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Full Length Research Paper

Study on serum fluorescence spectra based on wavelet transform

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Blood plays an important role in clinical diagnosis and treatment and as such, the analysis of blood spectrum will be of very important practical significance. Serum fluorescence emission intensity is closely related with the excitation wavelength; when the excitation wavelength is 230 nm, the blood lipid concentration and fluorescence intensity was significantly correlated. On the contrary, blood sugar was almost with no effect on the strength. Wavelet analysis was used in signal de-noising to get a wide range of applications. In this paper, fluorescence spectrum was divided into four layers by db4 wavelet, according to the principle of stein unbiased likelihood estimate.To choose the threshold, noise was removed and reconstruction signal received. This paper studied the correlation between blood lipid concentration and original fluorescence intensity, reconstruction fluorescence intensity and the fourth layer fluorescence strength. Some significant results were achieved, providing an experimental basis for further study on the fluorescence spectrum of blood.

Key words: Serum fluorescence spectrum, wavelet transform, blood lipid.

INTRODUCTION

Studies have shown that there are different kinds of fluorescence substances in chromophore tissue of biological moleculars since 1950s. Each fluorescence substance has certain concentration, absorption spectrum and fluorescence emission spectrum. Fluorescence spectrum of normal tissue might be different from that of same tissue with pathological changes. Therefore, the content of some components could be detected or distinguished according to the intensity of the fluorescence peak or the features of the spectrum.

Blood, the most important body fluid, can signify many life index of human body and provide different kinds of physical parameters. As it circulates in the body, pathological changes in the body could be reflected in the blood. Inspecting and studying the blood spectrum can facilitate researchers to inspect and analyze the physical index of blood, hence providing help for early diagnosis and cure for diseases. Consequently, inspecting and analyzing the blood spectrum has become an important trend for modern life sciences. Investigators have developed research on blood spectrum and have gained lots of meaningful achievements (Zhao et al., 2008; Zhu et al.,2007; Zhu et al., 2008a; Zhu et al., 2008b; Wang et al., 2002; Zhao et al., 2003; Guo et al., 2005; Lan et al., 2006; Ge et al., 2007; Shi et al., 2006; Rohleder et al., 2005; Runger, 2007; Tang, 1989; Rochkind et al., 2001; Gniadecka et al., 1997).

In recent years, the mathematical theory and method of wavelet transform have become the focus of many studies and have been widely applied in many fields such as mathematics, physics, image processing and spectrum analysis (Qin et al., 2006; Wang and Li, 2006; Li et al., 2006). In this paper, the wavelet de-noise has been used to study and analyze the fluorescence spectrum of the blood serum of body, as well as to research the correlation between blood lipid content and intensity of fluorescence spectrum.

In order to effectively separate the noise, the selected wavelet should have the following characteristics: (1) The effective support length of the wavelet function should be shorter; (2) The selected wavelet should be beneficial to highlight the spectral signal characteristics. Comprehensive consideration of all the characteristics of wavelet and through practice attempts, the db4 wavelet was used in

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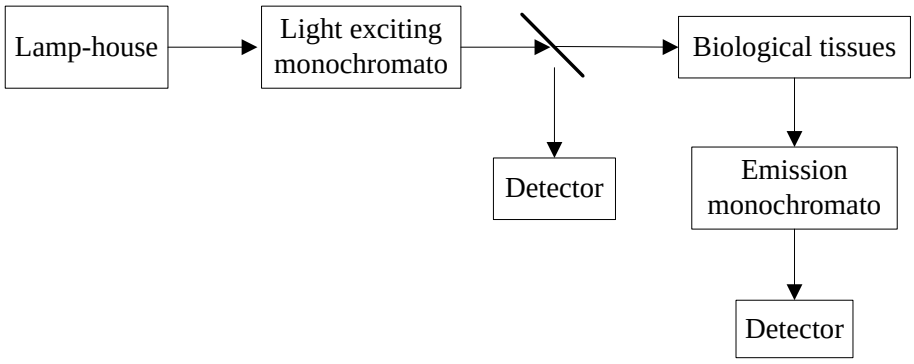

Figure 1. Diagram of the fluorescence spectrum detecting system.

