2013 IEEE/RSJ International Conference on
Intelligent Robots and Systems (IROS)
November 3-7, 2013. Tokyo, Japan

Multiple Microfluidic Stream based Manipulation for Single Cell Handling*

Yaxiaer Yalikun, Yoshitake Akiyama and Keisuke Morishima, *Member, IEEE*

***Abstract*—This paper proposed a Multiple Microfluidic Stream based Manipulation (MMSM) for bio-objects using micro hydrodynamics and Lab on Chip (LOC) technology. Our method can manipulate bio-objects without contact under open space, the advantages of which were experimentally confirmed in this paper. Compared with other conventional bio-manipulation methods, this method is not directly in contact with the target bio-object, and was considered as a non-invasive and soft manipulation via hydrodynamic fluids. The basic principle of this system is manipulating a micro object by controlling several micro water streams which are generated from orifices under the operating area simultaneously. By changing the parameters of the water stream such as flow rates, position and number of operating orifices, the direction and velocity of the object can be controlled. To verify this principle, we designed an open space fluid model for on-chip manipulation, and simulated force and direction of the water stream using CFD software. Then the prototype microchip with an array of 8 orifices of diameter 100 μm, and 3 types of channels of widths 100 μm, 200 μm, and 500 μm respectively were fabricated with glass. In experiments several kinds of rectilinear motion of insect ovum and micro beads were observed. The results presented in this paper showed that this multi micro fluidic stream manipulation system has the capability for MMSM.**

I. Introduction

In recent years, biotechnology, genetic engineering, and artificial insemination have advanced rapidly. As a result, single cell manipulations have become to the most popular technology which was widely used around the world.. For all of these current biotechnologies, more precise and more efficient handling operation at single cell level is indispensable.

A various types of bio micro manipulation methods have been reported so far, which basically can be divided into two main categories, namely the contact type method and non-contact type method [1]. For example, micro-grippers which is capable of high accuracy and power, is a typical representative for contact method. However, it may influence cell membrane and unnecessary stimulation [2][3] mechanically. On the other hand, a number of non-contact type manipulations, such as optical trapping force[4][5], electro kinetic force[6][7], acoustic force[8], dielectrophoretic force[9][10], and hydrodynamic force[11][12][13] have been reported.

However, except the method using hydrodynamic force, the others have influence on cell, such as thermal damage. It is important that the cell behavior is not disturbed by external field. Compared with the other methods, hydrodynamic force based bio manipulation is the method much more bio friendly, because this method is controlling a biocompatible fluid surrounding bio-object in closed micro environment [11][12][13].However, the application of these hydrodynamic force based methods is confined because of limited degree of freedom of fluidic streamline due to closed operating environment.

Furthermore, in some operations such as a micro injection of mouse embryos, high-cell orientation control is important for avoiding damage of cellular organelles [14].

However, most of the open space manipulation methods cannot implement the rotation without influence on cell.

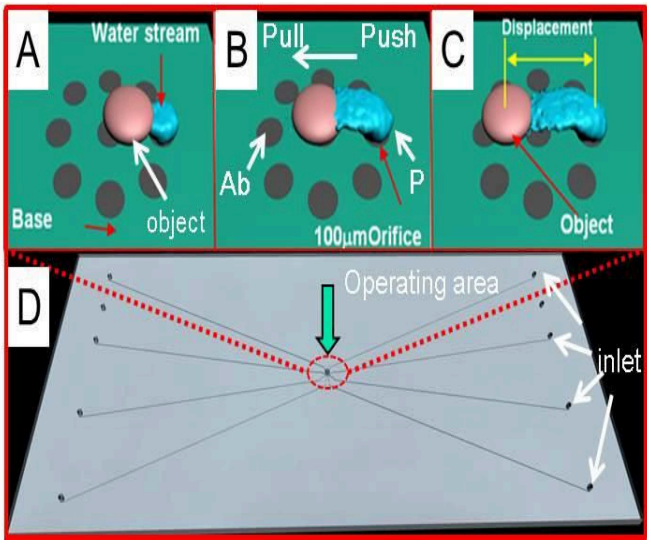

Figure 1. Concept of multiple microfluidic stream manipulation.
Fig.1. (A) the object which is suspending in operating area was captured by streams, manipulation will start when pumps start to generate microfluidic stream by pushing out and pulling in solution from orifices. (B) Object in operating area obtained a hydrodynamic force from the microfluidic stream then flow forward. (C) Object was pushed out and move forward. (C). Much more complex rectangle motion shall be obtained by repeating the above process. (D) is the MMSM device based on hydrodynamic force.

