Operation of Self-Sensitive Cantilever in Liquid for Multiprobe Manipulation

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We describe a novel and simple operation method of using a self-sensitive cantilever of an atomic force microscopy (AFM) system in liquid. As for operation of the cantilever in liquid, Al lines of an integrated piezoresistor patterned on the cantilever are easily damaged by electrochemical corrosion. To realize safe operation without the damage, an additional electrode was inserted into the liquid. By applying DC voltage and controlling the potential of the electrode, the Al lines of the piezoresistor circuit on the cantilever could be protected from the electrochemical corrosion. By using this method, AFM imaging of collagen fibrils was demonstrated in physiological saline. Furthermore, the technique allowed us to realize a multiprobe AFM system with a simple configuration. Two cantilever probes were successfully operated like a knife and fork for the manipulation of collagen fibers in liquid.

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1. Introduction

Scanning probe microscopy (SPM) is a well-known surface imaging technique with nanometer scale resolution. SPM can be used not only for surface observation but also for local manipulation such as modifications and fabrications of nanometer-scale structures of various surfaces. In such manipulations, it would be very convenient if multiple probes could be independently operated in comparison with a conventional operation using only a single probe. As for multiprobe techniques, there have already been several reports on multiprobe SPM systems.[1–8] Multiprobe systems were mainly developed for the conductive measurement of nanometer-scale structures, but they are applicable to many other applications in various fields. In particular, multiprobe atomic force microscopy (AFM) systems are promising because it is possible to use them for the imaging and measurement of nanometer-scale characteristics on various surfaces including those of insulating samples. Thus, in the biological field, multiprobe AFM can be expected for measurements and manipulations of biological samples. However, in the operation of an AFM system in biological applications, a liquid environment is required to maintain moisture in the sample.

To develop a multiprobe AFM system capable of being operated in liquid, an AFM unit should be constructed with a simple configuration. One of the difficult points in simplifying the design of a multiprobe AFM system is that the optical lever system, used in most of the present AFM systems to sense cantilever deflection, is complicated; it consists of a laser, a photodiode, and optical parts. In addition, the alignment of the laser spot on the cantilever using the system is not easy, especially in liquid.

Recently, a multiprobe AFM system with a self-sensitive cantilever has been developed and operated in air.[8] The self-sensitive cantilever is a microcantilever with an integrated piezoresistor as a deflection sensor; thus, the use of the self-sensitive cantilever reduces the complexity of the setup without any optical sensing elements.[9] However, it is very difficult to operate a self-sensitive cantilever in liquid because of damage to the electric circuit of the piezoresistor caused by electrochemical corrosion.

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In this paper, we describe a novel and simple method of using a self-sensitive cantilever in liquid. By inserting an additional wire electrode and applying a DC potential, it was possible to protect the electrical circuit lines of the piezoresistor on the cantilever from electrochemical dissolution. By using the protection technique, a multiprobe AFM system with self-sensitive cantilevers, capable of being operated in liquid, was constructed with a simple configuration. AFM imaging and manipulation of collagen fibrils using the multiprobe cantilevers were successfully performed.

2. Experimental Procedure

2.1 Multiprobe AFM

Figure 1 shows a schematic diagram of the multiprobe AFM system developed in this study. The total system consists of AFM units, controllers, piezoelectric transducer (PZT) drivers, haptic devices, and personal computers (PCs). The AFM unit was set on a sample stage of an inverted optical microscope; thus, it is possible to monitor the position of the probe using the microscope objective from underneath the probe. Each cantilever probe is independently positioned by its own positioning stage. To realize a simple AFM unit, we employed a self-sensitive cantilever. In this study, commercial self-sensitive cantilevers with spring constants of 4 and 40 N/m were selected for imaging and manipulation (SII Nanotechnology NPXICTP003, PRC-DF40P), respectively. A piezoresistor is integrated on the cantilever as a deflection sensor, which can easily detect deflection signals from the cantilever without other sensing devices such as optical lever systems. Sensing of the deflection signal was achieved through piezoresistors connected to a Wheatstone bridge circuit. The output deflection signal was then sent to a custom-built feedback controller.[10] Then, the output of the controller was sent to the *z*-axis PZT driver for feedback control of the *z*-axis PZT actuator. As for topographical imaging, output signals of the *xy*-axis PZT driver were connected to a sample-side PZT flat scanner (Nanonics Flat Scanner™); thus, the sample was moved and scanned against the probe. On the other hand, for manipulation operation, output signals of the *xy*-axis PZT driver were connected to cantilever-side *xy* PZT actuators, which move the probe; thus, two probes can be independently moved against the sample. In the manipulation, it is possible to move the probe using a haptic device connected to the AFM system. By

