Improving the precision of fluorescence lifetime measurement using a streak camera

Heng Li (李 恒), Yonghong Shao (邵永红), Yan Wang (王 岩), Junle Qu (屈军乐)*, and Hanben Niu (牛憨笨)

Key Laboratory of Optoelectronic Devices and Systems of Ministry of Education and Guangdong Province, Institute of Optoelectronics, Shenzhen University, Shenzhen 518060, China

**E-mail: jlqu@szu.edu.cn*

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Streak camera has high temporal resolution and high sensitivity, and is a powerful tool in biomedical study to measure fluorescence lifetime and perform fluorescence lifetime imaging. However, nonuniformity of the gain in the streak tube and nonlinearity of the sweeping speed limit the precision of fluorescence lifetime measurement, particularly when fluorescence lifetimes are short. We have constructed a two-photon excitation fluorescence lifetime measurement system that is based on a synchroscan streak camera and have developed accordingly a method to correct the effect of gain nonuniformity and nonlinearity of sweeping speed on the measurement precision. A continuous-wave laser of high stability is used to calibrate the gain of the streak camera, and a Fabry-Perot etalon is used to calibrate the nonlinearity of the sweeping speed. Fitting algorithms are used to correct the gain of the streak camera and nonlinearity of the sweeping speed respectively, which significantly improves the measurement precision of the system, as characterized through the fluorescence lifetime of the short-lived fluorescence dye, Rose Bengal. Experimental results show that the measurement fluctuation of the lifetime has been improved from more than 10% to 2% after correcting the effects of gain nonuniformity and sweeping speed nonlinearity.

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Fluorescence is a powerful tool in biomedical study. Analysis of fluorescence properties such as the emission spectrum and excited-state lifetime can provide novel insights of a sample with high molecular specificity and sensitivity[1−5]. Fluorescence lifetime is an important parameter which provides a unique contrast in biomedical imaging, particularly in differentiating fluorophores with overlapping spectra. In addition, the lifetime measurement is very sensitive to the microenvironment of the fluorophores. Therefore, such physiological parameters including pH, ion concentration ($[Ca^{2+}]$, $[Na^{+}]$, etc.), and oxygen pressure may be quantified from the fluorescence lifetime[6]. Fluorescence lifetime measurement and imaging have been applied widely in biochemistry, biophysics, and clinical diagnosis[7−9].

Various methods are available for fluorescence lifetime measurements in time domain[10] or frequency domain[11,12], e.g., time-correlated-single-photon counting (TCSPC)[13] and time-gated image intensifier[14]. Streak camera, which has high temporal resolution and high sensitivity, has been used in biomedicine to measure fluorescence lifetime and perform lifetime imaging[15,16]. High repetition rate streak camera and synchroscan streak camera are particularly useful for measuring very weak fluorescence signals. Another advantage of a streak camera is that it can provide information regarding the change of fluorescence intensity as a function of time, which enables the recording of the whole fluorescence decay process in a single shot manner.

In order to make the best use of the laser power, to increase the detection efficiency of weak signals, and to measure the lifetime shorter than 1 ns, we have previously developed a two-photon excitation fluorescence lifetime measurement system that is based on a synchroscan streak camera[17]. The temporal resolution of the system is 10 ps. When compared with the streak camera of repetition rate of approximately 1 MHz, the repetition rate of the synchroscan streak camera is as high as 76 MHz, which can increase the efficiency of excitation by 76 times. However, our data show that the fluctuation of the short lifetime measurement is greater than 10%. In this letter, we analyze the factors which contribute to the low precision of lifetime measurement and develop a method to improve it.

Figure 1 shows the schematic of the two-photon excitation fluorescence lifetime measurement system. The Ti:sapphire laser (Coherent, Mira 900F) provides mode-locked, ultrafast femtosecond pulses with the repetition rate of 76 MHz. The pulse width of the laser is 120 fs and the output wavelength is 800 nm. The output from the Ti:sapphire laser is split into two beams by a beam splitter: the excitation and the trigger beams. The excitation beam is directed in parallel into the entrance pupil of an objective. The objective lens then produces a tightly focused spot on the sample for simultaneous two-photon excitation. The emission from the sample is collected by the objective and focused onto the photocathode of the streak camera to produce photoelectrons which resembles the fluorescence signal. The PIN diode converts the trigger beam into an electric signal which is then filtered and amplified to produce a high voltage sinusoidal sweeping signal. The sweeping signal produces a sinusoidal deflection electric field between the pair of deflection plates inside the streak tube. The photoelectrons are deflected by the sweeping electric field according to their arrival time; therefore the fast fluorescence decay process is swept onto the phosphor screen, which is then recorded by a charge-coupled device (CCD) cam-

era. The spectral characteristics of the phosphor screen are matched to the spectral sensitivity of the CCD. Fitting of the light intensity profiles on the phosphor screen provides the lifetime of the fluorophore sample. Without special instructions, the fluorophore we used is Rose Bengal.

