JARQ 30, 213-220 (1996)

Robotization of Orchid Protocorm Transplanting in Tissue Culture

Tsuguo OKAMOTO

Department of Agricultural Engineering, The University of Tokyo
(Bunkyo, Tokyo, 113 Japan)

Abstract

Nowadays, orchid plants are propagated by mericlone culture. When the seed propagation method of this culture is used, a protocorm transplant is necessary during the growth stage. This study on robotization of the transplant for orchid protocorms was conducted to prevent biological contamination and to reduce labor costs. The image processing algorithm developed in the study was useful for recognizing protocorms about 0.5 mm in diameter, interspersed on the culture medium. A small, light gripper driven by a shape memory alloy actuator was constructed and was able to handle a very small object. The intelligent robotic system with machine vision and micro-handling mechanism that was developed could perform protocorm transfers automatically, with a higher accuracy and efficiency than humans.

Discipline: Agricultural machinery/Biotechnology
Additional key words: automation, end-effector, machine vision, micropropagation

Introduction

Mass production of orchid plants is commonly achieved by tissue culture. However, to promote the germination and growth of orchid seeds, the seed propagation method has become popular. The seed of the orchid plant is exalbuminous and the germination percentage is very low. The plant also grows very slowly. Using the seed propagation method, an orchid seed planted in agar growth medium generates a protocorm. If this protocorm is transferred to a fresh culture medium at the proper time during its growth stage, the germination percentage increases, and growth is improved.

The seed propagation technique is one of the sterile culture methods. About 1 to 3 weeks after planting an orchid seed, a slender ovule, about 0.5 to 1.0 mm in length appears and enlarges. The ovule tears open its seed coat and swells with a sphere at its center. The protocorm then becomes light green and, when transplanted to fresh growth medium, it germinates and takes root. A rooted plant that has grown to 2 to 5 cm, should be transferred to a pot. When the protocorm is cultured in a liquid rotary system it grows into an aggregation of protocorms. If the aggregation becomes too large, it should be divided into several pieces and propagated again in the liquid rotary system.

The size of the orchid protocorm is very small, about 0.5 to 1.0 mm in diameter. For the transfer, a technician must pick it up from the exhausted culture medium and gently place it into the fresh culture medium in another container. These operations which must be delicately and subtly handled under sterile conditions to avoid biological contamination are difficult and tedious even for a trained worker. Most of the biological contamination is caused by various microorganisms transmitted by workers' hands. The best way to prevent contamination is to remove the main contamination source, i.e. manual operation. The authors earlier developed a robotic transfer system[1-5] for plant tissues such as callus at the subculture stage. This system which was found to be effective did not enable to handle an object as small as an orchid protocorm. In this study, an intelligent robotic system was developed for efficiently transferring orchid protocorms, preventing biological contamination, and reducing labor costs. The robot employed an image processing and micro-handling technique to transfer minute and delicate objects such as protocorms.

Materials and methods

Protocorms used in the study were those of *Bletilla striata* Reichenbach Fil., with a standard size (Plate 1). Since the process of growth is also about the same, the robot system that was developed would be used to transfer most orchid plants. Fig. 1 shows an outline of the system. The robot consisted of a manipulator control, an end-effector control, an image processor and a computer control.

1) Manipulator

The manipulator used, a SCARA type robot (SONY SRX-4CH), is depicted in Plate 2. The length of the first arm was 350 mm and of the second arm 250 mm. The synthetic maximum velocity of the 2 arms was 5.2 $m \cdot s^{-1}$. Maximum velocity of the Z axis was 300 $mm \cdot s^{-1}$, and maximum rotating speed of the R axis was 1080° s^{-1}. The positional accuracy of the X, Y and Z axes was ± 0.05 mm. The rotational accuracy of the R axis was $\pm 0.05°$.

