The 2009 IEEE/RSJ International Conference on
Intelligent Robots and Systems
October 11-15, 2009 St. Louis, USA

On-Chip Fabrication and Assembly of Rotational Microstructures

Masaki ITO[*], *Masahiro NAKAJIMA*[*], *Hisataka MARUYAMA*[**], *and Toshio FUKUDA*[*]

***Abstract*—The microstructures are expected to be used as the components of on-chip single cell analysis system such as cell manipulation and measurement tools. In this paper, we propose the on-chip fabrication method of microstructures with minimum dimensions of 5 µm. The micro objects with arbitrary shape are fabricated by illumination of patterned UV-ray through the mask. The arbitrary shape objects are made of the photo-crosslinkable resin on the microchip under a microscope. The polymerized micro-objects, which we call as "Microtools", can be used for the single cell analysis. The microtools are fabricated at the desired place on the microchip in less than 1 second. Additionally, the microtools are manipulated by optical tweezers. By high-speed scanning of a single laser with galvanometer mirror, it is able to manipulate multiple points simultaneously. Using this technique, the rotational microstructure composed of the rotation axis and the microgear is assembled and rotated. We propose the two method of microstructure assembly. The assembled rotational microstructure is evaluated about the rotation speed depending on the laser power.**

I. Introduction

The group cell analysis has been widely used in biotechnology. Through the group cell analysis, only averaged information is obtained on each type of cells. Recently, the single cell analysis receives high attention to know more detailed information on the individual cell [1].

The microchip is effective to realize a stable environment for the single cell experiment. The microchip has advantages such as low-cost, high integration and high speed analysis. The elemental technologies for the on-chip cell analysis are the cell sorter which enables the division and retrieving of specified cells [2], the system of cell response measurement [3] and the keeping and immobilization technique of cells at specific space [4]. Additionally, the methods are expected to establish that are used for the research of the physical property of cell such as the stiffness measurement and local environment measurement and control around cell [5]. For example, the method that a micro object is pressed against a cell to measure the reactive force of the cell is proposed. The cell stiffness is measured in this method. It is considered that the cell-sized tool which can be manipulated on the microchip is effective for applying to the on-chip cell analysis.

It is possible to improve the functions of the microchip by building microstructures inside the channel. There are several researches to apply the microstructures. The 3D complicated shape microstructures can be fabricated by using two photon microfabrication with about 100 nm resolution. The stiffness measurement of the cell was realized using the microstructures [6], [7]. However, the expensive device is needed to fabricate these structures. The fabrication process is so troublesome that it takes long time. In this method, the rinse process, which flushes the non-polymerized resin out of the channel and inject the other solvent, is indispensable after the prefabricated microstructures are fixed on the microchip. It is difficult to fabricate the microstructure during the cell experiment and to transfer the prefabricated microstructure to other place. The work place of this microstructure is restricted on the microchip at predetermined spot. On the other hand, by using "Microtool" made of polystyrene beads or photo-crosslinkable resin, the single cell analysis and local environment control have been investigated [8], [9]. However, many shapes of these conventional microtools are too simple such as sphere and stick. So it is inadequacy to construct the microchip system for the cell analysis.

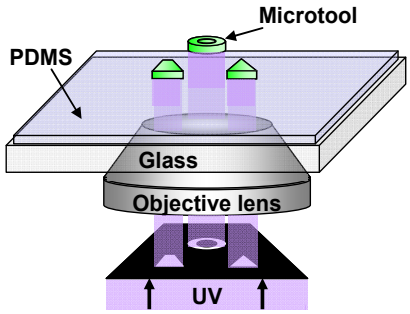

Fig.1 A schematic of on-chip fabrication of arbitrary shape microtools by UV irradiation though the mask

In this research, we propose the on-chip fabrication method of microstructures expected to apply to the single cell analysis system. As shown in Fig. 1, under the microscope observation, the patterned UV-ray is illuminated through the mask to photo-crosslinkable resin injected to the channel in the microchip made of polydimethylsiloxane (PDMS). The photo-crosslinkable resin, which is PEG-DA, has low toxicity [10]. This resin is polymerized and the arbitrary shape microtools are fabricated at the desired place on the microchip. There is no need to prefabricate microstructures or to inject the microtools from the outside into microchip. It is possible to fabricate only the required microtools at desired place. The fabricated microtools can be manipulated by optical tweezers [11], [12]. So the microstructures can be assembled by manipulating the microtools used as the parts of the microstructures. They are applied to the components of the microchip.

