A VISUAL SERVO SYSTEM FOR MICROINJECTION UNDER STEREOSCOPIC MICROSCOPE

Tetsuya Sano * Hideki Yamamoto *

* *Faculty of Education, Okayama University*
Tsushima Okayama, 700-8530, Japan
Phone +81-86-251-7723 Fax +81-86-251-7755
E-mail yamamoto@cc.okayama-u.ac.jp

Abstract: In this paper, an automatic microinjection system, which controls the micromanipulator so that the needle may pierce a target to the desired depth, is proposed. A method for prediction of the tip position of the needle head within the target is developed. The shape of the needle head is preserved as a reference pattern before the needle pierces the target. During the needle proceeds within the target, the tip position of the needle head is predicted by the correlation matching using the reference pattern. Experimental results indicate that the proposed system may be useful in microinjection of seeds.

Keywords: Visual, Feedback control, Microinjection, Micromanipulation, Image processing, Stereo vision, Microscope

1. INTRODUCTIOIN

In the area of biotechnology, microinjection system is widely used for such purposes as operating on genes and transferring biological materials into cells. For the some experiments, such as biochemical assay, a large number of cells has to be injected in a short time. The development of a computer-assisted and microprocessor-controlled injection system provides high injection rates. The AIS(automated injection system, Zeiss, Germany) is a automated microinjection system, and it permits reliable and simple microinjection into living cells by a system configuration of various hardware and software components that controls the injection procedure (Ansorge and Saferich, 1998).

However, the AIS cannot perform sufficiently with the cells in various thicknesses because the manipulator of the AIS moves constantly regardless of the distance between the injector and the cells. For achieving the stable microinjection, an automatic microinjection system that allows control of the injector so that it may pierce a target in the desired depth is needed.

In this paper, an automatic microinjection system under the stereoscopic microscope by using well-known techniques of visual feedback is realized. Lately, the several techniques of visual feedback are proposed (Castaño and Hutchinson, 1994; Feddeman *et al.*, 1992). However, these system have some difficulties in performing the microinjection. At first, it has difficulties in estimating parameters for the measurement of distance under the stereoscopic microscope. Secondarily, the significant problem for the stable microinjection is that the tip position of the needle head disappears when the needle head pierces the target. The problem makes it difficult to decide whether the needle head pierces the target in the desired depth.

Therefore, in this system, the measurement of the distance under the stereoscopic microscope is automatically carried out by image processing. In order to control the depth of the needle head

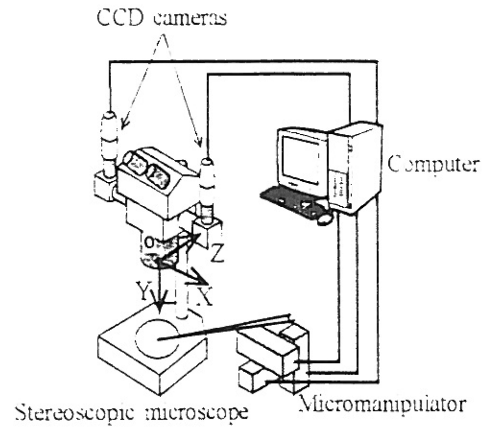


Fig. 1. Automatic microinjection system

within the target, a prediction method, which determines the tip position of the needle head within the target, is developed. Experimental results indicate that the proposed system may be useful in microinjection of seeds.

2. METHODS

2.1 *Microinjection system with visual feedback*

Figure 1 shows the microinjection system. The system consists of a stereoscopic microscope, two CCD cameras, a micromanipulator, and a personal computer. A target is set on the stage of the microscope. The cameras are mounted to the eyepieces of the microscope. The micromanipulator has three degrees of freedom. It moves linearly. The needle is set to the micromanipulator. The (X,Y,Z) coordinate is set as shown in Figure 1. The Y axis is perpendicular to the stage of the microscope. The XZ plane is parallel to the stage. The left image and the right image are inputted to the personal computer that performs image processings to detect the needle head and the target in the images. The distance between the needle head and the target is measured three dimensionally by the stereovision method. The microinjection system guides the needle head to reach the goal within the target. The measurement of the distance between the tip position of the needle head and the goal, and the movement of the needle head are carried out alternately to reduce the distance to 0.

