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DELAYED LUMINESCENCE AS AN OPTICAL INDICATOR OF TOBACCO LEAF QUALITY

© 2013 г. **Ping Chen[1]; Lei Zhang[1]; Song-Cheng Mao; Xing Li; Feng Zhang; Chang-Hai Shen[2]; Guo-Qing Tang; Lie Lin**

Institute of Modern Optics, Key Laboratory of Optical Information Science & Technology, Ministry of Education of China, Nankai University, Tianjin, China

E-mail: chping@nankai.edu.cn

In this paper, we present our study on the spectral discrimination between high and low quality tobacco leaves using a time-resolved ultraweak luminescence detection system. Photoinduced delayed luminescence (DL) is employed as a nondestructive and objective indicator of tobacco leaf quality. DL decay kinetics of tobacco leaf samples is measured, and the data are fitted by a hyperbolic cosecant function. Results show that the function's parameter A is significantly related to the quality grades of tobacco leaves – compared with the low quality tobacco leaves, an increase of the A value by a factor of 7 is obtained for the high quality tobacco leaves. Research from this work contributes to the development of a novel optical method applicable for the quality evaluation of agricultural crops and food products.

Keywords: *delayed, luminescence, optical indicator, ultraweak detection system, tobacco quality.*

OCIS Codes: 170.6280, 170.6510, 260.3800.

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Introduction

Tobacco is one of the most profitable industries in both developed and developing countries [1]. One important and fundamental step in cigarette production is the quality grading of tobacco leaves, which involves a combined work of visual inspection, chemical composition analysis, and sensory evaluation [2–4]. The analysis of the chemical compositions, such as total nitrogen, total alkaloid, reductive sugar, nicotine, and so on requires complicated sample preparation procedures, while sensory evaluation could cause health problems [5–7]. Consequently, the grading of tobacco leaves is often done manually via visual inspection by qualified technicians. However, due to the diverse origins, visually classifying tobacco leaves by the shape, size, texture, and color is a difficult work and is subject to the level of experience of different technicians.

To avoid the inconsistency of the analyses, equipment-based quality grading of tobacco leaves with a simple sample preparation procedure is increasingly attracting research attention. Considering that the above-mentioned properties (shape, color, etc) relate to human vision, it is not surprising that most of the current efforts take advantage of the computer vision to process the images of tobacco leaves [8–10]. Nevertheless, research has shown that the sensory quality of tobacco leaves is highly correlated with their chemical components [4, 5]. Therefore, it is demanded to evaluate the tobacco leaf quality by novel techniques, providing that the measurement results can reflect chemical component information.

Ultraweak self-bioluminescence, also called biophoton emission (BPE), is a type of biological chemiluminescence strongly correlated with cellular function and state of health [11]. However, the intensities of BPE were measured in extremely low levels from a few up to hundreds of photons per unit area (cm^2) per second [12], which is comparable to the intensities of candle emission recorded 10 km away. To facilitate the measurement of BPE signals, a photoinduced method has been utilized by shining light on the sample and recording the delayed luminescence (DL). The phenomenon of DL is known as the long-lived afterglow of biological systems after being illuminated with white or monochromatic light [13–15]. DL was first observed on green plants [16], while later investigations confirm that

[1] Corresponding authors.

[2] Present address: Peregrine Semiconductor, Inc., Shenzhen 518052, China.

all living systems display distinct DL [17–20]. As a sensitive indicator of interactions between photons and living matter [21], DL can be related to the changes of functional states of a number of biological systems [22–24]. In this work, we construct a time-resolved ultraweak luminescence detection system and carry out DL measurements toward nondestructive spectral discrimination between high and low quality tobacco leaves.

Materials and methods

Tobacco Samples

Tobacco leaves used in this study are Zhoukou B2F of Henan, Longyan C2F of Fujian, and Liangshan X3L of Sichuan, which are kindly provided by the Tianjin Tobacco Company. All of these samples are strips of tobacco leaves, which are further smashed into powder for experimental use.

Steady-State Spectral Measurements

Fluorescence emission spectra of tobacco leaves 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.

DL Decay Kinetics Measurements

To perform DL decay kinetics measurements, an ultraweak luminescence detection system was homebuilt. The experimental setup of the system for monitoring DL decay kinetics is shown in Fig. 1 [25]. 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 at constant temperature (22 °C). A bin width of 10.24 μs was used for all measurements.