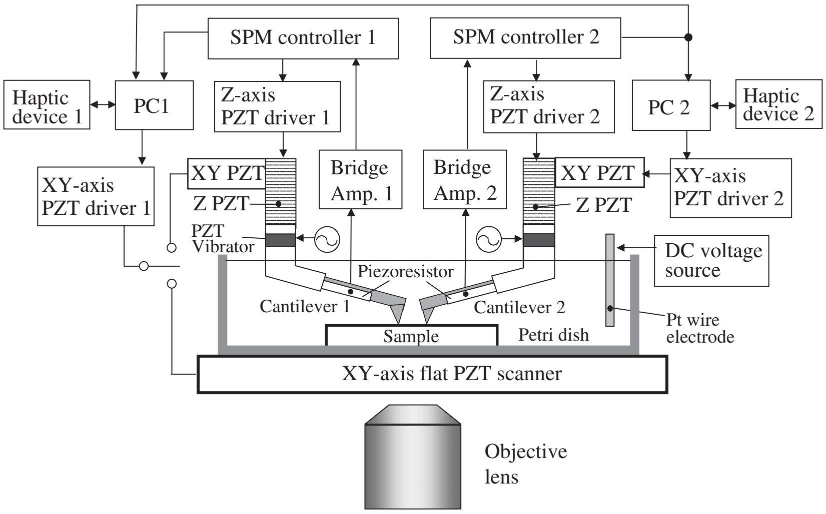

Fig. 1. Schematic diagram of multiprobe AFM system with self-sensitive cantilevers capable of being operated in liquid.

moving the handle of the haptic device, the operator can move the AFM cantilever probe at any position on the sample surface and feel the response from the surface according to the cantilever deflection. By pushing the handle, the probe pushes the sample surface. Other details concerning the haptic device manipulator are described elsewhere.[11] The experiments were carried out at room temperature in air and liquid.

2.2 Operation of self-sensitive cantilever in liquid

In this study, we developed an operation method of a self-sensitive cantilever in liquid. Figure 2 shows the commercial self-sensitive cantilever used in this experiment. As described above, the electric circuit for the piezoresistor (R_1) is integrated on the cantilever. The Al electric lines of the integrated circuit are patterned on the surface of the cantilever, as shown in Figs. 2(a) and 2(b). The piezoresistive cantilever is associated with a reference piezoresistor (R_2) to compensate for thermal drift. It can be compensated for by the reference resistor with the same thermal response as the one in the cantilever, but the reference resistor is not affected by the cantilever deflection. Thus, by connecting the Wheatstone bridge circuit, the deflection signal of the cantilever can be detected without the thermal drift. However, if the cantilever is immersed in the liquid and driving voltage is applied on the piezoresistor circuit on the cantilever, the Al line on which the most positive potential was applied becomes an anode in the liquid. As a result, the line is dissolved and damaged by electrochemical corrosion. To avoid the damage of the line, a Pt wire electrode is additionally inserted into the liquid, as shown in Fig. 2(c). By applying more positive potential on the Pt electrode, the Pt electrode becomes the anode instead of the Al line. Here, Pt is selected owing to its electrochemical stability, which produces chlorine without Pt dissolution under the electrochemical reaction. Thus, the Al line of the piezoresistor circuit can be preserved by cathodic protection.

The technique of using a self-sensitive cantilever in liquid has some advantages such as easy set up and multiple-probe operation. Furthermore, the simple configuration without an optical lever system allows an open space on the upper part of the cantilever, which can be combined with a phase-contrast microscope or differential interference contrast microscope to image living cells or tissues, since transmitted light can be irradiated directly onto the samples. In this experiment, the differential interference contrast microscope was combined with the multiprobe AFM system.

