Design and experimental study of minitype modularized fluorescence microscopic imaging system and focusing mechanism

Yang Zhang1, Weiwei Fu 1*, Min Liu1 and Hailong Zhu1

1 Suzhou Institute of Biomedical Engineering Technology, Chinese Academy of Sciences, Suzhou, Jiangsu, 215163, China

*Corresponding author’s e-mail: fuww@sibet.ac.cn

Abstract. In the global in vitro diagnostic (IVD) market, point-of-care testing (POCT) features the largest market share, while POCT in China features small market share. With the development of consumption upgrading, urbanization and medical reform of China, this kind of real-time detection without central laboratory features huge market demand and potential. POCT is a testing method, which can obtain test results in just a few minutes with portable equipment and supporting reagents. In this paper, a minitype modularized fluorescence microscopic imaging system is proposed, which is used for blood cells detection, microbial observation and pathological section scanning. In order to meet the needs of portable, miniaturized and chip-type, a linear objective lens switching and focusing mechanism (LOLSFM) has been designed and developed, which changed the rotating disk switching mode of existing microscope. Finally, the precision and verticality of the LOLSFM are verified by experiments. The imaging quality of minitype modularized fluorescence microscopic imaging system.

1. Introduction

The medical equipment with the features of miniaturization, rapidness and chip is the new trend of modern instrument development [1]. Instant test results can be obtained within a few minutes by using portable equipment and supporting reagents, which can reduce the dependence on large-scale and high-end equipment. It also can reduce the technical requirements for users, which is easy to popularize. POCT is especially suitable for blood cell detection, microbiological observation and pathological section scanning [2].

At present, the real-time detection technology in the field of blood cell detection is mainly used in hemoglobin concentration detection, white blood cell count and platelet count. Platelet transfusion is the main way of clinical blood transfusion. If there are residual white blood cells and red blood cells in the platelet, it will stimulate the immune system of the injected patient to produce isotype antibodies, which results invalid platelet transfusion. The mixing amount of white blood cells and red blood cells should be lower than $5 \times 10^6$ / bag and $8 \times 10^9$ / bag, which is one of the daily quality control items in blood stations. At present, the microscopy is used to count the red blood cell and blood count disk based on Nageotte is mainly used to count the white blood cell in blood stations. The manual counting method based on Nageotte blood count disk features large error, poor repeatability and without original records, which does not meet the modern requirements for record preservation and evidence...
chain. The detection threshold of cell counting method commonly used is 10^4/mL, which is impossible to detect the residual cells in platelets quickly and effectively.

The microscopic image method is used to count and classify cells morphologically based on image recognition and signal processing technology. The representative is HemoCue®WBC System that can achieve the function of white blood cell count in 2008. In 2010, based on the previous products, HemoCue improved the imaging system and developed the white blood cell classification and detection instrument HemoCue®WBC DIFF System. Chempaq XBC analyzer and dedicated chip can achieve full blood cell classify and count. Different disposable chips are needed for counting different kinds of cells, which contain different detection reagents. The disadvantage is that the classification and counting of three kinds of cells cannot be completed at the same time. Luna-FLTM dual fluorescent cell counter can measure the number, viability, diameter, concentration, and other indicators of 5 to 180 μm cells with 4X or 10X objective under bright field or fluorescence field.

In this paper, a method based on fluorescence labeling detection was proposed to integrate different use requirements, and a minitype modularized microscopic imaging system was designed to realize low sample capacity, automatic and rapid blood cell imaging analysis and counting. The instrument operating principle and the composition are introduced in Section 2. The linear objective lens switching and focusing mechanism is present in Section 3. Then the repeat positioning accuracy and verticality of LOLSFM and imaging quality of instrument are validated in Section 4. Conclusions are given in the last Section.

2. Formatting the title, authors and affiliations

Inverted fluorescence microscope structure is used, which is combined the advantages of ultrapak microscope structure and infinity conjugate optical system. The excitation light and fluorescence don’t interfere with each other, because they are opposite to the objective lens. The falling light and the viewing surface are on the same surface. The fluorescence image brightness is not lost, and the image quality is not affected. This structure is particularly suitable for thick section imaging, such as bacterial colony and tissue culture [3]. The imaging optical path can greatly reduce the external dimension of the instrument after three times of reflection. The optical path system is shown in Figure 1.

![Figure 1. Optical path of minitype modularized microscopic imaging system](image)

The minitype modularized microscopic imaging system is divided into four layers, which are chip layer, focusing layer, fluorescence layer and imaging layer. The closed structure with only one socket of microfluidic chip is adopted to effectively avoid external pollution and accidental damage to core parts in the process of handling, and improve the reliability of the instrument. The microfluidic chip is driven by a permanent magnet stepper motor to achieve two-dimensional motion. The linear objective lens switching and focusing mechanism is adopted to realize the high precision switching of multiple objective lenses and fast focusing. According to the field of view of the objective lens, every movement of 0.5mm, microfluidic chip is recorded by CCD camera. The full stroke is 15mm×50mm. Finally, a panoramic image of microfluidic chip is formed by image splicing on PC. Combined with
image processing technology, the morphological and quantitative parameters of cells or microorganisms can be quickly and accurately detected [4].

