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Spectral discrimination between normal and leukemic human sera using delayed luminescence

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Abstract: In this work, photoinduced delayed luminescence (DL) was used to distinguish serum samples of patients with acute lymphoblastic leukemia from those of healthy volunteers. DL decay kinetics of human serum samples was measured using a homebuilt ultraweak luminescence detection system. It was found a significant difference in the weight distribution of the decay rate between normal and leukemic serum samples. A comparison of the DL kinetics parameters including the initial intensity, the peak decay rate, and the peak weight value was used in making discrimination between normal and leukemic human sera. Results in this work contribute to the development of a novel optical method for the early diagnosis of leukemia.

OCIS codes: (170.0170) Medical optics and biotechnology; (170.4580) Optical diagnostics for medicine; (300.6280) Spectroscopy, fluorescence and luminescence.

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1. Introduction

Serum, the portion of blood plasma that consists of non-coagulating proteins, electrolytes, antibodies, antigens, hormones, and any exogenous substances, represents an important biological material for disease diagnosis [1–4]. To diagnose leukemia, numerous methods have been employed to acquire information about the changes of serum components that serve as disease markers. For example, serum levels of vascular endothelial growth factor of the patients with hematological malignancies were examined by enzyme-linked immunosorbent assay method [5]. The concentrations of trace and major elements in serum of patients with chronic myelogenous leukemia were determined by using total reflection X-ray fluorescence with synchrotron radiation [6]. With the development of optical technology, the intrinsic optical properties of serum have attracted the interest of spectroscopists and medical research scientists. One recent achievement is that absorption spectroscopy has been applied to discriminate between normal and leukemic human sera based on intensity ratio measurements [7]. It is a quick method for making preliminary evaluation, but its accuracy is only about 80%. To further improve the accuracy of disease diagnosis, a new method based on photoinduced delayed luminescence (DL) is worthy of attention and exploration.

DL, referring to a long-term light-emitting decay process after illumination, is a sensitive indicator of the functional states of many biological systems, including plants, animals, human cells and tissues [8,9]. Previous investigations have shown that there is a close correlation between the parameters of DL and the characteristics of organisms, such as the biological activity, injury, physiological and pathological changes [10–15]. Owing to this striking correlation and the advancement of ultraweak light detection technology, it is possible to obtain information about the changes of substance components and biochemical reactions in serum by using DL as a fast, sensitive optical indicator [16]. With the aim of developing a novel diagnostic method of leukemia, we report in this paper the use of DL to spectrally discriminate between normal and leukemic human sera.

2. Experimental section

2.1. Materials

All starting materials were purchased and used without further purification, unless otherwise specified. Serum samples from 12 healthy volunteers (38.8 ± 12.7 years) and 9 patients with acute lymphoblastic leukemia (ALL) (33.2 ± 14.9 years) were obtained from Tianjin Medical University General Hospital and Tianjin Hematonosis Hospital, respectively. To eliminate the

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quenching of DL by water, serum samples were vacuum-dried at room temperature and before their use in the spectral measurements.

2.2. Steady-state spectral measurements

Diffuse reflectance absorption spectra of human serum were recorded on a Shimadzu UV-2101 spectrophotometer. DL emission spectra of human serum were recorded with a CCD camera after passing through a monochromator (SpectraPro-300i, Acton Research, U.S.A.). Xenon flash lamp (L7685, Hamamatsu Photonics, Japan) was used as the excitation light source.

