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# Fast two-dimensional fluorescence correlation spectroscopy technique for tea quality detection

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**A fast two-dimensional fluorescence correlation spectroscopy technique based on light emitting diodes is developed, which uses light intensity and excitation wavelength as quickly changeable and easily controllable external perturbations. A compact and automatic system is set up to detect tea quality. A partial least square regression method is used to create predictive models for tea grades. Compared to the traditional fluorescence spectroscopy method, this convenient two-dimensional correlation spectroscopy technique is more accurate according to our experimental results and is promising for practical applications.** © 2015 Optical Society of America

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## 1. INTRODUCTION

Generalized two-dimensional (2D) correlation spectroscopy, which was first invented by Noda [1,2], is a powerful and versatile technique to resolve spectral characters. It is based on the correlation between different kinds of spectroscopies, such as x-ray [3], near-infrared [4], infrared [5], and Raman spectroscopy [6], of the investigated samples and the dynamic spectral bands caused by external perturbations, such as electric, thermal, and mechanical variables [7,8]. During the past two decades, the method has also been used on all kinds of occasions, such as prediction of ionic liquids in water [9], characterization of humic acids [10], and discrimination of different species of dendrobium [11].

The first application of the 2D fluorescence correlation spectroscopy technique was proposed by Roselli *et al.* [12], who utilized the excitation wavelength as a perturbation to investigate the metal-binding sites of proteins. Wang and Geng detected serotonin using fluorescence spectra under different pH levels [13]. Other useful external sources of perturbations for fluorescence spectroscopy include quenching [14], sinusoidally modulated excitation [15], polarization [16], and concentration [17]. However, these approaches are generally complex and take a long time to obtain dynamic spectra. Compact, simple, and online methods are always required in some practical applications, so a fast, automatic, and convenient system for measuring 2D fluorescence correlation spectra is proposed and developed in this paper. In our system, both the excitation intensity and wavelength perturbations could be controlled easily and quickly. To our knowledge, it is the first time that light source intensity is used as a perturbation variable to obtain enough fluorescence spectral differences for different samples.

Tea, as our investigated sample, is a healthy and popular beverage throughout the world [18]. Many kinds of methods, such as high-performance liquid chromatography [19], near-infrared spectroscopy [20,21], and fluorescence spectroscopy [22], are effective for tea quality evaluation. In our present work, instead of the original single fluorescence spectrum, 2D fluorescence correlation spectroscopy is used to extract more information about the samples. By combing the 2D correlation spectra with a partial least square regression (PLSR) method [23], a more reliable and accurate technique with compact configuration for tea quality evaluation is developed and verified.

## 2. EXPERIMENTAL SETUP

### A. System

The LED-based 2D fluorescence correlation spectroscopy system is shown in Fig. 1. There are four slots for different LEDs with a 5 mm diameter and 60 deg inclination angle as well as one Sub-Miniature-A port for a multimode fiber with a core diameter of 1.0 mm in the compact sensor head. A rotary filter wheel (FW1A, Thorlabs Inc., Newton, USA) is fixed on the horizontal flat slit of the sensor head, which is controlled by a step motor to select different filters according to different excitation wavelengths. Four LEDs with peak wavelengths of 400, 430, 450, and 500 nm were employed for detecting the tea leaves. Each LED is combined with a high-pass filter (OD 4

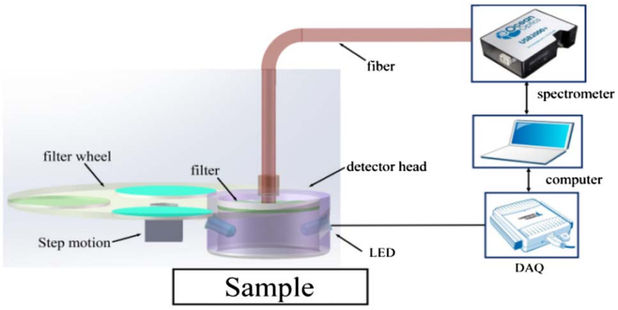

**Fig. 1.** Schematic of 2D fluorescence spectroscopy system based on LEDs.

High Performance Longpass Filter, Edmund Optics Inc., Barrington, New Jersey, USA) with cutoff wavelengths at 450, 525, 525, and 575 nm, respectively, to eliminate high-intensity excitation light. The intensities of the LEDs could be controlled by a data acquisition card (DAQ, National Instruments, USB 6008) connected to a computer. The excited fluorescence spectra were recorded by an optical spectrum analyzer (OSA, USB 2000, Ocean Optics Inc., Dunedin, Florida, USA). A LABVIEW (Labview 2010, National Instrument Co. Inc., Austin, Texas, USA) based program controls the DAQ, step motor, and OSA, by which the fluorescence spectra of tea samples at different excitation intensities or different excitation wavelengths could be automatically and quickly recorded. Further calculation and analysis was done with the help of these spectra to build 2D fluorescence correlation spectra.

