

High Precision Magnetically Driven Microtools with Ultrasonic Vibration for Enucleation of Oocytes

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Abstract:

This paper presents innovative driving method for the magnetically driven microtools (MMT) which is micrometer positioning accuracy by horizontally arranged permanent magnets. A piezoelectric ceramic is used to induce nanoscale vibration to the microfluidic chip to reduce the friction on the MMT. The enucleation process has been conducted by the dual arm MMTs. MMTs receive enough driving power supplied by permanent magnets and their positioning accuracy is adequate to cut oocytes precisely. It takes only 5 seconds to cut the nucleus and the removed area is less than 25 percent of the original oocyte.

1. INTRODUCTION

Cell manipulations in the confined space of a microfluidic chip are highly important in the field of biotechnology because of the low contamination capability, repeatability, and high throughput ability. Especially robots on a chip have great advantages for the treatment of biological cell instead of human handling due to its non-skill dependent, high throughput and high repeatability. Magnetic field can be suitable power source for the on-chip robot because of its non-contact drive, low invasiveness with respect to a cell, low production cost, and a considerable amount of research has been carried out on magnetic actuators [1-5]. The magnetically driven microtool (MMT) by a permanent magnet can output millinewtons force amount with keeping small size of the drive unit, and a permanent magnet has more than 10 times stronger magnetic field than an electromagnetic coil of the same size [6]. Therefore, an MMT driven by a permanent magnet can be applied to wide range of cell manipulations such as loader, sorter, droplet generation, etc. [7-9]

However, an MMT driven by a permanent magnet has also disadvantage of low positioning accuracy and response speed against the drive stage. The main reason of the low performance of an MMT is that applied friction force is relatively large comparing to the magnetic force in driving direction component. When an MMT is driven by a permanent magnet on the XY stages from beneath the chip, there is an area where the MMT is not driven when the magnet passes under the MMT; we call this area the “dead band”. The dead band interferes with the precise positioning of the MMT on a chip and deteriorates the effective control

of the MMT as a robot on a chip because of the slow response speed to the driver unit.

Under the low positioning accuracy, it makes difficulty of treating smaller cell than its accuracy. Even though the cell size is relatively large like an oocyte, precise accuracy is often required for the cell manipulation. For example in the enucleation process, removed part with nucleus has to be small as much as possible not to damage an oocyte and to prevent from failure in the following processes. Therefore, less than ten micrometer accuracy for the MMT is necessary for secure enucleation process. On the other hand, pairs of Helmholtz coils have been used in some research [10-12] in order to achieve positioning control, but the supplied force by magnetic coil is not sufficiently strong to manipulate a relatively large cell.

Hence, we present innovative drive method for the MMT driven by permanent magnets in order to achieve micrometer order positioning accuracy with keeping the benefit of permanent magnets drive and applied it to the oocyte enucleation process in a microfluidic chip by dual arm MMTs. Figure 1 shows the concept of oocyte manipulation by the dual arm MMTs. Owing to the high power output from permanent magnets and the precise positioning accuracy, MMTs can handle the oocyte to control its posture and remove the nucleus with less damage to the oocyte.

