Study of micromanipulation using stereoscopic microscope

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Abstract

In this paper, we describe a visual feedback system using a stereoscopic microscope that controls a micromanipulator so that a needle may pierce a target as much length as desired. At first, for achieving the manipulation at a realistic rate, we proposed a strategy of moving the needle head. Secondarily, we developed an algorithm for prediction of the tip position of the needle head within the target. Before the needle pierces the target, the shape of the needle head is preserved as a reference pattern. After the needle piercing the target, the shape of the needle head within the target is predicted using the reference pattern and the tip position of the needle head can be detected. Experimental results indicate that the proposed system may be useful in micromanipulation such as microinjection to seeds.

1. Introduction

A micromanipulation is widely used such as to operate on genes and to inspect integrated circuits using a stereoscopic microscope. In the area of agriculture, microinjection into seeds is done for the purpose of improvement of a species of vegetables. Usually, the microinjection is performed manually with a joystick. As such wok creates heavy burdens to operators, it is desirable to perform the microinjection automatically.

The automatic micromanipulation of the needle head is realized with well-known techniques of visual feedback. However, there are some difficulties in performing the microinjection with the visual feedback system. One is that the system requires many times of image measurement for micromanipulation. The other is that the tip position of the needle head disappears when the needle head pierces the target.

At first, we proposed a strategy of moving the needle head for achieving the manipulation at a realistic rate. Secondarily, we developed an algorithm for prediction of the tip position of the needle head within the target for control of the tip position of the needle head. Experimental results indicate usefulness of the proposed system.

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 microscope focuses on the target. The cameras are mounted to the eyepieces of the microscope, and they output the left and right images to the computer. The needle is set to the micromanipulator obliquely to the stage and the angle doesn't change while manipulation. The distance between the target and the needle head can be derived three dimensionally with the left and right images. The micromanipulator has three degree of freedom, and does linear motion. Each axis of motion is considered as X_m axis, Y_m axis and Z_m axis. The Y_m axis is vertical to the stage. The X_m axis and the Z_m axis are parallel to the stage. The X_m axis is equal to the needle head direction in the image. The micromanipulator is controlled so that the needle head may reach the target.

The target and the needle head are detected by their color and shape information. The shape information consists of the size and the normarized area. The normarized area is the square of the contour length over the size. As a first step of image processing, median filtering is performed for the reduction of noise. Pixels of

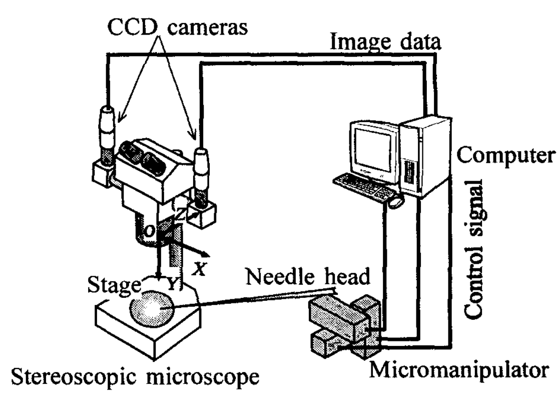


Fig. 1. Visual feedback system.

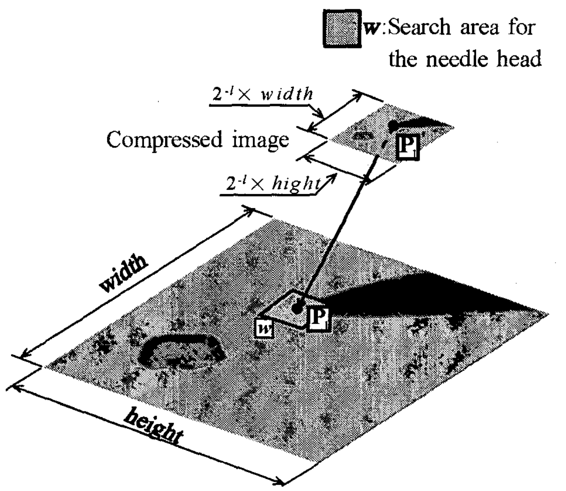


Fig. 2. **Image data compression.**

the target and pixels of the needle head are extracted by thresholding. Sizes and contour length of extracted pixels are estimated by labeling. The needle head is identified from its shape information. For reducing the image processing time, the image data is compressed as shown in figure 2. The original image data is divided into small regions. The intensities of the compressed image are medians, which are obtained from each small region. As the result, the image is compressed into 2^{-l}. The parameter l is decided due to the magnification of the microscope. After image data compression, the position of the needle head is detected in the compressed image data. The point $\mathbf{P}_1$ is predicted from the position of the needle head in the compressed image. A small search area is set around the point $\mathbf{P}_1$. The tip position of the needle head in the original image data is detected in a short time.

