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Fluorescence lifetime measurement from a designated single-bunch in the BEPCⅡ colliding mode*

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Abstract: Fluorescence lifetime measurement in the time domain requires excitation from a well separated single bunch using synchrotron light sources. In the colliding mode of the Beijing Electron Positron Collider Ⅱ (BEPCⅡ), a hybrid filling pattern was realized such that a single bunch was placed in the middle of a large gap between two multi-bunch groups. Detection of fluorescence lifetime, based on the excitation of the light pulse from this designated single-bunch, was established at Beamline 4B8 of the Beijing Synchrotron Radiation Facility (BSRF). The timing signal of the BEPCII was utilized as a trigger to gate this fluorescence event. L-Tryptophan amino acid, a known lifetime standard, was selected to assess the lifetime measurement performance. The measured lifetime was consistent in both colliding and single-bunch mode with the time resolution down to 450 ps. Moreover, both the bunch purity and the fine structure of the hybrid filling pattern were characterized.

Key words: fluorescence lifetime, time-correlated single-photon counting (TCSPC), synchrotron radiation, hybrid filling pattern, bunch purity

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1 Introduction

Fluorescence spectroscopy has been widely used in the characterization of biomacromolecules and materials. Time-resolved fluorescence lifetime measurement can provide more information than steady-state fluorescence measurement, so it has become a powerful tool for studying both protein dynamics and cellular imaging [1, 2]. Fluorescence lifetime is also important in the study of luminescent materials like scintillators and phosphors [3].

Synchrotron radiation provides an excellent light source for fluorescence lifetime study [4, 5], owing to its wide excitation range from UV to X-ray and its inherent pulse characteristic with duration around 100 ps. The fluorescence lifetime detection in the time domain has been realized in a single-bunch mode at the Beijing Synchrotron Radiation Facility (BSRF) [6]. The time-resolved experiments, including fluorescence lifetime measurement, usually require a single bunch or a few bunches, because they require a gap long enough for sample relaxation and detection electronic recovery. However, the beamtime available to single-bunch or a few bunches mode is rare as the steady-state experiment requires multi-bunch mode for the high beam current to get high flux. To solve this dilemma, the dedicated synchrotron sources generally operate in a hybrid filling mode [7–9]. In this mode, the bunches are not filled evenly but instead the multi-bunch groups are followed by a large gap, where a single bunch is placed. This single bunch can be selected for time-resolved experiment while the multi-bunch groups keep the beam current high enough for steady-state experiments.

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BSRF is a parasitic light source of the Beijing Electron Positron Collider Ⅱ (BEPCⅡ) and the majority of beamtime is allocated to colliding for high energy physics experiment. In the colliding mode, a large gap exists between multi-bunch groups, so it is desirable to implement a hybrid filling pattern and develop a technique to obtain a wanted fluorescence signal from multi-bunch excitation. This will make fluorescence lifetime measurement available in the colliding mode and lift the beamtime limitation due to single-bunch operation requirement. In this paper, a hybrid filling pattern, realized in the colliding mode, is described with a designated single bunch for fluorescence lifetime detection. A gating technique was developed to detect the fluorescence event excited by this special bunch with the time resolution down to 450 ps. In the meantime, both the bunch purity and the fine structure of the filling pattern were also characterized.

2 Experimental section

2.1 TCSPC instrumentation

Lifetime measurement was established using the time-correlated single-photon counting (TCSPC) method [10]. At present, the electronics for the TCSPC is based on NIM modules, as shown in Fig. 1. Sample fluorescence, excited by synchrotron light, is detected by a PMT (H7422P-40, Hamamatsu) with a pre-amplifier (C5594-44, Hamamatsu). The signal is input into a constant-fraction discriminator (CFD, ORTEC935) to generate a start signal triggering a time-to-amplitude converter (TAC, ORTEC567). The stop signal of the TAC can be generated by either directly using a timing signal from the BEPCII or detecting the excitation pulse with another PMT (XP2020Q, Photonis) [9, 11, 12]. The former was selected for fluorescence lifetime detection. TAC output is input into a multi-channel analyzer (MCA, ORTECTRUMP-PCI-2k) to count in different channels. After adequate accumulation, the fluorescence decay curve is restored.

The system time resolution can be optimized by choosing a proper delay and zero cross level of the CFD, while the signal to noise ratio (SNR) can be improved by setting both appropriate PMT gain and CFD threshold. The counting rate is less than 10^4cps, which is much lower than the repetition frequency of the selected pulse (1.26 MHz), so the pile-up effect is safely avoided [2]. The time scale of the TAC was calibrated by the time-delay module with each channel corresponding to 57 ps.