Therefore in this research, we forced on developing a powerful new full degrees of freedom manipulation without physical influence or other types of effect on bio-object, also capable for wide scope of application for different sizes and

*Research supported by JSPS,MEXT KAKENHI Grant Number 20034017, 21676002, 21225007, 21111503, 23111705, and the Industrial Technology Research Grant Program from the New Energy and Industrial Technology Development Organization (NEDO) of Japan.

Yaxiaer Yalikun is with the Department of Mechanical Engineering, Osaka University, 5670047 JAPAN. (e-mail:yaxr724@gmail.com).

Yoshitake Akiyama is with the Department of Mechanical Engineering, Osaka University, 5670047 JAPAN. (e-mail: akiyama@mech.eng.osaka-u.ac.jp).

Keisuke Morishima is with the Department of Mechanical Engineering, Osaka University, 5670047 JAPAN. (corresponding author, phone: 0668797343; fax: 0668797344 e-mail: morishima@mech.eng.osaka-u.ac.jp).

types of bio-object. For achieving the purpose of MMSM, we also utilized the lab on a chip (LOC) technology. The studies of cell-based micro fluidic biochip have rapidly advanced as a tool in drug discoveries, cell delivery, etc. It has been focused as a cheap, rapid and simplified method to replace the existing biochemical laboratory works.

It is possible to form miniaturized lab functionalities on a chip with development of MEMS technologies (16). The most ordinary usages of LOC are transports or analyzing in 2D limited space. However in this paper we confirmed the possibility of transporting and manipulating objects without full space limitation based on LOC micro fluidic technology. The system we proposed implemented common functions without contact object by separately control of micro water stream. In addition, for confirming the possibility of operating object, several kinds and sizes of objects been employed..

The concept and principle we proposed are shown in Figure 1. Manipulating micro object by microfluidic stream which generated from multiple orifices simultaneously can be realized. As shown in Figure.1(A), a steady fluidic stream is generated from an orifice. The trapped object is about to move forward. As shown in Figure.1(B), the streams are generated from single orifice by pushing out and pulling in extremely small amount of fluid simultaneously controlled by syringe pumps. Then, the object moves toward in an orifice pulling in fluid. As shown in Figure.1(C), finally, the object moved forward from original position. In addition, with precise control of the flow rate, there are possibilities to achieve a various kinds of function, such as holding of cell, rotation of cell, assembly of cell. To confirm the possibility of operating objects, several types and sizes of objects were experimentally demonstrated.

II. Basic Principe of MMSM

In this paper, a diameter of 350 μm biological cell as insect oocyte was selected as MMSM target. In previous work, a diameter of 150 μm cell was manipulated by Nano Newton level force[13], and the hydrodynamic force was proportional to the radius of an object[15], therefore, we estimated that for manipulating the insect oocyte, at least 2.5Nano Newton is needed. Based on this estimation, the proper flow rate could be calculated by equation (1).

Normally suspended insect oocyte in aqueous solution has to suffer several kinds of forces, so the motion of the individual object in solution shows as scalar equation (1)[16]:

$$F_e = F_h + F_b - F_g - F_{vdw} + F_a \qquad (1)$$

where F_e is all external force exerted on the object in the flow stream direction; F_h, F_b, F_g, and, F_{vdw} are the hydrodynamic force (the drag force), the buoyancy force, the gravity force and the van der Waals force , respectively; F_a represents the other additional forces exerted on object, depending on the flow condition and object properties. These forces include pressure gradient force, Basset force, virtual mass force caused by unsteady flow, Brownian force, and lift force due to shear, that is, Saffman's lift force. The object properties, such as size and density and the air flow field influence the magnitude of these forces. However, F_a and F_{vdw} are extremely small, so they are ignorable [17] if compare with F_h, F_b, F_g.

