Robotica (2005) volume 23, pp. 441–448. © 2005 Cambridge University Press
doi:10.1017/S0263574704000876 Printed in the United Kingdom

Cylindrical micro touch sensor with a piezoelectric thin film for microbial separation

Fumihito Arai*†, Kouhei Motoo*, Paul G. R. Kwon*,
Toshio Fukuda*, Akihiko Ichikawa* and Tohoru Katsuragi‡

SUMMARY

Isolation and separation of the target microbe or cell from a large heterogeneous population is quite important. We propose a new touch sensor that is used for the separation of the target microbe with a pipette. This sensor is sensitive enough to protect the fragile pipette tip from abrupt collision. Using this sensor, we developed a novel separation system for screening a target microorganisms from the randomly distributed samples in the dish with the local viscosity control of the thermosensitive hydrogel. With this system, the target yeast cell was extracted successfully.

KEYWORDS: Microsensor; Touch sensor; Piezoelectric thin film; Pipette; Separation; Screening; Microorganism.

1. INTRODUCTION

Recently, there has been great interest in the high throughput screening of microorganisms, for example, for finding new microbes. It is estimated less than 10% of all the microorganisms are known at present. In applied microbiology, single-cell selection of viable cells with the desired characteristics from a large heterogeneous population is needed.[1] To satisfy this demand, flow cytometry (FCM) is well-known method and fluorescence activated cell sorters (FACS) were developed for high-speed separation of cells.[2] However, conventional flow cytometers tend to be large. Recently, there appeared some works on miniaturization of the FACS or separation chips with sequential flow.[3–6] Microorganisms are separated by mechanical forces, hydrodynamic flow, ultrasound, magnetic forces, laser tweezers, and electric forces in the microchannel flow.[4] However, most of the conventional separation processes are sequential and it is impossible to compare multiple objects simultaneously before separation, and positional information is lost after separation.

To overcome these drawbacks of FCM, laser scanning cytometry (LSC) was proposed.[1] LSC is a microscope-based method. A large heterogeneous population can be treated on the microscope stage and individual cells can be examined repeatedly, measured automatically and later observed microscopically by the operator. However, automation of the target cell separation was still difficult mainly because of manual pipetting work with a mechanical micromanipulator. As for the extraction method of a microbe based on the parallel comparison of several samples, there have been proposed a lot of methods based on non-contact manipulation.[7,8] However, the mechanical micromanipulator with a pipette is commonly used, as illustrated in Fig. 1, to extract a microbe, since the system is quite simple.

In using such a system, it is difficult for the operator to recognize the distance between the pipette tip and the bottom plate since the focal depth of the commonly used microscope is narrow, much time is needed for the extraction of targets and the task is laborious. Moreover, the pipette tip is sharp and very fragile. Once the tip collides with the environment abruptly, it is easily broken, and then we have to replace it with the new one. This is the common problem in manipulating a microscopic object with a microscope. To solve this problem, a dynamic focusing lens was developed to enlarge the focal depth of the microscope image.[9] It is desirable to watch the manipulation environment in real-time, but the lens variation is limited, and a high resolution lens with a high numerical aperture has not been developed. On the other hand, we can avoid collision of the pipette tip by employing a high sensitive touch sensor at the tip of the manipulator. To improve such manipulation work with the pipette and microscope, we aim to develop a new touch sensor which is attached to the pipette tip to detect contact with the environment.

We have developed a micro tri-axial force sensor with semiconductor strain gauges for the mechanical micromanipulation of cells.[10] Although the sensor has high sensitivity, it is fragile and delicate without special attention in its design. Normally, the pipette is connected to the additional pump unit with the tube. In such cases, the rigidity of the tube will cause large disturbances to the flexible elements for sensing. So we have to isolate the effect of the tube rigidity from the sensor; however, this is difficult for the sensor based on detection of deformation of flexible elements. Here we propose a novel touch sensor for the pipette work based on detection of the mechanical impedance change after contact of the vibrating pipette tip with the plate. The principle of sensing based on the impedance change of the vibrating beam is well known.[11–18] The force between the object and vibrating probe tip changes the resonant frequency of the probe. The basic principle was applied to the SPM

---

* Department of Micro System Engineering, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8603 (Japan). http://www.mein.nagoya-u.ac.jp, E-mail: arai@mein.nagoya-u.ac.jp
† PRESTO, JST.
‡ Nara Institute of Science and Technology (Japan).

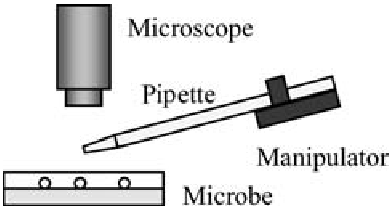


Fig. 1. Extraction of a microbe by the pipette.