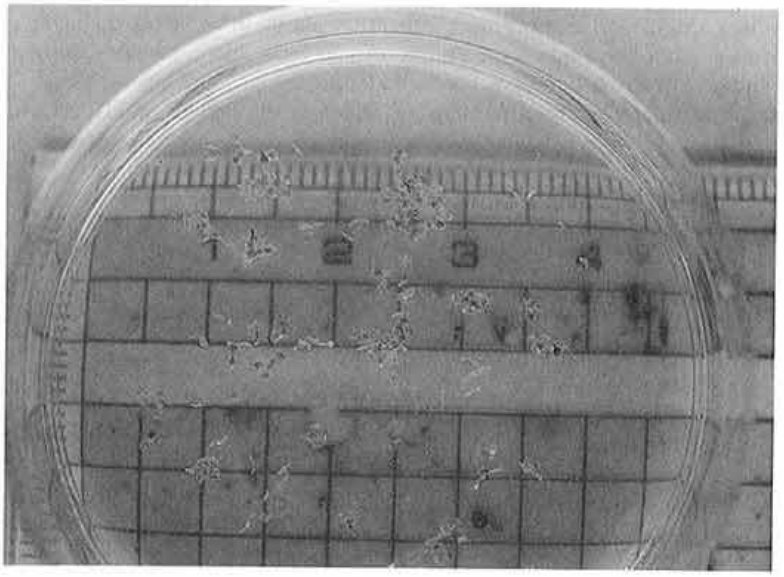

Plate 1. Protocorms of *Bletilla striata* Reichenbach Fil.

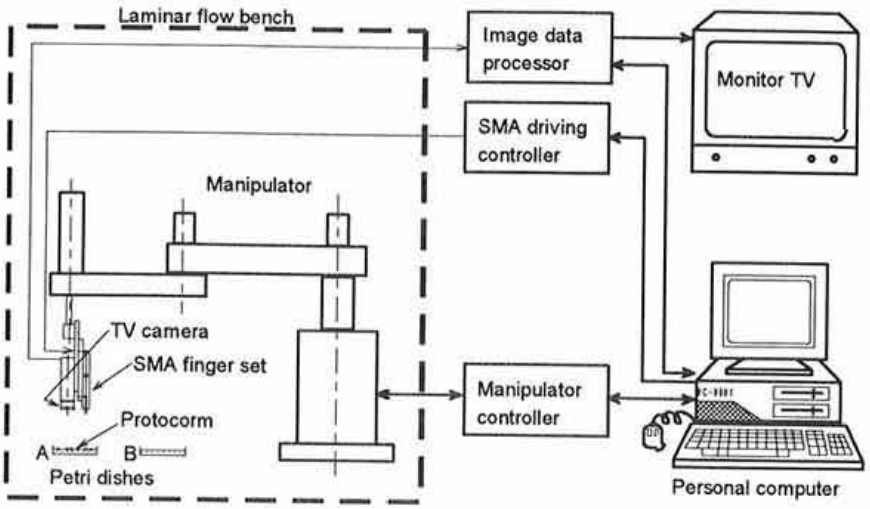

Fig. 1. Schematic diagram of a robotic system for transplanting of orchid protocorms in seed propagation