Figure 2 shows the XY plane of the visual feedback system. The y_p is the distance between the lens and the tip position of the needle head. By the stereovision method, the y_p is defined as

$$y_p = \frac{fd}{x_l - x_r} \quad (1)$$

where f is the focal distance of the left camera and the right camera, d is the distance between

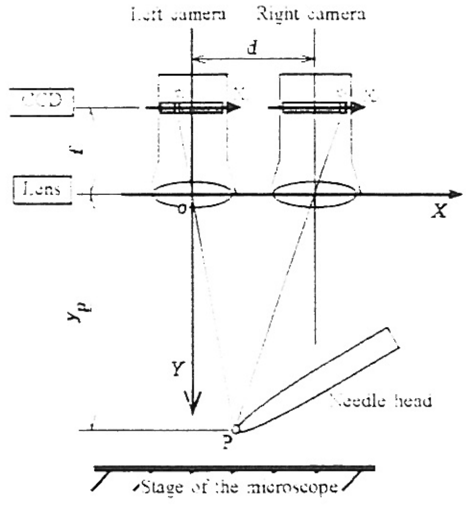
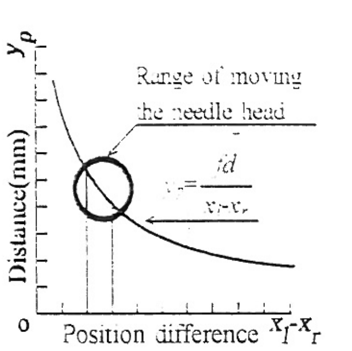
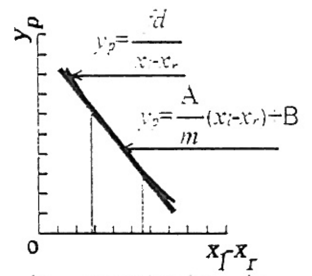


Fig. 2. Principle of stereovision method



a) Relation between the distance and the positoin difference



b) Approximation in the range of moving the needle head

Fig. 3. Linear approximation of the distance

the left camera and the right camera, and x_l and x_r represent the tip position of the needle head in the left CCD and in the right CCD, respectively. However, the estimations of f and d are difficult because the cameras are mounted to the microscope.

Figure 3 show the relation between the position difference $x_l - x_r$ and the distance y_p. A range of moving the needle head is is extremely limited because the focal depth of the camera is very narrow in the microscope. In this range, the position difference is experimentally derived as if it changes linearly according to the distance. Therefore, for simple processing. y_p is approximately estimated by the following linear function:

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

where m is the magnification of the microscope. The parameters A and B are determined by the least square method from the experimental result of the position difference of the needle head.

2.2 *Basic algorithm of the visual feedback system*

Figure 4 shows the path of the needle head until the needle head reaches the goal within the target. The path has three phases in order to realize the stable microinjection.

The first phase is the descending phase. The tip position of the needle head is positioned three dimensionally under the stereoscopic microscope. The needle head is guided to set the depth to that of the goal. Consequently, the tip of the needle head becomes in focus and the microinjection is carried out with the sharp image. After descending phase, the image of the needle head becomes clear as shown in Figure 5 a) where LL' is the centerline of the needle head. In this phase, the distance between the needle head and the target is measured three dimensionally by the stereovision method.

The second phase is the approaching phase. The needle head approaches horizontally to the neighborhood of the target. For the preparation of the piercing, the tip of the needle head is positioned either perpendicularly to the centerline LL' of the needle head or parallel to the the centerline LL' of the needle head so that the goal might be located on the axis of the needle. In this phase, the distance between the needle head and the target is measured two dimensionally with a single image. The image processing is carried out with a reduced image.

The final phase is the piercing phase, in which the needle head gradually proceeds within the target. For achieving the precise microinjection, the needle head should be moving horizontally for the ease of the position prediction. The tip position of the needle head is detected by an algorithm, which will be described later.

2.3 *Detection method of the needle head*

An image processing algorithm for detection of the needle head in the approaching phase is as follows:

- Noise reduction
- Extraction of the needle head
- Identification of the needle head

