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3D 6DOF Manipulation of Micro-object Using Laser Trapped Microtool

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***Abstract* - Laser tweezers is suitable for manipulation of a single microscopic biological object. It can manipulate micro bio-object without contact in the closed space. Single cell manipulation is important for biological research, and 3D 6DOF manipulation (Position control and Orientation control) is useful technique in many biological experiments. In this paper we propose 3D synchronized laser manipulation system that can manipulate multiple micro-objects along separate trajectories in 3D space. The Position as well as the orientation of microbeads can be controlled using this system. We succeeded in achieving orientation control of the microbead by using the laser trapped microtools. We demonstrate experimentally 3D 6DOF manipulation of microbeads.**

Index Terms – Micromanipulation, 3D, 6DOF, Laser tweezers, Optical trap, Microtool

I. INTRODUCTION

With recent developments in biotechnology, several methods for observing and manipulating bio-molecules and cells under the microscope have been developed [1]. The mechanical micromanipulator has been widely used for manipulation, but its operation is quite delicate and it requires considerable skills to use it. Both the tip of the micromanipulator and the object to be manipulate are small and fragile, thus there is a possibility of breaking the tip of the tool or damaging the object through a sudden collision.

On the other hand, non-contact micromanipulation employing dielectrophoretic force [2],[3] or laser trap [4]-[6] has been widely used, since it can manipulate the minute targets in the closed space. With these methods, there is no possibility of damage caused by collision and we can easily avoid mechanical damage to the object. Using dielectrophoretic manipulation techniques, it is possible to manipulate the object in three-dimensional space with six degrees of freedom. However, the dielectrophoretic force arises from an electric field gradient generated in the vicinity of an electrode in general, making it impossible to manipulate objects far from the electrode. Therefore, it is not suitable for manipulation of an object suspended in a chip.

Laser tweezers, on the other hand, is suitable for position control of individual objects suspended in the liquid, and a lot of work on this has been reported. Recently, attention has focused on 3D manipulation. For example, a technique using a spatial light modulator was proposed [8], and 3D manipulation of a laser-trapped object was achieved using it. However, to date, orientation control of the object using this method has not been reported. Two methods for achieving orientation control have been reported. One involved adjusting the laser beam profile in such a way as to apply a rotational moment to an object [8], while the other involved using an object having a specific shape [9]. However, these methods are not practical for our purposes since the direction of rotation is fixed for the first method, while the second method is only applicable to a trapped object having a specific shape.

Moreover, direct irradiation by a focused laser beam may damage the cell being manipulated by the laser tweezers [11]. To overcome this problem, we used a laser trapped object to manipulate the target object indirectly [12],[13]. The laser trapped tool used in this operation is called a "microtool". We can use two or more than microtools to realize dexterous manipulation of the target. Multiple targets can be manipulated independently using a single laser beam by changing the discrete laser scanning pattern. This is called "Synchronized laser micromanipulation: SLM"[14],[15].

We confirmed that it is possible to control the orientation of the target when two different points inside an object are trapped and then scanned along two independent laser trajectories in a circle [7]. However, it is not possible to avoid direct laser irradiation onto the object with this approach. It is possible to control the orientation of the target by using microtools without irradiating the object directly with the laser beam. Each trajectory of the microtool is controlled by SLM simultaneously. The target is sandwiched between two microtools and is rotated by the contact force. Although 2D orientation control in the focus plane was achieved in a previous report by us, 3D 6DOF manipulation of the target has not been achieved before.

For gene injection or nuclear transplantation, the 3D orientation control is effective in improving operation of the procedure when it is done in conjunction with 3D observation of the target. In this paper, we extend SLM to 3D SLM, making possible 3D control with 6DOF (position and orientation) of a microbead or microorganism. We verified the effectiveness of the position and orientation control method of the target using microtools in the Ray Optics regime (the object size was on the order of a μm).