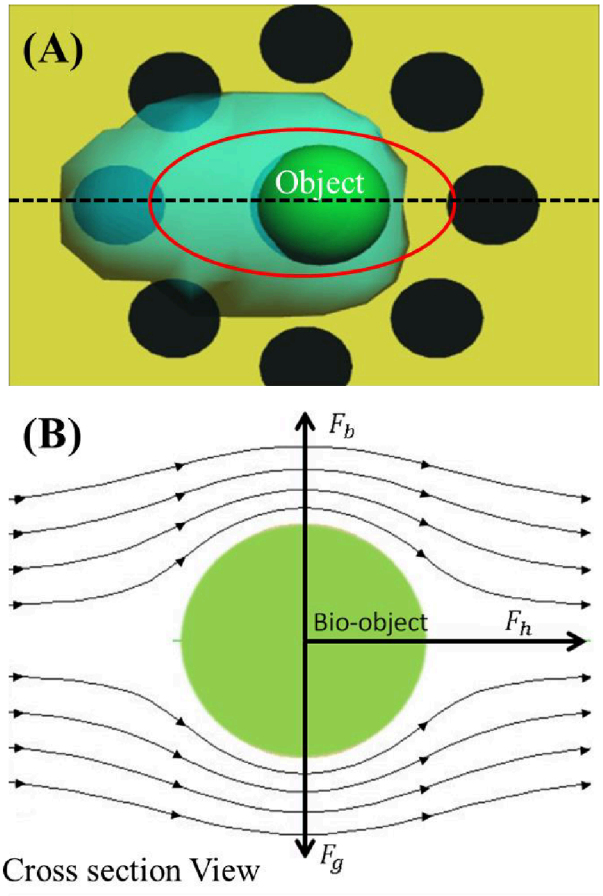

Figure 2. Schematic image of dynamics of suspended object in an aqueous solution when be manipulated by MMSM.
(B) is cross section view of (A). In (B), F_h, F_g, F_bare the drag force (the hydrodynamic force), the gravity force, the buoyancy force, respectively. When the microfluidic stream was generated, the object which trapped in the streams can be actively "force closured" by filed field forces from streams [18]and be lead to where it need to be in operate area.

where F_e is all external force exerted on the object in the flow stream direction; F_h, F_b, F_g, and, F_{vdw} are the hydrodynamic force (the drag force), the buoyancy force, the gravity force and the van der Waals force, respectively.The hydrodynamic force in operating area can be expressed by [17]:

$$F_h = \frac{1}{2}\rho_f C_d S(V_f - v)^2 \qquad (2)$$

In equation (2), ρ_f is density of fluid, C_d is hydrodynamic force coefficient, S is cross-sectional area of the insect oocyte,in this demonstration the possible deformation of oocyte caused by hydrodynamic forces is ignorable because of open space situation, therefore S was assumed as static parameter. V_f is stream velocity, v is object velocity which assumed as zero in an experiment in this study. The Reynolds number is generally small at micro scale, the hydrodynamic force coefficient can be approximately calculated as follow when the Reynolds number is up to 2×10^5[17];

$$C_d \approx \frac{24}{Re} + \frac{6}{1+\sqrt{Re}} + 0.4 \quad (3)$$

The gravity and buoyancy forces in equation (1) are given by the following equations:

$$F_g = \frac{4}{3}\pi r^3 g \rho_o \quad (4)$$

$$F_b = \frac{4}{3}\pi r^3 g \rho_f \quad (5)$$

where ρ_o, ρ_f is the density of insect oocyte cell and fluid respectively. Therefore $\rho_o = 1.007$ gram per cubic centimeter[18] and $\rho_f = 0.997$ gram per cubic centimeter are estimated when temperature is 25 degree. According to calculation result of (2), (3), the F_b, F_g is approximately equal and opposite direction. F_a represents the other additional forces exerted on object, depending on the flow condition and object properties. However, F_a and F_{vdw} are extremely small, so they are ignorable[16] compared with F_h, F_b, F_g. Therefore, in equation (1) the external force F_e numerically equals the hydrodynamic force F_h.

$$F_e \approx F_h \quad (6)$$

As results based on equation (2), (3), (4), (5), (6) if the hydrodynamic force F_h as 2.33 Nano Newton is required, the velocity of micro stream onto the insect oocyte is about 650 μm per second, minimum flow rate is about 7.11 μL per minute, the Reynolds number around the insect oocyte is less than 0.023[17]. This hydrodynamic force caused the insect oocyte to move forward along with the steady streams.

III. Design and Fabrication

A. *Design of micro fluidic device for MMSM*

According to the principle we proposed, the MMSM system consists of chip shape microfluidic device made of glass, several programmable syringe pumps, and a microscope. The prototype of microfluidic device of MMSM was designed and fabricated.

The microfluidic device in Figure 3 was fabricated with 2 layers of glass slide. The length and width of the chip is 70 millimeter and 30 millimeter. The through hole orifices array in center area and all channels were manufactured in first layer, by ultra-precision machining process. The first layer was bonded to anther glass slide. 500 μm, 200 μm, and 100 μm three types of widths of channels were fabricated; the depth of these channels is 200 μm. The lengths of the 500 μm, 200 μm, and 100 μm channels were 23 mm, 1 mm, and 200 μm respectively.

