EXPERIMENTAL STUDY FOR AUTOMATIC COLONY COUNTING SYSTEM BASED ON IMAGE PROCESSING

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Abstract: Colony counting in many colony experiments is detected by manual method at present, therefore it is difficult for man to execute the method quickly and accurately .A new automatic colony counting system was developed. Making use of image-processing technology, a study was made on the feasibility of distinguishing objectively white bacterial colonies from clear plates according to the RGB color theory. An optimal chromatic value was obtained based upon a lot of experiments on the distribution of the chromatic value. It has been proved that the method greatly improves the accuracy and efficiency of the colony counting and the counting result is not affected by using inoculation, shape or size of the colony. It is revealed that automatic detection of colony quantity using image-processing technology could be an effective way.

Key words: colony counting; image-processing; optimal chromatic value; automatic detection

1. INTRODUCTION

Colony counting is one of the most basic and frequent operation in colony cultivating experiments, and is both basic and important in agriculture, foods, medical analysis. At present, people usually adopt national standard detection method to count colony, which is ordinary nutrition agar pump method. When the number of sample is huge, the method becomes complex, time consuming, low efficiency, so it is necessary to ameliorate traditional counting method. With the promotion of computer technology, using computer as a tool to effectively reduce the intensity of production, improve labor productivity, and realize production automation has become a development trend. In recent years, using computer image processing technology to solve the quality detection task of agricultural products has been the general concern of scholars at home and abroad, so as to colony counting. The study developed a detection system based on computer image processing which found a recognition and rapid colony counting approach.

2. HARDWARE PLATFORM OF THE SYSTEM

Hardware composition of automatic colony counting system is shown in Fig.1; the system is mainly made up of light source box, CCD camera, image acquisition card, computer, monitor and other equipments. CCD camera is the color camera of Panasonic WV-CP410. Image acquisition card is DH-CG300 which is a product of China Daheng Group, Inc., maximum resolution of image acquisition is 768×576×24bit. Configuration of computer is PENTIUM-Ⅳ 1.7G CPU，256M memory，80G hard disk，64M display memory. Petri dish with cultured colony is placed under optical platform, optical platform is used to adjust amplitude and angle of optical radiation, so as to get a clear image which will be easy to process and identify. Colony image is acquired by CCD camera under preset illumination condition, then it was sent to a computer installed with image acquisition card, after that image processing software which runs in the computer will do a series of preprocessing, object segmentation and counting, then the number of colony is obtained.

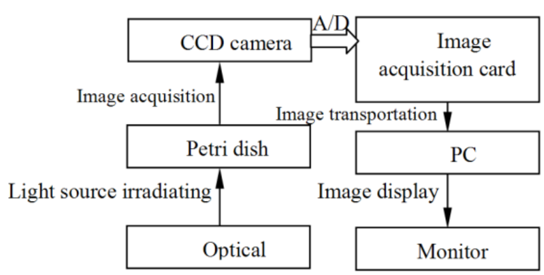

Fig.1 Hardware configuration of the system

3. IDENTIFICATION OF COLONY IMAGE

From gray image acquired from CCD camera as is shown in Fig.2, we can see that: colony image has a uniform gray value, although gray value inside and outside of Petri dish is different, both of them are uniform, what is more, gray value contrast between colony and background is large. Taking into account these characteristics, threshold segmentation method can be used to binarize the image which will separate colony from background.

Fig.2 Gray image

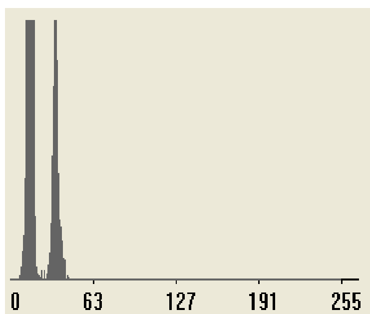

Fig.3 Histogram of gray image

3.1 Image Preprocessing

In order to enable easy target segmentation and enhance process accuracy, automatic counting system needs to preprocess acquired images. According to the system structure as is shown in Fig.1, system noise is mainly bring about from CCD circuit, threshold used to segment images is sensitive to noises, so the paper adopted grayscale, median filter, contrast enhancement and other preprocessing methods to remove noises and enhance image.

