Research on Reflectance Spectra Measurement of Chlorophyll-Containing Water in Laboratory

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Abstract. Chlorophyll is the important index to estimate the phytoplankton biomass. In order to research phytoplankton biomass and eutrophication condition of water, the spectroscopy method has been used usually now. A large number of spectrum experiments also need to be taken in the laboratory. In this article, the gray and the white diffuse reference scale are respectively used to measure the character reflectance spectrum of the chlorophyll-containing water. Then we analyze the differences of the data quality between these two ways. The result shows that, when measuring the water object which has low reflectance in the laboratory, using the white scale will cause a big data noise and the data quality will be poor. But when using the gray scale to take the experiment, the data noise will be small and the data quality will be good enough to find the character reflectance spectrum.

Keywords: chlorophyll; character reflectance spectrum; white scale; gray scale; data noise

1 Introduction

Phytoplankton such as algae is an important part of water ecological system. The concentration of chlorophyll which determines the photosynthesis of phytoplankton is an important indicator of the water quality monitoring. In recent years, research of getting water spectral information through remote sensing technology and inversing the concentration of chlorophyll has been more and more attention.

The type II water spectrum data is usually measured by the water-above method. The water-leaving radiance (L_w), the normalized water-leaving radiance (L_{wn}) and the remote-sensing reflectance can be computed form the data measured by spectrometer. [1] In order to get the downward radiation data of the sun, the data needs to be calibrated by a diffuse reference scale which has been calibrated. [2] But due to the strong absorption property of the water in the visible and near infrared bands, the reflectance of the water is usually very low. And the measurement is

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easily affected by the surrounding environment and the sky light. [3] Too much data noise will cover the real spectrum signal of the water, and the improper operation will cause a lot of uncertainty to the result of the experiment. [4, 5] Some researchers have done much research in the measurement and experiment of the water spectrum. Yu-rong Gao, etc [6] get the water spectrum of the Qiandao Lake in summer by using a 30% standard gray scale as the reference scale, and thus to compare the inversion results of chlorophyll concentration through the different methods. Ying Liu, etc [7] measured the water spectrum of the Qiandao Lake by using a standard white scale as the reference scale. Then they modeled and inversed the chlorophyll concentration through the ratio of the 701nm and the 516nm reflectance. Amr Abd-Elrahman, etc [8] used the hyper-spectral remote sensing to estimate the quality of the water in fish ponds. For enhancing the reflective signal of the water, they placed a calibrated white scale under the water as the reference scale. They established the relations between the chlorophyll and the ratio of two bands or three bands data. The relative variance was 0.975 and 0.982 respectively.

Normally, when measuring the target of high reflectance, the white scale is usually used as the reference scale, such as vegetation. And the gray scale is usually used as the reference scale when measuring the target of low reflectance, such as water [9, 10]. For analyzing the difference between the two types of reference scale in the spectrum experiment quantitatively, we design a spectrum observation scheme for the chlorophyll-containing water in the laboratory. The chlorella solution which concentration is determined is put into the distilled water step by step, and the spectrum of the water which has different chlorophyll concentration is measured through the white scale and the gray scale separately. In this experiment, the follow two questions are discussed: the first one is that, the comparison of the impact on the data quality between the white scale and the gray scale when measuring the spectrum of water. The other one is that, the different characteristics of the changing spectrum data measured between these two types of reference scale when the chlorophyll concentration of water changes gradually.

2 Preparation of Your Paper

In the nature water, the chlorophyll comes from the algae majorly. And the chlorella is the most common single-celled algae in the fresh water. The living chlorella solution of high concentration is cultivated to simulate the chlorophyll-containing water in the lab. The chlorophyll concentration of the initial chlorella solution is measured by the Acetone-Ethanol mixture method. The cells of the chlorella is collected by centrifugation method, and washed by deionized water three times. Then the cell supernatant is poured out, and 2ml mixture with acetone and ethanol of 1:1 proportion is added. The Mixture is placed for 24 hours in dark, and then dilute with water to 10ml. We colorimetric the solution in 537nm, 647nm and 663nm bands by ultraviolet spectrophotometer. The calculation formula for chlorophyll concentration is (Sims et al., 2002):

$$Chla(mg*L^{-1}) = 0.01373A_{663} - 0.000897A_{537} - 0.003046A_{647} \quad \text{(Formula 1)}$$

