INTRODUCTION

Scanning probe microscopes (SPMs) are well known as surface imaging tools with nanometer scale resolution. SPMs can be used not only for surface observation but also for local surface manipulation. Using the SPM probes, micro and nanometer-scale objects can be manipulated. Several studies concerning manipulations have been performed [1,2]. However, as for such nanometer-scale manipulation based on a conventional SPM, the manipulated scenes can not be monitored during the operation, which leads to damage of the sample or probe. Therefore, real-time monitoring systems have been desired during the SPM manipulations. One solution to the problem is to combine SPMs with other types of microscopes. As another solution, a haptic device can be coupled with SPMs. In the realm of force feedback control, haptic technology refers to technology that supplies an interface between the operator and a machine via the operator’s sense of touch while applying forces. This emerging technology has been widely used in various fields such as games, computer-aided design, medical simulations, and remote control robotics. Recently, haptic technologies have been introduced in nanometer-scale manipulations using AFMs [3,4]. Using a haptic device, an operator can directly move an AFM probe on a surface and feel the response from the surface being manipulated by the AFM. The haptic technique should be very useful for nanometer-scale manipulations and fabrications, particularly in the fields of biology and anatomy.

In this paper, we describe a nanometer-scale manipulator based on an atomic force microscope (AFM) which can be operated under observation using a scanning electron microscope (SEM). The design of the AFM unit was sufficiently compact, that a few units could be put on the sample stage for multi-probe operation. Nano manipulations of biological samples using the haptic control system of the AFM are demonstrated.

EXPERIMENTAL PROCEDURE

Compact AFM Manipulator

Figures 1 show a photograph of the AFM manipulator we constructed. The AFM body was compact enough to be installed into a sample chamber of a scanning electron microscope (HITACH S-3700). The gap between the objective lens and the sample holder was less than 10 mm; thus, there was no space to place an optical lever system to detect the deflection of the AFM cantilever. In our apparatus, a commercial self-sensitive type cantilever (NPX1CTP003, SII Nanotechnology) was employed. The self-sensitive cantilever includes piezo-electric resistive elements; thus, the deflection of the cantilever can be directly detected as an electric signal without optical lever systems. The self-detective cantilever can be inserted just under the objective lens of the SEM, which allowed us to design a compact AFM body for operation under SEM observation. The AFM head is a stand-alone type, which has a z-axial coarse positioning stage and x-, y-, z-axial fine-positioning mechanisms. As for z axial coarse positioning, a small z-axial stage was assembled with a miniature piezoelectric motor (30 nm / step, 4 mm displacement). In order to achieve a large scanning range for biological cell manipulation, flexure guide stages consisting of a parallel spring structure and a piezoelectric actuator were constructed for both the x and y axes; these stages mechanically amplify by three times the stroke of the piezoelectric actuator.
FIGURE 1 Compact and stand alone AFM manipulator. The manipulators are put on the sample stage in the SEM chamber (S-3700N, Hitachi).

As for z-axis, an external elliptical shell including a piezoelectric actuator was employed. The mechanism uses the elastic deformation of a rigid shell to increase the displacement of the PZT actuator. The maximum strokes of the x-, y- and z-axis scanners are 50, 50 and 60 um, respectively, which is within the range to be able to manipulate a biological cell. A couple of AFM manipulators can be put on the sample stage of the chamber for multi-probe manipulation as shown in Fig. 1 (b).

**Coupling with a Haptic Device**

Figure 2 shows the schematic diagram of the manipulation system. The total system consists of the AFM head, a controller, a PZT driver, a haptic device and a personal computer (PC). The compact and stand-alone AFM head was put on the sample stage in the SEM chamber (S-3700N, Hitachi). We employed a commercial haptic device that has a pen-like handle with a serial link mechanism (PHANTOM Desktop; SensAble Technologies). The operator can move the AFM cantilever directly on the sample and feel the response from the surface. A homemade controller and software were modified to couple the haptic device for the manipulation. With respect to lateral movements in the x and y directions, a signal from the haptic device was sent to a personal computer, and then the signal was sent to the piezo drive circuit passing through a digital-to-analog converter. The topographical signal detected in the AFM controller was fed to the personal computer, and then the signal was converted to the displacement in the z-direction of the pen handle of the haptic device. Using this process, nanometer-scale topographical information detected by the AFM can be scaled up to millimeter movements of the pen handle of the haptic device. Thus, the operator could move the AFM cantilever at any position on the sample surface and feel the response from the surface according to the cantilever deflection. During the manipulation, if
the operator pushes the pen handle haptic device against the response, a signal is sent to the personal computer to calculate and convert the force feedback signal; then the converted signal is sent to the feedback controller to change the set point of the feedback control. The system changes the force between the probe and the surface in response to pushing by the operator. By this sequential process, the operator can change the applied force for manipulation or fabrication of the surface by feeling the response from the surface via the haptic device.

