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Micromanipulation using Stereoscopic Microscope

Tetsuya Sano
Okayama University

Hideki Yamamoto
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Tetsuya Sano Hideki Yamamoto
Okayama University
Faculty of Education
Tsushima Okayama, 700-8530, Japan
yamamoto@cc.okayama-u.ac.jp

Abstract— **In this paper, we developed a visual feedback system that controls a micromanipulator so that a needle head may reach a target. The system consists of a stereoscopic microscope, a micromanipulator, two CCD cameras and a personal computer. The positions of the target and the needle head under stereoscopic microscope are measured three dimensionally by using two CCD cameras which are set to eyepieces of the microscope. In order that the micromanipulation can be carried out at a realistic rate, it is necessary that the image processing is employed be fast. The image processing time for detecting the target and the needle head is greatly reduced by using image data compression.**

I. INTRODUCTION

A micromanipulation is widely used such as to operate genes and to inspect integration circuits by using a stereoscopic microscope. As such works creates heavy burdens to operators, it is desirable to perform the micromanipulation automatically. In this paper, we propose a visual feedback system for micromanipulation with a stereoscopic microscope to make a needle head attached to the micromanipulator reach a target. It is highly demanded for micromanipulation at a realistic rate that the image processing is employed be fast. The image processing time for detecting the target and the needle head is greatly reduced by using image data compression.

II. METHODS

Fig. 1 shows the visual feedback system. The system is composed of a stereoscopic microscope, two CCD cameras, a micromanipulator and a personal computer. The cameras are mounted to the eyepieces of the microscope. A target is set on the stage of the microscope. Fig. 2 shows the right image. The size of the image is 640pixels × 480pixels. At the beginning of micromanipulation, the needle head is guide to reach an end of the target and then it proceeds to the centroid of the target.

The target and the needle head are detected by their color and shape informations. The shape informations are the size and the normalized area. The normalized area K is given by

$$K = \frac{L^2}{S} \quad (1)$$

where, L is the length of the outline and S is the size of the target. The normalized area of the target is usually lager than the normalized area of the needle head. The needle head is detected by the algorithm as shown in Fig. 3. After thresholding, the center line of the needle head is obtained by thinning as shown in Fig. 3 a). The point **P** of the needle head is an end of the needle head. The point **P** is searched on the center line. Fig. 3 b) shows the differential values on the center line. At the point where the differential value becomes maximum, a boundary can be fixed between the needle head and the back ground. The point is detected as the needle head **P**.

By the stereovision method, the distance between the needle head and the object lens of the microscope is estimated. Fig. 4 shows the principle of the stereovision method. The point x_l and the point x_r are the needle head **P** in the left and the right images respectively.

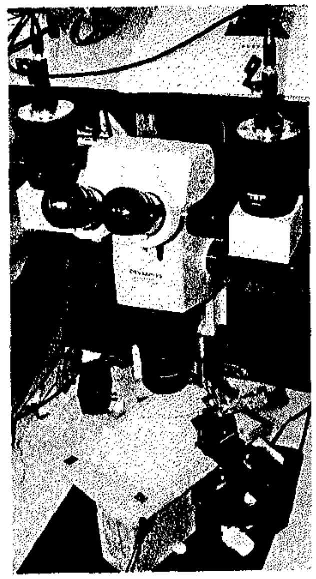


Fig. 1. Visual feedback system

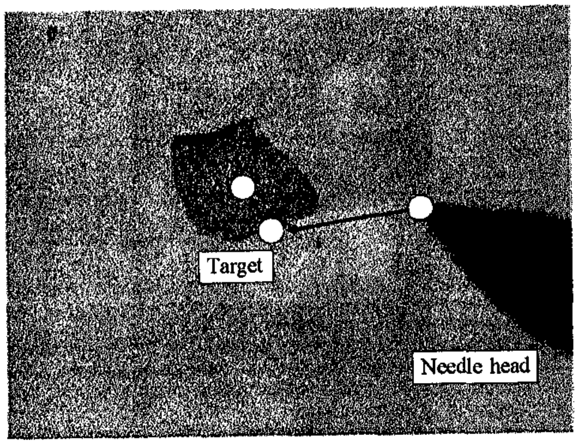
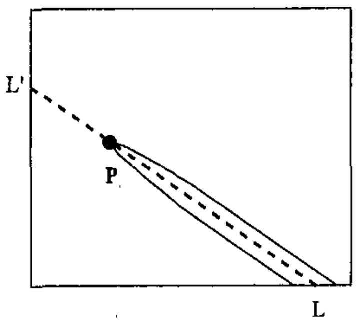
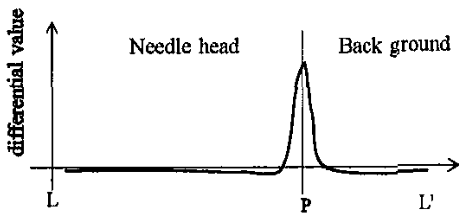


