

3D Micro Image Recognition for in Bio-Cell Operation by Micro Robots

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1. Introduction

With the demand for microscopic operation such as micro parts handling and bio cell manipulation, it becomes much more important to control the micro robots system with fine tools on the desktop. For these years, many tiny robots have been developed and they have provided several unique accurate applications such as fine surface machining, micro parts conveying and surface roughness measurement in our group [1]. In order to provide such microscopic operations automatically under the microscope by multiple micro robots, we have investigated the navigation strategy and the positioning method based on microscopic image recognition in 2-dimensional space [2]. However, in order to achieve more advanced microscopic biological operations in the bio cell, more advanced image recognition and fine tool guidance in 3 dimensions are required. For example, when conveying a micro injection tool and carrying out such a nuclear transplantation under a microscope while preventing the breakage of the bio cell, we must check the position for insertion and maneuver the small robots to approach from the specified direction.

In this report, a unique non-contact rotating mechanism in-situ is proposed at first. When the thin pipette on the small robot is vibrated near the bio cell, the micro flow can be generated around it and then the cell can be rotated without any mechanical contact. And then several cell images at every rotating angle can be monitored by a microscope with a CCD device and a 3D image can be reconstructed after the image processing computation to provide the precise location of the bio cell elements. After the identification of each element in the bio cell, the small robots can be controlled to execute the fine operation. We aim to develop the micro robots system which can provide the automatic bio cell operation combined with the 3D image recognition property.

2. System Configuration

Fig.1 shows the flexible micromanipulation system by using versatile micro robots under a microscope which is ongoing development in our laboratory. These micro robots can be controlled using a PC in real-time analyzing of microscopic images. All micro robots

are set on a steel table. In this basic setup, this system can execute flexible microscopic tasks with easy operation. All positioning facilities are given by the micro robots' movement so all mechanical functions are simply divided. This unique arrangement allows the system good flexibility and high mechanical stability although sophisticated control is required. This may be a good application for micro robots practically. We can easily attach micro robots to micro processing instruments. This flexibility meets the requirements of users, and the use of small robots avoids the repair of microscopes and easily implements processing with low cost.

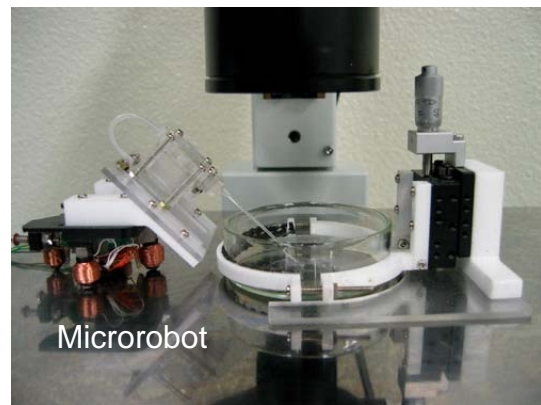


Fig.1 The flexible micromanipulation system by using versatile micro robots under a microscope.

3. Versatile micro robot

In microscopic operations, an operator needs to operate a tool intricately. Fig.2 shows the motion patterns required during microscopic operation. Because there is no surplus space in such a narrow area, such movement was difficult for the old micro robot [3]. To realize these motion patterns at the same time, a structure was needed that could move in XY directions and in rotation in θ independently, namely holonomic behavior. Fig.3 shows the structure of the versatile micro robot which is proposed to satisfy the requirements above. Two U-shaped electromagnets which are arranged to cross each other are connected by four piezo elements so that the micro robot can move in any direction like an inchworm. Also we designed the special joint at one of the 4 legs to ensure

all legs smooth simultaneous contact on the surface. This layout of actuators can allow it to move precisely in a holonomic locomotion manner.