2.2 Gating configuration for single-bunch signal selection

The timing signal of the BEPCⅡ is used to gate the signal from the designated single-bunch. The BEPCII timing system is an event timing system, which mainly consists of an event generator (EVG) and an event receiver (EVR). It generates a series of timing signals, providing accurate synchronization for the accelerator and synchrotron [13].

To detect the signal from the designated single bunch, a timing sub-station was established at BSRF equipped with EVR (VME-EVR-230RF) and an input output controller (IOC, MVME51005E-0163). As shown in Fig. 1, the timing signal of the designated bunch is obtained by setting the EVR with the event code from the EVG of the BEPCII timing main-station. This signal triggers a BNC8010 gate and delay generator to output a gating signal as a gate input of the CFD. By adjusting the delay and width of the BNC8010 output, the CFD will only accept a fluorescence signal excited from the designated single-bunch, so the fluorescence event from this single bunch can be detected. In addition, the timing signal can also be used as the stop signal for the TAC. The stop signal is synchronized with the designated single bunch using a timing single-channel analyzer (SCA, ORTEC552), which is triggered by the timing signal. The jitter of the timing signal is around 50 ps, measured by an oscilloscope (DSA70404, Tektronix). In the meantime, the hybrid filling pattern was monitored by detecting the scattering of the excitation light from the beamline entrance slit using the PMT XP2020Q.

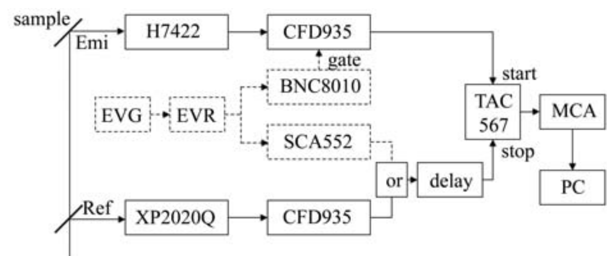

Fig. 1. Block diagram of the fluorescence lifetime system. The solid line is for the TCSPC set-up, Ref for synchrotron radiation reference pulse, Emi for sample emission fluorescence, H7422 and XP2020Q for PMT detector, CFD 935 for constant-fraction discriminator, TAC 567 for time-to-amplitude converter, MCA for multi-channel analyzer. The dashed line is for single bunch selection, EVG for event generator, EVR for event receiver, BNC 8010 for gate and delay generator and SCA 552 for the timing single-channel analyzer.

2.3 Beamline and samples

Measurement was performed at Beamline 4B8 designed for vacuum ultraviolet (VUV) spectroscopy, which provides fluorescence detection with excitation wavelengths ranging from 125 nm to 360 nm, and the emission from 200 nm to 800 nm. All measurements were undertaken at room temperature.

The reflected light of $BaSO_4$ was used to measure the instrument response function (IRF), which represents the system time resolution. L-Tryptophan amino acid, a well-known lifetime standard, was selected to assess the performance of the lifetime measurement. It was purchased from the Sigma-Aldrich Company and used without further purification. L-Tryptophan was dissolved in distilled H_2O at a concentration of 8.2×10^{-4}M with pH slightly less than 7.

The fluorescence lifetime was obtained by fitting the fluorescence decay curve using CFS software based on nonlinear least square method. CFS is free online software [14].

3 Results and discussion

3.1 Gating profile for the designated single bunch

The bunch revolution period is ∼800 ns for the BEPCⅡ ring. In the colliding mode, there are 80 bunches in the electron ring of the BEPCⅡ, grouped into two multi-bunch groups, and the groups are separated asymmetrically with a large gap of 136 ns, as shown in Fig. 2. As the gap within a multi-bunch group is only 8 ns, it is not long enough for most of the fluorescence lifetime measurement. For example, the fluorescence lifetime of an amino acid is in the range of a few nanoseconds, so it requires tens of nanoseconds gap to restore the fluorescence decay profile. Therefore, a larger gap between multi-bunch groups can be utilized. A hybrid filling pattern was realized in the colliding mode such that a single-bunch (7.5 mA of peak current) was filled into a designated bucket to be placed in the middle of this large gap. This single-bunch, corresponding to an excitation light pulse of ∼150 ps duration, is well separated from the multi-bunch groups, as shown in Fig. 2. It leaves an 80 ns interval from the first multi-bunch group and it is long enough for sample relaxation after being excited by multi-bunch. A 56 ns interval that follows, from the second multi-bunch group, is used to detect the fluorescence lifetime. Under this hybrid filling pattern, an adequate gap is obtained for lifetime measurement, while the overall beam current keeps high enough (the peak beam current is up to 550 mA during measurement) for colliding and steady-state synchrotron experiments. It should be noted that this single bunch has no impact on both the colliding and synchrotron experiments.

