Development of a Novel Spectrophotometer for Biochemical Analyzer Based on Volume Holography Transmissive Grating and Linear CCD

Zhong Ren*1, Guodong Liu1, Longmin Dai2, Zhen Huang1, Lvming Zeng1
1Key Laboratory for Optoelectronics and Communication of Jiangxi Province, Jiangxi Science & Technology Normal University, Nanchang 330013, China
2Department of Engineering Technology, Eastern Airlines Co. Ltd, Pudong, Shanghai 201202, China
Email: renzhong0921@163.com

Abstract. The classical surface-embossed plane and concave grating are usually used as the diffraction grating in some spectrophotometers. But the minute cracks are produced on the surface of the gratings' grooves, which leads to generate the stray-light and decrease the efficiency of instrument. Therefore, a novel custom-built spectrophotometer for BCA is developed in this paper. Meanwhile, the volum holography transmissive(VHT) grating is used as the diffraction grating in this spectrophotometer. Additionally, a high resolution CCD and data acquisition (DAQ) card with combined the virtual software platform based on LabVIEW are used to design the spectral acquisition and analysis system. Experimental results show that the spectral range and the diffraction efficiency of the spectrophotometer for BCA are greatly increased. The spectral range of the spectrophotometer for BCA can reach 300-1000 nm, its wavelength resolution can reach 1nm. And, it uses the back-splitting-light technology and multi-channel parallel analysis. Compared with other same types, this spectrophotometer has many advantages, such as, higher efficiency, simpler algorithm, higher accuracy, cheaper cost and fewer stray-light and higher imaging quality, etc. Therefore, this spectrophotometer for BCA based on VHT grating will has the greatly potential values in the fields of the biochemical or medical research.

1. Introduction

* Corresponding author. Email: renzhong0921@163.com, Phone: +86-0791-3831968.
Biochemical analyzer (BCA) is a kind of instrument based on the theory of Lambert-Beer. It is often used to measure the clinical and chemistry indexes of blood, such as, the glucose, bilirubin, albumin, uric-acid, cholesterol, HGB, etc. Because it can truly and rapidly offer test data for doctor or chemistry identifier, it plays an important role in clinic diagnose and chemistry inspection. Spectrophotometer is the key component in the BCA, the performance of the BCA is connected with the performance of the spectrophotometer[1-2]. From the arising of the spectrophotometer up to now, some classical plane grating splitting-light system, e.g. Czerny-Turner (C-T) system, and the concave grating splitting-light system have been used into some spectrophotometers or spectrometers[3-4]. But these classical surface-embossed gratings will not avoid producing some minute cracks on the surface of the gratings’ grooves at the time of manufacture, these cracks will lead to generate the stray-light and decrease the efficiency. Moreover, the conventional reflection gratings work in the Littrow geometry, in which the diffracted light can retro-reflect back toward the source of incident light and the off-axis geometry makes it difficult to correct aberration efficiently. These factors impact the performance of spectrophotometer. However, the VHT grating is a novel grating which is made by the photosensitive polymer material exposed by two periodically-changed beams by virtue of the holography technique, it will not produce the scattered light due to the absence of groove of classical surface-relief gratings. So, its spectral range can be greatly increased and its theoretical diffraction efficiency can approach 100%. The VHT grating has been used into some fields[5-6]. Therefore, a novel custom-built spectrophotometer for BCA is developed in this paper. Meanwhile, the VHT grating is used as the splitting-light apparatus of the spectrophotometer. Experimental results show that some performances of the custom-built spectrophotometer for BCA based on the VHT grating are improved, such as, the wavelength range is widened, the resolution, accuracy and efficiency are all increased, the range of the wavelength can reach 300-1000nm, its resolution can reach 1nm.

2. Theory

2.1. The theory of Lambert-Beer
The spectrum analysis theory of the spectrophotometer for BCA is based on the Lambert-Beer theory[7]. That is, when a beam of parallel monochromatic light vertically passes through a homogeneous and non-absorbing or scattering material, the absorbance A is proportional to the concentration of the substance \( c \) and the thickness of the absorption layer \( b \).
The Lambert-Beer theory can be expressed as follows:

\[
A = \log \left( \frac{I_0}{I} \right) = \log (I_0) - \log (I) = \epsilon bc
\]

(1)

Where, \( A \) is represented to medium absorbance of the incident light; \( I_0 \) is intensity of the incident light; \( I \) is intensity of the transmission light; \( \epsilon \) is Moore absorbance parameter of the medium; \( c \) is concentration of the medium; \( b \) is thickness of the medium.