(scanning probe microscope).[11–13] The PZT thin film was deposited on the flat body of the cantilever and a very sensitive touch sensor was developed; however, it was not designed for mechanical micromanipulation with the pipette. There is no research work on the touch sensor which can be set easily at the tip of the pipette. Since the glass pipette has a cylindrical shape, here we propose a cylindrical shaped touch sensor. The PZT thin film was deposited on the surface of cylinder by the hydrothermal method,[15–21] and a driving and sensing method of pipette tip was studied. The prototype sensor has high sensitivity and high rigidity and of excellent durability.

Moreover, here we propose a novel separation method based on the fixation of the target cell using a viscosity control around the target by the thermal gelation technique.[22–24] Once we fix or trap the target somewhere by heating and remove the obstacles around it, we can access near the target easily by the mechanical micromanipulator with the pipette. The fixed target is released by naturally cooling the temperature. Then the target is collected by the pipette. We employed a sol-gel transformation material to fix and release the target. Basic experiment results have shown the effectiveness of our proposed separation method.

2. MATERIAL OF TOUCH SENSOR

2.1. *Material for the sensor*

As a material of the touch sensor with high sensitivity that can be used for micromanipulation, piezoelectric ceramics, semiconductor strain gauge, etc. can be considered. Here, conditions required for the touch sensor for our system are summarized as follows:

(i) Its size is small so it could be set near the microscope.
(ii) It has high sensitivity to detect contact with the substrate, such as an agar culture medium or glass plate.
(iii) It has high durability to be used several times.

As the touch sensor that fulfills the above conditions, we decided to use the piezoelectric thin film which is deposited on a titanium substrate.

2.2. *Hydrothermal method*

As the deposition method of a piezoelectric thin film, there have been proposed sol-gel, sputtering and hydrothermal procedures,[15–21] etc. We used the hydrothermal method for the PZT thin film deposition. The advantage of this method is described below.

(i) A PZT thin film is deposited at a relatively low temperature (below 150 °C).
(ii) A PZT thin film is deposited on the surface of the three-dimensional structure.

The process condition of the hydrothermal method employed in this paper has been reported elsewhere.[17]

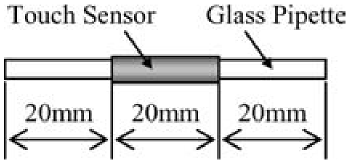


Fig. 2. Schematic of touch sensor with the pipette.

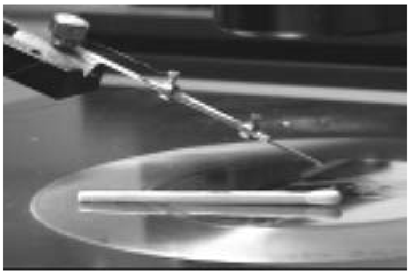


Fig. 3. Photograph of touch sensor with the pipette.

3. PIPETTE WITH A TOUCH SENSOR

3.1. *Structure*

Generally, an extraction of a microbe is performed using a glass pipette with a diameter of 1 mm. Here, we designed and produced a cylindrical shaped touch sensor (inner diameter: 1.5 mm, outer diameter: 1.6 mm, length: 20 mm), which can be easily attached to the pipette (diameter: 1 mm, length: 60 mm). Figure 2 shows the schematic of the touch sensor with the pipette. Figure 3 shows the photograph. Since the sensor is cylindrical, the glass pipette is easily inserted and fixed. Maintenance of the fragile pipette is quite easy.

The structure of the touch sensor is shown in Fig. 4. The PZT thin film is deposited on the titanium (Ti) pipe by the hydrothermal method. There are two electrodes on the PZT thin film. One is used for the actuation at the resonant frequency of the structure. The other is used for sensing vibration. The process of detection of touch is explained below.

(i) First, an alternating current is applied to vibrate the touch sensor with its appropriate resonance frequency. Then the sensor vibrates with the pipette.
(ii) When the pipette contacts the environment, the output of the touch sensor changes because of the mechanical

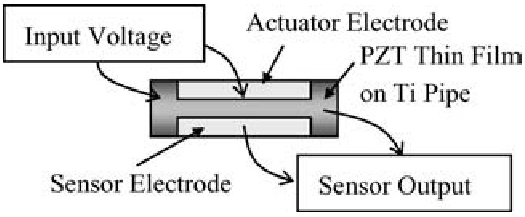


Fig. 4. Structure of the touch sensor.

impedance change. Then, contact is judged by measuring the change of sensor output.

3.2. *Analysis of stress distribution and sensor output*

A vibration mode can be properly selected to maximize the sensor output. In order to obtain higher sensitivity, we did analysis to investigate the effect of the vibration mode on the sensor output, and the validity of the analysis was checked by experiment.