2.3. DL decay kinetics measurements

The experimental setup of the system has been described previously [17]. A xenon flash lamp (L7685, Hamamatsu Photonics, Japan), with a pulse width of 5 μs and adjustable pulse energy and flash frequency, was chosen for illumination. The detection system was composed of a highly sensitive PMT (R943-02, Hamamatsu Photonics, Japan) cooled down to −20 °C, a preamplifier (SR445, SRS, U.S.A.), a photon counter (Multichannel Scaler/Averager, SR430, SRS, U.S.A.), two shutters and a sequential control unit. The two shutters are located in the excitation and signal light paths and are able to complete open and close actions respectively one after the other within 15 ms interval, which can eliminate the disturbance by the afterglow of light source and ensure the safe measurement of the luminescence signal by the PMT. DL decay kinetics measurements were carried out under the same excitation conditions (mean power: 30 mW, illumination time: 16 s) at constant temperature (22 °C) and humidity (40% RH). A bin width of 1.31 ms was used for all measurements. For each sample, three repeated measurements were performed.

3. Results and discussion

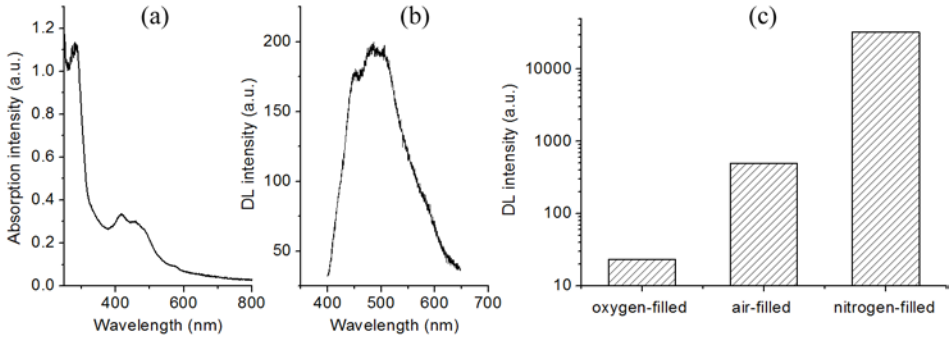

Fig. 1. (a) Absorption spectra of human serum. (b) DL spectra of human serum. (c) DL intensities of human serum in the sample chamber filled with oxygen, air, and nitrogen.

Figure 1(a) shows the absorption spectrum of human serum. The absorption features are consistent with the literature [18], while the dominant absorption peak at 280 nm is mainly due to the proteins in the serum [5,19]. Figure 1(b) shows the DL spectrum of human serum, which exhibits similar emission characteristics comparing to previously reported fluorescence spectra [18,20]. It is reported that the main fluorophores present in human serum are identified as tryptophan, NAD(P)H, pyridoxic acid lactone, pyridoxal phosphate Schiff base, and protein-bound bilirubin [21]. Our extended experiments showed that the DL intensities of human serum decreased when exposed to an increasing amount of oxygen (Fig. 1(c)). This suggests that oxygen shows remarkable quenching effects on the DL emission. Since oxygen is known as an effective quencher for long-lived luminescence [22], we postulate that the DL of human serum is mainly originated from the excited triplet state of biomolecules.

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The DL decay curves of normal and leukemic human sera in the duration of 20~1000 ms after illumination are shown in Fig. 2. A good repeatability for the measurements of the same sample could be found, as the percentage standard deviation corresponding to each time point within 1 s is less than 6%. In addition, the system noise was evaluated by using magnesia powder as the photon scatterer. The background count is about 32 c/s in the magnitude, which could be neglected when compared to the DL intensities of serum samples within 1 s after illumination. The outstanding signal-to-noise ratio of this detection method provides the basis for reliable measurements of ultraweak luminescence signals.

