Supplementary materials

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

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S1. Preface

This document provides supplementary materials for the paper “A novel fit-flexible fluorescence imager: Tri-sensing of intensity, fall-time, and life profile”. Different sections here provide details of the cross-references in the main paper. These sections also refer to the paper body where required.

S2. Mean lifetime for infinite-exponential decay

Consider the most general infinite-exponential decay as:

$$v(t)=\sum_{i=1}^{\infty}A_i e^{-\frac{t}{\tau_i}}. \tag{S1}$$

We define $A \triangleq \sum_{i=1}^{\infty} A_i$. Dividing (S1) by A gives:

$$v(t)=A\sum_{i=1}^{\infty}\alpha_i e^{-\frac{t}{\tau_i}}, \tag{S2}$$

where $\alpha_i \triangleq \frac{A_i}{A}$, $0<\alpha_i<1$, $\forall i$, and $\sum_{i=1}^{\infty}\alpha_i = 1$. In fluorescence lifetime imaging microscopy (FLIM), the time of arriving the first photon can be considered as a random variable; hence, the histogram of photon-count arrivals will be a non-normalised approximation of probability density function of the temporal random variable. Consider T as the random variable of photon arrival time, for which the probability density function of T is calculated from:

$$f_T(t)=\frac{v(t)}{\int_{-\infty}^{\infty}v(t)dt}. \tag{S3}$$

Define the denominator in (S3) as a constant as $d \triangleq \int_{-\infty}^{\infty}v(t)dt$, where it is:

$$d=A\sum_{i=1}^{\infty}\alpha_i\underbrace{\int_0^{\infty}e^{-\frac{t}{\tau_i}}dt}_{=\tau_i}=A\sum_{i=1}^{\infty}\alpha_i\tau_i. \tag{S4}$$

The expected value of T gives mean lifetime as:

$$\mathbb{E}(T)=\int_{-\infty}^{\infty}tf_T(t)dt=\frac{A}{d}\sum_{i=1}^{\infty}\alpha_i\underbrace{\int_0^{\infty}te^{-\frac{t}{\tau_i}}dt}_{=\tau_i^2}. \tag{S5}$$

By replacing d, solving the integral from integration by parts, and simplifying, the mean lifetime is finally determined by:

$$\tau_{\text{mean}}=\frac{\sum_{i=1}^{\infty}\alpha_i\tau_i^2}{\sum_{i=1}^{\infty}\alpha_i\tau_i}. \tag{S6}$$

Mono- and bi-exponential decays are both spacial cases of (S2). Consequently, one can simply show that the mean lifetime for the famous mono- and bi-exponential models are respectively as:

$$\tau_{\text{mean}}=\tau, \tag{S7}$$

$$\tau_{\text{mean}}=\frac{\alpha\tau_1^2+(1-\alpha)\tau_2^2}{\alpha\tau_1+(1-\alpha)\tau_2}. \tag{S8}$$

S3. Life models

A. Mono-exponential life model

If in the equivalent RLC circuit of Fig. 1 (a) the winding resistance approaches $R_w \rightarrow \infty$, a mono-exponential RC circuit will be determined as shown in Table II. This circuit is a LTI system. The time constant of a RC circuit is defined as $\tau_{\text{RC}} \triangleq R\times C$, where for the life circuit, it is $\tau_{RC}=\tau\times 1=\tau$. This reveals the fact that the concept of the time constant and the average fluorescence lifetime is the same.

Theorem 1 (Mono-exponential life model): Consider the mono-exponential circuit shown in Table II. The response of the circuit leads to mono-exponential life model (Mo-xp).

Proof: KCL in the mono-exponential circuit from Table II gives:

$$C\frac{dv(t)}{dt}+\frac{v(t)}{R}=i(t). \tag{S9}$$

Substituting components $R=\tau$ and $C=1$, and taking bilateral Laplace transform from both sides of (S9) yields:

$$sV(s)+\frac{1}{\tau}V(s)=I(s), \tag{S10}$$

where $I(s)=\frac{A}{K}\sum_{k=0}^{K-1}e^{-skT}$. If $T\rightarrow 0$, then $I(s)\approx A$. By substituting the value of $I(s)$ in (S10), we have $V(s)=\frac{A}{s+\frac{1}{\tau}}$. Taking inverse Laplace transform results in $v(t)=Ae^{-\frac{1}{\tau}t}u(t)$, where A and τ denote amplitude and lifetime, respectively. ∎

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B. Rayleigh life model

Equivalent circuit of Rayleigh life model is shown in Table II. This circuit is a LTV system. The resistor $r_2(t)$ acts as a negative resistance[1], as a natural property of fluorescent lamps[2] or molecules here.

Theorem 2 (Rayleigh life model): Assume the Rayleigh circuit shown in Table II. The response of the circuit leads to Rayleigh life model (Rayl.).

Proof: Starting from the Weibull circuit in Table II, KCL in it yields:

$$C\frac{dv(t)}{dt} + \left[\frac{1}{r_1(t)} + \frac{1}{r_2(t)}\right] v(t) = i(t). \tag{S11}$$

Its corresponding homogeneous equation by substituting Weibull circuit' components will be:

$$\frac{dv(t)}{dt} + \left[\frac{bt^{b-1}}{\tau} + \frac{(1-b)}{t}\right] v(t) = 0. \tag{S12}$$

By separating variables, we have:

$$\frac{dv(t)}{v(t)} = \left[\frac{(b-1)}{t} - \frac{bt^{b-1}}{\tau}\right] dt, \tag{S13}$$

where integrating from both sides of it gives $\ln[v(t)] = \ln\left(t^{b-1}\right) - \frac{1}{\tau}t^b + c$, in which c is an integration constant. Finally, taking exponential of that results in $v(t) = At^{b-1}e^{-\frac{1}{\tau}t^b}u(t)$. Repeating the above proof for $b = 2$ obtains Rayleigh model. ■

C. Weibull life model

Equivalent circuit of Weibull life model is shown in Table II. In this LTV circuit, the variable $b \in \mathbb{R}^+$ is defined as a flexible shape parameter. For the special case of $b = 1$, the equivalent circuit of Weibull life model is simplified to the mono-exponential equivalent circuit. Also, if $b = 2$, the circuit is exactly converted to the equivalent circuit of Rayleigh model. The same property is held for the system response, as shown in Table II.