II. MICROTOOL

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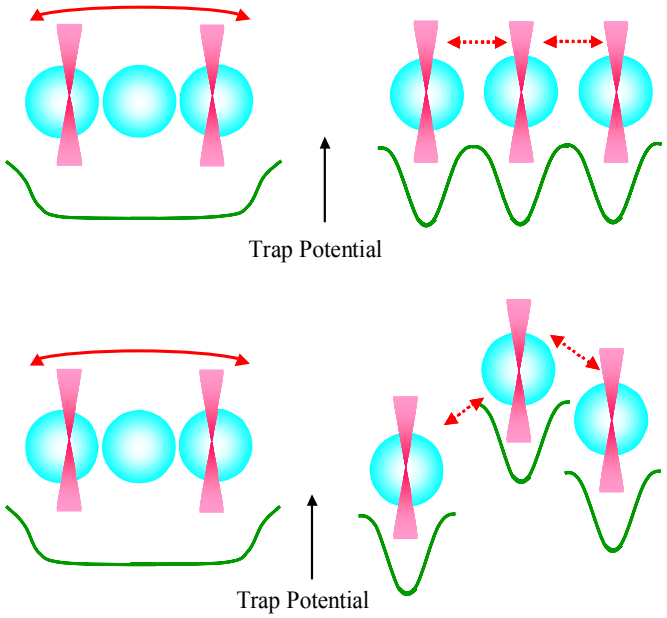
The advantages of using microtool with laser tweezers are as follows. (1) Firstly, we can avoid heat damage caused by direct laser irradiation of the target. it has been reported that direct irradiation by a focused laser may damage the trapped object [11]. This phenomenon depends on the target, wave length, irradiation time, and power of the laser. We can avoid this problem by using the indirect manipulation of the target by using laser trapped microtools. (2) Secondly, we can trap the microtool even if the refractive index of the target is the same as that of the environment. When the refractive index of the object is similar to the surrounding solution, the object cannot be trapped by laser because the laser trap power is very small under such condition. We can move the target indirectly by using the microtool which has a different refractive index. (3) Thirdly, we can control the orientation of the laser trapped object, and much more dexterous manipulation is possible than with other techniques.

III. Synchronized Laser Manipulation

Position of each laser trap potential can be controlled in the 2D or 3D space by changing the discrete laser scanning pattern. We can manipulate multiple targets or points with the specific trajectories, if the laser irradiation time and power is sufficient to move each object to the instantaneous focus point and to keep the particle moving along the desired trajectory. We call this multiple trajectory control method as the Synchronized Laser Micromanipulation (SLM). Figure 1 shows an example of a laser scanning pattern. A single laser having a high speed laser scanning system is required. The laser irradiation time is discrete, however, if the trapping time is long enough and trap stiffness is strong enough to realize the given trajectory. In this case, each target will move along its desired trajectory.



(a) Laser scanning micromanipulation (b) Synchronized laser micromanipulation

Fig.1 Comparison of laser scanning micromanipulation and SLM

IV. 3D Position Control System

Figure 2 shows a schematic diagram of the optical system used in this study. An image of the target is obtained using microscope equipped with a x100 (numerical number 1.35) oil-immersion objective lens and CCD camera. Nd:YVO_4 laser emission (1064 nm, continuous wave, max: 4.98W) for laser trapping is introduced into the microscope. The X-Y stage and object lens (Z axis) are actuated by a stepping motor. The laser is scanned in 2D observation plane by controlling the angle of a pair of galvanometer mirrors with DC motors. In addition, a piezo stage (PIFOC P-721, PI-Polytec) was used to drive an objective lens for scanning laser in the Z axis. These actuation units are controlled by a computer. As a result, high-speed movement in 3D space is possible. The response speed is much better than a system having stepping motors. In our system, high speed laser manipulation in 3D space synchronized at the focus plane is possible.