First, the relation between stress and electric field was derived. The relation between stress and electric field of the piezoelectric material is expressed as follows:

$$P = \varepsilon\varepsilon_0 E + d\sigma \tag{1}$$

P: Polarization	σ: Stress
E: Electric field	ε: Dielectric constant
d: Piezoelectric constant	

Here, since voltage is not applied to the output electrode, equation (1) is written as follows:

$$P = d\sigma \tag{2}$$

The electric field generated at the output electrode is written as follows by eqs. (1) and (2).

$$E = \frac{P}{\varepsilon\varepsilon_0} = \frac{d}{\varepsilon\varepsilon_0}\sigma \tag{3}$$

From eq. (3), since the piezoelectric and dielectric constants are constant and fixed, the electric field is proportional to the stress.

Next, by assuming the pipette with the touch sensor shown in Fig. 2 as a cantilever beam, we performed a modal analysis to obtain modal functions and stress distribution. The simplified model for the analysis is shown in Fig. 5. First, the equation of motion of the pipette with the touch sensor is expressed as follows:

$$\rho A\frac{\partial^2 u}{\partial t^2} + \frac{\partial^2}{\partial x^2}\left(EI_0\frac{\partial^2 u}{\partial x^2}\right) = 0 \tag{4}$$

ρ: Density	E: Young's modulus
A: Cross section	I_0: Moment of inertia

Here, x-axis and u-axis are defined in Fig. 5. Then, the solution of this equation is given as follows:

$$u = \sum_{r=1}^{\infty}\phi_r\left(a_r\cos\omega_r t + b_r\sin\omega_r t\right) \tag{5}$$

ϕ_r: Modal function	ω_r: Natural angular frequency

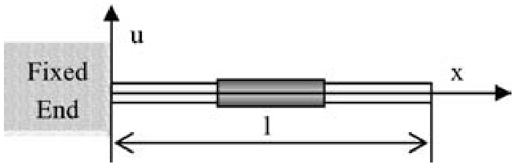


Fig. 5. Model for vibration analysis.

The boundary condition is given as follows:

$$u = 0, \quad \frac{\partial u}{\partial x} = 0 \tag{6}$$

Then, the modal function is obtained as follows:

$$\phi_r = (\sin\lambda_r + \sinh\lambda_r)\left(\cos\frac{\lambda_r x}{l} - \cosh\frac{\lambda_r x}{l}\right) - (\cos\lambda_r + \cosh\lambda_r)\left(\sin\frac{\lambda_r x}{l} - \sinh\frac{\lambda_r x}{l}\right) \tag{7}$$

$$\lambda_r = l\sqrt{\omega_r\sqrt{\frac{\rho A}{EI_0}}} \tag{8}$$

l: Full length of the pipette

The amplitude of oscillation in each mode is given by eqs. (5) and (7).

Next, the stress distribution in each mode is derived. First, the stress distribution along the position of x is expressed as follows:

$$\sigma_x = \frac{M}{I_0}u \tag{9}$$

σ_x: Stress at the position of x, M: Moment

With the following relation,

$$EI_0\frac{d^2u}{dx^2} = -M \tag{10}$$

the stress is written by the following equation.

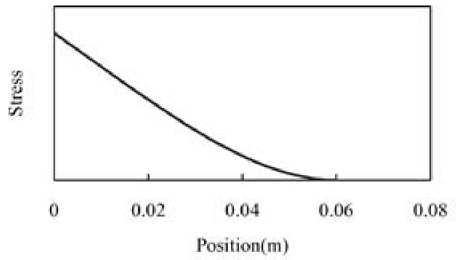
$$\sigma_x = -Eu\frac{d^2u}{dx^2} \tag{11}$$

The stress distribution of the pipette with the touch sensor can be derived by substituting eq. (5) into eq. (11). The results are shown in Fig. 6.

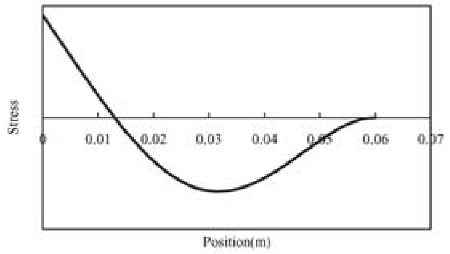
From eq. (3), the electric field is proportional to the stress. Hence the stress distribution along the sensor is quite important in order to obtain a large output. As a result of the analysis, the 2nd mode and the 4th mode are suitable to realize high sensitivity. Figure 7 shows the analytical result of the stress distribution of the 3rd mode. In this case, the output is reduced, since whether the stress is positive or negative depends on the location along the sensor. Figure 8 shows the analytical result of the stress distribution of the 4th mode. In this case, the output is not reduced since the stress is positive along the sensor.

