Ohmic load like a light bulb model as a representative of the resistor \(R\) [32]. Therefore, each unit of the sample is modelled by a linear, parallel RLC circuit.

<table>
<thead>
<tr>
<th>Terminology</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth phase</td>
<td>The duration in a time series starting from the time zero to reaching the maximum intensity</td>
</tr>
<tr>
<td>Decay phase</td>
<td>The duration in a time series starting from the maximum intensity to the asymptotic dissipation</td>
</tr>
<tr>
<td>Life cycle</td>
<td>The sum of the growth phase and the decay phase</td>
</tr>
<tr>
<td>Life profile</td>
<td>The shape or envelope of a time series regardless of any growth or decay local fluctuations</td>
</tr>
<tr>
<td>Life model-set</td>
<td>A set of mathematical functions describing different time series</td>
</tr>
<tr>
<td>Life pattern map</td>
<td>An image arrangement of different life profiles in a 2D space visualised by distinct colours</td>
</tr>
<tr>
<td>Fall-time</td>
<td>The time at which a representative life profile falls 1/2 its maximum intensity</td>
</tr>
</tbody>
</table>

https://doi.org/10.7488/ds/7662

2https://doi.org/10.7488/ds/7662

TABLE I: Terminology and definitions used in the paper

1st-...
C. Laser modelling

A pulsed laser, as an illumination source [53], generates short-duration focused light pulses [14]. It can be generally modelled by a current impulse train plus a DC shifter as:

\[
i(t) = c_1 \sum_{k=0}^{K-1} \delta(t-kT) + c_2 u(t). \tag{5}
\]

Currents \(i_1(t)\) and \(i_2(t)\) model pure periodic laser impulses and a residual average power spread in-between the pulses as an imperfection, respectively. The operator \(u(\cdot)\) and \(T\) denote the Heaviside step function and the laser repetition rate, respectively. The arbitrary constants \(c_1\) and \(c_2\), and \(K\) respectively represent the amplitude of the impulse, the DC shift, and the number of excitation pulses per spot. In current lasers, the repetition rate is between nano- and micro-second respectively. Hence, they are being established independently. Consequently, three models are derived from the \(1\)-order circuits that solve the differential equation corresponding to the highest derivative order is unity [56]. A passive analogue RLC circuit is in nature a \(2\)-order system. Three possible responses exist based on the position of the roots of the characteristic equation, namely over- (equivalent to bi-exponential), critically-, and under-damped [47]. We derive \(2\)-order circuits that solve their ODEs resulting in the definition of three proposed bi-exponential, critically-, and under-damped life models. In the under-damped case, it is notable that decaying oscillations are from the nature of stochastic dynamical systems such as FLIM [57]. The periodic fluctuations are seen as an aggregation of the dynamics, including: sample behaviour, intrinsic characteristics of detector hardware (e.g., the undesirable effect of afterpulsing in TCSPC electronic circuits [58] or the chaotic avalanches in SPAD electronic circuits [59, 60]), and other unobservable latent variables such as vibrational relaxation and internal conversions [61]. Our experimental observations confirm these fluctuations have a periodic meaningful wave-like pattern, which are somewhat different from random noise or outlier points. An equivalent resistor in the under-damped (U-dmp) circuit exists which represents the consuming fluorescent components. So, it damps the amplitude of oscillations of the interchangeable energy between \(L\) and \(C\) over time. We extract the fall-time from the envelope of fluctuations of U-dmp model, maintaining it appropriate for FLIM information extraction.

D. Analogue electronic measurements modelling

The measurement equipment in time-resolved imaging can be modelled by an AC voltmeter recording a circuit’s response. Fig. 1 (a) embodies our modelling. We consider the capacitance of the capacitor as

\[C = \frac{Q}{V},\]

which reduces to

\[C = \frac{\text{charge}}{\text{voltage}} = \frac{\text{energy}}{\text{voltage}^2} \text{ (for a parallel plate capacitor)}\]

where \(Q\) is the total charge stored on the capacitor, \(V\) is the voltage across the capacitor, and \(E\) is the energy stored. The relationship between the charge, voltage, and energy is given by

\[E = \frac{1}{2}CV^2\]

These models can be considered as special cases of Weibull’s function with the integer time exponents \(b = 1\) and \(b = 2\) in Table II, respectively. Hence, they are being established independently. Consequently, three models are derived from the \(1\)-order modelling. Finally, six comprehensive \(1\)st- and \(2\)nd-order

Fig. 4: A representation of the proposed fit-flexible fluorescence sensing for the application of time-resolved imaging on the video frame #6 of a microscopic biological bacteria sample containing 16 frames. On the right side, the histograms of photon counts at four distinct pixels are visualised. The table attached to each histogram shows the Misfit-percent for different models.
### III. Algorithmic Implementation