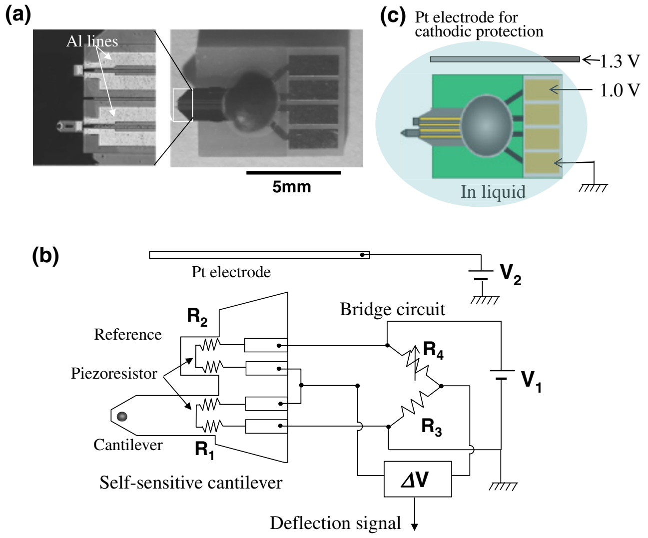

Fig. 2. (Color online) Operation method of a self-sensitive cantilever in liquid. (a) Optical micrograph of the self-sensitive cantilever. Al electric lines of the integrated piezoresistor are seen on the surface of the cantilever. (b) Integrated piezoresistor included in the self-sensitive cantilever. DC voltage of V_1 is applied on the electric lines of the piezoresistor sensor for driving a Wheatstone bridge. DC voltage of V_2 is applied on a Pt electrode wire. V_2 is positively higher than V_1. (c) Cathodic protection of the Al lines is achieved by inserting the Pt electrode wire, and applying a positive DC potential, as an anode in the liquid. In this study, V_1 and V_2 were 1.0 and 1.3 V, respectively.

3. Results and Discussion

3.1 Simultaneous observation

The two probes of an AFM system were successfully operated simultaneously when the probes were independently controlled during the sample scanning. Here, a tip-view-type cantilever, with a spring constant of 40 N/m, was selected because the probe is mounted at the edge of the cantilever; thus, the probe position could be easily observed

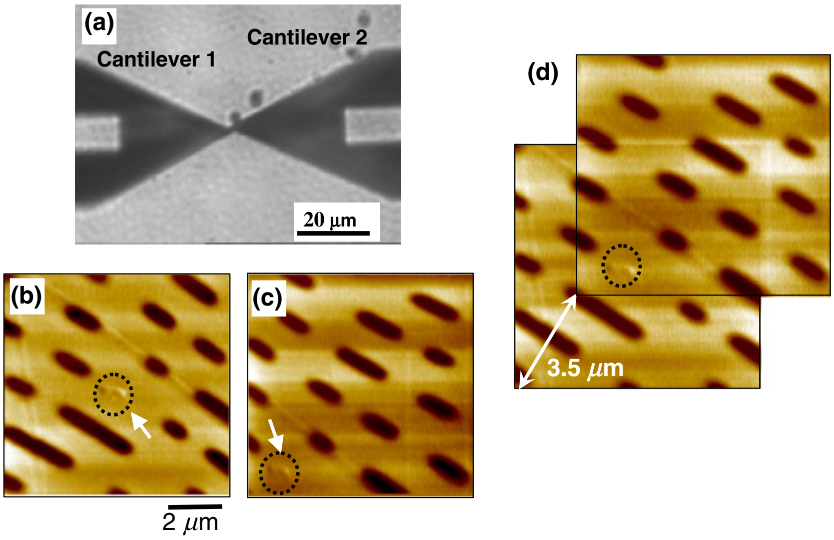

Fig. 3. (Color online) Simultaneous observation by the multiprobe AFM. (a) Optical microscopy image of the two cantilevers close together. (b) Compact disk (CD) image obtained with left-side cantilever. (c) CD image obtained with right-side cantilever. (d) Superimposed image of the left and right images.