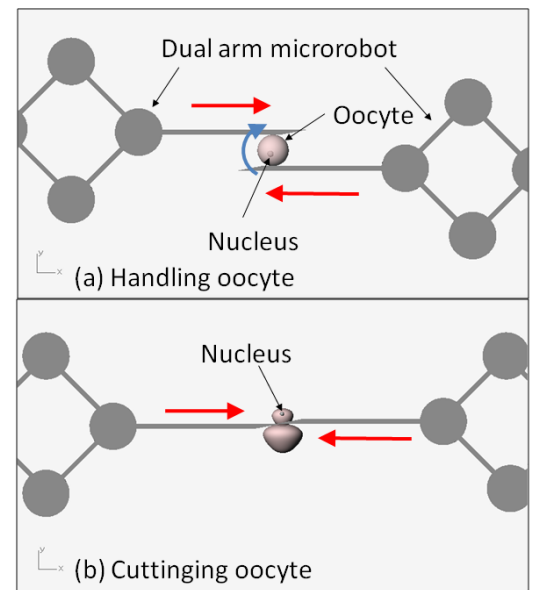


Fig. 1. Concept of oocyte manipulation by dual arm microrobot

2. HORIZONTAL POLAR MAGNETIC DRIVE

In order to reduce the friction on an MMT and supply more magnetic force into driving direction, we have developed horizontal polar drive (HPD) [6]. Figure 2 shows the driving concept and FEM result of the conventional drive and HPD for an MMT. As shown in the figure, the direction of the magnetic flux density around the Ni MMT is vertically aligned for the conventional drive, and therefore, a large magnetic force is applied in the downward direction. This increases the friction on the MMT in the case of a conventional driving unit. On the other hand, when the MMT is set such that the permanent magnet pole is parallel to the driving direction of a magnet that has the same size as the MMT, the magnetic force in the downward direction is considerably reduced. As a result, the friction on the MMT is significantly decreased compared to that shown in Figure 2 (a). Figure 3 shows the experimentally obtained following response ability of the MMT against the linear stage with the permanent magnet. The stage moves with 1 degree of freedom (DOF) with a sine wave of 0.5 Hz; the stroke is ± 1.5 mm. In the case of conventional drive, the movement of the MMT against the stage is delayed by 0.3 sec; the maximum difference between the MMT and the stage is 1.0 mm. On the other hand, the response of the MMT in the HPD is more than 10 times faster and the difference between the stage and the MMT is considerably smaller than in the conventional drive. Figure 4 shows the multi-degree of freedom (DOF) MMT with HPD. Two pairs of magnets under the HPD conditions are set with the polar axis normal to each other. The cell manipulation is conducted on the head of the extended part. Figure 4 (c) shows the FEM result of the magnetic flux density for the 2-DOF MMT. It can be seen that the drive magnets independently actuate the circular disc part of the MMT as it is shown in Figure 2 (b). However, the effect of the friction still large and the positioning accuracy is dozens of micrometer.

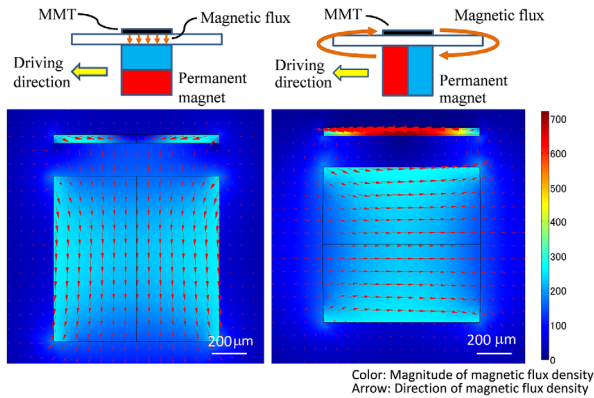


Fig. 2. FEM results of magnetic flux density of (a) conventional drive unit and (b) horizontal polar drive unit

On the other hand, it is known that the effective friction decreases significantly when ultrasonic vibration is applied to the sliding surface of the moving object. Here, we employ this phenomenon to the microrobot by vibrating the microfluidic chip in order to reduce friction on the microrobot and improve the positioning accuracy.

3. FRICTION REDUCTION BY ULTRASONIC VIBRATION

When ultrasonic vibration is applied to the sliding surface of the moving object, the direction of the friction on the object switches with ten thousands to millions times per second and as a result, the effective friction decreases significantly [13-16]. Littman et. al. [14] and their following work [15]

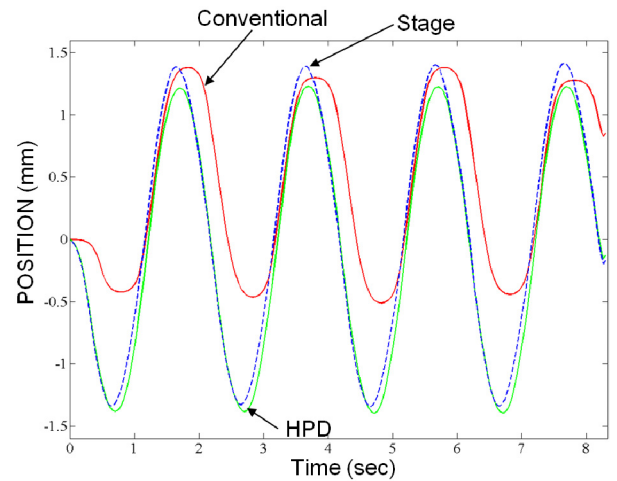


Fig. 3. Following response of MMT actuated by conventional drive and horizontal polar drive against a linear stage