3.3. *Measurement of sensor output*

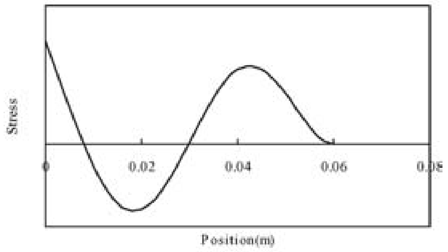
In order to confirm the validity of the analysis, we did the experiment to measure the sensor output at each resonant frequency. In the experiment, the pipette with a touch sensor is firmly fixed as shown in Fig. 5. An alternating current (20 Vp-p) is applied to the touch sensor, and the output is measured. The resonant vibration frequency was adjusted by measuring displacement near the tip of the sensor using a laser Doppler meter, as shown in Fig. 9. The experimental



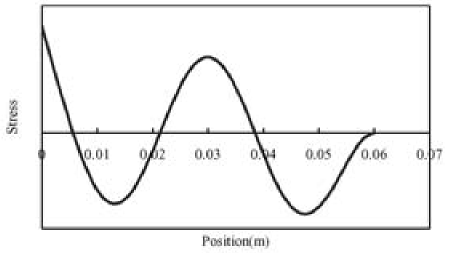
(a) First mode



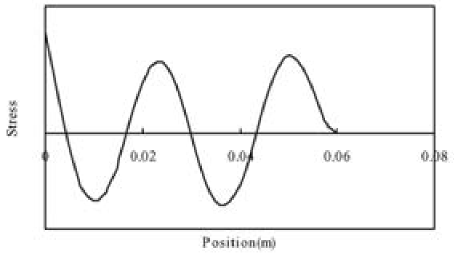
(b) Second mode



(c) Third mode



(d) Fourth mode



(e) Fifth mode

Fig. 6. Analytical result of stress distribution.

result of the sensor output is shown in Fig. 10 and Table I.

From the experimental result, it is seen that a large output was obtained at the 2nd and the 4th resonant frequency, as expected from the analytical result.

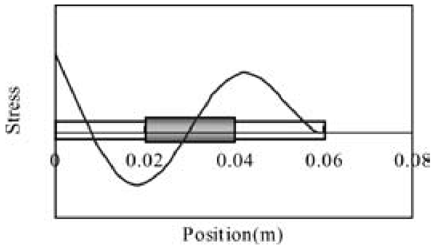


Fig. 7. Stress distribution (The 3rd Mode).

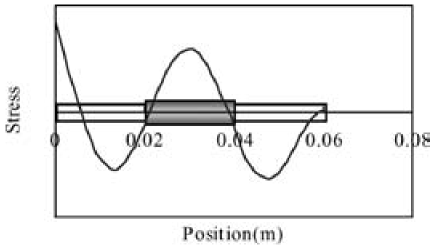


Fig. 8. Stress distribution (The 4th Mode).

Fig. 9. Measurement of sensor output.

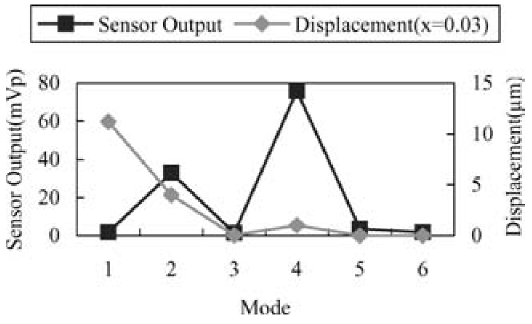


Fig. 10. Sensor output and displacement at each resonant frequency.

Table I. Experimental result of sensor output.

Mode	Frequency (Hz)	Displacement (μm) ($x = 0.03$)	Sensor Output (mVp-p)
1	122	11.20	1.76
2	1,158	4.00	33.00
3	2,171	0.03	1.36
4	6,711	1.00	76.00
5	7,836	0.01	3.60
6	8,577	0.01	1.75

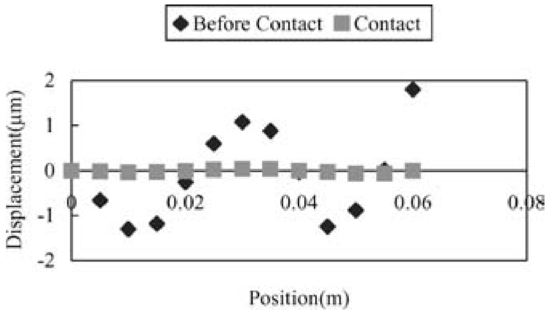


Fig. 11. Deformation of the pipette with the sensor at the 4th resonant vibration before and during contact.