Theorem 3 (Weibull life model): Assume the Weibull circuit shown in Table II. The response of the circuit leads to Weibull life model (Weib.).

Proof: See Theorem 2. ■

D. Bi-exponential life model

A bi-exponential function contains two different fluorescence lifetimes. These fluorophores are modelled in the load of our proposed RLC circuit as two distinct parallel light bulbs. Table II portrays equivalent circuit of bi-exponential life model.

Theorem 4 (Bi-exponential life model): Assume the bi-exponential circuit shown in Table II. The response of the circuit leads to bi-exponential life model (Bi-xp).

Proof: KCL in the bi-exponential circuit from Table II gives:

$$C\frac{dv(t)}{dt} + \left(\frac{1}{R_1} + \frac{1}{R_2}\right) v(t) + \frac{1}{L}\int_{-\infty}^{t} v(\lambda)d\lambda = i(t). \tag{S14}$$

Components' substitution and derivative from both sides yield:

$$\frac{d^2v(t)}{dt^2} + \left(\frac{1}{\tau_1} + \frac{1}{\tau_2}\right)\frac{dv(t)}{dt} + \frac{v(t)}{\tau_1\tau_2} = \frac{di(t)}{dt}. \tag{S15}$$

Taking bilateral Laplace transform obtains:

$$s^2V(s) + \left(\frac{1}{\tau_1} + \frac{1}{\tau_2}\right)sV(s) + \frac{1}{\tau_1\tau_2}V(s) = sI(s), \tag{S16}$$

where $I(s) = \frac{A}{K}\sum_{k=0}^{K-1} e^{-skT} + \frac{A}{s}\left(\frac{\alpha}{\tau_2} + \frac{1-\alpha}{\tau_1}\right)$. If $T \to 0$, then $I(s) \approx A + \frac{A}{s}\left[\frac{\alpha}{\tau_2} + \frac{(1-\alpha)}{\tau_1}\right]$. By substituting the function $I(s)$ in (S16) and after simplifications, we rewrite $V(s) = A\left[\frac{\alpha}{s+\frac{1}{\tau_1}} + \frac{(1-\alpha)}{s+\frac{1}{\tau_2}}\right]$. Inverse Laplace transform results in $v(t) = A\left[\alpha e^{-\frac{1}{\tau_1}t} + (1-\alpha)e^{-\frac{1}{\tau_2}t}\right]u(t)$, where $A \in \mathbb{R}^+$, $0 < \alpha < 1$, $\tau_1 \in \mathbb{R}^+$ and $\tau_2 \in \mathbb{R}^+$ signify initial amplitude, pre-exponential factor, short lifetime and long lifetime, respectively. ■

E. Critically-damped life model

If the two light bulbs in Bi-xp circuit are identical, i.e., $\tau_1 = \tau_2 \triangleq \tau$, the equivalent circuit of critically-damped life model is determined. It is shown in Table II.

Theorem 5 (Critically-damped life model): Assume the circuit shown in Table II. The response of the circuit leads to critically-damped life model (C-dmp).

Proof: KCL in the critically-damped circuit from Table II gives:

$$C\frac{dv(t)}{dt} + \frac{v(t)}{R} + \frac{1}{L}\int_{-\infty}^{t} v(\lambda)d\lambda = i(t). \tag{S17}$$

Components' substitution and derivative from both sides yield:

$$\frac{d^2v(t)}{dt^2} + \frac{2}{\tau}\frac{dv(t)}{dt} + \frac{v(t)}{\tau^2} = \frac{di(t)}{dt}. \tag{S18}$$

By taking bilateral Laplace transform, we have:

$$s^2V(s) + \frac{2}{\tau}sV(s) + \frac{1}{\tau^2}V(s) = sI(s), \tag{S19}$$

where $I(s) = \frac{A}{s}$. By substituting the function $I(s)$ in (S19) and after simplification, we obtain $V(s) = \frac{A}{\left(s+\frac{1}{\tau}\right)^2}$. Inverse Laplace transform results in a critically-damped response as $v(t) = Ate^{-\frac{1}{\tau}t}u(t)$. ■

F. Under-damped life model

With the same light bulbs, the equivalent circuit of under-damped life model is described in Table II.

Theorem 6 (Under-damped life model): Assume the under-damped circuit shown in Table II. The response of the circuit leads to under-damped life model (U-dmp).

[1] D. K. Roy, "Tunnelling and negative resistance phenomena in semiconductors," 2014.

[2] W. Elenbaas, Fluorescent lamps. Macmillan International Higher Education, 1971.

Proof: KCL in the under-damped circuit from Table II gives:

$$C\frac{dv(t)}{dt}+\frac{v(t)}{R}+\left(\frac{1}{L_1}+\frac{1}{L_2}\right)\int_{-\infty}^{t}v(\lambda)d\lambda=i(t). \quad \text{(S20)}$$

Components' substitution and derivative from both sides yield:

$$\frac{d^2v(t)}{dt^2}+\frac{2}{\tau}\frac{dv(t)}{dt}+\left(\frac{1}{\tau^2}+\omega^2\right)v(t)=\frac{di(t)}{dt}. \quad \text{(S21)}$$

Applying bilateral Laplace transform determines:

$$s^2V(s)+\frac{2}{\tau}sV(s)+\left(\frac{1}{\tau^2}+\omega^2\right)V(s)=sI(s), \quad \text{(S22)}$$

in which $I(s)=\frac{A\omega}{s}$. By substituting the function $I(s)$ in (S22) and simplifying, we have $V(s)=\frac{A\omega}{\left(s+\frac{1}{\tau}\right)^2+\omega^2}$. The response is obtained by taking inverse Laplace transform as $v(t)=Ae^{-\frac{1}{\tau}t}\sin(\omega t)u(t)$, where ω signifies the natural frequency. ■