As shown in Fig.4, the robot is 35mm in length, 35mm in width and 15mm in height. We use the stacked type PZT elements of 5mm x 5mm x 10mm. Each piezo element is connected to each electromagnet with a plastic insulator. In experiments, we confirmed that the micro robot can move in XY directions. We also design the spherical 3D micro manipulator as a microscopic manipulator [4] as shown in Fig.5. The manipulator is composed of three piezo elements with a small accurate sphere and a large accurate sphere on the top of them and can be set on this micro robot.

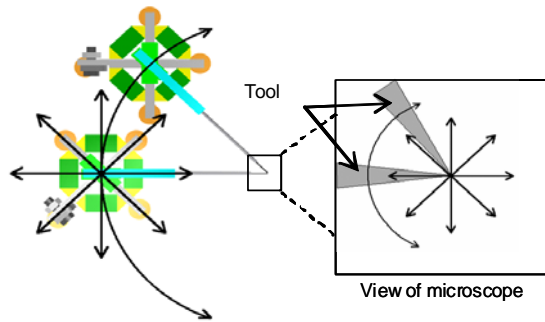


Fig.2 Motion pattern required during microscopic operation.

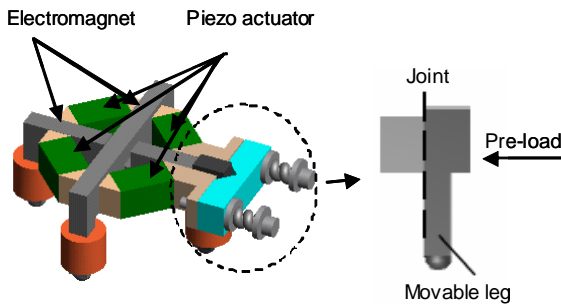


Fig.3 Structure of versatile micro robot

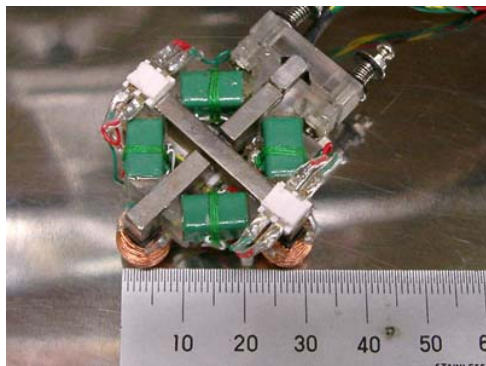


Fig.4 Versatile micro robot provided for microscopic operation.

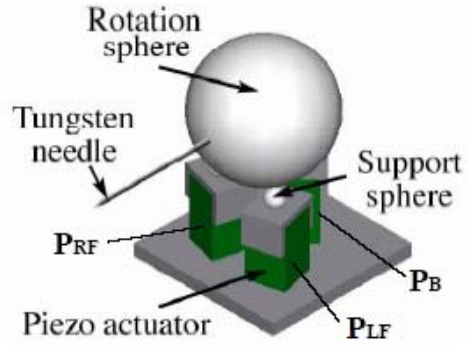


Fig.5 Structure of spherical 3D micro manipulator

4. Non contact rotation positioning of the egg cell by pipette vibration

To perform more precise cell operation, the operation of direction of Z as well as the operation of XY is needed. Three-dimensional micro operations are difficult to operate by using a 2D image from the CCD camera. In order to construct the 3D information, it is necessary to observe the object from any direction by rotating a CCD camera centering on an object, or rotating the object itself. In the microscopic operation under microscope, because it is difficult to move a camera by spatial constraint, constructing 3D information for precise micro manipulation is carried out by fixing a camera and rotating an object. Rotating an object mechanisms have been studied from the former. Those systems using an electromagnetic field may damage bio-samples which are sensitive to environmental change and are not suitable in order to handle a bio cell [5].

In this paper we proposed and used the non contact rotation positioning of an egg cell by pipette vibration, which makes a rotational flow near the pipette. In our system, this rotational flow is generated by using the versatile micro robots. First, as shown in Fig. 6, a robot's electromagnet 1 is always adsorbed and electromagnet 2 is always not adsorbed. And the position Fig. 6 (a) and (b) is repeated by expanding and contracting the piezo-electric element between both electromagnets. By this motion, the pipette on a micro robot can be vibrated with the amplitude of tens of microns. This proposed mechanism can be carried out non contact rotation operation, without using some additional actuators for a micro robot. This is a very important factor, in order to construct a simple and low cost microscopic operation system.