Water spectrum is measured with ASD Field-Spec HandHeld2 spectrometer. The view angle is 25 degree. The band range is from 325nm to 1075nm. The wavelength accuracy is ±1nm. The sampling interval is 1.5nm. The spectral resolution is 3nm@700nm. An optical fiber which view angle is 2.5 degree is used in the experiment. In order to compare the influence of different types of reference scale in the water spectrum experiment. A white scale which reflectance is nearly 1 and a gray scale which reflectance is nearly 0.2 is used respectively in the experiment.

In order to induce the influence on the optical field from the lab environment, the experiment is finished in a dark room built by black suction light cloth. The incident light source is a short arc xenon lamp. The illuminace of the lamp is beyond 105lx within a 6×6 cm^2 exposure area. The irradiance of the lamp is beyond 1000W/m^2 within the 400nm~1000nm band. The height and angle of the lamp are adjustable in order that the illumination geometry is relatively stable when the water level changes. The target solution is placed in a cylindrical bucket of 27cm height and 23cm diameter. In order to induce the influence by the bottom and wall of the bucket, we paint black extinction smoothly and uniformly on the bottom and wall of the bucket. The bucket is placed on the table which is also paint black. We simulate the illumination geometry as the sun of 1pm in July. The incident height angle of the light is set as 68 degree through adjusting the height of lamp.

The spectrometer probe is placed beyond the center of the target solution, and clip 40 degree angle with the surface normal. The probe direction clips 45-90 degree angle with the light direction.

The spectrometer is calibrated strictly before the experiment. But due to the artificial light source and the spectrometer easily affected by initial unstable current, we preheat the lamp and the spectrometer for 40 minutes respectively at the beginning of the experiment. The measuring job is done after the lamp and the spectrometer are stable. The integration time of the spectrometer is set as 1.09s, the dark current is set as 25, and the white average data is set as 15. Fifteen spectral curves are measured one time, then the abnormal curves are eliminated, and the average is taken as the final result.

In order to induce the influence on the result by the wall shadow of the bucket, 7500ml distilled water is put into the bucket at first so as to make the distance less than 10cm between the surface of the water and top edge of the bucket. And the algae solution of certain concentration is added into the bucket step by step. Then the spectrum is measured after stirring and the surface static. (Fig.1)

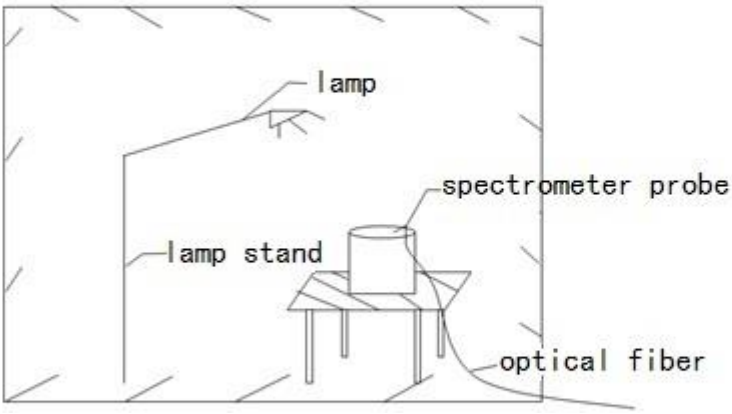

Fig.1. Experiment scene

The experiment is divided into two groups. In the first one, the white scale is taken as the reference scale. The chlorophyll concentration of the water is between 0 and 371μg/L. Seven concentration gradients are set. In the second experiment, the gray scale is taken as the reference scale. The chlorophyll concentration of the water is between 0 and 419μg/L. Six concentration gradients are set. A distilled water group which chlorophyll concentration is 0is set as the contrast test. The step of the reflectance measurement shows as follow (Fig.2).