S4. Lower bound of fall-time for bi-exponential

Consider the model of bi-exponential decay as a spacial case of (S2) as:

$$v(t)=A\left[\underbrace{\alpha_1}_{\triangleq\alpha}e^{-\frac{t}{\tau_1}}+\underbrace{\alpha_2}_{\triangleq(1-\alpha)}e^{-\frac{t}{\tau_2}}\right]. \quad \text{(S23)}$$

At the time $t=\tau_{\mathrm{f}}$, we have $v(t)=\frac{A}{e}$. substituting this and simplifying yield:

$$\frac{1}{e}=\alpha e^{-\frac{\tau_{\mathrm{f}}}{\tau_1}}+(1-\alpha)e^{-\frac{\tau_{\mathrm{f}}}{\tau_2}}. \quad \text{(S24)}$$

Solving (S24) requires the mathematical task of isolating τ_{f}. This can be realised by Maclaurin series approximation of $e^x=1+x+\frac{x^2}{2!}+\frac{x^3}{3!}+\cdots$. For all x, there exists $e^x\geqslant 1+x$. Hence, we can rewrite (S24) by ignoring the higher order terms as:

$$\frac{1}{e}\geqslant\alpha(1-\frac{\tau_{\mathrm{f}}}{\tau_1})+(1-\alpha)(1-\frac{\tau_{\mathrm{f}}}{\tau_2}). \quad \text{(S25)}$$

By using some simplification, we will:

$$\tau_{\mathrm{f}}\geqslant\frac{(1-\frac{1}{e})\tau_1\tau_2}{(1-\alpha)\tau_1+\alpha\tau_2}. \quad \text{(S26)}$$

S5. Different cases in fall-time determination

Below lists five main possible cases which may occur in real fitting scenarios:

- A strictly monotonic decreasing function, which is a normal case such a mono-exponential decaying function.
- A strictly monotonic growth function, which shows an unstable behaviour with a negative lifetime. For such an exception, any fall does not exist; therefore, we truncate $\hat{\tau}_{\mathrm{f}}=0$.
- A function with first growth and then decay trend, e.g., Rayleigh life model, as that shown in the curve of Fig. 1 (b).
- A curve with first decay and then growth trend. This case may happen in combination of two different decay and growth exponential terms in a bi-exponential model due to some specific estimated parameters. In such a case, we consider falling edge of the response but not its rising edge and correspondingly measure the fluorescence fall-time.
- A flat fit without any rise or fall. In this case, we set the span value as $\hat{\tau}_{\mathrm{f}}=\Delta N$.

S6. Penalising rules

Important rules are as follows:

- By identifying the control-theoretic property of the dominant pole between two real poles on the left side of s-plane from a stable system, we can ignore the effect of the farther pole than the imaginary axis and basically reduce a bi-xp model to a mono-xp counterpart. So, if $j^\star=4\wedge\frac{\min(s_1,s_2)}{\max(s_1,s_2)}\geqslant R_{\mathrm{DP}}$, then assign $\phi_{r,c}\leftarrow 1$. In implementations, we considered the ratio $R_{\mathrm{DP}}=10$.
- If the absolute value of the imaginary part of complex-conjugate poles in a detected U-dmp model is negligible, it can be replaced by a C-dmp model. So, if $j^\star=6\wedge\omega\leqslant\epsilon$, then $\phi_{r,c}\leftarrow 5$. We set $\epsilon=0.1$.
- A detected Weibul model with $b\approx 1$ is assigned to a mono-xp model. So, if $j^\star=3\wedge 1-\delta_1\leqslant b\leqslant 1+\delta_1$, then $\phi_{r,c}\leftarrow 1$. We set $\delta_1=0.05$.
- A detected Weibul model with $b\approx 2$ is singled out as an individual non-fractional Rayleigh. So, if $j^\star=3\wedge 2-\delta_2\leqslant b\leqslant 2+\delta_2$, then $\phi_{r,c}\leftarrow 2$. We set $\delta_2=0.2$.

S7. Unknown class assignment

In practical scenarios, a process may encounter with some unknown inputs that demand appropriate handling. In our problem, examples that can take an unknown label are: 1) an undefined life outside the already defined normal range of life model set; 2) fitting error at a location exceeds a tolerable threshold; 3) intensity of a pixel is below or above a predefined value; and 4) an uninformative content related to scene background. To be responsible in such situations, we define an extra unknown class #7 in the life pattern map. In Algorithm 1, a passive function is considered, where user can configure if-then rules and activate it if required. As a result, the user can take further notices and actions on unknown labels.

Figures S1 and S2 show results of Frame 5, Band 1 of Sample C_1 before and after an unknown class assignment, respectively. In Fig. S2, we assigned both Classes 5 and 6 mainly related to background lung tissue as the unknown class to be able to more clearly visualise and single out potential microbeads.

