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Massive Parallel Assembly of Microbeads for Fabrication of Microtools Having Spherical Structure and Powerful Laser Manipulation

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Abstract— **Production of functional microtools having an arbitrary shape by self-assembly of microparticles and heat treatment above the glass transition temperature of the microparticles was developed. Polystyrene microbeads were used as a material of the microtool. A solution including microparticles was dispersed onto the silicon substrate having microtool patterns fabricated by photolithography and etching. Dispersed particles were introduced to the pattern by gravity force. Microparticles in the pattern aggregate autonomously by surface tension through evaporation of the solution. Aggregated microparticles were fused by heating above the glass transition temperature (100 Celsius degrees). Fused microparticles were detached from the pattern by ultrasonic treatment and used as microtools. Produced microtool has spherical part since the microtool is made of microparticles. Spherical part is suitable for trapping point of optical tweezers. We demonstrated production of microtools using self-assembly and manipulation of the fabricated microtool on a chip. Position and attitude accuracy of the fabricated microtool controlled by TSS were evaluated by image processing. Finally, the transport speed of the fabricated microtool was compared with that of the photofabricated microtool. We confirmed the improvement of the transport speed and the effectiveness of our proposed microtool.**

I. INTRODUCTION

RECENTLY, manipulation and measurement of single cell have been important for cell analysis. Several researches have done with contact manipulation, which uses micropipettes. However, this manipulation method needs skilled technique and has a possibility to injure the cell. And it causes disturbance and contamination. Hence optical tweezers is thought as a suitable method for cell manipulation in a closed space [1-2]. Single optical trapping was first achieved using two laser beams in 1970 [3]. Then, single optical trapping was achieved with one laser in 1986 [4]. In optical tweezers, a laser beam is introduced into an inverted microscope and is focused with the objective lens for generating radiation pressure. In such a way, we can trap dielectric objects at the focus [5]. However, direct irradiation of the focused laser is harmful to the cells in some cases. Therefore, we proposed indirect cell manipulation by laser manipulated microtools as shown in Fig. 1 [6, 7].

In our previous studies, indirect cell manipulation using microparticles as microtool was performed [8]. In 1999, we manipulated rod-shaped bacterium (bacillus) as a tool. This is the first microtool. In 2001, we produced 3D microtool by SEM manipulation and electron-beam-induced-deposition. In 2004, we produced arbitrary shape microtool by binding microsphere using local photo fabrication. We manipulated nano-scale biomaterials such as DNA and virus using microtool made of thermosensitive hydrogel [9]. From 2006, we produced microtool having the arbitrary shape using photolithography because the productivity of conventional microtools was low. Moreover, smart microtools having pH and temperature sensitivities were developed for simultaneous use for manipulation and measurement in 2006 [10]. On control technique, Multi-beam optical tweezers such as a time-shared approach and spatial-shared approach were developed in 2002. By combination use of microtool and multi-beam optical tweezers, 6-DOF manipulation of microparticle was achieved in 2006.

There are some methods for constructing micro-scale objects such as photolithography and two-photon stereolithography [11, 12]. Although photolithography can achieve the mass production of the microstructure, shape of the structure was 2-dimensional pattern because production of the 3-dimensional structure is difficult. Two-photon stereolithography can produce the 3-dimensional nano-scale structure. However, throughput of production was low compared with photolithography.

In this paper, we proposed a new fabrication method of microtool having spherical structure to achieve mass production of microtool of high trap efficiency. We fabricated microtool having the spherical structure by the self-assembly of microspheres. We employed step-by-step deposition method for aligning the microparticles into the substrate having the photo fabricated patterns. Aligned microspheres are connected by heat to glass transition temperature. Fabricated microtools are released from the substrate and manipulated by Integrated Optical Tweezers (IOT) [13]. We demonstrated the self-assembly of the microtool and laser manipulation of the fabricated microtool. We confirmed that the trap efficiency of self-assembled

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microtool was improved better than the conventional photo fabricated microtool.

