Automatic Micromanipulation System using Stereoscopic Microscope

Tetsuya Sano
Okayama University

Hidekazu Nagahata
Okayama University

Hideki Yamamoto
Okayama University

Automatic Micromanipulation System using Stereoscopic Microscope

Tetsuya Sano
Okayama University,

Hidekazu Nagahata
Okayama University,

Hideki Yamamoto
Okayama University,

Automatic Micromanipulation System using Stereoscopic Microscope

Tetsuya Sano, Hidekazu Nagahata and Hideki Yamamoto
Faculty of Education Okayama University, Tsushima Okayama, 700-8530, Japan
ged97701@edrsews1.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 position 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. It is necessary the image processing employed be fast so that the micromanipulation can be carried out at a realistic rate. The image processing time for detecting the target and the needle head is greatly reduced by using image compression.

1. 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. While a measurement accuracy of the visual feedback system is improved by using a microscope of high magnification, it takes a great deal of time for image processing. A region of interest for detecting the needle head in images is narrowed by using image compression so that the micromanipulation can be carried out at a realistic rate.

2. Methods

Figure 1 shows the visual feedback system. The system consists of a stereoscopic microscope, two CCD cameras, a micromanipulator and a personal computer. The cameras are mounted to the microscope. The position of the needle head and the target are measured three dimensionally by using the cameras. The target and the needle head in the left and right images have characteristics in their color and shape information. The color information is derived as intensities of red, green and blue color components. The shape information is derived as the size and the normalized area. The normalized area K is given by

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

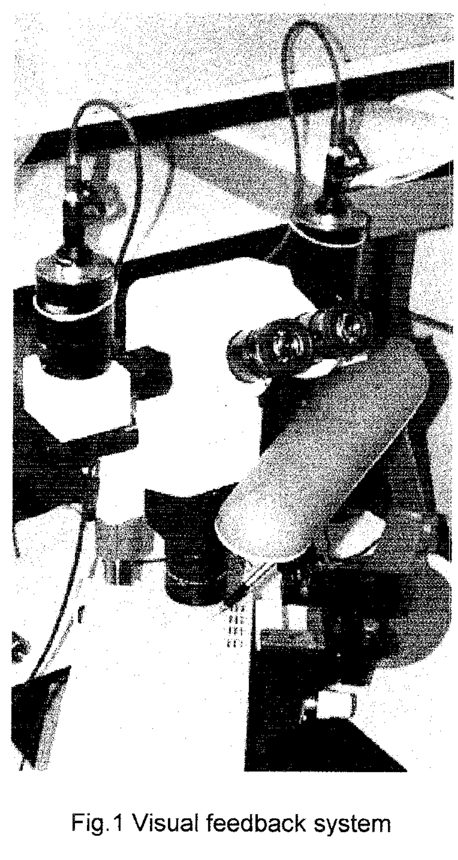
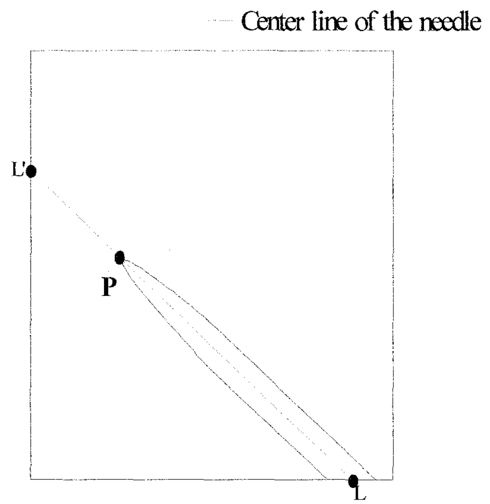
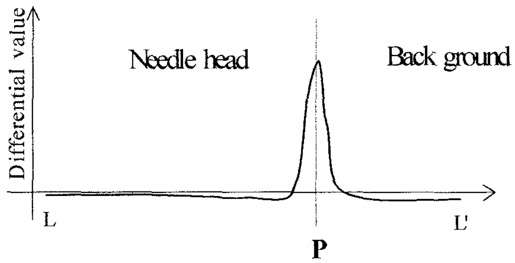