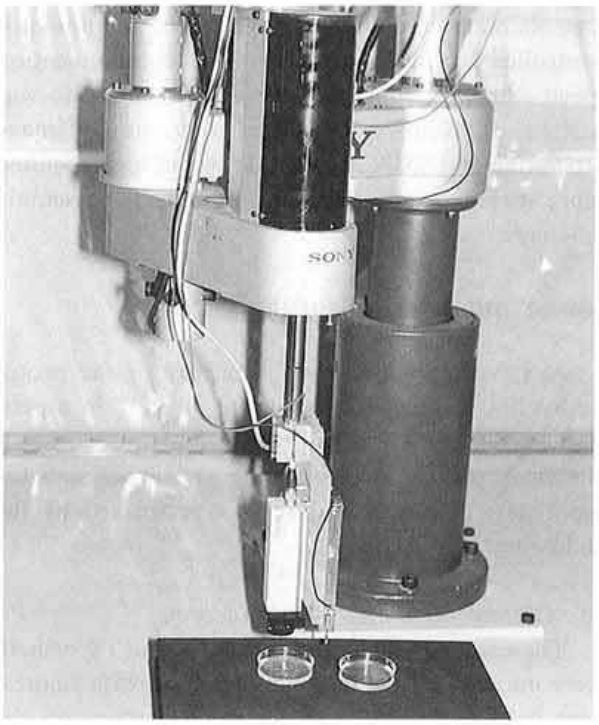

Plate 2. Robotic transplanting of orchid protocorms in mericlone culture

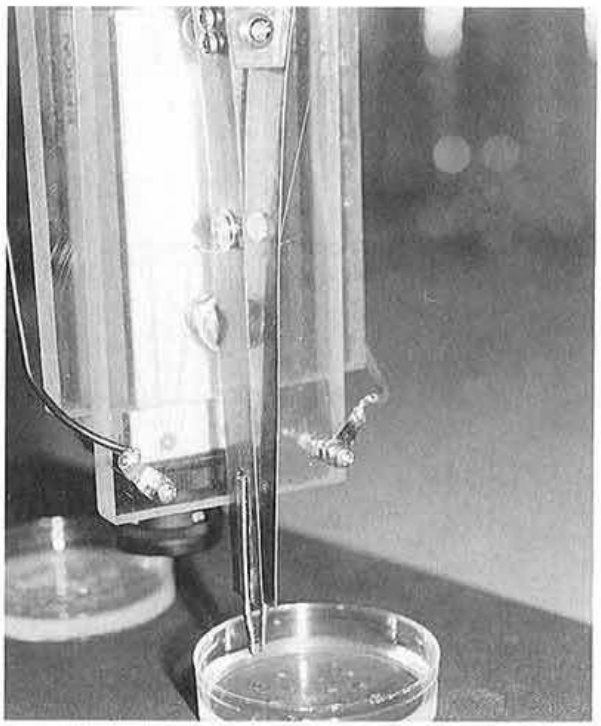

Plate 3. Gripper for protocorm transplanting

2) Machine vision system

A monochromatic TV camera was used for imaging. With its image sensor of the metal oxide semiconductor type, it took 2-dimensional image data of the protocorms dotted on the agar growth medium. The composite video signal output from the camera was changed from an analog signal to a digital signal by a 4-bit A-D converter. The image data of a field were stored in the field memory. Video image data in a single field consisted of 64,000 sampling points in an area of 320 × 200 pixels. The data stored in the field memory were loaded onto the memory of the computer in response to an order. It took about 0.3 s to transfer 32 Kbyte of data. The TV camera was installed with its back toward a gripper in the *R* axis of the manipulator as shown in Fig. 1. The camera enabled to observe a given work area. A magnifying ring for the camera was attached to the lens system. To obtain the resolution necessary to recognize a protocorm, the focal length of the TV camera was adjusted to 15 mm (length) and 24 mm (width) on the surface of the agar culture medium. As a result, the size of a pixel in the picture was equivalent to 75 × 75 μ^2 on the work surface.

3) Soft-handling gripper

Various grippers have been studied for soft handling of plant tissues[1-6], microcutting[6] and plantlets[7]. Here, a gripper was developed to handle a very small object. The structure of the gripper that has a pair of finger-like tweezers is shown in Plate 3. It is operated by a shape memory alloy (SMA) actuator. A SMA wire, 0.15 mm in diameter and 270 mm in length, is stretched tightly so that it passes the A, C, E, F, D and B points shown in Fig. 2. An electric current applied between terminal A and terminal B heats the SMA wire. As the temperature of the SMA rises, the length of the wire decreases. The result is that point C and point D are pulled apart, causing the fingers to open. The gripper is normally in a closed position.

The transformation temperature of SMA is controlled by heating with an input electric current using the pulse width modulation (PWM) method and by cooling in the atmosphere. The pulse frequency of the current is 5 kHz and the height of the current pulse, supplied by a constant current regulator, is 400 mA. The SMA material used was a Ni-Ti alloy that had a resistivity of about 42 $\Omega \cdot m^{-1}$. The phase transformation temperatures of SMA, *As*, *Af*,