This work was supported by MEXT KAKENHI (17076004).

First, second and fourth Authors are with Department of Micro-Nano Systems Engineering, Nagoya University, Furo-cho, Chikusa-ku, Nagoya, 464-8603 JAPAN (e-mail: itom@robo.mein.nagoya-u.ac.jp)

Third Author is with Department of Bioengineering and Robotics, Tohoku University, 6-6-01, Aramaki Aza Aoba, Aoba-ku, Sendai, Miyagi, 980-8579 JAPAN

In this paper, the property conditions of the Z axial position of objective lens in microtool fabrication are revealed. Then, the arbitrary shaped microtools such as "Microhand" are fabricated on the microchip. Additionally, the rotational microstructures are assembled by manipulating these microtools which is "Microgear" on the microchip. We propose two assembly methods of these microstructures. The particular microchip is needed to assemble the microstructures. The rotation speed of this microgear depending on the laser power is evaluated. We succeed in meshing the microgears by laser manipulation.

II. Laser Manipulation System

The used on-chip laser manipulation system is shown in Fig. 2. The laser (Ytterbium fiber laser) for optical tweezers is built into inverted microscope (IX-71, Olympus). For the motion to change the observation range and manipulate the objects, X-Y stage and height of the objective lens (Z-axis) are controlled by manual. The oil immersion objective (UPLFLN 100XOI2, Olympus) is used. The magnification is 100 and N.A. was 0.6~1.3. The laser is scanned on X-Y coordinate by controlling the angle of galvanometer mirror (LSA-10A-30, Harmonic Drive Systems). UV-ray is illuminated by the mercury lamp (USH-103tems) using DC m0L, Olympus) controlling the shutter (BSH-RIX, Sigmakoki). The microchip is put on the stage. The experiment and observation are performed using the CCD camera (XC-555, Sony).

III. On-chip Fabrication of Microtools

This section describes the fabrication method of microtools on the microchip and the procedure for fabrication of the microchip used for microtool fabricaition.

A. Method of Microtool Fabrication

As shown in Fig. 2, the mask made of PET (polyethylene terephthalate) is set in front of the shutter on the optical axis. The arbitrary shape patterns are printed on the mask. As shown in Fig.3, the UV-ray is illuminated through this mask from bottom of the objective lens to photo-crosslinkable resin in the channel of PDMS (SILPOT 184 W/C, Dow Corning Toray) microchip. In this way, the photo-crosslinkable resin is polymerized on the microchip and the arbitrary shape microtools are fabricated [13]. In the first fabrication, the position of the microtool is confirmed. In the second fabrication, the microtool is positioned at the desired place by calibrating the first position. When the surface of wall in the channel is the glass, the fabricated microtool adheres on it. However, when the surface of wall in the channel is the PDMS, the fabricated microtool can move in the non-polymerized resin freely without adhering. Because the oxygen layer which exists near the PDMS surface inhibits the polymerization of photo-crosslinkable resin [14]. PDMS has the permeability to air and it penetrates oxygen. This oxygen molecule inhibits the radical polymerization by the strong reaction to a radical species. Therefore, the polymerization

