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Image Processing Based Colony Identification Research

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Abstract

Aiming at the shortcomings of the existing automatic colony counter, a set of algorithms based on the principle of image chromatic aberration to achieve colony identification is proposed, and a colony identification device is developed on this basis. The colony identification method is mainly based on the fact that different kinds of colonies and different concentrations of the same kind of colonies have different light-absorbing characteristics, and the judgement of colony types and concentrations is achieved through the method of image processing. The main features of the developed colony recognition equipment are high working efficiency, short recognition and detection time, and the potential of mixed recognition ability of multiple colonies. Therefore, the identification method and equipment have good application and promotion value in agriculture, food, medicine and other industries.

Keywords

Colony Identification, Colony Counting, Image Recognition, Colony Identification

1. Introduction

At present, there are many kinds of instruments for colony identification at home and abroad. One is the cheaper colony counter equipment, which can simply count the number of colonies and is simple to operate. However, the use of high cost, long detection cycle, error, cannot detect the number of colonies in a timely and effective manner. In addition, there is an ultraviolet spectrophotometer. It determines the density of colonies by measuring the value of OD (Optical Density). Compared to colony counters, it has higher accuracy and shorter testing cycles. Its disadvantages are expensive price and large equipment. The current

conventional colony identification instruments have single function, poor economy, difficult to popularise and other problems. So there is an urgent need for a colony identification equipment that can solve these difficulties.

Currently, colony identification research has begun to receive widespread attention and rapid development. For example, Ximin Liu carried out the development of a plate counting medium for simultaneous detection of coliforms and total colony counts, and established a coliform detection method based on characteristic enzymes [1]. Zhou Donghai *et al.* used EasyDisc to detect the total number of colonies in water with the traditional plate counting method and performed a comparative analysis [2]. Chen Xinping *et al.* developed a machine vision algorithm for colony segmentation and counting based on Python language, which can segment and count colonies accurately and systematically [3]. Yongxiang Jiang *et al.* addressed the problems of uncertainty and low efficiency of manual counting of colonies, and conducted research on industrial cameras, image processing, feature comparison, and counting algorithms, and proposed a colony counting algorithm based on machine vision [4]. He Jianxian *et al.* proposed a colony segmentation counting algorithm based on target colour base and gradient direction matching. The algorithm firstly uses the colour feature of the colony in the image as a base and transforms the image into the base space to enhance the difference between the colony and the background. Secondly, the gradient direction is filtered by using the gradient magnitude feature of the colony image, and then it is matched by the gradient direction, and then the adherent colonies are segmented, and finally, the colonies are screened and counted by using the method of non-extremely large value suppression [5]. Yixiang Cui *et al.* based on artificial intelligence combined with small target detection algorithm for image recognition and processing, which can improve the accuracy of the detected targets and achieve the automation of colony counting [6]. SMS *et al.* carried out the study of population optimised microbial colony counter [7]. Shuvam B *et al.* proposed a fast and simple method of counting the colony forming units [8]. Fan Xiangyu *et al.* proposed a model to improve YOLOv5. The model was modified by adding a small target detection layer and replacing the K-means algorithm with the K-means ++ algorithm, to better accommodate different sizes of eyes [9]. Sun Hongchang *et al.* conducted research on industrial cameras, image processing and counting algorithms and proposed a colony counting system based on improved Otsu [10]. Gong Youtong *et al.* tested the accuracy of the automatic colony counter and explored its application in healthcare with good results [11].

Despite the abundance of related research and certain progress, the existing colony counters, when used, need to put the solution of the colony to be tested into the petri dish for cultivation in advance, and can only be labelled and counted after the appearance of obvious white spots, resulting in a long period of detection. In the process of detection, the cultivation of colonies and the labelling of white spots are both completed manually, with high labour costs. To address this, we propose a set of colony identification algorithms based on image processing based

on the light-absorbing characteristics of the colony and the principle of chromatic aberration of the projected image, and develop a colony identification device based on this algorithm.