We use the (X, Y, Z) coordinate system. The Y axis is perpendicular to the stage of the microscope. The XZ plane is paralell to the stage. Figure 3 shows the XY plane of the visual feed back system. The left CCD is assumed to be the X_l coordinate system and the right CCD is assumed to be the X_r coordinate system respectively. The x_l and x_r represent the points which are the needle head position $\mathbf{P}$ to be projected to the left and right CCD. The distance y_p is estimated by the stereovision method as follows.

$$y_p = \frac{fd}{x_l - x_r} \tag{1}$$

where the f is the focal distance of the cameras and the d is the distance between the cameras. However, it is difficult to estimate the f and the d from the actual

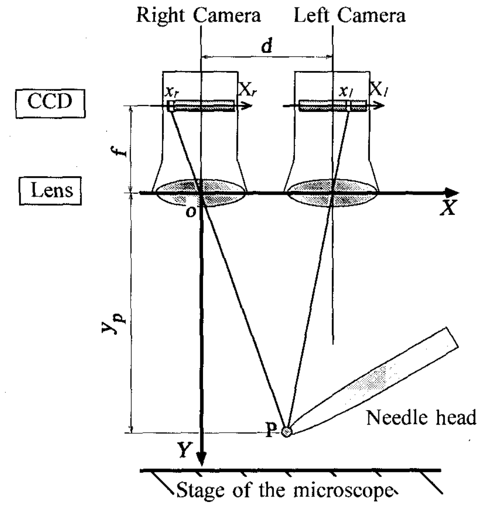
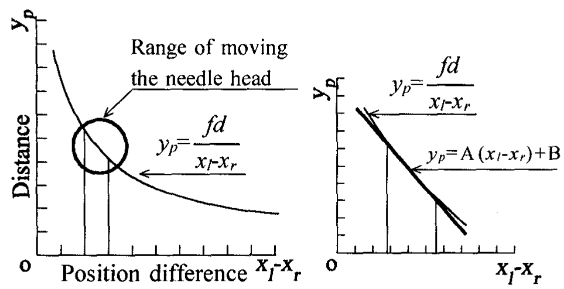


Fig. 3. **Principle of stereovision method.**



a) Relation between the distance and the positioin difference

b) Approximation in the range of moving the needle head

Fig. 4. **Approximation of the distance.**

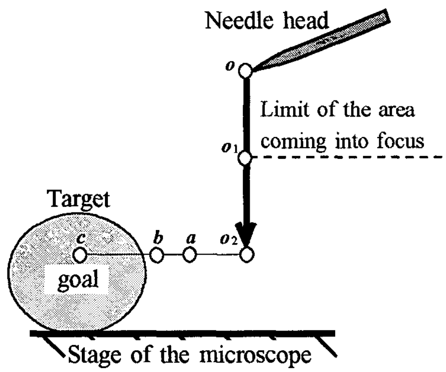
measurement. Although the $(x_l - x_r)$ is inversely proportional to the y_p as shown in figure 4 a), it is found that the distance y_p can approximately be estimated by a linear function (figure 4 b)). For simple processing, we estimate the distance as follows.

$$y_p = \frac{A}{m}(x_l - x_r) + B \tag{2}$$

where the m is the magnification of the microscope. The constant A and B are experimentally determined from the actual measurement of the position difference of the needle head, which is moving near the target.

2.1. Strategy of moving the needle head

We propose three ideas in the strategy of moving the needle head.



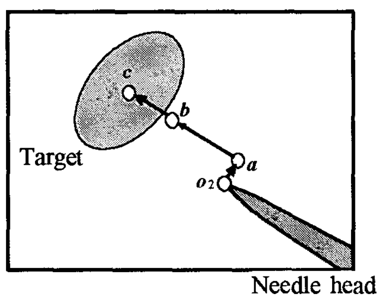


Fig. 5. Path of the needle head.

1) A path of the needle head
2) Estimation of moving length of the needle head.
3) Selection rule of a size of the image data

We set a path of the needle head as shown in figure 5. The point $\mathbf{o}$ is a position of the needle head at the beginning of manipulation. The point $\mathbf{o}_1$ indicates a limit of the area coming into focus. The limit of the area is experimentally estimated. Since the image of the microscope is shallow in focal depth, the needle head in the image is unclear at the beginning of the manipulation. The needle head should be guided to be in focus. The needle head descends vertically to the height of the goal(between $\mathbf{o}$ and $\mathbf{o}_2$). After that, the needle head moves horizontally(between $\mathbf{o}_2$ and $\mathbf{c}$). With this path, the most part of manipulation is performed with the needle head clear in the image.