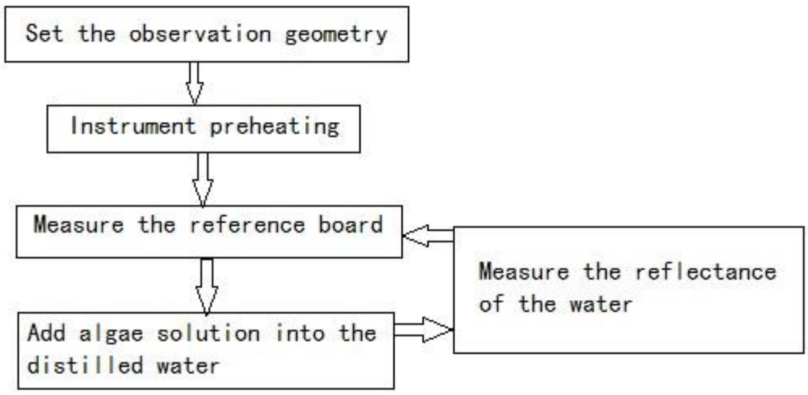

Fig.2. Step of reflectance measurement

3 Results and Analysis

3.1 The reflection performance comparison between the white and gray scale

In order to avoid the inaccurate result from the error of the white and gray scale, the two scales are measured under the same condition. The result is compared through DN from the spectrometer.

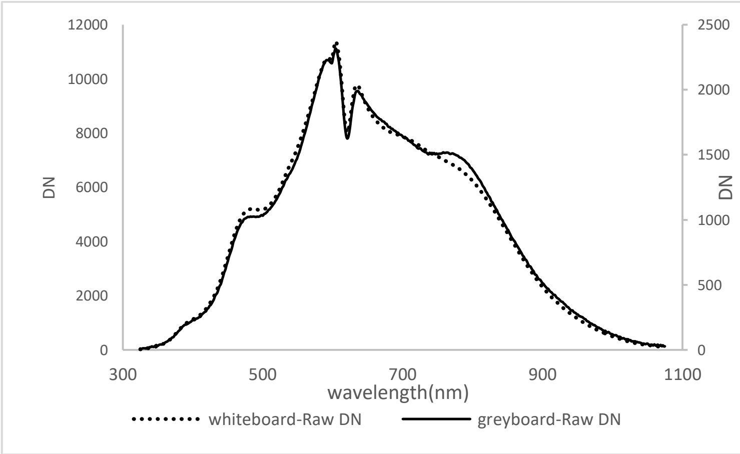

Fig.3. DN result of the white and gray scale

The result shows that, under the artificial light source condition, the white and gray scales have a similar reflection performance. But DN response range between them is different. DN response range of the white scale is from 0 to 12000, but DN response range of the gray scale is from 0 to 2500.

Because of this difference, Coefficient of variation (C_v) is used to analyze the result. The calculation formula of C_v is:

$$C_v = \frac{S}{\bar{x}} \times 100\% = \frac{\sqrt{\frac{1}{n-1}\sum_{i=1}^{n}(x_i - \bar{x})^2}}{\bar{x}} \qquad \text{(Formula 2)}$$

S: Standard deviation of the measured data. $\bar{x}$: Average of the measured data. The greater the value of C_v is, the greater the discrete degree is.

