Dual-beam laser micromanipulation for sorting biological cells and its device application

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ABSTRACT

Laser manipulation system combining microfluidic and microimaging devices was developed, in which a cell sorting in a transparent microchip was successfully demonstrated. The microchip containing two microchambers was prepared with laser microfabrication, in which a solution containing yeast cells was injected as a sample. In the microchip, a cell transfer from one chamber to another one was performed by using single, fixed trapping laser beam. Furthermore, to realize an efficient cell sorting, the trapping laser beam was split into two by a polarizing beam splitter and each beam was modulated independently; one trapping beam was used to trap individual cells and to move them, which is freely controlled by a mouse pointer, and another was used to store the selected yeast cells with its linear scanning. In this method, the cells on a locus of the scanned beam were isolated to transfer in the microchip. From these results, it is concluded that shortening of the cell sorting time in microchip by a few time was realized by using dual-beam laser manipulation.

Keywords: cell sorting, laser manipulation, laser microsurgery, microimaging, microchip

1. INTRODUCTION

In the recent rapid progresses in biotechnology, single cell manipulation using laser and microscope has been attracting significant attention, in which laser trapping and laser ablation have been applied as micro-tweezers and micro-scissors, respectively\textsuperscript{1-4}. By using these techniques, not only separation and fusion of cells but also extraction/injection of organs from/into cells will be realized. In order to place such a laser technique as a step of biological protocol, it is important to establish the method to pull laser-manipulated cells out of the solution under a microscope. In conventional methods, such an extraction has been realized by a pipette with volume from microliter to picoliter, however, it is difficult to combine it with laser trapping system installing microscope objective with large numerical number and short working distance. Furthermore, the operation of the pipette is usually not so reliable that its automatic method has been strongly desired.

As a method to overcome the problem, microchip and microfluidic devices are worth noting\textsuperscript{5,6}. When the microchip was made of transparent materials, it is possible to manipulate cells in the microchannel by using lasers. In the present work, a cell manipulation system installing microfluidic devices was developed and an active operation of cells was demonstrated by conducting dual-beam laser manipulation in a transparent microchip including two chambers, whose volume is less than microliter. Furthermore, by combining laser micromanipulation with real-time microimage analysis, an easy and convenient cell manipulation system can be constructed. Here, as a first step, such an automatic cell manipulation system is demonstrated.

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2. EXPERIMENTAL SETUP

The experimental setup is shown in Fig. 1. CW Nd:YAG laser beam (1064 nm) was split into two by a polarizing beam splitter. The each beam was modulated by two sets of computer-controlled galvano mirrors, led to an up-light microscope, and focused on a microchip with microscope objective lens (magnitude is 100x, numerical number is 1.25). The laser manipulation processes were monitored in real time by CCD camera set on the microscope, which is connected with computer to realize mouse control of galvano mirrors. The microchip was prepared with laser microfabrication and photopolymerization, which is shown in Figure 2A, and set on the microscope stage. Three syringes are equipped with homemade pump (pumps A, B, and C) and connected with the microchip. There are two chambers in the microchip, which are like wings of a butterfly, and they are connected with pumps A and C. They are connected through a microchannel with 100 µm width, which crosses a large microchannel connected with pump B and used as a drain. Thickness of the chambers is 100 µm. An yeast cell, *Schizosaccharomyces pombe* (h-) was prepared in usual as a representative sample.

![Fig. 1: An experimental setup of dual-beam laser manipulation and microfluidic devices.](image)

![Fig. 2: (A) A microchip fabricated by laser polymerization technique (left) and its schematic representation (right). (B) The micropictures at the cross point of the channels in the microchip, where the culture medium is injected independently by the microfluidic device.](image)
3. NONCONTACT TRANSFER OF YEAST CELLS BETWEEN CHAMBERS IN MICROCHIP

The cell transfer from left to right chambers was performed by using single, fixed trapping laser beam and controlling an electrically movable microscope stage. At first, the microchip was fulfilled by the culture medium independently as follows. Firstly, neat culture medium was injected to the right chamber using pump C, whose velocity of a flowing fluid is less than 1 µl/s, and stopped at the cross point of the channels. In Fig. 2B(b), the right channel is only fulfilled by the culture medium and the residual channels are empty. Secondly, the culture medium including the yeast cells was injected and stopped by using pump A (Fig. 2B (c)). Finally, neat culture medium is injected to the long channel from pump B and the microchip was fulfilled by the culture medium (Fig. 2B(d)). In this condition, the yeast cells was only existing at the left chamber.

