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MICROMANIPULATION SYSTEM USING STEREOSCOPIC MICROSCOPE

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Abstract: In this paper, we describe a visual feedback system using a stereoscopic microscope. The system controls a micromanipulator so that a needle head may pierce a target as much length as desired. The tip position of the needle head and the target under the microscope are measured three dimensionally with two CCD cameras that are set to the eyepieces of the microscope. However, the visual feedback system has difficulties in detecting the tip position of the needle head within the target. We developed a method to predict the tip position of the needle head in the target. Before the needle head pierces the target, the end of the needle head is cut as much length as desired for piercing the target. The reminder of the needle head is set as a reference pattern. After the needle head piercing the target, a part of the needle head that is the same shape as the reference pattern is searched in the image. An invisible part of the needle head can be predicted and the tip position of the needle head in the target is detected.
Keywords: visual feedback, stereoscopic microscope, image processing

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 developed a visual feedback system using a stereoscopic microscope. The system controls a micromanipulator so that a needle head may pierce a target as much length as desired. The tip position of the needle head and the target under the microscope are measured three dimensionally with two CCD cameras which are set to the eyepieces of the microscope. However, if the needle head pierces the target, the visual feedback system has difficulties in detecting the tip position of the needle head within the target. As a first step, we developed a method to predict the tip position of the needle head in the target. The end of the needle head is cut as much length as desired for piercing the target. The reminder of the needle head is set as a reference pattern. A part of the needle head that is the same shape as the reference pattern is searched in the image. An invisible part of the needle head can be predicted and the tip position of the needle head in the target is detected, Next, we developed a strategy of moving the needle head in order to reduce the repetition number of the image processing and the needle head moving. In our method, the needle head could reach the target in a short time.

2 VISUAL FEEDBACK SYSTEM

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 eyepieces of the microscope. A target is set on the stage of the microscope. The *(X,Y,Z)* coordinate system is used as the world coordinate system. The Y axis is equal to the light axis of the cameras.

Figure 2 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 **P** to be projected to the left and right CCD. The distance y_p is estimated by the stereovision method as follows.

$$y_p = \frac{f\,d}{x_l - x_r} \tag{1}$$

where the *f* is the focal distance of the cameras and the *d* is the distance between the cameras. Although the $x_l - x_r$ is inversely proportional to the y_p, it is found that the distance y_p can approximately be estimated as follows when the needle head is closest to the target.

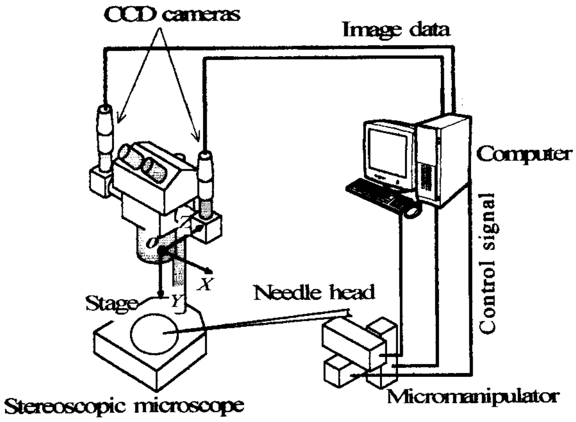

Figure 1. Visual feedback system

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

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

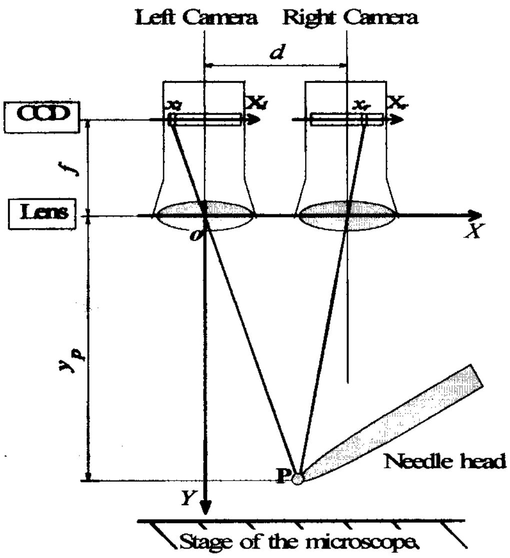

Figure 2. Principle of stereovision method

The needle head approaches the target gradually with repetition of the processes as follows.

1) Image processing
2) Estimation of the vector of moving the needle head
3) Needle head moving

As a first step of the image processing, the images from the CCD cameras are inputted into the computer using a video capture board. Then, the image is binarized with its color information. The areas of the needle head and the target are extracted. Finally, the needle head is extracted from its shape information. The target that is fixed to the stage is eliminated from the image at the same time.