2. Selection and Cultivation of Strains

The selection of strains is the key and necessary prerequisite for this study. The strains were selected to better understand the basic principles of biology and to promote research progress in related fields. To this end, the strains we selected mainly contain the following characteristics. Firstly, the strains are widely distributed, usually prevalent in the natural environment and easy to collect. Secondly, easy-to-cultivate strains should have a fast growth rate, simple cultivation requirements and less demanding environmental adaptability. Finally, strains are genetically mature and strains should have a sound genetic research base.

Based on the above requirements and combining the laboratory conditions and resources, we selected three strains of *Bacillus subtilis*, *Escherichia coli* and S*accharomyces* cerevisiae for the study. One of them, *Bacillus subtilis*, is shown in Figure 1(a). It is a species of the genus Bacillus. It is sporulating and the endospore formed in the body can survive against harsh external environment. It is a Gram-positive aerobic bacterium and is commonly found in soil and on the surface of plants and in animals. *Escherichia coli*, as shown in Figure 1(b). It is an obtuse-rounded, motile, non-budding, Gram-negative, partially anaerobic, rod-shaped, *Escherichia coli* bacterium of the genus Escherichia. *Accharomyces*, as shown in Figure 1(c). It is a commonly used eukaryotic receptor cell in gene cloning experiments, and it is as easy to culture *accharomyces* as it is to culture *Escherichia coli*. It belongs to the fungal group of higher microorganisms. It has nucleus, cell membrane, cell wall, mitochondria, same enzymes and metabolic pathways.

(a) (b) (c)

Figure 1. Selected strain. (a) Cultivated *Bacillus subtilis*; (b) Cultivated *Escherichia coli*; (c) Cultured *Accharomyces*.

The strains were cultured using the traditional culture method. In order to ensure the activity of the bacterial solution, we used freshly cultured bacterial solution to carry out the experiment each time, as shown in Figure 2.

(a) (b)

Figure 2. Related pictures from the scene. (a) Laboratory Shaker Activation; (b) Cultured Bacterial.

3. Basic Principles of Colony Identification

Image processing based colony identification technology is a study based on the different degree of absorption of light waves by different colonies. Firstly, light is projected so that light of different wavelengths is transmitted through the bacterial liquid, and the image of the transmitted light spot is collected through the receiving screen. Due to the different degree of absorption of light waves by different colonies, the colour of the image will be significantly different, making the colour of the spot image become diversified. Secondly, based on the imaging of each colony at different concentrations, a standardised image library is established through image processing. This library is mainly used for image comparison of the bacterial fluids to be tested. Third, the images of the bacterial fluids to be tested were collected and subjected to standardised image processing. Fourth, the image library was used for image comparison of the samples of the bacterial fluids to be tested, and the final determination of the species and concentration of the bacterial fluids was made.

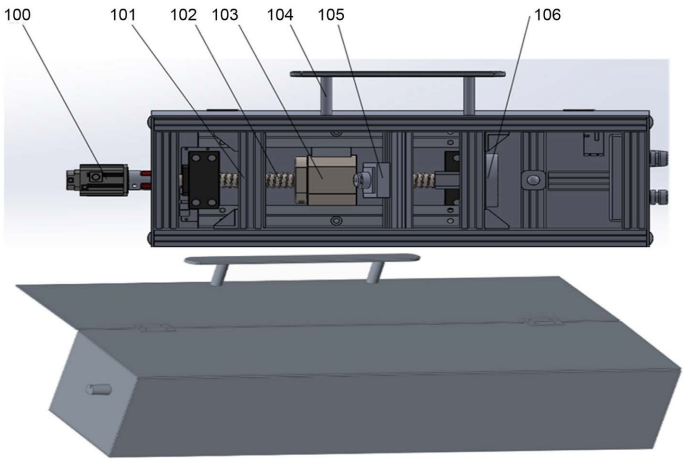
4. Design and Prototyping of a Colony Identification Apparatus

4.1. Structural Design

Based on the above principles, we carried out the design of the colony identifier. Based on the use of the environment and experimental requirements, we designed the colony identification instrument should ensure that people can easily carry and use, and the weight is less than 25 kg. we have gone through a lot of practice and research, the preliminary determination of the design of the colony identification instrument and the relevant parameters, and its three-dimensional and two-dimensional diagrams are shown in **Figure 3** and **Figure 4**.

