Development of Biological Cell Manipulation System using Visual Tracking Method

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Abstract

Conventionally, biological manipulations have been performed manually with long training and pretty low success rates. To overcome this problem, a novel biological manipulation system has been developed to manipulate biological cells without any interference of a human operator. In this paper, we demonstrate a development of tele-autonomous Cell Manipulation System (CMS) using an image processing at a remote site. The CMS consists of two manipulators, a plane stage, and an optical microscope. We developed deformable template-model-matching algorithm for micro objects and pattern matching algorithm of end effect for these manipulators in order to control manipulators and the stage. Through manipulation of biological cells using these algorithms, the performance of the CMS is verified experimentally.

I. Introduction

Recently, there is much attention around the technology in handling micro object. The examples of biological objects that are manipulated in biotechnology by a human operator are embryo, cell, nucleus, chromosome, and DNA. Dimensions of these objects are on the order of micron or nanometer. Because trained operators perform theses tasks manually, training of such operation may take several years and the long hour of manipulation may weary operators. Operators that performed in the field of biology and bioengineering manage to manipulate the biological objects by the micromanipulators with the two-dimensional image from the optical microscope [1-5]. These data have much noise because images of biological cells through an optical microscope are obtained through high magnification. The images are, moreover, easily influenced by external lighting effects. Putting cells into various solutions during manipulation brings about dispersion by mediums.

To overcome these problems, we developed a Cell Manipulation System (CMS) using visual tracking to manipulate biological cells. In this paper, we present the development of the tele-autonomous CMS with image processing functions. The developed CMS has several advantages of the following. First, anyone can easily manipulate biological cells without long training. Second, by using the CMS, contamination rate due to the human factors can be greatly reduced in the course of manipulation. Third, the CMS can improve the precision and repeatability. Finally, the human operator can monitor the process in detail through the vision system composed of microscope and three CCD cameras with different magnifications.

This paper is organized as follows. In Chapter 2, the conceptual design and prototype of the system are presented. This CMS consists of two micromanipulators, motorized microscope stage, optical microscope, and CCD cameras. The visual tracking process of the system is discussed in Chapter 3, where the recognition and auto-centering algorithm for the tracking process is described. The efficiency test for the CMS is shown in Chapter 4, where it is confirmed that the experimental results verify the performance of the CMS through manipulation of biological cells using these algorithms. Finally, this paper is concluded in Chapter 5.

II. Architecture of the CMS

The schematic diagram of the CMS is shown in Fig. 1. The CMS embodies a visual tracking processing based on error information between the target point of unfertilized eggs of a mouse and spot coordinates of biological instruments on the image plane. As in the following Fig. 1, the functional structures of the CMS are classified into master part and slave part. The master control computer (Pentium Ⅲ, Memory 500) delivers the master command and posture information of the haptic device to the slave computer through the TCP/IP. The master GUI of this computer displays actual images of the process for cell manipulation and geometric data of the slave part. (See Fig. 2) In addition, each manipulator can be controlled by the haptic device, which sends human operator's reference command and can reflect the injection force in the slave part.

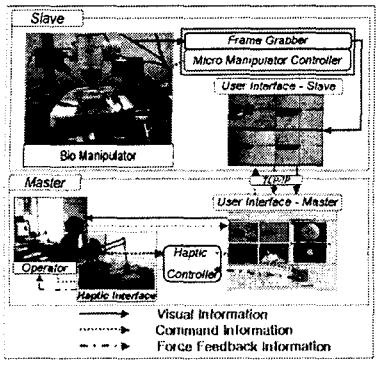


Fig. 1 Architecture of the CMS

The slave part performs autonomous executions for cell manipulation such as cell recognition, cell identification, and auto-centering. The execution result was transmitted to the master PC through TCP/IP. This slave system of the CMS consists of the slave PC, two manipulators, a plane stage, an optical microscope, and CCD cameras as shown Fig. 1 and 3.