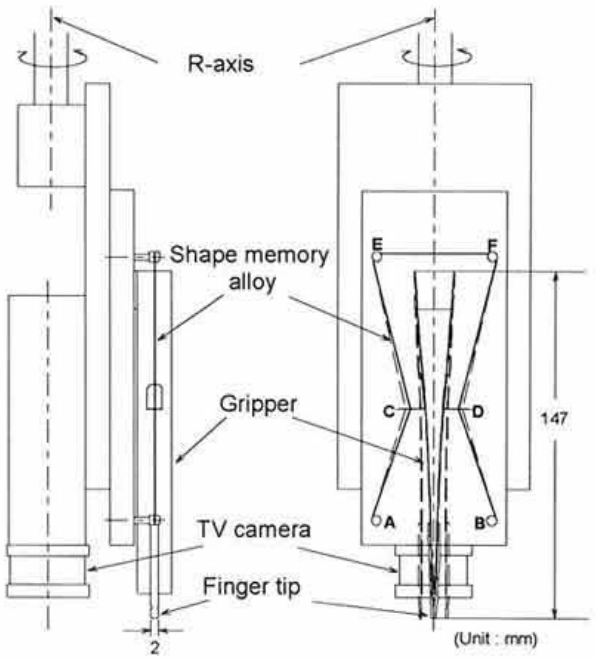

Fig. 2. Finger pair driven by shape memory alloy actuator for handling protocorms

Ms and *Mf* were 90, 120, 90 and 70°C, respectively. The shape memory recovery ratio of the alloy is generally equal to or less than 3% of the full length. To magnify the displacement of the fingers, the SMA wire was tightened by winding as shown in Fig. 2.

The SMA actuator, without mechanical parts, allows the gripper mechanism to be smaller and lighter than in other actuators, and the movement is smooth and quiet. The gripper fingers were made of a phosphorus bronze plate, 0.4 mm thick. A fingertip made of stainless steel 2 mm wide and 0.5 mm thick was attached to each finger. Only after the gripping motion had begun, was the normally closed pair of fingers opened by the SMA actuator. To grip an object, the applied current to the SMA was shut off and the pair of fingers closed by the bending force of the finger plates. The force required to grip a protocorm was set at about 0.1 N. The bending force of the finger plate acts as a bias force that extends the SMA material to its original length when not active. SMA under the shape memory non-recovery condition cannot generate power. That is, the SMA can generate a shrinking force itself just by opening the fingers.

4) Computer

The computer (NEC PC-98RL) used here had a 32-bit CPU with a clock speed of 20 MHz. The manipulator was controlled through a serial interface RS232C cable that connected the manipulator controller to the computer. The communication speed was 9,600 bps, and the control program was written by N88BASIC. The programs of image processing and SMA driving that would have required more time to process were written in assembly language.

Image processing algorithm

A TV camera first took a picture of the protocorms dotted on the agar culture medium in a petri dish. The procedure of recognizing protocorms is shown in the flow chart in Fig. 3. Processing the main part of the procedure was performed by the following algorithm.

1) Getting an image of protocorms

The contrast and the brightness of the TV camera were initially adjusted. By illumination with fluorescent light from above, specular reflectance on the agar growth medium was minimized. Protocorms pictured were growing on semitransparent agar growth medium and their color, when ready for transfer, was light green. A petri dish with protocorms was put on top of a black sponge. The background intensity was then lowered so that the contrast in the image obtained was sufficient for the computer to