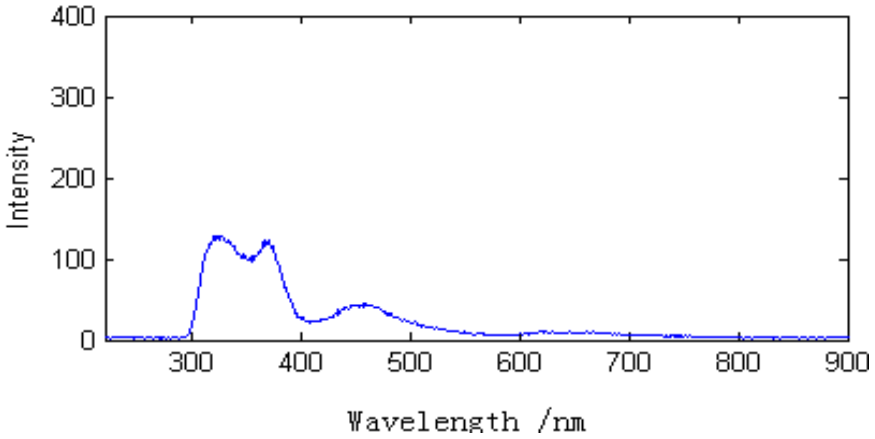

Figure 2. Fluorescence emission spectrum of sample 1.

the blood spectral signal processing.

MATERIALS AND METHODS

Detecting system of fluorescence spectrum

The blood serum fluorescence spectrum was detected by using the system shown in Figure 1. The equipment used was the RF 5301 fluorescence spectrophotometer made by Japan's SHIMADZU Corporation. Wavelength ranged between 220 – 900 nm, excitation light side (EX) and emission light side (EM) aperture were 5 nm.

When the exciting light passed through the grating monochromator or filter, it projects on the sample groove with quartz cell and transparent four sides and then it radiates fluorescence to all directions. The emission lights vertical to the exciting light were decomposed by monochromator and then were received by photomultiplier and transformed to electrical signal, which was processed and displayed on the computer.

Experimental samples

The experimental sample used was human blood from the Hospital of Nanjing University of Aeronautics and Astronautics. Persons, whose blood were collected, were not permitted to have breakfast. 0.2 ml blood serum was mixed with 2 ml distilled water. The mixture of proper volume was injected into quartz cell and spectrometer was used in detecting the fluorescence spectrum.

RESULTS AND DISCUSSION

Decomposing and restructuring of blood serum fluorescence spectrum

The fluorescence emission spectrum of normal blood serum (noted as sample 1, whose glucose content is 4.81mmol/L, while blood lipid content is 0.51 mmol/L) is shown in Figure 2. It was discovered from Figure 2 that the fluorescence spectrum of serum, excited with 230 nm exciting light, had strong fluorescence in the wave band from 300 to 550 nm. The spectrum signal was comparatively clear and contained certain noise information. In order to attain better spectrum curves, the db4 wavelet was used to decompose the signals into 4 layers, and then the threshold was selected by the principle of stein unbiased likelihood estimate. Lastly, reconstruction signal was synthesized by using the treated signals. Figure 3 showed the signals of layers after decomposition by db4 wavelet. It is known from Figure 3 that the difference among the low- frequency signals of layers is small. In contrast, there are large differences in high-frequency signals of layers. The noise phenomena were very serious in the first and the second layer and mainly focused on the parts with relatively strong original signals