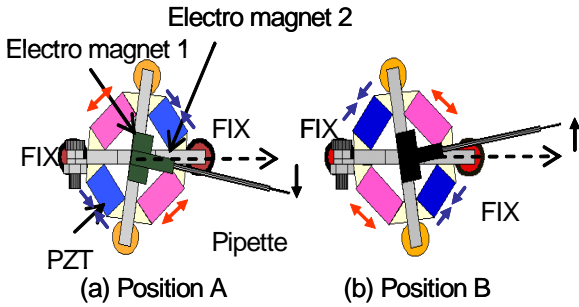


Fig.6 The flow generating mechanism by using a versatile micro robot.

In this experiment, the rotational flow in water of schale was generated using the holding pipette of the bore 15 [μm] and an outside diameter 100 [μm] at a tip of micro pipette. The pipette tip was bent to 45 [$^\circ$], and it placed in parallel to the schale bottom, and observed from the schale bottom. To tracing partial flows, water mixed with black sumi was used. The flow pattern when vibrating a piezo-electric element by the sine wave of pitch 200 [Hz] and the seal-of-approval voltage ± 45 [V] is shown in Fig. 7 (a) and (b).

These experimental results show that pipette vibration can generate constant flows, which shows a fixed flow pattern near the pipette. When changing the pitch and seal-of-approval voltage of a piezo-electric element, the flow velocity changed, but this flow pattern was about fixed. Therefore, the proposed mechanism may be effective in order to control rotation of a miniature ball such as an egg cell for bio cell operations.

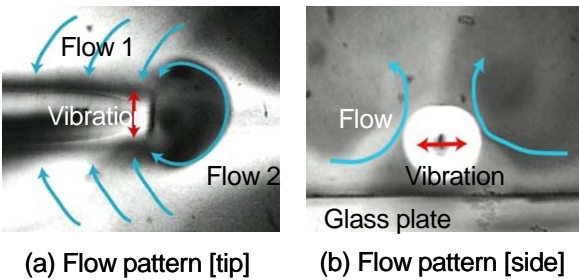
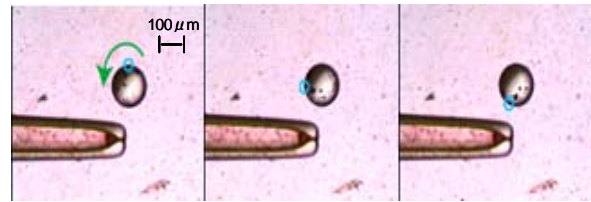


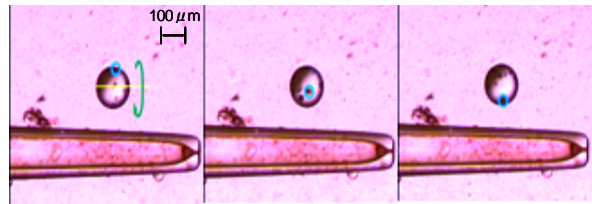
Fig.7 Flow pattern near a micro pipette. [Scale: $\times 200$]

As a basic experiment, the rotation positioning of the minute ball placed around a pipette was tried using our proposed mechanism. The glass minute ball of a diameter 130 [μm] (UB-67L Union Co. Ltd) was used for this experiment. When vibrating a piezo-electric element by the sine wave of pitch 200 [Hz] and the seal-of-approval voltage ± 45 [V], a minute ball rotates counterclockwise in the same position at the pipette tip as shown in Fig. 8 (a). This motion occurs based on the straight flow and circle flow shown in Fig.7 (a). Rotational movement is shown in Fig.8 (b) is also occurs based on the flow pattern in Fig.7 (b). The