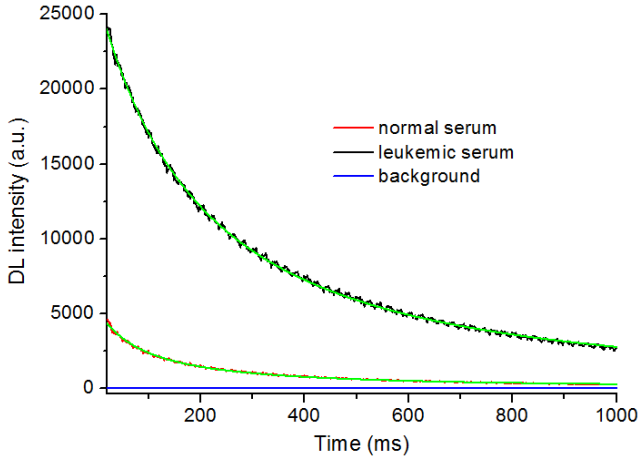

Fig. 2. DL decay curves of normal (red) and leukemic (black) human sera. Each curve represents the mean values of three individual measurements. The data are fitted using hyperbolic function Eq. (1) (green). For normal serum, I_0 = 5347 c/1.31 ms, t_0 = 111.92 ms, m = 1.276, R^2 = 0.997. For leukemic serum, I_0 = 26325 c/1.31 ms, t_0 = 315.04 ms, m = 1.574, R^2 = 0.999. The background (blue) was obtained by replacing the serum sample with magnesia powder.

A significant difference in DL decay features between normal and leukemic serum samples is observed (Fig. 2). To quantitatively characterize the difference, it is necessary to find a suitable mathematical model to describe the DL decay curves. Previous investigations have shown that for different biological systems, there are a variety of mathematical models available [12–14]. In this study, the DL decay curves of both normal and leukemic sera were characterized by a hyperbolic function (Eq. (1)) with a high degree of fitting (0.999 and 0.997):

$$I(t) = \frac{I_0}{\left(1 + t / t_0\right)^m}, \tag{1}$$

where I_0 and $I(t)$ respectively denote the DL intensities at the initial time t = 0 and time t after illumination, and m is the index factor. DL characteristics are described by I_0, t_0, and m, which can be obtained by fitting the experimental data. I_0 is dependent on various factors, such as the sample composition, the surface morphology, the excitation conditions, and so on. Compared to I_0, the DL decay kinetics can be better characterized by t_0 and m, which can provide us information reflecting the intrinsic nature of the biological systems [12].

It is found that the DL decay kinetics of different kinds of biological systems are well in line with the hyperbolic function [12,23]. Based on this phenomenon, it is proposed that the hyperbolic relaxation of biological systems is a characteristic active response of an ergodic coherent state [8,9]. However, there are also viewpoints stating that this decay can be also generated by the non-coherent superposition of various exponential relaxations with different decay rate constants. That is, the DL is a comprehensive embodiment of various decay

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processes of luminescence in the biological systems [23]. In the case of human serum, our previous results implied that its DL contains a variety of light-emitting decay processes [17]. Therefore it is reasonable to describe the DL decay kinetics as the superposition of exponential functions with different decay rate constants and weight coefficients, as expressed by Eq. (2):

$$I(t) = \int_0^\infty Af(v)e^{-vt}dv, \tag{2}$$

where A is the normalized constant and is set to be I_0 to eliminate the impact of the initial intensity of DL, v is the decay rate, and $f(v)$ refers to the weight distribution for the factor v. From the viewpoint of mathematical analysis, the distribution function $f(v)$ is the Laplace transform of $I(t)/I_0$. Therefore, $f(v)$ can be obtained through anti-Laplace transform processing:

$$f(v) = t_0^m v^{m-1} e^{-vt_0} / \Gamma(m), \tag{3}$$

where the gamma function $\Gamma(m) = (m\text{-}1)!$. Based on Eq. (3), the weight distribution of the decay rate v for the DL of each sample is obtained and is shown in Fig. 3(a).

It may be mentioned that the above method has been applied to analyze the DL decay kinetics of human cells and skin [12,13]. Compared to the reported biological systems, the DL decay rate v of human serum moved to a lower range indicating much longer DL lifetimes. From Fig. 3(a), it can be noticed that the DL parameters of serum samples belonging to the same group also exhibited a noticeable individual difference, which should be correlated with the variations in sex, age, health status, and other factors of serum providers. Figure 3(a) reveals a significant difference in the weight distribution of the decay rate v between normal and leukemic serum samples. To make a quantitative analysis of the difference, the peak parameters of the weight distribution $f(v)$, including the peak decay rate v_{max} and its corresponding peak weight value F_{max}, were used to describe the DL decay kinetics.