2.2. The theory of the VHT grating
The mainly manufacturing process of the VHT grating is represented as follows: firstly, a film of photorefractive polymer material is exposed by two beams of coherent light irradiated from a laser
source to take on a holographic fringe pattern. Then, two pieces of glass or silica substrate are merged on two surfaces of the film. To enhance the efficiency of the light, the material of the ultraviolet-enhanced-aluminum coating is coated on the surface of glass or silica substrate.

The structure of the VHT grating is shown in Figure 1.

![Figure 1. The structure of the VHT grating](image)

In order to save the pattern on the grating, the desired refractive modulation can be got by Equation (2)

\[ n(x, z) = n + \Delta n \cos\left(x \cdot \frac{2\pi}{\Lambda}\right) \tag{2} \]

Where \( n \) is the average value of the modulated refraction, \( \Lambda \) is the fringe spacing of the grating equal to \( 1/\nu \) for fringe planes orthogonal to the grating surface (\( \nu \) is the grating frequency) and \( \Delta n \) is the refractive modulation.

Supposed that two interference beams irradiate into the photorefractive crystal with the thickness of \( d \) and the incident-light with the reference-light lie on the same side of the grating, the VHT grating is formed by the interaction of two interference beams and the generation of the diffraction-light should be satisfied with the Equation (3),

\[ 2n\Lambda \sin \theta = \lambda \tag{3} \]

Where \( \Lambda \) is the period of the VHT grating, \( \theta \) is the angle of the incident light, \( \lambda \) is the wavelength of the incident light.

When the angle of incident light \( \theta \) is approached to the angle of Bragg, the diffraction efficiency can reach 100%. The Bragg condition can be represented in the Equation (4),

\[ m\lambda = \Lambda 2n \sin \theta_0 \tag{4} \]

Where \( \theta_0 \) is the Bragg angle in the grating medium.

Kogelnik[8] developed a two-wave, first-order, coupled wave analysis that can be used to approximately estimate the first-order efficiency of a VHT grating. The peak diffraction efficiency \( \eta \) of s-polarization light is represented in the Equation (5),

\[ \eta_s = \sin^2\left(\frac{\pi \Delta nd}{\lambda \cos \alpha}\right) \tag{5} \]

From Equation (5) we know that, for the VHT grating, when the angle of incident light equal to the angle of Bragg, the change of the diffraction efficiency \( \eta \) is the square of the function of \( \sin(\cdot) \) following the increasing of the thickness \( d \) and the refractivity.

3. System design of spectrophotometer for BCA based on VHT grating

The spectrophotometer for BCA system mainly consists of the irradiation unit, splitting-light and detection unit, signal processing unit, control unit, etc. where the irradiation unit includes the lamp.
source, collimation lens, cuvette and objective lens. The splitting-light and detection unit includes the slit, collimation lens, VHT grating, focusing lens, CCD, driver and trigger module of CCD. The signal acquisition and processing unit includes the off-DC amplifier, adjustable-gain amplifier, signal filter, the A/D data acquisition (DAQ) card and the data register. The control unit includes the computer and the software. The basic principle of this system is shown as follows: Firstly, the composite light with certain power is emitted by the lamp source and is collimated by the collimation lens, then castes into the cuvette in which the sample, e.g. blood, is loaded. According the Lambert-Beer theory, parts of light are selective-absorbed by the sample, then the transmitted light is focused by the focalizing lens into the splitting-light unit, the light pass through the slit, collimation lens, VHT grating and objective lens in turn. At this time, the composite light is separated by the VHT grating into the monochromatic light and is focused by the objective lens to become the spectrum-band to cast onto the surface of CCD. The light is transformed into electricity signal by CCD based on the effect of the photo-electricity transform. Then, the electricity signals are amplifier by the off-DC amplifier, the adjustable-gain amplifier and is filtered by filter to remove some noise, the signal is acquired by A/D-DAQ card and transformed into the digital signals, the digital signals are stored by the data register and sent into personal computer through PCI bus to be analyzed or displayed by computer and software. The schematic diagram of the spectrophotometer for BCA is presented in Figure.2. where, the splitting-light unit, CCD detector and CCD driver module are fixed into a sealed and inside-blacked aluminium-alloy box, its size is 10×8×8cm.