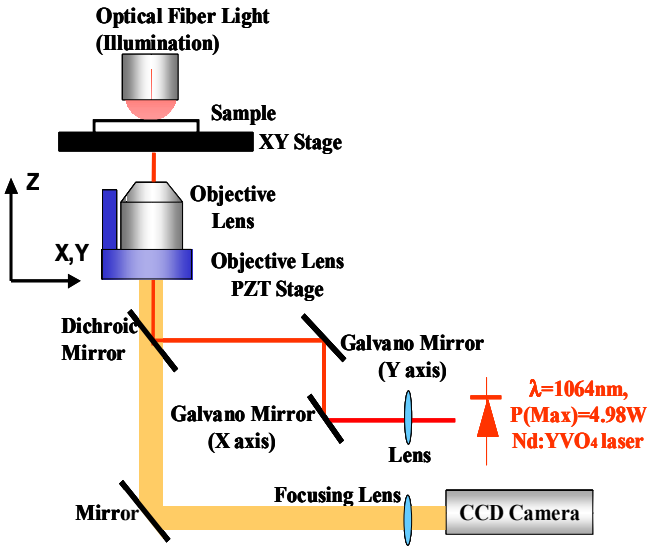


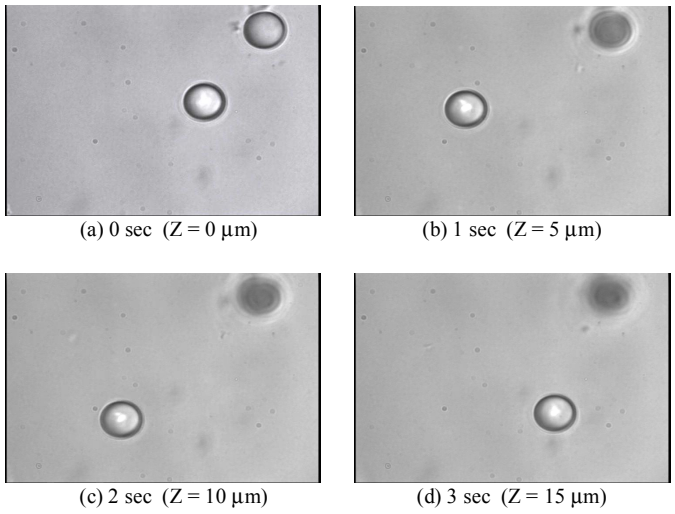
Fig.2 Schematic diagram of optics for 3D laser trapping

V. 3D Position Control Experiment

We manipulated the position of a polystyrene bead (7 μmϕ) in 3D space. In Fig. 3, the laser trapped microbead was moved along the quadrangle trajectory in the laser focus and observation plane. At the same time, it was moved in Z direction by moving the objective lens with the Z axis piezo stage. As it can be seen from the figure, 3D manipulation of the trapped microbead was achieved. The observation plane synchronizes with the manipulation plane. The change in height of the manipulated target (near the center of the photo) can been seen by comparing it with the microbead, which is immobilized on the bottom (near the top right of the photo). Initial value of the Z coordinate of these two beads was the same.

When the height of Z axis is set at around 100 μm, the laser trapped object is defocused in the image. This indicates that the height of the focus plane and the laser trap plane is

different at higher coordinates. In the future we intend to adjust the difference by adjusting the optical system. If the height of the target is not far from the bottom, it will not be a serious problem.



(a) 0 sec (Z = 0 µm) (b) 1 sec (Z = 5 µm)

(c) 2 sec (Z = 10 µm) (d) 3 sec (Z = 15 µm)

Fig.3 Experiment of 3D position control

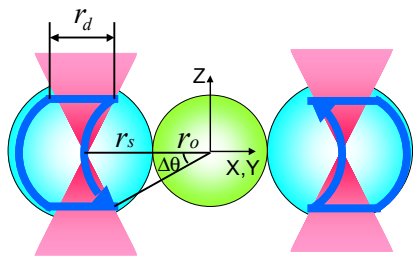


Fig.4 Laser scanning trajectory

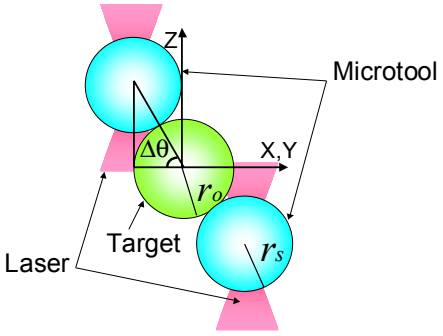


Fig.5 Schematic of 3D orientation control using microtools

VI. Three-Axis Orientation Control with Contact

In previous work, we have demonstrated orientation control of the target in the focus plane by using SLM with two laser trapped microtools. The laser was scanned in the 2D focus plane, making it impossible to control its orientation in 3D space. In this paper, we propose a manipulation technique that can control orientation of the target in 3D space using 3D SLM.

