Development of a nano manipulator based on an atomic force microscope coupled with a haptic device: a novel manipulation tool for scanning electron microscopy*

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Summary. We developed a novel nano manipulator based on an atomic force microscope (AFM) that can be operated inside the sample chamber of a scanning electron microscope (SEM). This AFM manipulator is also coupled with a haptic device, and the nanometer-scale movement of the AFM cantilever can be scaled up to the millimeter-scale movement of the pen handle of the haptic device. Using this AFM manipulation system, we were able to observe the AFM cantilever and samples under the SEM and obtain topographical images of the AFM under the SEM. These AFM images contained quantitative height information of the sample that is difficult to obtain from SEM images. Our system was also useful for positioning the cantilever for accurate AFM manipulation because the manipulation scene could be directly observed in real time by SEM. Coupling of the AFM manipulator with the haptic device was also useful for manipulation in the SEM since the operator can move the AFM probe freely at any position on the sample surface while feeling the interaction force between the probe and the sample surface. We tested two types of cutting methods: simple cutting and vibration cutting. Our results showed that vibration cutting with probe oscillation is very useful for the dissection of biological samples which were dried for SEM observation. Thus, cultivated HeLa cells were successfully micro-dissected by vibration cutting, and the dissection process could be observed in real time in the SEM. This AFM manipulation system is expected to serve as a powerful tool for dissecting various biological samples at the micro and nanometer-scale under SEM observation.

Introduction

Scanning probe microscopes (SPMs) are a family of microscopes which scan a probing tip over the sample surface. SPMs have been mainly used as surface imaging tools with nanometer scale resolution, but they can be also used for local surface manipulation. Manipulators based on SPMs are expected to become powerful tools not only for material science but also for biology because micrometer and nanometer scale objects can be moved or fabricated in various (i.e. vacuum, air or liquid) conditions by them. Indeed, several studies involving manipulation have been performed by SPMs (Decossas et al., 2003; Yongda et al., 2007). However, as for such nanometer scale manipulation using conventional SPMs, the manipulation itself cannot be viewed during the operation. The operator therefore has to move the probe in a blind condition, which often induces damage to the...
scale manipulation and fabrication, not only for material sciences but also in biological fields.

We recently developed a nanometer-scale manipulator based on an AFM coupled with a haptic device that can be operated under the SEM. Using this system, operators can monitor the manipulation by SEM not only visually but also tactilely by the haptic device in real time conditions. In the present paper, we introduce the details of this system and show its applicability to the manipulation of biological samples under SEM observation.

Material and Methods

Compact AFM manipulator

Figures 1 shows the schematic diagram of an AFM manipulator which was constructed by us in the present study. The AFM manipulator should be compact enough to be installed into the SEM specimen chamber. Because the gap between the objective lens and the sample holder is less than 10 mm in the SEM chamber, there is no space to place an optical lever system for the AFM to detect the deflection of a cantilever. Therefore, a commercial self-sensitive type cantilever (NPX1CTP003, SII Nanotechnology, Chiba) was used in our apparatus. This cantilever includes piezo-electric resistive elements, and...
Manipulation tool for SEM

As for the z axis, an external elliptical shell (APA-60S, CEDRAT Technologies, France) including a piezoelectric actuator was used; this also mechanically amplifies the stroke of the piezoelectric actuator three times. The maximum strokes of the x, y and z axis scanners are 50, 50 and 60 μm, respectively.

Coupling with a haptic device

Figure 2a shows a schematic diagram of the manipulation system. The entire system consists of the AFM manipulator, a controller, a PZT driver, a haptic device and a personal computer (PC). The compact and stand-alone AFM manipulator was put on the sample stage in the SEM chamber (S-3700N, Hitachi, Tokyo).

We employed a commercial haptic device that has a pen-like handle with a serial rink mechanism to interact with the AFM system. The deflection of the cantilever can be directly detected as an electric signal without any optical lever systems. Thus, the insertion of the self-detective cantilever under the objective lens of the SEM allowed us to design a compact AFM manipulator for operation under SEM observation.

The AFM manipulator is a stand-alone type AFM, which has a z axial coarse positioning stage and x, y, z axial fine positioning mechanism. For the z axial coarse positioning, a small z axial stage was assembled with a miniature piezoelectric motor (Picomotor, New Focus Technologies, USA). For the fine positioning and scanning mechanism, a large stroke movement is required for the manipulation of biological samples. In order to achieve a large scanning range, flexure guide stages consisting of a parallel spring structure and a piezoelectric actuator were constructed for each axis in the x and y direction which mechanically triples the stroke of the piezoelectric actuator. As for the z axis, an external elliptical shell (APA-60S, CEDRAT Technologies, France) including a piezoelectric actuator was used; this also mechanically amplifies the stroke of the piezoelectric actuator three times. The maximum strokes of the x, y and z axis scanners are 50, 50 and 60 μm, respectively.

Fig. 2. a: Schematic diagram of the experimental setup of the AFM manipulator. The AFM is coupled with a haptic device, so the operator feels the response from the sample during scratching. b: Photograph of the manipulator system.
Signal is sent to the feedback controller to change the setting point of the feedback control. The system then changes the force between the probe and the surface in response to pushing by the operator. Through this sequential process, the operator can change the applied force for the manipulation or fabrication of the sample while feeling the response from the sample surface via the haptic device.