by monitoring the optical microscopy image. The operation was carried out in the tapping mode in air. Two cantilevers were brought closer to each other, while the distance between the two edges of the cantilevers was monitored using the inverted optical microscope so as not to avoid contact, as shown in Fig. 3(a). Then, each cantilever was independently brought closer to the sample surface. Here, a compact disk (CD) made of polycarbonate was used as the sample. Figures 3(b) and 3(c) show AFM images obtained simultaneously by cantilevers 1 and 2, respectively. Pit structures of the CD surface can be seen clearly. In both images, the circle indicates the same dimple on the surface. Figure 3(d) shows a superimposition of the two images. From the shift amount between the two images, the edge of the probe distance was estimated to be 3.5 µm. The probe stands perpendicularly at the end of the cantilever. However, the cantilever is held with tilting to avoid hitting the holder socket of the cantilever on the sample surface; therefore, the actual probe edge contacting the surface is positioned slightly inside from the edge of the cantilever. In this experiment, the length of the probe was 8 µm and the tilt angle was 12°; thus, the probe edge position is calculated to be 1.7 µm inside from the edge of the cantilever. Therefore, the tips cannot approach each other within a distance shorter than approximately 3.4 µm in this case, which should be considered when multiple cantilevers are operated simultaneously.

3.2 Operation and imaging in liquid

To use the self-sensitive cantilever for biological applications, it should be operated in liquid. However, the self-sensitive cantilever does not work owing to electrochemical dissolution of the electric lines of the piezoresistors on the cantilever surface. Figures 4(b) and 4(c) respectively show optical micrographs of the electrode on the self-sensitive cantilever before and after operation in physiological saline. In this system, a DC voltage of 1 V, the driving voltage of the piezoresistor of the deflection sensor, was applied between the upper electrode and the lower electrode in the image. However, as shown in Fig. 4(c), the upper electrode wire on which 1 V was applied was completely dissolved 15 min after applying the DC bias voltage. Therefore, it is impossible to use the self-sensitive cantilever without any protection of the electric lines in liquid. In this study, cathodic protection of the electric lines was achieved by inserting a Pt wire electrode in the liquid and applying a positive potential higher than the driving voltage of the piezoresistor circuit on the cantilever, as shown in Fig. 4(d). In this experiment, 1.3 V was applied on the Pt wire electrode, which is a slightly higher positive potential than the driving voltage of the piezoresistor circuit of 1 V. Figure 4(e) shows an optical micrograph of the cantilever after 2 days of operation in liquid. It can be confirmed that the Al lines were successfully preserved without any damage even after 2 days of operation in liquid.

To confirm the practicality of the protection technique, AFM imaging was carried out in liquid, and the results were compared with those obtained by conventional imaging in air. As a biological sample, collagen fibrils taken from rat tail tendons were prepared on coverglasses using tweezers; then, they were fixed with glutaraldehyde. After rinsing with ethanol and water mixed solution, one sample was immersed in physiological saline in a petri dish for imaging in liquid, and another sample was dried for imaging in air for comparison. Figure 5(a) shows the topography of the collagen fibrils obtained by the conventional method in air. The observations were carried out in the contact mode using a self-sensitive cantilever with a spring constant of 4 N/m. The structure of fibrils, well preserved by the glutaraldehyde fixation, was clearly observed. On the other hand, Fig. 5(b) shows the collagen fibrils obtained in physiological saline using the protection method of the Al electric lines. As shown in the image, the topography of the fibrils was successfully obtained even in liquid. Therefore, by protecting the Al electric lines from electrochemical corrosion, AFM imaging can be safely carried out using the self-sensitive cantilever in liquid as well as by conventional imaging in air.