Fig.1 Visual feedback system



a) Detected needle head and its center line



b) Differential value on the center line

Fig.2 Detection of the needle head

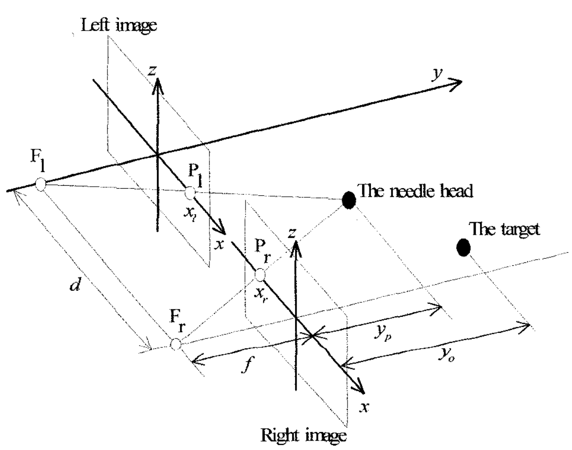


Fig. 3 Principle of stereovision method

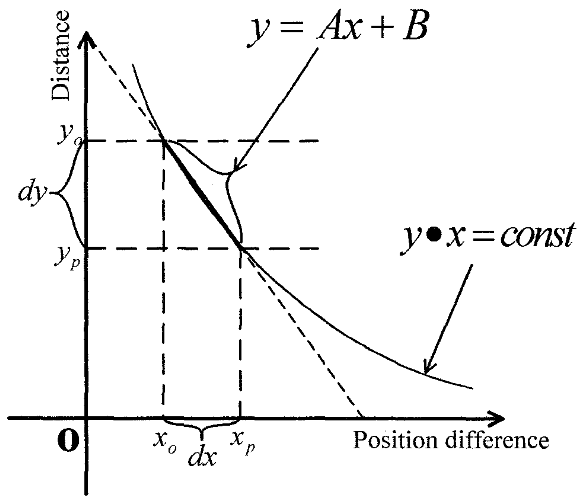


Fig. 4 Approximation by the linear expression of the distance between the target and the needle head

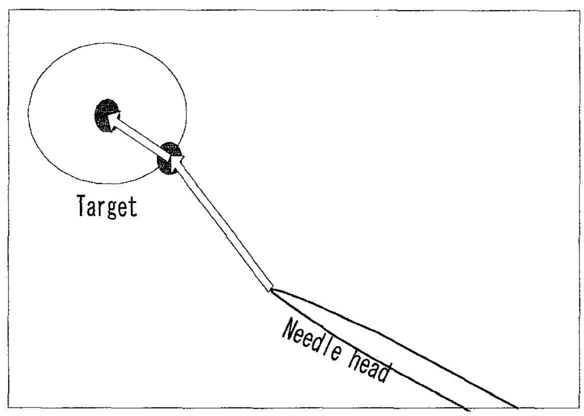


Fig.5 The path of the needle

where, L is the length of the outline and S is the size of the object. The normalized area of thin shape object is larger than the normalized area of round shape object. The needle head and the target in the images are detected by color information, and are identified by their shape information. The center of the target and the tip of the needle head in the images are detected respectively.

Binarization with a fixed threshold has an advantage at its speed, but it has difficulty to detect the tip of needle head if the brightness of the images changes. By using region segmentation, the region of the needle head is extracted.