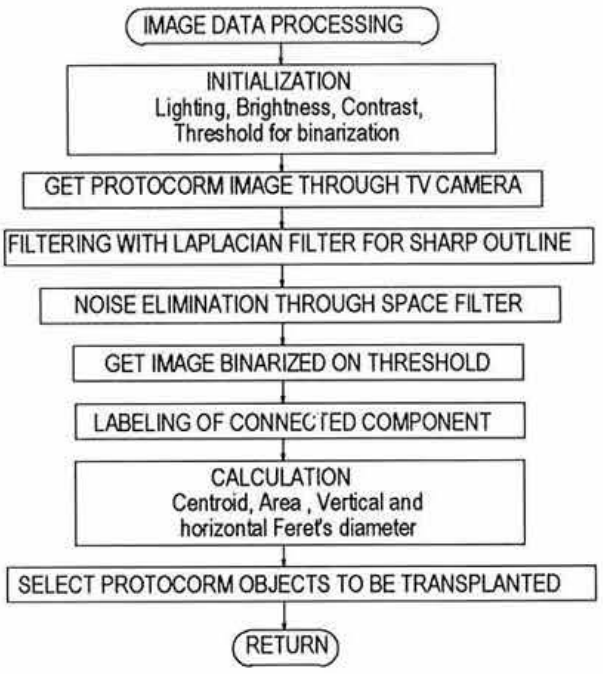

Fig. 3. Image processing procedure

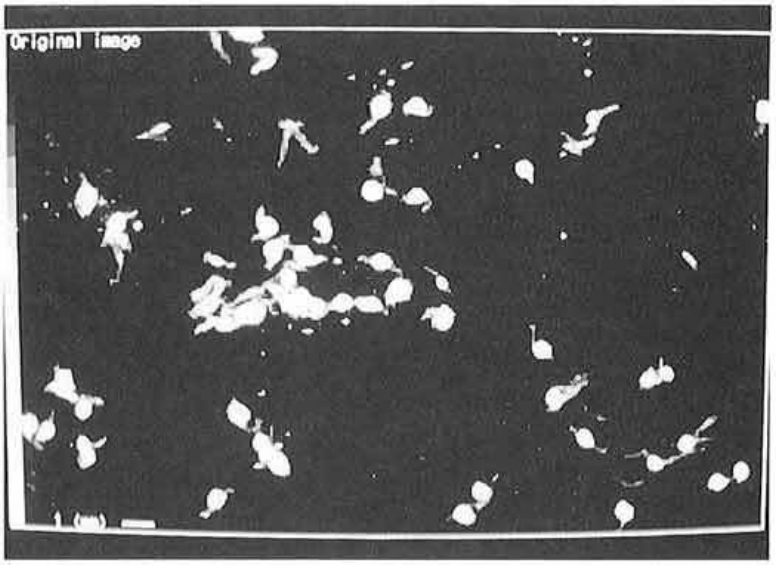

Plate 4. Print of an original picture image of orchid protocorms

recognize individual protocorms.

Input image data were sent to the computer memory. The data were displayed as a monochrome picture with a gray scale of 16 intensity levels. It was also possible to display a 16-pseudo-color image. Plate 4 is an example of an original image. A pixel with an intensity of 0 displayed a black dot while a pixel with an intensity over 1 displayed a white dot. Some protocorms were in close contact and overlapped seed coats. The picture also shows noise due to specular reflection on the agar growth medium.

2) Image filters

Various spatial filters were used to enhance the image and extract characteristics efficiently. Picture noise caused by irregular reflections on the medium surface occurred in the original image. As the image of the seed coat surrounded the globular protocorm image, the edge of the latter was indistinct. To remove noise and make the picture edge clear, a low pass filter and a Laplacian filter were used.

To make the edge clear, the result of the Laplacian filtering process was deducted from the original image data. The intensity inclination in the edge then became steep. This computational process is described by the equation,

$$\begin{aligned} Y_{i,j} &= X_{i,j} - (X_{i-1,j} + X_{i+1,j} + X_{i,j-1} \\ &\quad + X_{i,j+1} - 4X_{i,j}) \\ &= 5X_{i,j} - X_{i-1,j} - X_{i+1,j} - X_{i,j-1} - X_{i,j+1} \end{aligned}$$

where $X_{i,j}$ is the original intensity of pixel (i, j), and $Y_{i,j}$ is the value of pixel (i, j) after the filtering process. The expression in parentheses refers to the Laplacian process.

The low pass filter for smoothing operates the processing shown in the next equation.

$$Y_{i,j} = \frac{1}{12}\left[\sum_{m=i-1}^{m=i+1}\sum_{n=j-1}^{n=j+1} X_{m,n} + 3X_{i,j}\right]$$

3) Binarization

It was necessary to identify individual protocorms that were close together. To locate the centroid of a protocorm, the image data were analyzed. Before binarization, processing of the original image was performed using a Laplacian filter to clarify it. After noise was removed by the low pass filter,

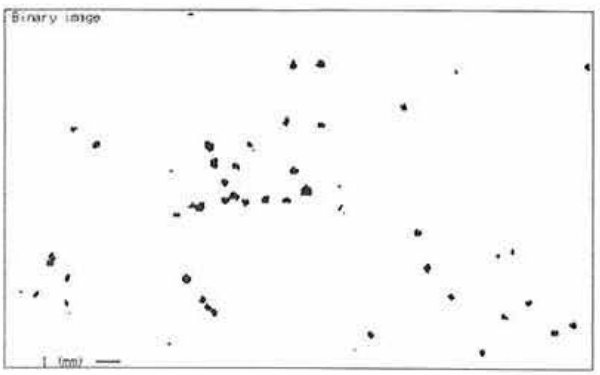

Fig. 4. Binary image of figured components based on the image in Fig. 5

a binary image was obtained using a global threshold that was experimentally determined.