#### A. Real-world digital measurements

In actual measurements, the number of counted photons at each time bin of the histogram of photon counts is a non-negative value \([13]\). This means that unquantised amplitudes of a circuit voltage response should satisfy the real constraint \(v(t) \in \mathbb{R}^+\). If any deviations exist, the negative parts of the signal model should be treated by rectification. Specifically, the situation is seen for the sinusoidal response of the underdamped model in Table II (See also Fig. 3 in \([32]\)). This can be electrically interpreted as passing the response through a representative full-wave rectifier implementable by schemes including two or four perfect diodes. Generally, the rectified response can be mathematically modelled by:

\[
v(t) \leftarrow |v(t)|, \tag{6}
\]

where \(| \cdot |\) means absolute function. Hence, for the underdamped model, the new assigned version of \(v(t)\) is applied for parameters estimation. Additionally, photon counting is done in practice at discrete time bins. If variables \(\Delta \) and \(N\) are

<table>
<thead>
<tr>
<th>Model</th>
<th>Equivalent circuit</th>
<th>Components</th>
<th>Input/output equations</th>
<th>Specifications</th>
<th>Application areas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mo-S</td>
<td>( i(t) ) [ R \ C \ \pm \</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rayl</td>
<td>( i(t) ) [ R \ C \ \pm \</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Web</td>
<td>( i(t) ) [ R \ R \ L \ C \ \pm \</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bi-S</td>
<td>( i(t) ) [ R \ L \ L \ C \ \pm \</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-dmp</td>
<td>( i(t) ) [ R \ L \ L \ C \ \pm \</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U-dmp</td>
<td>( i(t) ) [ R \ L \ L \ C \ \pm \</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TABLE II: A summary of our developed fluorescence life circuits

models constitute our life model-set. Table II summarises our developed life circuits, and Fig. 3 illustrates synthesised life profiles. Their corresponding functions can approximate well different shapes from histogram of photon counts. We have motivated the choice of a set of life models are physically meaningful rather than an arbitrary choice of waveforms, as often seen in model selection problems. We have also spotted applications of life circuits. Section S3 from the SMs provides proofs of life models.
respectively the bin width and the number of bins for a histogram, the discrete representation \(\tilde{v}[n], \forall n = 0, 1, \ldots, N-1\), of the continuous response \(v(t)\) can be generated by replacing \(t\) with \(n\Delta\) in life models. The signal \(v[n]\) is characterised in the column of "Specifications" from Table II for each model. An algorithmic implementation of our method, depicted in Fig. 1(b), is summarised in Algorithm 1.

**Algorithm 1 Our fit-flexible fluorescence soft imager**

1. **Inputs:** The \(\mathbb{Z}^{h \times w \times N}\) fluorescence tensor data including a time-resolved histogram \(\tilde{v} = [\tilde{v}_0, \tilde{v}_1, \ldots, \tilde{v}_{N-1}]^T\) at each pixel \((r,c), \forall r = 0, 1, \ldots, h-1, c = 0, 1, \ldots, w - 1,\) and the \(M\)-element life model-set \(\mathcal{M}\).
2. **Outputs:** Maps of intensity \(\lambda = [\lambda_{r,c}] \in \mathbb{Z}^{h \times w}\), fall-time \(\Psi = [\psi_{r,c}] \in \mathbb{R}^{h \times w}\), life pattern \(\Phi = [\phi_{r,c}] \in \mathbb{Z}^{+ \times h \times w}\).

3. for \(r \leftarrow 0, h - 1\) do:
4. for \(c \leftarrow 0, w - 1\) do:
5. Acquire the histogram \(\tilde{v}\) belonging to point \((r,c)\).
6. for \(j \leftarrow 1, M\) do:
7. Estimate \(\hat{\theta}^{(j)}\) in Table II for \(\mathcal{M}\{j\}\) by LS fit.
8. Recover \(\hat{\psi}(j)\) in (8) by replacing parameters.
9. Obtain Misfit-percent \(e_j = e(\hat{\psi}, \hat{\theta}^{(j)})\) by (9).
10. end for
11. Compute \(j^*\) in (10).
12. Estimate intensity by (14) as \(\lambda_{r,c} \leftarrow \hat{I}_{\text{out}}\).
13. Feed \(\hat{\psi}(j^*)\) to FDP to estimate fall-time \(\hat{\tau}_{r,c} \leftarrow \hat{\tau}_t\).
14. Initialise life profile label as \(\phi_{r,c} \leftarrow j^*\).
15. Update the life profile label using penaliser.
16. Assign unknown class where required.
17. end for
18. end for

**B. Stochastic modelling**

Measurements in the real world are noisy, but not deterministic as modelled in Section II. This means the deterministic life model of \(v[n] \in \mathbb{R}^+\) should be contaminated by a representative random component. Various dependent and independent noise sources from photon counting equipment and instrument ambient disturbances exist [13]. Their collective effect can be considered as additive noise by a Poissonian distribution of \(\eta[n] \sim P(\lambda)\), in which the parameter \(\lambda \in (0, \infty)\) denotes the mean rate of shot noise photons. Hence, actual measurements for each model can be rewritten as:

\[
\tilde{v}[n] = v[n] + \eta[n] = s[n] + \eta[n],
\]

where \(\tilde{v}[n] \in \mathbb{Z}^+\) and \(\eta[n] \in \mathbb{Z}^+.\) The symbol \(\mathbb{Z}^{+}\) represents the set of all positive integers. The operator \([\cdot]\) means round function to mimic physical quantised measurements, where \(s[n]\) is defined the original signal.

