

There are three main methods of measuring fluorescence lifespan; they are: the data fitting method, analogue phase-lock method, and digital phase-lock method. Among them the data fitting method can not only measure the fluorescence lifespan, but also make analyses of the signals. The data fitting method of measuring fluorescence lifespan primarily covers the method of Marquardt, the method of Prony, logarithms fitting (log-fit) method etc (Zhang *et al.*, 1996). In order to improve the signal-to-noise ratio, we need a kind of more effective fitting method. Since fluorescence signals are of single-valued exponential decay, we can apply the FFT to this kind of signal, and then calculate the fluorescence lifespan from the non-zero terms in the FFT transform result. The deviation of the fluorescence lifespan using the method is far smaller than using the Prony method or logarithms fitting method, but near to the result from the method of

Marquardt (Sun *et al.*, 2004). It is not subjected to the influence of the background noise, but at the same time the fitting time is greatly shortened by using the FFT. After the stimulating light stops, the fluorescence cannot disappear at once, but fades away exponentially. The time that fluorescence fades away calls the fluorescence lifespan. The fluorescence lifespan is a function of temperature, and is independent of light intensity. The fluorescence-attenuating signal can be expressed as

$$f(t) = A \exp(-t/\tau) + B, \quad (1)$$

Where A denotes the start light intensity, B represents the background noise originating from the black radiation or dark current of the circuit carrying the electric current, which is the direct current component. When digitally sampling by using digital sampling cards or other means, we obtain the following expression

$$f_k(t) = A \exp(-k \cdot \Delta t / \tau) + B, \quad k=0, 1, \dots, N-1. \quad (2)$$

Where Δt is the interval of sampling time. We can perform the fitting to the signal to work out the fluorescence lifespan τ .

The n th term of FFT in formula (2) is

$$F_n = \sum_{k=0}^{N-1} f_k \exp\left(-j \frac{2\pi n}{N} k\right) = \sum_{k=0}^{N-1} A \exp\left[-\left(\frac{\Delta t}{\tau} + j \frac{2\pi n}{N}\right) k\right] + B \sum_{k=0}^{N-1} \exp\left(-j \frac{2\pi n}{N} k\right) \quad (3)$$

$$n=0, 1, \dots, N-1.$$

The zeroth term is:

$$F_0 = A \frac{1 - \exp(-N\Delta t / \tau)}{1 - \exp(-\Delta t / \tau)} + NB, \quad (4)$$

Which is dependent on the background noise. While the other items ($n \neq 0$) can be simplified.

$$F_n = A \frac{1 - \exp(-N\Delta t / \tau)}{1 - \exp(-\Delta t / \tau) \exp(-j2n\pi / N)}, \quad n=0, 1, \dots, N-1. \quad (5)$$

Except the zeroth term, the other terms are independent of the background noise. We use the first non-zero term to calculate the fluorescence lifespan. Applying the FFT to the formula (5) with , the formula (5) can be changed into

$$F_1 = A [1 - \exp(-N\Delta t / \tau)] / \{ [1 - \exp(-\Delta t / \tau)] \cos(2\pi / N) + j \exp(-\Delta t / \tau) \sin(2\pi / N) \}. \quad (6)$$

This term is complex, and its tangent function of the phase angle is expressed as follows

$$Q_1 = \text{tg} \varphi_1 = \frac{\text{Im} F_1}{\text{Re} F_1} = \frac{-\exp(-\Delta t / \tau) \sin(2\pi / N)}{1 - \exp(-\Delta t / \tau) \cos(2\pi / N)} \quad (7)$$