Since the shape of a protocorm is globular, the intensity of the top part is greater than that of other parts. The center of a protocorm could thus be identified by discriminating the high intensity part. Fig. 4 is a binary image based on the original image in Plate 4.

4) Labeling

In a binary image, the value of each pixel is 0 or 1. An area where every pixel has the same value and connects is designated as a 'connected component'. There were many connected components segmented in the central part of a protocorm that had a value of 1 in a binary image. To recognize each individual component, a unique label must be given to each. A labeling process assigned different names to them. Various algorithms can be used for the labeling. In this study, pixel data were observed from the top left to the bottom right on the image. Four connected pixels of a pixel were examined and a label was given to the pixels in the areas with a value of 1. After all the pixels were examined, the identity of the label was determined. The same labels were given to every pixel of the connected component.

5) Feature calculation

Centroid, bounding rectangle and area were calculated as the features of the connected components. The detected centroid positions are indicated by the + marks in Fig. 5. To calculate these parameters most efficiently, a rectangle that bounded the connected component was drawn. Only the small area inside the rectangle was used when the features were calculated.

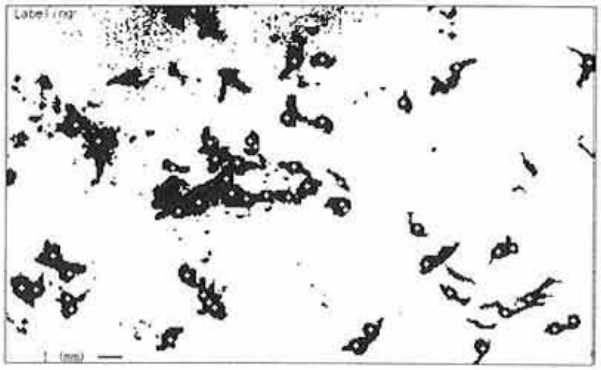

Fig. 5. Detection of centers of protocorm image with labeling

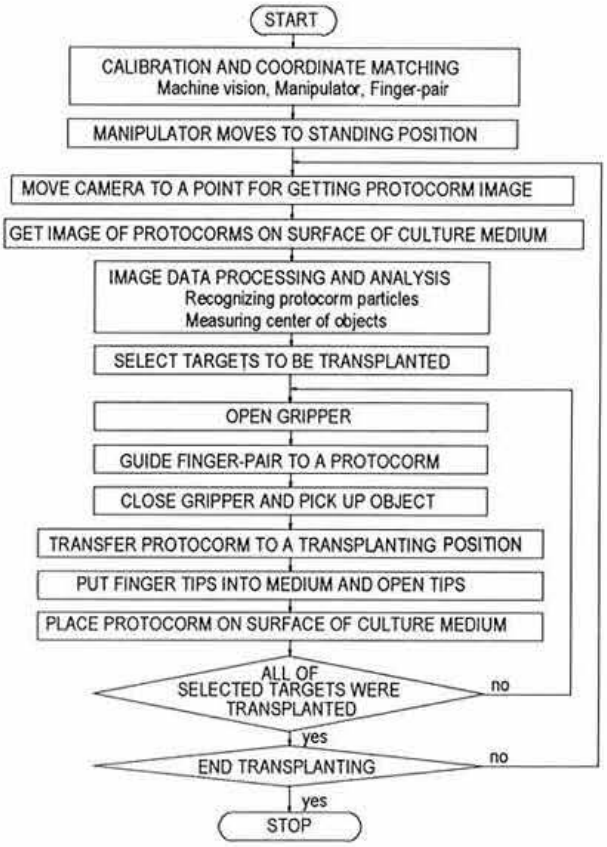

Fig. 6. Flow chart of robotic transplanting of orchid protocorms

Robotization of protocorm transplant

1) Transplant procedure

A protocorm should be transferred to fresh agar growth medium within 2 to 3 weeks after the seed is planted. The robot picked up protocorms from exhausted culture medium, transferred them individually to a petri dish containing a fresh culture medium, and placed them in a pin grid array at regular intervals of 7 mm. The robotic transfer procedure is shown in Fig. 6.