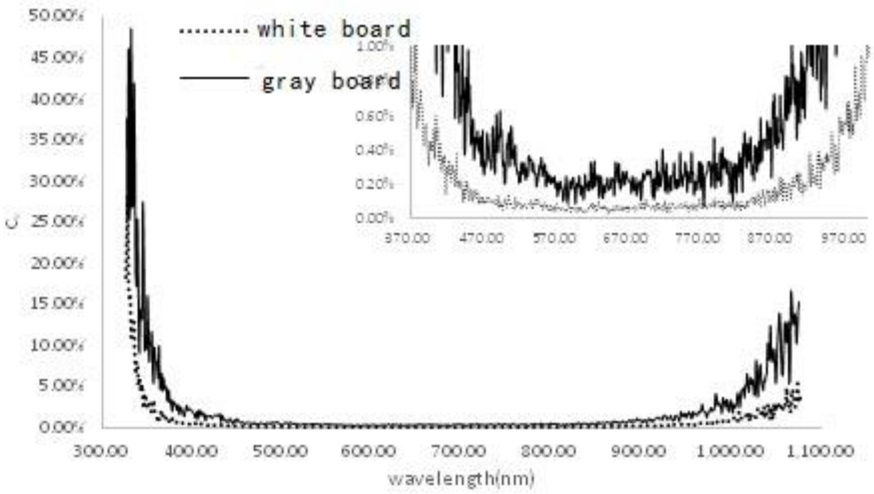

Fig.4. C_v of the white and gray scale

The Fig.4 shows that, in 325-1075nm, C_v of the white and gray scale present the same trend. The value of C_v is much greater in blue-violet (325-400nm) and near-infrared (950-1075nm). But much smaller in 400-950nm.In 370-1000nm, the C_v value of the gray is greater than the white scale.

	Median	Average	Max	Min	Variance

White scale	0.001011	0.007303	0.280791	0.000196	0.000484
Gray scale	0.00427	0.022683	0.484913	0.00069	0.00261

Table 1 C_v of the white and gray scale

Through the statistical analysis, all the index of the white scale is smaller than the gray scale. It shows that the white scale is superior to the gray scale as a whole. But in 400-1000nm, the C_v value of them are all under 5%, and most close to 0. This shows that both the two types of reference scale have a good reflection performance.

3.2 Impact analysis on the spectrum data of chlorophyll-containing water through the different reference scale

Due to the stability problem of the artificial illuminant, data near 300nm and 1100nm measured by ASD have a big error. So the data between 444nm and 878nm which $C_v$of the spectrum is less than 1 is selected to make analysis. The two groups of experiment samples set are measured among several concentration gradients by using the white and gray scale respectively.