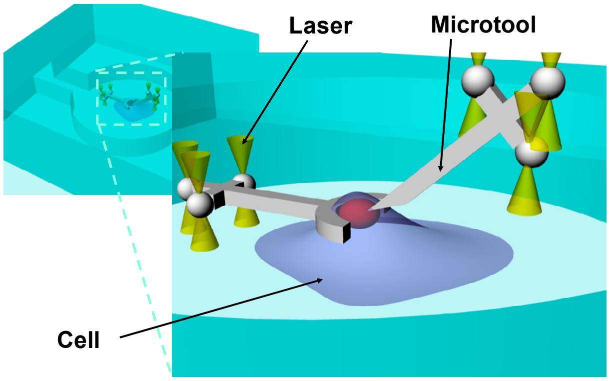


Fig. 1 Schematic diagram of the cell surgery using microtool.

II. MANIPULATION OF MICROTOOL BY OPTICAL TWEEZERS

A. *Integrated Optical Tweezers*

We used optical tweezers for manipulation of the microtool. Figure 2 shows integrated optical tweezers (IOT) optical system [13]. The bottom part is time shared scanning (TSS) optical system [11]. In this part, we used polarized Nd: YVO_4 & Nd:YAG laser (Laser1) of which wave length is 1064 nm. The deadened laser with ND filter (ND) was introduced into mirror (M1, M2) and biaxial galvano mirrors (GM: Harmonic Drive Systems). We scanned the laser quickly with these galvano mirrors to trap and manipulate multiple objects. This scanned laser acts like the multi-beam laser. Dichroic mirror (DM) of an inverted microscope reflected the infrared laser beam and the beam is introduced into the objective lens (OL: Olympus x100 NA=1.4). The laser beam was focused with high numerical aperture.

The upper part is generalized phase contrast optical system. Generalized Phase Contrast (GPC) is the extension of Zernike phase contrast scheme [14]. With the phase contrast scheme, we can convert phase distribution of lasers to intensity distribution. If we make phase distribution like multi-beam, we can obtain the complete multi-beam. In GPC system, we used Yb fiber laser (Laser2) the wave length of which was 1064 nm. The laser was the incident on a Spatial Light Modulator (L-COS: Hamamatsu Photonics). The L-COS can input phase information into the coherent laser beam. The reflected laser from SLM was introduced into 4f optical processing setup, which had two Fourier lenses (L5, L6) and Phase Contrast Filter (PCF). Phase distribution was processed by Fourier transform and converted to the frequency domain at the back focal plane (Fourier plane). GPC system uses these two different regions to obtain intensity distribution from second Fourier lens (L6). With this system, phase information was converted into intensity distribution. Lens (L7) and Objective lens (OL) scaled the intensity distribution in the manipulation plane. Small objects can be trapped at the high intensity spot at the microscopic plane.

To integrate TSS and GPC, we used the Polarization Beam Splitter (PBS). The PBS fused the two polarized laser beams with small losses of the power.

In TSS system, we controlled the laser position with biaxial galvano mirrors. Control instruction was generated in Personal Computer (PC) and transferred into the motor driver via DA converter. The images of the manipulation results were obtained to PC from CCD camera mounted in the microscope via the image capture board. For these processes, we developed control program, which had user-friendly Graphical User Interface (GUI). Figure 3 shows the developed control system. This system used two manipulators for controlling laser position, and captured image was displayed on GUI for manipulability.

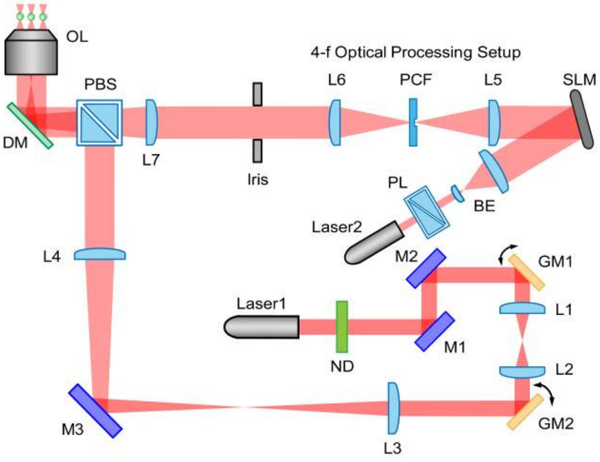


Fig. 2 Optical system of IOT.