![Figure.2 The schematic diagram of the spectrophotometer for BCA](image)

3.1. Light source
According the different demand, there are many light sources can be used into the BCA, such as, the halogen lamp, laser diode, solid laser, etc. In this work, the halogen-tungsten lamp(Philip6605, ± 12v/20W) is chosen as the light source of the spectrophotometer for BCA, its spectrum range is from 0.3 μm to 2.5 μm. It has some characteristics, such as, full-waveband, cheap cost, etc. Its spectrum has the characteristics of continue, no-peaked, the middle of its spectrum is very flat. And its energy is all strong in the range of waveband from 200nm to 1000nm, the waveband is usually used in the bio-chemical analyzer of clinical diagnose or chemical checking.

3.2. Design of the splitting-light and detection unit based on the VHT grating
The splitting-light unit is a key part of the spectrophotometer for BCA, its performance is directly association with the performance of the BCA. In past years, the plane and the concave grating have been used in the classical spectrophotometer, As we all know, these conventional gratings are worked
by the groove, these grooves are surface-embossed on the surface or inside of the grating at the time of manufacture, So, there has a problem exists in these grating, that is, the embossed mode will generate some minute cracks on the surface of the gratings’ groove. These minute cracks will produce the stray-light in the splitting-light system to decrease the performance of the spectrophotometer for BCA. And, this problem is very difficult to avoid in the conventional grating. Therefore, the VHT grating is used in this work. The splitting-light schematic diagram of spectrophotometer for BCA based on the VHT grating is shown in Figure.3.

![Figure 3](image3)

**Figure 3** The splitting-light diagram of spectrophotometer for BCA based on the VHT grating

In the unit of splitting-light, the slit, collimation lens, VHT grating lie on the same optical axis, the VHT grating, focusing lens and CCD lie on another optical axis. The angle of two lines is about 38.4°, and the curvature of the collimation lens and the focusing lens is 26.33mm and 6.24mm respectively, the material of two lenses are fused quartz with the surface ultraviolet-enhanced-aluminum coating. The CCD is custom-built photodiode array, its efficient pixels are 21, the size of each pixel is about 1mm×5mm, the spectral width is about 53mm. The diagram of the custom-built linear CCD is shown in Figure.4. The working frequency of CCD is about 4MHZ, but it can be adjusted by changing the level of trigger in CCD driver module to get more integration time of CCD. In this unit, since the VHT grating is adopted, the efficiency of the spectrophotometer is more than the spectrophotometer based on plane and holography concave grating.

![Figure 4](image4)

**Figure 4**. The diagram of the custom-built linear CCD

3.3. Design of signal acquisition and processing Unit
The biochemical signals acquired by CCD is usually very weak analog signals, it must be amplified by the amplifier. So, the off-current amplifier and the adjustable-gain amplifier are used. In addition, the acquired signals are often polluted by noises, so, the filter must be used. The filtered signals are transformed into the digital signals by the A/D-DAQ card and stored by the data register through the bus trigger unit and the data arrangement unit. Finally, the stored signals are sent into CPU to be processed. The schematic diagram of the signal acquisition and processing unit is shown in Figure.5.
3.4. Design of the virtual-spectrophotometer for BCA based on LabVIEW

Virtual instrument (VI) is a trend of the future instrument development. In this paper, we use the VI technology based on LabVIEW [9] to acquire and analyze the spectrum. Compared with the traditional spectrophotometer controlled by SCM, DSP chips or other digital circuits, this signal acquisition and processing unit for spectrophotometer based on VI technology have many advantages, such as, higher development efficiency, shorter designing time, cheaper cost and more function, etc. The front panel of the virtual-spectrophotometer for BCA is shown in Figure 6. The virtual-spectrophotometer for BCA has many functions, such as, spectrum acquisition, data storing, threshold estimation, auto-adjustment of the integration time of linear CCD and multi-window displaying, etc.

4. Experiments and results

4.1. Materials

Halogen-tungsten lamp (Philip6605, ± 12v/20W) is chosen as the lamp source of the spectrophotometer for BCA, its spectrum range is from 0.3 μm to 2.5 μm. The high-pressure mercury lamp is used to as the calibration light source. The curvature of the collimation lens and the focusing lens is 26.33mm and 6.24mm respectively. Four lenses are made by the fused quartz with surface coated ultraviolet-enhanced-aluminum coating. The quartz colorimetric ware is chosen to as the cuvette of the spectrophotometer, its size is 12.4×12.4×45mm, its optical path is 10mm. The material of the splitting-light unit’s shell is aluminum, the width of the slit is 60μm. The VHT grating(1200lines/550nm, incident angle is 19.2°) is made by the synthetic photopolymer with high refractive index modulation, and its encapsulation is the quartz glass with surface coated the ultraviolet-enhanced-aluminum coating, the thickness of the VHT grating is about 10mm, the
efficiency is about 80% at the wavelength of 340nm and 750nm. The focusing lens is F-θ lens, its angle of the imaging is about 35.6°. The CCD (TCD1251UD, TOSHIBA, Japan) is glass capsulated, its size is 41.6×9.65×3.22mm, the length of the photosensitive section is 29.7mm, the efficient pixels is 2700, the size of each pixel is 11×11×11μm. The resolution of the A/D-DAQ card (QY-PCI-S12H, Co.QIYAO, Tianjin, China) is 14bits, its work frequency is about 5MHZ. The control unit is Lenovo Computer(Windows professional XP) and software is LabVIEW(Version.8.0 student edition, NI). The photography of the custom-built setup for is shown in Figure.7.