In order to guide the needle head approaching the target in safety and speedy, we set a path of the needle head as shown in figure 3 and figure 4. Figure 3 shows the descending process of the needle head from the point **o** to the point **o$_2$**. At the beginning of the micromanipulation, the needie head is out of the focus and it is unclear in the image. As a first step of micromanipulation the needle head descends to the point **o**$_2$ where is the same as the height of the center of the target. The point ol shows the limit of focal depth. Because the needle head is out of focus when the needle head is in the area between **o** and $\mathbf{o}_1$, the needle head should be moving slowly. The moving length of the needle head per a manipulation is set as follows.

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

where M is the moving length, D is distance between the needle and the destination and R is constant which value is less than 1.0. In this case, the vector of moving the needle head is parallel to *Y* axis.

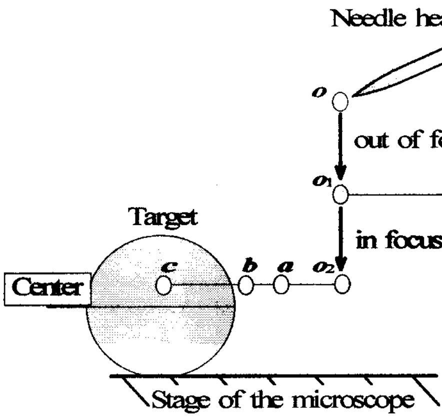

Figure 3. Descending processes of the needle head

After the needle head descends to the point $\mathbf{o}_2$, the needle head approaches to the target as shown in figure 4. The point **a**, **b** and **c** are the destination of the needle head. At first, the needle head approaches to the point **a**. Then the needle head approaches to the point **b**. When the needle head is between **b** and e, the target obstructs the needle head. In this case, the tip position of the needle head is predicted stated later.

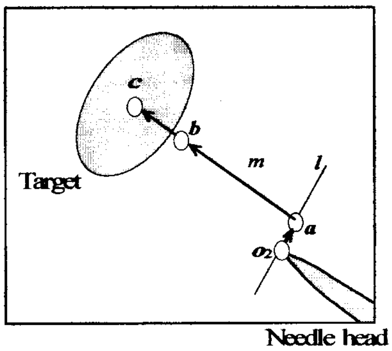

Figure 4. Path of the needle head to approach the target

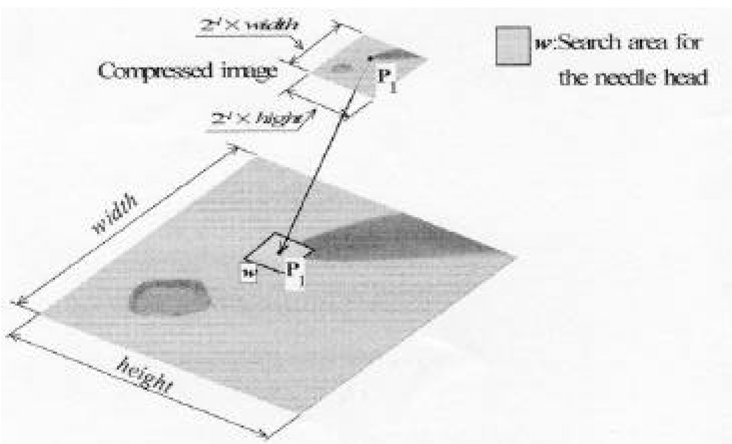

Figure 5. search area for the needle head

The image data is compressed as shown in figure 5 to reduce the image processing time. 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 tip 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 is redetected in the original image data.

3 PREDICTION OF THE NEEDLE HEAD POSITION IN THE TARGET

When the needle head proceeds inside of the target, it is difficult to detect the tip position of the needle head. In order to control the length of the needle head within the targets, we developed an image processing method to predict the tip position of the needle head.

Before the needle head pierces the target, we set the reference pattern of the needle head as shown in figure 6. The *R* is a desired length of the target piercing the target. The end of the needle head is cut by *R.* It is predicted that the shape of the needle head will be deformed as shown in the gray area when the needle head reaches to the center of the target. The reminder of the needle head is set as a reference pattern.

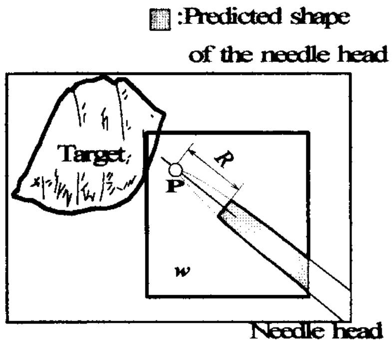

Figure 6. Prediction of needle head

Figure 7 shows the process of determine the position of the needle head. At first, the center line of the needle head is estimated. Then, in order to search a part of the needle head which is as the same shape as the reference pattern, regions of interest $f_k\,(i, j)$ are set on the center line as shown here. When the needle head reaches the center of the target, the shape of the needle head will be almost equal to the reference pattern. In order to estimate the similarity between the shape of the needle head and the reference pattern, the correlations r_k between $f_k\,(i,j)$ and $g\,(i,j)$ are estimated as follows.