**C. Life-model’s parameters estimation and selection**

Consider the multi-parameter models in Table II as the set \(\mathcal{M} = \{\text{Mo-xp}, \text{Rayl}, \text{Weib}, \text{Bi-xp}, \text{C-dmp}, \text{U-dmp}\}\). Any other uncertainties are assigned to an extra "unknown class". For the \(j^{th}\) model, parameters can be represented by the vector \(\theta^{(j)} = [\theta_0, \theta_1, \ldots, \theta_{K_j-1}]^T\), where \(K_j, \forall j = 1, 2, \ldots, M\), denotes the number of parameters of \(j^{th}\) model. Also, \(M \geq |\mathcal{M}| = 6\) means the number of models of the life model-set, where \(|\cdot|\) represents the cardinality of a set. Our method can be expanded to other candidate models. The unknown parameters are identified from available measurements of histogram of photon counts. The problem can be formulated by a parameter estimator. We utilised the optimised nonlinear LS with the “trust region” algorithm [46] for estimating the unknown vector as \(\hat{\theta}^{(j)}\). Once the vector \(\theta^{(j)}\) was determined for the \(j^{th}\) model, its related fitted curve can be calculated by replacing the estimated parameters into its corresponding discrete response, definable as the vector of:

\[
\hat{\psi}(j) = [\hat{\psi}_0^{(j)}, \hat{\psi}_1^{(j)}, \ldots, \hat{\psi}_{N-1}^{(j)}]^T.
\]

Afterwards, our method contains a mechanism of model selection [19] below.

**Misfit-percent criterion:** To select an optimal curve describing the best data trend, various badness-of-fit (BoF) or goodness-of-fit (GoF) objective functions may be employed [62]. Generally, BoF criteria such as the two-sample Kolmogorov-Smirnov (K-S) difference [63], Kullback-Leibler (K-L) divergence [64], chi-square [13], mean squared error (MSE) [13], normalised root mean square error (NRMSE) [65] and symmetric mean absolute percentage error (SMAPE) [66], or GoF metrics such as the correlation coefficient (CC) [64] can be used. However, these metrics suffer from two main problems: 1) being limited in terms of fidelity and robustness, or 2) being non-fully normalised. The former causes inefficient model selection in noisy situations; e.g., MSE may only work well for head (bins with higher intensities) fitting of the skewed life distributions, whereas the chi-square measure is loyal more to tail fitting [13]. The latter hardens understanding the rate of a criterion; e.g., consider the task of thresholding on a non-normalised value, which would not be straightforward by user (See (14) for a problem of thresholding.). To tackle them, we have proposed a novel, simple yet efficient error metric for model selection, called the Misfit-percent. This calculates the sum of absolute error between the actual histogram of photon counts and an estimated curve on all bins and normalises the result to the union of the curves as the whole possible photons space. Generally, Misfit between the estimated \(\hat{\psi}\) and actual \(\psi\) vectors is defined in \% as:

\[
\text{Misfit-percent} = \frac{100 \sum_{i=0}^{N-1} |\hat{\psi}_i - \psi_i|}{\sum_{i=0}^{N-1} \max(\hat{\psi}_i, \psi_i)}.
\]

We redefine the entry \(e(\tilde{\psi}, \hat{\psi}^{(j)})\), \(\forall j = 1, 2, \ldots, M\), as the error of Misfit-percent between the vectors of actual histogram \(\tilde{\psi} = [\tilde{\psi}[0], \tilde{\psi}[1], \ldots, \tilde{\psi}[N-1]]^T\) and \(j^{th}\) estimated model \(\hat{\psi}^{(j)}\) and arrange it over all models as \(e = [e(\tilde{\psi}, \hat{\psi}^{(1)}), e(\tilde{\psi}, \hat{\psi}^{(2)}), \ldots, e(\tilde{\psi}, \hat{\psi}^{(M)})]^T\). The label of optimal life model for a pixel can be detected by minimising:

\[
j^* = \arg\min_j(e).
\]

The minimised Misfit-percent model is referred to intensity and fall-time estimators as well as life profile detection.
D. Intensity estimation

A summation on bin-wise photons is considered as intensity per histogram in FLIM [2] (called an “empirical mode”) as:

$$\hat{I}_{\text{usual}} = \sum_{i=0}^{N-1} \hat{v}_i = \mathbf{1}^T \hat{\mathbf{v}},$$  \hspace{1cm} (11)

which $\mathbf{1}$ denotes a column-wise vector of all ones. Although this sort of integration has an inherent smoothing property due to the summation, the intensity still is calculated from a mixture of signal and noise, where we have from (7) that:

$$\hat{\mathbf{v}} = \mathbf{s} + \eta.$$  \hspace{1cm} (12)

It can be shown that the noisy regime propagates a bias shift of $N\lambda$ photons and a standard deviation of $\sqrt{N\lambda}$ photons in estimating the intensity. So, one ideally desires to determine the intensity from the original signal $\mathbf{s}$ alone, i.e., $\hat{I} = \mathbf{1}^T \hat{\mathbf{s}}$. Practically, this necessitates a histogram of photon counts denoiser for alleviating the effects, so that:

$$\hat{\mathbf{I}} = \mathbf{1}^T \hat{\mathbf{G}}(\hat{\mathbf{v}}),$$  \hspace{1cm} (13)

in which the operator $\hat{\mathbf{G}}(\cdot)$ is the denoiser.