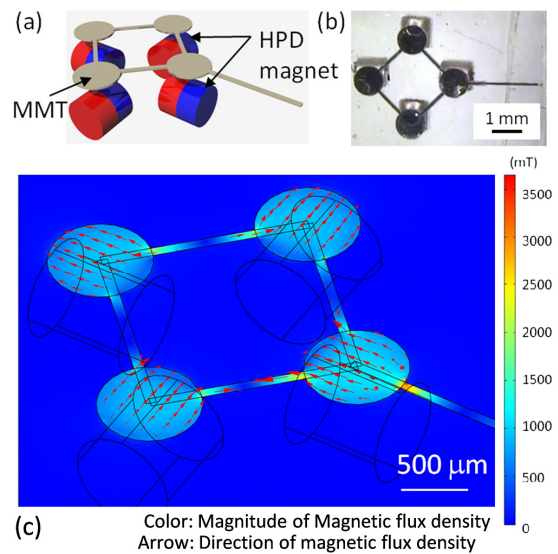


Fig. 4. Multi-DOF MMT with HPD magnet. (a) Concept, (b) actual design and (c) FEM result of magnetic flux density (magnet: neodymium, ϕ 1.0 mm \times 1.0 mm, MMT: Ni).

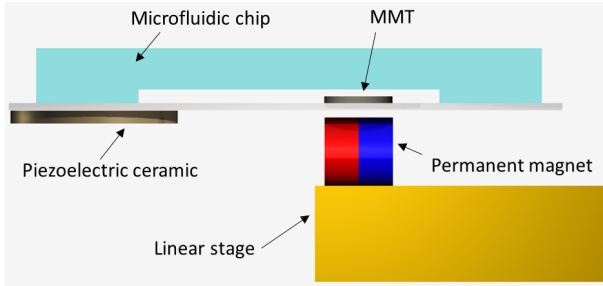


Fig. 5. Driving concept of the MMT by HPD with ultrasonic vibration

developed analytical model to explain the phenomena of the friction reduction and resulted that the reduction ratio depends on the velocity ratio of the moving object and the sliding surface. Kumar et al. also developed analytical modeling and expressed the friction reduction ratio as follows [16];

$$\frac{F_a}{F_0} = \frac{2}{\pi} \sin^{-1} \frac{V_s}{a\omega} \approx \frac{2 \cdot V_s}{\pi \cdot a\omega} \quad (1)$$

where F_0 is the frictional force in the absence of vibration, F_a is resultant average frictional force with vibration, V_s is the velocity of the sliding object, a and ω are the amplitude and the angular frequency of the vibration respectively.

In microscale, friction is the dominant contributor for the deterioration of the positioning accuracy due to the scale effect. In addition, much less energy is required to cause the vibration due to its small size. Therefore, the friction reduction by the vibration can be expected to have a considerable impact on the positioning accuracy in microscale.

4. EXPERIMENTAL EVALUATIONS

4.1 Experimental setup

Figure 5 shows the driving concept of the MMT by HPD with ultrasonic vibration. Radially displaceable piezoelectric ceramic is attached to the glass substrate under the microfluidic chip and oscillates the sliding surface of the MMT. The MMT is actuated by HPD and the permanent magnet is set on the 2-DOF linear stage. At the experiments, commercially available piezoelectric ceramic (W-40, MKT Taisei Co.), whose size is $\phi 42.0 \times 3.5$ mm, the resonance frequency is 55 kHz, and the electrostatic capacitance is 4600 pF, is used. As a drive magnet, four neodymium (Nd2Fe14B) magnets (diameter: 1.0 mm, grade: N40) are used and for the MMT, Ni based multi-DOF MMT, which is designed in Figure 4, is used for the evaluation.