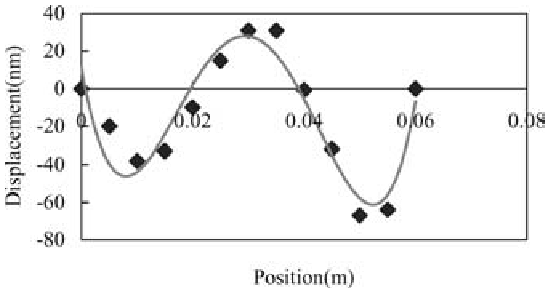


Fig. 12. Deformation of the pipette with the sensor at the 4th resonant vibration during contact (magnified figure).

Fig. 13. Experiment of force measurement.

We also measured the deformation of the pipette with the sensor at the 4th resonant vibration before and during contact with the soft agar. The result is shown in Figs. 11 and 12. In this case, the sensor input level was 20 Vp-p.

3.4. Measurement of force

In order to investigate the sensitivity of the pipette with the touch sensor, we evaluated the force sensing property of this sensor. First, a pipette with a touch sensor is fixed to the micromanipulator, and an alternating current is applied to the touch sensor to vibrate it at the effective resonant frequency. Next, the pipette tip is put on an electronic balance and the output of the touch sensor is measured, as shown in Fig. 13.

In this experiment, the pipette with the touch sensor was vibrated at the 4th resonant frequency (6,711 Hz), and the contact force was measured by the electronic balance of 0.1 mg resolution. We tested two kinds of input voltage to the touch sensor, that is, 20 Vp-p and 5 Vp-p. The output of the touch sensor was amplified 300 times and was measured successfully by the computer system.

Figure 14 shows the sensor output after amplification when the input voltage to the sensor is 20 Vp-p, while Fig. 15 shows the sensor output when the input voltage is 5 Vp-p. By reducing the input voltage to the touch sensor, the sensor becomes more sensitive to detect the small weight change.

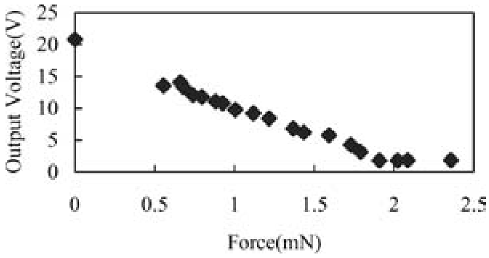


Fig. 14. Force measurement (Input voltage: 20 Vp-p).

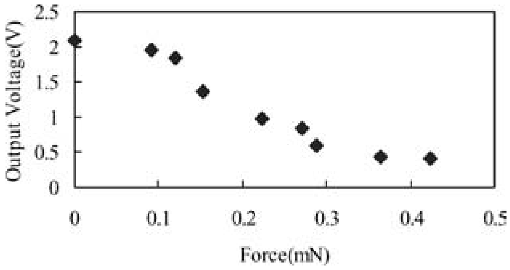


Fig. 15. Force measurement (Input voltage: 5 Vp-p).

4. EXTRACTION OF MICROBE ON SOFT AGAR BY THE PIPETTE WITH TOUCH SENSOR

The extraction of the target microbe was performed using the pipette with the touch sensor. The target microbe is *chlorella* (diameter: about 10 μm) placed on the soft agar culture medium. We used an inverted microscope. The magnification of the object lens was 10 times. The pipette with the touch sensor was vibrated at the 4th resonant frequency (6,579 Hz), and the input voltage to the touch sensor was 10 Vp-p.

The result of extracting the target is shown below.

(i) First, sample objects are distributed on the agar culture medium.
(ii) The pipette is vibrated. Before the tip of the pipette contacts the agar culture medium (Fig. 16), the output of the touch sensor is 35 mVp-p (Fig. 17).
(iii) When the tip of the pipette contacted the agar culture medium (Fig. 18), the output of the touch sensor decreased and became 16 mVp-p (Fig. 19).
(iv) Finally, the target was sucked by the pipette (Fig. 20).

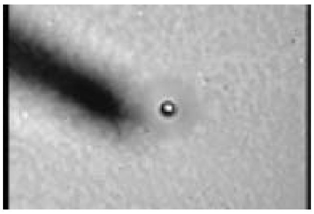


Fig. 16. Before contact.

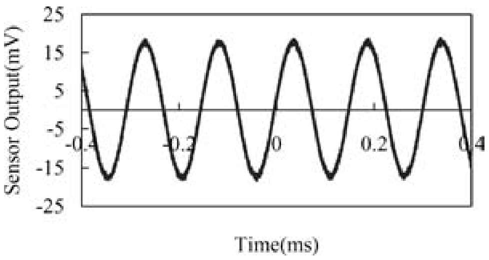


Fig. 17. Touch sensor output (Before contact).