During the needle head is unclear in the image, we should perform the manipulation carefully. For realization of it, we set a moving length of the needle head. After the needle head moving the length, the image measurement is performed. The moving length M is estimated as follows.

$$M = r \times D \tag{3}$$

where M is the moving length, D is the distance between the needle head and the destination and r is constant which value is less than 1.0. While the needle head is unclear in the image (between $\mathbf{o}$ and $\mathbf{o}_1$), the needle head should be guided slow in order to achieve fully control of the needle head position. When the needle head is in focus, the needle head is clear in the image. We set the r as 1.0 while the needle head is clear in the image(between the $\mathbf{o}_2$ and the $\mathbf{b}$).

It takes a lot of time for image processing in visual feedback. For reduction of the image processing time, we use two sizes of image data. One is full-size of CCD image data and the other is small one. When the needle head is between $\mathbf{o}$ and the $\mathbf{o}_1$, the small image data is used for the image measurement. The small image is used when the needle head is between the $\mathbf{o}_2$ and the $\mathbf{a}$ too. In each case, it is not so important to measure the distance at high resolution because the needle head is far from the stage or is far from the target. Furthermore, during the needle head moves parallel to the stage, the distance between the needle head and the target is measured two dimensionally with the right image. The needle head can approach the target in a short time.

2.2. Prediction algorithm of the shape of the needle head

Next, we describe about the prediction methos of the tip position of the needle head within the target.

As a first step of prediction, when the needle head reaches to the $\mathbf{b}$, a region of interest is set as shown in figure 6. The region is a square and the tip position of the needle head is on a diagonal line of the region. The shape of the needle head is preserved as a reference pattern. The preserved shape of the needle head is divided into two areas as shown in the figure. One is an invisible area and the other is visible area. The parameter R is a desired length of piercing the target. When the needle head reaches the goal, the shape of the needle head will be almost equal to the visible area. The visible area is used to detect the position of the reference pattern in the image. The invisible area is used to predict the tip position of the needle head.

Figure 7 shows the determination process of the tip position of the needle head. At first, the centerline of the needle head is estimated. Then, we set regions of interest $f_k(i,j)(k = 1, 2, \cdots, n)$ on the centerline. Each region's upper left corner is on the centerline. One of the regions indicates the position of the reference pattern. In order to estimate the similarity of the shape of the needle head between the regions and the reference pattern, the correlations r_k between $f_k(i,j)$ and $g(i,j)$

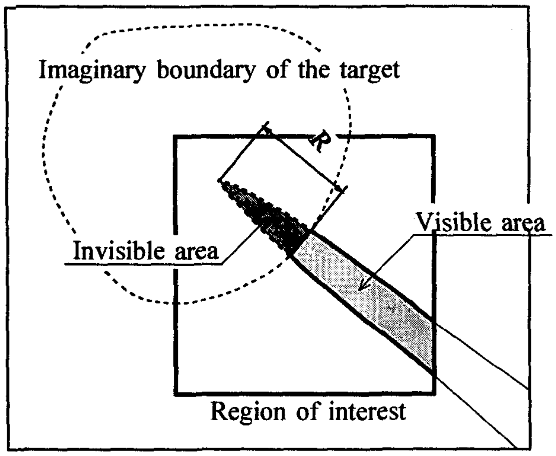


Fig. 6. The reference pattern.

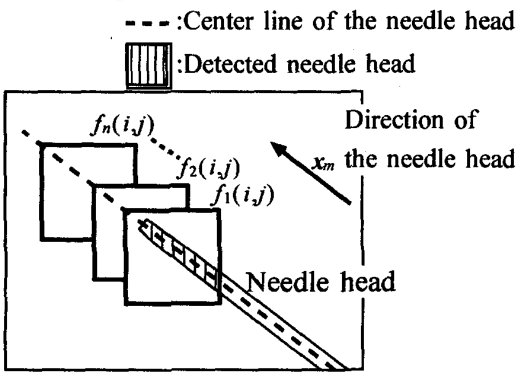


Fig. 7. Determination process of the tip position of the needle head.

are estimated as follows.

$$r_k = \frac{\sum_i \sum_j (f_k(i,j) - \bar{f}_k)(g(i,j) - \bar{g}))}{\sqrt{\sum_i \sum_j (f_k(i,j) - \bar{f}_k)^2 \sum_i \sum_j (g(i,j) - \bar{g})^2}} \tag{4}$$

where $\bar{f}_k$ is an average of $f_k(i,j)$ and $\bar{g}$ is an average of $g(i,j)$. The region of interest $f_k(i,j)$ where the correlation r_k is closest to 1.0 is chosen.

The invisible part of the needle head can be predicted from the reference pattern. As a result, the position of the needle head within the target is detected.