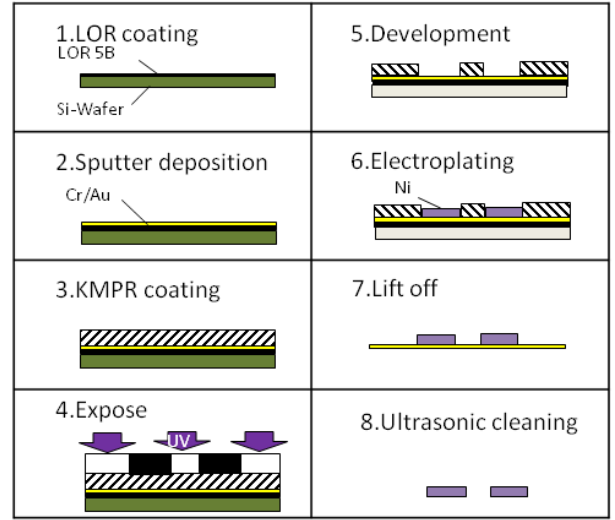


Fig.6. The fabrication process of Ni based MMT

4.2 MMT fabrication process

The Ni based MMT fabrication process is shown in Figure 6. At first, the sacrificial layer (LOR 5B, Tokyo Ohka Kogyo Co., Ltd.) is coated on Si wafer. Then Cr-Au is sputtered on this wafer (thickness = 300 nm). Next, the photoresist (KMPR 3035, Nippon Kayaku Co., Ltd.) is coated on the substrate. After the exposure, the KMPR pattern is developed. Finally the Ni is grown by the electroplating (= 50 μ m). Then, removing the photoresist and sacrificial layer and the Ni parts can be collected and cleaned by ultrasonic.

4.3 Evaluation of the effect of the ultrasonic vibration

In order to evaluate the effect of the ultrasonic vibration and the improvement of the MMT positioning accuracy, the experiment has been conducted. The linear stage was actuated in circular trajectory with the constant drive velocity and the corresponding MMT position was measured by CCD camera. Figure 7 shows the MMT positioning accuracy without vibration from piezoelectric ceramics against the target trajectory of the circular (radius: 0.5 mm) and the linear stage drive velocity was 0.785 mm/sec in x-y direction respectively. The measurement was conducted in 100 points and the average error of MMT positioning from the target trajectory is 84.0 μ m, and the standard deviations is 28.0 μ m and thus the total positing error is 112 μ m. On the other hand, when the vibration was applied to the microfluidic chip, the MMT movement was significantly improved. Figure 8 shows the MMT positioning accuracy when 300 Vp-p is applied to the piezoelectric ceramic with

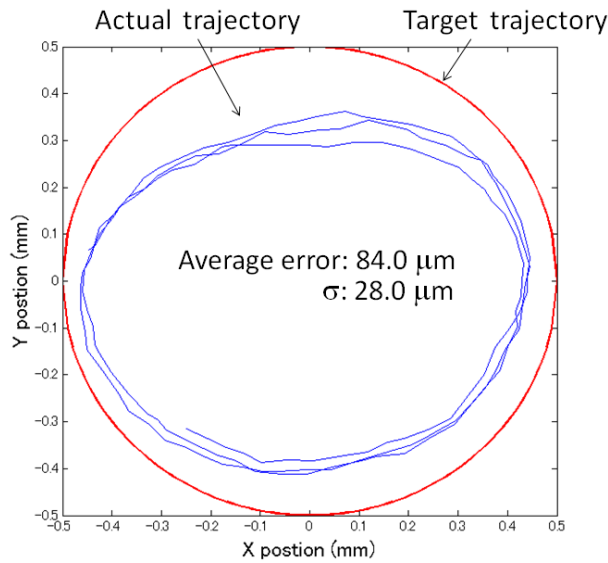


Fig. 7. Positioning accuracy of the MMT without vibration from piezoelectric ceramic (drive frequency of the stage: 0.5 Hz)

