A Colony Picking Control System Based on Machine Vision

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Abstract. With rapid growth of the number of genomic sequences, researchers are faced with the task of managing huge clone libraries. A single experiment may involve classifying tens of thousands of bacterial, yeast or phage colonies. While, repetitive manual operation is followed by a lower efficiency and accuracy of colony picking and inoculating, leading to the fail in high throughput. In order to improve the efficiency and accuracy of colony picking process, this paper introduces a prototype, which picks colonies automatically. After analysis of colony picking process, we develop a colony picking control system based on industrial computer and CCD camera. Beyond that, we put forward the design of hardware and software of system. Finally, picking and inoculating colonies, as well as cleaning and heating needles come true.

Introduction

Traditionally, colony picking and inoculating is accomplished by hand [1]. While, repetitive operation, followed by a lower efficiency and accuracy of colony picking and inoculating, will lead to mistakes of colony picking, which has an impact on downstream experiments. There is no doubt that manual picking cannot meet the demand of high throughput in biological experiments, due to its inefficiency and low accuracy. Therefore, efficient colony picking machine comes into being.

Research abroad in colony picking system began from 1990s. Many scientific research institutions have developed machines, which pick colonies automatically [2-5]. Foreign market is occupied by companies, such as Molecular Devices [6] and KBiosystems [7], whose colony picking machine picks colonies at the throughput of 3000 col/hr. However, the domestic research on colony picking system began in 2000, but it has not yet been commercialized. Because of the high price in purchasing foreign machine and long maintenance cycle, it is necessary to develop a colony picking machine independently. Bacteria colonies are randomly spaced throughout the plate and each colony has a different size, shape and morphology. Hence, the robot cannot just follow a preprogrammed set of movements [8], leading to a demand of vision system. Recently, vision system is divided by working platform into two categories, PC-based machine vision system and embedded vision system. PC-based machine vision system consists of five parts, illuminating system, camera, image acquisition card, industrial computer and control mechanism. Image acquisition card is not used in vision system with one camera gradually, as interface technology developing, and inserted into computer directly to make digital image signal transmission through simple high-speed interface. With the enhancement of microprocessor capability, manufacturers embedded image acquisition cards and computer functions into cameras, forming embedded systems represented by intelligent cameras. Embedded visual systems have the advantages of simple use and small size, but are relatively weak in processing capacity and functionality, leading to a limitation in application, such as...
multiplexer processing applications with the same objectives and requirements for use of software tools with large computing volume. At this time, PC-based machine vision system can better meet the technical requirements. With the increasing speed of PC processors, PC-based machine vision system has strengthened its flexibility and scalability in visual applications. Moreover, the standardization of interface also enhances the usability of PC-based system and reduces the cost. As a result, PC-based machine vision systems continue to occupy an important position in high-end applications. The processing ability of embedded vision system is too weak to process the image of colony, so PC-based vision system is adopted.

This paper presents a colony picking prototype developed based on industrial computer and motion control card, which picks and inoculates colonies in 96-well plate automatically. In order to clear away residual colonies on needles after picking colonies, the function of cleaning and heating needles is added to prototype. To achieve functions stated above, machine vision is used to recognize and locate colonies, and open-loop control strategy is adopted for the control system.

**Introduction of Colony Picking Control System**

**System Composition**

Colony picking control system based on machine vision and colony picking prototype is showed in Fig. 1. Colony picking control system includes CCD camera, backlight, industrial computer, motion control card, turntable, mobile platform and picking needles. The whole control system has two levels, upper and lower computer. The core of upper computer is industrial computer. System acquires images by CCD camera, and recognizes desired colonies by image processing software. After that, position coordinates gained are transferred to lower computer. The core of lower computer is motion control card. Through controlling two mobile plates, turntable and picking needles, picking and inoculating colonies, as well as cleaning and heating needles are realized.

![Figure 1. Colony picking control system (a) CAD model (b) Prototype.](image)

**Characterization of Colonies to be Selected**

1) Colonies are randomly distributed on the surface of solid culture dishes, in which the thickness of each culture dish is consistent.

2) Colonies to be selected are bacteria colonies, with a diameter of about 1-2 mm, gathered by bacteria. As shown in Fig. 2.

![Figure 2. The Petri dish and 96-well plate (a) Petri dish, (b) 96-well plate, (c) Colony image.](image)
Colony Picking Process

As shown in Fig. 3, the X and Y mobile platform drives the Petri dish to the bottom of the picking position, and 10 picking needles are installed on the turntable. The picking needles installed cooperate with the X and Y mobile platform to realize colony picking. After rotating the turntable, the selected colonies are transported to the inoculating position. Another mobile platform, Z and A, cooperates with picking needles to inoculate colonies into 96-well plate. After inoculation, the turntable turns to the cleaning and heating position in turn. Cleaning position includes two cleaning tanks filled with distilled water and 70% alcohol respectively to achieve the cleaning of picking needles. The heating position is equipped with a heater, where heating temperature can reach 400°C to remove all bacteria remaining on the needle.

Picking Inoculation
Washing1 Heating
Washing2

Figure 3. A diagram of the picking process.

In the whole process, the turntable rotation time is set to 0.5 s, the intermittent stop time is set to 1s, and picking speed can reach 2400 col/hr. The cleaning step is an actual speed limit in the whole process. If the cleaning effect is guaranteed, the intermittent stop time can be reduced by increasing cleaning times and reducing the single cleaning time, then the picking speed could continue to improve.