S8. Setting the number of photons per histogram

In generating synthesised data, we desire the number of photons per histogram (or equivalently the number of photons per pixel) remains constant for all pixels related to a given model before adding any noise. We determine the amplitude of the given model to meet the target. To do this, consider the deterministic life model of j^{th} as:

$$v_j[n]=A_jf_j[n],\forall j=1,2,\ldots,M. \quad \text{(S27)}$$

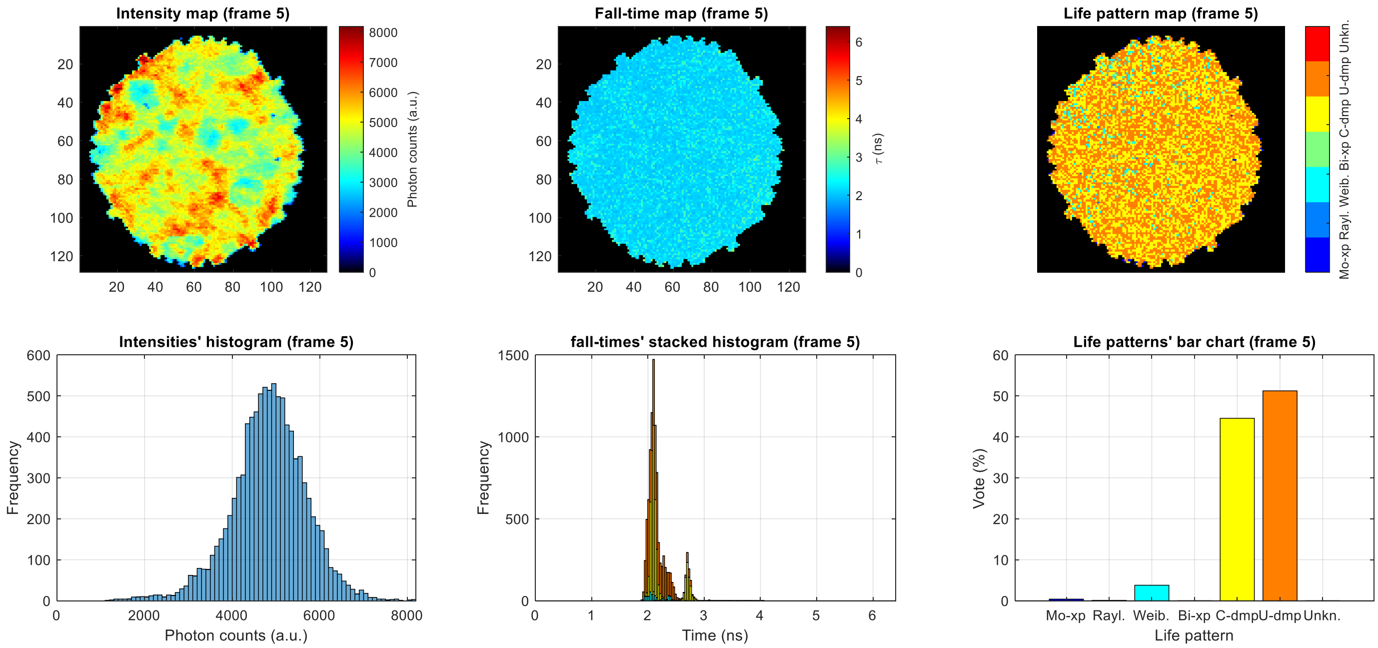

Fig. S1: Original results of Frame 5, Band 1 from Sample C_1 before assigning unknown class in comparison to Fig. S2 having unknown class assignment.

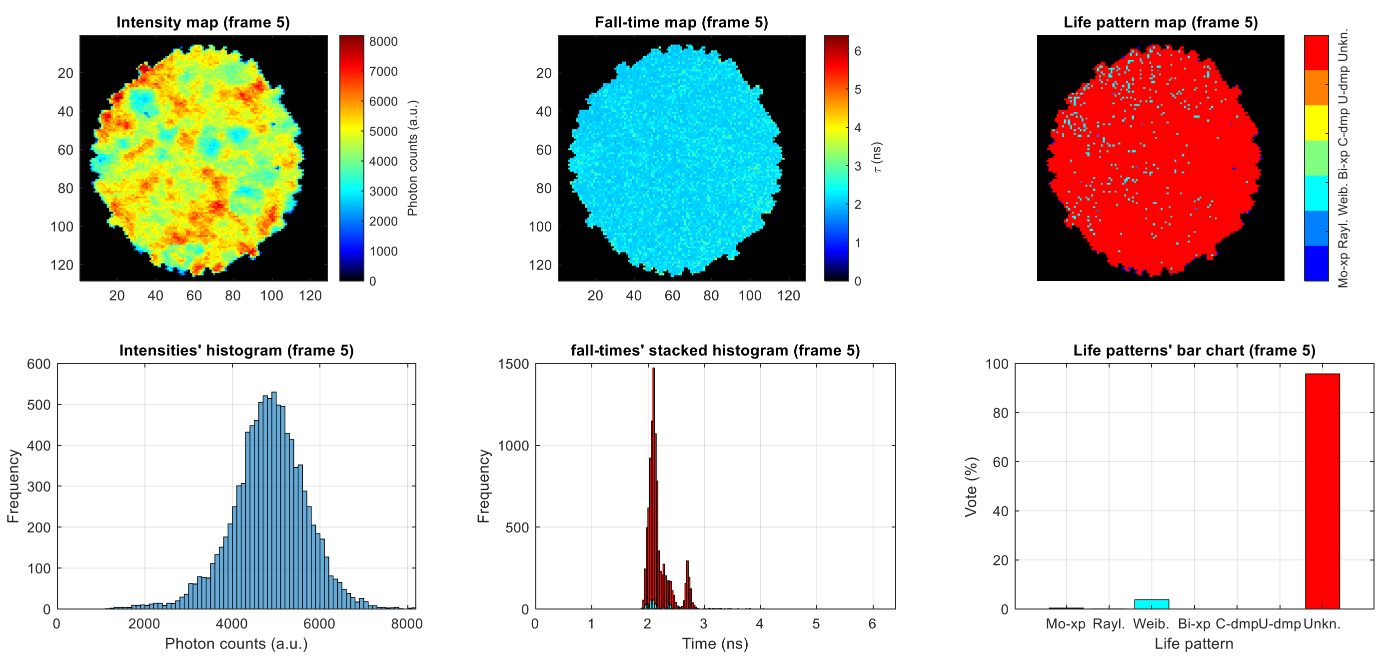

Fig. S2: Results of Frame 5, Band 1 from Sample C_1 after assigning Classes 5 and 6 as unknown class to visualise foreground microbeads.