EXPERIMENTAL RESULTS

AFM Imaging under SEM Observation

As a system demonstration, AFM topographical imaging was carried out under SEM observation. Figure 3(a) shows an SEM image of a HeLa cell, which is a cell line of human cervical cancer cells widely used in medical research. As shown in the image, it is easy to recognize the probe edge. The square area of the SEM image was scanned for AFM imaging. In this operation, the probe was easily positioned at a certain area for AFM imaging; then scanning could be carried out in contact mode under SEM observation. Figure 3(b) shows the AFM image obtained under SEM observation. The AFM image of the HeLa cell is exactly corresponding to the square region of the SEM image shown in Fig. 3(a). The topographical detail information of the cell can be seen in the AFM image. In general, SEM imaging with secondary electrons provides high contrast; however, it does not correspond to the exact surface topography. For instance, the edge line of the HeLa cell is brighter in the SEM image even though this part is topographically lower. On the other hand, the middle of the cell is darker even though that part is topographically higher. Therefore, by coupling SEM imaging with AFM measurement, it becomes possible to analyze the topographical information.

Scratching Fabrication

As a demonstration of the manipulator, nano dissection of a HeLa cell was performed. Using the haptic device, the HeLa cell was scratched with the AFM probe. The spring constant of the cantilever was 4 N/m. In general, biological samples exhibit complicated scratched behavior due to the sliding interaction between the AFM probe and the viscoelastic surface, making it difficult to cut soft material surfaces smoothly for micro or nanometer-scale fabrication. For modification of viscoelastic surfaces, it is known that vibration cutting, i.e., scratching with an oscillating probe, is effective at cutting such soft material surfaces without distortion [5]. To cut the HeLa cell surface smoothly and effectively, we carried out vibration cutting with probe oscillation. A modulation signal of 9 kHz with an
amplitude of 50 nm was added to the y-axis scanner. The average loading force was 2 μN. Figure 4(b) shows the cell surface fabricated by vibration cutting the letter “S”, which is the first letter of “Shizuoka University”. As shown in the image, the curved line is relatively smooth. During the process, we could cut the surface while sensing a smooth response in the haptic device.

Performance of Multi-probe Manipulation
As a demonstration of multi-probe AFM manipulators, biological samples were dissected using the two probes. Figures 5 shows the dissection scenes of a crystalline lens taken from a rat eye. Because the crystalline lens consists of a fibrous texture, if the sample is scratched by only one AFM probe applying a strong loading force, it was easily moved without dissection. Therefore, in order to dissect the sample without moving the surface, two AFM manipulators were employed. Figure 5(a) shows an SEM picture of the rat crystalline lens before the dissection. The spring constant of the employed cantilevers was 40 N/m. The right cantilever was used to dissect the sample by scratching the probe toward the right side of the image. The left cantilever was used to hold on the sample to avoid moving against the scratching force. Figure 5(b) shows the crystalline lens after the dissection. The crystalline lens was successfully dissected. Thus, using a multi-probe system, even such complicated and sophisticated manipulations could be achieved. Therefore, the manipulator system will be a very convenient technique, especially in the fields of micro and nanometer-scale anatomy and engineering.

CONCLUSION
We developed a compact nano manipulator based on an AFM system for multi-probe operation under SEM observation. The probe of the manipulator was operated using a haptic device. The manipulator could be put on the SEM sample stage. Thus, it was possible to operate the manipulator while observing the manipulation scene using SEM. As demonstrations, a HeLa cell was successfully scratched, and the crystalline lens of a rat eye was manipulated and observed by a multi-probe system. These results show the ready availability of this technology for application to various fields, such as micro or nano engineering and anatomical fields.

REFERENCES