Colony Recognition

Image Acquisition

Image acquisition has two steps, illumination and imaging. Illumination system is a relatively key part of machine vision system. Good lighting structure could enhance contrast between colonies to recognize and background. In addition, it could also improve image resolution, which will simplify image processing software and increase system efficiency. Via comparison of imaging effect under three conditions, without light, red backlight and blue backlight, showed in Fig. 4, we draw a conclusion that blue backlight provides a higher contrast compared to two other conditions. Therefore, blue backlight is adopted.

Figure 4. Comparison of imaging effect under different illumination
(a) Without light (b) Red backlight (c) Blue backlight.

Imaging process is completed by lens and camera working together. The higher image resolution is, the more features within a unit pixel could be provided, which results in convenience of image
processing and analysis. This paper adopts German Imaging Source DMK 23G445 camera, which has 1.2 million pixel of image resolution to single out colonies with a diameter of 1-2mm. The CCD camera and the illuminator used in system are shown in Fig. 5. Specific parameters of the hardware are exhibited in Table 1.

![Figure 5. Pictures of the CCD camera and the illuminator used, (a) CCD camera attached to system, (b) Illuminator.](image)

| Device       | Item                      | Specifications  |
|--------------|---------------------------|-----------------|
| Camera       | Power                     | 12VDC,400mA     |
|              | Type                      | monochrome      |
|              | Effective pixel           | 1280 × 960      |
| Camera Lens  | Focal length[mm]          | 16              |
|              | Max aperture ratio        | 1:1.4           |
|              | Iris range                | 1.4-16          |
| Illuminator  | Power                     | 24V             |
|              | Size[mm]                  | 158 × 106       |

**Image Processing**

Image processing software is developed on basis of HexSight toolkit copyrighted by LMI Technologies. HexSight uses the principle of geometric contour positioning. The linear repetition accuracy is 1/40 pixels and the rotational repetition accuracy is 0.01 degrees. HexSight is a comprehensive library of machine vision tools, available as ActiveX controls, C++ DLL libraries, or a .NET DLL assembly, which is used to construct a complete high-performance 2D machine vision system to save the development time of the whole system greatly. In this paper, we use HS AcquisitionDevice and HS Locator function modules in HexSight. HS AcquisitionDevice module is used to import the original colony image into the image database and use it as the input image of the next module. HS Locator module is used to establish the feature template and determine whether the image inputted by HS AcquisitionDevice contains the desired features. Finally, the central coordinate information of colonies desired is outputted. The program flow is shown in Fig. 6.
In order to provide the position of colonies in the world coordinate system for the lower computer and complete the colony picking function of the system, we should calibrate the camera, that is, coordinate conversion, after obtaining the position of colonies in the image coordinate system by HexSight. Because of the manufacturing and assembly errors of the system, it is necessary to compensate the mechanical errors of the actual coordinates transformed after calibration. Eq. 1 represents the relationship between actual coordinates and image coordinates. The parameter matrix $M$ can be obtained by camera calibration, and the mechanical error compensation matrix $H$ can be determined by precision measurement.

$$E = MF + H$$  \hspace{1cm} (1)

In Eq. 1, $E$ denotes the world coordinate matrix, $F$ denotes the image coordinate matrix, $M$ denotes the parameter matrix of the system, and $H$ denotes the mechanical error compensation matrix of the system.

**Control System**

**Hardware Design of Control System**

Control system adopts the open motion control method of "industrial computer + motion control card + CCD camera". The core part of control system is the motion control card, which realizes functions including driving the motor, receiving the input signal and sending the output signal. By sending pulse signals, the card drives the motor to move and controls its speed, acceleration and run time. The output signal includes the on-off of magnetic valve and frequency converter, as well as the start-up and shut-down of the illumination. Picking needles and turntable are controlled respectively through the output signal stated above. The industrial computer is responsible for analyzing, processing and recognizing the images collected from the CCD camera, setting the operation parameters of the motor, and coordinating the work of the hardware components. The whole control system realizes the communication between motion control card and industrial computer through PCI bus. Then, the motion control card executes the instructions issued by industrial computer to complete the control of the executing mechanism. The control system structure is shown in Fig. 7.
In this paper, GTS-800-PV(G)-PCI motion control card copyrighted by Googol Technology is used to realize high-speed point motion control, whose core is composed of DSP and FPGA, which can realize high performance control calculation.

**Software Design of Control System**

The main task of the software part of the control system is to realize the program control of each hardware part on the VC platform, and to realize the integration of each hardware part. The industrial computer provides a good interface for management, operation and observation. According to the actual needs of the system, programming on the industrial computer can change the running state of the system in real time. The workflow of the control system is shown in Fig. 8.

According to the workflow of colony picking control system, the application program is developed by using C++ language and Visual Studio 2008 software development platform. The application
interface is shown in Fig. 9. The program is divided into two operation modes, manual operation and automatic operation. In manual mode, it debugs single axis motion, general output, illumination and CCD camera, as well as sets features of colonies recognized by HexSight. Motion status, information prompt and recognition results are common areas in two modes, which are used to display motion status and position of each axis during operation, information feedback when there are running errors, template matched, as well as the number and specific position coordinates of desired colonies.

Figure 9. Application interface.

**Conclusion**

This paper presents a colony picking control system based on machine vision. After recognizing and locating colonies, system achieves picking, inoculating, cleaning and heating simultaneously. The prototype can be used in biological laboratory automation system to pick and inoculate bacterial colonies quickly.

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