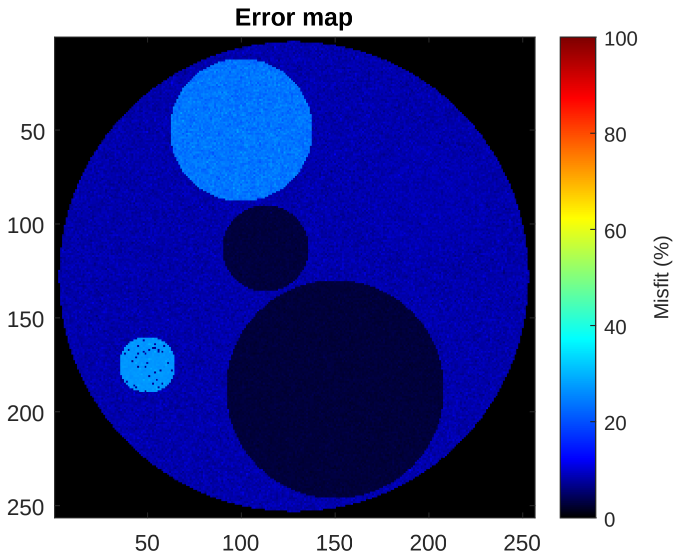

Fig. S3: Error map of the experiment related to The Parameters Set 1.

Taking summation on all bins from both sides of (S27) gives:

$$A_j = \frac{\sum_{n=0}^{N-1} v_j[n]}{\sum_{n=0}^{N-1} f_j[n]} \triangleq \frac{N_\mathrm{p}}{\sum_{n=0}^{N-1} f_j[n]}, \tag{S28}$$

where the constant N_p means the photons per histogram which is set by user.

S9. Information of The Parameters Sets 1 to 4

Figure S3 shows error map from the experiment related to The Parameters Set 1.

Figures S4, S5, S6 and S7 provide detailed information about The Parameters Set 2, which include: life profiles, visualised results of the proposed imager, confusion matrix and Misfit error map, respectively. Similarly, Figs. S8, S9, S10 and S11 show the information for The Parameters Set 3, and Figs. S12, S13, S14 and S15 for The Parameters Set 4, too.

S10. Our imaging raw data format

Figure S16 depicts a false-colour data format of the utilised imaging system represented as a 5D tensor arrangement consisting of a matrix of cubes as $(x, y; t; \lambda; i)$. The dimensions along $(x; y)$, t, λ, and i denote spatial coordinate, time, wavelength, and frame sequence index, respectively.

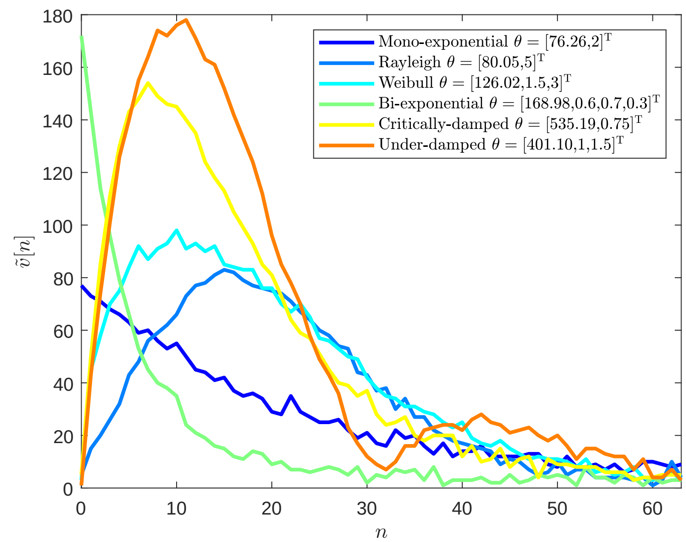

Fig. S4: Life profiles related to The Parameters Set 2.

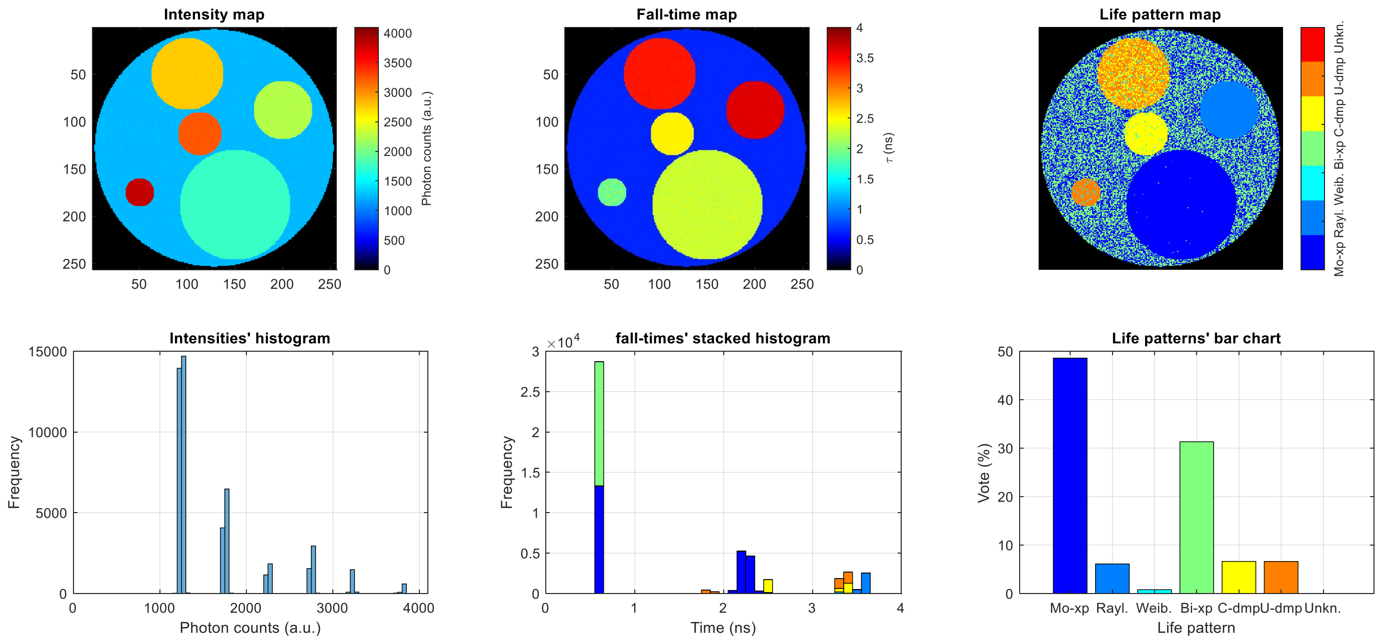

Fig. S5: GUI related to The Parameters Set 2.