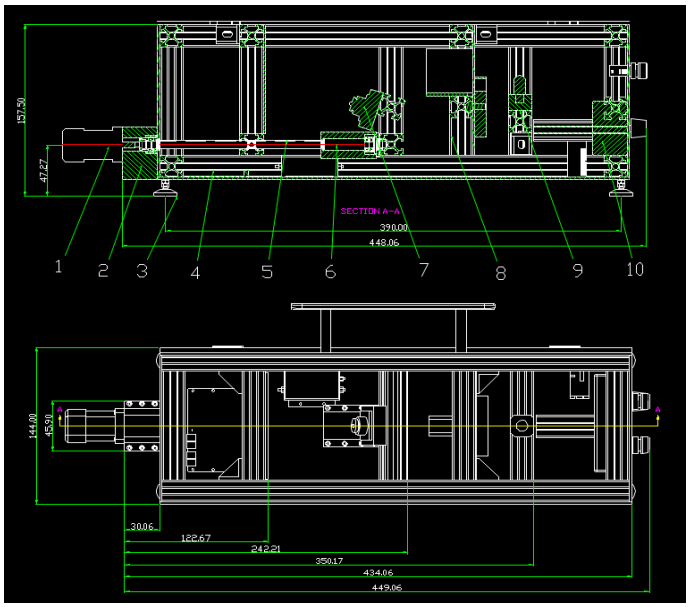
The colony automatic counter is mainly composed of motor, ball screw, camera screen, camera, handle, cuvette device, filter turntable. When in use, hold the handle and open the lid of the instrument, rotate the filter dial to select the appropriate filter, adjust the camera, put the cuvette into the cuvette slot, snap the lid of the instrument, rotate the power switch, the cuvette collection device through the

movement of the silk rod to reach the appropriate position and then wait for 2 - 3 minutes, press the button for data processing on the computer, wait for 2 - 4 minutes, save the data, concentration. In the production design in order to reduce the error in the process of image acquisition, so that the colony automatic counter device has better portability, stability and aesthetics. We use 20 new materials as the skeleton material of the device, pp board as the sealing plate of the device, so that the device has a certain degree of flexibility, and further reduce its weight, and beautify the appearance of the device, enhance the comfort of the use of the device.



100—servo motor 101—camera screen 102—ball screw 103—petri dish collector 104—handle 105—camera 106—filter turntable.

Figure 3. 3D Design drawing of the colony identification device.



1—Servomotor 2—Fixture 3—Standing foot 4—Stand 5—Screw 6—Push block 7—Camera 8—Collector unit 9—Filter dial 10-Power.

Figure 4. Two-dimensional design drawing of the colony identification device.

One of the more critical designs of the machine is the use of a petri dish collector that moves back and forth through a ball screw mechanism. The main principle of operation is that the ball screw acts as the active body, and the nut is converted into a linear movement according to the angle of rotation of the screw and the corresponding specification of the lead. The passive workpiece can be connected to the nut through the nut holder and the nut, thus realising the corresponding linear motion. Both the screw and the nut have semi-circular helical grooves which, when fitted together, form the nut raceway for the balls. The nut has a ball circuit pipe that connects the ends of several turns of the nut raceway to form a closed loop rotation, thus forcing the nut to move axially with essentially rolling friction.

4.2. Development of Prototypes

Based on the above structural design and combined with the actual processing conditions, we mainly considered the following factors when making the prototype. Firstly, the movement speed of the petri dish device of the colony counter has strict requirements, and its movement speed should be not less than 0.5 m/s. Secondly, the structure of the colony counter needs to be firm, reliable and safe enough to ensure that there will be no loosening during the working time, and there will be no leakage of electricity and so on, so as to ensure the safety of the operators. Third, the volume and weight of the automatic colony counter cannot be too high, to ensure that people can easily carry and use, its length, width and height of the basic size range in 0.5 m × 0.2 m × 0.2 m or less, the weight should be less than 25 kg, the specific parameters of its requirements as shown in Table 1. The prototype is shown in Figure 5.

Table 1. Table of requirements for colony identification parameters.