Fig. 3 Bilateral control of microtool by TSS

B. *Microtool for optical manipulation*

We have proposed microtool for stable and dexterous manipulation of cell in a microfluidic chip as shown in Fig. 4. In this figure, this method uses two microtools, which have been disk-shaped rotating base and arrow edge for contact of a cell. These two microtools grasp and manipulate a cell. Because GPC is suitable for static laser trap, it traps the rotating base of the microtools. The static laser trapping with

GPC avoids microtools from dispersing. Because TSS is suitable for dynamic laser trap, it traps and manipulates the edge of the microtools to rotate. Three-dimensional laser trapping point such as the sphere is desired for strong trapping force. And not only the sphere but also different shapes and functions for different purposes are favorable as the microtool design. Figure 5 shows the photograph of photofabricated microtool. Figure 5(b) in manipulation of the microtools by TSS and GPC as shown Fig. 4. However, manipulation was not stable because trap efficiency of plate structure is not good. Microtool having structure of high trap efficiency is required for dexterous manipulation by optical tweezers.

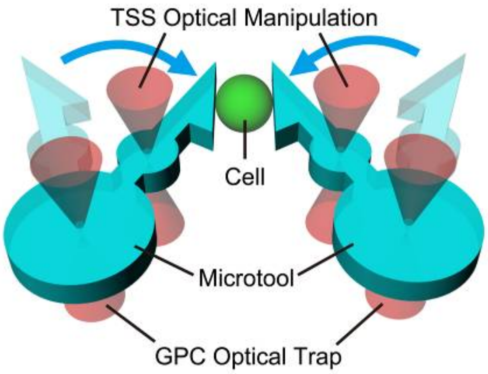
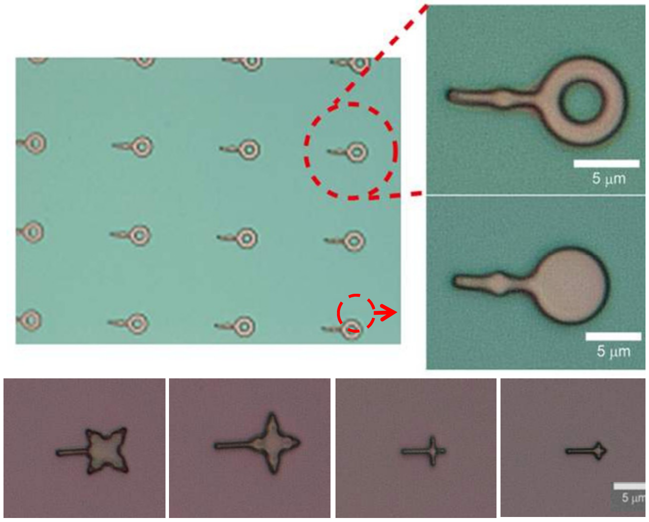
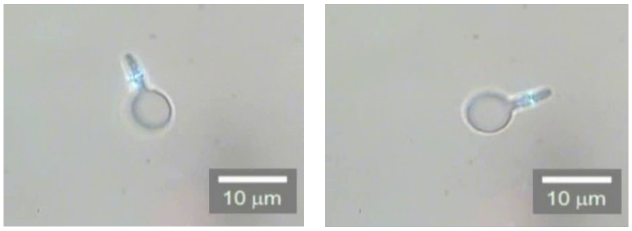


Fig.4 Concept of cell manipulation with microtools.



(a) Photographs of microtool fabricated by photolithography



(b) Trap of microtool (c) Rotation of microtool

Fig. 5 Microtool fabricated by photolithography.

III. SELF-ASSEMBLY OF MICROTOOL HAVING SPHERICAL STRUCTURE

A. Effect of the Structure of Microtool to Trap Force

Trap efficiency of conventional photo fabricated microtool is weak because the microtool is 2-dimensional shape. First, we compare the trap force between the cylindrical shape microtool made of SU-8(diameter: 5 µm, thickness: 5 µm, refractive index: 1.6) and microsphere (diameter: 5 µm, refractive index: 1.59) as shown in Fig. 6. Laser power was 35 mW. Trapping forces for microtool was measured with the drag-force method [15]. The microtool was trapped to move in the water. When the moving speed increase, the drag forces increase proportionally and become equal to the trap force as shown in Fig. 7. When the microtool escaped from the trap, the current drag force was equal to the trap force. Using the Stoke's law, the drag forces on microtools to viscous fluid flow are shown in equations 1 and 2.