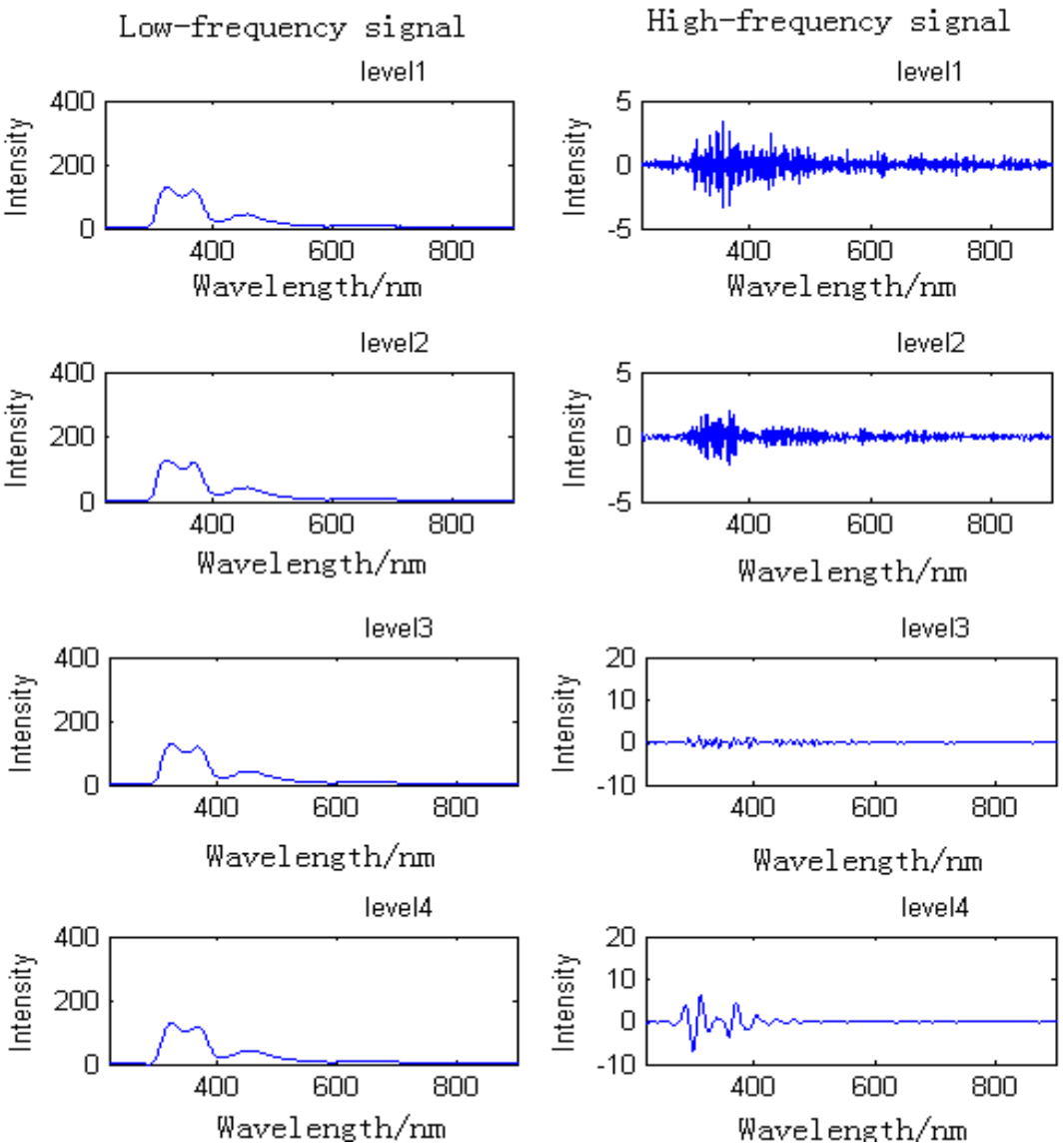

Figure 3. Signals of different layers decomposed by wavelet of serum fluorescence spectrum for sample 1.

of fluorescence. There were light noises and clear signals in the third and fourth layers.

The signals of the different layers processed by the threshold value are shown in Figure 4. It can be inferred from Figure 4 that the differences among low-frequency signals were small compared to the unprocessed one, whereas the differences among high-frequency were obvious. Most high-frequency signals in the first and the second layers were wiped off while those of the third and fourth layer were reserved. Figures 5 and 6, respectively, showed the reconstruction signals and the general noise signals that have been wiped off. It could be observed from Figure 5 that there could be a little change in signals after it has being processed. This is due to the small noise in the original signals. However, the noise signals included in reconstruction signals in 300 – 550 nm were decreased, making the fluorescence peak position much clearer and the data more reliable.