Figure 4 shows the conceptual diagram explaining how the target can be rotated indirectly using two microtools in contact with the target. The figure shows an example of a pair of scanning trajectories that can be generated by the 3D scanning system.

To realize stable manipulation of two microtools, we have to be careful in designing the scanning trajectory so as to avoid interference between objects, which depends on the relative position of the target and tools. For example, if we trap the tools as shown in Fig. 5, the tools will interfere with the target, since the tool overlaps with the target. It will happen depending on the relative position of the tool, target and laser focus position. To avoid this, Δθ which determines the laser scanning trajectory must satisfy the following condition.

$$(r_o + r_s)\cos\Delta\theta > r_o + \frac{4\lambda}{\pi}F \tag{1}$$

This expression was derived geometrically from Fig. 5. Here r_o and r_s are the radius of the target and microtool respectively. The second term on right-hand side of eqn. (1) is the radius of the laser spot. λ is the wave length of the laser radiation. F is the focal ratio of the microscope. When the target comes into contact with the rotating microtool, a resistance force is imparted to the microtool. Here we assume that the influence of fluid flow is negligible, and that there is no slipping between the microtool and the target. With these assumptions the force balance equation is given by the following.

$$f = \mu \cdot K\Delta r + 6\pi\eta r_s V + 8\pi\eta r_s^{\,2} \cdot \omega + f_o \tag{2}$$

Here, µ is coefficient of friction between the microbeads and the target, K is the trap stiffness of the laser trap, Δr is the amounts of pushing, V is the speed of the microtool, and f_o is the force exerted by the Brownian motion and other effects. These effects are assumed to be small and were neglected. To rotate the target through contact with the tools, the trapping force must be larger than the resistance force, and the necessary condition for rotation of the target is given as follows.

$$\begin{aligned} K \cdot 2(r_o + r_s)\Delta\theta > f \quad & (r_s \le 2(r_o + r_s)\Delta\theta) \\ K \cdot r_s > f \quad & (r_s > 2(r_o + r_s)\Delta\theta) \end{aligned} \tag{3}$$

Here, it was assumed that K is the maximum at the edge of the bead.

When the microtools move along the trajectory shown in Fig. 4 and the above condition is satisfied, and if the influence of the fluid flow is neglected and there is no slipping between the microtool and the target, then rotational speed ω of the target can be written as follows.

$$\omega = 2\Delta\theta \cdot \frac{V}{2r_d + 2(r_o + r_s)\cdot 2\Delta\theta} \tag{4}$$

In the case of using microbead of 10 μmϕ as a microtool being used to rotate the target of 12 μmϕ. The rotational speed ω and rotation condition are estimated as follows.

$$0 < \Delta\theta < 0.88 \quad \text{rad}, \qquad 0 < \omega < 1.55 \quad \text{rad/s} \qquad (5)$$

Here we used following values.

$r_s = 5$ μm, $r_o = 6$ μm, $\lambda = 1.064$ μm, $F = 0.74$, $V = 47.39$ μm/s, $r_d = 4$ μm, $\mu = 0.1$, $\Delta r = 0.1$ μm, $\eta = 1.002$ MPa*s, $K = 10.1$ pN/μm

In reality, it is difficult to measure the coefficient of friction μ. So, in the above calculation, we used a tentative value for μ.

VII. Three-Axis Orientation Control Experiment with Contact

A. *Rotation control in observation plane*

To verify the derived equation (5), we measured the experimental rotational speed ω using the 10 μmϕ polystyrene bead as a microtool to control the 12 μmϕ bead. This was restricted to the X-Y plane (2D plane), as this made it easier to measure the rotational speed from a video signal. Figure 6 shows the comparison between the calculated data and the experimentally measured data of rotational speed ω for different rotation angles Δθ. In this experiment, we used a 12 μmϕ bead which has a 500 nmϕ bead inside it. The 500 nmϕ bead was put inside the 12 μmϕ bead made of photocrosslinkable resin (ENTP), and it was cured using UV irradiation [16].