Fig. 2. The needle head and the target in the image



a) The needle head and its center line



b) The differential values on the center line

Fig. 3. Algorithm for detecting the needle head

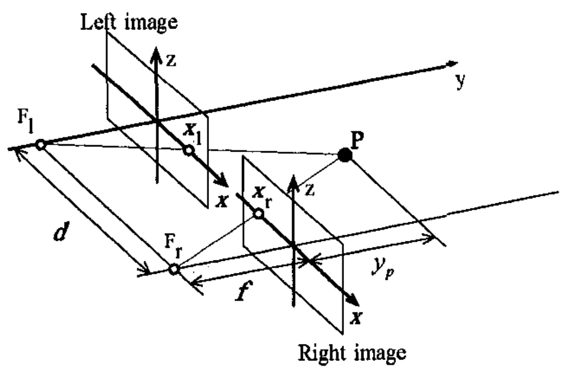


Fig. 4. Principle of stereovision method

There is a position difference between the point x_l and the point x_r. The position difference is inversely propotional to the distance between the needle head and the object lens. The distance y_p is derived from the position difference $(x_l - x_r)$ as follows.

$$y_p = \frac{f \cdot d}{x_l - x_r} \tag{2}$$

where the f is the focal distance of the cameras and the d is the distance between the cameras. If the needle head is closest to the target, the distance y_p is estimated as follows.

$$y_p = A \times (x_l - x_r) + B \tag{3}$$

where B is the distance between the stage and the object lens. The constant A is measured from the movement of the needle head near the target before micromanipulation.

Because the next position of the needle head can be predicted, the region of interest (ROI) in the image is set as shown in Fig. 5. The point $\mathbf{P}_1$ is predicted from the movement lengths of the needle head Δx and Δz and the point of the needle head before moving $\mathbf{P}_0$. The 128pixels $\times$ 128pixels of ROI is set around the point $\mathbf{P}_1$. Furthermore, by the image data compression, the region of interest is reduced into 16pixels $\times$ 16pixels. By using needle head position in the compressed image, the region of interest in the image can be reduced into 32pixels $\times$ 32pixels as shown in Fig. 6.

The needle head begins to move from 1.0mm over the stage. The needle head paths through the point **a** and the point **b**, and reaches to the centroid of the target **c** as shown in Fig. 7. The point **b** is a end of the target. The point **a** is over the point **b** in 1.0mm. The sizes of image are as shown in TABLE I. At the beginning of the micromanipulation, the needle head moves toward the point **a** to avoid the needle head be in collision with

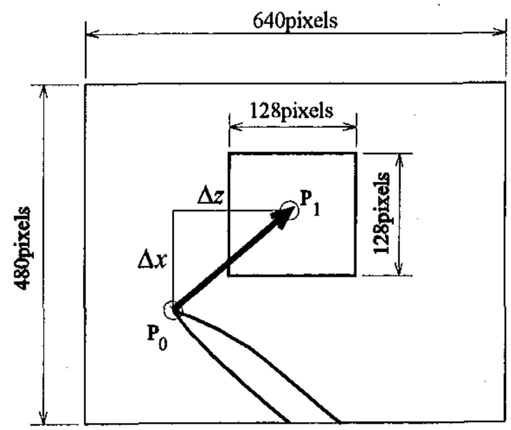


Fig. 5. Region of interest(ROI)

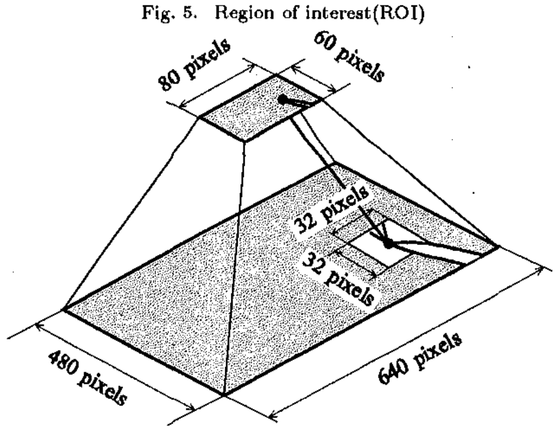


Fig. 6. Reduced ROI by image data compression

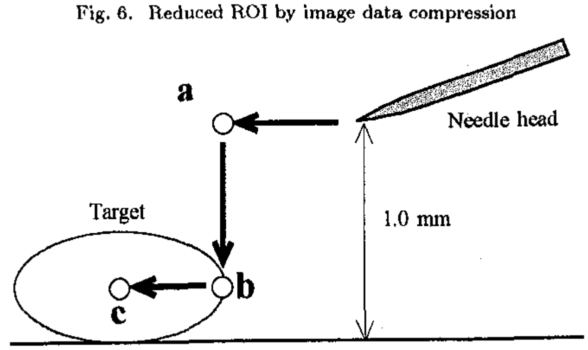


Fig. 7. Path of the needle head

TABLE I

THE SIZE OF THE IMAGES

	Input images	Size of images (pixel)
i)	right	80 × 60
ii)	left and right	640 × 480
iii)	right	640 × 480