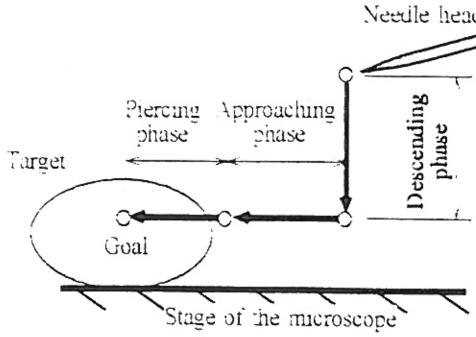
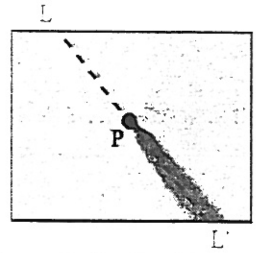
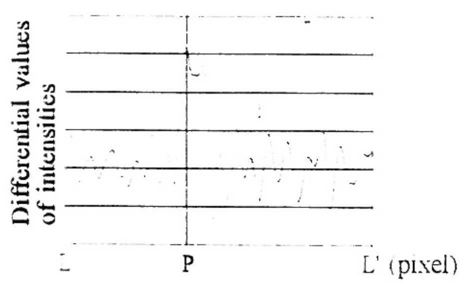


Fig. 4. Path of the needle head



a) The needle head and its center line



b) The differential values of intensities on the center line

Fig. 5. Detection of the tip position of the needle head

At first, the noise reduction is performed with the median filter. Secondarily, the pixels of the target and the pixels of the needle head are extracted by thresholding. The value of the threshold is an average intensity of the image. For identification of the needle head or the target, the size and the normalized area of the extracted objects is derive. The sizes of the extracted objects are estimated by labeling, then the contours of the area are detected by an algorithm based on a tracing of the contours. The normalized area is the square of the contour length over the size. The target and the needle head are detected by their feature parameters of size and normalized area, which are derived by the operator.

If the tip of the needle is out of focus, the precise outline of the needle head is hardly detected. The tip position of the needle head is detected by an algorithm as follows: Figure 5 shows the method for the tip position of the needle head. After the needle head is detected, the centerline of the needle head LL' is derived by thinning, as shown

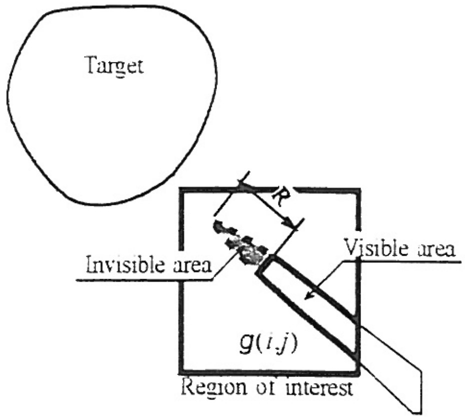


Fig. 6. Reference pattern

in Figure 5 a). Then the differential value of LL' is traced. Figure 5 b) shows differential values of intensities. At the tip position of the needle head, the intensities greatly change. Therefore, the tip position is detected by searching for the position that indicates the peak of the differential value.

2.4 *Prediction method for the needle head in the piercing phase*

A method for the prediction of the tip position of the needle head in the piercing phase is as follows:

Figure 6 shows the needle head after the approaching phase. A square region of interest is set automatically, after the needle head is detected. The tip position of the needle head is on a diagonal line of the square region. The binary image of the needle head in the region is preserved as the reference pattern. The parameter R is the desired depth for piercing the target. The reference pattern is considered as two areas. When the needle pierces the target to the depth desired, the tip part of the needle becomes invisible. This part is referred as an invisible area. The remaining part is referred as a visible area. The visible area indicates the shape of the needle head after the needle head reaches the goal. The visible area is used to detect the position of the reference pattern in the image after the needle head pierces the target. The invisible area is used to predict the tip position of the needle head.

After the needle head pierces the target, the area of the needle head and the target overlap with each other. The area of the target is eliminated by comparison with the image which is kept before the piercing phase. Because the target is fixed on the stage, immovable are is deleted. Then the part of the needle head corresponding to the visible part of the reference pattern is searched as shown in Figure 7. Because the tip of the needle is on the enter line of the needle head, at first, the centerline of the needle head is estimated. Secondarily, in

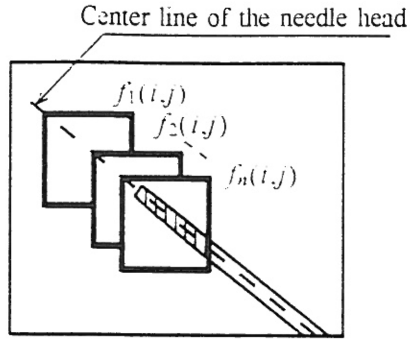


Fig. 7. Search method for the tip position of the needle head within the target.