Project Name	Basic Requirements for parameters	
	Unit (of Measure)	Numerical Value
Colony Counter External Dimensions	m	Less than 0.5 × 0.5 × 1
External Dimensions of Colony Collecting Dish Device	m	0.05 × 0.04
Ball Screw Motor Power	KW	0.01 - 0.02
Speed of Movement of the Colony Collecting DishUnit	m/s	Greater than 0.5

Figure 5. Colony recogniser prototype.

5. Experiments and Analyses

5.1. Prototype-Based Image Acquisition and Analysis

A large number of experimental tests and validations were carried out using the prototype. As the baffle of the prototype is movable, the experimental parameters can be changed at any time for verification, and the cover on the top of the device is also divided into "blocks". Therefore, it is convenient to take and put the bacteria liquid, so as to achieve the convenience of repeated experiments. In this experiment, we compared the images of different strains of bacteria. In addition, different dilution concentrations of the same colony were also compared.

After acquiring the images using the prototype, we used MATLAB software for image processing, including preprocessing, multiple filtering and denoising. We found that the images obtained under different strains can obtain better results after being processed by the algorithms of R, B and G, respectively. Including the matrix plots obtained after their processing can be significantly different, and the related images are shown in Figure 6.

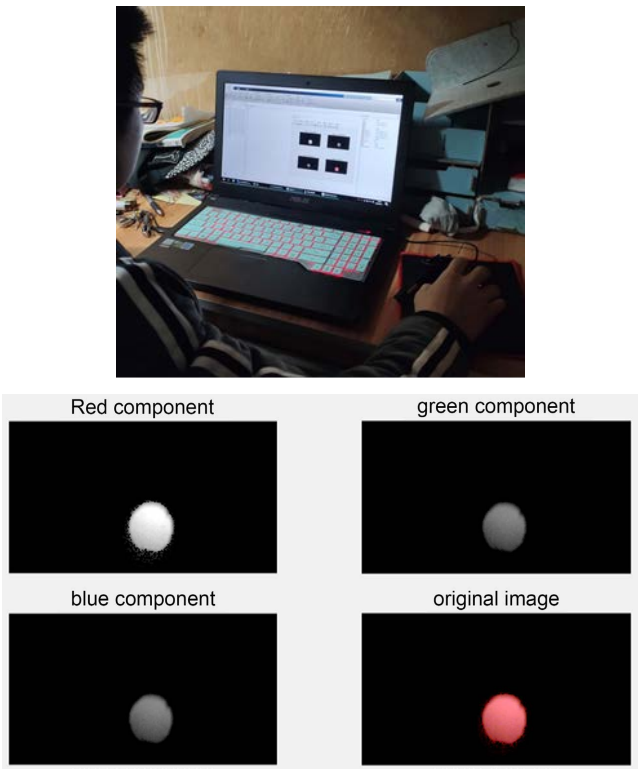


Figure 6. Correlation diagram for image processing.

5.2. Prototype-Based Colony Identification Experiment

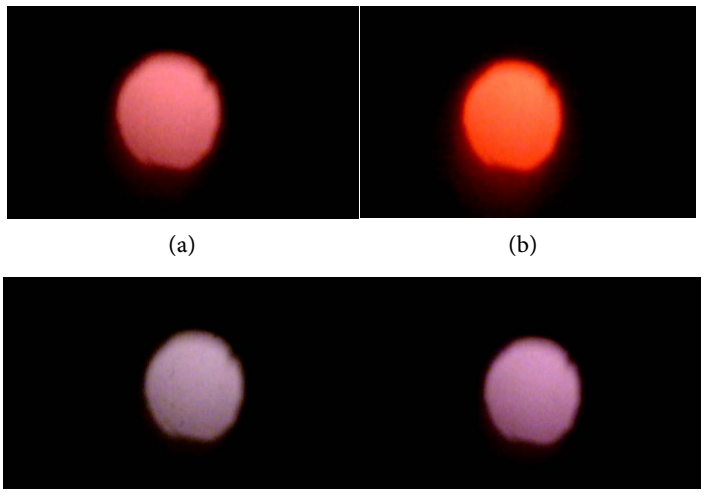
We found that different colonies will absorb light and light waves to different degrees under the irradiation of infrared visible light with a certain light wave. Based on this experimental phenomenon, we carried out a series of enquiries and experimental verification. We found that Bacillus subtilis has an obvious biostimulation effect on the light wave of 600 nm, and has a very good light absorption effect

compared with other light waves. Saccharomyces cerevisiae has a good physiological effect on light with a light wave of 660 nm, and has a good absorption effect compared to other segments of light waves, as shown in Figure 7. With the above findings and validation results, we will be able to identify the species of the colony by the different colours of the light spots presented by the colony.