3. Results

We choose a sesame seed as a target. The target is 3.3mm in length, 1.7mm in width, and 1.5mm in height. The sesame seed is soaked in water about 30 minutes, and it is fixed on the stage. The magnification

TABLE I
Measurement resolutions

X direction	0.0136 (mm/pixel)
Y direction	0.0548 (mm/pixel)
Z direction	0.0135 (mm/pixel)

TABLE II
Resolutions of positioning about the manipulator

	+direction	−direction
X	0.0485 mm/s	0.0529 mm/s
Y	0.0421 mm/s	0.0358 mm/s
Z	0.0465 mm/s	0.0571 mm/s

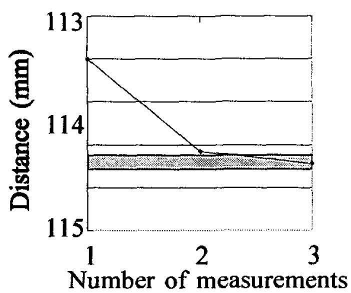


Fig. 8. Result of manipulation in Y_m axis.

of the stereoscopic microscope is 7 times. The distance between the object lens and the stage is 115mm. From an actual measurement of distance and position difference, the coefficient A of the equation (2) is estimated to -0.384. The coefficient m is 7. The coefficent B is estimated to 223. The measurement resolutions are derived as shown in table I. Table II shows the resolution of the manipulator. It is high resolution enough to perform the micromanipulation. Since the needle head is dark in the image, we decide the threshold as 133 in red color component, 131 in green color component, and 113 in blue color component. An area of the needle head is extracted by thresholding.

In this experiment, the needle head is set 2.0mm over the stage. Table III shows the sizes of image data and r.

Figure 8 shows the result of manipulation in Y_m axis. The needle head is positioned to the same height of the goal with 3 times of image measurement. The error is 0.02mm. Total time to reach the target is about 180 seconds. It is enough speedy to operate the fixed target.

The needle is guided so that it pierces the target

TABLE III

The values of the strategy

	Sizes of image data (pixels)	Dimensions of measurement	r
$\mathbf{o} \to \mathbf{o}_1$	60×80	3D	0.82
$\mathbf{o}_1 \to \mathbf{o}_2$	480×640	3D	0.82
$\mathbf{o}_2 \to \mathbf{a}$	60×80	2D	1.0
$\mathbf{a} \to \mathbf{b}$	480×640	2D	1.0
$\mathbf{b} \to \mathbf{c}$	480×640	2D	0.82

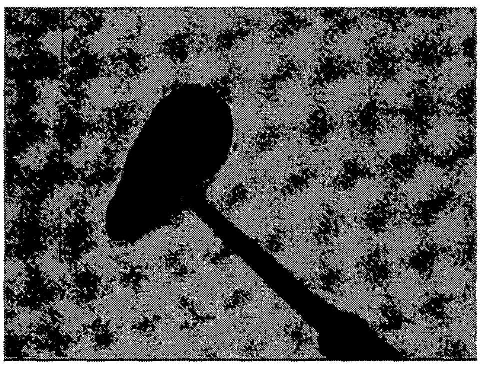


Fig. 9. **The needle head reaches the goal.**

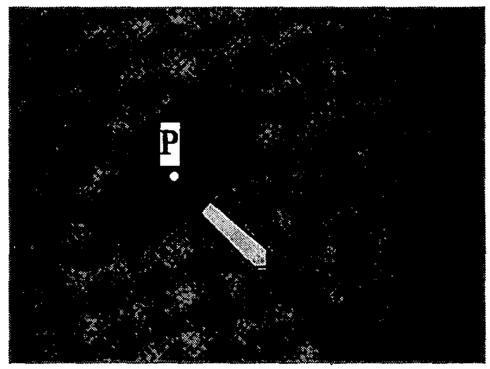


Fig. 10. **Result of prediction of the needle head position in the target.**

0.8mm in length. Figure 9 shows the result of manipulation. Figure 10 shows the result of prediction about the needle head position. The needle head, the target and the background are distinguished by thresholding and by comparison with the reference pattern as shown in figure 6. The correlation r_k is estimated as 0.99. The needle head position is predicted in the point **P**. The invisible area of the needle head is predicted by comparison with reference pattern. The needle head position **P** is detected as shown in the figure.

4. Conclusions

In this paper, we propose a visual feedback system for micromanipulation with the stereoscopic microscope to make the needle head pierce the target as much length as desired. At first, for achieving the manipulation at a realistic rate, we proposed a strategy of moving the needle head. Secondarily, since the system has difficulties in detecting the needle head position within the target, we developed the method to predict the needle head position within the target. The length of the needle head, which is within the target, is fully controlled. As a result, a needle head is guided to reach a goal within the target in a short time. This system may be useful in the micromanipulation such as microinjection into seeds.

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