The denoising operator can be generally designed of various forms. For instance, it can be a non-parametric smoothing filter such as Savitzky-Golay filter [13] (called a “smoothed mode”), or a parametric fitting model (called a “fitted mode”). Our approach, as a type of the latter, leverages the capability of life recovery to estimate the intensity, too. We define $\hat{\mathbf{v}}_{(j^*)} = \hat{\mathbf{G}}(\hat{\mathbf{v}})$. In practice, to control any failed fitting, if Misfit stays below the fitting failure threshold $T_{AM}$, we rely on the integral of optimal fitted curve as a filtered, smoothed signal; otherwise, it is estimated as usual via (11). The proposed estimator is formulated to:

$$\hat{I}_{\text{out}} = \begin{cases} \mathbf{1}^T \hat{\mathbf{v}}_{(j^*)}, & e(\hat{\mathbf{v}}, \hat{\mathbf{v}}_{(j^*)}) < T_{AM} \\ \mathbf{1}^T \hat{\mathbf{v}}, & \text{otherwise} \end{cases}.$$ \hspace{1cm} (14)

E. Fall-time determination procedure

Conventional FLIM assumes a monotonically decaying curve for applying tail fitting, whereas underlying life distributions may be generally left or right skewed, or even symmetric. A skewed distribution has three characteristics of mode (its peak point), median, and mean (also defined as centre of mass [8] or the first moment). For imaging in the right manner, distinguishing the features is crucial. To this intent, we have measured fall-time as graphically explained in Fig. 1 (b). The value of distributions characteristics can be determined mathematically or computationally. The former requires to analytically derive an equation for each life mode as a function of model parameters as presented in Table II. If during the fitting process, a failure in estimating one or more parameters exists due to lack of control on noisy data, computations are being wrong, physically meaningless. For example, a lower bound for bi-exponential fall-time in terms of parameters $(\alpha, \tau_1, \tau_2)$ is:

$$\tau_1 \geq \frac{(1 - \frac{1}{e})\tau_1 \tau_2}{(1 - \alpha)\tau_1 + \alpha \tau_2},$$  \hspace{1cm} (15)

where it is derived in Section S4 of the SMs. However, in the latter, the parameter $\tau_1$ can be graphically computed from profile’s shape with less sensitivity to parameters $(\alpha, \tau_1, \tau_2)$.

As seen from the curve of Fig. 1 (b), at most two points cross the red line corresponding to the amplitude $\frac{1}{\varepsilon}$, one corresponding to the rising edge, and another due to the falling edge. To improve the tail fitting overall, the estimated fall-time, $\hat{\tau}_1$, is determined at falling edge of the response. To analyse the intersection point for a given life model, we calculate the slope at crossing points. For the rising and falling edges, the slope is identified respectively positive and negative. Nevertheless, for a measurement window, it may happen that such a crossover does not exist in the falling edge, for example, because of slow damping. In this case, we quantise the fall-time to a predefined span value such as $\hat{\tau}_1 = N\Delta$. In terms of estimated parameters, the profile of a fitted model may not always follow from a reasonable shape such as the first growth and then decay trend shown in the curve of Fig. 1 (b). Generally, five main possible rise and fall forms may occur in real scenarios, which are controlled in the fall-time determination procedure (FDP) of Algorithm 1 for obtaining $\hat{\tau}_1$. Section S5 from SMs provides details of these cases. It is notable that, for the fall-time determination in the U-dmp model, the envelope of the rectified sinusoidal response, namely $\hat{\mathbf{v}}(t) \rightarrow Ae^{-\hat{\tau}u(t)}$, is used as the input. Although the valid $\hat{\mathbf{v}}_{\text{m}}$ is calculated from the original rectified version in (6).

F. Life profile extraction and parsimony

A decision about detecting a fluorescence life profile relies on selecting the model with minimum Misfit-percent in (10). Due to following reasons, this alone will not lead to accurate outcomes. A model from a “model set” may have different shapes, but can also take parameters from an infinite interval. Therefore, in practice, different mathematical functions from the model set may be equivalent to each other for some specific vectors of parameters, and consequently generate similar functional forms [67]. In our model set, this can be seen between the naturally flexible model of Weibull and other models. As clear examples, see specifications of mono-exponential and Rayleigh models in Table II. This reveals that further rules are required to investigate model parameters and improve the chance of deciding the right profile. We check consistency of estimated parameters with physical constraints such as those mentioned in (1) and stability criterion. If we observe any inconsistencies, the corresponding model is penalised to be able to select the best descriptive label for a life profile. To this intent, consider the matrix of fluorescence life pattern map as $\Phi = [\phi_{r,c}]_{H \times W}$, $\forall \phi_{r,c} \in \mathbb{Z}^+$. We first initialise $\phi_{r,c} \leftarrow j^*$. Then, the entry $\phi_{r,c}$ is updated using a penaliser if necessary. In addition to the parameters control mechanism, we considered a parsimonious strategy in establishing penalising rules; namely, if the difference of Misfit-percent between two models is less than a threshold, a 1st-order system is preferred than a 2nd-order one. We set the rules according to our optimisation procedure. Nonetheless, important rules that may change the current state of a fluorescence life profile in (10) are itemised and detailed in Section S6 from SMs. Each
A. Evaluation of imaging on synthetic samples

expressed in Section S7 from SMs. We have also defined the unknown class of Class #7 for more control on uncertainties in the proposed profile detection, as expressed in Section S7 from SMs.

