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Magnetically Modified Polymeric Microsorter for On-chip Particle Manipulations

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***Abstract*—The paper describes a novel non-contact manipulation system using an on-chip, magnetically driven micro-tool (MMT) rather than conventional biomanipulation by hand, which has a higher risk of contamination and lower success rate and repeatability. MMTs can sort particles individually, and have the unique feature that they can be installed directly in a microchannel (width = 150 µm), unlike conventional cell sorting systems. The drive unit was significantly downsized by amplifying the magnetic power using a permanent magnet. Lower power consumption could also be realized, because no energy is required to keep MMTs stationary at a particular position. Sorting was performed using real-time sensing images of microbeads, where the system successfully sorted beads of different sizes at switching speeds up to 18 Hz. The MMT developed in this study is unique with respect to its flexibility and biocompatibility; in addition, since the PDMS (Polydimethylsiloxane) microchip is disposable, it can be applied to cell sorting without any risk of contamination. Potential fields of application of this technology include cloning techniques, which require sorting of oocytes with and without nuclei.**

I. Introduction

MICROCHIP production has progressed remarkably with the development of micro/nano fabrication techniques in the fields of chemistry and biology. For example, an integrated microchip system for chemical analysis has been developed that effectively performs chemical reactions in the microspaces of microchannels. A microchip that can culture cells has been developed to maintain the desired environment by controlling oxygen, nutrients, and waste materials in the microchannel. In such microchannels, the transportation medium can be a single-phase as well as a multiphase flow, typically containing microbeads, chemicals, or cells. Sorting of microparticles in such microchannels offers great potential in biology, chemistry, and environmental analysis.

In general, when microparticles are sorted in microchannels, the effects of centrifugal force and gravity on the particles of the order of several tens of micrometers are relatively small, compared to large-scale sorting. Therefore, large-scale sorting techniques are not applicable to sorting of microparticles in a microchannel. Instead, the fluid force, electrostatic force, and optical force are commonly used to sort microparticles in microchannels.

Many sorting methods are based on fluid force, such as field-flow fractionation (FFF)[1][2], which uses thermal and fluid forces, and capillary hydrodynamic fractionation (CHDF)[3], where microparticles are sorted according to the differences in motion of colloidal fluid in microchannels. Also, SPLITT fractionation can achieve high performance in sorting particles by adding a particle-laden cross-flow into the main stream [4]. These techniques can sort particles accurately based on the velocity distributions in microchannels, and can sort a wide range of microparticles (1–100 µm in diameter); however, it is difficult to make fine adjustments in the pump flow rate with high accuracy. Moreover, it is time consuming to design and fabricate microchips to sort particles of arbitrary sizes.

Cell sorters, which are based on flow cytometry, can sort cells in a continuous cell-laden flow, and can collect the sorted cells in cell suspensions[5]. Cell sorting is generally performed either electrically (by charging cells) or by mechanically moving the receiving dishes after a shot of laser light. The laser pulse is controlled such that a single cell is placed in a single droplet by thrusting the diluted cell suspension into an air phase. However, because this system tends to be large and expensive, a low-cost cell sorter has been developed on a microchip. There are many methods of microscale cell sorting, such as dielectrophoretic sorting [6]-[8], laser trap [9], magnetic isolation, and switching in microfluidic channels using microvalves [10]. It is necessary to design the system to fit the characteristics of the sorting object (e.g., size), the condition of the carrier liquid, and the sorting speed.

The most popular microactuators that can be applied in the confined space of microchannels are electrostatic microactuators, optical tweezers [11], and magnetic microactuators [12][13]. The Coulomb force has been often used in manipulating cells of the order of 10 µm, whereas it is necessary to apply a high voltage to manipulate particles of the order of 100 µm, which risks damaging cells by heat generation. The dielectrophoretic force can be adjusted by varying the squared value of the gradient of the electrical

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field; however, it is controllable only in the limited region adjacent to the electrodes, and requires higher voltages to sort larger objects. Optical tweezers can manipulate cells indirectly by non-contact actuation of microtools, thus reducing the risk of damaging cells during manipulation; however, the generated force is of the order of several pN, which is not suitable for manipulating cells of the order of 100 µm. On the other hand, the magnetic sorting offers a limited risk of cell contamination, and it has been used in many studies because of its low cost [15]-[26].