### B. Samples

Shifeng Longjing tea samples with seven different grades were provided and evaluated by a Longjing tea company in Hangzhou, Zhejiang Province, China. These seven groups of tea leaves were picked from the Longjingcun tea plantation and stored in air-tight packaging. Their grades were marked by experts from one to seven. The higher the grade number, the better its quality and the higher its price.

### C. Data Processing

The principle of the 2D correlation method was put forward by Noda [2,7]. In this paper, the dynamic spectra could be defined as $y_j = y(v, k_j)$, where $v$ is the spectral variable and $k_j$ is the $j$th external perturbation. As the measured spectral data consist of discrete sets of numbers, the synchronous 2D correlation spectroscopy intensity, $\Phi$, is also in discrete form and could be expressed as [24]

$$\Phi(v_1, v_2) = \frac{1}{m-1}\sum_{j=1}^{m} y_j(v_1)\cdot y_j(v_2), \tag{1}$$

where $v_1$ and $v_2$ are the two spectral components of synchronous 2D correlation spectroscopy, $y_j$ stands for the $j$th dynamic spectrum, and $m$ is the number of dynamic spectra. A more detailed explanation of synchronous 2D correlation spectroscopy can be found in [24].

The PLSR technique is used to establish predictive models for tea grade evaluation. Assume that $X_{np}$ stands for the spectra of tea samples with different grades and $Y_{n1}$ represents the corresponding tea grades, where $n$ is the number of spectra and $p$ is the number of $X$ variables. PLSR decomposes both the predictor $X$ and objective $Y$ into several principal components. The principal components of $X$, denoted by $T$, is correlated with $Y$ as [23]

$$Y = TC + E = XW^{*}C + E, \tag{2}$$

where $E$ is the residual matrix, and $C$ and $W^*$ are weights of $Y$ and $X$, respectively.

To verify the effectiveness of the model, a strict statistic criterion, namely the root mean square error of the validation set (RMSEV), is used. The leave-one-out cross-validation method is utilized to build the validation set, by which a sample can be predicted by building up a PLSR model with the spectra excluding the sample's spectrum. The RMSEV is deduced as

$$\text{RMSEV} = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(Y_i' - Y_i)^2}, \tag{3}$$

where $n$ is the number of spectra, $Y_i'$ is the predicted grade value of the $i$th sample spectrum through the model, and $Y_i$ is the real grade. All the above analysis methods are implemented through a self-written program based on MATLAB (The MathWorks, Matlab R2008b).

## 3. RESULTS AND DISCUSSION

### A. Intensities as a Perturbation Variable

Shifeng Longjing tea leaves with seven grades were measured by the LED-based system. Each sample was measured six times at each intensity value. The excitation intensity of the 400 nm LED has four different values (21, 24, 27, and 29 mW), which were automatically changed. The integration time of each measurement was 800 ms, and thus the fluorescence spectra of samples could be quickly recorded. The average fluorescence spectra for a tea of grade seven at four different excitation intensities are shown in Fig. 2. The spectra are normalized to the

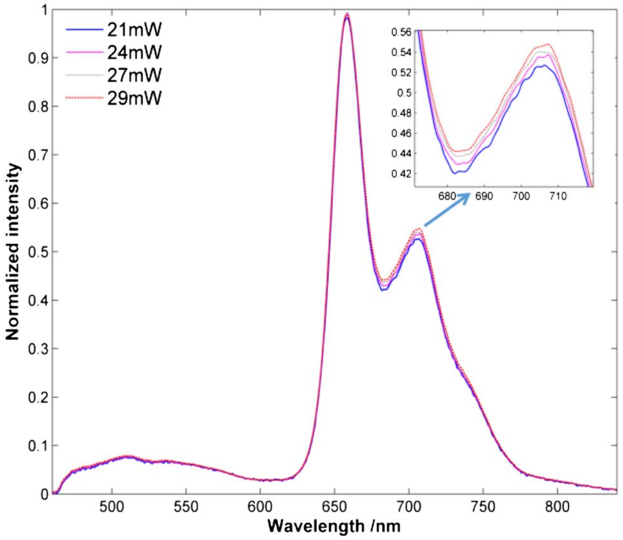

**Fig. 2.** Normalized fluorescence spectra for a tea of grade seven at four excitation intensities. The inset is the magnified spectra around 710 nm.