In the streak tube, the gain may not be uniform across the microchannel plate (MCP) and the phosphor screen, which, taking intuitive explanation, would distort the light intensity on the phosphor screen and its temporal response. The distortions will affect the precision of lifetime measurement. We experimentally analyze the effect of gain nonuniformity on the lifetime measurement. For a single component fluorophore, the fluorescence decay is described as

$$I(t) = I_0 \mathrm{e}^{-t/\tau}, \quad (1)$$

where I_0 is the light intensity at $t = 0$, $I(t)$ is the fluorescence intensity at t after being excited by the pulsed light, and τ is the fluorescence lifetime which is a characteristic of fluorophores and indicates the period when the fluorescence intensity decays to 1/e or 37% of its maximum. Nonuniformity in the streak tube would cause fluctuation in the light intensity on the phosphor screen. The fluctuation will affect the fitting of the fluorescence decay described by Eq. (1). We use a continuous-wave (CW) semiconductor laser as the light source. The fluctuation of the output is less than 1%. The light output from the CW laser irradiates the photocathode of the streak tube while it is working in a dynamic mode, i.e., the sweeping circuit is on. The recorded intensity on the phosphor screen along the sweeping direction is shown in Fig. 2. Ideally, the recorded light intensity should be uniform as a stable CW light is used in the experiment. The fluctuation of intensity shown in Fig. 2 indirectly represents the gain of the streak camera. This profile is used as a reference for gain nonuniformity correction. All the original data are divided by the reference before the fitting process.

We measure the fluorescence lifetime of a short-lived fluorescence dye, Rose Bengal, which is often used as a lifetime standard[18]. The fluorescence decay profile is shown in Fig. 3. From Fig. 3, we can see that there is significant fluctuation in the fluorescence decay. When the experimental data are corrected for gain nonuniformity by dividing the reference from the raw data, the result is shown in Fig. 4, which shows that after gain nonuniformity correction, the fluorescence intensity decay curve becomes smooth and the fitting precision is greatly improved.

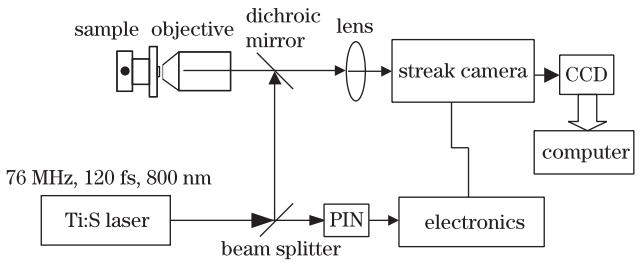

Fig. 1. Schematic diagram of the two-photon excitation fluorescence lifetime measurement system based on a synchroscan streak camera.

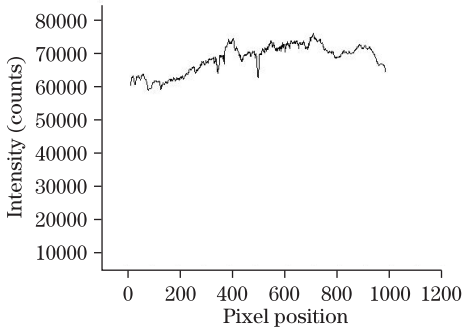

Fig. 2. Light intensity on the phosphor screen along the sweeping direction recorded by CCD, which represents the gain of the streak camera.

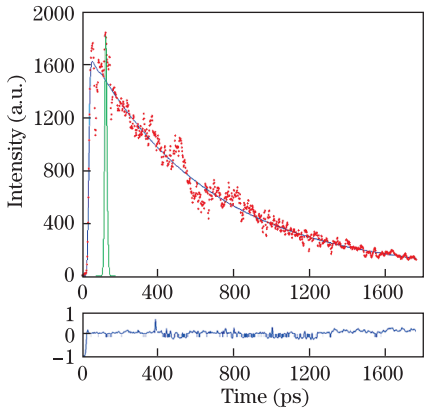

Fig. 3. Fluorescence intensity decay and fitting result before gain nonuniformity correction.