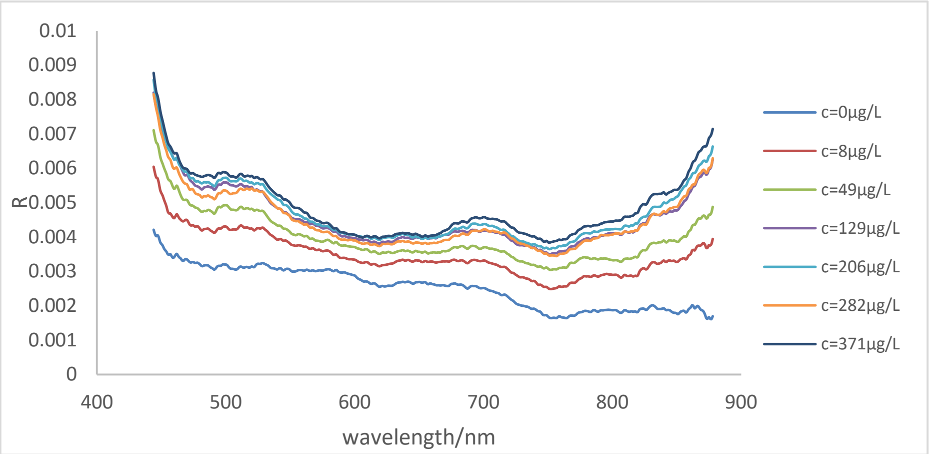

Fig.5. Reflectance measured by white scale

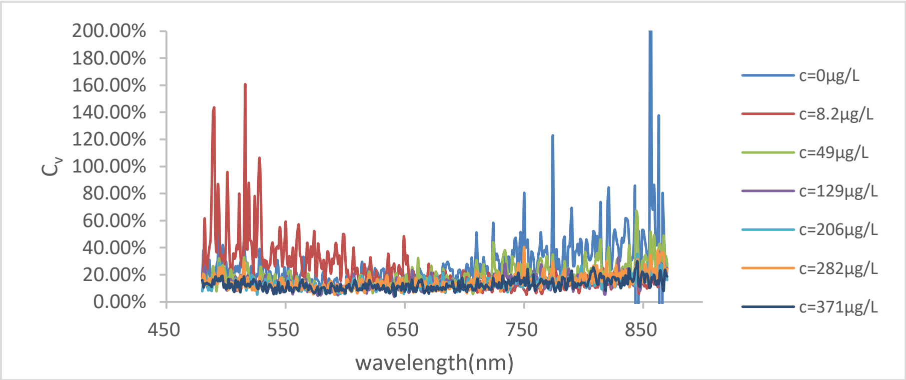

Fig.6. C_v of the reflectance measured by white scale

Fig.5 and Fig.6 show that, when the chlorophyll concentration change gradually within the scope of 0-371μg/L, C_v of the spectrum data shows a decrease trend along with the increase of the chlorophyll concentration. But the reflectance of the water does not change obviously. The spectrum line looks likely as the distilled water, and does not show the characteristic of chlorophyll-containing water.

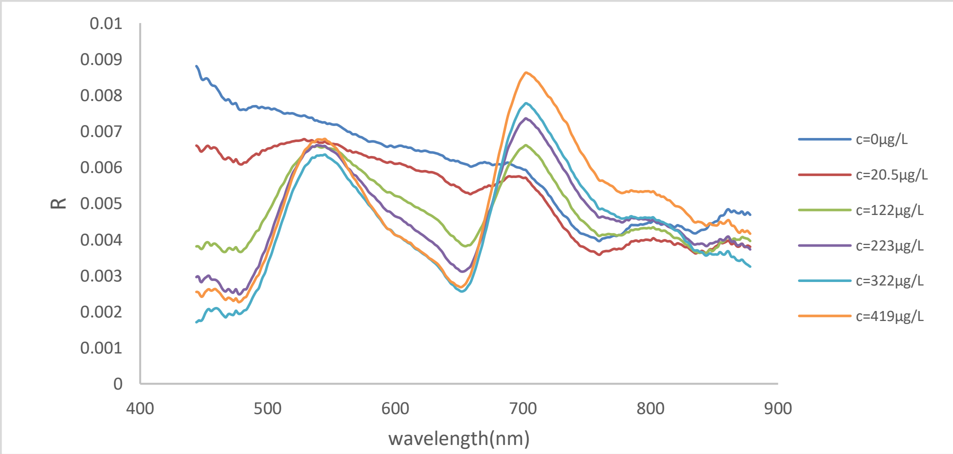

Fig.7. Reflectance measured by gray scale

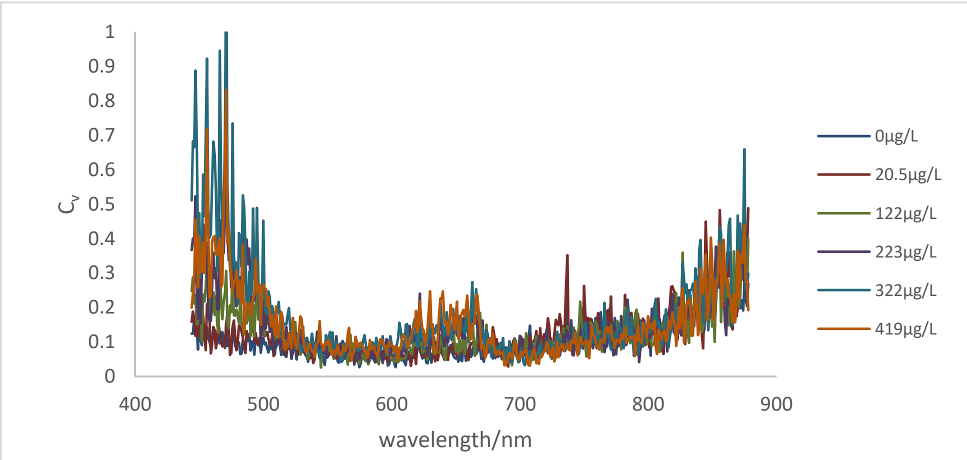

Fig.8. C_v of the reflectance measured by gray scale

Fig.7 and Fig.8 show that, C_v of the spectrum data is lowest to 10% near 550nm and 685nm, and 20% near 645nm. The reflectance shows a obvious absorption valley near 645nm. But within the low reflectance area, the reflective spectrum may be easily affected by other stray lights. This increase the data noise, and discrete data.