The trapping laser was irradiated in the left chamber and a yeast cell was trapped and transferred to the right chamber. A representative demonstration is given in Fig. 3. The position of the trapped yeast cell was controlled by the electrically movable microscope stage with the velocity of 20 µm/sec. The single yeast cell was successfully transferred from left to right chambers at the rate of 26 sec.

Fig. 3: Cell transfer in microchip using laser trapping. A trapping laser beam is fixed at the center of each picture, by which a particle is trapped and transferred from left to right chambers. The micropictures of (b), (c), and (d) are taken at 6, 17, and 26 sec after that of (a), respectively. The position of each picture is shown in the bottom inset corresponding to dashed area in Fig 3 (b).

4. COLLECTION AND ALIGNMENT OF YEAST CELLS IN MICROCHIP

When the system is applied in a biological protocol, transfer time should be shortened. It is better to close these chambers and to shorten the combining channel, however, the possibility that the cells move from left to right chambers spontaneously will be increased because of their living motion and/or Brownian motion. For improving the efficiency, a method to transfer many cells simultaneously using dual-beam laser manipulation was suggested. The scheme to transfer many cells with dual-beam laser manipulation is shown in Fig. 4. One trapping beam A was scanned on a line with the rate faster than motions of cells (Fig. 4a). We have been reported that many microparticles can be trapped by scanning the forces point 7. The other trapping beam B was used to trap an independent cell and to move it to the line sequentially (Fig. 4b). Finally, by moving microscope stage, many particles trapped at the scanning line can be transferred to another chamber (Fig. 4c).
The example is shown in Fig. 5. The trapping beam A was scanned on a line with the rate of 15 Hz whose length is 30 \( \mu \text{m} \). The trapping beam B was used to trap the yeast cell and to move it to the line sequentially, which is controlled by a mouse pointer. The cells stored on the line were successfully isolated. Here, three cells can be collected within 15 sec. Furthermore, the cells could be transferred with the velocity of 20 \( \mu \text{m/sec} \) by controlling the microscope stage.

Fig. 4: A schematic illustration of efficient collection and transfer of cells based by dual-beam laser manipulation.

Fig. 5: Collection and alignment of yeast cells using dual-beam laser manipulation. The micropictures of (b), (c), and (d) are taken at 3, 12, and 15 sec after that of (a), respectively.
5. CONCLUSION

Firstly, an operation to isolate specified cells was demonstrated by conducting single, fixed laser trapping in a transparent microchip including two chambers. A yeast cell was successfully transferred from the left to right chambers at the rate less than 30 sec/cell. Secondly, to realize further efficient cell transfer, the system was expanded to dual-beam laser manipulation system and combined with microimaging. It is considered that the collection rate will be improved by several times when we realize automation of the image recognition and laser manipulation. Finally, it is predicted that shortening in the sorting time by a few tens times will be realized by using dual-beam laser manipulation and the ability of the cell sorting will be larger than 10 particles per min.

In near future, the cells transferred to right chamber will be extracted as follows. The cells in the right chamber can be isolated by using pump B to insert air in the microchannel connecting both chambers. The isolated culture medium will be pulled in the syringe of pump C. This demonstration will be reported very soon elsewhere.

Recently, flow cytometer (cell sorter) has been attracted much attention as a selection system of biological cells, however, it is difficult to perform additional cell manipulation because the selection is executed in a flowing channel. In comparison with the cell sorter, the present system is superior as it combines cell manipulation using lasers not only in the routine of the cell sorting but also in cultivation before and after the sorting. As a further application, it will be possible to combine polymerase chain reaction system. Although it is difficult to compare both systems, the present cell manipulation system combining laser trapping with microimaging and microfluidic devices is expected to provide new possibilities for biotechnology.

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