obstacles. Because the needle head moves horizontally, the distance between the needle head and the target is measured by the right image. And the needle head should be approaching to the target fast, the size of image is reduced to 80pixels × 60pixels. After the needle head approaches to the point **a**, the needle head is descending to the point **b**. Finally, the needle head proceeds to the centroid of the target horizontally.

III. RESULTS

The magnification of the microscope is set at 20 times. The size of visual field is 3.0mm × 2.3mm. A measurement accuracy is derived as shown in TABLE II. We choose a plastic particle as a target as shown in Fig. 8. The target is 0.8mm long, 0.6mm wide, and 0.4mm tall. It is considered that the needle head reaches the centroid area which is shown in the slant line area in the figure. The movement speed of the manipulator is as shown in TABLE III. The needle head is located by control the time for micromanipulating. As the unit of time is 1 second, the accuracy of locating the needle head is enough accurate to make the needle head reach the target. Fig. 9 shows the needle head under control. The region of interest is set to include the needle head. Fig. 10 shows the histograms of the region of interest. As the needle head is dark in the image, the pixels that the intensities are under 160 for each colors are detected as the needle head. The needle head is detected as shown in Fig. 11. The needle head is shown as the intersection of the cross hairs.

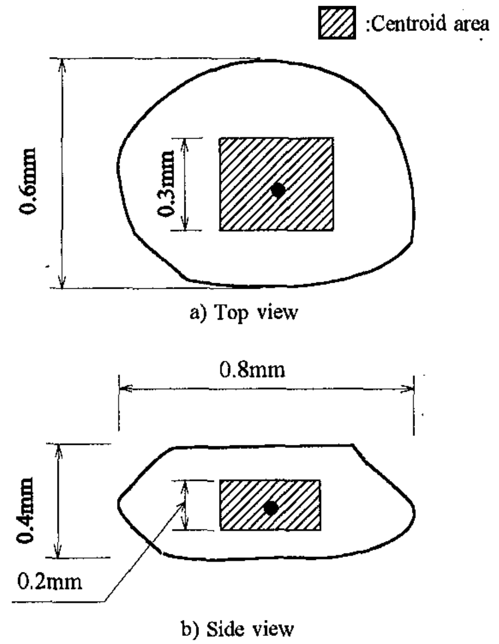


Fig. 8. Target

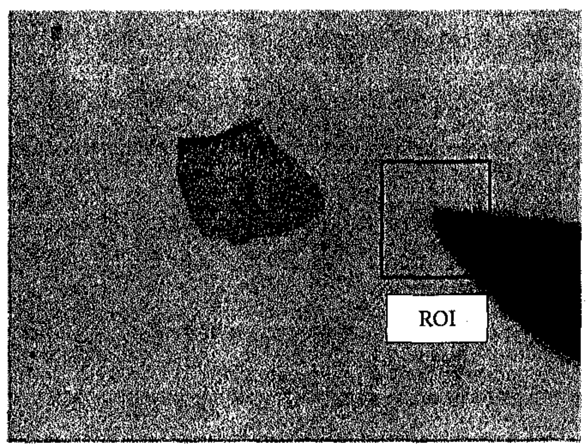


Fig. 9. Right image

TABLE II

MEASUREMENT ACCURACY

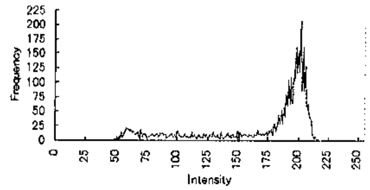
X direction	4.2×10^{-3} (mm/pixel)
Y direction	16.7×10^{-3} (mm/pixel)
Z direction	4.1×10^{-3} (mm/pixel)

TABLE III

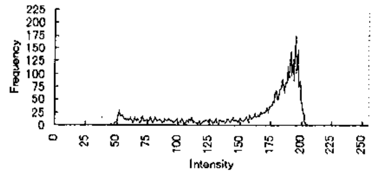
THE MOVEMENT SPEED OF THE MANIPULATOR

	+direction	−direction
X	3.783×10^{-2} mm/s	4.167×10^{-2} mm/s
Y	3.967×10^{-2} mm/s	3.500×10^{-2} mm/s
Z	4.267×10^{-2} mm/s	4.817×10^{-2} mm/s