3.2 Image Extraction

From gray image as is shown in Fig.2 and corresponding histogram as is shown in Fig.3, we can see that gray value of Petri dish and colony is so close that ordinary bimodal law can not separate them but withdraw them together.

Having compared several image segmentation methods, the paper used the follow method: divide image to be detected into several small blocks first, use iterative threshold method to determine optimal threshold of each block, compare these thresholds, select the largest one and then subtract a small compensation named B (B=10 in the paper) which is used as global threshold, binarize the preprocessed colony image (shown in Fig.4). Gray value of colony in the image is higher (black represent gray value is 0, white represent gray value is 255), so this iterative threshold method can automatic find the most suitable threshold. Optimal threshold value of each block image is determined as separate pixels of the small block image into foreground and background by an initialized switching function, carry out integral computing on them separately and averaging the two number to get a new threshold value, separate the image into foreground and background using the new value, then a new switching function is generated. Iteration repeated until switch function does not change any more, that means the irritation convergence in a stable value which will be optimal threshold of the small block image.

Mathematical expression of irritation threshold method is as follows:

$$T_{i+1}=\frac{1}{2}\left[\frac{\sum_{k=0}^{T_i} h_k \cdot k}{\sum_{k=0}^{T_i} h_k}+\frac{\sum_{K=T_i+1}^{L-1} h_k \cdot k}{\sum_{K=T_i+1}^{L-1} h_{k_i}}\right] \quad (1)$$

Where: L is the number of gray level, h_k is the number of pixel with gray value of k, T_{i+1} is optimal threshold value of the small block image.

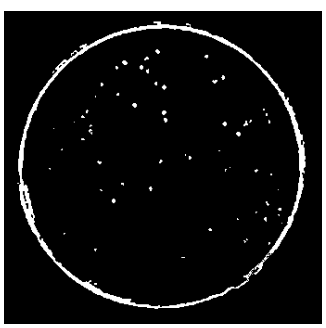

Fig.4 Binary image

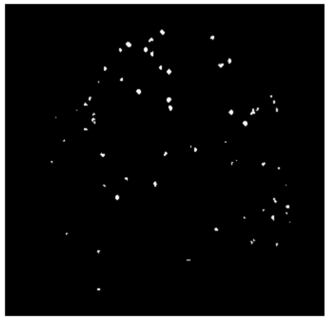

Fig.5 Image removed Petri dish edge

3.3 Remove Petri Dish Edge

From binarized colony image as is shown in Fig.4, we can see that there is still obvious Petri dish edge besides colony image which will affect later counting approach. Edge image of Petri dish is always connected region with the most number of pixels, so scan the image to find the largest region and turn it into black (set gray value to 0), result is shown in Fig.5.

4. COLONY COUNTING ALGORITHM

The task to count the number of colony is to count the number of connected regions in binary image. Traditional connected region labeling algorithm is simple, its principle is: find a white point, scan its neighborhood from bottom to top, from right to left to get connected region and label it, so time complexity of the algorithm is O(n^2)(n is size of the image), when n is big, calculation speed was very slow. This paper presented a new labeling counting method, its time complexity is only O (n) (n is size of the image), thereby improved the calculate speed on the basis of accuracy. Details of the algorithm is as follow: Scan the image from left to right, from up to bottom, when come across a white pixel A, use A as a seed point, set its value with 1, find other white pixels that are eight neighborhood linked with it, set the found pixel value with 1, continue the search process with these seed point until there is no unlabeled white pixels. Now, a connected region labeling has finished. Find next unlabeled white pixel as new seed point, set its label value with 2, repeat above process until the whole image has been scanned, the largest region label is the number of colony.

5. CONCLUSION

The system used VC++ to program. Total counting time is less than 1 second; average relative error is 2.5%. Experiment shows that above image processing method is feasible for white colony counting, it does not affect by colony shape, size and inoculation method, but sensitive to illumination condition and background color. So, a closed light source system will be a good choice which may avoid the impact of changes in natural light. Besides, using black background will get a more accurate counting result.

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