The timing profile was measured as shown in Fig. 3 indicating the timing signal of the storage ring, the pulse signal of the synchrotron excitation light, the gating signal of CFD and the stop signal of the

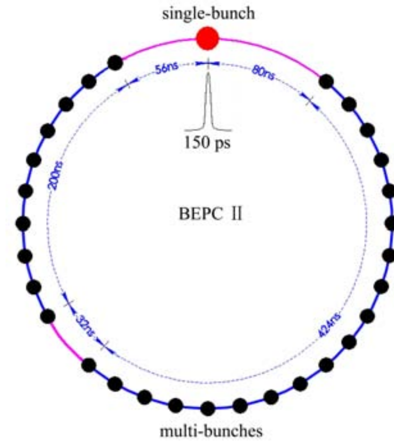

Fig. 2. The schematic of a hybrid filling pattern in BEPCⅡ colliding mode. Two multi-bunch groups (200 ns and 424 ns long, respectively) are separated asymmetrically with a large gap of 136 ns. A single bunch (the bigger circle) is placed in this large gap for fluorescence lifetime measurement. The number of bunches in the multi-bunch group is indicative.

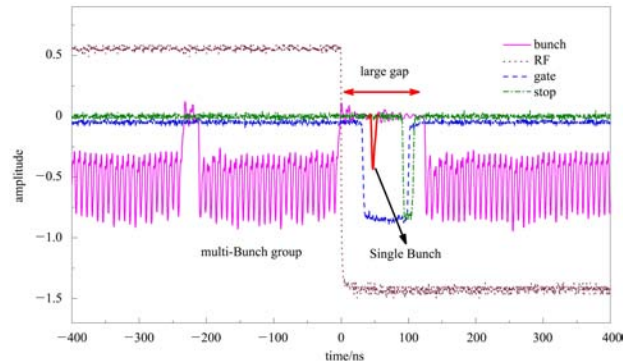

Fig. 3. The timing profile of lifetime measurement based on a designated single bunch. The hybrid filling pattern in a revolution period is revealed by the pulse signal of synchrotron light (solid); RF (dot) is the revolution frequency of the storage ring, i.e. the timing signal; Gate (dash) is the gating signal of CFD generated by BNC 8010; Stop (dash-dot) is the stop signal of TAC generated by SCA552. The RF signal is offset intentionally to make the profile clear.

TAC. The gating signal, triggered by the timing signal and input to the CFD, was adjusted in delay and width to catch the fluorescence event excited by the light pulse from the designated single-bunch, so the CFD will only accept the desired fluorescence signal. Besides this colliding mode with the hybrid filling pattern, a single-bunch mode was also applied to compare the fluorescence lifetime results.

3.2 Fluorescence lifetime measurement

As an intrinsic fluorescence probe of protein molecules, the decay lifetime of L-Tryptophan is subject to local environmental impact, so it can be used to indicate the protein conformation change and has become a powerful tool to study the protein folding and enzyme catalysis [2]. Here L-Tryptophan is chosen to assess the performance of the fluorescence lifetime measurement based on the designated single bunch in the colliding mode. As shown in Fig. 4, the lifetime of L-Tryptophan is bi-exponential decay and the short-lifetime component (τ_2) is less than the system time resolution (450 ps, as discussed below), so the long-lifetime component (τ_1) will be compared with the result measured in the single-bunch mode. It is noted that there exists a large background counts in the raw data in the colliding mode. The background counts were mainly caused by the afterpulses of PMT, which is much higher in the colliding mode than that in the single-bunch mode due to PMT afterpulse contributed by multi-bunches. However, after the background removal, the fitted lifetime was consistent with that measured in the single-bunch mode and the fit qualities were very close to each other. The lifetime value was also consistent with the reported value [15], thus validating the fluorescence lifetime measurement based on the excitation from the designated single-bunch in the colliding mode.

The FWHM of the IRF, representing the time resolution of the entire system, is 450 ps with the timing signal as the stop input. IRF mainly depends on the light pulse width and the transit time dispersion (TTS) of the PMT H7422. Alternatively, the excitation pulse signal, detected by XP2020Q for filling pattern measurement, can be used as the stop input, however, the IRF degrades to 800 ps due to the ~300 ps TTS of the XP2020Q, which is much larger than the ~50 ps jitter of the timing signal.