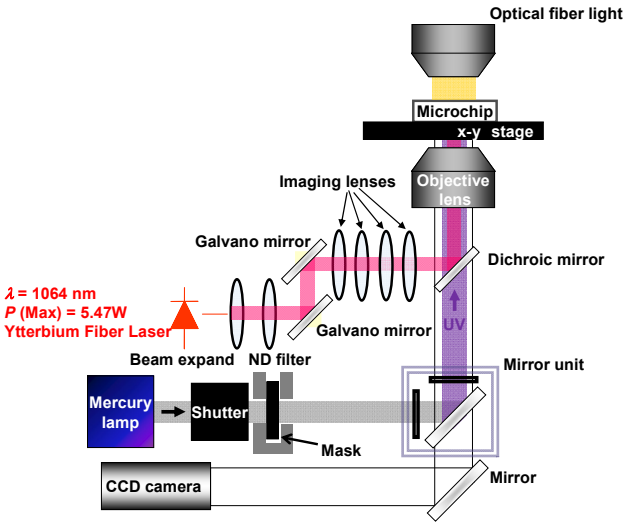

Fig. 2 A schematic of on-chip laser manipulation system

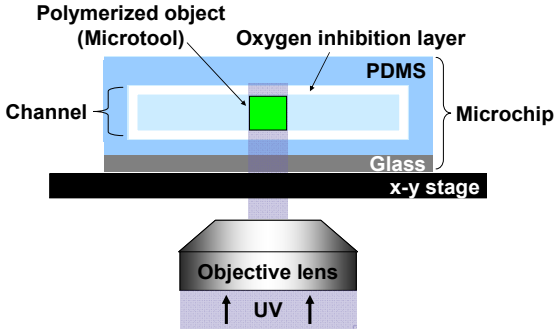

Fig. 3 A schematic of on-chip microtool fabrication

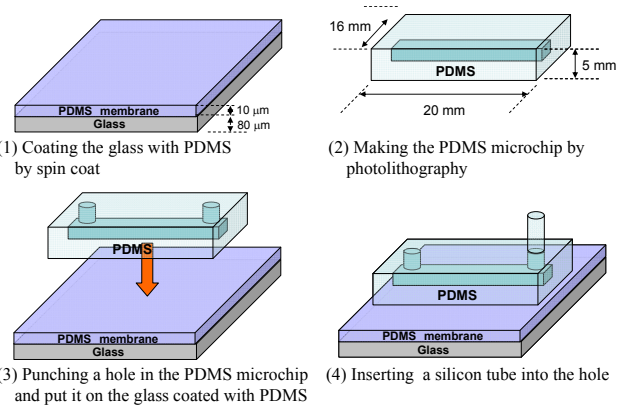

Fig.4 Schematics of fabrication process of PDMS microchip

does not occur near the PDMS surface and the fabricated microtool moves in the channel freely.

B. Procedure for Microchip Fabrication

As mentioned in section 3-A, it is necessary to coat the surface of wall in the channel of microchip with PDMS, in order for the polymerized microtools to move freely in the channel. The PDMS microchip was fabricated by the procedure in Fig. 4.

(1) PDMS is spin coated on the glass (thickness of 80 μm) used as a bottom of the microchip. The 5000 rpm spin is kept for 30 seconds after 300 rpm spin for 15 seconds.
(2) PDMS (16 mm×20 mm×5 mm) which has the channel (14 mm×0.5 mm×10 μm) is put on the glass. This channel is formed by a mold which is made by photolithography technique with SU-8.
(3) The PDMS with the channel is bonded to the glass by PDMS. In order to put the tube and inject the resin, holes are made on the ends of the channel.
(4) The silicon tube is put in the hole and bonded by PDMS.

In this procedure, the microchip was fabricated. The resin was injected to the channel by the negative pressure.

IV. Microtool Fabrication by UV-ray Irradiation

In this microtool fabrication method, there is the property Z axial position of objective lens. Because the light focus density of UV-ray illuminated to the resin depends on the position of objective lens. This section revealed this fabrication condition by experiments.

A. Focal Position in Microtool Fabrication

When the microtool is fabricated, it is impossible to fabricate it if the objective lens is focused on the bottom of the channel. Because the light focus density of UV-ray is not enough to polymerize the resin. So the property condition is revealed by the experiment that the microtools are fabricated changing the position of the objective lens.

The photo-crosslinkable resin which contains PEG-DA (poly ethylene glycol diacrylate (400), Polysciences) and the 6 % polymeric initiator is injected into the microchip which has the PDMS surface of wall in the channel. The microtool is fabricated illuminating the UV-ray for 0.2 s by using the long focus objective lens (LUCPlanFLN, Olympus). The magnification is 60 and N.A. is 0.7. The used mask had the circle pattern which is 0.5 mm in diameter. Since the illuminated UV-ray through the objective lens is focused, the light focus density and area of UV illuminated to the resin is changed depending on the position of objective lens. As shown in Fig. 5, the microtool is fabricated changing the focal position based on the bottom of the channel. The diameters of these microtools were measured. The experimental result is shown in Fig. 6 and Fig. 7. Fig. 7 shows that the light focus area of UV illuminated to the resin was the smallest and the density was the highest when the position of objective lens was 30 μm (The bench mark was bottom of the channel). And the light focus area of UV illuminated to the resin became smaller and the density became lower with distance from 30 μm. Therefore, it is appropriate condition that the microtool is fabricated in the 30 μm focal position based on the bottom of the channel.