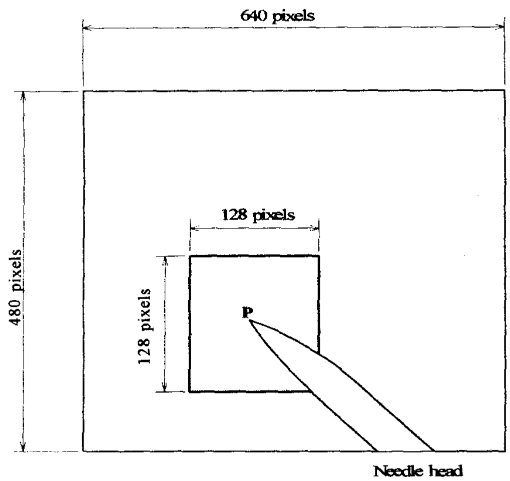


Fig.6 The region of interest for the needle head

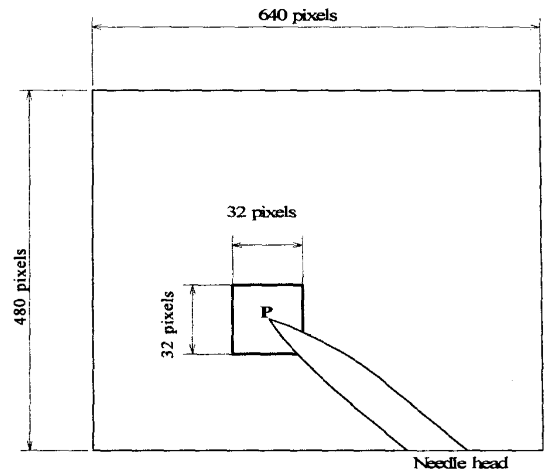


Fig.8 The reduced region of interest

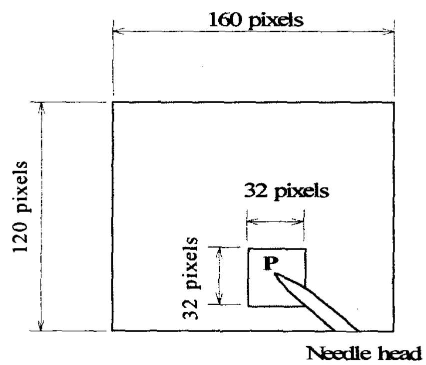


Fig.7 The compressed image

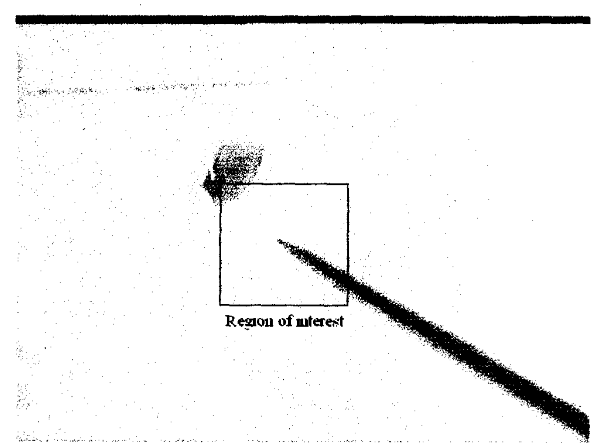


Fig.9 The needle head at control

Figure 2 shows the process of detecting the position of the needle head. After thinning, the center line of the detected needle head is obtained as shown in Fig.2 a). The point P of the needle head is a tip of the needle head. The point P is searched on this center line. Figure 2 b) shows the differential values on the center line. The maximum differential value on the center line is searched to detect the point P.

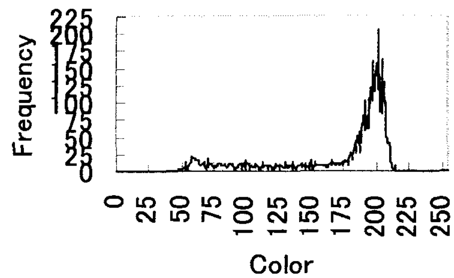
By using the stereovision method, the distance y_p between the needle head and the object lens of the microscope is given by the position difference $x_p(=x_l-x_r)$ of the needle head in the right and left images as shown in Fig.3. The distance $dy(=y_o-y_p)$ between the needle head and the target is inversely proportional to the position difference $dx(=x_o-x_p)$ in two images as shown in Fig.4. If the needle head is closest to the target, the distance dy can be approximated in the linear expression of the position difference dx.

To avoid accidents such as piecing the target, the needle head approaches to the target gradually. If the color of the needle head is similar to the target and the needle head is above the target, it is difficult to detect the needle head. To avoid such overlapping of the needle head and the target, we first make the needle head reach the end of the target and then make it proceed to the centroid position as shown in Fig. 5.

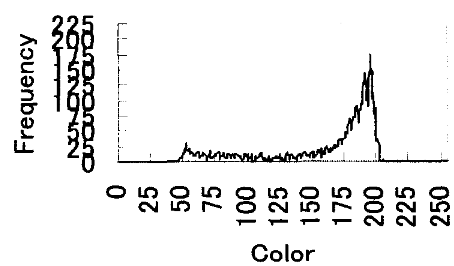
The speed of the needle head becomes slow as the needle approaching to the target. It takes a great deal of time for image processing time, and it occupies a great part of the

Table 1 Three cases of the visual feedback system experiments

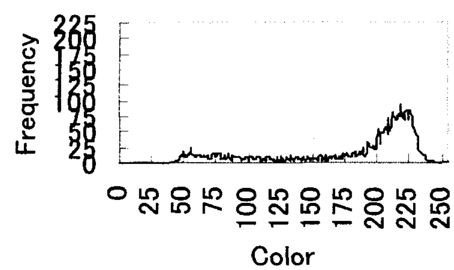
	case a)	case b)	case (c)
Magnification (power)	7	20	20
Size of ROI (pixel)	128×128	128×128	32×32



a) Red color component



b) Green color component



c) Blue color component

Fig.10 Histograms of the needle head

time for micromanipulation. During the micromanipulation, the visual feedback system traces the position of the needle

Table 2 Measurement accuracy of case a)

X direction	1.4×10^{-2} (mm/pixel)
Y direction	5.4×10^{-2} (mm/pixel)
Z direction	1.3×10^{-2} (mm/pixel)