In Fig. 6, there is a significant difference between the calculated results and the experimental results. We consider three major reasons for this error. (1) Firstly, the microtool may not make secure contact with the target in the experiment. (2) Secondly, the effect of slip was neglected in the analysis. (3) Thirdly, even when the microtools make secure contact with the target, reverse rotation of the target occurs in the interval during which the microtools return to the point of contact. This had the effect of reducing the total rotation angle of the target.

The reverse motion of the target occurs even though there is no contact between the tool and the target. Figure 7 shows the reverse motion of the target. In this figure, the target rotates about 0.12 rad in the reverse direction during the time interval from t = 2 sec to t = 3 sec. This observed reverse motion demonstrates that even when the microtool is not in direct contact with the target, the influence of its motion on the target is not negligible. The viscous force of the water is large enough to rotate the minute target; it is remarkable that the viscous force exerts such a strong effect on an object as small as the target. This effect is similar to the viscous force produced by the motion of shear stress flow of an incompressible fluid near a wall and is known as Couette flow.

Figure 8 shows a plot of rotation angle of the target as a function of contact angle of the microtool for microtools having different diameters. In this figure, distance r_d (see Fig. 4) was varied, and the relation between the contact angle of the laser scanning trajectory cycle Δθ and the rotation angle of the target Δϕ was measured.

The distance between the target and the tool has a remarkable effect on the rotation angle of the target. From these results, we will be able to improve the accuracy of the rotation speed model. The results demonstrate that it is important to consider the viscosity of water when calculating the rotation speed. It is our intention to improve the modeling accuracy by considering the effect of reverse rotation on the rotation of the target.

We also used a yeast cell as the target. We were able to rotate it as shown in Fig. 9.

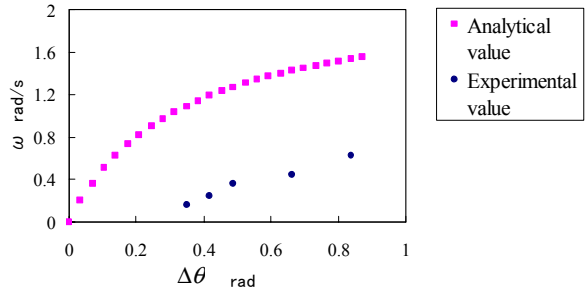


Fig.6 Comparison of analytical value with experimental value

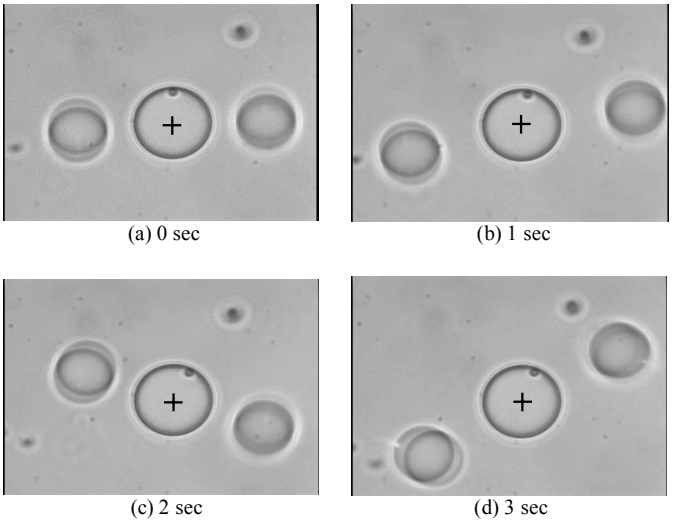


Fig.7 Experiment of noncontact rotation by Couette flow
(+ sign is the center of sphere)