![Figure 7](image)

**Figure 7.** The photography of the custom-built setup

### 4.2. Calibration of the mercury lamp

In the experiment, we firstly used the mercury lamp as the calibration light source, the linear CCD with combined the A/D-DAQ card as the spectral detector and the virtual-spectrophotometer for BCA based on LabVIEW to test the resolution of the spectrophotometer for BCA. The characteristic spectrum of the mercury lamp is gotten and shown in the Figure.8. From Figure.8, we can see that the wavelength of 365nm, 404.7nm, 407.8nm, 435.8nm, 546.1nm, 577nm, 579nm can be easily distinguished. So, it is adequately indicate that the spectral resolution of the spectrophotometer for BCA is less than 2nm, wavelength accuracy is satisfactory and the structural design of the spectrophotometer is quite ideal.

![Figure 8](image)

**Figure 8.** The characteristic spectrum of the mercury lamp

### 4.3. The experiment of the full wavelength spectrum

Then, to do the experiment of the full-wavelength, the Halogen-tungsten lamp is used to as lamp source of the spectrophotometer for BCA, the custom-built linear CCD with combined A/D-DAQ card to as the spectral acquisition system. The virtual-spectrophotometer for BCA based on the LabVIEW is used as the software platform to control the spectral acquisition and analysis. The full wavelength spectrum of the spectrophotometer for BCA is got, and the spectrum at the wavelength of 340nm, 380nm, 405nm, 420nm, 450nm, 460nm, 480nm, 505nm, 520nm, 546nm, 578nm, 600nm, 620nm, 650nm, 700nm,
750nm, 800nm, 850nm, 900nm, 950nm and 1000nm can be clearly distinguished.

Acknowledgements

This work has been supported by the Chinese National Foundation for Natural Scientific Research grant (60767001), the Science and Technology Pillar Program of Jiangxi Province (2009BSA12700), the Natural Science Foundation of Jiangxi Province (2008GQW0013), and the Scientific Research Foundation of Jiangxi Provincial Education Bureau (GJJ09307,GJJ10243), and the Natural Science Fundation of Jiangxi Science and Technology Normal University (No.KY2009ZY10).

References

[1] Massey, P., Strobel, K., Barnes, J. V., & Anderson, E. 1988 Spectrophotometric standards. Astrophysical Journal, Part 1, vol.328, 315-333.
[2] SHI Junfeng, HUI Mei. 2003 Micromation and applications of spectrometers. Optical Technique,29(1).13-16.
[3] KM Rosfjord, RA Villalaz and TK Gaylord 2000 Constant-Bandwidth Scanning of the Czerny-Turner Monochrometer Applied optics. Vol. 39, Issue 4, 568-572.
[4] Takashi Onaka 1995 Aberration-corrected concave grating for the mid-infrared spectrometer aboard the Infrared Telescope in Space. Applied Optics, Vol. 34, Issue 4, 659-666.
[5] SC Barden, JA Arns, WS Colburn, JB Williams 2000 Volume Phase Holographic Gratings and the Efficiency of Three Simple VPH Gratings. The Publications of the Astronomical Society of the Pacific, Vol. 112, Issue 772, 809-820.
[6] SC Barden, JA Arns 1998 Volume-Phase Holographic Gratings and their Potential for Astronomical Applications. Optical Astronomical Instrumentation. Proc. SPIE Vol.3355, 866-876.
[7] LUO Q Y, DENG Y ZH 1992 A analyze with photometric. Science Press, Beijing. 57-60.
[8] Kogelnik and Herwig 1969 Coupled wave theory for thick hologram gratings. The Bell System Technical Journal, Vol. 48, No. 9, 2909-2947.
[9] Robert H. Bishop 2006 National Instruments, USA.