Fig. S6: Confusion matrix related to The Parameters Set 2.

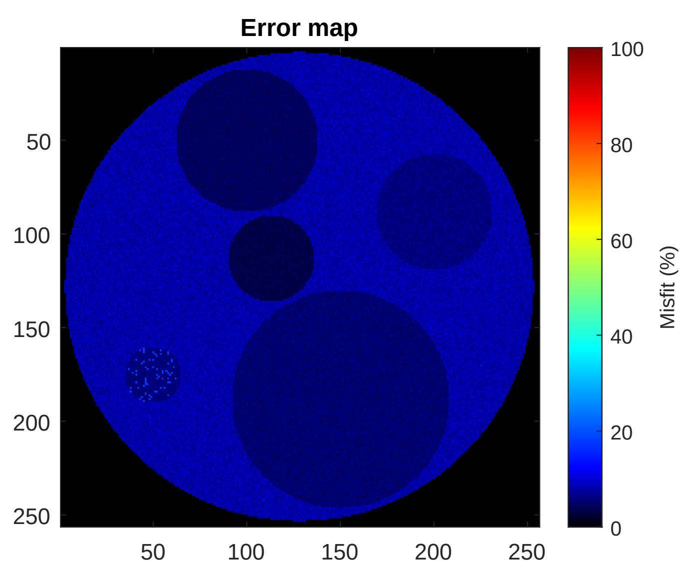

Fig. S7: Error map of the experiment related to The Parameters Set 2.

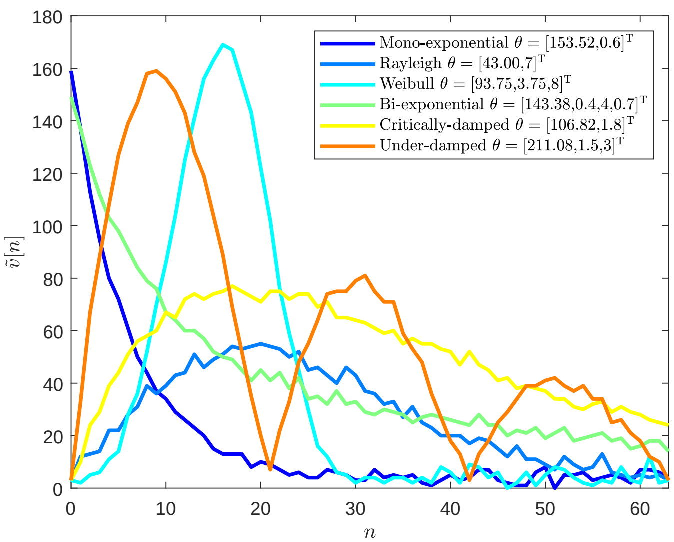

Fig. S8: Life profiles related to The Parameters Set 3.

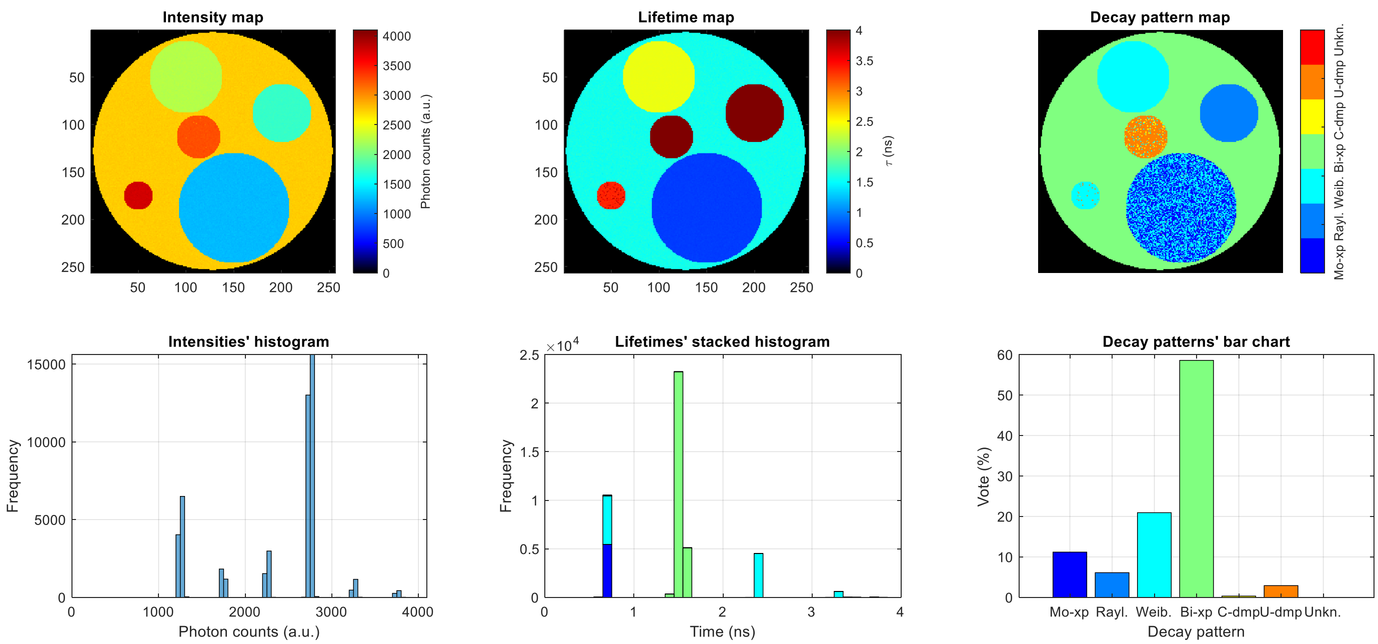

Fig. S9: GUI related to The Parameters Set 3.

