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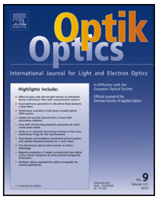

Research on opto-mechatronic biological microscope design

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ABSTRACT

The monitoring method of 1–40 layer cell factories is limited in China. In order to observe the planting status of cell in the cell factories in a real-time, in-situ and efficient way, we propose an observing method which adopts to tilt observation cells through transparent side-wall of cell factories and it will improve the production efficiency of vaccines and other biological medicine. An Cassegrain reflective long working distance microscope objective has been designed so as to monitor the cultured cells in the cell factories. The magnification is 5 and working distance is 85 mm. The numerical aperture can reach 0.3 and the minimum distance resolution is 1.1 μm. At the same time, a servo-control system has been researched by utilizing single neuron adaptive PI control algorithm which aimed at the observation device. The servo-control system has high control precision and good repeatable performance. The system can fill the blank of domestic technology and have a great market prospect.

1. Introduction

Cell factory is a kind of multi-plated cell culture equipment which is applied to large-scale cell culture. The distance between the adjacent two layers of culture plate is only 17 mm, and the special design increases the cell culture superficial area of per unit volume and guarantees the asepsis operation. It also decreases patch differences up to the hilt, and makes the amplification of production scale of vaccine, monoclonal antibod and biological medicine simple and practicable. The application of cell factory realizes the operating procedures of cell culture [1,2].

In recent years, cell factory culture technology is developing rapidly in our country. Therefore, manufacturing enterprises need the real-time quality monitoring for cell factory culture technology. The traditional cell culture technology adopts replacement-bottle culture, makes use of ordinary bottom-up microscope to observe and evaluates the qualification of the cells in replacement-bottle. However, cell factory is multi-plated cell culture equipment and bottom-up microscope can't observe it. At present, only Japanese Shikoku Precision Instruments Corporation owns automatic monitoring technology related to cell factory, and unit prices are above 3 million. But the production can only observe a line area of cell factory and the observation area is very small. At present, there is no domestic related technology and production.

Given this, this paper comes up with a kind of cell observation. This method adopts a long working distance micro optical system and aslant observes transparent sidewall of cell factory. This paper also respectively designs and tests the optical system and servo-control system, independently researches and develops new observation equipment realizing real-time automatic monitoring for the cell in plated cell factory culture dishes. This new equipment makes up for the domestic blank.

2. The observation theory for cell factory

Because of the limit of cell factory structure, the traditional observation method can't observe cells which grow adhered to the sidewall inside cell factory. In order to conform to the specified transpex in USP, the sidewall of cell factory material is transparent. In this case, though the transparent sidewall of cell factory, adopting a microscopic observation device with large tilt angle of observation can observe cells growing adhered to the bottom of upper layer culture dish. The observation theory is shown in Fig. 1.

When observing with the microscope lens diameter of 60 mm at angle of 30°, in order to make that the horizontal observable distance x is greater than 50 mm, the working distance of microscope lens is demanded greater than 80 mm. And the optical systems are demanded to have large depth of field due to the large viewing angle.

Controlling the movement between the cell factory and aslant long working distance microscope by servo-control system, realizes the purpose of automatic observation and respectively

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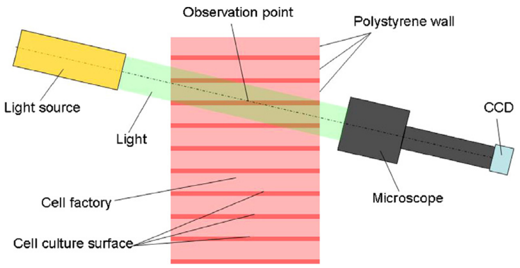

Fig. 1. Schematic diagram of microscope observation principle.

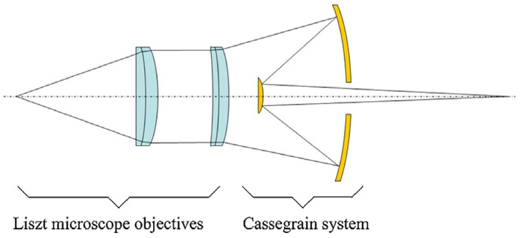

Fig. 2. Initial structure diagram.

observes cell growth condition in a rectangle field from the first to the fortieth layer of cell factory sidewall. Rotating one side of cell factory can observe cell growth condition on the other side of rectangle field and increase the observable area. The width of the cell factory culture surface is 200 mm, when the horizontal observable distance x is 50 mm, observable area of cell factory can reach 50% and observation rate is greater than the Japanese similar products.