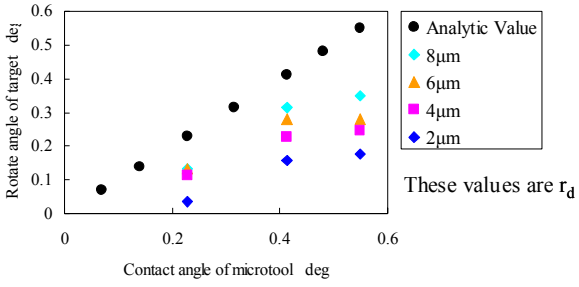
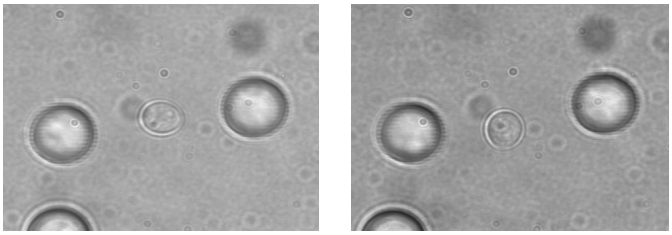


Fig. 8 Rotation angle of target by changing trajectory of mictotools



(a) Angle 0 deg (b) Angle 90 deg

Fig. 9 Experiment of yeast cell rotation

B. Rotation control around an axis in the observation plane

We rotated the target around an axis (the X or Y axis) in the observation plane. A laser trajectory was generated by synchronizing the Galvanometer mirrors and PZT actuator that drives the objective lens. The width of the scanning in the Z direction was adjusted between 1 to 1.5 μm. Here, as in the previous experiment, we used a 12 μmϕ bead which had a 500 nmϕ bead inside it.

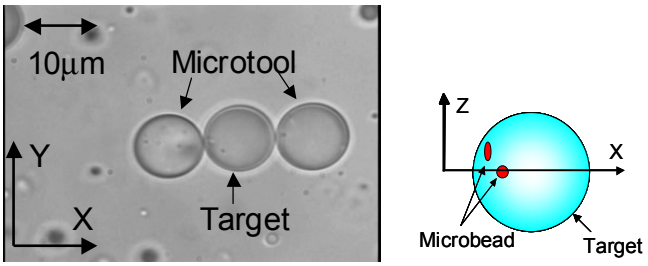
Figure 10 shows some video stills of the experiment. It can be clearly seen that the nano bead put inside the target is defocused in Fig.10(b) while it is in focus in Fig.10(a) and (c), indicating that its position had changed. Thus, we can confirm rotation of the target around an axis in the observation plane using this technique. In addition, we were able to confirm that the rotational speed of the target was adjusted by changing the manipulation speed of the microtool. Figure 11 shows the plot of the average rotation speed after rotating through 180 degrees. These results confirm that the rotation speed of the target varies in proportion to the manipulation speed of the microtool. This is an important result since it demonstrates that 3D orientation control of the target is possible.

Orientation control of a yeast cell was also possible by using this technique. However, the yeast cells are small and had irregular shapes, and it was difficult to observe their rotation in the observation plane since the images is blurred.

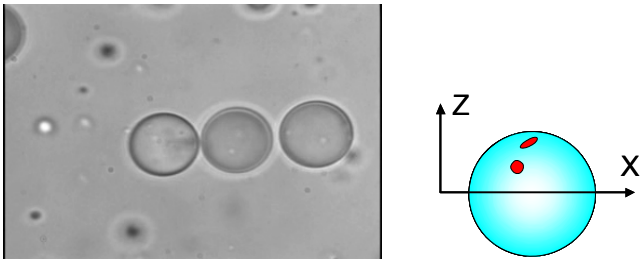
The rotation speed measured by experiment was only about 1/8 of that obtained by calculations. The reasons for this discrepancy are similar to those given earlier. There was not sufficient contact between the tool and the target. The gap between the tool and the target was estimated from the images obtained from the microscope. Moreover, there was reverse rotation caused by the viscous effect. The orientation control in 3D is much more difficult than that in the observation plane. The laser is focused along the Z direction, and there will be interference between the tool and target, since the distance between the laser and the target is closer compared to that when the rotation is restricted to the observation plane. The stability of the target in z direction will be worse depending on the laser scanning trajectory. Moreover, current optical system gives vibration by driving the object lens.

When the output of the laser was increased, the target became unstable and it was impossible to control it. In addition, if the scanning speed of the laser was increased, the trap power is not sufficiently large to control the trajectory. Therefore, in our experiment, it was difficult to control the microtools when they were rotated at a rate faster than 0.45 rad/s.