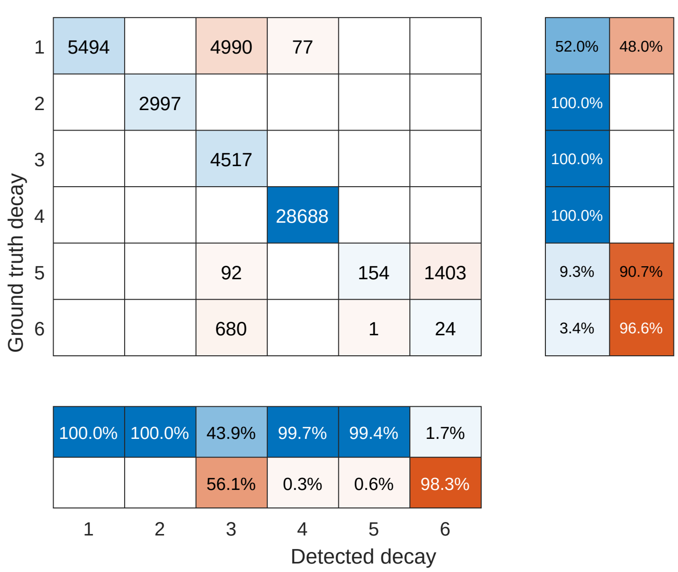

Fig. S10: Confusion matrix related to The Parameters Set 3.

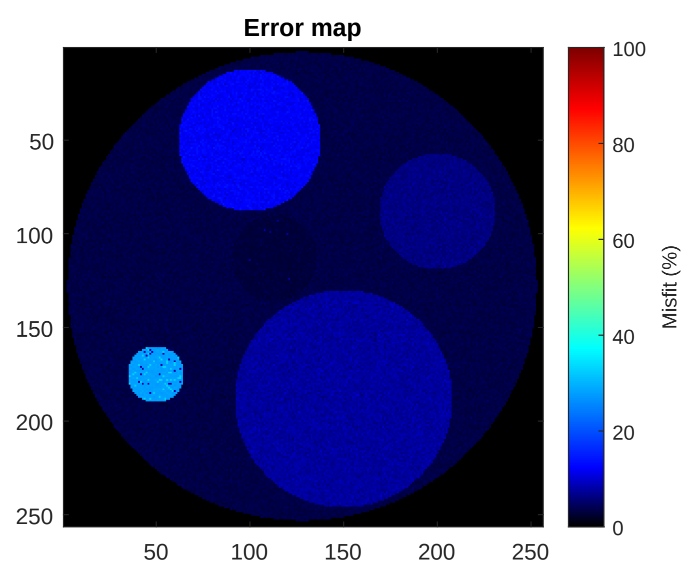

Fig. S11: Error map of the experiment related to The Parameters Set 3.

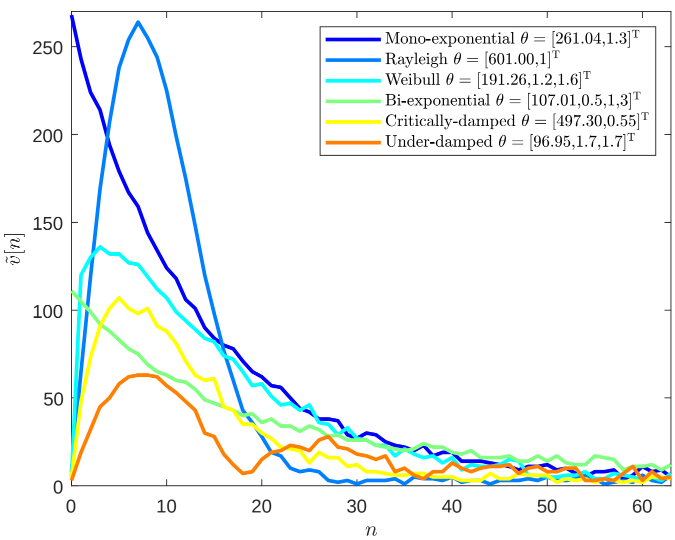

Fig. S12: Life profiles related to The Parameters Set 4.