Effect of blood lipid upon serum fluorescence spectrum

In the first experiment, glucose contents of samples a and b were 5.48 and 4.74 mmol/L, respectively, and their blood lipid contents were 2.55 and 2.57 mmol/L. Fluorescence emission spectra excited by 230 nm of sample a and b denoised by the wavelet were displayed as shown in Figure 7. It was explored from Figure 7 that the fluorescences of the two samples were of nearly similar intensities despite the obvious difference in glucose contents. Therefore, excited by 230 nm, the relationship

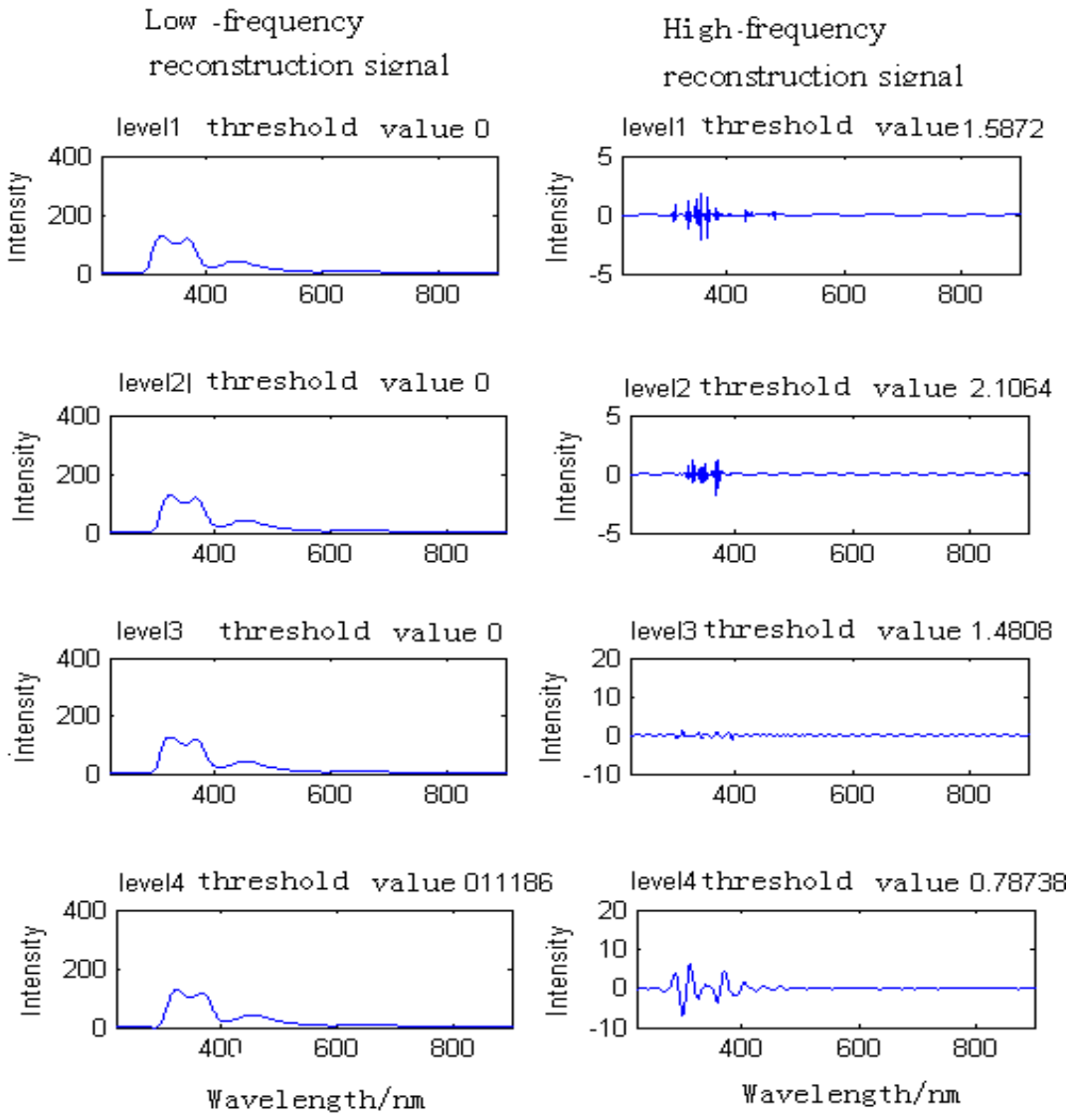

Figure 4. Reconstruction signals of different layers.