B. *Simulation of flow field Streamlines based on designed MMSM fluidic device*

Next, to confirm the principle we proposed, CFD simulation of a prototype of MMSM was performed in order to verify field streamlines surrounding the insect oocyte at the calculated flow rate conditions.

In this simulation, field streamlines in a cylinder area (600 μm × 600 μm) where hydrodynamic manipulation mainly perform were modeled in three dimensions, using the finite element method (FLUENT 14.0, ANSYS Inc.). The Navier–Stokes, Euler–Lagrange equations were used to model microfluidic stream with viscous and gravity terms. The generated microfluidic stream was assumed as a three-dimensional, continuous incompressible and laminar flow. The mesh size for simulation is 10 μm.

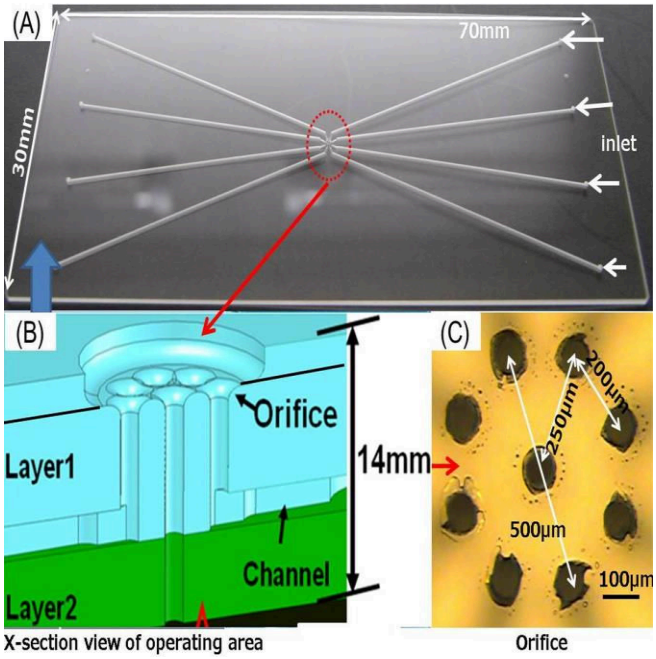

Figure 3.Design detail of the micro fluidic device.
(A) A photo of double layers prototype chip made of glass, with individual 8 channel input and output ports on the surface of the lay1. The size of this chip is 70 mm ×30 mm. Thickness of layer 1 is 1000 μm and layer 2 is 400 μm(B) The cross-section view of diagram of center area. 8 micro channels which connected to through hole orifices were manufactured. Liquid was transported through these channels and orifices to the surface for manipulating. The arrangement considered optimized design of channel–orifice connection of the center area. Three types of width of channel were fabricated. (C) The detail of the orifices in the center area. The diameters of orifices are about 100μm. These orifices were placed at vertices of an octagon.

When assuming the velocity of fluidic stream surrounding insect oocyte to about 650 μm per second according to our estimation explained before, an average velocity at the inlet orifice and outlet orifice, as shown in Figure. 4, is calculated as 15 mm per second, as inlet boundary conditions. The side walls of the computational domain were set to outflow. The bottom of the computational domain was set to wall in room temperature. The internal solution was set to liquid water in this simulation. The result of the streamlines colored by velocity was indicated in Figure 4. According to this simulation results, in the center area of designed prototype device, the steady streams with velocity of about 650 μm per second that flow from the orifice pushing out to the orifice pulling in, were confirmed. The result of streamlines indicated that it is possible to offer proper hydrodynamic force onto operating object.