$$F_s = 6\pi\mu r V_s \tag{1}$$

$$F_c = \frac{8\pi\mu l V_c}{1/2 - \gamma - \ln(8/(V_c \cdot d/\eta)} \tag{2}$$

where is F_s is drag force on spherical microtool, F_c is drag force on cylindrical microtool, μ (= 1.002 [mPa∗s]) is the viscosity at 20 Celsius degrees, r is radius of the spherical microtool, l is length of cylindrical microtool, γ is Euler's constant (= 0.557) , d is a characteristic linear dimension, η (= 1.004 [m^2/s]) is the kinematic viscosity at 20 Celsius degrees, V_s is the speed of the spherical microtool, and V_c is the speed of the cylindrical microtool.

As a result, trap force of the spherical microtool is about 5 times stronger as shown in Table 1. From this result, we developed an arbitrary shape microtool by the self-assembly of microspheres for producing microtool having high trap efficiency as shown in Fig. 8.

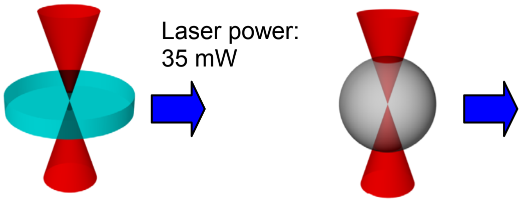


Fig. 6 Schematic of comparison of trap force between spherical too and cylindrical tool.

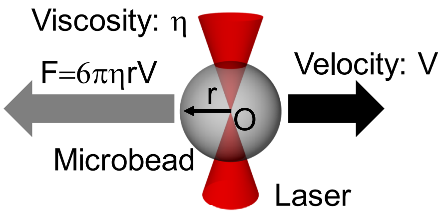


Fig. 7 Concept of cell manipulation with microtools.

Table 1 Comparison result of trap force between spherical tool and cylindrical tool.

Shape of microtool	Trap force pN
Cylindrical structure	5.7
Spherical structure	22.7

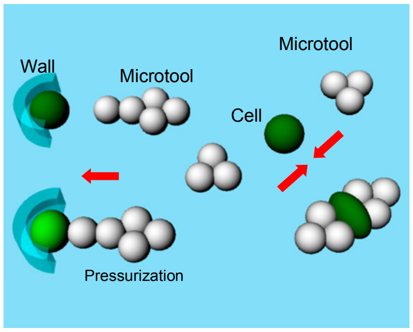
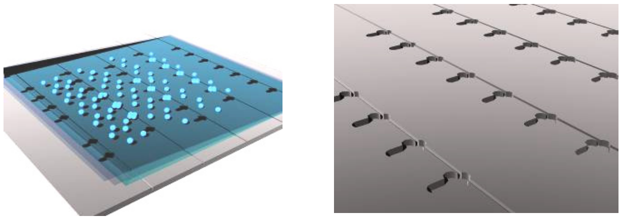


Fig. 8 Concept of cell manipulation with self-assembled microtools.

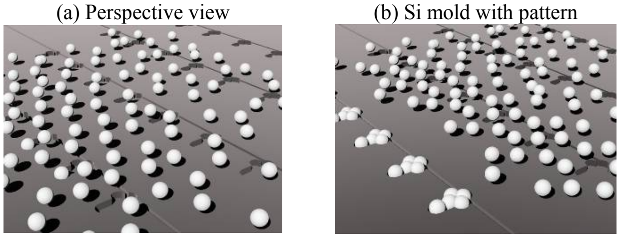
B. *Fabrication of Microtool by Step-by-Step Deposition Method and Heat Treatment*

A schematic diagram of fabrication of microtool using self-assembly was shown in Fig. 9. First, the substrate having the pattern is fabricated by photolithography. The solution including the microsphere is dropped onto the substrate and the microspheres are aligned along the pattern by step-by-step deposition method as shown in Fig. 10 [16, 17]. During the evaporation of the solution, microspheres are injected in to the pattern by the gravity force and controlled move of the water surface by the cover glass. This method can align the microspheres by controlling the shape and size of the pattern. The aligned microspheres in the pattern are formed close-packed structure by surface tension during the evaporation. Finally, microspheres are connected by heating at glass transition temperature. Fabricated microtools are released from the substrate by ultrasonic treatment.