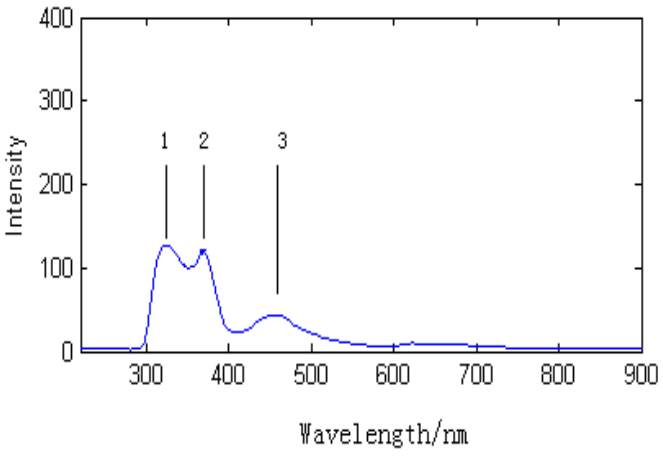

Figure 5. Reconstruction signal of sample 1.

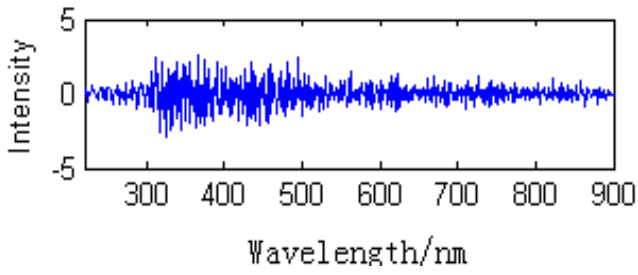

Figure 6. Noise signal of sample 1.

between the intensity of fluorescence and the glucose content was inconspicuous. The blood lipid contents of the two samples were almost the same. It was supposed that the intensity of fluorescence may have greater relativity

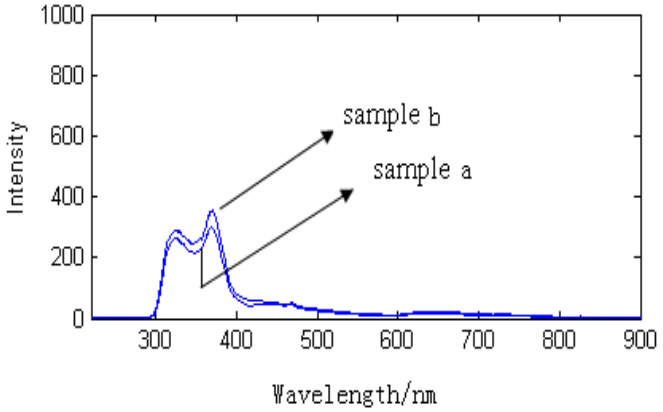

Figure 7. Fluorescence spectra of sample a and sample b.

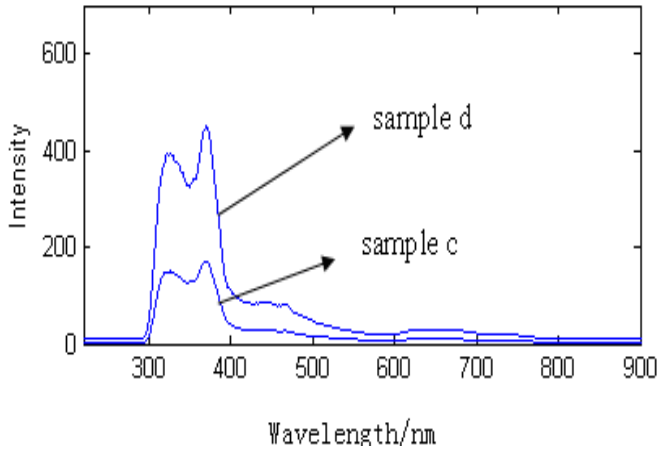

Figure 8. Fluorescence spectra of sample c and sample d.

with blood lipid content. That gave bases for experiment two.

In experiment two, the glucose contents of samples c and d were 4.24 and 4.37mmol/L and the blood lipid contents were 0.78 and 2.87mmol/L, respectively. Figure 8 showed the fluorescence emission spectra of the two samples excited by 230 nm. From Figure 8, the difference between the intensity of fluorescence of the two samples was quite obvious. The glucose contents of the two samples were almost the same while the blood lipid contents were much different. Thus, considering the result of experiment one above, the blood lipid contents had great effect on intensity of fluorescence excited by 230 nm. In order to determine the relationship between blood lipid content and intensity of fluorescence, experiment three was conducted.