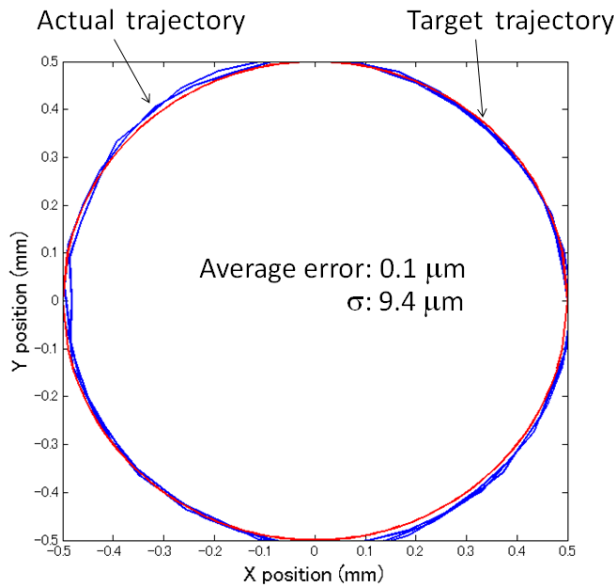


Fig. 8. Positioning accuracy of the MMT with vibration from piezoelectric ceramic (frequency of the piezoelectric ceramics: 55 kHz, drive frequency of the stage: 0.5 Hz)

55 kHz frequency. The drive stage configuration was same as Figure 7 but the average error and the standard deviation against target trajectory are 0.1 and 9.4 μm respectively, and thus the total positioning error is 9.5 μm which is more than 10 times higher accuracy than in the case of without vibration.

Now, we achieved the micrometer order positioning accuracy on the MMT and there is another benefit of the ultrasonic vibration applied to the microfluidic chip. The response speed is also significantly increases because of the significantly decreased dead band. Figure 9 shows the high

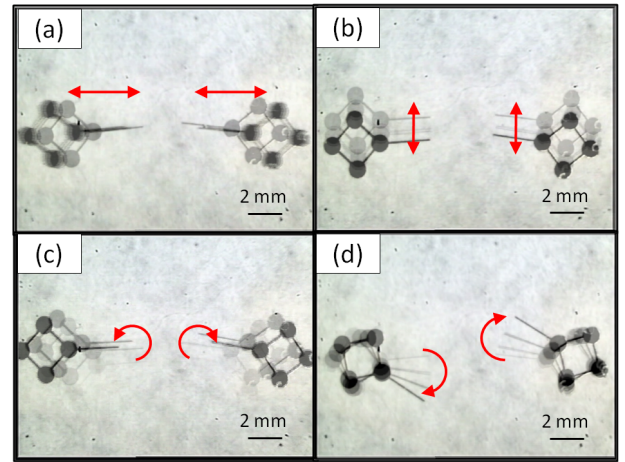


Fig. 9. High speed 3-DOF movement of MMTs

speed MMTs in 3-DOF movement with ultrasonic vibration. Owing to the high response speed of the MMT against the drive stages, it is confirmed that the MMT can follow the drive stage up to 5 Hz, which is the upper limit of the drive stage used in the experiments.

5. OOCYTE ENUCLEATION PROCESS

5.1 Introduction of oocyte enucleation process

The embryo manipulation is one of the breakthrough techniques for the amelioration of domestic animals, preservation of genes of rare animal. Especially the enucleation of oocyte techniques is actively studied for cloning process. At present, manually operated micromanipulators with glass capillaries are used to remove a nucleus under a microscope. However, the conventional manual manipulation tends to have problems of contamination, low success rate and low repeatability, and thus complicated cell manipulation could be carried out only by skilled people. Now, we present the enucleation of oocytes process in a microfluidic chip by dual arm MMTs applying ultrasonic vibration in order to achieve high speed processing with less damage to the oocyte by removing the area with nucleus from the oocyte as small as possible.

