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Auto-phase-locked time-gated luminescence detection for background-free upconversion spectra measurement and true-color biological imaging

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ABSTRACT

Time-resolved technique is widely used in biological detection and imaging, since this technique can eliminate the background signals from scattering and short-lived autofluorescence and greatly increase the signal-to-noise ratio. However, the relative apparatus always require pulse source, gated detector and electronic phase matching circuitry, which are expensive to implement and maintain. Herein, a simple method for time-gated upconversion luminescence spectra measurement and biological imaging was developed. By adjusting the exciting and detecting optical paths to pass through the same chopper wheel, only one mechanical chopper was needed, which simultaneously acted as pulse generator and detecting shutter. The phases of each excitation and time gate were synchronized and locked automatically as the optical paths fixed. Therefore, no complex electronic phase matching circuitry or control system was needed. By equipping with a 980 nm CW laser as exciting source, the time-gated spectra of upconversion lanthanide luminescence free from the scattering light was measured. Moreover, smart phones or the human eyes could easily detect the delayed luminescence of these materials, promising the true-color biological imaging with common cameras. This approach could be used in many other time-gated luminescence detection for long-lived luminescence materials and probes excited by other light sources.

1. Introduction

Lanthanide-doped upconversion nanocrystals have been widely used in biological assays and imaging [1,2]. Since these materials exhibit visible luminescence when excited by NIR light [3,4], this technique offers low autofluorescence background and high penetration depth comparing with the downconversion. However, due to the low absorption cross-section of the lanthanide ions, the power density of the exciting light was very high for imaging experiments [5]. This directly increased the scattering signals [6]. To overcome these challenges, time-gated luminescence (TGL) measurement is a usefull technique. It usually operates with a delay time and detects the events that occur at much longer timescales [7–14]. Therefor this technique can eliminate the background signals from scattering and short-lived autofluorescence and greatly increase the signal-to-noise ratio. It plays important roles in high-contrast biological detection and imaging for the observation of functional and molecular recognition events [15–22].

For most time-gated luminescence measurements, a pulsed excitation source and a gated detector are the basic requirements in relative apparatus [7–14]. In addition, the luminescence lifetimes of the probes should be long enough to be distinguished from background fluorescence in practical application. The most used probes in time-resolved detections are phosphorescence transition-metal complexes [15–21]. Their typical luminescence lifetimes are in the scale of about 10^{-6} to 10^{-3} s. To detect their delayed luminescence, shutter of the "gated" detector, as well as pulse cycle, should be operated within the approximately same time range. Although some high-speed cameras, such as ICCD (Intensified Charge-Coupled Device)[23], EMCCD (Electron-Multiplying CCD) [6] or stream camera [24–26], can photograph with a delay time from nanosecond to microsecond, they are prohibitively expensive. In order to reduce costs, a mechanical chopper or ferro-electric shutter was utilized as a gate in some detectors [27]. Besides,

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mechanical choppers were also used to generate pulsed excitation to replace some expensive laser sources [28,29]. However, all these systems require that the phase of each excitation and detecting shutter are precisely synchronized with both the excitation source and the detector acquisition state. This requires complex electronic phase matching circuitry and control systems [23]. In addition, phase jitter of mechanical chopper and circuit delay should be avoided to reduce the scattering signals. These directly make the instrument expensive to implement and maintain. Therefore, a low-cost time-gated luminescence system that is simple to both implement and use is highly demanded. So far as we know, time-gated luminescence imaging was mainly used though downconversion exciting [15–23,30–33]. Only a few group reported some work on time-gated upconversion luminescence imaging [4,6,25,34]. And in order to obtain the delayed luminescence with high sensitivity, gateable photomultiplier tube (PMT) or monochrome camera was used in these systems, which inevitably lost the color information. The challenge remains to develop a true-color time-gated imaging system for upconversion luminescence detetction with low cost and high sensitivty.

Herein, we developed a simple method to realize the detection of time-gated luminescence. By adjusting the exciting and detecting optical paths to pass through the same chopper wheel, only one mechanical chopper was needed, which simultaneously acted as pulse generator and detecting shutter. The phases of each excitation and time gate can be synchronized and locked automatically as the optical paths fixed. Therefore, no complex electronic phase matching circuitry or control system was needed. The pulse cycle and gate time can be adjusted to approximate values by setting the speed and duty ratio of the chopper to detect the delayed luminescence. And this method can effectively reduce the scattering signals even if the chopper frequency jitters. This simple assembled system can equip with any CW excitation source and measure the delayed spectra of many kinds of luminescent materials such as lanthanide-doped upconversion materials. This auto-phase-locked approach could also be used for naked-eye detection of delayed luminescence and true-color time-gated luminescence biological imaging.