To implement magnetic sorting, we used a magnetic material in the microchannels, which can be controlled by electromagnets mounted externally on the chip. This technique provides many microchannel functions, such as those of a valve, stirrer, and loader [19]-[23]. The microdevices actuated by magnetic force have a simple structure, and they are low in cost and easy to integrate with the microchip. For the present research, we developed a novel sorting system that effectively and precisely sorts microparticles by mounting a magnetically driven microactuator in a microchannel. This sorter provides a robust and reliable system that is not affected by the characteristics of the sorted objects. It is particularly important to note that it can sort relatively large particles (of the order of 100 µm).

We had developed magnetically driven microtools (MMT) in a previous research. MMTs allow sorting with high accuracy and the ability to be mass produced and fabricated in arbitrary shapes. For the present research, we developed an MMT for sorting along with its actuation system. The developed MMT is flexible and biocompatible, and can be applied to cell sorting applications where there is a risk of contamination, by fabricating the microchannel out of a disposable material. By analyzing the image around the separation point, the particles flowing in the microchannel were sorted individually and in real time based on particle size. While developing the MMT drive unit, it was difficult to downsize the electromagnetic coil, because of the large number of windings required to produce sufficient actuation power [19]-[22]. Permanent magnets have been reported to have significant advantages in miniaturizing systems effectively [12]; therefore, we used a permanent magnet that operated indirectly, and were able to downsize the drive unit significantly. Automating the sorting of different sizes of polystyrene beads was completed using a real-time image processing system.

II. Production of MMT and Microchannels

A. Fabrication of MMT

Figure 1(a) ([20]-[23]) shows the fabrication process of the MMT, which may be summarized as follows: (i) a thick nega-resist (KMPR1050, KAYAKU MicroChem, Japan.) was spread over the silicon substrate, and an MMT mold was produced by photolithography, (ii) a mixture of PDMS and magnetite (Fe_3O_4, 50 wt%) was spread over the patterned mold and baked in an oven (110℃, 10 min), and (iii) the MMT was obtained after the treatment with a stripper liquid, KMPR (Remover PG, 70℃, KAYAKU MicroChem, Japan.)

B. Fabrication of Microchannel

Figure 1(b) ([20]-[23]) shows the microchannel fabrication process, summarized as follows: (i) a thick nega-resist (SU8-3050, KAYAKU MicroChem, Japan.) was spread over the silicon substrate and the microchannel mold was produced by photolithography, (ii) the PDMS was poured on the patterned mold and baked in an oven (90℃, 15 min), and (iii) the silicon substrate was removed from the PDMS. The PDMS and cover glass were bonded together after installing the MMT in the microchannel.

Figure 2 shows the SEM images of the produced MMT and an MMT set in the microchannel. Figure 3 shows a schematic of the sorting operation. The base of the arrow shaped MMT (Young's Modulus ~ 5 MPa) was fixed using UV-curable resin, and the particles were sorted by moving the MMT tip across the width of the microchannel.

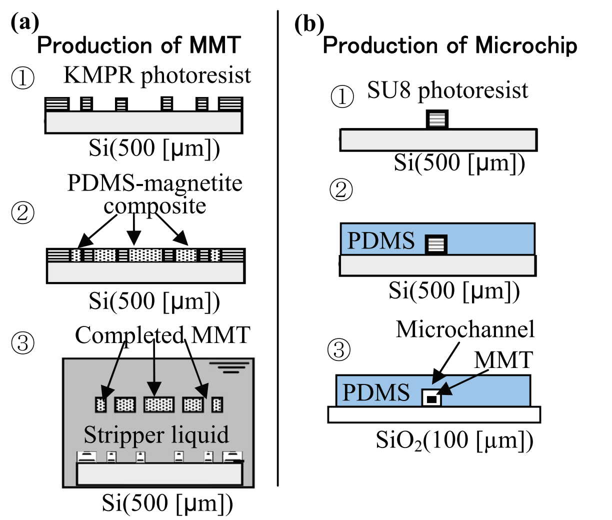


Fig. 1: Fabrication processes of MMT and microchannel.