2) Coordinate matching between image and manipulator

First, the manipulator moved to the machine origin of the manipulator base coordinate system and then moved to the task origin. This position was also the original position of the manipulator. The origin of the image coordinate system was in the center of the image field. The optical axis of the TV camera passed through this point. The optical axis of the camera lens and the center line of the

gripper were parallel to the rotational axis, R, but there was a space between them. Therefore, the gripper and image coordinate systems were related to the task coordinate system geometrically.

3) Selection of object

After protocorms were recognized in the image, their spatial positions were determined. Since orchid plant seeds are too small and light to be planted at regular intervals on the surface of agar growth medium, the generated protocorms were planted close together randomly and even formed a cluster in places. As a result, it was difficult to pick up just 1 protocorm with the gripper. The robot sometimes gripped a couple of protocorms. Even a trained worker may occasionally transfer more than 1 protocorm at a time.

A work area for the gripper had to be provided with enough space for the pair of fingers to operate. The area selected was rectangular as shown in Fig. 7. Even if more than 1 protocorm was present in that area, the protocorm in the center marked by a large +, as shown in Fig. 4, was selected as the only object to be transferred. Starting from the top left and moving to the bottom right of an image, work areas were identified. An object in the center of one area was present only in that particular area, and not in any other area.

Fig. 8 shows the motion of the pair of fingers transferring a protocorm. First, a shape memory alloy actuator made the fingers open only by 1 mm by applying an electric current. The open fingers were led to a position right over the protocorm and lowered by the manipulator as shown in Fig. 8(a). The fingers were dipped into the agar growth medium to a depth of 1 mm. Next, the electric current running through the shape memory alloy element was shut off so that it was free from the shape memory state. Then, the spring force of the finger plates caused the pair of fingers to close around the protocorm. Since the protocorms must be gently and carefully handled, the gripping force of the fingers was adjusted to about 0.1 N so that it would not damage the protocorms.