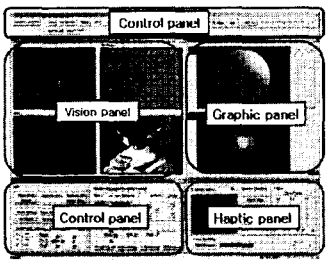


Fig. 2 Master GUI

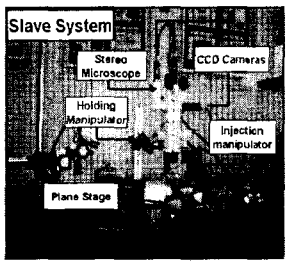


Fig. 3 Slave System

The left micromanipulator equipped with holding pipette moves or holds the cell, while the right micromanipulator with injection pipette is utilized to inject bio-material in the cell. Diameter of holding pipette and injection pipette is about 100 ㎛, and 5 ㎛ respectively. The left micromanipulator (Eppendorf, InjectMan NI2) drives a Cartesian movement by three micro-stepping motors. This manipulator has the workspace of 20*20*18 ㎣ and the resolution of about 2 ㎛. High resolution is required for the right micromanipulator (Sutter MP285) to achieve exact injection. The required resolution (roughly 40 ~ 200 ㎛/step) is derived by ensuring the requirement larger than the diameter of cells. The drive mechanism uses the method of precise worm gear cap-stan drive. The travel range of the right micromanipulator is 25.4 * 25.4* 25.4 ㎣. The planer stage (PI, M-126 DG) implements the function of automatic centering that means an error between a current position of micro objects obtained by visual processing and the center of the field of view of the microscope. Extremely high precision motion control is required for the successful auto-centering. A two DOF stage is used in which the X-Y axes have 25 ㎜ with a step resolution of 100 ㎚. The planer stage is installed as shown Fig. 3. CCD cameras (SONY XC55) and an optical microscope (below a vision part) implement the function of monitoring biological instruments and micro objects. The microscope (Leica, MZ-12.5) has the magnification rate of 1408*1056 pixels. Three CCD cameras are mounted on the top of the microscope and give cell images with three different magnification rates.

III. Visual Tracking Process

For successful biological manipulation, we developed a vision algorithm for cell recognition and auto-centering.

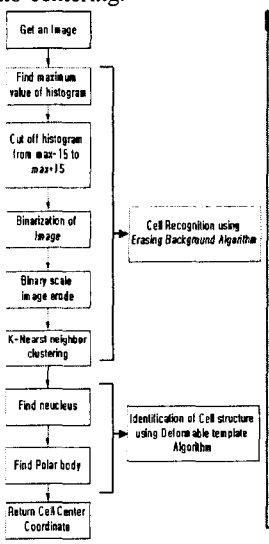


Fig. 4 Recognition Algorithm

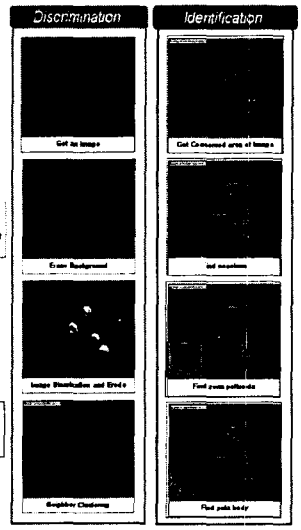


Fig. 5 Recognition Process

3.1 Cell Recognition Algorithm

Fig. 4 is a flow chart of the cell recognition algorithm for a biological cell manipulation. The recognition algorithm divides the process into two parts: cell discrimination and cell identification. The cell discrimination algorithm distinguishes biological cells from a background. The cell identification algorithm examines cellular structure using deformable template after cell discrimination. First of all, histogram segmentation in cell discrimination sharply distinguishes between cells and background from the obtained image. The second step is to binarize the image, which transforms the segment of cells into white segment. Third, the location of each cell is recognized using the nearest-neighborhood algorithm. For the cell

identification, a deformable-template-model-matching-algorithm is applied. This algorithm is to model a curve which forms many parameters on featured points and then to recognize the predetermined shape from the image using these controlled parameters - (x, y) : coordinate of X- and Y-axis, r : radius, θ : rotation angle, and l :length (See Fig. 6). The deformable template in Fig. 6 is applied to search the nucleus and the polar body which have different outlines.

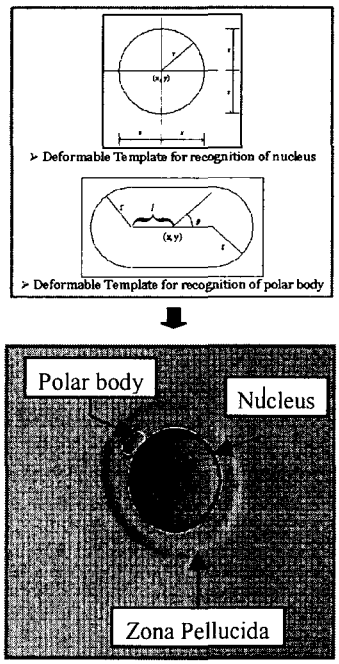


Fig. 6 Deformable Template

Although the top of Fig 6 is a simple circle, the template can be used to search a nucleus. The deformable template for the polar body is more complicated in configuration than that for the nucleus as shown in the bottom of Fig. 6. From now on, we describe the energy function for edge detection of the nucleus and polar body, which is defined as

$$E_{total} = E_{edge} + E_{deviation} \quad (1)$$

where E_{edge} is the boundary energy function and $E_{deviation}$ is the variation of the boundary. In Eq. (1), E_{edge} has the following form

$$E_{edge} = \frac{1}{255 \times n} \sum_{i=0}^{n} \phi_{edge}^{i}(x, y) \quad (2)$$

where n is the total number of pixels for the outline of a template, ϕ_{edge} is the potential field which corresponds to the image of outer boundary which can be abstracted from the original image. Since the nucleus and polar body do not exactly match with the templates, $E_{deviation}$ is necessary to make the value of E_{total} stabilize at the boundary of the template. While, $E_{deviation}$ is represented as follows:

$$E_{deviation} = 1 - \frac{1}{255} \sqrt{\frac{1}{n} \sum_{i=0}^{n} (ave - \phi_{edge}^{i}(x, y))^2} \quad (3)$$