Experiment three showed that the blood lipid contents of samples 1 - 11 were 0.51, 0.78, 0.94, 1.44, 1.86, 2.29, 2.55, 2.57, 2.87, 3.02 and 4.47 mmol/L, respectively. The blood lipid contents gradually increased in turn from sample 1 to 11.

The fluorescence spectra of all the eleven samples excited by 230 nm are shown in Figure 9. It was unveiled from Figure 9 that the intensity of fluorescence increased along with increasing concentration of the blood lipid in blood serum from sample 1 to 11. In Figure 9, the samples of No. 6 to No. 11 were all hyperlipidemia serums. The intensities of fluorescence and the peak values at 328 and 370 nm both exceeded those of normal serums numbered 1 to 5. Therefore, we can judge whether the blood lipid content is normal in serum according to the intensity of serum fluorescence excited by 230 nm.

According to the analysis above, the higher layer high-frequency signals of different samples were relatively clear and contain more minutiae information. In order to know the relationship between fluorescence spectrum and blood lipid content, the fourth layer high-frequency signals were studied further. Figure 10 showed the high-frequency signals of the fourth layer.

It could be seen from Figure 10 that the fourth layer high-frequency signals were stronger and grew stronger with increasing blood lipid contents of the samples. There

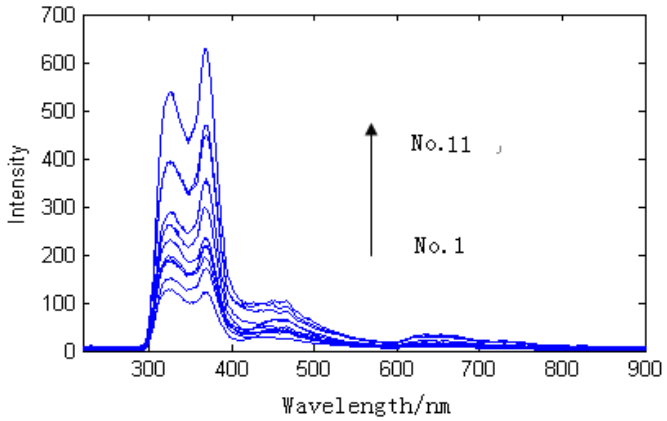

Figure 9. Fluorescence spectra of sample 1 to sample 11.

were five relatively strong peaks at 287, 313, 344, 370 and 404 nm for the fourth layer high-frequency signals of each sample (as No. 10 shown in the Figure 10).

To better understand the relevance between lipid content and fluorescence intensity, Figures 11 and 12 showed respectively the linear fitting curve between the lipid content of 34 samples and the original fluorescence intensity, reconstruction fluorescence intensity, fluorescence intensity of the fourth layer at 370 nm and the average fluorescence intensity of 287, 313 and 370 nm. As could be seen from Figures 11 and 12, in the 230 nm excitation, the serum lipid concentration and fluorescence intensity had a strong correlation. The correlation coefficients were respectively, 0.842, 0.844, 0.870 and 0.889. In which lipids had the largest relevance with the fourth layer average fluorescence intensity of 287, 313 and 370 nm. This showed that the information on lipid content distributed in the spectral signal, using intelligent algorithms such as neural networks to perform the full spectrum regression analysis may be more accurate to predict the blood lipid content from the fluorescence spectra. In addition, the filtered spectrum and the fourth