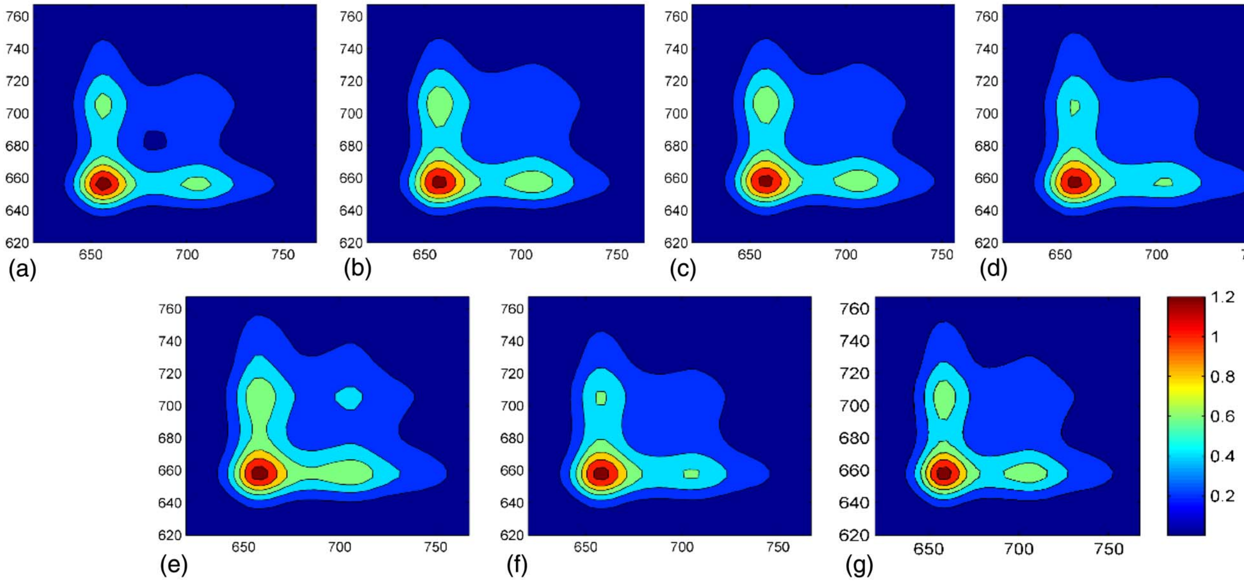

**Fig. 3.** 2D synchronous spectra of seven tea samples with different grades and varying intensities: (a)–(g) correspond to tea grades 1–7, respectively.

highest peak around 670 nm. The peak at 670 nm is caused by chlorophyll, and the peak at 720 nm is attributed to the energy transfer from Photosystem II to I. The spectral profile between 450 and 600 nm is a synthetic reflection of catechins, theaflavins, and anthocyanins. Figure 2 indicates that nonlinear fluorescence responses are produced during varying excitation intensities, and the higher excitation intensity corresponds to a higher second peak at about 710 nm.

The spectra between 620 and 770 nm of each sample are used to calculate the 2D correlation fluorescence spectra. Contour maps of the average 2D synchronous spectrum for tea samples with seven grades are shown in Fig. 3, in which different grades have apparently different maps. A relationship model between 2D synchronous correlation spectra and their corresponding actual tea grades are established with PLSR. The evaluated tea grades are calculated with the leave-one-out cross-validation method as shown in Fig. 4. The RMSEV value is only 0.1659. This result suggests that 2D correlation spectroscopy based on intensity perturbation is a powerful and effective tool for tea quality evaluation.

To further test the effectiveness of this 2D fluorescence correlation spectroscopy technique, the predictive models based on traditional fluorescence spectra with the same spectral region at four different intensities were created. The spectra of tea samples with seven different grades at the maximum excitation intensity (29 mW) are shown in Fig. 5. It indicates that the spectral differences are less obvious than the 2D spectra

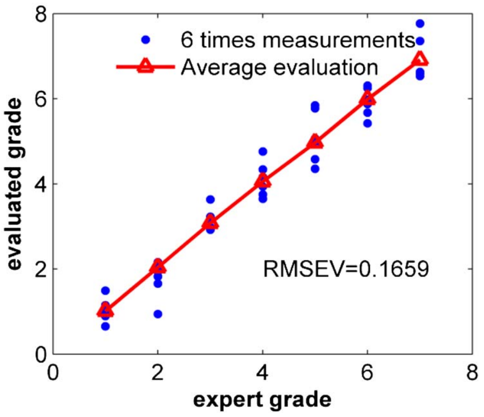

**Fig. 4.** Relationship between expert grades and evaluated grades of Shifeng Longjing tea with 2D fluorescence correlation spectroscopy based on intensity perturbation.