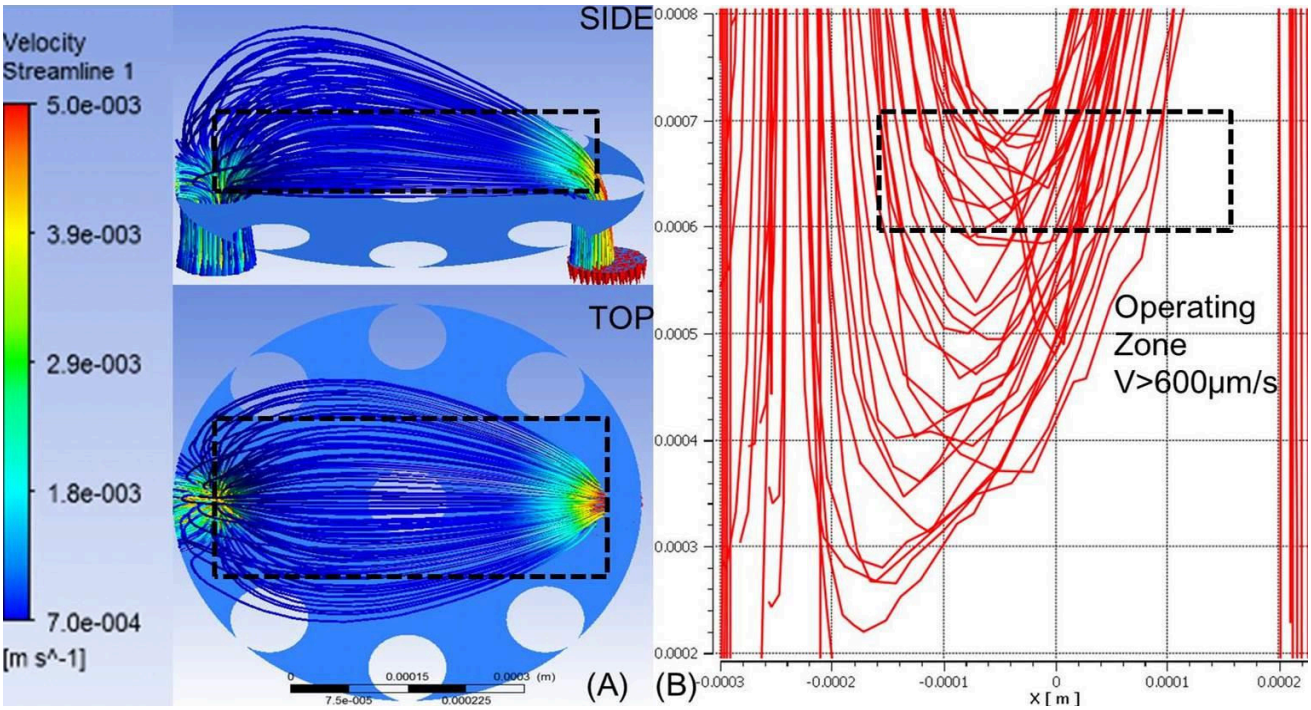

Figure 4. Simulated field streamline of MMSM.

(A)Side and top view of 3 dimensional simulated streamlines which were generated from the orifice pushing out toward the orifice pulling in the center area of the chip. (B) The graph of velocity distribution in center area. The simulation result indicated that steady and continuous micro fluidic streams were generated from the orifice pushing out toward the orifice pulling in. The area marked by black dotted line indicted the area which can offer more than 650 μm/s. The manipulation was implemented in this area.

IV. Experiment and Result

A. Experimental system

Figure.5 shows fully assembled operating chip with 8 input channels and one output channel. 8 inlets were directly connected to the syringe pump with peek tubing. In order to drive several syringes with the exactly same flow rate, these syringe pumps (PHD 2000, Harvard Apparatus Massachusetts, United States) were mounted on the multi-Rack upgrade kit. Blue plastic film was stuck on back surface of the glass chip for avoid stress concentration on the surface of inlet connection.

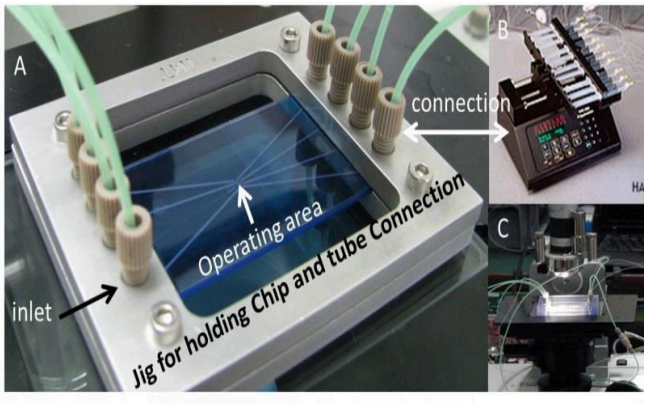

Figure 5. A photo of full assembled MMSM fluidic device, with 8 channel input, and output ports.(A)The peek tubing was connected between the syringe pumps and micro channels. The chip was settled on the stage of the microscope for observing.(B)The pump was used in this system. (C)Fully assembled chip under microscope stage.

This assembled micro fluidic device of MMSM was placed on the stage of a microscope and the pumps were placed separately but nearby the stage within 30 centimeter. For theoretical verification of MMSM, in this simple demonstration, all these syringe pumps were manually controlled. The solution media for manipulating was 0.9 percent saline solution.

B. Result and Discussion

To compare with result of simulation, trace trap experiment by using fluorescent beads was also conducted to observe the actual streamline generated from the orifice pushing out to the orifice pulling in solution on the surface of the microfluidic device. The fluorescent beads that were 2 μm in diameter were used to visualize the generated streamline.