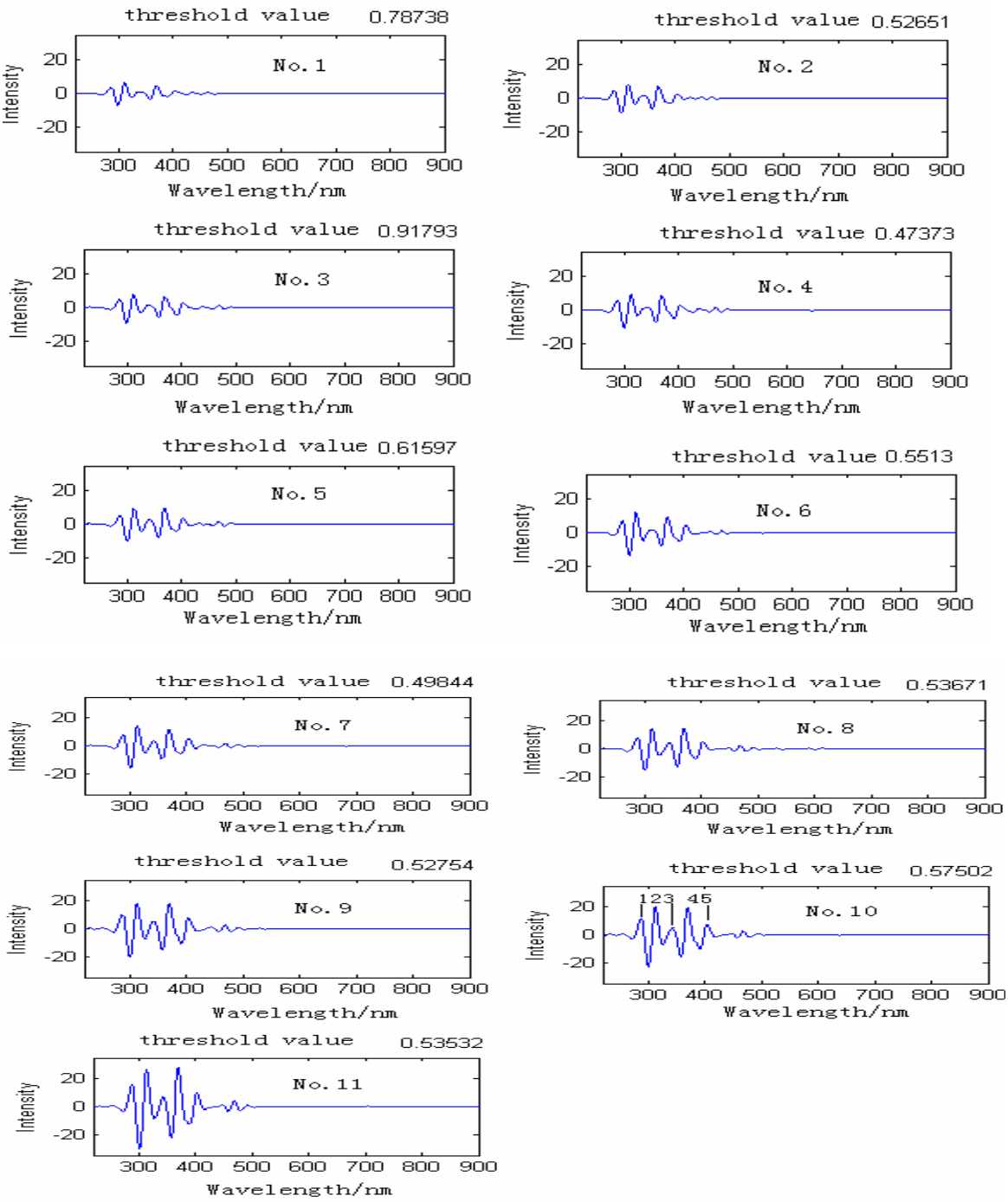

Figure 10. The fourth layer high-frequency signals of sample 1 to sample 11.

layer spectrum is of higher relevance with blood lipid content than that between the original spectra and the blood lipid, suggesting that de-noising using wavelet decomposition is conducive to useful signal acquisition.

Conclusions

The shapes of the low-frequency signals decomposed by db4 wavelet were similar to original signals and the high-frequency signals included more noise information and minutiae features. The noises were separated effectively by using different thresholds for the decomposition signals of different layers and reconstruction signals were established.

Excited by 230 nm, the concentration of blood lipid obviously affected the intensity of serum fluorescence spectrum in wave band from 300 to 400 nm; the fluorescence