IV. EXPERIMENTS

A. Synthesised imaging on synthetic samples

1) Synthesised-data generation and visualisation: We have generated synthetic data for simulation of sensing biological specimens based on a fibre bundle-based imager [2, 68]. Fig. 5 depicts a ground truth (GT) image of a synthesised life pattern map. As shown in the colour bar of Fig. 5, each colour represents an individual life model. The histograms of photon counts for each pixel are obtained using the generative model in (7). Fig. 3 plots the shape of life profiles at six separate locations of the fluorescence life regions in Fig. 5 from a random run. Section S8 from SMs explains setting of the number of photons per histogram for a model. We added Poissonian noise with the rate \( \lambda = 4 \). The vectors of parameters of models are reported in the legend of Fig. 3. Other parameters are: \( N = 64 \), \( \Delta = 0.1 \) ns, and \( T_{AM} = 10\% \).

Fig. 6 visualises our imaging framework (See also Fig. S3 from SMs that visualises Misfit-percent error.). The representation contains an interesting example with the following two cases:

- Case I: Weib. and Bi-xp share the same intensity but different fall-times. These are respectively equivalent to the numbered regions 3 and 4 in Fig. 5. As seen in Fig. 6, the regions are not separable in the intensity map. Instead, the fall-time map reveals the differences. This demonstrates how time-resolved fluorescence fall-time/lifetime imaging can surpass steady state intensity sensing.

- Case II: As a generalised case, Mo-xp and C-dmp models respectively corresponding to the numbered regions 1 and 5 in Fig. 5, expose both the same intensity and fall-time but have different life profiles in Fig. 6. Neither the intensity map nor the fall-time map cannot discriminate. However, they are separated in the life pattern map. This demonstrates the added value of our proposed life profile sensing, providing complementary information for high-level interpretations.

The visualisation in Fig. 6 contains 6 subplots, where from top to bottom and left to right include respectively: maps of intensity, fall-time and life pattern, intensities’ histogram, a stacked histogram of fall-times that accounts for the distribution of each life model across time bins, and a bar chart which represents models portion in percent. A fall-times’ stacked histogram can generally provide multi-modal distributions that make our model attractive for higher level analyses such as segmentation by valley thresholding. For instance, see the valley at \( t \approx 2.3 \) ns between the two peaks in the fall-times’ stacked histogram of Fig. 4. The pixel-wise classification capability of life pattern map can reveal microscopic structures of a specimen. It provides complementary contrast information as coherent shapes such as distinct islands. The information can also be employed in other tasks like co-registration, fluorescence data classification, and image-to-image translation.

2) Bias and variance of estimating intensity and fall-time: Bias and variance (\( \sigma^2 \)) are two metrics for measuring efficiency of an estimator as the indicators of Accuracy \( \alpha_{\text{Pred}} \) and Precision \( \alpha_{\text{Pred}} \), respectively. For practical analyses, Table III reports metrics of the GT, mean (\( \mu \)), standard deviation (\( \sigma \)), Bias, and SNR of both estimated intensity and fall-time values for different regions of Fig. 6. We measured Bias in \( \% \) by:

\[
\text{Bias} = \frac{100}{\max(\mu, \text{GT})} \left| \frac{\mu - \text{GT}}{\mu} \right|
\]  

as well as signal-to-noise ratio (SNR) [69] in dB by:

\[
\text{SNR} = 20 \log \left( \frac{\mu}{\sigma} \right)
\]  

Fall-time GT information was calculated from the computational procedure described in Section III-E in the noise-less case. Generally, comparing GT values to overestimated results notifies acceptable Accuracy and Precision. Specifically, for intensity estimators, bold values in the columns of “Usual” and “Our” highlight better results from their point-by-point comparisons. Our estimator in (14) exposes lower Bias due to the alleviated \( \mu \) shift with controlled variance than the usual approach in (11), capable of better balancing in-between. We adjusted the threshold \( T_{AM} = 25\% \) based on examining the information of error map (See Fig. S3 from SMs on this).

Also, for our fall-time estimator, the desirable results among models with the lowest Bias and the highest SNR are in bold type. The best Bias and SNR belong to models of U-dmp and C-dmp, respectively. Section S11 from SMs also repeats our experiments for Gaussian noise.