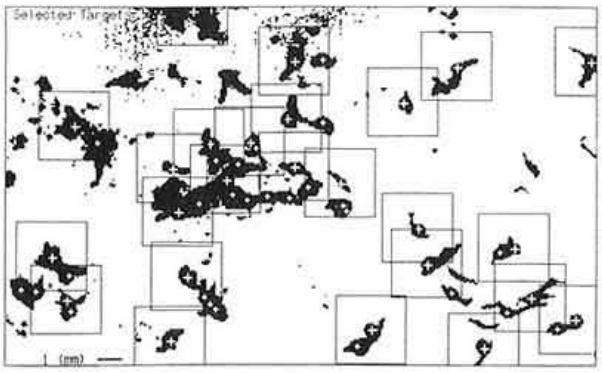

Fig. 7. Selected objects to be transferred

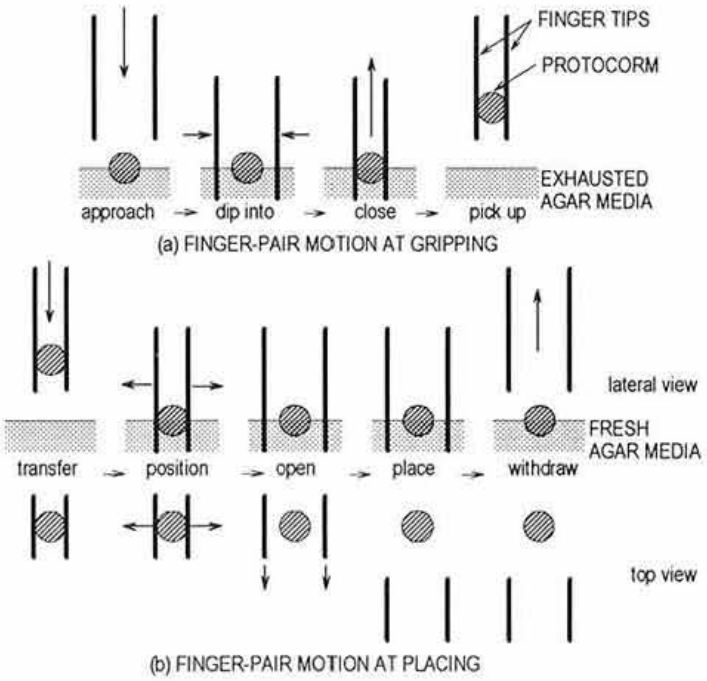

Fig. 8. Finger pair operation for transplanting of orchid protocorms

The gripper was then led to a pre-programmed position on the petri dish holding the fresh culture medium. The fingers immersed the protocorm into the medium to a depth of 1 mm, then opened (2 mm wide) as shown in Fig. 8(b). To ensure that the object was released into the culture medium, the manipulator shifted the fingers 2 mm to the right of the area where the protocorm was released.

4) Performance

Although the time of one image processing depended on the number of protocorms transferred, it took less than 4 s from obtaining the image to determining the centroid of objects. The selected protocorm was then transferred to a culture medium in a pin grid array at 7 mm intervals. About 8 s were required for each transfer. After all selected protocorms in the image frame had been transferred, the TV camera then gave on image of an adjacent area of the culture surface of the petri dish and the transfer procedure was repeated.

In contrast, it takes 5 to 10 s for a trained worker to transfer a protocorm. In a manual operation, much time is required for preparation because of the need to sterilize and disinfect instruments. Also, it is difficult for a worker to place a protocorm on the medium at regular intervals by hand and to continue such a tedious task for many hours. The robotic system developed in this study was more efficient than a technician performing the work manually.

Conclusion

The transfer of orchid protocorms is still performed manually by technicians skilled in biological routines, and is very costly. Most of the biological contamination is caused by various microorganisms transmitted by workers' hands. The best way to prevent contamination is to remove the main contamination source, manual operation. The current study on the robotic transfer of these orchid protocorms was performed to prevent biological contamination and to reduce labor cost.

The image processing algorithm developed in this study was useful in recognizing protocorms about 0.5 mm in diameter. A small, light gripper driven by a shape memory alloy actuator was constructed and enabled to handle a very small object successfully. An intelligent robotic system with machine vision and micro-handling mechanism was developed. The robot picked up protocorms from exhausted culture medium, transferred them individually to a petri dish containing fresh medium, and placed them in a pin grid array at regular intervals of 7 mm. It took about 8 s to transfer each protocorm. The efficiency was higher than in the case of manual operation.

References

1) Okamoto, T. & Kitani, O. (1989): Studies on robotics for biotechnological operations. I. On callus handling robot. *J. Jpn. Soc. Agric. Mach.*, **51**(5), 37–45 [In Japanese with English summary].
2) Okamoto, T. & Kitani, O. (1990): Studies on robotics for biotechnological operations. II. Intelligent robot for transplanting of calluses in subculture. *J. Jpn. Soc. Agric. Mach.*, **52**(5), 79–85 [In Japanese with English summary].
3) Okamoto, T., Kitani, O. & Torii, T. (1991): Studies on robotics for biotechnological operations. III. Fuzzy control of robot finger set driven by shape memory alloy actuators. *J. Jpn. Soc. Agric. Mach.*, **53**(5), 85–91 [In Japanese with English summary].
4) Okamoto, T., Kitani, O. & Torii, T. (1992): Tissue proliferation robot in plant tissue culture. ASAE paper No. 923516.
5) Okamoto, T., Kitani, O. & Torii, T. (1993): Robotic transplanting of orchid protocorm in mericlone culture. ASAE paper No. 933090.
6) Simonton, W. (1991): Robotic end effector for handling greenhouse plant material. *Trans. ASAE*, **34**(6), 2615–2621.
7) Ting, K. C., Giacomelli, G. A., Shen, S. J. & Kabala, W. P. (1990): Robot workcell for transplanting of seedlings. II. End effector development. *Trans. ASAE*, **33**(3), 1013–1018.

(Received for publication, November 6, 1995)