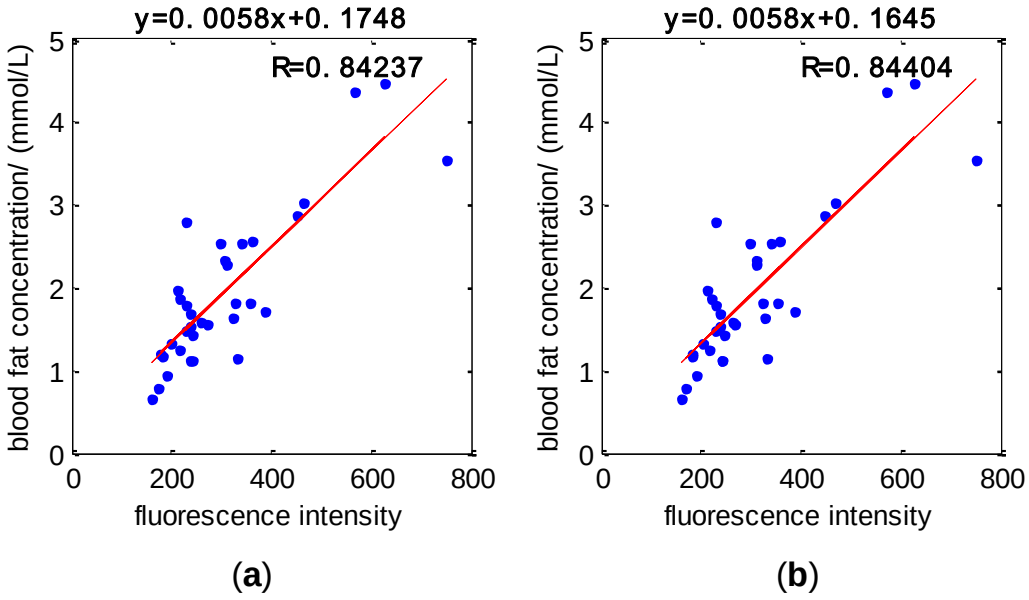

Figure 11. The correlation diagram between the lipid content and the original fluorescence intensity (a) and the reconstruction fluorescence intensity at 370 nm (b).

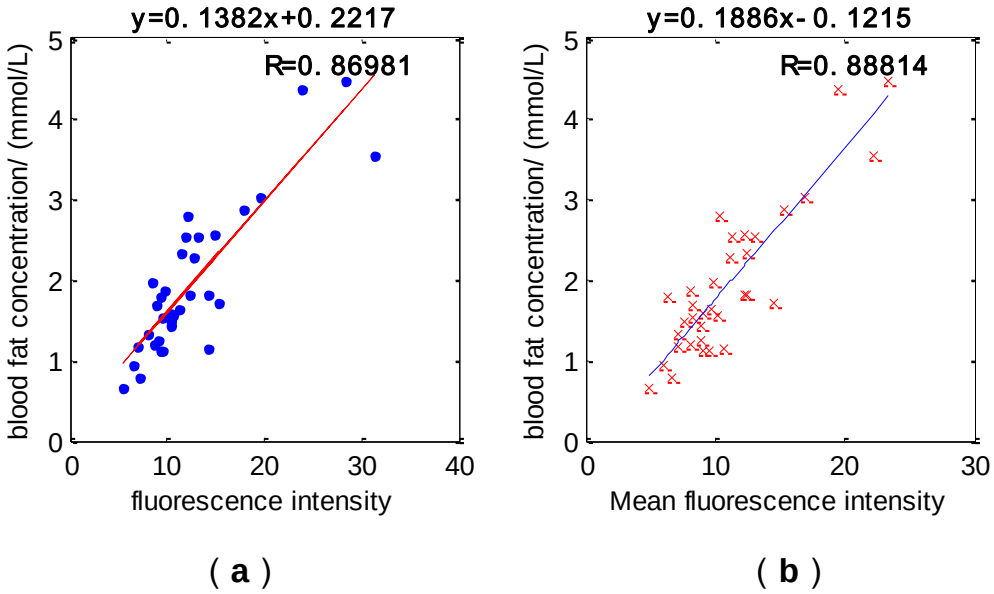

Figure 12. The correlation diagram between the lipid content and the fluorescence intensity at the fourth layer at 370nm (a) and the average of the fourth fluorescence intensity at 287, 313 and 370 nm (b).

intensity gradually increased with the enhancement of the concentration of blood lipid and the blood sugar had little effect on fluorescence intensity.

Lipids had a significant correlation with the average fluorescence on the fourth layer of 287, 313 and 370 nm, which indicated lipid information of the content distributed in the spectra signal, providing an experimental basis for the full spectrum regression analysis to predict more accurately the blood lipid content from the fluorescence spectra.

ACKNOWLEDGMENTS

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