3) Confusion table of life profile detection: Here, for a more comprehensive evaluation of the proposed method, in addition to the parameters set marked in Fig. 3 (called “The Parameters Set 1”), we designed three other parameters sets resulting in diverse profiles. Section S9 from SMs provides details of The Parameters Sets 2 to 4. The confusion matrix of life profile detection for The Parameters Set 1 has reported in the core table of Fig. 7. For classes 1 to 3 and almost class 4 (with only 2 misclassified pixels as Mo-xp), the classification is perfect. However, more misclassification errors mainly between classes 5 and 6 are seen as is confirmed in the upper right image of Fig. 6. The origin of errors is the similarity of their estimated distributions. A number of pixels of individual classes of 5 and 6 had been misclassified as class Weib. as well. The side vertical and horizontal tables in Fig. 7 report recall (accuracy)
Fig. 6: Visualisation of our imager. This shows both the regions discriminability of Weib. and Bi-xp in the fall-time map (Case I), and Mo-xp and C-dmp in the life pattern map (Case II).

TABLE III: Efficiency of intensity and fall-time estimators

<table>
<thead>
<tr>
<th>Model</th>
<th>Intensity</th>
<th>Fall-time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GT (photons)</td>
<td>Usual</td>
</tr>
<tr>
<td></td>
<td>$\mu \pm \sigma$ (photons)</td>
<td>Bias (%)</td>
</tr>
<tr>
<td>Mono-exponential</td>
<td>3000</td>
<td>(3256 ± 16)</td>
</tr>
<tr>
<td></td>
<td>Rayleigh</td>
<td>2000</td>
</tr>
<tr>
<td></td>
<td>Weibull</td>
<td>1000</td>
</tr>
<tr>
<td></td>
<td>Bi-exponential</td>
<td>1000</td>
</tr>
<tr>
<td></td>
<td>Critically-damped</td>
<td>3000</td>
</tr>
<tr>
<td></td>
<td>Under-damped</td>
<td>3500</td>
</tr>
</tbody>
</table>

Fig. 7: The chart of confusion matrix of life profile detection.

TABLE IV: Characteristics of the human lung experiment

<table>
<thead>
<tr>
<th>Sample</th>
<th>Label</th>
<th>Probe/dye (relative intensity)</th>
<th>Shutter open</th>
<th>$N_f$</th>
<th>Red</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beads in saline</td>
<td>A1</td>
<td>InSpek$^{TM}$/Green (0.3 %)</td>
<td>Blue</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>Beads in saline</td>
<td>A2</td>
<td>Sphero$^{TM}$/Red (low)</td>
<td>Blue, orange</td>
<td>18</td>
<td>1, 2</td>
</tr>
<tr>
<td>The lung alone</td>
<td>B1</td>
<td>-</td>
<td>Blue</td>
<td>15</td>
<td>1, 2</td>
</tr>
<tr>
<td>The lung alone</td>
<td>B2</td>
<td>-</td>
<td>Blue</td>
<td>15</td>
<td>1, 2</td>
</tr>
<tr>
<td>The lung+beads</td>
<td>C1</td>
<td>InSpek$^{TM}$/Green (0.3 %)</td>
<td>Blue, orange</td>
<td>19</td>
<td>1</td>
</tr>
<tr>
<td>The lung+beads</td>
<td>C2</td>
<td>Sphero$^{TM}$/Red (low)</td>
<td>Blue, orange</td>
<td>15</td>
<td>1, 2</td>
</tr>
</tbody>
</table>

$^*$Red stands for spectral Band(s) of Interest in investigation.

4) Fidelity and robustness of Misfit-percent criterion: This section investigates how the criteria stay stable by increasing noise levels. Here, we have compared Misfit-percent in (9) to the metric of Neyman’s chi-square test [13], a proposed

63.89%, 85.25%, and 97.07% for The Parameters Sets 1 to 4, respectively, which demonstrate reproducibility of results over the diversity of profiles’ shapes and parameters. Averaging on all sets gives a promising 86.15% accuracy.
stable behaviour across rates. In intense noises of Parameters Set 3. Misfit exposes competitive results with (19). Also, in (20), the symbol \( \chi^2 \) acts as a specific type of GoF metric, normalised between rate. To overcome the issue, we converted it back into a BoF polyed in our framework, because of using the error percentage remains outlier-robust in comparison to Misfit-percent proposed that spike outliers appear, our criterion outperforms others. Our levels with mean rates of \( \lambda \) life profile detection for different metrics vs various noise Identification Toolbox [65]. Fig. 8 plots the total accuracy of the Parameter Set 1 with the combined noise sources. reports detailed numerical comparisons of competing methods for The Parameter Set 1 with the combined noise sources. Highlighted values show the superiority of our approach, where other ones cannot consider the dynamics of the GT properly. Also, for the photon budgets, Fig. 9 (b) reports accuracy of life profile detection, as a unique property of our imager, in presence of three Poissonian noise alone, Gaussian noise alone, and both. As shown, it only fails in the low-photon budget (i.e., the minimum energy), where the intense distortion level destroys discriminability of profiles.