(a) (b)

Figure 7. Imaging of different colonies after fluoroscopy. (a) *Bacillus Subtilis*; (b) *Saccharomyces.*

On the basis of the colony light absorption technique, we found that different concentrations of the same species of colonies under the irradiation of infrared visible light of a certain light wave will have different degrees of absorption of light and light wave, and the phenomenon of imaging colour variability occurs. Based on this phenomenon, we carried out similar experiments for *Bacillus subtilis*, *Saccharomyces cerevisiae* and *E. coli*. After experimental verification, we found that *E. coli*, *Saccharomyces cerevisiae*, and *Bacillus subtilis* colonies have different degrees of absorption of light waves in different concentrations when other conditions are the same, and there are some differences in their imaging, as shown in Figure 8 below.



(a) (b)

(c) (d)

Figure 8. Comparison of images of the same colony at different concentrations. (a) *Saccharomyces* diluted 20 times; (b) *Saccharomyces* diluted 80 times; (c) *E. coli* plots diluted 80 times plots; (d) *E. coli* plots diluted 100 times plots.

According to the obtained images, it is not difficult to find that different dilution concentrations correspond to significant differences in the images after

absorption of light. Therefore, we separately carried out image analysis of E. coli with different dilution concentrations and found that there were significant differences in the grey values of the obtained images, as shown in Figure 9.

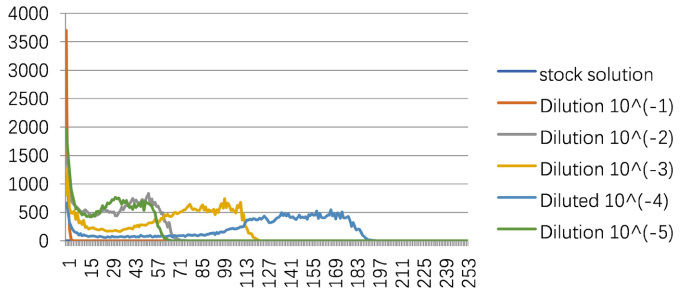


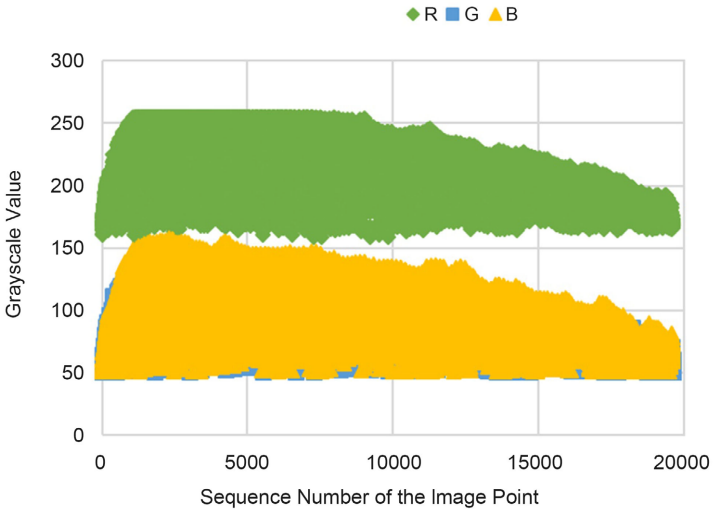
Figure 9. Distribution of grey values of images with different dilution concentrations.

In addition, we also found that the images obtained under different strains of bacteria can obtain different results by elimination of R, elimination of B and elimination of G, respectively. Therefore, the novel colony identification equipment and identification method proposed in this paper can be used to deduce the species and concentration of bacterial fluids using the established standard image library, which has strong feasibility and promotion value.