Automation of the cloning technique is required for high-throughput production of processed cells with high quality and homogeneity [17]. The approach to enucleate oocytes by hybrid MMT [18] were conducted previously, but it was difficult to handle the oocyte to control its posture and remove the only nucleus part. Here, we improved design of the microfluidic chip and have conducted the high speed enucleation process of swine oocytes on a chip by dual arm MMTs driven by HPD with ultrasonic vibration. The target throughput time is 5 seconds, which is far superior to the conventional operations and the cutting accuracy is less than 25 % of the original oocyte size.

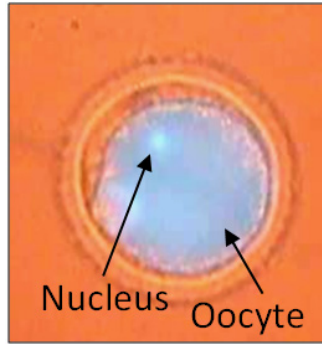


Fig. 10. Experimental setup for the enucleation of oocyte

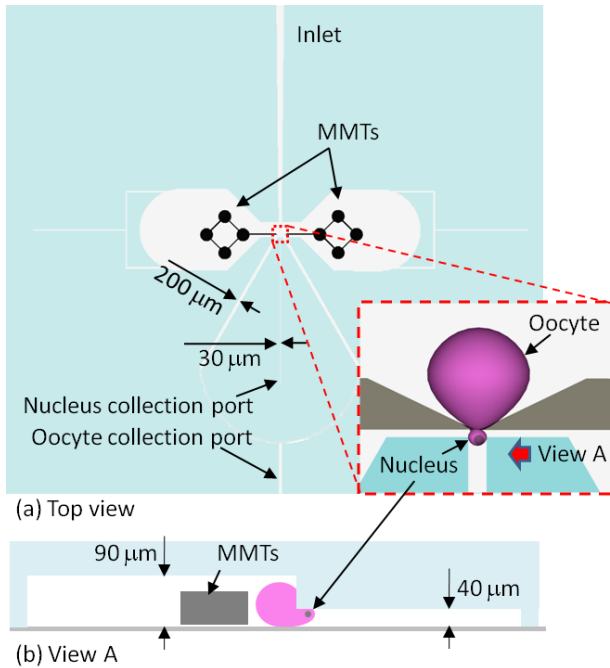


Fig. 11. Design of the microfluidic chip for the enucleation of oocyte

5.2 Experimental setup

In advance of oocyte enucleation process, the swine oocyte has to be prepared with hyaluronidase (0.1 % of TCM 199) for 10 minutes in order to remove cumulus cells surrounding the oocytes and pronase (0.5% of PBS) for 10 minute to remove zona pellucida. Then, Hoechst 34580 is applied for staining the nucleus of the oocyte. Figure 10 shows the stained oocyte by Hoechst 34580. The nucleus part was shield when mercury lamp was exposed.

Figure 11 shows the design of the microfluidic chip for the enucleation of oocytes. The cutting blade has to have enough power to cut the oocytes and thus two MMT blades made by Ni, whose young's module (50 GPa) is enough higher than oocyte's, is set in polydimethylsiloxane (PDMS) chip to cut off the nucleus part of oocytes at the middle of

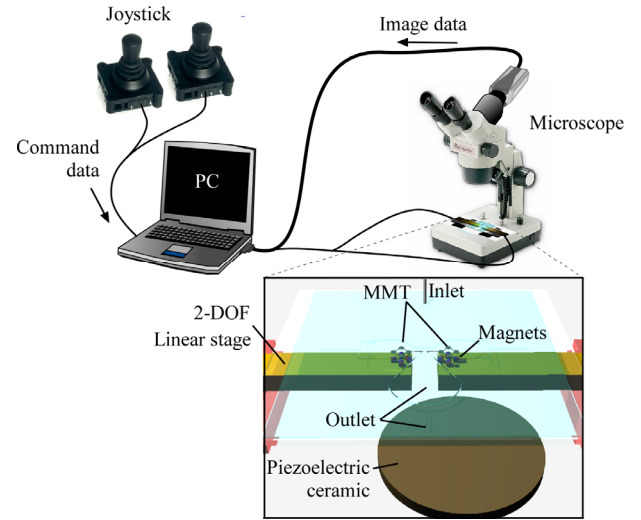


Fig. 12. Experimental setup for the enucleation of oocyte

the chip. The oocytes are put in from inlet port and flow in 90 μm height and 200 μm width of the channel. The dual arm MMTs then rotate the oocyte and the height of the channel to the nucleus collection port is set lower (30 μm) and the width is narrow (30 μm) so that nucleus part is withdrawn into the channel.