B. Fabrication of Arbitrary Shape Microtools

The complex shape microtools such as the microhand and microgear are fabricated under the same experimental conditions as section 4-A. The results are shown in Fig. 8. The microtool fabricated by using the long focus objective lens of ×60 (LUCPlanFLN, Olympus) was about 1/56 size compared with the pattern of the mask. The one by using the oil immersion objective lens of ×100 (UPLFLN 100XOI2, Olympus) was about 1/108 size compared with the pattern of the mask. These results show that the size of microtool

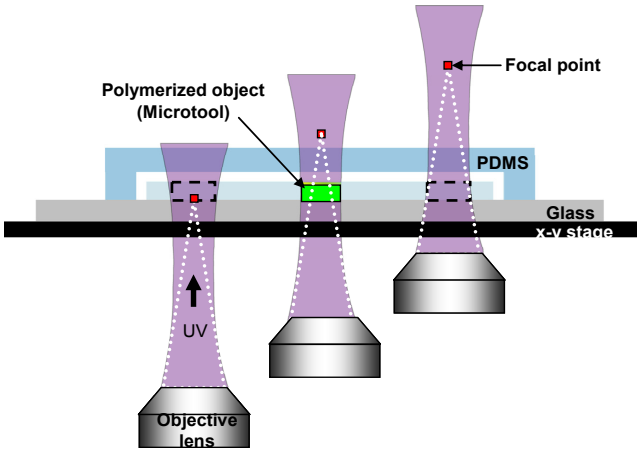

Fig. 5 A schematic of microtool fabrication depending on the focus position

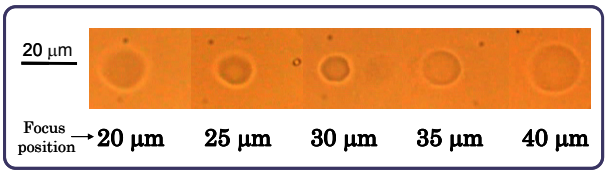

Fig. 6 Fabricated microtools depending on the focus position

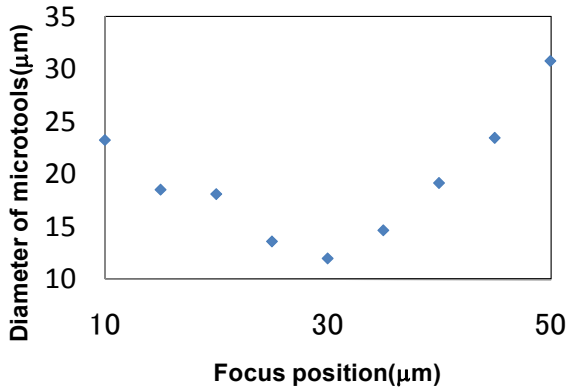

Fig. 7 Diameter of microtools by different focus positions

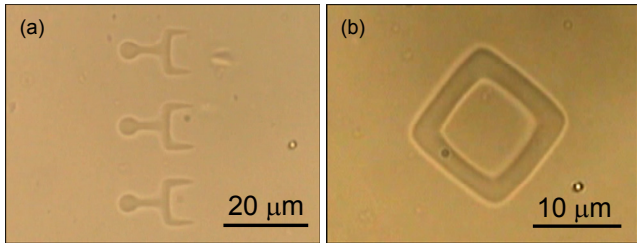

Fig. 8 Fabricated complex shape microtools:
(a) Microhand fabricated by ×60 objective lens, (b) Microframe fabricated by ×100 objective lens