$$r_k = \frac{\sum_i \sum_j \left(f_k\,(i,j) - \overline{f_k}\right)\left(g\,(i\,,j) - \overline{g}\right)}{\sqrt{\sum_i \sum_j \left(f_k(i,j) - \overline{f_k}\right)^2 \sum_i \sum_j \left(g\,(i,j) - \overline{g}\right)^2}} \tag{4}$$

where $\overline{f_k}$ is an average of $f_k\,(i,j)$ *and* $\overline{g}$ 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. An invisible part of the needle head can be predicted from the reference pattern. As a result, the tip position of the needle head in the target can be detected.

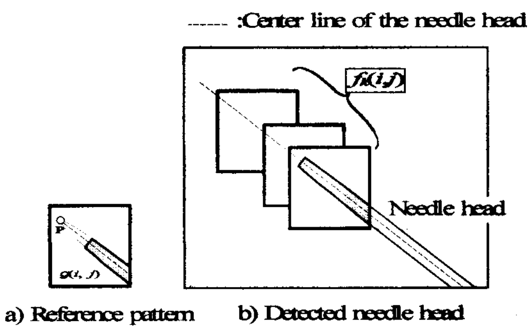

Figure 7. Detecting method of the needle head

4 RESULTS

We choose a sesame seed as a target. The target is 3.3 mm in length, 1.7 mm in width, and 1.5 mm in height. Figure 8 shows the position difference of the needle head and the distances between the needle head and the object lens. The magnification m of the stereoscopic microscope is 7 times. The distance between the object lens and the stage is 115 mm. The position differences are measured at 0.1 mm intervals from the stage to 1.0 mm over the stage. The parameter A of the equation (2) is estimated to -0.0548. B is estimated to 223. The measurement resolutions are derived as shown in table 1. Table 2 shows the resolution of the manipulator. It is enough resolution to micromanipulation to the sesame seed. Figure 9 shows the result of micromanipulation between the point **o** and $\mathbf{o}_2$. Since the needle head is dark in the image, the pixels that the intensities are 133 or less for red color component, 131 or less for green color component and 113 or less for blue color component are detected as the needle head.

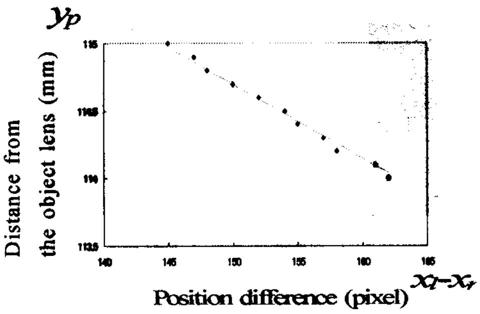

Figure 8. Linear approximation of distance y

Table 1. Measurement resolution

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

Table 2. The moving speed of 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

Figure 9 and figure 10 show 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 7. The needle head position is predicted as shown in figure 10. However the tip position of the needle head is cut by the target, the completed shape of the needle head is predicted by comparison with reference pattern. The needle head position **P** is detected as shown in figure 10. Total time to reach the target is about 180 seconds. It is enough speedy to operate the fixed target.

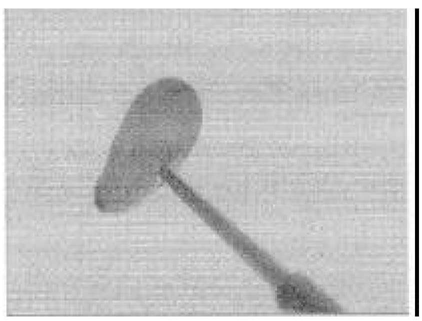

Figure 9. The needle head reaches the center of the target

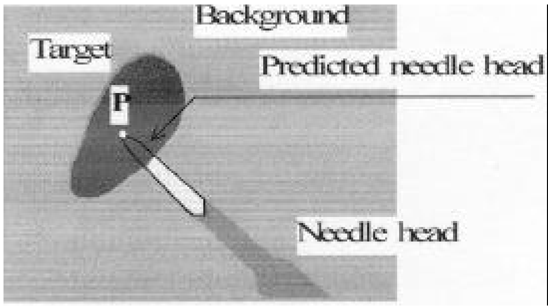

Figure 10. Result of prediction of the needle head position in the target

5 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. Since the system has difficulties in detecting the needle head position in the target, we developed a method to predict the needle head position in the target. In our method, the length of the needle head within the target is fully controlled. This system may be useful in the micromanipulation such as microinjection to the cells.

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