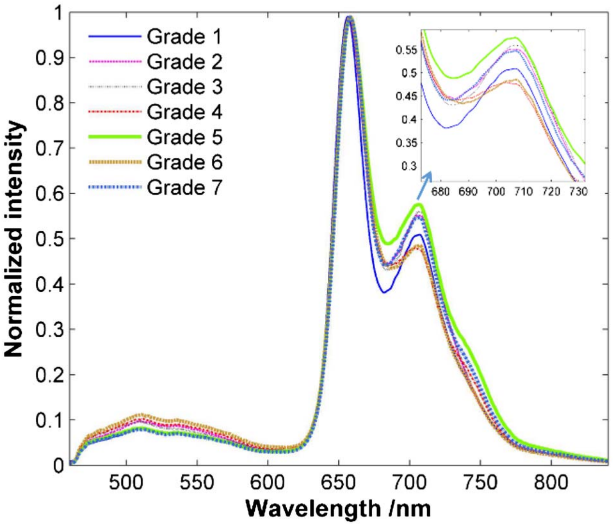

**Fig. 5.** Normalized fluorescence spectra for tea samples with seven different grades at the maximum excitation intensity. The inset is the magnified spectra around 710 nm.

**Table 1. Tea Grade Evaluation Results Based on Different Spectra**

| Spectroscopy method | Spectra recorded at 21 mW | Spectra recorded at 24 mW | Spectra recorded at 27 mW | Spectra recorded at 29 mW | 2D correlation spectra |
|---|---|---|---|---|---|
| Evaluation result (RMSEV) | 0.4820 | 0.3670 | 0.4007 | 0.2676 | 0.1659 |

in Fig. 3. By using the same analysis method, the tea grade evaluation results (RMSEV values) with four different excitation intensities are obtained as shown in Table 1, which shows that the fast 2D correlation spectroscopy technique we developed is more accurate for tea quality evaluation than the traditional fluorescence spectroscopy, because the 2D synchronous spectra contain more spectral information and resolve spectral details more effectively.

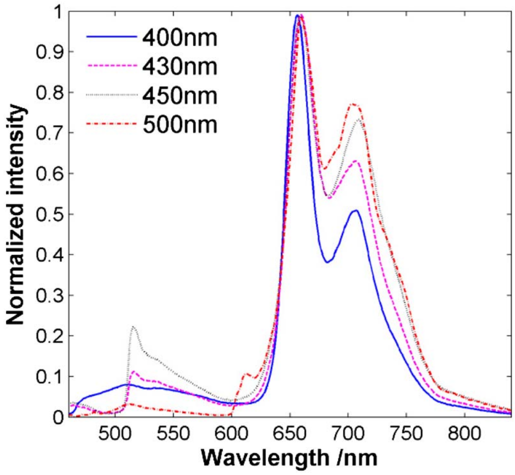

**Fig. 6.** Normalized fluorescence spectra of the tea at grade 1 with different excitation wavelengths.

### B. Excitation Wavelengths as a Perturbation Variable

Four LEDs with peak wavelengths around 400, 430, 450, and 500 nm were turned on with maximum intensity in sequence to excite the fluorescence spectra of seven tea samples with different grades. Each tea sample was measured six times. The average normalized spectra of the tea at grade 1 excited by four different LEDs are shown in Fig. 6, in which the fluorescence spectra of the tea are quite different for different excitation wavelengths.

Similarly to Section 3.A, the spectra between 620 and 770 nm are used for 2D synchronous correlation spectra calculation. Contour maps of the average 2D synchronous spectrum are shown in Fig. 7. The relationships between expert grades and evaluated grades with 2D fluorescence correlation spectroscopy are shown in Fig. 8. Using the same analysis process, the RMSEV values based on traditional fluorescence spectra excited by LEDs of their wavelengths at 400, 430, 450, and 500 nm are 0.2676, 0.2766, 0.3339, and 0.3417, respectively. The RMSEV of the 2D correlation spectra is only 0.0921, which verifies that the 2D correlation spectroscopy based on excitation wavelength perturbation is a powerful and effective tool for tea quality evaluation.