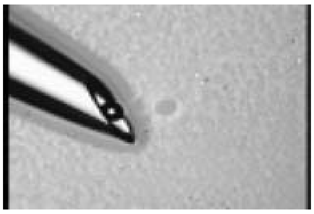


Fig. 18. Contact with sof.

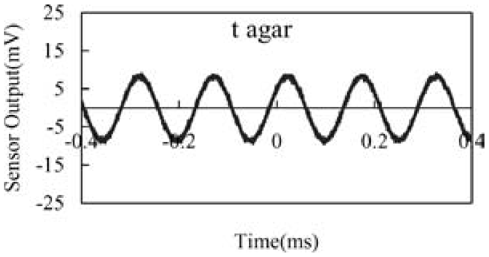


Fig. 19. Touch sensor output (Contact).

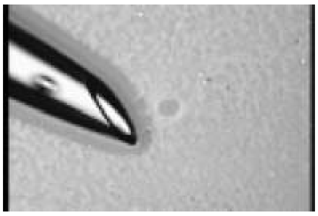


Fig. 20. Suction.

5. ISOLATION OF THE MICROBE IN LIQUIDS BY THERMAL GELATION AND EXTRACTION OF THE MICROBE BY THE PIPETTE WITH A TOUCH SENSOR

Next, we designed a novel separation system to pick up the target in the liquid using a pipette. Here we used the thermal sol-gel transformation of a methyl cellulose solution for the fixation and isolation of the target microbe. The principle of isolation is shown in refs.[22–24] Methyl cellulose is known as a thermosensitive hydro gel. Once we fix or trap the target somewhere and remove the obstacles around it, we can access the target easily by the mechanical micromanipulator. The disturbance of the liquid flow caused by moving the pipette tip is negligible and none of the obstacle adheres the pipette. By employing the thermal gelation method, we can overcome the drawback of the mechanical manipulation of the single cell in the liquid. The target is picked up by the mechanical micromanipulator with the pipette quite easily.

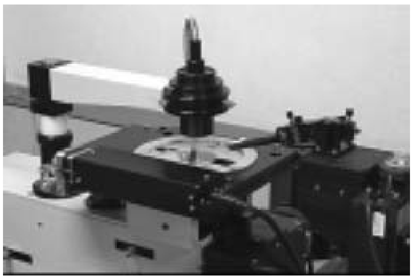


Fig. 21. Inverted microscope and micromanipulator.

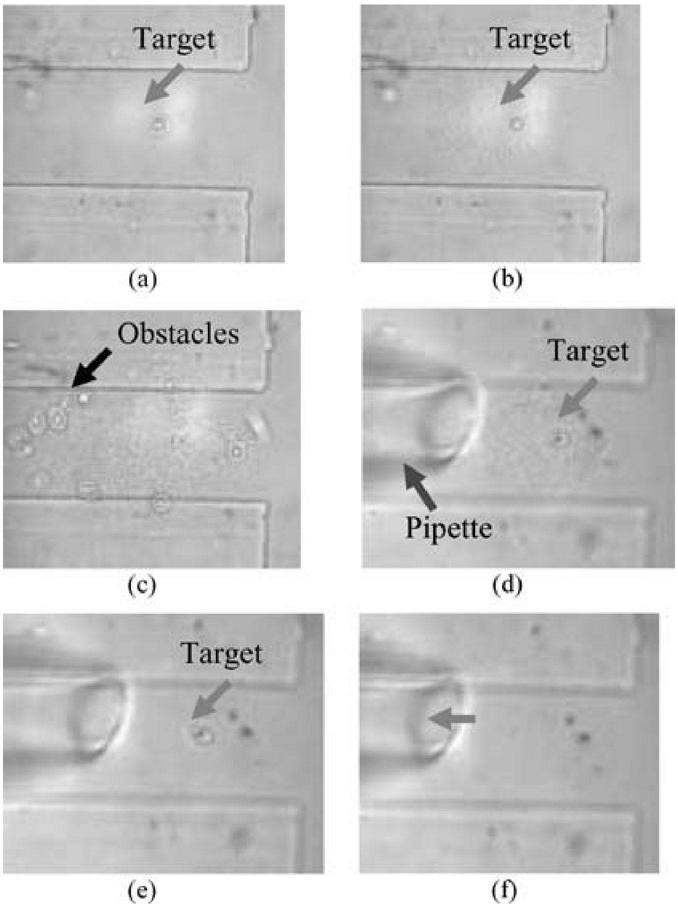


Fig. 22. Separation of the yeast cell with the pipette. (a) The samples are suspended in the dish with the 6 wt% methyl cellulose solution. The ITO (indium tin oxide) electrode is preheated below gelation level. The target yeast cell is located in the center. (b) The target is fixed by gel after laser irradiation near the target. Once the target is fixed by gel, it is kept as long as the microheater is put on. (c) In the process of cleaning, obstacles drifting near the target are observed. They flow away easily by the sufficient cleaning flow. (d) After cleaning the obstacles, the micropipette is positioned near the isolated target. (e) The microheater is cut off, and the target is released from the gel. (f) The target is sucked into the pipette immediately.