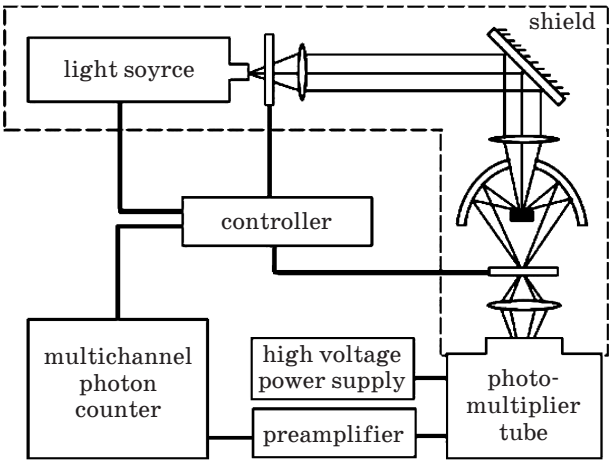

Fig. 1. Schematic view of the experimental setup.

Results and Discussion

Three kinds of tobacco leaves (Zhoukou B2F, Longyan C2F, and Liangshan X3L) are used in this study. In China, the classification of tobacco leaves follows a naming scheme of letter number letter. The first and last letters respectively indicate the stalk position and the color of tobacco leaves, while the middle number specifies the grade in this particular group of tobacco leaves [26]. As the reference information, it is already known that Zhoukou B2F and Longyan C2F tobacco leaves are high quality, while Liangshan X3L tobacco leaves is low quality.

To check the optical properties of tobacco leaves, steady-state fluorescence emission spectra were measured. To first examine if there is an excitation wavelength-dependent fluorescence behavior, Zhoukou B2F sample was excited at 390 and 450 nm, respectively. The results are shown in Fig. 2a. Two dominant peaks centering at *ca.* 580 and 680 nm can be identified unambiguously. As the excitation wavelength changes, the relative intensity ratio of these two peaks varies remarkably. This indicates that there are multiple species responsible for the fluorescence of tobacco leaves. The following extensive study, as can be seen in Fig. 2b, shows that all three kinds of tobacco leaves exhibit the same fea-

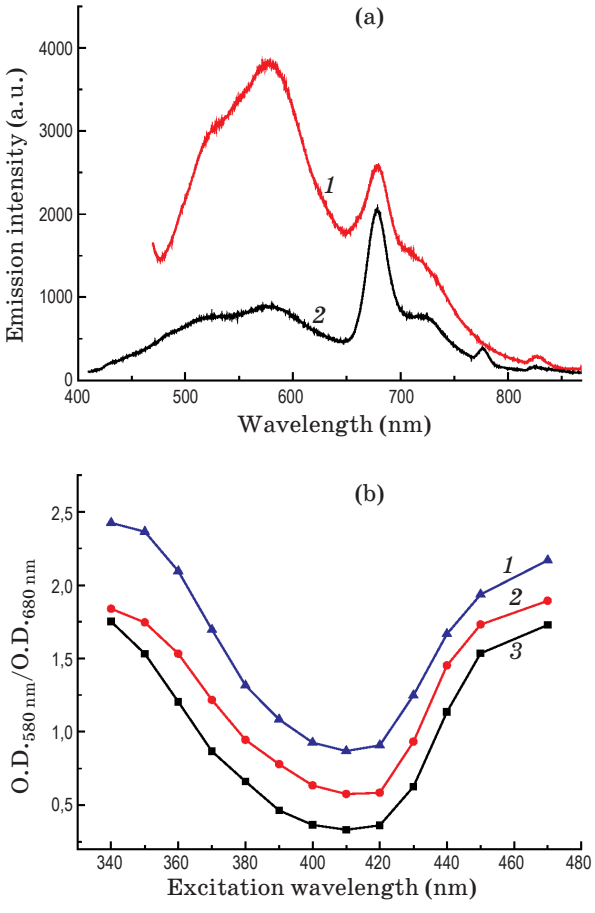

Fig. 2. (a) Fluorescence emission spectra of Zhoukou B2F tobacco leaves. Excitation wavelength: 390 nm (*3*); 450 nm (*2*). (b) Plot of the relative optical density ratios ($O.D._{580\ nm}/O.D._{680\ nm}$) at different excitation wavelengths. Tobacco leaves: Zhoukou B2F (*1*); Longyan C2F (*2*); Liangshan X3L (*3*).

ture of excitation wavelength-dependent fluorescence emission. However, we can also notice from Fig. 2b a varied ratio of the fluorescent species, since the relative optical density ratios ($O.D._{580\ nm}/O.D._{680\ nm}$) are evidently different when excited at the same wavelength. The steady-state spectral study can disclose certain optical information regarding the quality of tobacco leaves, but it is not informative in making a quality discrimination.