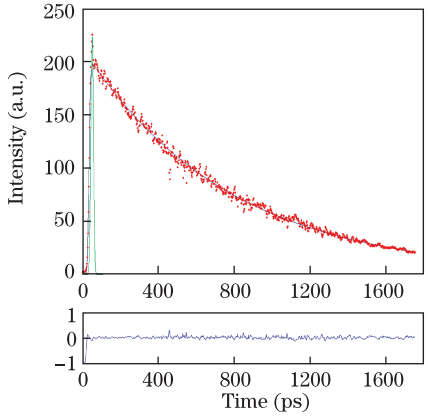

Fig. 4. Fluorescence intensity decay and fitting result after gain nonuniformity correction.

In the synchroscan streak camera, high voltage sinusoidal sweeping signal is applied to the pair of deflection plates to produce sweeping electric field. Generally, the sweeping signal is considered to be linear within $\pm\pi/4$ at zero cross point of the signal. But in reality, this approximation causes problems for short lifetime measurement. We use a Fabry-Perot etalon to calibrate the nonlinearity of the sweeping speed, i.e., to determine the time for each pixel (channel time). The result is shown in Fig. 5, which shows the spacing in pixel numbers between two continuous pulses from the etalon at different positions along the sweeping direction. Figure 5 shows that time

channels on the edge of the phosphor screen are smaller than those at the center of the phosphor screen, i.e., there is a nonlineariy of sweeping speed. In order to obtain the exact time channel for each pixel, we perform a fitting to the data in Fig. 5 using an experimentally obtained equation:

$$y = 83.61 + 0.1x - 2.04 \times 10^{-4}x^2 + 1.998 \times 10^{-7}x^3 - 1.087 \times 10^{-10}x^4. \quad (2)$$

For the measurement of long lifetime (>1 ns), the average time channel is used to reduce nonlinearity. But for the short lifetime measurement, this method does not work well. To obtain a consistent time channel, we derive the deviation of each channel,

$$k_i = 10.39 - 0.1x_i + 2.04 \times 10^{-4}x_i^2 - 1.998 \times 10^{-7}x_i^3 + 1.087 \times 10^{-10}x_i^4, \quad (3)$$

where i is the index of pixel number. Time channel normalization is achieved by adding the deviation value to each pixel.

We measure the fluorescence lifetime of Rose Bengal using different delay settings, which allow the fluorescence decays (as a function of delay) appear at different positions on the phosphor screen along the sweeping direction. The results are shown in Fig. 6. In Fig. 6, C1 and C2 are the lifetime data and the corresponding fitting curve without correction. The lifetime error is more than 10%. B1 and B2 are the lifetime and the corresponding fitting curve only after the gain nonuniformity is corrected, which shows that the lifetime error has been reduced to 3.24%. When both the nonuniformity of gain and the nonlinearity of sweeping speed have been corrected, the lifetime and the fitting curve are shown as A1 and A2. The lifetime is 0.76 ns and its error is 2%, which shows that the precision of lifetime measurement has been improved significantly.

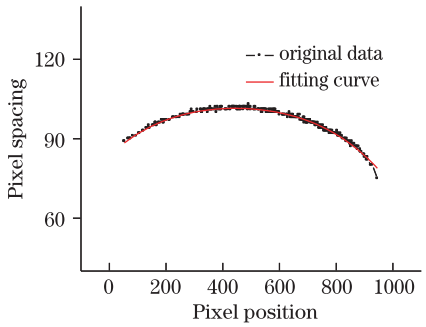

Fig. 5. The spacing in pixel numbers between two continuous pulses from the etalon at different positions along sweep direction.

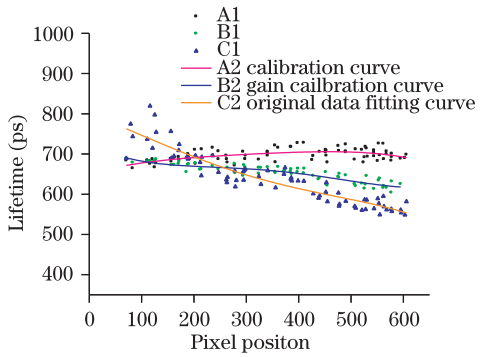

Fig. 6. Fluorescence lifetime of Rose Bengal before and after nonlinearity and nonuniformity correction.

In conclusion, we have built a two-photon excitation fluorescence lifetime measurement system that is based on a synchroscan streak camera. The system has high temporal resolution and high sensitivity. It is particularly suitable for the measurement of short lifetimes in biomedical research. We show that gain nonuniformity and nonlinearity of sweeping speed are the main factors affecting the precision of lifetime measurement. We analyze the effects and develop methods to correct the gain nonuniformity and sweeping speed nonlinearity. Our experiment with Rose Bengal fluorescent dye shows that the fluctuation of fluorescence lifetime measurement has been improved from more than 10% to 2%.

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