Figure 2. Structure of minitype modularized microscopic imaging system

A fluorescence module is composed of fluorescent light source, excitation filter, emission filter, dichroscope and spectrooscope, as shown in Figure 3. The Led source is arranged in parallel with the aspheric lens. The emitting light is the horizontal and uniform parallel light. The emitting filters can filter out stray light to form a specific band of light. The dichroscope is placed at 45° with the horizontal plane to reflect emitting light to the sample surface and ensure the excitation light of specific band passes completely. The end of the led source is a thermal slug, which can effectively emit heat generated by the light source. The instrument is equipped with three sets of fluorescence modules, which can be switched in the X-axis direction to observe different staining samples. The fluorescent module is compact, stable and substitutable, which can be used to match the corresponding optical elements according to different test samples and expand application.

Figure 3. Structure of minitype modularized microscopic imaging system

To ensure optical alignment and imaging effect, the optical devices are mounted on a plate, which the flatness is 1μm. The optical devices includes three reflectors, infinite sleeve lens, CCD and diaphragm.

Figure 4. Structure of imaging module
3. The linear objective lens switching and focusing mechanism

The focusing mechanism is an important part of the microscopic imaging system. The turntable objective lens converter is used in traditional microscope, which realize the focusing by the movement of slide platform [5]. It is difficult to achieve miniaturization of the instrument in this way. A linear objective lens switching and focusing mechanism is adopted, which is composed of HB screw motor, clearance nut, guide slider, cross roller guide, encoder, position sensor, base plate, objective lens switching plate, focusing moving plate, focusing fixing plate and motor fixing plate, as shown in Figure 5. The two screw motors provide power for the vertical and horizontal motion of the objective lens respectively. The screw motor is used to eliminate the empty travel caused by coupling and reduce the coaxiality requirement for installation [6-7]. The clearance nut is used to eliminate the empty return error, which improves the motion accuracy [8-9].

![Figure 5. Structure of linear objective lens switching and focusing mechanism](image)

The 10X and 20X objective lens are used as examples to calculate the load torque. The load torque equation can be formulated by

\[ T_L = \frac{(m_1 + m_2 + m_3)P_B}{2\pi \eta} \times \frac{1}{i} \times 2 \]  

where \( m_1 \) is the mass of 10X objective lens, \( m_2 \) is the mass of 20X objective lens, \( m_3 \) is the mass of focusing moving plate, \( P_B \) is lead of screw rod, \( \eta \) is the transmission efficiency, \( i \) is the transmission ratio. \( T_L = 1.3 \times 10^{-3} \text{Nm} \).

The depth of field equation of digital microscope with infinite optical path can be formulated by

\[ \sigma_x = \frac{1.22n\lambda}{NA^2} + \frac{ne}{NA\beta_r} \]  

where \( n \) is refractive index of the microscope space, usually 1 for dry lens and 1.515 for oil-soaked lens. \( \lambda \) is wavelength of the most sensitive line for the human eye, \( NA \) is the numerical aperture of the objective lens, \( \beta \) and \( \beta_r \) are the transverse magnification of the objective lens and the adapter lens respectively, \( e \) is the pixel size of 2-times charge-coupled device (CCD). 20X objective lens is used as examples to calculate, that \( \sigma_x \) is 5.33μm. According to engineering experience, the minimum step size of the motor should be less than \( \sigma_x/4 \)[10, 11].

4. The experiment

The accuracy and verticality of the focusing mechanism are the reliable guarantee for the image quality of the minitype modularized microscopic imaging system. Therefore, the focusing accuracy and verticality of the linear objective lens switching and focusing mechanism are tested. The laser interferometer was used to measure the repeated positioning accuracy [12,13], as shown in Figure 6(a). The perpendicularity of the linear objective lens switching and focusing mechanism is measured by TRIOPTICS autocollimator [14], as shown in Figure 6(b). The beam of the autocollimator reaches the reflector through 90° refraction of the pentaprism. The deviation of the reflected cross wire and the
emitted cross wire is used as the deviation angle between the axis of motion and the reference axis. The final deviation value is the geometric mean of the deviation value in the $x$ direction and the $y$ direction.

The position deviation relationship is obtained as shown in figure 7. The maximum repeatable positioning accuracy is 0.779μm. The vertical deflection angle of the focusing mechanism is less than 10°, which can meet the requirements of optical design and ensure the collimation of optical path.