2. Material and methods

2.1. Materials

Tm/Yb doped $NaYF_4$ nanocrystals (Tm 2%, Yb 18%, Fig. S9), Er/Yb doped $NaYF_4$ nanocrystals (Er 2%, Yb 18%, Fig. S9) and water-dispersed Er/Yb doped $NaYF_4$ nanocrystals (Er 0.02%, Yb 18.8%) were synthesized using the reported routes [35,36].

2.2. Time-gated luminescence system for spectra detection

A typical time-gated luminescence system for spectra detection is illustrated in Fig. S1. The optical chopper (MC2000B-EC, Thorlabs, Inc.) was equipped with an adjustable duty cycle blade (MC1F10A, Thorlabs, Inc.) at a radius of 10.2 cm, consisting of 10 slots with a duty cycle setting to 20% (Fig. S2). A Continuous-Wave 980 nm (adjustable power) laser, reflected by a mirror, was focused on the sample to excite upconversion materials. A collimating lens, couple with a fiber spectrometer (PG2000-Pro, Ideaoptics, Inc.), is used to collect the light in the particular direction. The whole system was shaded by an opaque cloth when detecting the luminescence of the samples.

The exposure time of the fiber spectrometer was set to a constant value when measuring the same sample in time-gated mode. Although the shutter time in each cycle shortened as the chopping frequency increased, the number of the shutters increased, making the real gate time (= exposure time × cycle duty) a constant value. However, the delay time was inversely proportional to chopping frequency. So if the delayed luminescence was detectable in this system, its intensity would increase significantly as the chopping frequency increased, while the scattering signals was irrelevant to the chopping speed.

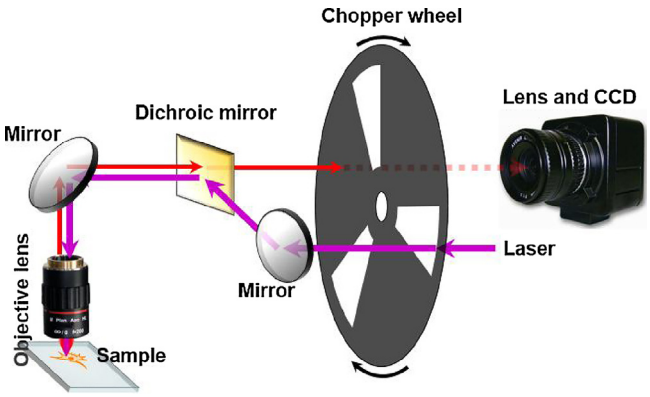

Fig. 1. Schematic of the time-gated luminescence microscope.

2.3. Time-gated luminescence microscope

The time-gated luminescence system for cell imaging is illustrated in Fig. 1. Similar to the system for spectra detection, the same optical chopper, blade and laser were used. The 980 nm laser, reflected by a mirror, a short pass dichroic mirror and a mirror successively, was focused on the sample on a 2D displacement platform by an objective lens (20 ×, NA.= 0.4). The diameter of the excitation laser focused was about 50 μm, estimated by an ocular micrometer. The upconversion luminescence, reflected by the mirror, could pass though the dichroic mirror and the chopper blade, then enter in the CCD color camera (Sony ICX282). A yellow LED was placed under the sample for bright field imaging.

2.4. The principle for time-gated detection

The principle and process for time-gated luminescence detection is illustrated in Fig. S1. As the chopper wheel rotates continuously, the continuous laser is converted to pulse train to excite the sample. Simultaneously, the same chopper acts as a shutter to switch on and off the optical path from the sample to the collimating lens. Therefore, the excitation and the detecting gate have the same cycle, and the pulse width equals to the gate time in each cycle. However, the exciting and detecting paths intersect the chopper wheel at different points, making the two paths open at different phases (Fig. S2 and S3). With a time delay caused by the phase difference (Fig. S1B), the delayed luminescence can be detected without the interference from the excitation source. The principle with more details is shown in supporting information (Fig. S1). To be brief, the delay and the shutter is auto-synchronized with excitation pulse, leading to a simple and low cost time-gated system that needs no gated detector, pulsed light source or phase lock circuit.

3. Results and discussion

The temporal resolution of this system is highly dependent on the chopper speed. In order to detect the delayed luminescence efficiently, the excitation pulse should switch off faster than the luminescence decay. The maximum rotation rate of the chopper (MC2000B-EC, Thorlabs, Inc.) is 100 RPS. With a 10 slots chopper blade, the highest frequency can reach 1 k Hz. Under this constant