The DL decay curves of Zhoukou B2F, Longyan C2F, and Liangshan X3L tobacco leaves are shown in Fig. 3. A notable disparity in the DL decay features, especially the relative initial intensities, is observed. To quantitatively characterize the difference, it is necessary to find a suitable mathematical model to describe the DL decay curves. It is noteworthy that a model based on the quantum theory of radiation-matter interaction has been developed to boost our understanding of DL [27, 28]. This model starts from a general Hamiltonian, which leads to a master equation of the density operator and yields various analytical expressions for the radiation intensity as a function of the time. According to this model, it is found that Eq. (1) is able to accurately characterize the course of DL emission for many kinds of biological systems [28, 29]. Therefore, in this study, the DL decay curves were characterized by Eq. (1),

$$I(t) = A\mathrm{csch}^2(t/B + C), \qquad (1)$$

where $I(t)$ denotes the DL intensity at time t after illumination. DL characteristics are described by the parameters A, B, and C, which can be obtained by fitting the experimental data. Table gives a summary of the fitting results. A high degree of fitting (higher than 99.9%) was achieved for all analyses. Although it seems at risk of reaching any conclusions from the parameters B and C, the parameter A is significantly related to the quality grades of tobacco leaves – compared with the low quality Liangshan X3L

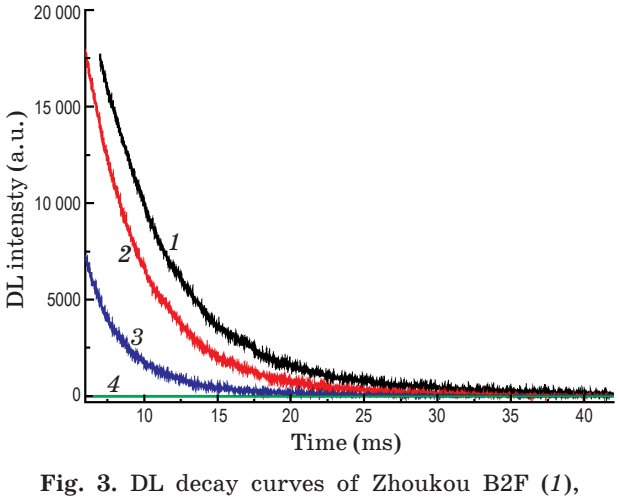

Fig. 3. DL decay curves of Zhoukou B2F (*1*), Longyan C2F (*2*), and Liangshan X3L (*3*) tobacco leaves. The background (*4*) is also measured.

Table

	A	B	C
Zhoukou B2F	21422.5	12.12	0.43142
Longyan C2F	23124.7	9.68	0.34829
Liangshan X3L	3129.4	8.42	–0.09677

(A = 3129.4), an increase of the A value by a factor of 7 is obtained for the high quality Zhoukou B2F (A = 21422.5) and Longyan C2F (A = 23124.7). This result confirms the effectiveness of using DL as an important indicator to spectrally discriminate between high and low quality tobacco leaves. We expect that this DL measurement method can become a powerful tool for applications in a variety of fields, such as food/water quality analysis and disease diagnosis.

Conclusions

In this work, photoinduced DL was used as a nondestructive and objective indicator for distinguishing tobacco leaves of different quality grades. DL decay kinetics of tobacco leaf samples was measured using a homebuilt ultraweak luminescence detection system. Fitting the DL decay curves by a hyperbolic cosecant function, a remarkable difference in the values of the function's parameter A was found, which supports the correlations existed between DL characteristics and tobacco leaf quality. Ultraweak self-bioluminescence detection provides a simple, yet sensitive approach that will find increasing use in optical and spectroscopic research. Future works will try to identify the fluorescent species that are responsible for the ultraweak self-bioluminescence.