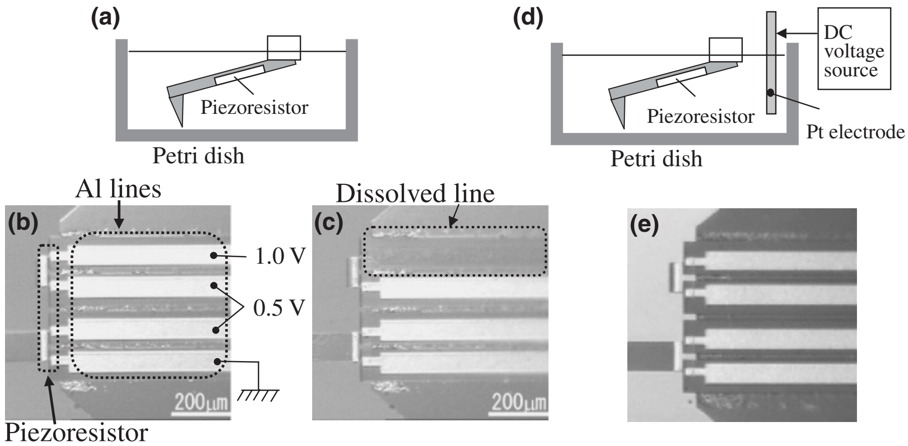

Fig. 4. Observation of Al electric lines on the cantilever operated in liquid. (a) A self-sensitive cantilever was immersed for operation in liquid. (b) Optical micrograph of the electrode on the self-sensitive cantilever before operation in physiological saline. (c) Optical micrograph of the electrode on the self-sensitive cantilever after a 15 min operation in physiological saline. Al lines were completely dissolved owing to electrochemical corrosion. (d) Protection of Al lines by inserting the Pt wire electrode into the liquid and applying DC voltage. (e) Optical micrograph of the cantilever after 2 days of operation in the liquid. The Al lines were successfully preserved without any damage.

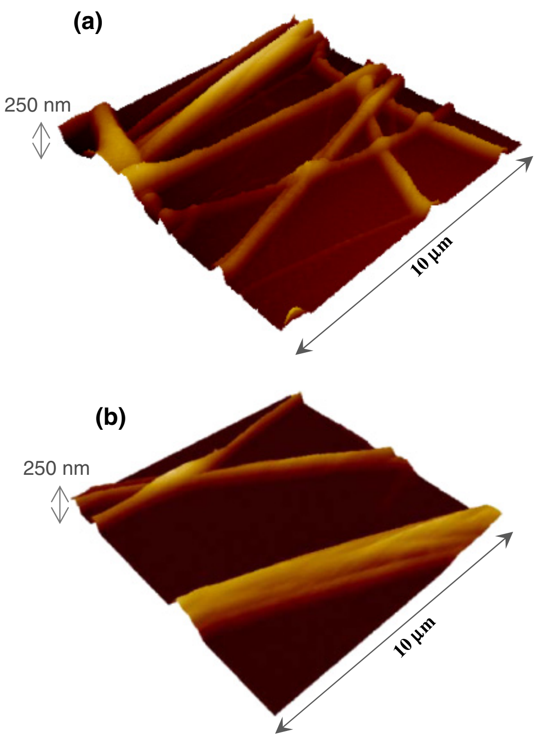

Fig. 5. (Color online) AFM images of collagen fibrils. (a) Topographical image of the collagen fibrils observed in air. (b) Topographical image of the collagen fibrils observed in physiological saline.

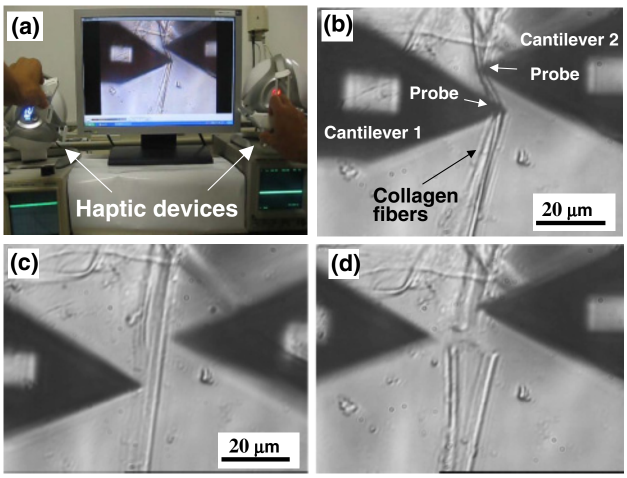

Fig. 6. (Color online) Manipulation of collagen fibers using the multiprobe AFM system in liquid. (a) Operation of the multiprobe system developed in this research. Two cantilevers were independently operated by the two haptic devices connected to the system. The operator can realize flexible and sophisticated movement of the cantilevers like a knife and fork for the manipulation. (b) Optical microscopy image of manipulation of collagen fibers. (c) Optical microscopy image of the collagen fibers before dissection. (d) Collagen fibers after dissection by AFM scratching through probe oscillation.