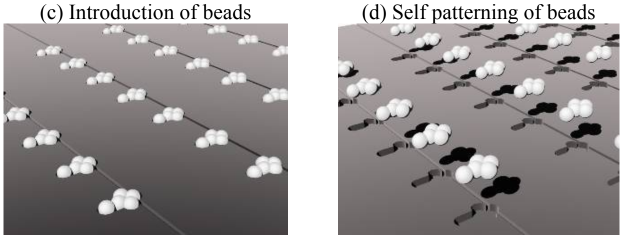
In this research substrate for alignment of microspheres was fabricated by two methods. One method was based on fabrication of the mold pattern by photolithography as shown in Fig. 11. Positive photoresist OFPR-800 (Tokyo Ohka) was spin-coated on the Si wafer. After exposure, mold pattern was fabricated by development. Another method was based on fabrication of the mold pattern by fabricating Si wafer as shown in Fig. 12. First, the OFPR pattern was fabricated by the same process of previous method. This OFPR pattern was used as resist mask for Si etching. Si wafer was etched by reactive ion etching. Finally, cleaning the Si wafer. Each mold has merit and demerit against another mold. In the mold made of photoresist, process is easy and precision of the mold is good. However, extraction of the fabricated microtool is difficult since the smoothness of the OFPR mold is not so good. On the other hand, extraction of the microtool from the Si mold is easy; however, fabrication process is complicated and precision of the pattern is not so good. However, both molds can fabricate self-assembled microtool. These molds can be selected according to the shape of the microtool and required precision of the tool shape.



(a) Perspective view (b) Si mold with pattern



(c) Introduction of beads (d) Self patterning of beads



(e) Assembled microtools (f) Remove microtools

Fig. 9 Concept of self-assembly process

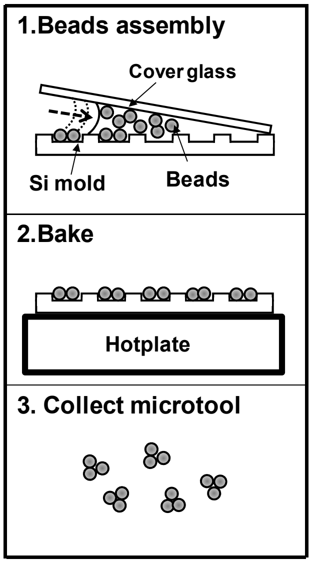


Fig. 10 Schematic diagram of alignment and connection of the microsphere.

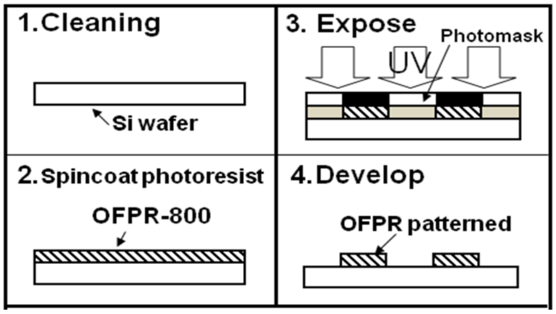


Fig. 11 Fabrication process of OFPR mold.

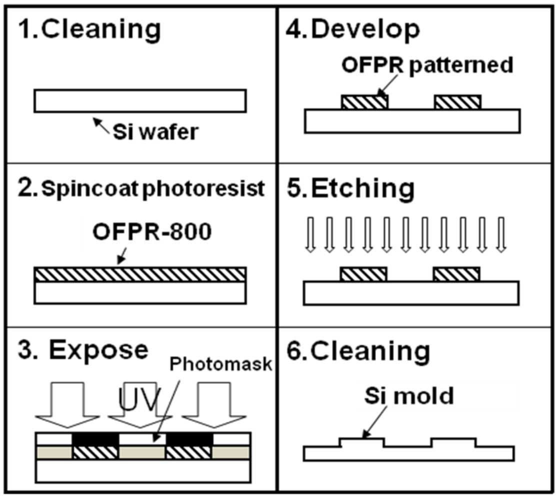


Fig. 12 Fabrication process of Si mold.