5) Performance under different photon budgets: Here, an experiment is designed to evaluate the performance under low, mid, and high photon-count regimes. We have compared our approach to CMM [8], Poisson MLE [5], RLD-OW [7], and Robust RLD [13]. To be able to compare the proposed fall-time imaging to the single-exponential-based benchmark methods, we first set a GT lifetime of about 2 ns for the region 1 as the representative of single-exponential. Then, we forced fall-times of the remainder regions of 2 to 6 into almost the same lifetime. Across all regions, the number of photons per histogram (i.e., noise-less intensity) was adjusted by about 350, 1800, and 6900 for the low, mid, and high photon-count budgets [13], respectively. Afterwards, the fluorescence signals were contaminated by a selective combination of additive Poissonian (\( \lambda = 2 \)) and Gaussian noise (the same mean and variance equal to 2). Fig. 9 (a) represents outcomes of fall-time/lifetime estimation. In mid- and high-photon budgets, our approach exhibits the most reliable results in terms of the bias and variance, whereas other methods act with a wrong model, except for the region 1. This means their results are not generalisable. To investigate the generalisation, Table V reports detailed numerical comparisons of competing methods for The Parameter Set 1 with the combined noise sources. Photon-count budget

<table>
<thead>
<tr>
<th>Photon-count budget</th>
<th>Estimated fall-time/lifetime (ns)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMM</td>
<td>Poisson MLE</td>
</tr>
<tr>
<td>Low</td>
<td>Ground truth</td>
</tr>
<tr>
<td>Mid</td>
<td>Ground truth</td>
</tr>
<tr>
<td>High</td>
<td>Ground truth</td>
</tr>
</tbody>
</table>

Fig. 9: Performance evaluation under different photon-count budgets. (a) A comparison among fall-time/lifetime estimation of different approaches; the "I-shape" red line on each bar denotes the extent of standard deviation of its corresponding estimator, where generally shorter is better. (b) Life profile detection accuracy in three noisy environments. In the low, mid, and high photon-count budgets, the number of photons per histogram before adding noise is about 350, 1800, and 6900, respectively.
fluorescence imaging from an ex vivo human lung model [2], with the alveolar space spiked with fluorescent microspheres as a surrogate for fluorescently labelled bacteria. This was designed as an experimental mimic of recently reported optical endomicroscopy based imaging of cases of suspected ventilator associated pneumonia in a clinical setting [70]. A challenge in its data is the spectral overlap between the labelled bacteria and lung autofluorescence, limiting the imaging sensitivity. We estimated fluorescence intensity, fall-time, and life profile to determine whether additional features could be identified with our approach than the steady-state intensity imaging.

All experimentation using human samples were performed following approval of the appropriate regional ethics committee (REC), NHS Lothian, and the South East Scotland Research Ethics Committee 02 (reference 11/SS/0103), and with informed consent. The human lung was obtained from a solid organ donor after being declined by all UK transplant centers as being unsuitable for transplantation. The lung was prepared and ventilated as described in [71]. InSpek™ Green ($\lambda_{ex} = 505$ nm, $\lambda_{em} = 515$ nm) 6 µm Beads, 0.3% intensity (ThermoFisher, I14785), and Sphero™ 1.7-2.2 µm Fluorescent Purple Particles ($\lambda_{ex} = 590$ nm, $\lambda_{em} = 620$ nm), low intensity (Sphero™ Tech, FL-2062-2) were each diluted 1:10 into sterile 0.9% NaCl (Baxter). An amount 100 µL of each dilution was instilled to a defined region of the lung by needle and syringe, and imaging was performed by bespoke FLIM system and endoscopy imaging fibre [2, 13, 71]. Also, an amount 100 µL of the prepared beads in saline were imaged.
Fig. 11: A visualisation of fitting results and temporal signals. The most left figure shows marking the points of bead (the location x) and lung tissue (the locations y and z) on the life pattern map of Sample C₁ (Band 1, Frame 5). Fitting tables and histograms of photon counts from the second left to the most right figures are for the pixels in x, y, and z with rows and columns of (43, 40), (37, 45), and (67, 108), respectively. The numbers in each plot show differences of Misfit-percent between the best fitted model and the baseline mono-exponential as well as that one with the second rank.

Fig. 12: Comparison of average histogram recovery error.

under the same parameters. We fed samples to our imager as well as conventional FLIMs.

2) Samples’ imaging, outcomes and comparison: We used a confocal [72, 73] laser scanning endomicroscope to acquire spatio-temporal-spectral fluorescence data. Each spot of a specimen is first excited and then sensed with the same focal points. Raster scanning all spots provides an in-focus time-resolved image. Our imaging system consists of an integration of a laser, an electro-mechanical-optical scanner, a flexible optical fibre bundle [68] for in vivo imaging of organs tissue (the lung), an electronic unit containing photon sensing and detection hardware, and a PC [2]. Data were captured with an image size of $128 \times 128$ pixels, 85 $\mu$s acquisition time per pixel, 400 ps bin width, and 16 time bins. Laser excitation was at 480 nm and 590 nm, with collection in spectral bands of green (Band 1: 498 $\sim$ 570 nm) and red (Band 2: 594 $\sim$ 764 nm). Each sample contains $N_1$ video frames. Fig. S16 in SMs shows a data format of the imager. Table IV summarises information and acquisition data regarding the samples.