The same configurations as shown in Figure 5 were used in experiment. The results of microscopic images are shown in Figure 6. The fluorescent beads in center area released from the orifice pushing out and moved toward to the orifice pulling in. The trajectory of fluorescent beads was observed and we confirmed that it matched the simulated streamlines.

To demonstrate bio manipulation using generated streamline without contact, single insect oocyte of moth manipulation was conducted using the MMSM with same configurations as shown in Figure 5 in simulation. Two laminar inlet streams of which flow rate was 7.11 μL/min are used to generate the micro stream flow. Due to controlling the different orifice pushing out and pulling in, several different motion of insect oocyte were observed. Typical mode of motion shows in Figure 7.

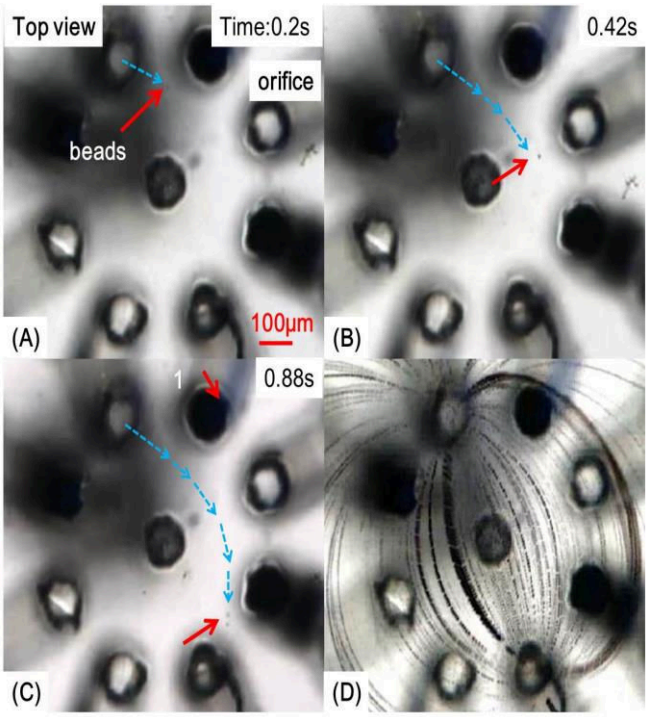

Figure 6. Microscopic images of trajectory of a fluorescent bead.
(A) Fluorescent beads were released from the orifice which push out solution continuously and trapped by stream generated from this orifice, the orifice at the opposite turn on to pull solution in simultaneously, which directed the motion of beads to move toward the orifice pulling in. (B), (C) Fluorescent beads were moved to orifice by the stream at an approximate average velocity of 568 μm per second. (D)Result image after Background subtraction. The traces of fluorescent beads can be easily recognized. The steady and continuous micro fluidic streams were generated from the orifice pushing out toward the orifice pulling in was observed.

The hydrodynamic force caused insect oocyte to move forward at the distance of 759 μm, 439 μm, and 263 μm in different mode respectively. The control of velocity and direction of insect oocyte's motion was succeeded. In this experiment, MMSM of insect oocyte was also confirmed.

To confirm possibility of repeatable manipulation of MMSM, the trapping- transportation- holding experiment was conducted by implementing the control of 2 pumps simultaneously. Experimental result of 3 steps manipulation of glass beads which diameter is 200 μm is shown in Figure.8. First, the bead was trapped to absorbing orifice by pulling solution in. In Figure 8 (A) then absorbing orifice changes to push out solution and next orifice starts to pull in to generate streams toward orifice 2.In Figure 8(B), when the beads arrived at orifice, it change to push out solution and orifice 3 starts to pull in until the beads arrived at orifice, then it was hold in (C) then be transport back in (D).

The MMSM is not only capable of manipulating object using microfluidic stream in near field of orifices, but also capable of manipulate object by using far field streams. In our other paper[19] MMSM also rotated and trap insect oocyte and micro beads on the specific area, where the eddy be generated by microfluidic streams. Because the speed of rotation and rotating position of the insect oocyte and micro beads could be precisely controlled by tuning flow rate by using appropriate orifice of MMSM, more complex motion and manipulation has been confirmed.