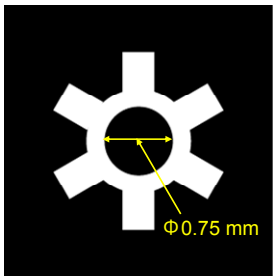

Fig. 9 Designed pattern of microgear

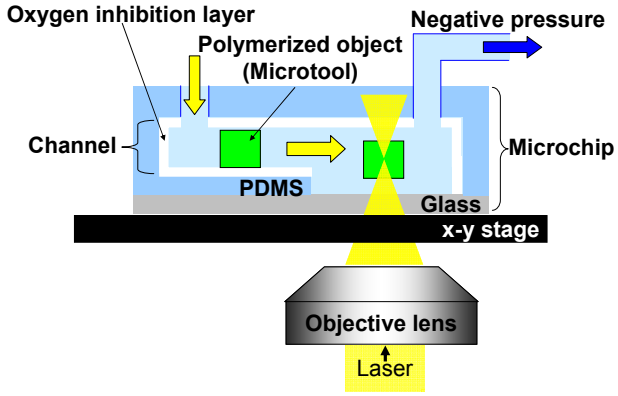

Fig. 10 Laser manipulation and assembly of microtools, which are fabricated on PDMS surface, on glass surface. The microtools are transferred from PDMS to glass surface by negative pressure

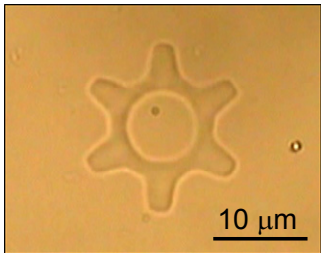

Fig. 11 Fabricated microgear on the PDMS surface

depends on the objective lens.

V. Microstructure Assembly by Fabricated Microtools

The rotational microstructure is assembled to demonstrate our assembly technique. This rotational microstructure is composed of the rotation axis and the microgear. This section describes the two methods of microstructure assembly.

A. Microchip for Microstructure Assembly

The microgear is designed which dose not only rotate, but also transmit a force to objects in the microchip. The microgear design is shown in Fig. 9. This microgear is engaged to the rotation axis which is fixed to the surface of the channel. The fixed rotation axis is needed to assemble the microstructure. Therefore, we propose the new microchip which has both of the glass surface and the PDMS surface of the bottom in the channel as shown in Fig. 10. As shown in