Figure 21 shows a system to pick up the target by the mechanical micromanipulator with the pipette. The micromanipulator with 3-DOF is set near the microscope. The positioning accuracy of the tip is about 1 μm in 3D space.[25] At first, the samples are suspended in the dish with the 6 wt% methyl cellulose solution. The dish is set on the stage of the inverted microscope. The microelectrode is attached to the bottom plate of the dish. The ITO (indium tin oxide) thin film is patterned to make a transparent microheater. The laser is guided through the objective lens. Here we used the Nd: YVO$_4$ laser (maximum output 4.98 W, wave length 1,064 nm, TEM$_{00}$, M^2 < 1.1). The microelectrode is heated widely by the applied voltage and locally by the energy absorption of the irradiated laser power. Then the liquid near the focused area is heated by thermal conduction, and the target is fixed by thermal gelation. By cutting off the microheater and laser, we can release the fixed target immediately, since the sol-gel transformation is fast.[24]

Figure 22 shows the result of separation experiment by this system. The samples are yeast cells (diameter on the average: about 6 μm) distributed in the dish. The target located near the center was fixed in the gel and obstacles were washed out, as shown in Fig. 22 (d). Then, the pipette tip is positioned properly near the isolated target, and it was quite easy to suck the target by the pipette, as shown in Fig. 22 (f). Here, a touch sensor was installed near the tip of the pipette to avoid collision. This system can be easily combined with the LSC.[1]

6. CONCLUSIONS

In this paper, a novel touch sensor was proposed. We used a PZT thin film deposited on the surface of the titanium cylinder by the hydrothermal method. The prototype sensor has high sensitivity and of excellent durability. The relation between the vibration mode and the output of the touch sensor was analyzed, and the validity was examined by experiment. When the pipette with the touch sensor is used at the 2nd or the 4th resonant frequency, it has high sensitivity.

The extraction of the target microbe (*chlorella*: diameter of about 10 μm) was performed using the pipette with the touch sensor. The change of the touch sensor output by contact could be monitored, and we succeeded in the target extraction without damaging the pipette tip.

Moreover, we employed a novel separation method based on the fixation and isolation of the target using local viscosity control by the thermal gelation technique. The target yeast cell was extracted successfully. At present, safety against heating is a remaining problem. However, there are another thermosensitive hydrogels with low sol-gel transformation temperature, such as a poly(N-isopropylacrylamide) hydrogel.[26] Thus a thermal gelation control with proper gelation temperature will be realized after extensive research work.

In the future, we will improve the property of the PZT thin film to improve the sensitivity of the touch sensor. We plan to build an automatic extraction system of the target microbe based on a mechanical micromanipulator.