The dual arm MMTs cut the oocyte by the tip of the blade and the small removed part with nucleus from the oocyte flows to the nucleus collection port and the other part of the oocyte flows to oocyte collection port to be sent to the post process.

Figure 12 shows the experimental setup for the enucleation of oocyte including the linear stage for the magnet actuation, the microscope with CCD camera, PC for remote controlling the stage movement by joysticks and capturing image data from CCD camera. The same piezoelectric ceramic as previous experiment attached to the microfluidic chip designed in Figure 11 and applied AC 20 V with 55 kHz to vibrate the sliding surface of MMTs. The same MMTs and the drive magnets as the previous evaluation experiment were used as well.

5.3 Experimental result

Figure 13 shows the experimental result of swine oocyte enucleation process. The oocyte inserted from the inlet flew to the narrow channel and stuck there since the oocyte size was not small enough to get into the channel with 30 μm width and height (Fig.13 (a)). After the nucleus position was confirmed, the oocyte was rolled by the MMT (Figure 13 (b)) and pushed back to the narrow channel so that the nucleus part was trapped to the channel (Figure 13 (c)). Then the tip of the MMT pressed oocyte (Figure 13(d)) and cut the oocyte (Figure 13 (e)). It can be seen that the nucleus was successfully removed and the oocyte shape kept circle

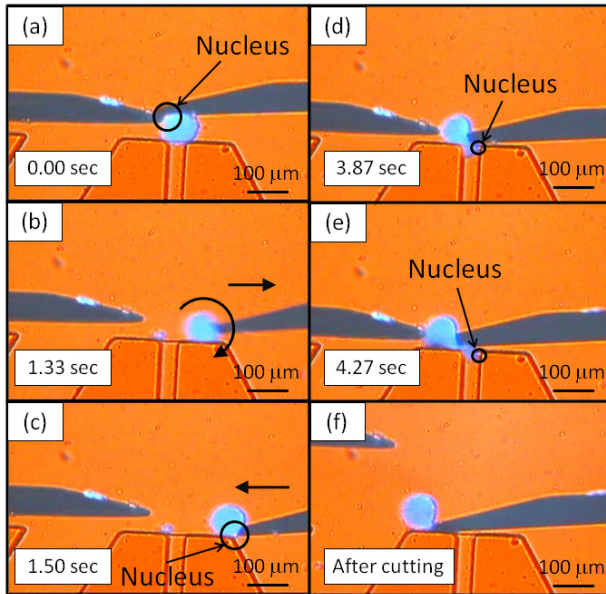


Fig. 13. Experimental result of oocyte enucleation process by dual arm MMTs with ultrasonic vibration

even after the cutting (Figure 13 (f)). The processing time, which is the duration from the time when oocyte reached to the narrow channel to the time when oocyte was cut, was less than 5 seconds and the oocyte removed rate is about 20 % of the original size.

6. CONCLUSION AND FUTURE WORK

In this paper, we presented the innovative drive method using HPD with ultrasonic vibration applied to the microfluidic chip for the MMTs. The positioning accuracy improved by 10 times than in the case of only HPD is applied and by 100 times than in the case of the conventional drive. Since the MMT is actuated by permanent magnets, the output force is significantly higher than the case of the electromagnetic coil is applied. Now that the MMT can be actuated without contact but with high power output (mN order), high precision (μm order), and high speed (up to 5 Hz), we can expect the great deal of applications for cell manipulations in a microfluidic chip. Here we demonstrated the on chip enucleation process by MMTs with ultrasonic vibration and achieved high speed processing far superior to the conventional operation and less than 25 % of the removal accuracy

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