order to perform the correlation matching, regions of interest $f_k(i,j)$(k=1, 2, ..., n) are set on the centerline. Each region's upper left corner is on the centerline. One of the regions will indicate the position of the reference pattern. The correlations r_k between $f_k(i,j)$ and $g(i,j)$, which indicates similarity between the regions and the reference pattern, are defined as

$$r_k = \frac{s_{fg}}{s_f s_g} \qquad (3)$$

$$s_{fg} = \sum_i \sum_j (f_k(i,j) - \bar{f}_k)(g(i,j) - \bar{g})$$

$$s_f^2 = \sum_i \sum_j (f_k(i,j) - \bar{f}_k)^2$$

$$s_g^2 = \sum_i \sum_j (g(i,j) - \bar{g})^2$$

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, indicates the position of the reference pattern. The shape of the needle head in the invisible part can be predicted by using the invisible area of the reference pattern and the tip position of the needle head is position of the needle head is detected by the same algorithm used when the needle head is outside the target.

2.5 *Image data compression*

In order to reduce the required time for the microinjection, an algorithm to detect the tip position of the needle head with a compressed image is proposed. At first, a compressed image is derived from the original image. The compressed image is 2^{-2l} of the original image in size. The value of the compressibility l is that the needle head and the target can be observed in the compressed image. The intensities of the compressed image are derived as the medians of the small areas, which are set on the original image. Secondarily, the tip position of the needle head is detected

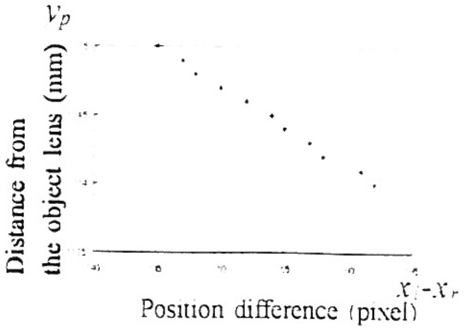


Fig. 8. The position difference of the tip position of the needle head and the distance between the tip position and the object lens.

Table 1. Resolutions of the image measurement

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

using the compressed image. Finally, based on the tip position of the needle head in the compressed image, the tip position of the needle head P in the original image can be predicted. A small search area for detection of the needle head is set in the original image. The tip position of the needle head is detected precisely in the search area. AS a result, the size of the image data is much smaller than the size of the image data without image data compression.

3. RESULTS

A microinjection system with a stereoscopic microscope having a magnification of 7 times is performed. A steel needle is attached to a micromanipulator. At first, the position difference of the needle head is measured for the calibration. Figure 8 shows the position difference of the needle head and the distances between the needle head and the object lens. The position difference is measured when the distance between the needle head and the object lens is between 115mm and 114mm at 0.1mm interval. Based on the result, the coefficient A of the equation 2 is estimated to be -0.384. The coefficient B is estimated to be 223. The resolution of the XY plane is measured by a measure putting on the stage of the microscope. By counting pixels for 1.0mm, the measurement resolutions are derived as shown in table 1.

A sesame seed is chosen as a target. The experiment is performed for the purpose of piercing the needle head so that the needle head reaches to the center of the target. The target is 3.3mm in length, 1.7mm in width, and 1.5mm in height. The sesame seed is fixed on the stage. When the needle head reaches the center of the target, the distance between the tip of the needle head and the object lens should be 114.25mm. According to the

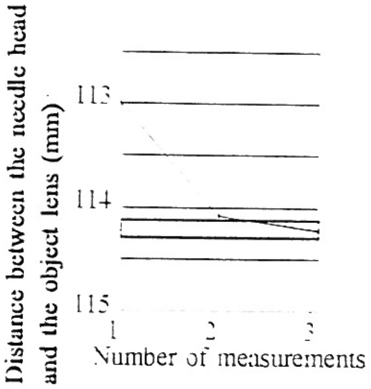


Fig. 9. Microinjection procedure in the descending phase

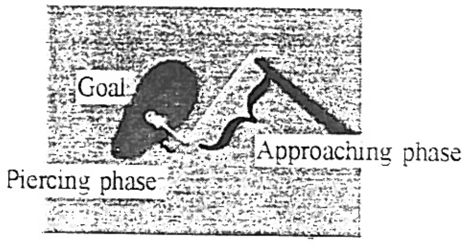
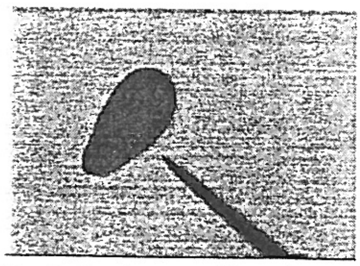


Fig. 10. Microinjection procedure in the approaching phase and in the piercing phase