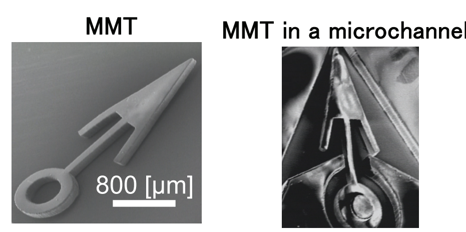


Fig.2: Scanning electron microscope image of MMT (left) and MMT installed in a microchannel (right). (Fe_3O_4+PDMS)

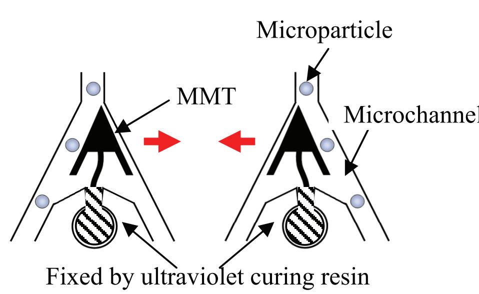


Fig.3: Actuation method of MMT.

III. ACTUATION METHOD OF MMT

A. Actuation Method of MMT

Figure 4(a) shows the conventional configuration of an MMT that consists of a microchannel module and an actuation module containing a drive unit. Figure 4(b) shows the improved design proposed in this study. The system consists of two modules—an upper module containing a disposable microchannel and a lower actuation module. In the present configuration, the actuation module is composed of a magnetic circuit unit containing an electromagnetic coil and a permanent magnet unit. The magnetic force generated by the electromagnetic coil is amplified by the permanent magnet (neodymium) unit mounted between the microchannel and the magnetic circuit, and the MMT is moved by non-contact actuation.

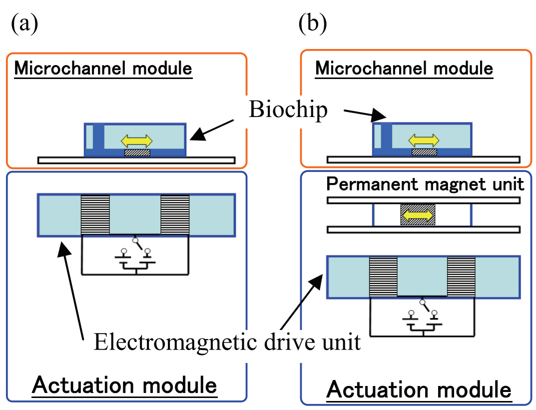


Fig.4: Schematic of experimental setup

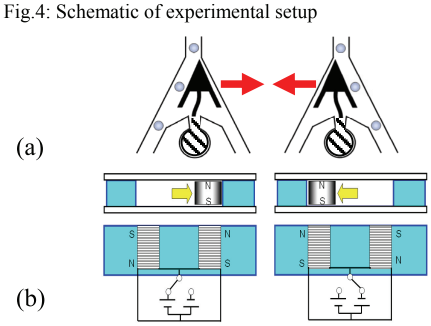


Fig.5: Actuation method of magnet

Figure 5 shows the mechanism of the magnetic circuit and the permanent magnet unit in the actuation module. The direction of the current in the coil of the magnetic circuit can be switched to reverse the electromagnet's polarity, resulting in translatory motion of the permanent magnet. The magnetic force generated by the electromagnetic coil is amplified, and an adequate magnetic force (maximum = 316 mT, voltage applied to electromagnet = 1.5 V) is transmitted to actuate the MMT in the microchannel. The magnetic force for actuation was approximately 110 times that of the conventional setup without a permanent magnet. It is important to note that the MMT remains in position even after the magnetic circuit is switched off. Thus, it enables lower power consumption, because no energy is consumed in keeping the MMT fixed in the same position.