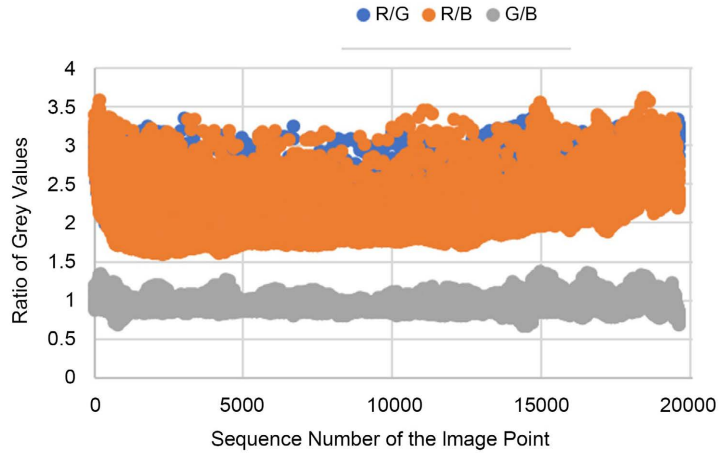
5.3. Data Analysis

We conducted a number of experiments using the prototype and found that the experiments were highly reproducible. Taking yeast as an example, we conducted a number of experiments using yeast solutions of different concentrations and obtained consistent data. Figure 10 shows the data points for 40 images of yeast diluted 20 times, with 19,627 image points collected. The average values of R, G and B are 219.9, 101.7 and 107.4 respectively. The standard deviations of R, G and B are 25.0, 22.1 and 25.6 respectively. The average values of R/G, R/B and G/B are 2.22, 2. 12, 0.95, and the standard deviations of R/G, R/B, and G/B are 0.289, 0.343, and 0.0678, respectively. Figure 11 shows the image points of the yeast after being diluted 80 times. 8691 image points were collected, from 20 experimental images. The average values for R, G and B are 253.9, 110.7 and 66.0 respectively. The standard deviations for R, G and B are 1.2, 10.5 and 8.9 respectively. The average values for R/G, R /B and G/B are 2.31, 3.92 and 1.69 respectively, and the standard deviations of R/G, R/B and G/B are 0.221, 0.535 and 0.116 respectively.

Because of the strict sealing requirements inside the prototype, the images obtained by projection are almost unaffected by external influences, so the image quality is high. From the above experimental (yeast) data, we found that although there is a certain deviation in the grayscale values of R, G, and B and the standard deviation is relatively high, the stability of R/G, R/B, and G/B is very high and the standard deviation is also very small. Moreover, there are significant differences in the R/G, R/B, and G/B of the perspective images of yeast liquid with different concentrations.

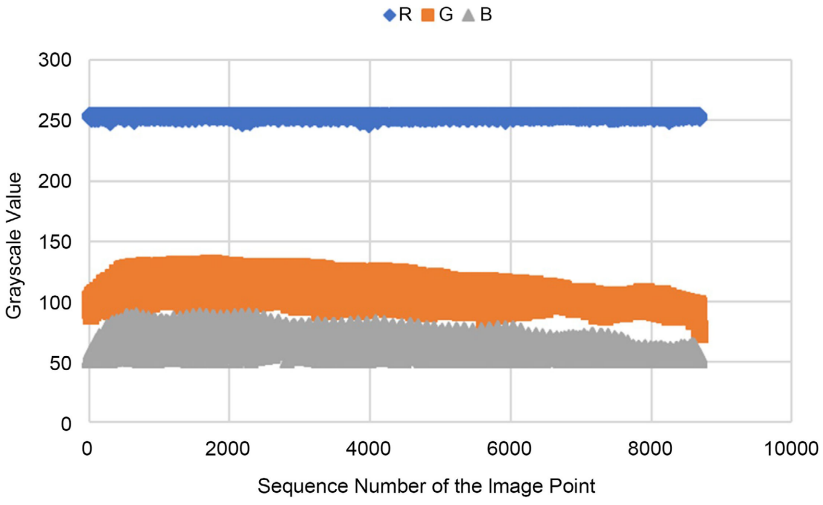


(a)