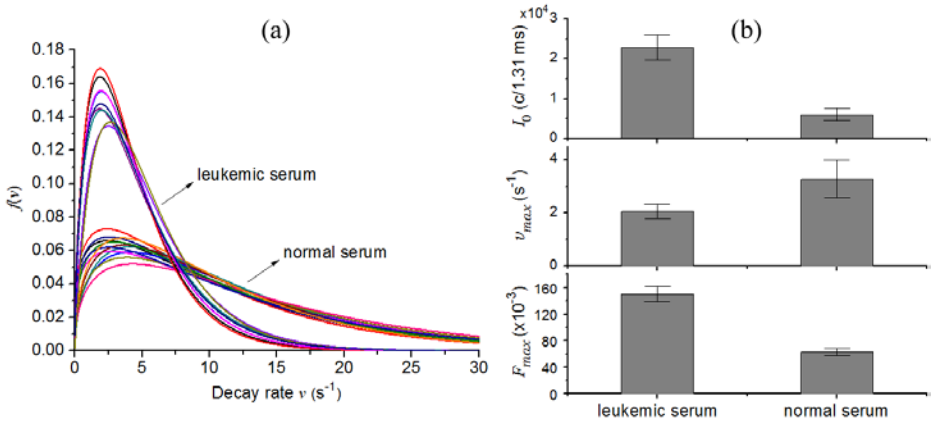

Fig. 3. (a) Weight distribution of the decay rate v for the DL of normal and leukemic serum samples. (b) Comparison of I_0, v_{max}, and F_{max} between normal and leukemic human sera.

For $df(v)/dv = 0$, we can obtain v_{max} and F_{max} as follows:

$$v_{max} = \frac{m-1}{t_0}, \tag{4}$$

$$F_{max} = f(v_{max}) = t_0(m-1)^{m-1}e^{1-m} / \Gamma(m). \tag{5}$$

According to Eqs. (1), (4), and (5), three characteristic parameters (I_0, v_{max}, and F_{max}) of the DL can be obtained. Comparison of these parameters between normal and leukemic serum

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samples is shown in Fig. 3(b). As a result, the initial intensity I_0 (22857 ± 3209 c/1.31 ms) and peak weight value F_{max} (15.03 ± 1.10 × 10^{-2}) of DL from leukemic serum samples are respectively 278% and 139% higher than those of the healthy group (I_0 = 6045 ± 1470 c/1.31 ms, F_{max} = 6.29 ± 0.55 × 10^{-2}). In addition, the peak decay rate v_{max} (2.06 ± 0.28 s^{-1}) for leukemic serum samples is noticeablely less than that of the healthy group (3.28 ± 0.71 s^{-1}). To make a statistical analysis of these differences, bilateral *t*-test was used for the comparisons. In consequence, *p*-values for I_0, v_{max}, and F_{max} between the two groups are 4.82 × 10^{-8}, 1.01 × 10^{-4}, and 4.41 × 10^{-10}, respectively [24]. These *p*-values ($p < 0.01$) indicate that the differences in the three parameters between normal and leukemic serum samples were statistically significant. Therefore, a comparison of these parameters can be considered as a way to distinguish leukemic serum samples from healthy controls.

4. Conclusions

In this paper, we measured DL from both normal and leukemic human sera using a homebuilt ultraweak luminescence detection system. Outstanding signal-to-noise ratio of the detection system was achieved providing the basis for reliable DL measurements. We found a significant difference in the weight distribution of the decay rate between normal and leukemic serum samples. A comparison of the initial intensity (I_0), the peak decay rate (v_{max}), and the peak weight value (F_{max}) can be used in making explicit discrimination between normal and leukemic human sera. Future works will try to identify the biological species that are responsible for the variation of the kinetics parameters in the DL of diseased human plasma/serum.

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