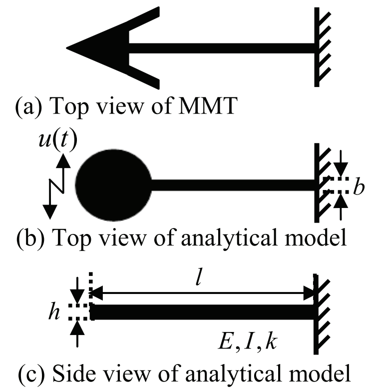


Fig.6: Analytical model of MMT

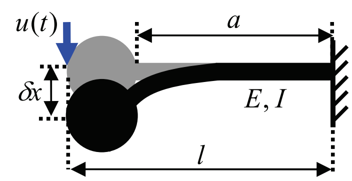


Fig. 7: Top view of analytical model for evaluating k

IV. DESIGN OF MMT

A. Vibration Analysis of MMT

A simple model was adopted for analyzing MMT vibration. We assume that a concentrated mass m is located at the edge of a beam of length l. For simplicity, the MMT is approximated as a cantilever beam attached to a column at one end (one end free and the other end fixed; Fig. 6(b)), with a uniform cross-section (Fig. 6(c)). In addition, we assume that there is no flow in the microchannel, and that the viscosity of the fluid can only affect the column. The equation of motion of the MMT can be expressed as

$$m\ddot{x} + c\dot{x} + kx = u(t) \qquad (1)$$

where c is the viscous damping coefficient and k is the spring coefficient. Further,

$$c = 8\pi\eta h/(0.5 - 0.558 - \ln(8/Re)) \qquad (2)$$

where η is the coefficient of viscosity and h is the height of the column. Consequently, the transfer function for equation (1) can be expressed as

$$G(s) = \frac{X(s)}{U(s)} = \frac{1}{m}\frac{1}{s^2 + 2\varsigma\omega_n s + \omega_n^2} \qquad (3)$$

$$\varsigma = c/2\sqrt{mk}, \omega_n = \sqrt{k/m} \qquad (4)$$

To determine k, we assume a concentrated mass m at the edge of the cantilever beam, as shown in Fig. 7. When the Young's modulus of the beam is E, and the section modulus is I, the deflection δx for the static load $u(t)_{const}$ at the edge is expressed as

$$\delta x = u(t)_{const}\left\{l^3 - (l-a)^3\right\}/3EI \tag{5}$$

where

$$u(t)_{const} = k\delta x \tag{6}$$

Combining equations (5) and (6), we obtain the following equation:

$$k = 3EI/\left\{l^3 - (l-a)^3\right\} \tag{7}$$

Thus, the natural frequency of the system can be expressed as

$$\omega = \sqrt{1-2\varsigma^2}\,\omega_n \tag{8}$$

V. Performance and Evaluation of MMT

A. Sorting Experiment with Fixed Base

Figure 8 shows an overview of the experimental setting, and Fig. 9 shows the actuation module developed in this study. The base of the MMT was fixed, its length was 2.25 mm, a was 1.03 mm, and its thickness was 80 µm. The spring coefficient k was 0.02 mN/µm (equation 7), and the natural frequency was 66.0 Hz (equation 8).

Fig.8: Experimental setup

Fig.9: Actuation module

Figure 10 shows the maximum magnetic force along the center line of the coil as a function of input voltage. The permanent magnetic force (316 mT) produced by an input voltage of 1.5 V was applied to the MMT. The force (3.2 mT) can be calculated from $F = B^2S/(2\mu)$, where B is the density of magnetic flux, µ is the magnetic permeability of air (1.26 × 10−6 H/m), and S is the area of the MMT. The magnetic force can displace the MMT by 159.05 µm, which is sufficient for switching in the microchannel, whose width is 150 µm, thus enabling sorting of particles. During the experiment, we confirmed that the MMT can swing across the full width of the microchannel.