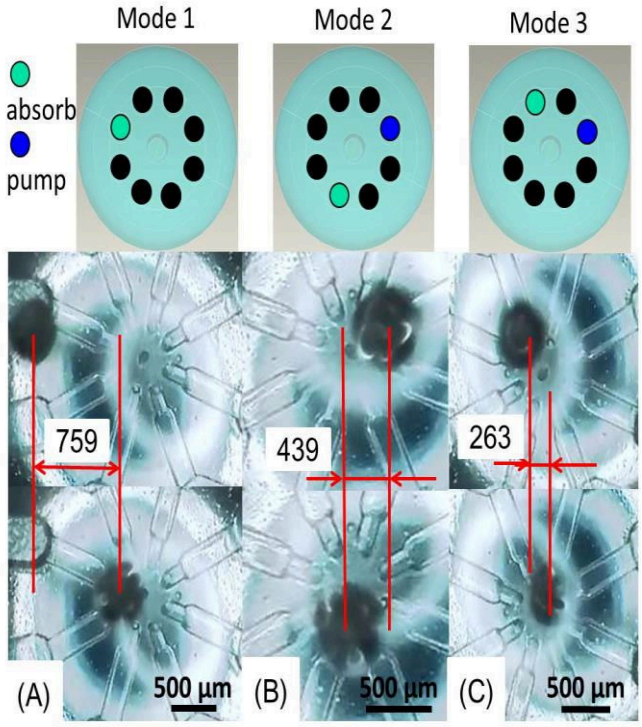

Figure 7. The manipulating mode based on different positions of working orifices, and displacements of insect ovum.
(A), (B), (C) show different mode of generating microfluidic stream respectively; Blue solid circles represent the orifice pushing out solution. Green solid circles represent the orifice pulling in solution. Black solid circles represent the orifice not be activated in this experiment. The top picture of (A), (B), (C) show the initial position of insect ovum when3 microfluidic stream was not generated, respectively; the under picture of (A), (B), (C) show the final position of insect oocyte when microfluidic stream was generated, respectively.

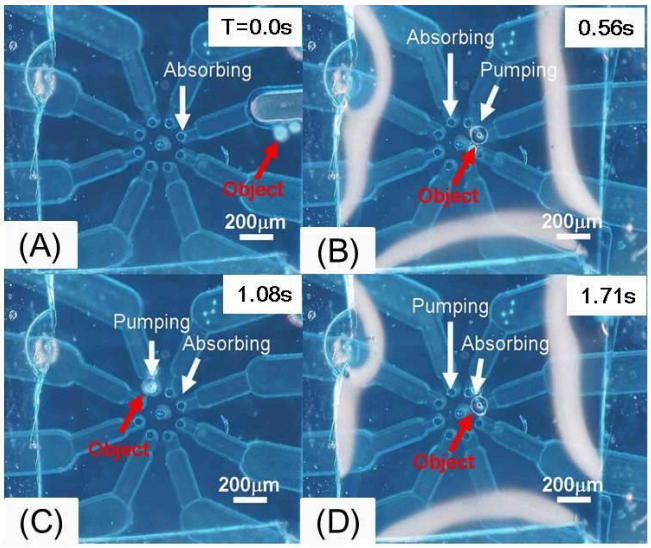

Figure 8. Repeatable driving process of glass beads. In (A)Targeting the object which is far from orifice; turn the nearest absorbing orifice to pull water in to generated fluidic stream toward to it. (B) Trap and transport target; the target beads be trapped by the pull in orifice, the next absorbing orifice turn on to pull in water and the absorbing orifice in (A) turn to push out water simultaneously to generated a directed stream from orifice 1 toward orifice 2 ,beads be trapped by stream moved to absorbing orifice , the process be repeated, beads be transport back to orifice which trapped it in (D).

In summary, continuous pushing out and pulling in extremely small amount of solution through orifices array can generate steady micro fluidic streams, of which direction and velocity can be controlled by tuning the flow rate and number of operating orifice. As a result, MMSM we proposed in this study was successfully demonstrated.

However, to apply this method as high throughput bio-object manipulation, we have to solve several problems. First, to manipulate much smaller micro bio-objects, much smaller orifice is essential. Second, to manipulate object with smaller orifice, which conducted pressure loss more significantly, much more powerful pump source is necessary. Third, realizing the automatic system of MMSM may greatly improve the performance of this method, because the effect of pumping delay which is caused by fluid inertia force, and undirected streams are not ignorable. Therefore, prediction of motion of target object and direction is necessary [19]. Highly automatic MMSM system with vision feedback and high speed response pump is needed.

V. CONCLUSION

We proposed non-contact Multiple Microfluidic Stream based Manipulation (MMSM) system for bio-objects using micro hydrodynamics, and experimentally confirmed the capability of MMSM. We have designed the glass prototype chip based on the principle of dynamics of suspended object in an aqueous solution when be manipulated by MMSM, and calculated flow profile by using CFD simulation, which approximately matched the motion of the bio-object.