As a comparison, the spectrum of distilled water is measured by using the white and gray scale respectively. The C_v value is also computed.

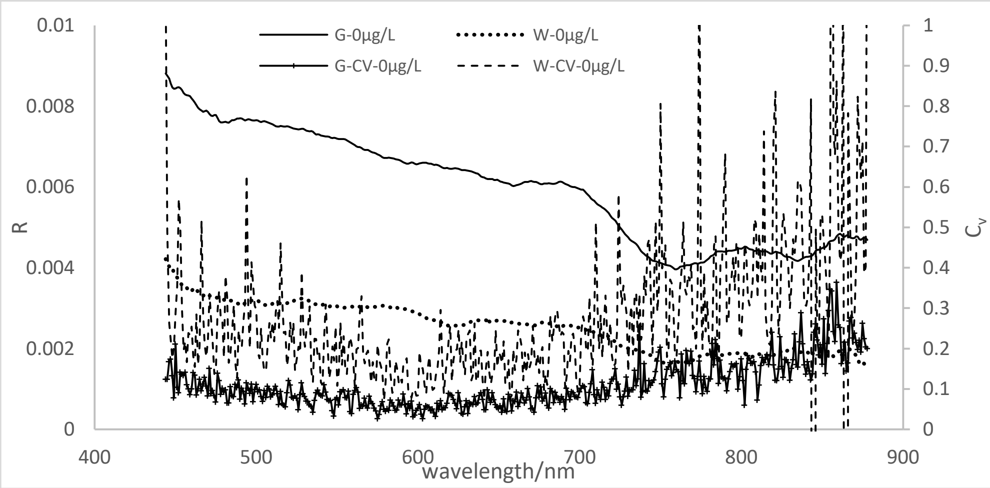

Fig.9. reflectance and C_v of distilled water

Fig.9 shows that, the reflectance of the distilled water by white scale is much lower than the reflectance by gray scale. And C_v of the data measured by white scale is about 20%-30%, even can reach 100%, and fluctuate greatly. But C_v of the data measured by gray scale is about 10%, at most 33%, and fluctuate slightly.

Chlorophyll concentration of the water becomes much bigger by adding chlorella solution into the water gradually. The two groups contrast experiments are taken by using white and gray scale respectively. The results show as the Fig.10.