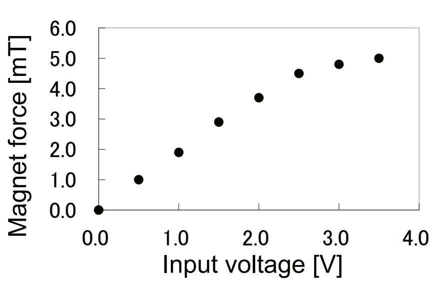


Fig. 10: Measured electromagnetic force

Images were captured using a CCD camera attached to an optical microscope. The MMT was triggered based on the size of the particle images; it was able to sort polystyrene beads of the order of 100 µm. Figure 11 shows the operation of the MMT using the actuation module. It was confirmed that the edge of the MMT can be used to switch the microchannel. The depth of the channel was 150 µm. The MMT was installed in the microchannel of the PDMS chip before the bonding between the cover glass and the PDMS chip. The base was fixed with UV-curable resin, as shown in Fig. 3. Two problems need to be addressed. One is the complicated fabrication process of fixing with UV-curable resin. The other is the difficulty in fixing the MMT symmetrically at the base.

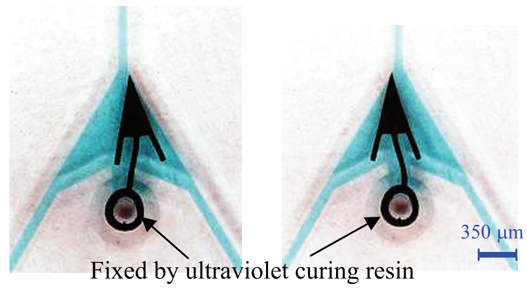


Fig.11 Magnetic microsorter in biochip

B. Optimization of MMT Fixation

To solve the problems discussed in Section V. The method of fixing the MMT was changed from a fixed support to a pinned support that is free to rotate, as shown in Fig. 12. This method simplifies the fabrication process and reduces the risk of asymmetric installation. (the fluid in the microchannel was dyed with red food coloring).

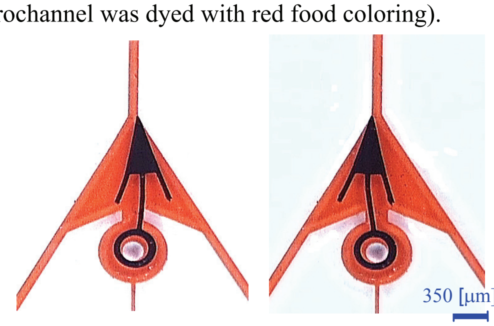
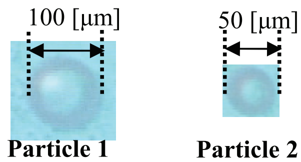


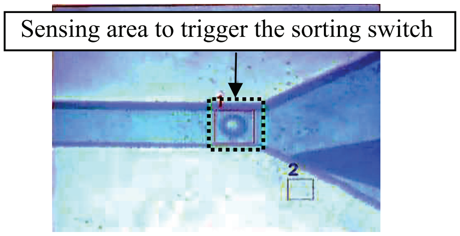
Fig.12 Magnetic microsorter in biochip

C. Results and Discussions of the Experiments with Pinned Support

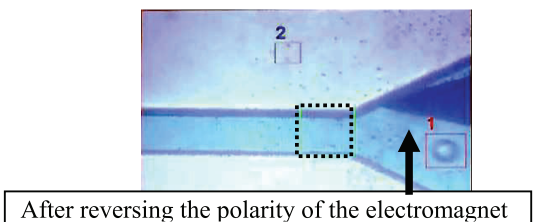
The experiment was performed in the same manner as that in Section V. Figure 13 shows the improved MMT configuration and the reduction in deformation at the fixation point. When sorting polystyrene beads, we confirmed that sorting could be performed at high precision. Therefore, changing the manner of fixing the MMT did not affect sorting performance. Consequently, the fabrication process was simplified by omitting the process of fixing it with UV-curable resin. A similar experiment was performed with swine oocytes after removal of zona pellucida, and was also successful [23].