In these experiments we only demonstrated simple motions of single cell manipulation because of limitation of actuators we currently used. The MMSM may also be applied to conduct complex 3D motions such as rotation, transportation, holding in an open space by precisely controlling these multi microfluidic streams.

REFERENCES

[1] T. Fukuda, F. Arai, "Prototyping design and automation of micro/nano manipulation system," *Proceedings of ICRA*, vol.1, pp.192–197,2000

[2] F. Beyeler, S. Member, A. Neild, S. Oberti, D. J. Bell, Y. Sun, J. Dual, "Monolithically Fabricated Microgripper With Integrated Force Sensor for Manipulating Micro objects and Biological Cells Aligned in an Ultrasonic Field," *JMEMS*, Vol 16(1), pp.7–15,2007

[3] B. Solano, D. Wood, "Design and testing of a polymeric micro gripper for cell manipulation. Microelectronic Engineering," *Microelectron Eng* Vol 84 , pp.1219–1222,2007.

[4] A. Ashkin, "Optical Trapping and Manipulation of Neutral Particles Using Lasers: A Reprint Volume With Commentaries," *World Scientific Publishing Company*, 2006.

[5] K. C. Neuman, S. M. Block, "Optical trapping," *RSI*, Vol 75, pp.2787–2809,2004.

[6] P. Y. Chiou, A. T. Ohta, M.C. Wu., "Massively Parallel Manipulation of Single Cells and Micro particles Using Optical Images," *Nature*, Vol 436 , pp.370–372,2005.

[7] A. E. Cohen, "Control of nanoparticles with arbitrary two-dimensional force fields", *PRL*, Vol 94, 118102,2005.

[8] H. M. Hertz, "Standing‐wave acoustic trap for nonintrusive positioning of micro particles," *J. Appl. Phys.,* Vol 78, pp.4845‐4849,1995.

[9] S. Grilli, P. Ferraro, "Electrophoretic trapping of suspended particles by selective pyroelectric effect in lithium niobate crystals," *J. Appl. Phys*, Vol 92 , pp.232902–232902–3,2008.

[10] S. K. Srivastava, A. A. Gencoglu, R. Minerick, "DC insulator dielectrophoretic applications in microdevice technology: a review," *ABC*, pp.301–321,399,2010.

[11] D. D. Carlo, L. Y. Wu, L. P. Lee, "Dynamic Single Cell Culture Array," *LOC*, Vol 6 , pp.1445–1449,2006.

[12] B. R. Lutz, J. Chen, D. T. Schwartz, "Hydrodynamic Tweezers: 1. Noncontact Trapping of Single Cells Using Steady Streaming Microeddies," *Anal. Chem.*, Vol 78, pp.5429–5435.2006

[13] M. Hagiwara, T. Kawahara, F. Arai, "Local streamline generation by mechanical oscillation in a microfluidic chip for noncontact cell manipulations," *APL*, Vol 101 , pp.074102–074102-4,2012.

[14] X.Y Liu, Yu Sun, "Visually Servoed Orientation Control of Biological Cells in Microrobotic Cell Manipulation," Experimental Robotics,STAR, Volume 54, 2009, pp 179-187

[15] M. Tanyeri, E. M. Johnson-Chavarria, C. M. Schroeder, "Hydrodynamic trap for single particles and cells," *APL*, Vol 96, pp. 1786-1794,2010.

[16] Z. X. Liu, Z. Q. Chen, M. H. Shi. "Thermophoresis of particles in aqueous solution in micro-channel," *APPL THERM ENG* 29, Vol 5–6 : pp.1020-1025,2009.

[17] J. P. Owen, W. S. Ryu, "The effects of linear and quadratic drag on falling spheres: an undergraduate laboratory," *European Journal of Physics*, Vol 26, pp.1085–1091,2005.

[18] R. Wayne, M. P. Staves, "The density of the cell sap and endoplasm of Nitellopsis and Chara," *Plant Cell Physiol*, Vol. 32, No. 8 pp. 1137-114,1991.

[19] Y.Yalikun, Y.Akiyama, T.Hoshino, K.Morishima "A Bio-ManipulationMethod Based on the Hydrodynamic Force of Multiple Microfluidic Streams," J. Robotics Mechatronics, Vol.25,No.4, pp.10-18, 2013.

[20] S. Iwaki, H. Morimasa, T. Noritsugu, M. Kobayashi, "Contactless manipulation of an object on a plane surface using multiple air jets," *Proceedings of ICRA*, pp.3257–3262, 2011.