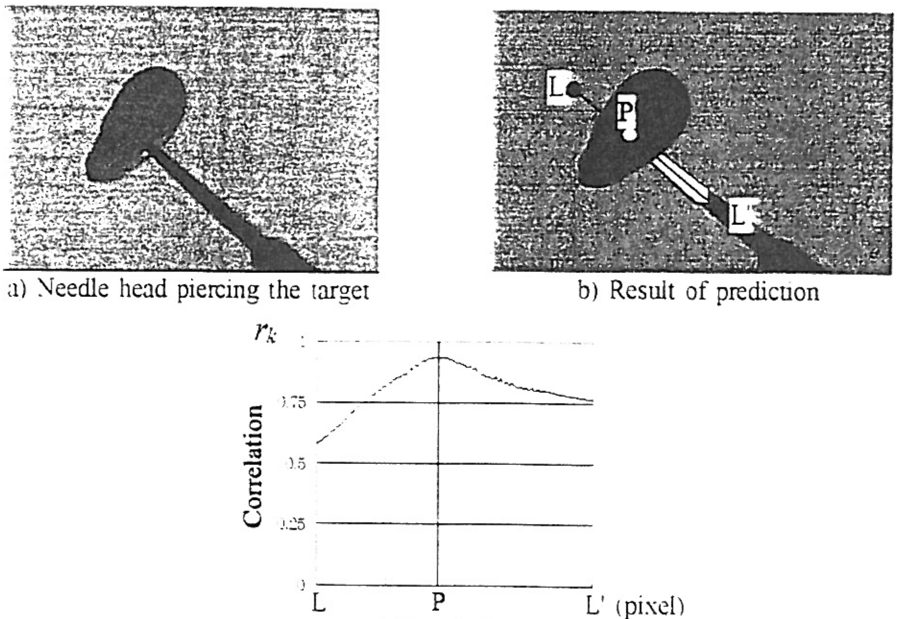
a) Needle head approaching the target

b) Reference pattern

Fig. 11. The reference pattern

resolutions of the image measurement, the needle head is considered to reach the same height of the center of the target when the distance between the needle head and the object lens is between 114.25mm and 115.75mm in the descending phase. The needle head is considered to reach the center of the target when the depth to which the needle head pierces the target is estimated between 0.773mm and 0.827mm.

Figure 9 shows the distance between the needle head and the object lens in the descending phase. The needle head is positioned precisely so that it corresponds to the goal of depth within the target. This result indicates that this system is of sufficiently high resolution to carry out the three-dimensional injection with this target. Figure 10 shows the result of the microinjection procedure in



a) Needle head piercing the target

b) Result of prediction

c) Correlation

Fig. 12. Result of the prediction.

the approaching phase and in the piercing phase. The approaching phase is performed with 4 times of image measurement. Figure 11 a) shows the needle after the approaching phase. The binary image of the needle head is derived by the image processing algorithm. As a result, the reference pattern is preserved, as shown in Figure 11 b). The black pixel area indicates the invisible area and the gray pixel area indicates the visible area after the needle head pierces the target. The prediction of the tip position of the needle head is carried out 3 times in the piercing phase. The needle head piercing the target as shown in Figure 12 a). Figure 12 b) shows the result of the prediction. After the detection of the needle head, the centerline of the needle head LL' is detected. The correlation matching with the reference pattern is performed on the center line near to the tip of the needle head. Figure 12 c) presents the values of the correlations. The horizontal axis presents imaginary tip position of the needle head. By the search for the tip position where the correlation value becomes maximum, the position of the reference pattern is detected. In this case, the maximum value of the correlation is 0.99. The tip position of the needle head within the target **P** is detected with the reference pattern, as shown in Figure 12 b). The depth to which the needle head pierces the target is estimated as 0.779mm.

4. CONCLUSIONS

In order to realize the stable microinjection into cells in various thicknesses in the area of biotechnology, an automatic microinjection system allowing control of the micromanipulator so that the needle may pierce a target to the desired depth is needed.

In this paper, an automatic microinjection system for use with a stereoscopic microscope that includes visual feedback is proposed. A thickness of the target on the microscope is automatically derived by the image processing. A method for prediction of the tip position of the needle head within the target is developed. During the needle proceeds within the target, the tip position of the needle head is predicted by the correlation matching using the reference pattern. As a result, the tip position of the needle head within the target is fully controlled and effectively guided to reach a goal within the target.

Experimental results indicate that the proposed system may be useful in microinjection of seeds.

5. REFERENCES

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