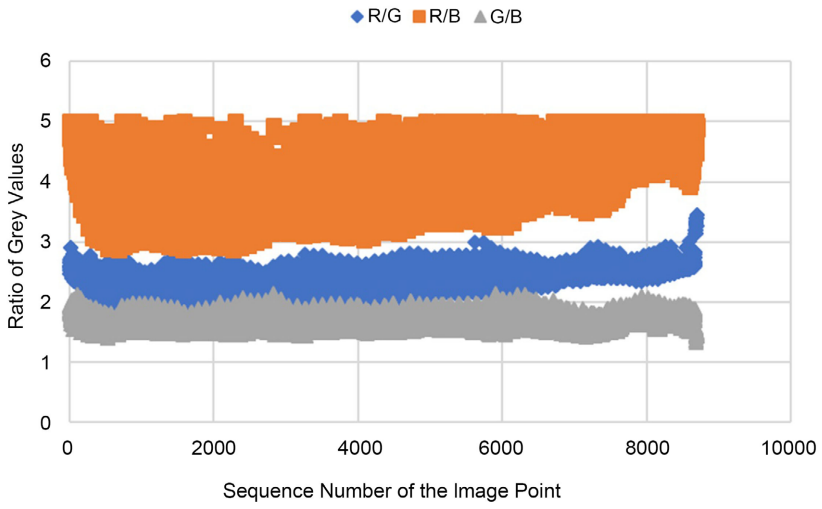


(b)

Figure 10. Data analysis of yeast dilution 20 times. (a) Grey value distribution; (b) Ratio distribution of grey values.



(a)



(b)

Figure 11. Data analysis of yeast dilution 80 times. (a) Grey value distribution; (b) Ratio distribution of grey values.

6. Conclusion

We have developed a colony identification device based on image processing. The device breaks through the short board of the existing technology and makes use of the principle of differential absorption of different colonies to various types of light waves and the principle of differential absorption of light waves by different

Table 2. Functional comparison table for prototypes.

Project Name	The Methodology Proposed in This Paper	Traditional Method		
		Counting method of OD colony identifiers	Calculation of artificial colony concentration	Artificial colony identification methods
Rationale	In the confined space, using light to project the bacterial liquid, part of the light wave is absorbed by the bacterial liquid, so according to the light spot after transmitting the bacterial liquid imaging map special judgement of the bacterial liquid type and concentration.	Within a certain concentration range, bacterial suspensions can be considered as homogeneous particle solutions whose optical density is proportional to the concentration of bacteria. Therefore, the number of bacteria is estimated by measuring the OD at a specific wavelength.	The bacterial solution was diluted in a certain proportion and then counted using a snowball counting plate.	Cultivation was carried out on solid medium, followed by observation of morphological characteristics of colonies and extraction of DNA for genetic identification by sequencing.
Identification of the type of bacterial fluid	Yes	No	No	Yes
Judgement of the concentration of bacterial fluid	Yes	Yes	Yes	No
Test speed	Fast (takes a few minutes)	Fast (takes a few minutes)	Slow (takes a few hours)	Very slow (takes a few days)

concentrations of the same colony to carry out the identification of different colonies and the identification of different concentrations of the same colony through the projected image of the characteristic light spot. We use MATLAB software for image processing, mainly including preprocessing, data analysis and processing of R, G, and B. We use the colony characteristic images that have been tested to establish a standard library. By using this standard library, it is possible to quickly compare with the images of the bacterial solution to be tested, thereby achieving efficient identification and rapid counting of the bacterial solution. The method described in this paper has obvious advantages over the traditional OD value colony counter counting method, manual colony concentration calculation method and manual colony recognition method, as shown in Table 2.

From Table 2, it is not difficult to find that the prototype can not only perform the important functions of species identification and bacterial liquid concentration determination simultaneously, but also in a very short time. By comparison, the advantages of the method and equipment described in this paper, such as comprehensive functions and short identification and detection times, can to some extent promote the development of automated colony identification technology, compared with other traditional methods.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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