direction of rotation of the minute ball in the experimental result of Fig. 8 is the same as the direction of a flow observed in Fig. 7. Therefore, it is guessed that rotation movement is caused by this rotational flow. From these experimental results show that the proposed mechanism can be used non-contact rotation positioning in three dimensions. In the conditions of dealing with a minute object, viscous power acts on a minute object greatly rather than force of inertia. Therefore, it is possible to stop rotation of a minute ball immediately by suspending vibration of a pipette. The degree of rotation angle of a minute ball can be determined with the resolution of several degrees by controlling the oscillating time of a pipette. This is very effective function in order to carry out precise microscopic operations.



(a) Rotational movement of a minute ball [Pipette tip]



(b) Rotational movement of a minute ball [Pipette side]:

Fig.8 Non contact rotation positioning of the minute ball using vibration by pipette vibration [Scale: $\times 160$]

When the egg cell of the mouse of a diameter 120 [μm] was used as an object of microscopic operations, the non contact rotation positioning of the egg cell by pipette vibration of a micro robot was also available in a similar manner. A general sequence of microscopic operations (rotation positioning of an egg cell in a pipette tip, movement of a pipette and aspiration fixation) required for an operation can be carried out by using only this mechanism without some special additional actuators.

5. 3D Micro Image Recognition for in Bio-Cell Operation

Using the non-contact rotation positioning mechanism, the object of microscopic operation is recognized in three dimensions. A minute glass ball is used as an object of a basic experiment for bio cell operations.

This ball is a diameter 130 [μ m] (UB-67L Union Co. Ltd). The diameter of this ball is equivalent to the egg of a mouse.

To a preparation of 3D micro image recognition, the 3D sphere model corresponding to the size of this ball is generated on a computer based on the information on the scale factor of a microscope, and the diameter of the circle on a screen. The sphere on a computer and an actual sphere are rotated 360 degrees by the resolution of a unit 10 degrees towards being shown in Fig. 8 (b). The circle shape on the surface model of a sphere of the boundary for recognition of front area is extracted.

Pre-processing of recognition of the object of circle shape assigns a label number to a continuous pixel area, after binary-izing the bit map image of a CCD camera. The circular value (Value shows circle-likeness) is calculated to each labeling area. A circular domain is extracted using this value and threshold value. The circular value e is calculated by the following equation using the neighboring length l and the area S of a area.

$$e = \frac{4\pi S}{l^2} \quad (0 < e < 1)$$

When a local area is a perfect circle, e is set to 1.0. This value becomes small, so that area is no like a circle. When a circle is extracted, the position and size are measured and the result plotted on the surface-of-a-sphere model is shown in Fig. 9.

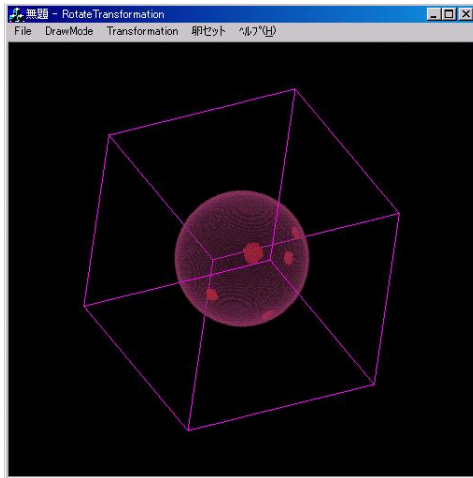


Fig.9 The 3D model for microscopic operations based on the non contact rotation positioning mechanism

To generate this 3D surface model in the computer previously is useful to execute more precise operation and this model can be used creating control signal of micro robots.

6. Conclusions and Future Works

Flexible micro-processing organized by versatile micro robots under microscopes was proposed and developed 3D model for microscopic operations based on the non contact rotation positioning mechanism.

In future work, our spherical micro manipulator which can control the bio operation tool in Z direction as well as angles precisely and this 3-D image recognition approach will be combined, and then we're going to develop a more precise bio operation system.

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