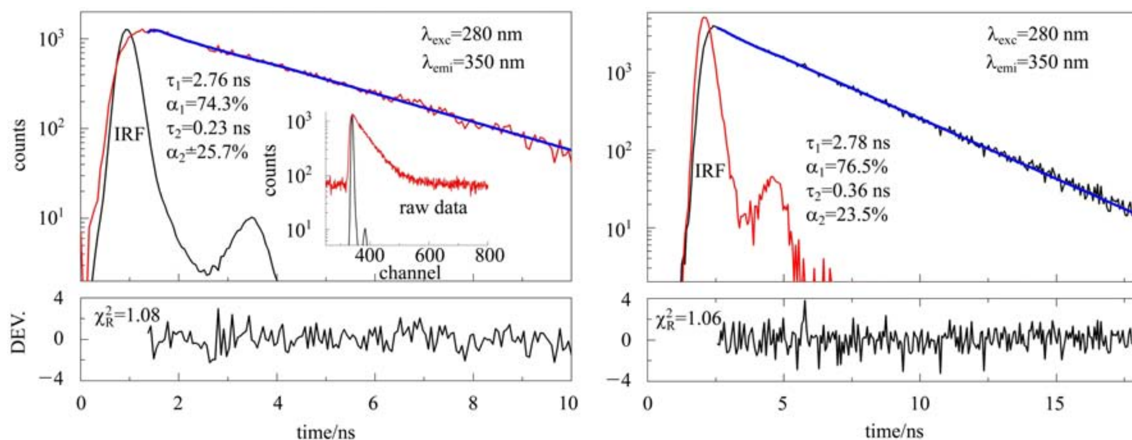

Fig. 4. The lifetime fitting results of L-Tryptophan measured in colliding (left, the inset is the raw data) and single-bunch mode (right). The colliding mode: τ_1=2.76 ns, α_1=74.3%; τ_2=0.23 ns, α_2=25.7%. The single-bunch mode: τ_1=2.78 ns, α_1=76.5%; τ_2=0.36 ns, α_2=23.5%. The excitation wavelength is 280 nm and the emission wavelength is 350 nm. IRF is for the instrumentation response function. χ^2 is the goodness-of-fit.

3.3 Characterization of filling pattern and bunch purity

The hybrid filing pattern, usually used for time-resolved experiments, needs to be monitored to check bunch filling performance. Using the TCSPC method, the fine structure of filling pattern [16] was also characterized, as shown in Fig. 5(a), with bunch spacing 8 ns within a multi-bunch group. It is consistent with the filling bucket distribution.

Time-resolved experiments generally require high bunch purity, defined as the ratio of electron populations between the wanted and unwanted bunches [16, 17]. The TCSPC method can be used to monitor the bunch purity by counting the photon signal from the wanted and unwanted bunches, so the designated single-bunch purity in hybrid filling mode was measured (Fig. 5(b)). The single-bunch purity is better

than 5×10^3, which is adequate for lifetime measurement. At present, the measured purity sensitivity is limited by the background noise caused by the afterpulse of PMT.

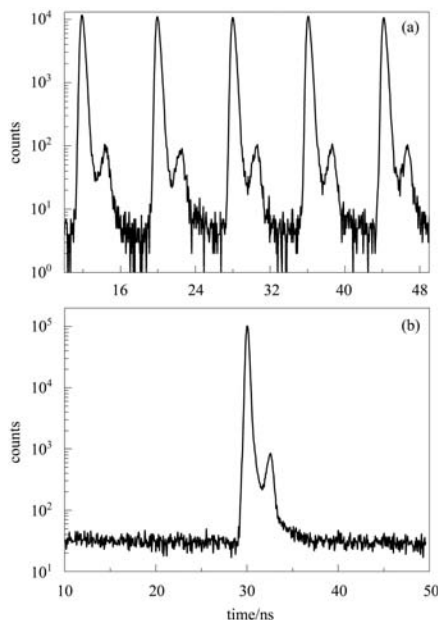

Fig. 5. The bunch filling pattern and bunch purity measured in the colliding mode with the hybrid filling pattern. (a) The fine structure of BEPCⅡ filling pattern, 10 seconds counting; (b) the single-bunch purity measurement, 100 seconds counting. The side-peak is caused by photoelectrons reflected at the first dynode of the PMT, an inherent characteristics of PMT.

4 Conclusion

In the colliding mode of the BEPCII, a hybrid filling pattern was exploited to provide a well separated single bunch between multi-bunch groups. The timing signal from the storage ring is used to trigger the gating of the fluorescence event excited by the light pulse from this designated single bunch. The lifetime measurement of L-tryptophan validated this method, making fluorescence lifetime measurement available in colliding mode other than just being available in single-bunch mode, as was the case previously. To our knowledge, this is the first exploitation of fluorescence lifetime measurement in colliding mode. In the meantime, the TCSPC method has been applied to measure the bunch purity and the fine structure of the filling pattern. It provides a convenient way to monitor bunch filling performance.

A hybrid filling pattern can be applied in dedicated synchrotron mode to make more beamtime available for time-resolved experiment. Both the timing signal and the developed gating technique can be used for other time-resolved methods. There is also a large potential to improve the fluorescence lifetime system. For example, the time resolution can be pushed down to less than 200 ps using a MCP-PMT. At present, the sample is excited in the UV and VUV range, but this method can be readily implemented in X-ray excited optical luminescence (XEOL) study, which will make a wider range of materials accessible to fluorescence lifetime study.

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