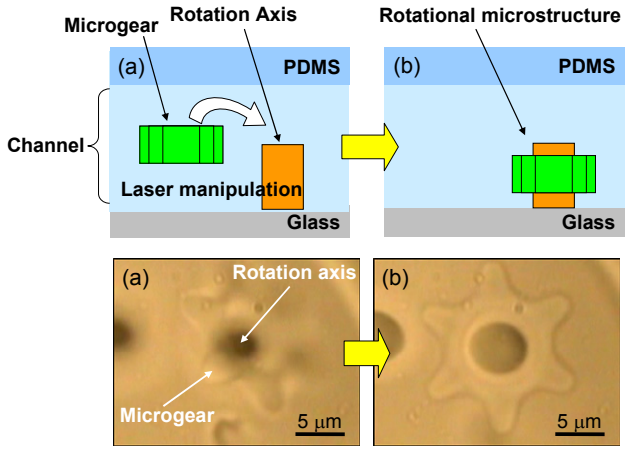

Fig. 12 Result of laser manipulation assembly

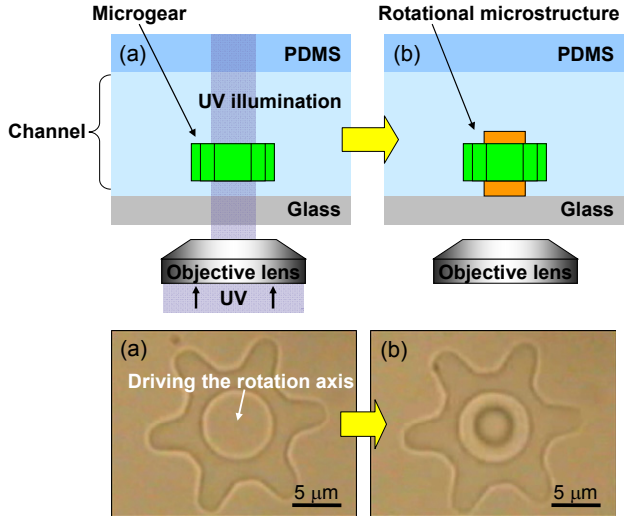

Fig. 13 Result of no-laser manipulation assembly method

section 3-A, the fabricated microtools on the PDMS surface can move in the non-polymerized resin freely. On the other hand, when the microtools are fabricated on the glass surface, they adhere on it. Using these behaviors, the microgear is fabricated on the PDMS surface and the rotation axis is fabricated on the glass surface. In this method, the fabricated microgear can be manipulated by optical tweezers and the fabricated rotation axis is fixed on the bottom of the channel. The following is the procedure before assembling the microstructure. Firstly, the microgear is fabricated on the PDMS. The fabricated microgear is shown in Fig. 11. Secondly, this microgear is transferred from PDMS to glass surface by negative pressure with the non-polymerized resin as show in Fig. 10. After that, microgear is moved to arbitrary place by optical tweezers and assembled.

B. Two Methods of Rotational Microstructure Assembly

There are two methods of the rotational microstructure assembly. One method is laser manipulation assembly as show in Fig. 12. In this method, the microgear is manipulated by optical tweezers and engaged to the rotation axis which is

prefabricated near the microgear. There is a space over the fabricated rotation axis because the oxygen layer which exists near the PDMS surface over the rotation axis inhibits the polymerization of photo-crosslinkable resin. Therefore, the microgear can be inserted above the rotation axis. Thickness of the oxygen layer is approximately 2 μm. The following is the procedure of this laser manipulation assembly. Firstly, the rotation axis is fabricated by illuminating the UV-ray near the microgear on the glass surface. This rotation axis is fixed on the bottom of the channel because it is fabricated on the glass. Secondly, the microgear is put above the rotation axis by laser manipulation. When the objective lens is moved in the direction of Z axis keeping the microgear trapping by optical tweezers, the microgear is manipulated upward in the direction of Z axis because the laser focal point is moved upward simultaneously in the channel. After that, the microgear is engaged to the rotation axis. Another method is no-laser manipulation assembly as show in Fig. 13. In this method, the rotation axis is driven into the hole of microgear. There is no need for laser manipulation assembly. The following is the procedure of this no-laser manipulation assembly. Firstly, the microgear is located at the arbitrary place. Secondly, the rotation axis is fabricated in the hole of the microgear by controlling the stage position.

The two methods, which are the laser manipulation assembly and no-laser manipulation assembly, are compared about the time, workability and versatility. The laser manipulation assembly takes more time than the no-laser manipulation assembly. It takes several minutes to engage the microgear to the rotation axis. In the workability, the no-laser manipulation assembly is easier than the laser manipulation assembly. There is no need to move the objective lens in the direction of Z axis. In the no-laser manipulation assembly method, there is no versatility because the assembled microgear is not movable. In contrast, the assembled microgear by laser manipulation can be disassembled. It is able to exchange a part with another one or reassemble a microstructure by the laser manipulation assembly.

VI. Evaluation of Assembled Microstructures

In order to apply the microstructure to microchip, it is important to know the manipulation performance of the microstructure. In this section, the assembled rotational microstructure is evaluated by measuring the rotation speed depending on the laser power. Moreover, we succeeded in meshing the microgears assembled by the no-laser manipulation assembly method.

A. Rotation Speed Depending on the Laser Power

The rotation speed of microgear depends on the laser power, the shape of microgear and the viscosity of solvent. In this experiment, the dependence on the laser power is focused. When the laser power is turned down gradually, the constant rotation speed laser trap is departed from the trap point at a laser power. From this result, we show the relation between the rotation speed of microgear and the laser power. The microgear shown in Fig. 11 is used in this experiment. The laser trap field is formed into arbitrary pattern by high-speed scanning of a single laser with galvanometer mirror [15]. In this way, the multiple points can be trapped. By this technique,