3.3 Dissection by multiprobe manipulation

By using the operation method of a self-sensitive cantilever in liquid, two self-sensitive cantilevers could be employed as a multiprobe manipulator of a biological sample. Here, a tip-view-type self-sensitive cantilever with a spring constant of 40 N/m was selected for dissection manipulation. The operation using the two probes is shown in Fig. 6(a). In the manipulation experiments, each cantilever was independently operated using the haptic devices by the operator's hands[11] like a knife and fork for the microdissection of collagen fibers. Figure 6(b) shows an optical microscopy image of the manipulation of the collagen fibers in physiological saline. As shown in this image, a collagen fiber was held on the substrate by pushing it with the right-side probe; then, the fiber was scratched with the left-side probe. However, the fibers could not be cut by conventional scratching. In a liquid, such collagen fibers might be very difficult to cut owing to their strength and viscoelasticity. Thus, we attempted to cut the fibrils by scratching through probe oscillation, which is effective for cutting such viscoelastic materials.[12] Figures 6(c) and 6(d) respectively show fibers before and after dissection by AFM scratching through probe oscillation. In this study, a y-axis PZT actuator was modulated with oscillation frequency to realize oscillation scratching. The frequency and amplitude were 200 Hz and 50 nm, respectively. The average loading force for cutting the fibers was 400 nN. As shown in this figure, the collagen fibers were successfully cut, even in liquid. Thus, the technique would be very useful and effective for various applications such as cell manipulation and nanometer-scale fabrication. In the cutting process, the oscillation conditions such as amplitude and phase might be changed owing to the cut resistance reflected by the viscosity and elasticity of the sample. As a future work, it would be interesting to observe the oscillation condition of the cantilever during the dissection of biological samples.

4. Conclusions

We developed a multiprobe AFM manipulator with a self-sensitive cantilever capable of being operated in liquid. By inserting a Pt electrode into the liquid and controlling the potential of the electrode, the self-sensitive cantilever could be safely used without causing electrochemical damage to the integrated piezoresistor on the cantilever. AFM images of collagen fibrils were successfully obtained in liquid, and the effectiveness of this operation method was confirmed. By using two AFM cantilevers, the manipulation of the collagen fibers was successfully performed in liquid. Therefore, the multiprobe AFM using the presented operation of the self-sensitive cantilever in liquid would be very effective for biological manipulation and nanometer-scale fabrication.

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1) I. Shiraki, F. Tanabe, R. Hobara, T. Nagao, and S. Hasegawa: Surf. Sci. **493** (2001) 633.
2) T. Kanagawa, R. Hobara, I. Matsuda, T. Tanikawa, A. Natori, and S. Hasegawa: Phys. Rev. Lett. **91** (2003) 036805.
3) M. Ishikawa, M. Yoshimura, and K. Ueda: Jpn. J. Appl. Phys. **44** (2005) 1502.
4) K. Takami, M. Akai-Kasaya, A. Saito, M. Aono, and Y. Kuwahara: Jpn. J. Appl. Phys. **44** (2005) L120.
5) H. Okino, I. Matsuda, R. Hobara, Y. Hosomura, S. Hasegawa, and P. A. Bennett: Appl. Phys. Lett. **86** (2005) 233108.
6) O. Kubo, Y. Shingaya, M. Nakaya, M. Aono, and T. Nakayama: Appl. Phys. Lett. **88** (2006) 254101.
7) E. Tsunemi, N. Satoh, Y. Miyamoto, K. Kobayashi, K. Matsushige, and H. Yamada: Jpn. J. Appl. Phys. **46** (2007) 5636.
8) N. Satoh, E. Tsunemi, Y. Miyamoto, K. Kobayashi, S. Watanabe, T. Fujii, K. Matsushige, and H. Yamada: Jpn. J. Appl. Phys. **46** (2007) 5543.
9) C. Lee, T. Itoh, and T. Suga: Sens. Actuators A **72** (1999) 179.
10) A. Sasaki, F. Iwata, A. Katsumata, and J. Fujiyasu: Rev. Sci. Instrum. **68** (1997) 1296.
11) F. Iwata, K. Ohara, Y. Ishizu, A. Sasaki, H. Aoyama, and T. Ushiki: Jpn. J. Appl. Phys. **47** (2008) 6181.
12) F. Iwata, M. Yamaguchi, and A. Sasaki: Wear **254** (2003) 1050.