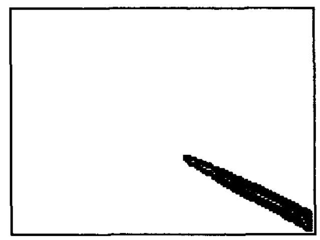


Fig. 11 The detected needle head

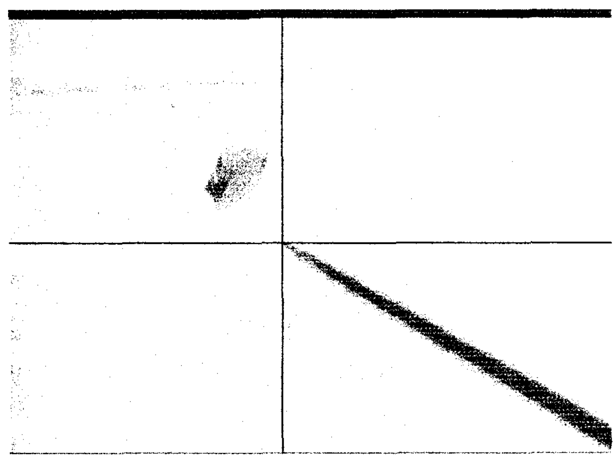


Fig.12 The position of the needle head

head. As the next position of the needle head can be predicted, the region of interest in images can be set to 128 pixels × 128 pixels as shown in Fig.6. The detection of the needle head is performed in a compressed image. Figure 7 shows the size of the compressed image and the region of interest. The region of interest is narrowed into 32 pixels×32 pixels. By using the position information of the needle head in the compressed image, the region of interest in the image is narrowed into 32 pixels × 32 pixels as shown in Fig.8. Thus, the point P is detected from the narrowed region of interest.

Table 3 Measurement accuracy of case b) and case c)

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 4 Comparison of the required time

	case a)	case b)	case c)
Total (s)	75.5s	180.3	106.5
measurements (times)	4	11	11
Image processing in total (s)	30.7s	130.2s	53.3s
Image processing (s)	7.7s	12.8s	4.8s

3. Results

We choose a plastic particle as a target. The particle is 1.2mm in length, 0.8mm in width, and 0.5mm in height. The micromanipulator is controlled automatically so that the needle head may reach the centroid of the target. Table 1 shows the three cases of the visual feedback system experiments. Case a) uses a 7-power microscope. The size of visual field is 8.5mm×6.5mm. A measurement accuracy is derived as shown in Table 2. It is considered that the needle head reaches the target when the needle head approaches to the target within ±0.027mm in the x and z direction, and within ±0.11mm in the y direction.

Figure 9 shows the needle head at control. The region of interest is set to include the needle head. Figure 10 shows the histograms of the region of interest. As the intensity of the needle head is lower than the intensity of the background, the threshold values for color components are determined. The needle head is detected as shown in Fig.11. The white dots in the center of the needle head are derived by thinning. The center line of the needle is estimated from the dots by using the least square method. Figure 12 shows the detected needle head. The needle head is shown as the intersection of the cross hairs.

Case b) and case c) use a 20-power microscope. A measurement accuracy is derived as shown in Table 2. It is considered that the needle head reaches the target when the needle head approaches to the target within ±0.0083mm in the x and z direction, and within ±0.033mm in the y direction. The measurement accuracy is improved in proportional to the magnification of the microscope. Table 4 shows the comparison of the required time for each cases. Case b) requires 2.4 times of time for image processing in comparison with case a). By image compression, the region of interest of case c) is reduced to 32pixel × 32pixel. As a result, the required time for image processing is reduced to 60 percent as much as the required time for image processing at case a).

4. Conclusion

In this paper, we propose a visual feedback system for micromanipulation with a stereoscopic microscope to make a needle head reach a target. The measurement accuracy of the visual feedback system can be improved in proportional to the magnification of the microscope. The required time for image processing is greatly reduced by image compression so that the micromanipulation can be carried out at a realistic rate.

This proposed visual feedback system may be useful in the micromanipulation such as microinjection to the cells.

References

[1] Huang T.S., "Image sequence analysis", Springer-Verlag, 1981.

[2] Zhang Z. and Faugeras O., "3D dynamic scene analysis", Springer-Verlag, 1992.

[3] Sankar K.P., Dwijesh K., and Dutta M., "Fuzzy Mathematical approach to pattern recognition", John Wiley & Sons, 1986.

[4] Liu J. and Yang Y., "Multiresolution color image segmentation", IEEE Transactions on Pattern Analysis and Machine Intelligence, Vol.16,No.7(1994),689-700.

[5] Castano A. and Hutchinson S., "Visual compliance:Task-directed visual servo control", IEEE Transactions on Robotics and Automation, Vol.10,No.3(1994),334-341.

[6] Volpe R. and Khosla P., "Analysis and experimental verification of a fourth order plant model for manipulator force control", IEEE Robotics & Automation Magazine(1994),4-13 Vol.10,No.3(1994),334-341.