References

1. T. Katsuragi and Y. Tani, "Review: Single-cell sorting of microorganisms by flow or slide-based (including laser scanning) cytometry", *Acta Biotechnol.* **21**, 99–115 (2001).
2. M. R. Melamed, T. Lindmo and M. L. Mendelsohn, *Flow Cytometry and Sorting*, second edition (Wiley-Liss, New York, USA, 1991).
3. G. Fuhr, R. Hagedorn, T. Mueller, B. Wagner and W. Benecke, "Linear Motion of Dielectric Particles and Living Cells in Microfabricated Structures Induced by Traveling Electric Fields", *Proc. IEEE MEMS* (1991) pp. 259–264.
4. T. Schnelle *et al.*, "Paired Microelectrode System: Dielectrophoretic Particle Sorting and Force Calibration", *J. of Electrostatics* **47**, 121–132 (1999).
5. A. Wolff *et al.*, "Rare Event Cell Sorting in a Microfluidic System for Application in Prenatal Diagnosis", *Proc. of the Micro Total Analysis Systems* (μ-TAS1998) (1998) pp. 77–80.
6. J. Krueger *et al.*, "Development of a Microfluidic Device for Fluorescence Activated Cell Sorting", *J. Micromech. Microeng.* **12**, 486–494 (2002).
7. F. Arai, A. Ichikawa, M. Ogawa, T. Fukuda, K. Horio and K. Itoigawa, "High Speed Separation System of Randomly Suspended Single Living Cells by Laser Trap and Dielectrophoresis", *Electrophoresis* **22**, No. 2, 283–288 (2001).
8. F. Arai, A. Ichikawa *et al.*, "Separation of Target Microbe by Laser Manipulation and Flow Control", *Journal of Robotics and Mechatronics* **14**, No. 2, 133–139 (2002).
9. T. Kaneko, N. Mitsumoto and N. Kawahara, "A New Smart Vision System Using a Quick Response Dynamic Focus-ing Lens", *Proc. IEEE Micro Electro Mechanical Systems(MEMS)* (2000) pp. 461–465.
10. F. Arai *et al.*, "3D Bio Micromanipulation (Bilateral Control System Using Micro Tri-axial Force Sensor)", *IEEE Proceedings of the 1999 International Symposium on Micromechatronics and Human Science* (1999) pp. 71–77.
11. G. Binnig, C. F. Quate and C. Gerber, "Atomic force microscope", *Phys. Rev. Lett.* **56**, 930–933 (1986).
12. S. M. Harb and M. Vidic, "Resonator-based Touch-Sensitive Probe", *Sensors and Actuators* **A50**, 23–29 (1995).
13. C. Lee, T. Itoh and T. Suga, "Self-exited Piezoelectric PZT Microcantilevers for Dynamic SFM – with Inherent Sensing and Actuating Capabilities", *Sensors and Actuators* **A72**, 179–188 (1999).
14. Y. Miyahara, M. Descheler, T. Fujii, S. Wataneba and H. Bleuler, "Non-contact Atomic Force Microscope with a PZT Cantilever Used for Deflection Sensing, Direct Oscillation and Feedback Actuation", *Applied Surface Science* **188**, 450–455 (2002).
15. T. Kanda, T. Morita, M. K. Kurosawa and T. Higuchi, "A Rod-Shaped vibro touch Sensor using PZT thin film", *IEEE Transaction on Ultrasonics, Ferroelectrics, and Frequency Control* **46**, No. 4, 875–882 (1999).
16. F. Arai, T. Amano *et al.*, "Mechanical Micro-dissection by Microknife Using Ultrasonic Vibration and Ultra Fine Touch Probe Sensor", *Proc. 2001 IEEE Int. Conf. Robotics & Automation* (2001) pp. 139–144.
17. G. Kwon, F. Arai, T. Fukuda, K. Itoigawa and Y. Tshukaha, "Micro Touch Sensor Array Made by Hydrothermal Method", *Procs. of 2001 Int. Symposium on Micromechatronics and Human Science (MHS2001)* (2001) pp. 81–86.
18. G. Kwon, F. Arai, T. Fukuda, K. Itoigawa and Y. Tshukaha, "Microtouch-Sensor Array Fabricated by Hydrothermal Method", *Journal of Robotics and Mechatronics* **14**, No. 6, 633–639 (2002).
19. Y. Ohba, M. Miyauchi, T. Tsurumi and M. Daimon, "Analysis of Bending Displacement of Lead Zirconate Titanate Thin Film Synthesized by Hydrothermal Method", *Jpn. J. of Appl. Phys.* **32**, 4095–4098 (1993).
20. T. Morita, M. K. Kurosawa and T. Higuchi, "A Cylindrical Micro Ultrasonic Motor Using PZT Thin Film Deposited by Single Process Hydrothermal Method (ϕ2.4 mm, $L = 10$ mm Stator Transducer)", *IEEE Transaction on Ultrasonics, Ferroelectrics, and Frequency Control* **45**, No. 5, 1178–1187 (1998).
21. T. Fukuda, H. Sato, F. Arai, H. Iwata and K. Itoigawa, "Parallel Beam Micro Sensor/Actuator Unit Using PZT Thin Films and Application Examples", *Proceedings of the IEEE ICRA* (1998) **Vol. 2**, pp.1498–1503.
22. F. Arai, A. Ichikawa *et al.*, "Fixation and Isolation of Microorganisms by Local Viscosity Control of Methyl Cellulose Solution", *Procs. of the Micro Total Analysis Systems* (*μ*-TAS2002) (2002) pp. 928–930.

23. F. Arai, A. Ichikawa *et al.*, "High Throughput Separation of Microorganisms by Pinpoint Gelation Controlled by Local Heating for Parallel Incubation on a Chip", *Procs. IEEE MEMS-03* Kyoto (2003) pp. 219–222.
24. F. Arai, A. Ichikawa *et al.*, "Isolation and extraction of target microbes using thermal sol-gel transformation", *Analyst* **128**, 547–551 (2003).
25. A. Kawaji, F. Arai and T. Fukuda, "3D Calibration for Micro-manipulation with Precise Position Measurement", *J. of Micromechatronics* **1**, No. 2, 117–130 (2001).
26. F. Arai, A. Ichikawa *et al.*, "Continuous Culture and Monitoring of Selected and Isolated Microorganisms on a Chip by Thermal Gelation", *Procs. of the Micro Total Analysis Systems* (μ-TAS2003) (2003) pp. 757–760.