Figs. 10 (a) and (b) visualise outputs of our imager and compare them to competing methods for two different image representations. As seen, diverse representations with and without the same colour bars lead to visually different figures. The usual and fit-flexible intensity maps seem to be visually identical, although we expect slight improvement in bias for our method, as was confirmed already on data with the GT in Section IV-A2. The distinguishable granular points on Samples $A_1$ and $C_1$ (places with beads’ presence) show flow traces of microbeads on both saline and more importantly human lung tissue, which are not detectable by conventional systems of FLIM$^3$. The mechanism is without prior labelling or staining. Fig. S2 from SMs shows results of Sample $C_1$ after assigning an unknown class for segmenting foreground beads. Fig. 11 illustrates fitting results and temporal histograms of photon counts for three different pixels of Sample $C_1$. For each location, Misfit-percent differences to the baseline single-exponential model as well as that one with the 2nd rank are reported. To compare results of Fig. 10 (a) more quantitatively, Fig. 12 reports average error of recovering histogram of photon counts calculated via (9). The proposed approach achieves the lowest error on all samples with around 7% improvement on average than the best competing result from Robust RLD, as a benefit of our model selection mechanism. The CMM approach was omitted from comparisons list; because, it only estimates the lifetime but not the amplitude of mono-exponential and consequently incapable of a full histogram recovery [13].

V. DISCUSSION ABOUT COST-BENEFIT ANALYSIS

This section provides a cost-benefit analysis on our approach, where one can decide what is right for a real application based on values and weaknesses, as reported in Table VI. In our method, the number of life models, $M$, is the most influential parameter in determining computational complexity among others as the number of parameters $K_j$, or the number of bins $N$. In terms of this independent factor, for a single-model FLIM system, the theoretical complexity is from a unique constant order, i.e., $O(1) = 1$. However, it is $O(M) = 6$ for fit-flexible approach with negligible overhead related to the model selection mechanism. Complementarily, practical average run-time for The Parameters Set 1 in Fig. 6 is reported in Table VI. The run-time of fit-flexible approach is consistently about six times of the average of the six life models (28.27 ms). Codes were implemented in MATLAB R2022a and run in a 64-bit Windows 10 OS with hardware of an Intel Core i7 2.1 GHz processor and 32 GB RAM.

Fit-flexible approach is currently appropriate for detailed examination of a specimen in a software forensic mode.

$^3$This problem is similar to the proverb “finding a needle in a haystack”.
Despite the complexity, our method can be parallelised by HPC mechanisms for real-time demand; because model fitters work independently from each other. As an optimality benefit, it always minimises probability of error of Misfit as:

\[
P_{\text{fit-flexible}} = \min_{\theta} \left( \sum_{j} e_{j}^{2} \right)^{1/2},
\]

Fig. 12 proves the fact. This also helps characterise statistical differences among fluorescence life models. Even small differences may provide valuable structural information, such as those illustrated in Fig. 11. So, conventional FLIM techniques can be a special case of fit-flexible approach if one ignores the discrimination or the differences are not significant (e.g., tendency towards a specific model) for a given sample in an environment. This is done by selecting just a single model from the set of life models. Considering the discrepancy makes added values to current FLIM systems such as extracting life profile and measuring rise- and fall-time characteristics.

VI. CONCLUSION AND FUTURE STUDIES

This paper first proposed a model for investigation of time-resolved fluorescence imagers. We modelled the complex photonic system of confocal microscopy by understandable white-box electrical models. Afterwards, we derived life models for fluorescence techniques, with beyond applications. Then, an algorithm called a fit-flexible approach, was developed for soft sensing of fluorescence intensity, fall-time, and life profile from the hardware capacity of time-resolved fluorescence imaging. Supported by mathematical insights, we demonstrated capabilities of our method to visualising the information. Experiments on real data demonstrated sharper images and the potential for discriminating beads from lung tissue. The ability of beads detection can find attractive potential applications such as detection of microplastics in the lung, drug carriers efficacy, and bacteria detection.

Other than the subject of fluorescence data “analysis”, our modelling can be useful for biological “synthesis” and “control” purposes; e.g., it may open up research avenues towards characterising molecular and cellular structures of living organisms. This would be of great importance in real-world scenarios because of potential applications to disease diagnosis and drug discovery. Due to the capabilities of stacked fall-time histogram and life profile detection, our method can be employed in higher level biomedical images analysis tasks like registration, segmentation, and classification. One can also extend the search engine to more useful mathematical models describing real phenomena. It is also possible to extract other temporal markers from our framework such as a fluorescence rise-time. Other developments could be incorporating spatial or spectral correlations, and denoising operations.

REFERENCES


### TABLE VI: A comparison among different fluorescence life models both isolated and in fit-flexible setup

<table>
<thead>
<tr>
<th>Model</th>
<th>Complexity</th>
<th>Rise-time (ns)</th>
<th>Com.</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>McP</td>
<td>O(1)</td>
<td>16.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rayl.</td>
<td>O(1)</td>
<td>37.96</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bolt.</td>
<td>O(1)</td>
<td>37.78</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bi-xp</td>
<td>O(1)</td>
<td>19.26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>U-Rfl</td>
<td>O(1)</td>
<td>18.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>U-Rep</td>
<td>O(1)</td>
<td>40.51</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- The symbol \( e_{j} \) represents the number of parameters for the \( j \)-th model.
- The symbol \( e_{k} \) denotes probability of Misfit error of the \( k \)-th model.