The RMSEV value based on excitation wavelength perturbation is lower than the one based on intensity perturbation. This may be because the 2D correlation spectrum obtained with different wavelengths of light contains more effective information than the one obtained with different intensities of one wavelength.

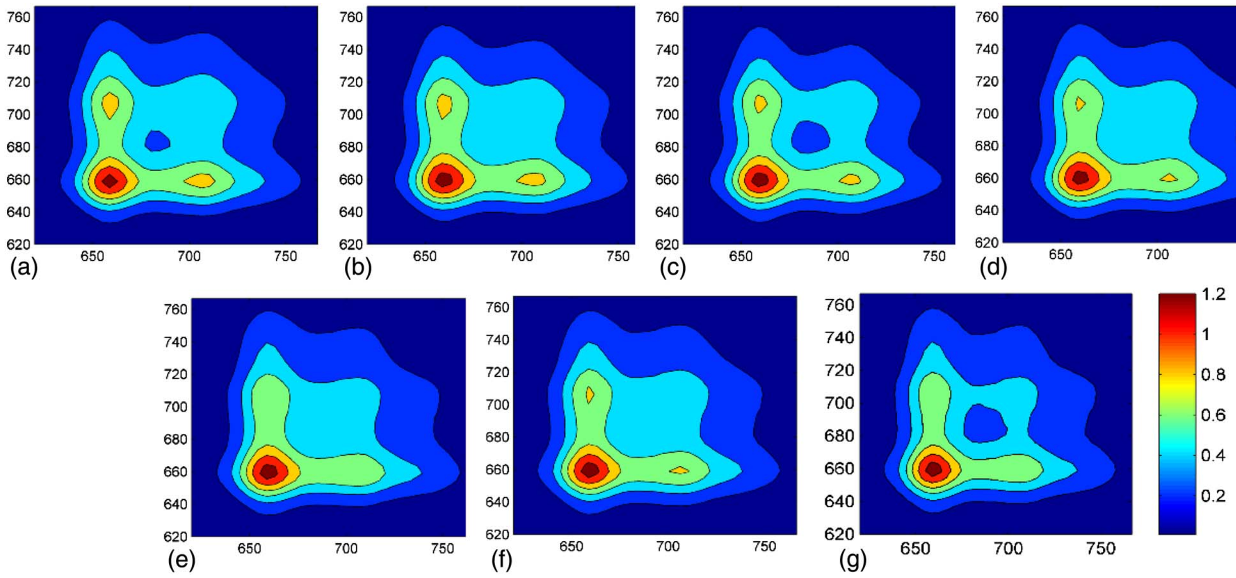

**Fig. 7.** 2D synchronous spectra of seven tea samples with different grades and varying excitation wavelengths: (a)–(g) correspond to tea grades 1–7, respectively.

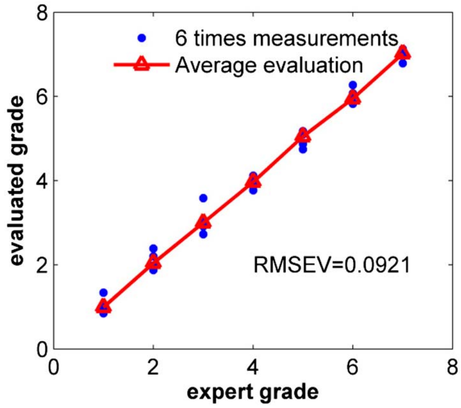

**Fig. 8.** Relationship between expert grades and evaluated grades of Shifeng Longjing tea with 2D fluorescence correlation spectroscopy based on excitation wavelength perturbation.

## 4. CONCLUSION

In this paper, a fast LED-based 2D fluorescence correlation spectroscopy technique is presented for tea quality evaluation. Synchronous correlation spectra using intensity and wavelength as perturbations are used to create PLSR models for Shifeng Longjing tea with seven grades, and the corresponding RMSEV values for each perturbation method are 0.1659 and 0.0921. Compared to the traditional fluorescence spectroscopy technique for tea quality evaluation, our method is more accurate and has lower RMSEV values. To our knowledge, this is the first compact, automatic, and online system based on the 2D correlation spectra measurement method by using excitation wavelength or intensity as an easily controllable external perturbation variable. It could be expected that this fast, convenient, and powerful technique could be developed for additional applicable fields, such as food quality evaluation and so on.

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