(a) Polystyrene beads for size calibration



(b) Sensing area to trigger the sorting switch



(c) MMT was moved by the trigged signal.

Fig.13 Details of automation of sorting system

VI. Automation of Sorting by Image Processing

A. Experimental Setup

After evaluating the results of experiments in Chapter 5, we automated the sorting system using image analysis. The system is first calibrated using an image of representative sorting particles (Fig. 13(a)). The particles can then be sorted (Fig. 13(b)) at a threshold, based on the results of the calculation of the coefficient of correlation between the CCD image data and the sample image data. A sensing area is then set in the CCD image. A current, amplified by a power amplifier, is used to change the magnetic polarity of the electromagnet coil, and the output of a D/A (Digital to Analogue) circuit is varied. This process is controlled by the results of image processing in the sensing area (Fig. 14(3)). We used a pin-supported MMT for the experiment, and sorted polystyrene beads of two sizes—50 and 100 µm.

B. Evaluation of Sorting Automation

Figure 14 shows the sorting operation for 50- and 100-µm polystyrene beads. The 100-µm beads were set to be transported to the lower microchannel, and the 50-µm particles were to be sent to the upper microchannel. The figures on the left show a 100-µm bead passing through the sensing area (dashed square), and triggering the sorting module. The bead was successfully transported to the lower microchannel by moving the MMT to the upper wall of the microchannel. Similar results were observed for the 50-µm particles. We thus succeeded in automating the sorting of beads by size. The total sample size for the experiment was 20; all the particles were successfully sorted, and a switching speed of 18 Hz was achieved.

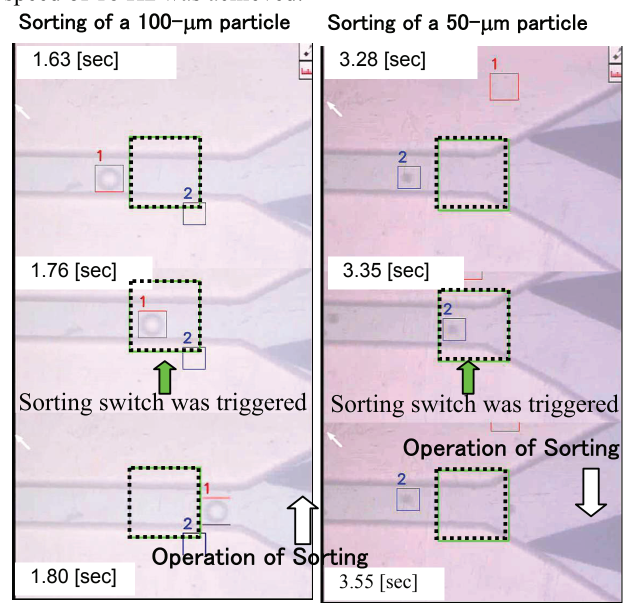


Fig.14 Demonstration of automatic sorting system (square 1: recognized as a 100-µm particle, square 2: recognized as a 50-µm particle, dashed square: sensing area for triggering the sorting switch).

VII. Magnetic Modifications of MMT

A. Magnetization of MMT

It is important to integrate the MMTs in a limited small area to miniaturize the microchip. One of the solutions of the miniaturization is the magnetization of MMTs. Once the MMTs become "small magnets", the size of the actuation module could be smaller dramatically. For the present study, two kind of magnetic powder (which is the conventional magnetite (Fe_3O_4) and neodymium powder ($Nd_2Fe_{14}B$)) were mixed with PDMS and magnetized.