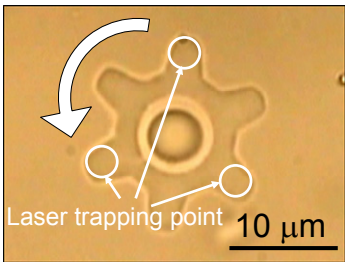

Fig. 14 Rotational manipulation by trapping multiple points

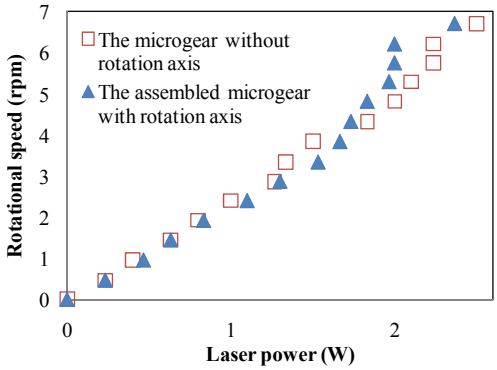

Fig. 15 Rotation speeds depending on the laser power for the microgear without rotation axis and the assembled microgear with rotation axis

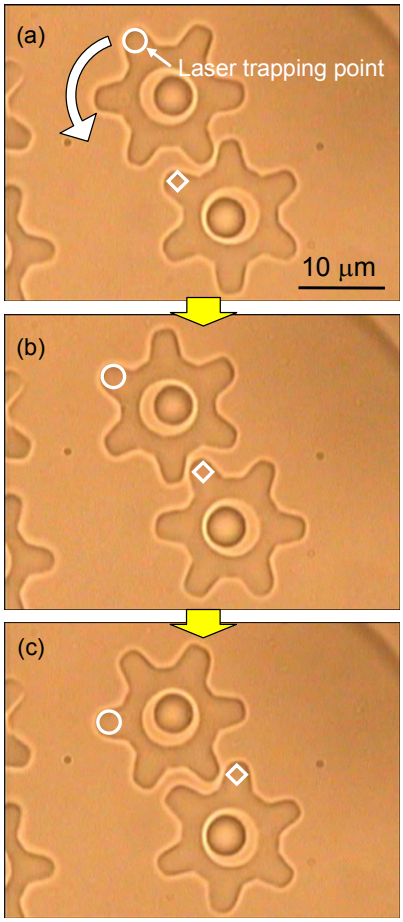

Fig. 16 Driving the 2nd microgear by rotation of 1st microgear

a single laser is formed into 3 points and the fabricated microgear is rotated as shown in Fig. 14. The rotation diameter is 15.8 μm. The laser trapping points are teeth of the microgear and the rotational manipulation is performed by trapping these teeth. The experiment is performed in the two cases. One is only the microgear without rotation axis and another is the assembled microgear which is engaged to the rotation axis. The assembled microgear is fabricated by no-laser manipulation assembly method shown in section 5-B. Fig. 15 shows the rotation speeds of the microgear and the assembled microgear. The rotation speed is almost proportional to the laser power. Additionally, the rotation speeds of the microgear without rotation axis and the assembled microgear with rotation axis are almost the same. This result shows that the manipulation performance of microgear does not change even if it is assembled as the microstructure.

B. Driving the 2nd Microgear by Rotation of 1st Microgear

We succeeded in driving the 2nd microgear by rotation of 1st microgear. The microstructure which rotate microgears is easy to be assembled by the no-laser manipulation assembly method. The following is the procedure of this microstructure. Firstly, a rotational structure is assembled as shown in the section 5-B. Secondly, 2nd microgear is arranged to mesh the preassembled microgear by laser manipulation. After that, the arranged 2nd microgear is assembled by the no-laser manipulation assembly method. The assembled microgear structure is manipulated by single laser trap as shown in Fig. 16. The microgears are rotated at 0.63 rpm. When the two microgears are rotated, much viscosity resistance is acted on the microgears. Therefore This result of the rotational speed is much lower than the result in Fig. 15. Using this microstructure, it is possible to transmit the motion with high accuracy in the microchip.

VII. CONCLUSION

We showed that the methods of the microtool fabrication with photo-crosslinkable resin and the assembly of microstructures with a view to fabricating the functional microstructure applied to the on-chip single cell analysis system. The fabrication of the arbitrary shape microtool was realized at the desired place on the microchip in a short time. The optimal Z axial position of objective lens was revealed experimentally to fabricate the microtools with arbitrary shape. Additionally, we succeeded in assembling the rotational microstructure and manipulating it by optical tweezers. We proposed two methods of the rotational microstructure assembly. One method is laser manipulation assembly that the microgear is manipulated by optical tweezers and engaged to the rotation axis which is prefabricated near the microgear. Another method is no-laser manipulation assembly that the rotation axis is driven into the hole of microgear. The manipulation performance of the microgear was evaluated. The rotation speed of microgear is almost proportional to the laser power. The manipulation performance of microgear does not change even if it is assembled as the microstructure. Moreover, we succeeded in driving the 2nd microgear by rotation of 1st microgear.