3. The design of long working distance micro optical system

The cell factory single size (335 mm × 205 mm × 17 mm) determines that the microscope lens need to equip with high resolution, high magnification and longer working distance, in order to observe the cell clearly. Calculating the related parameters needs that the working distance of optical system should be greater than 80 mm. The overlong working distance makes optical design more difficult.

Therefore, the design adopts Liszt microscope objectives as the initial configuration and the rear is Cassegrain system. The optical system structure is shown in Fig. 2. When choosing two sets of doublets lens center of Liszt microscope objectives as axis of symmetry, the optical system is approximate symmetry. The vertical axis aberration, namely coma, distortion and vertical chromatic aberration, decreases effectively. The first group of negative power doublet diaphragm bended to lens in initial configuration, can revise astigmation effectively [3–7].

This paper chooses CCD, Industrial high resolution digital camera. Its pixel size is 3.75 μm, the highest resolution is 1280 × 960 and the pixel is 1.2 million. Calculating can get parameters related to optical system as below.

$$\text{Height of image}: \quad 2h = 1.4 \times \sqrt{1280^2 + 960^2} = 6\,\text{mm}$$

The formula $N = \left(1000/(2 \times a)\right)$ (N stands for limiting resolution, and a stands for pixel size), can obtain the barrier frequency of system modulation transfer function: $\left(1000/(2 \times 3.75)\right) =$ 133 lp/mm.

According to experiences, when the field of height $2y$ is less than 1/20 of lens focal length, the CCD camera image quality is satisfying. This design chooses 1/3 in. CCD sensor. According to the request of formula, linear field of view $2y = 1.2$ mm, the lens focal length of 60 mm can meet the above condition [8].

Analyzing and revising aberration of the initial configuration in optical system, after optimizing can obtain the final result as shown in Fig. 3. This working distance of optical system is 85 mm, magnifying power $\beta = 5\times$, numerical aperture NA = 0.3, object height of linear field of view $2y = 1.2$ mm, and the abscured radio of system is 30%. The optical system imaging curve is shown in Fig. 4.

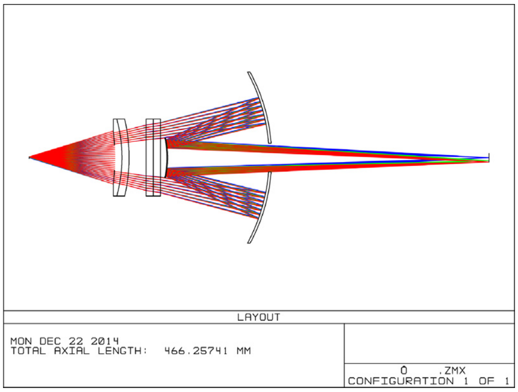

Fig. 3. Final structure diagram.

As the MTF curve in Fig. 4(a) shows, MTF values greater than 0.5 at the medium frequency of 66 lp/mm and MTF value is reduced to about 0.3 at the high frequency of 133 lp/mm, which is the barrier frequency of the CCD. And the meridian plane and the sagittal plane coincide well in each field of view, which means the image quality is high then.

In Fig. 4(b), the system spot diagram shows the RMS of confusion disc in each field of view is no larger than 3.75 μm, which is the pixel size of the chosen CCD. The diameter size of confusion disc in this optical system is also very close to the airy spot. The optical system has high resolution and good imaging quality. It can still give clear cell images even under the influence of stray light, because the confusion disc will not expand too much.

In Fig. 4(c), we can find in the System energy diagram that the optical system is of high energy concentration and the light energy through the system to the detector is enough for the optical system to get clear image.

Thus, the designed long working distance microscope optical system is suitable to be used to observe Cell factory as an optical–mechanical–electrical integrated Biological Microscope.

4. Design of servo control system

4.1. Mechanical structure of the automatic monitoring device for cell factories

During the observation of cell factories, the relative position of the head and tilted optical microscopy illumination system is fixed. In order to keep the testing target in the observed field while moving, mechanical system must have high drive stability and high drive precision, which demand closed loop control of transmission. The mechanical structure diagram of monitoring device and every shaft is defined as shown in Fig. 5

Four shafts of the monitoring device respectively are called: the horizontal shaft (X), the vertical shaft (Y), the feed shaft (Z) and the rotation shaft (W). In mechanical structure of the monitoring device, the base act as the bottom base of the monitoring device. Rotation structure and vertical moving structure are installed on the base. By controlling the movement of these four shafts, we can change the relative position between the optical system and the cell factory as we need.

Feed mechanism moves the cell factory to the view field of the optical systems and we can make the optical system reach the level needed for cell factory observation location by controlling vertical movement mechanism. Horizontal moving mechanism will move the optical system to the observing the position of the cell factory in that chosen level. We can move the optical system in the