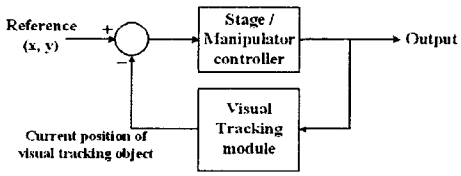
where ***ave*** is the average of total pixels of the template, and ***n*** is the number of total pixels.

On the other hand, the purpose of the pattern-matching algorithm is to search and detect holding pipette and injection pipette. The image-processing program was developed by using the MIL image library.

3.2 Auto-centering Algorithm

The auto-centering algorithm is to move a biological cell detected by the cell recognition algorithm to the center of the field of view. The closed-loop is constructed as shown Fig. 7. The reference input is the center of the field of view: (320,240) in pixel coordinates. The recognition module determines the coordinates of cells and pipettes, respectively and then transmits them to the controller module, where the error between the reference input and detected coordinates is computed. If the control error comes in the range of a permitted value, the control loop is stopped. Fig. 9 (c) shows that the holding pipette approaches the auto-centerized cell using the auto-centering algorithm.

Fig. 7 Auto-centering Algorithm



IV. Efficiency Test for the CMS

The process of cell manipulation for the CMS is represented in Fig. 8. First of all, the recognition process selects a cell to be manipulated and discriminates cellular structure such as zona pellucida, polar body, and cytoplasm using the deformable template-model-matching algorithm. Second, the cell is moved to the center of the field of view by auto-centering process. Third, "moving and holding cell" is to make the holding micromanipulator approach the cell automatically and then hold the cell by suction. Finally, "cell injection with tele-operation" is to penetrate the cell using the injection pipette which is controlled by the injection micromanipulator.

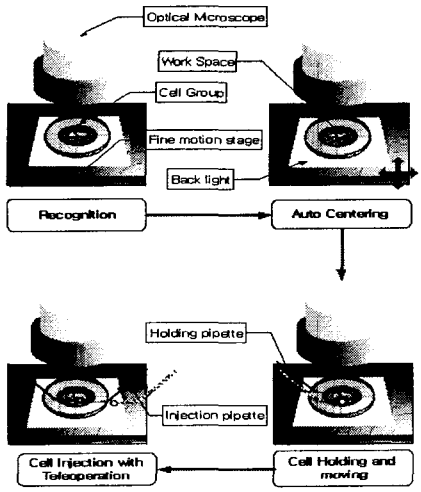


Fig. 8 Manipulation Processes of the CMS

The experimental result for mouse embryo cells is displayed in Fig. 9, where respective pictures are divided into four parts with three magnification windows and an image processing result window.

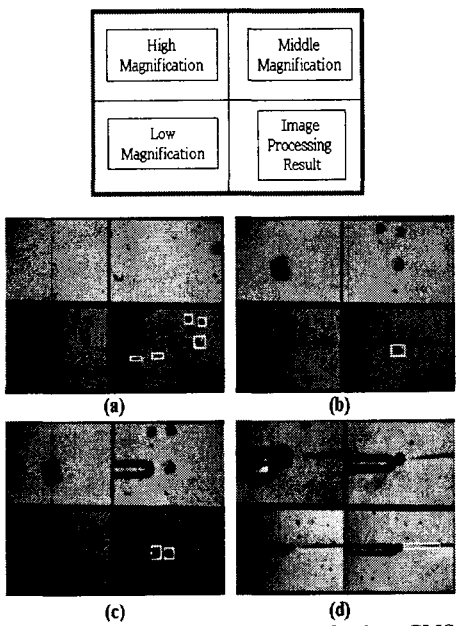


Fig. 9 Experimental results of the CMS: (a) autonomous selection of a cell using deformable template-model-matching algorithm, (b) auto-centering, (c) holding pipette approach to the cell, (d) injection into the zona pellucida

V. Conclusions

Biological CMS using visual tracking method is developed. For the visual tracking of the cell, the deformable-template-model-matching algorithm and pattern-matching algorithm are employed. Through manipulation of biological cells using these algorithms, the performance of the CMS is verified experimentally. Consequently, advantages for the CMS can be claimed as follows: anyone can easily manipulate biological cells without special training; the CMS can improve the precision and repeatability in biological cell manipulations.

Acknowledgement

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