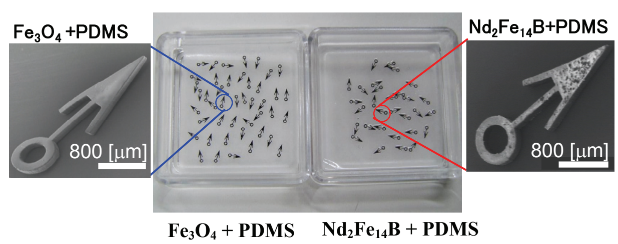


Fig.15. Magnetized MMT based on PDMS (left: Fe_3O_4 , right: $Nd_2Fe_{14}B$)

Figure 15 shows the magnetized MMT. It was confirmed that the density of magnetic flux of MMT increased for both composite of Fe_3O_4 (0.01 mT→0.10 mT)and $Nd_2Fe_{14}B$ (0.04 mT→4.4 mT), and found that MMT with $Nd_2Fe_{14}B$ was magnetized more effectively.

B. Focusing the Magnetic Field for MMT

Figure 16 shows the focusing of the magnetic field of MMT by mounting a couple of fine needle (ϕ= 500 μm) just above the electromagnetic coils. The density of magnetic flux at the tip was 6.8 mT. It was observed that MMT sorter was controlled directly by the electromagnetic coil and operated successfully. Figure 17 shows the magnetic interaction region of previous setting with permanent magnet (Figure 5) and current setting with pins. It was confirmed that the size of interaction for the current setting was one twentieth of previous setting. Therefore the entire size of the sorting chip can be reduced remarkably.

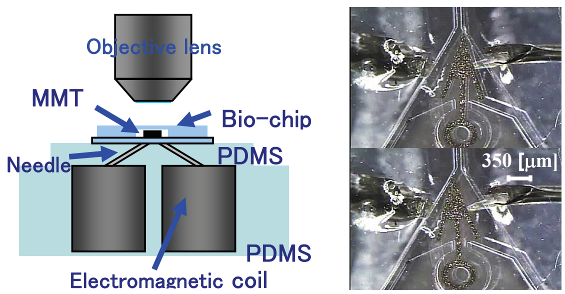


Fig.16. Operation of Magnetized MMT ($Nd_2Fe_{14}B$ + PDMS) focusing the magnetic field actuated by electromagnetic coil to the tip of a couple of needles.

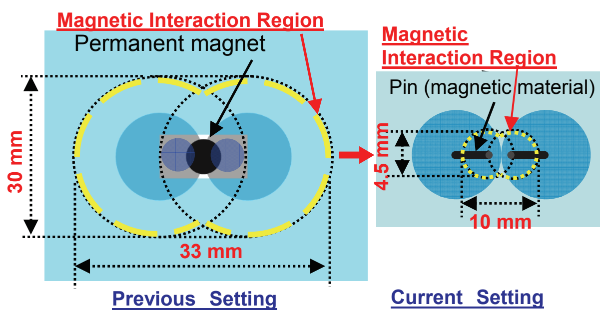


Fig.17. Reduction of Magnetic Interaction Region (not-scaled)

VIII. Conclusions

We developed an MMT, for application in sorting, along with its actuation module. The sorting operation was automated through image processing, and it was confirmed that the system could effectively sort polystyrene beads by size. The magnetization of MMT recognized increase of the density of magnetic flux of MMT, and MMT with $Nd_2Fe_{14}B$ was magnetized more effectively. Also, the focusing of the magnetic field was achieved by mounting a couple of pins above the electromagnetic coils and reduced the magnetic interaction region one twenties of the previous setting, and which contribute to the miniaturization of the sorting chip.

In future, application of the present system to fluorescent image analysis, and sorting halved oocytes with and without nuclei—an important technology for cloning—should be attempted. Furthermore, our sorting MMT could be combined with MMTs performing other diverse functions, possibly allowing automation of the entire cloning process on a chip.

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