![focusing mechanism setup](image)

**Figure 6.** The experiment setup of linear objective lens switching and focusing mechanism

![deviation graph](image)

**Figure 7.** The repeated positioning accuracy of focusing mechanism

| position(mm) | x direction(°) | y direction(°) | square root(°) |
|--------------|----------------|----------------|----------------|
| 0.2          | 1.49           | 5.52           | 5.72           |
| 0.4          | -0.52          | 6.73           | 6.75           |
| 0.6          | 1.32           | 7.47           | 7.59           |
| 0.8          | -1.44          | 6.67           | 6.82           |
| 1.0          | -1.14          | 4.41           | 4.56           |
| 1.2          | 0.74           | 5.18           | 5.23           |
| 1.4          | 1.71           | 7.23           | 7.43           |
| 1.6          | -0.88          | 7.65           | 7.7            |
| 1.8          | 0.58           | 5.62           | 5.65           |
| 2.0          | 0.31           | 4.74           | 4.75           |
The test was carried out to verify the imaging quality of the designed minitype modularized microscopic imaging system. The detection of residual white blood cells in platelet products was targeted for the simulation experiment. According to the characteristics of blood cells, markers were selected as CD45 and nuclear DNA of white blood cells. The samples were fluorescent microspheres with diameter of 10 μm. CD45 fluorescent antibody and DAPI specific nuclear dye were stained. The corresponding fluorescence module selected monochromatic LED light source with wavelength of 546nm and 365nm. The test results are shown in the Figure 8. The minitype modularized microscopic imaging system features good imaging effect in brightfield and fluorescent field.

![Figure 8. The imaging effect of minitype modularized microscopic imaging system](image)

5. Conclusion
In this paper, minitype modularized microscopic imaging system is developed to realize low sample capacity, automatic and rapid blood cell imaging analysis and counting. The linear objective lens switching and focusing mechanism is first developed, which can realize objective lens switching, effectively reduce the size of the instrument and improve the focusing accuracy. The linear objective lens switching and focusing mechanism has been verified by experiments that maximum repeatable positioning accuracy is 0.779μm and vertical deflection angle is less than 10°. Finally, the detection of residual white blood cells in platelet products was targeted for the simulation experiment.

Acknowledgments
This work was supported by Natural Science Foundation of Jiangsu Province (No.BK20190197) and Post-doctoral Research Funding Program of Jiangsu Province (No.2018K021C).
References

[1] Manz A, Graber N, Widmer H M. Miniaturized total chemical analysis Systems: a novel concept for chemical sensing [J]. Sensors and Actuators B: Chemical, 1990, 1(1-6): 244-248.

[2] Lü M, Li C, Chen F, et al. Research progress of POCT blood cell counting equipment[J]. Medical and Health Equipment, 2018, 39(7): 91-95.

[3] Yang GL. Fluorescence and fluorescence microscopy [J]. Optical Instruments, 2001, 23(2):18-29.

[4] Xu W, Lang T. Application of LED light source and CCD on fluorescence microscopy [J]. Optical Instrument, 2015, 37(4): 363-370.

[5] Tian P, Gu CC, Hu Jie, et al. Review of microscopy autofocus methods [J]. Optical Technology, 2014, 40(1): 84-88.

[6] Lin WC, Wang J. Design of a high precision focusing mechanism [J]. Journal of Changchun University of Science and Technology, 2010, 33(4): 39-42.

[7] Xue LT, Chen T, Xu T, et al. Design of a high precision and high reliability focusing mechanism [J]. Journal of Changchun University of Science and Technology, 2012, 35(2): 9-11.

[8] Wu FY, Hu CC. Anti-shake auto-focus modular structure: 7881598[P]. 2011-02-01.

[9] Xu XX, Li Y. Design of high precision small volume focusing platform for visible light TV [J]. Optics and Precision Engineering, 2017, 25(6): 1526-1533.

[10] Guo XH, Zhao CX, Zhou P, et al. Depth of field and depth of focus of zoom optical system and its analysis [J]. Optical Technique. 2019, 45(3): 263-268.

[11] Ren LQ. Research on depth of field and autofocus method in fluorescence microscope [D]. Tianjin: Tianjin University, 2012.

[12] An Y, Qi YC. Design of linear camera focusing mechanism for space camera [J]. Optics and Precision Engineering, 2009, 17(3): 609-614.

[13] Jiang ZQ, Jia JJ. Design and test of focusing mechanism of space camera lens [J]. Optics and Precision Engineering, 2018, 26(12): 2956-2962.

[14] Jia XZ, Zhang L, An Y, et al. Design and experiment of precision focusing mechanism for space optical remote sensor [J]. Journal of Mechanical Engineering, 2016, 52(13): 25-30.