IV. EXPERIMENT

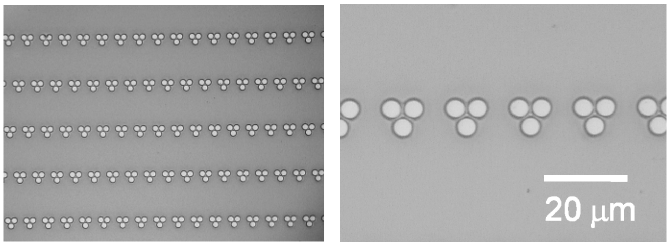
A. Self-assembly of microtool having spherical structure

Figure 13 shows the molds for the patterning the microspheres. We fabricated both OFPR mold and Si mold. In this experiment, microtools with two different shapes were fabricated. Triangle shape microtool was aimed for stable and precise manipulation of the cell as shown in Fig. 8 and was fabricated using OFPR mold. Cross shape microtool is aimed for mechanical stimulus to the cell by pushing by the edge of the microtool as shown in Fig. 8.

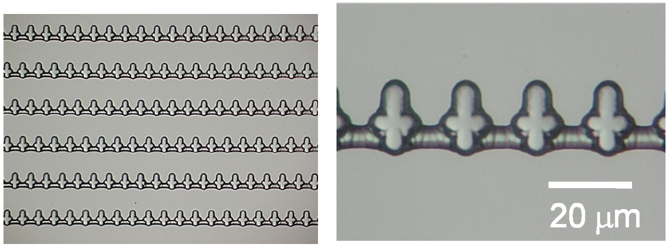
Figure 14 shows the fabrication results of the microtools using self-assembly. In this research, polystyrene (PS) (glass transition temperature: 100 degrees C) microsphere was used. Diameter of the bead was 5 μm. These microspheres were dropped and self-aligned in the pattern using step-by-step deposition method. In this experiment, pattern of cross shape microtool was fabricated on Si wafer. The size of long axis was 20 μm and that of short axis was 13 μm. Thickness of the mold was 5 μm. In OFPR mold, pattern of cross shape microtool was fabricated. The size of each hole was 5 μm in diameter. Thickness of the mold was 2 μm. Angle of the cover glass was adjusted 2 degrees in experiment. As a result, microspheres were aligned in both patterns. After evaporation of the solution, substrate was heated at 130 degrees C on the hot plate for 15 minutes. After heating, microtools were extracted as shown in Figs. 14 (f), (g).

B. Manipulation of self-assembled microtool

All experiment was performed in pure water, of which refractive index was 1.33. Figure 15 shows manipulation of the triangle microtool. In this experiment, two triangle shape microtools were manipulated by TSS. Triangle microtool is composed of three microspheres. Control of position and attitude of microtools was performed by trap of each microsphere. Unexpected rotation of the microtool was avoided because the microtool was trapped by three points. Automation with force feedback control of the microtool will be achieved by employing image processing and it is our future work. Comparison of the transport speed of triangle shape microtool fabricated by the self-assembly with that of photofabricated microtool was shown in Fig. 16. The shape of photofabricated microtool was like combined three cylindrical objects as shown in Fig. 16. The diameter of the cylindrical object was 5 μm and thickness of it was 5 μm. Laser power was 35 mW. In this experiment, we compared