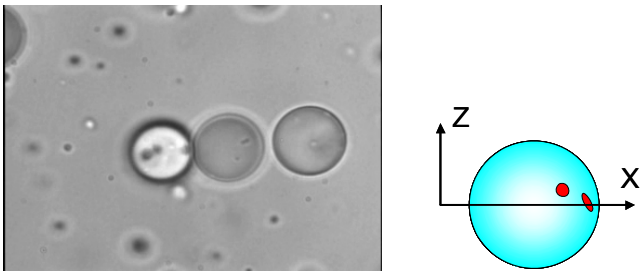
In evaluating control performance, it is difficult to observe the orientation of the target. We can improve the measurement system by employing the confocal laser scanning microscope. It should be possible to solve this problem in the near future by using existing technology.



(a) Initial state 0 sec, Top view (left) and side view (right)



(b) Intermediate state 60 sec, Top view (left) and side view (right)



(c) Final state 180 sec, Top view (left) and side view (right)

Fig.10 Experiment of 3D orientation control

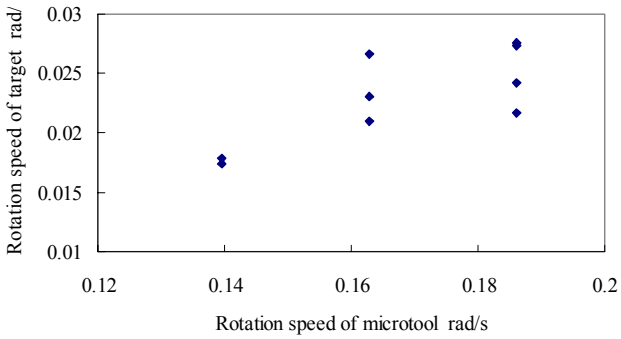
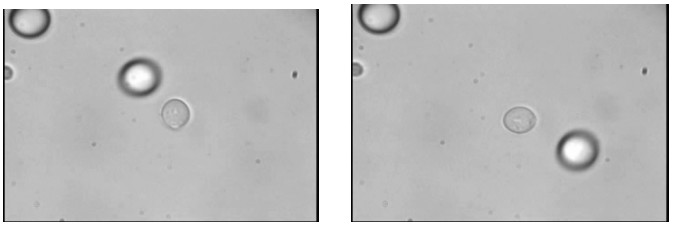


Fig.11 Rotation speed of target by changing laser scan speed

C. *Orientation control using fluid force*

In the previously described experiment, we demonstrated that the target rotated due to the fluid between the microtool and the target, even when there was no contact between the tool and the target. Rotation torque is generated on the target by the Couette flow. We utilized this phenomenon positively to achieve the orientation control. Figure 12 shows that we can rotate a yeast cell by moving a single 7 μmϕ microtool in a circular trajectory around the cell. In this case, since we are using fluid force, there is no need for contact between the tool and the target. Thus it is possible to realize stable manipulation. Another advantage is that interference with the laser will not occur. However, the yeast cell is put in the vicinity of the bottom of the holder, so it was impossible to generate proper trajectory around it. If we have 3D free space surrounding the target, it should be relatively easy to utilize fluid force to rotate the target.



(a) 0 sec (b) 2 sec

Fig.12 Experiment of noncontact rotation using a single microtool

VIII. CONCLUSION

In this paper, we proposed the method for achieving position and orientation control of micro-objects in 3D space using laser tweezers and microtools. We developed the system for 3D SLM by using 3D laser scanning system with Galvanometer mirrors and a PZT actuator which drives an objective lens. We demonstrated 3D 6DOF control of microbead in the Ray Optics regime (object size on the order of a μm). It was confirmed that the rotational speed of the target can be adjusted by changing the trajectory of the microtool. In addition, we succeeded in rotating the target by rotating the microtool in its vicinity, without any contact between the two. The rotation efficiency varied depending on the distance of the microtool and the target. Using this approach, we can achieve much more stable control of the orientation, since there is no need for contact between the microtool and the target. This is a new method for manipulation a target, and will be suitable for manipulation of small objects having irregular shapes such as microorganisms. If we have 3D free space around the target, it should be straightforward to utilize fluid force to rotate the target.

ACKNOWLEDGMENT

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