As future works, we assemble the functional microstructures which are applied to the on-chip single cell analysis system.

ACKNOWLEDGMENT

This work was partially supported by MEXT KAKENHI (17076004).

REFERENCES

[1] I. Inoue, Y. Wakamoto, H. Moriguchi, K. Okanob and K. Yasuda, "On-chip Culture System for Observation of Isolated Individual Cells", *Lab on a Chip*, Vol.1, (2001), pp.50-55

[2] A. Y. Fu, H. P. Chou, C. Spence, F. H. Arnold, and S. R. Quake, "An Integrated Microfabricated Cell Sorter", *Anal. Chem.* Vol.74, (2002), pp.2451-2457

[3] E. Tamaki, K. Sato, M. Tokeshi, K. Sato, M. Aihara, and T. Kitamori, "Single-Cell Analysis by a Scanning Thermal Lens Microscope with a Microchip: Direct Monitoring of Cytochrome c Distribution during Apoptosis Process", *Anal. Chem.* Vol.74, (2002), pp.1560-1564

[4] M. Yang, C. Li, and J. Yang, "Cell Docking and On-Chip Monitoring of Cellular Reactions with a Controlled Concentration Gradient on a Microfluidic Device", *Anal. Chem.* Vol.74, (2002), pp.3991-4001

[5] O. Thoumine, A. Ott, O. Cardoso and J-J. Meister, "Microplates: a New Tool for Manipulation and Mechanical Perturbation of Individual Cells", *J. Biochem. Biophys. Methods*, Vol.39, (1999), pp. 47-62

[6] S. Maruo, O. Nakamura, and S. Kawata, "Three-dimensional Microfabrication with Two-photon-absorbed Photopolymerization", *Optics Letters,* Vol. 22, (1997), pp.132-134

[7] H. Inoue1, S. Maruo,"Development of an Optically Driven Disk Micropump", *2007 JSME Conference on Robotics and Mechatronics*, (2007), pp.2A1-N02(1)-(3)

[8] H. Maruyama, F. Arai, T. Fukuda, "Gel-tool Sensor Positioned by Optical Tweezers for Local pH Measurement in a Microchip", *IEEE International Conference on Robotics and Automation*, (2007), pp.806-811

[9] F. Arai, T. Endo, H. Maruyama , T.Fukuda, T. Shimizu, and S. Kamiya, "3D Manipulation of Lipid Nanotubes Using Laser Trapped Functional Gel Microbeads", *Proc. of the 2007 IEEE/RSJ International Conference on Intelligent Robots and Systems*, (2007), pp.3125-3130

[10] P. Panda, S. Ali, E. Lo, B. Geun Chung, T. Alan Hatton, A. Khademhosseini and P. S. Doyle, "Stop-flow Lithography to Generate Cell-laden Microgel Particles", *Lab on a Chip*, Vol.8, (2008), pp. 1056-1061

[11] D. G. Grier, "A Revolution in Optical Manipulation", *Nature*, Vol. 424, (2003), pp.810-816

[12] A. Ashkin, "Atomic-beam Deflection by Resonance-radiation Pressure", *Phys. Rev. Lett*., Vol. 24, (1970), pp.1321-1324

[13] D. Dendukuri, Daniel C. Pregibon, J. Collins, T. Alan Hatton and Patrick S. Doyle, "Cntinuous-flow Lithography for High-throughput Microparticle Synthesis", *Nature Materials*, Vol.5, (2006), pp.365-369

[14] C. Decker and Aubrey D. Jenkins, "Kinetic Approach of O_2 Inhibition in Ultraviolet and Laser –induced Polymerizations", *Macromolecules*, Vol.18, (1985), pp.1241-1244

[15] F. Arai, K Yoshikawa, T. Sakami, and T. Fukuda, "Synchronized Laser Micromanipulation of Multiple Targets along Each Trajectory by Single Laser", *Applied Physics Letters,* Vol.85, (2004), pp.4301-4303