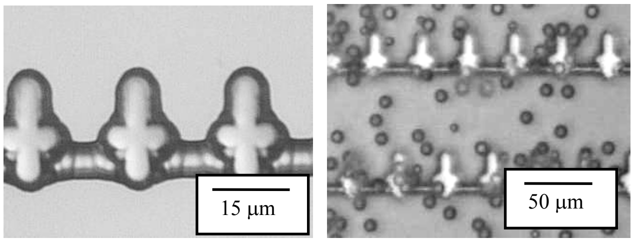


(a) OFPR mold

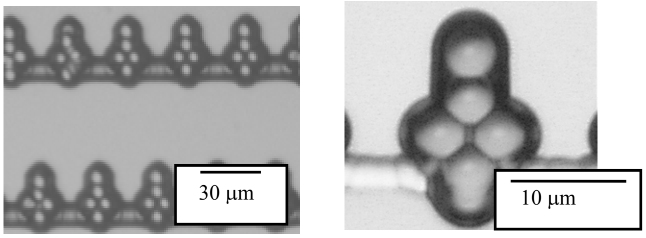


(b) Si mold

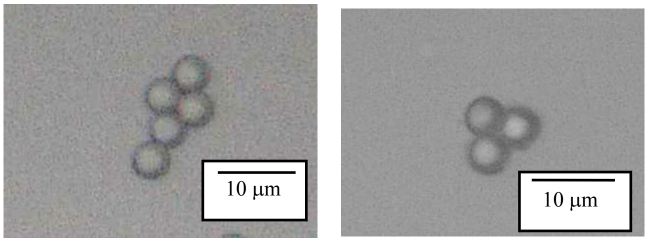
Fig.13 OFPR and Si mold



(a) Si mold (b) Injection of 5 μm PS beads

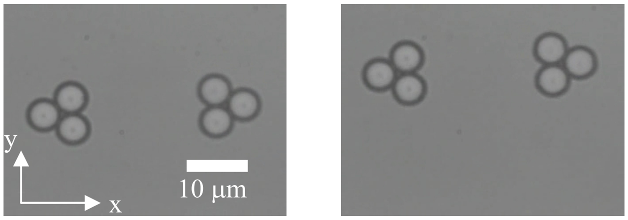


(c) Alignment of 5 μm PS beads (d) Aggregated microbeads

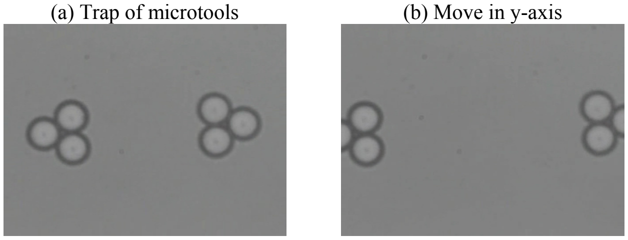


(e) Cross shape microtool (f) Triangle shape microtool

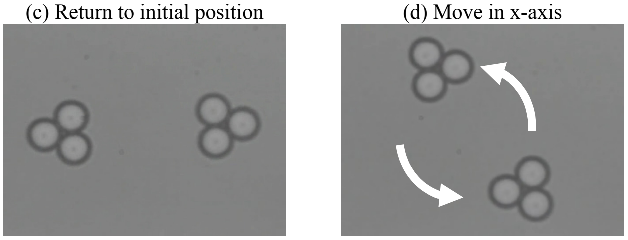
Fig. 14 Fabrication of the microtools using self-assembly the transport speed. Measurement of trap force of such microtools from equations 1 and 2 was difficult because calculating force of stokes drag of such complicated structure was difficult. Measurement of trap force is future work. Transport speed of triangle shape microtool was about 6 times faster than that of photofabricated microtool as shown in Table 2. From these results, effectiveness of self-assembled microtool on the manipulation was confirmed.



(a) Trap of microtools (b) Move in y-axis



(c) Return to initial position (d) Move in x-axis



(e) Return to initial position (f) Rotation of microtool

Fig.15 Images of manipulation of microtools

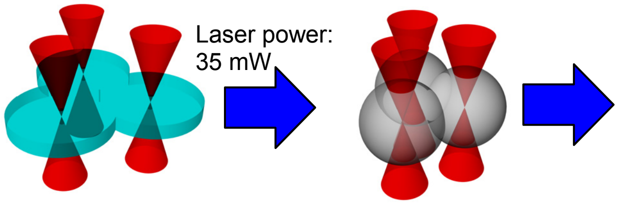


Fig. 16 Schematic of comparison of transport speed between photofabricated microtool and microtool fabricated by self-assembly.

Table 2 Comparison of transport speed between photofabricated microtool and microtool fabricated by self-assembly.

Shape of microtool	Transport speed µm/s
Photofabricated tool	1.5
Self-assembled tool	8.3

V. CONCLUSION

In this paper, we proposed self-assembled microtool having the spherical structure using step-by-step deposition method and connected by heat to glass transition temperature. And we demonstrated the fabrication of the microtool and manipulation of the microtool by IOT. We confirmed that the trap efficiency of the self-assembled microtool was improved from conventional photo fabricated microtool. Fabricated microtool can be controlled precisely by optical tweezers.

Moreover, this fabrication method can achieve mass production of arbitrary shape microtool. This technique will make a great contribution to cell biology.

ACKNOWLEDGMENT

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