# TWO-PHOTON PHASE-RESOLVED FLUORESCENCE LIFETIME MEASUREMENT METHOD USING ULTRASHORT PULSE LASER

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**KEYWORDS**: ultrashort pulse laser, phase-resolved fluorescence-lifetime, two-photon microscopy, temperature sensor

Fluorescence lifetime imaging microscopy (FLIM) is an invaluable tool for medical and biological applications. We propose a new FLIM measurement technique with a combination of a two-photon phase-resolved fluorescence lifetime measurement method using ultrashort pulse laser. Here, a pulsed laser is used for the excitation of two-photon process. The lock-in amplifier performs a multiplication of a modulated pulsed laser as reference signal and fluorescence signal emitted by the sample as input signal. The output signal is expressed in Eq. (1). The lock-in amplifier is capable in controlling the phase difference φ between the reference signal and the fluorescence signal. Fig. 1(a) shows detected signal intensity of lock-in amplifier to fluorescence lifetime with three different phases and were used to find value of x in the Eq. (2). Fig. 1(b) shows the curve graph of Eq. (2). These equations mathematically support the idea in calculating the fluorescence lifetime τ corresponding to the value of x.

$$I(\tau) = \int_0^T \alpha \exp(-t/\tau) \times \cos(\omega t + \varphi) dt \tag{1}$$

$$x(\tau) = \frac{I_0(\tau) - I_{\pi/4}(\tau)}{I_0(\tau) - I_{\pi/2}(\tau)} \tag{2}$$

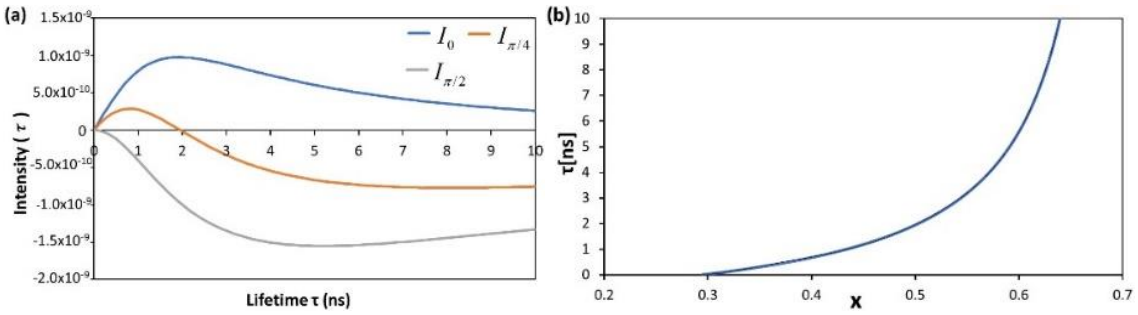


*Figure 1 a) Relationship between fluorescence lifetime, τ and output I (t) (α = 1, repetition freq 81.5 MHz); b) x - τ curve*

We used rhodamine B as the first fluorescence dye to be tested in this experiment. The fluorescence lifetime measurement results of rhodamine B showed quite consistent result with average value and the standard deviation were 2.15 ± 0.096 ns which matches with previous studies [1]. We have developed and investigated the performance measurement method using ultrashort pulse laser using method is desirable to measure the fluorescence lifetime of a biological sample in a short time and without requiring complex architecture.

## References

[1] R. W. K. Leung, S.-C. A. Yeh, and Q. Fang, "Effects of incomplete decay in fluorescence lifetime estimation"*Biomed. Opt. Express* 2, 2517 (2011).