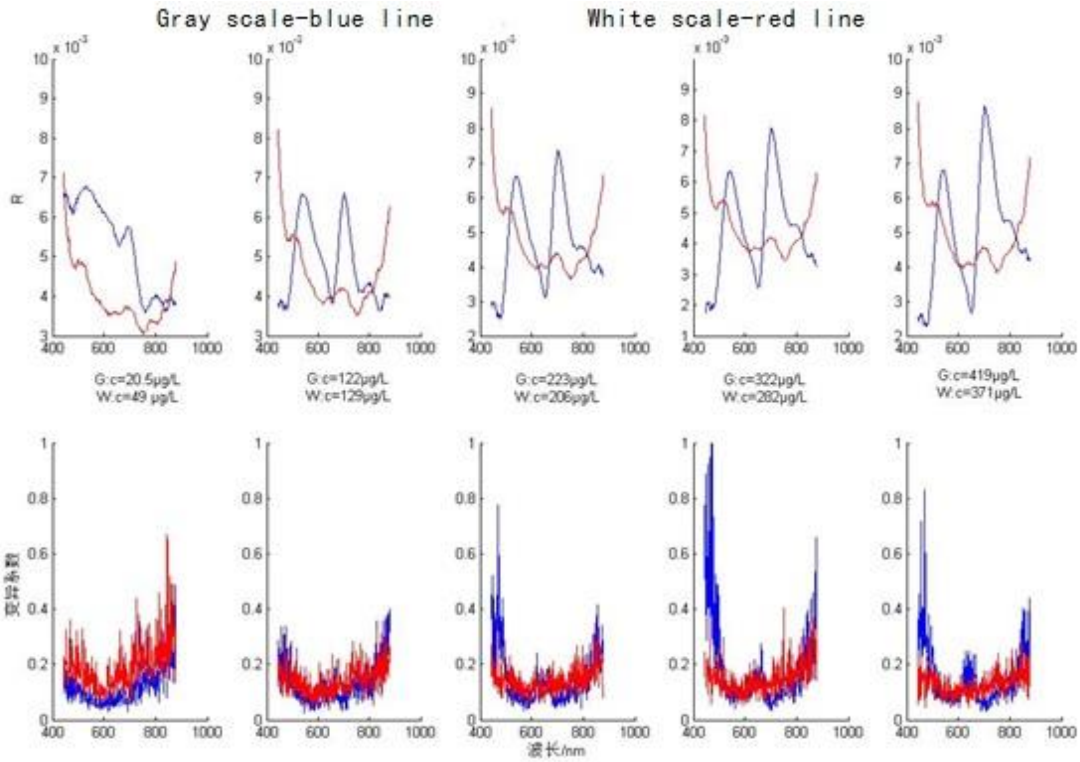

Fig.10. Results of the contrast experiment

In the gray scale experiment, when the chlorophyll concentration reaches 20.5μg/L, the chlorophyll characteristic spectrum at 680nm appears. But in the white scale experiment, the chlorophyll characteristic spectrum does not appear even when the chlorophyll concentration reaches 49μg/L. And the reflectance measured by the white scale is less than the reflectance measured by the gray scale as a whole.

While the chlorophyll concentration reaches 125μg/L, the characteristic spectrum of chlorophyll-containing water appears obviously in the gray scale experiment. There are two reflection peaks at 550nm and 695nm, and an absorption valley at 665nm. But in the white scale experiment, this phenomenon does not appear, and the spectrum line looks like the characteristic of distilled water. This result show that the spectrum signals of the chlorophyll is hidden by the spectrum signals of water at this time. The similar phenomenon appears while the chlorophyll concentration reaches 200μg/L, 300μg/L and 400μg/L.

In addition, C_v of the two groups of data shows that while the absorption valley appears at 655nm, C_v has a small rise. But as the chlorophyll concentration increases, the reflectance at 655nm reduces, and the C_v rises at the same time. But this does not affect the result largely. Accordingly, C_v reduces slightly as the chlorophyll concentration increases, but it has little help to measure the reflective spectrum of water accurately.

When the chlorophyll concentration of water increases gradually, the spectrum signal of chlorophyll hide the spectrum signal of water gradually, the reflection peak becomes higher and the reflection valley becomes lower. The gray scale experiment verifies this phenomenon, but the white scale experiment does not reflect.

4 Conclusions

The data noise of the spectrum experiment in the lab mainly comes from the instrument and illuminant. When measuring the reflectance, the performance of diffuse reference scale could affect the data quality greatly. It is very important to use an appropriate diffuse reference scale which has been calibrated accurately for the experiment. Because the low reflectance characteristic of the water in the visible bands. The spectrum data quality must be good enough to extract the information such as chlorophyll concentration from the water. The big data noise will cover the true target signal. Research in this article shows that, when taking spectrum experiment for chlorophyll-containing water in the lab, the data noise will be smaller when measured by the gray scale. The data quality will be better. And the spectrum characteristic of the target is also more significant. At the same time, the regularity of the characteristic spectrum is more obvious as the changes of chlorophyll concentration in water. In a word, it will be beneficial to use a gray scale as the reference scale when taking spectrum experiment for chlorophyll-containing water in the lab.

In this article, we compare the experiment data between the white scale which reflectance is 1 and the gray scale which reflectance is 0.2. The target is chlorophyll-containing water which concentration has a certain range. More experiments should be taken to analyze that whether the data quality measured by the gray scale of different reflectance will change as the changes of the chlorophyll concentration in future.

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