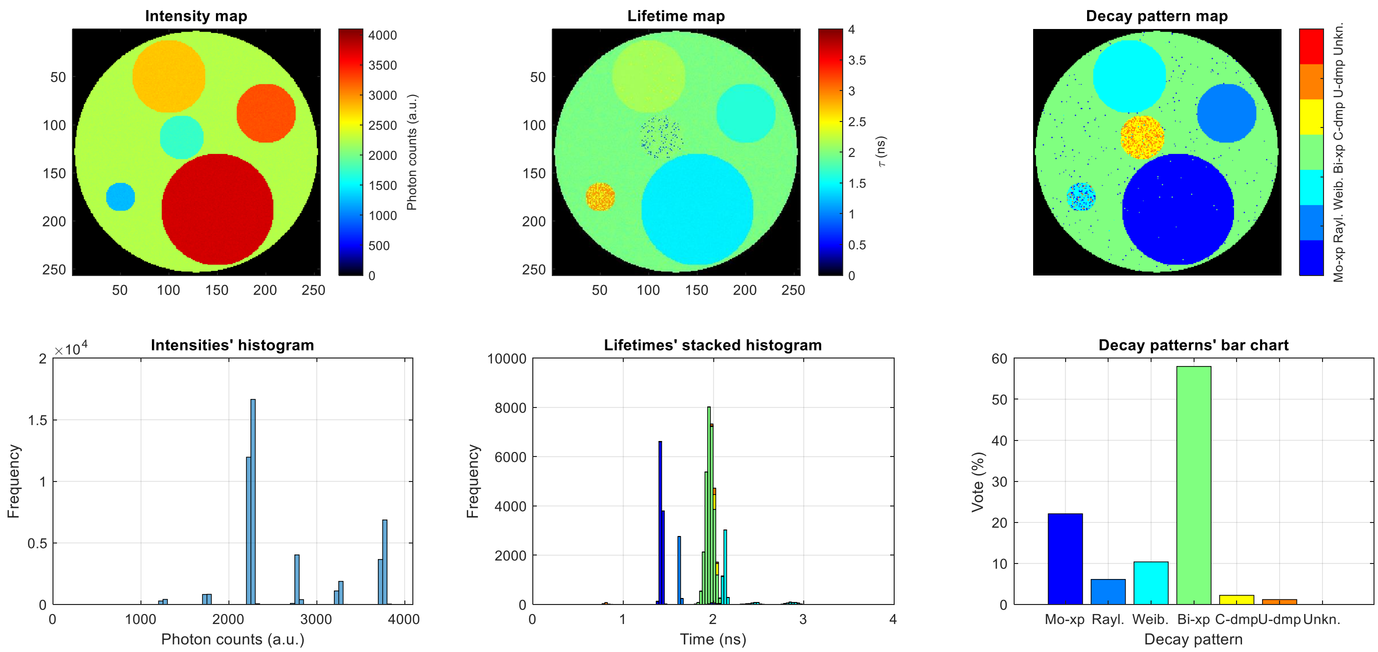

Fig. S13: GUI related to The Parameters Set 4.

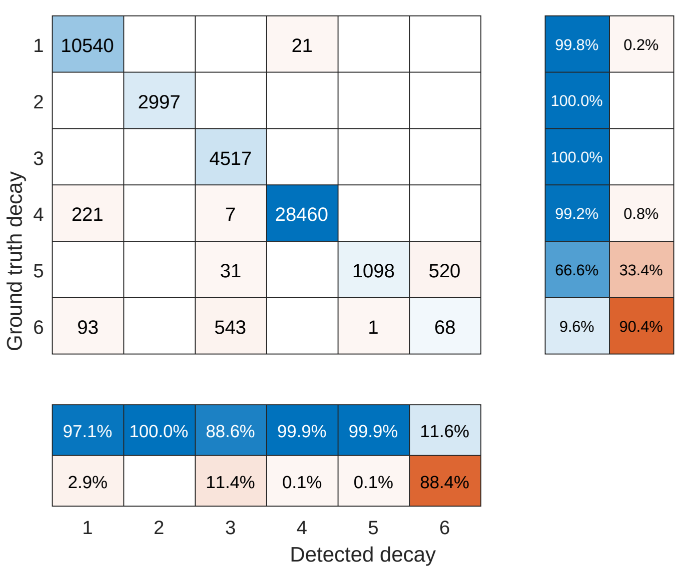

Fig. S14: Confusion matrix related to The Parameters Set 4.

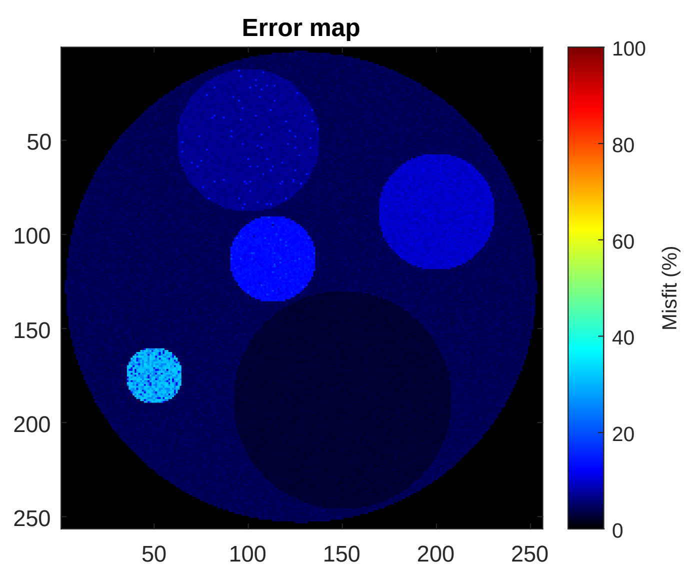

Fig. S15: Error map of the experiment related to The Parameters Set 4.

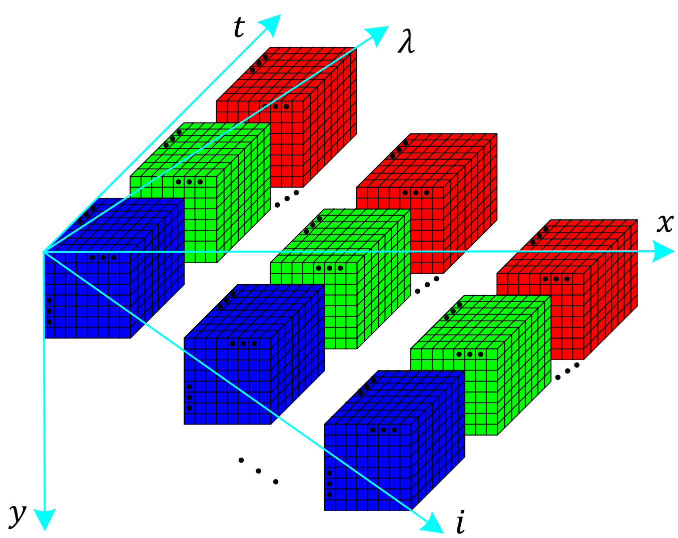

Fig. S16: Tensor data formatting of our multi-spectral fluorescence imaging system. The dimensions along $(x;y)$, t, λ, and i represent spatial coordinate, time, wavelength, and frame sequence index, respectively.