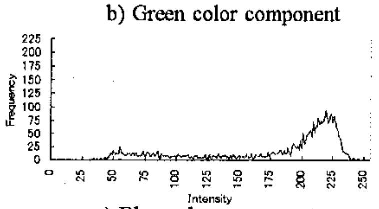
The micromanipulator is controlled automatically so that the needle head should reach the centroid of the target as shown in Fig. 12. In this case, the number of image processing is 17. The first 6 times of image processing is for approaching to **a**, the second 7 times of image processing is for descending to **b** and the third 4 times of image processing is for proceeding to **c** in details.Fig. 13 shows the comparison of the required time for image processing at approaching, descending and proceeding. The 1.6 times to 6.2 times speed-up is done. The visual feedback system with image data compression is 2.1 times faster than the system without image data compression as shown in Fig. 14.



a) Red color component



b) Green color component



c) Blue color component

Fig. 10. Histograms of ROI

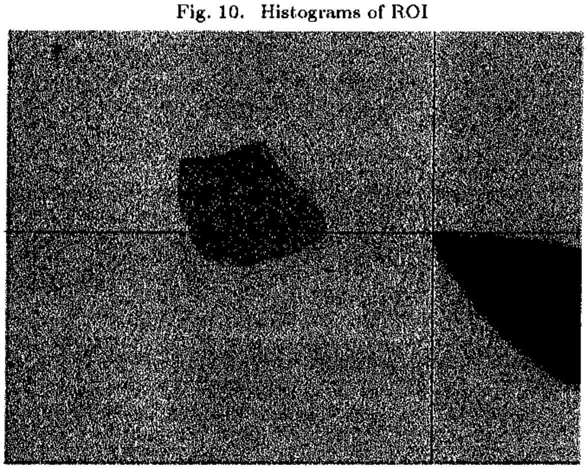


Fig. 11. Detected needle head

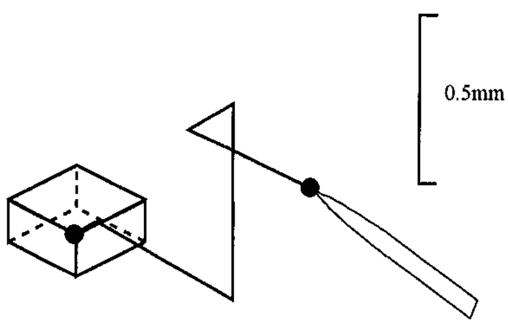


Fig. 12. Path of the needle head under control

IV. CONCLUSIONS

In this paper, we propose a visual feedback system for micromanipulation with a stereoscopic microscope to make a needle head reach a target. The required time for micromanipulation is reduced in 1/2.1 with image data compression. This proposed visual feedback

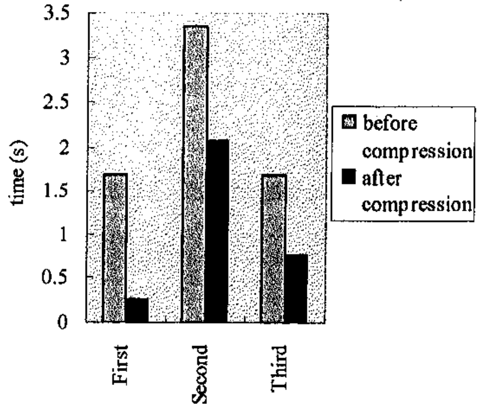


Fig. 13. Required times for image processing

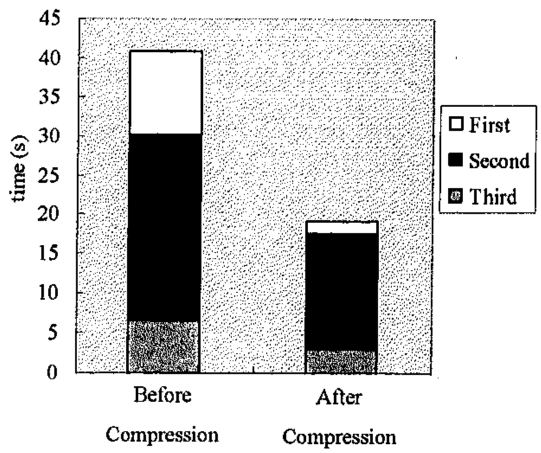


Fig. 14. Comparison of the time for image processing

system may be useful in the micromanipulation such as microinjection to the cells.

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