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REFERENCES

1. *Yach D., Bettcher D.* Tob. Control 9, 206 (2000).
2. *Shao Y.N., He Y., Wang Y.Y.* Eur. Food Res. Technol. 224, 591 (2007).
3. *Shen H.F., Qian B.J., Yang L.T., Liang W.Q., Chen W.W., Liu Z.H., Zhang D.B.* Eur. Food Res. Technol. 231, 143 (2010).
4. *Sun J.G., He J.W., Wu F.G., Tu S.X., Yan T.J., Si H., Xie H.* Agric. Sci. China 10, 1222 (2011).
5. *Leffingwell J.C.* Rec. Adv. Tob. Sci. 2, 1 (1976).
6. *Fenner R.A.* Rec. Adv. Tob. Sci. 14, 82 (1988).
7. *Qin S., Wang Z.Y., Shi J.X.* Plant Nutr. Fert. Sci. 13, 443 (2007).
8. *Zhang F., Zhang X.H.* Sensors 11, 2369 (2011).
9. *Zhang H.M., Han L.Q., Wang Z.* Int. Conf. Mach. Learn. Cybern. 4, 2582 (2003).
10. *Zhang J., Sokhansanj S., Wu S., Fang R., Yang W.* Comput. Electron. Agric. 16, 231 (1997).
11. *Creath K.* Proc. SPIE 7057, 705708 (2008).
12. *Kim H.W., Sim S.B., Kim C.K., Kim J., Choi C.H., You H.R., Soh K.S.* Cancer Lett. 229, 283 (2005).
13. *Popp F.A., Yan Y.* Phys. Lett. A 293, 93 (2002).
14. *Yan Y., Popp F.A., Sigrist S., Schlesinger D., Dolf A., Yan Z.C., Cohen S., Chotia A., Photochem J.* Photobiol. B 78, 235 (2005).
15. *Wang C.L., Xing D., Zeng L.Z., Ding C.F., Chen Q.* Luminescence 20, 51 (2005).
16. *Strehler B.L., Arnold W., Gen J.* Physiol. 34, 809 (1951).
17. *Forbus W.R., Senter S.D., Wilson R.L., Food J.* Sci. 50, 750 (1985).
18. *Triglia A., Malfa G.La, Musumeci F., Leonardi C., Scordino A., Food J.* Sci. 63, 512 (1998).
19. *Musumeci F., Applegate L.A., Privitera G., Scordino A., Tudisco S., Niggli H.J., Photochem J.* Photobiol. B 79, 93 (2005).

20. *Costanzo E., Gulino M., Lanzano L., Musumeci F., Scordino A., Tudisco S., Sui L.* Eur. Biophys. J. 37, 235 (2008).

21. *Jursinic P.A.* in Light Emission by Plants and Bacteria, Ed. by Govindjee, J. Amesz, and D.C. Fork (Academic Press, New York, 1986).

22. *Baran I., Ganea C., Scordino A., Musumeci F., Barresi V., Tudisco S., Privitera S., Grasso R., Condorelli D.F., Ursu I., Baran V., Katona E., Mocanu M.M., Gulino M., Ungureanu R., Surcel M., Ursaciuc C.* Cell Biochem. Biophys. 58, 169 (2010).

23. *Mik E.G., Johannes T., Zuurbier C.J., Heinen A., Houben-Weerts J.H.P.M., Balestra G.M., Stap J., Beek J.F., Ince C.* Biophys. J. 95, 3977 (2008).

24. *Katsumata M., Takeuchi A., Kazumura K., Koike T., Photochem J.* Photobiol. B 90, 152 (2008).

25. *Bai H., Chen P., Lin L., Chang S.J., Tang G.Q., Mu G.G.* Proc. SPIE 7182, 71820K (2009).

26. National Standards of the People's Republic of China. GB 2635-92. Flue-cured tobacco.

27. *Popp F.A., Li K.H., Gu Q.* Recent Advances in Biophoton Research and Its Applications (World Scientific, Singapore, 1992).

28. *Gu Q., Popp F.A.* Experientia 48, 1069 (1992).

29. *Gu Q.* Radiation and Bioinformation (Science Press, Beijing, 2003).