**FIB cut cantilever for nano manipulation**

In order to perform an accurate manipulation, the probing tip of the AFM manipulator should be directly observed under the SEM. However, the probe of the commercial cantilever is not observed in the top view image (because the probe is present on the lower side of the cantilever (Fig. 3a, b). In order to solve this problem, the end of the cantilever was partly cut by using focused ion beam milling (Fig. 3c, d). This enabled the probing tip to be recognized in the top view SEM image as shown in Fig. 3d, which allows us to access the probe precisely and accurately to the target position on the sample surface.
it was easy to recognize the probing tip of the FIB fabricated cantilever. After we decided on an area to be studied, we could move and approach the cantilever for AFM imaging in the SEM. In this operation, the probe was easily positioned at the area for AFM imaging, and then scanning could be carried out in a contact mode under the SEM observation. The sequential operation was very smooth and convenient for AFM observation, and, during the AFM operation under SEM observation, there was no cross-talk and no electrical-noise between both the SEM and the AFM systems. Thus, we were able to obtain the topographical image of AFM under SEM observation (Fig. 4b). In Figure 4, the AFM image of the HeLa cell (b) exactly corresponds to the square region of the SEM image (a). This enabled details of the surface topography of the cell with height information to be distinguished in the AFM image.

**Dissection of HeLa cells under SEM observation**

To verify the performance of our manipulation system, we dissected HeLa cells with the AFM manipulator under SEM observation. The haptic device used in our system enabled the dissection of HeLa cells with the AFM probe, as if we could directly feel the loading force of the cantilever with the operator's hand. When the cells were cut by a simple movement of the AFM probe with the average loading force of 10 μN, the cut line was not smooth but irregular, as shown in Figure 5a. During the process, we also felt any irregularity in the dissecting

**Sample preparation**

In this experiment, HeLa cells were used in the present study. They were grown on glass coverslips according to the standard procedure of the cell culture. Briefly, the cells were cultivated on the coverslip in a culture medium (RPMI 1640, Invitrogen Japan, Tokyo) containing 10% fetal bovine serum and antibiotics, at 37°C in a 5% CO₂ incubator for 1–3 days. The cells on the coverslip were fixed with 1.5% glutaraldehyde in a 0.1% phosphate buffer (pH 7.4) for 2 h or more and then conductive-stained by treatment with a 0.5% tannic acid solution and a 1% osmium tetroxide solution. They were dehydrated in a series of ethanol, transferred to isoamyl acetate and dried in a critical point dryer using liquid CO₂. The coverslips with dried cells were mounted on aluminum stubs with double-sided adhesive carbon tape and coated with platinum-palladium in an ion coater for SEM observation.
response with our fingers via the haptic device. This is probably because dried biological samples are not uniform but rather fragile in structure, which resulted in a cracking or collapsing of the sample surface as if the samples had been scratched with a blunt needle.

To cut the HeLa cells smoothly and effectively, we then 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. Figure 5b shows the scratched lines from cutting with probe vibration. The direction of the probe vibration was parallel to the long axis of the cantilever. The average loading force was 2 $\mu$N. Using this method, the cells were successfully dissected with a smooth cutting line. The cutting edge was sharp and there was no surface distortion in the periphery of the cutting region; even the loading force was lower than in the case of cutting without a probe vibration. Figure 5c shows scratched lines by cutting with and without probe vibration. In both cases, the same loading force of 2 $\mu$N was applied. Figure 5d shows an example of cell dissection by vibration cutting: the letter "S" was carved on the surface of the HeLa cell.

**Fig. 5.** SEM images of HeLa cells cut by the AFM manipulator using the haptic device. a: Fabrication by cutting without probe vibration. The broken line indicates the trajectory of the probe. It is difficult to cut the cell smoothly. b: Fabrication by cutting with probe vibration. The HeLa cell was successfully dissected. c: Cutting of the cell process with and without probe vibration. In both cases, the same loading force of 2 $\mu$N was applied. d: Carving a letter "S" on the surface of the HeLa cell.
Discussion

The present study has introduced a compact AFM which can operate in the sample chamber of the SEM, demonstrating its applicability to the biological fields not only as an imaging tool but also as a novel manipulation tool for SEM.

SEM has been widely used for obtaining three-dimensional information of samples at the millimeter to nanometer scales. However, there is no quantitative height information in conventional SEM images. For instance, the edge line of the HeLa cell is bright in the SEM image due to the edge effect produced by secondary electrons, even though the actual height of this part is topographically low. On the other hand, AFM images contain quantitative information in three dimensions, and the sample profile containing height information can be easily obtained. Therefore, by coupling SEM with AFM, it becomes possible to analyze the sample height from AFM imaging while observing the detail of the surface topography by SEM. In addition, the combination of AFM and SEM is useful for AFM imaging because the probing tip of the cantilever can be easily and accurately approximated to the sample of interest under SEM observation. Our system is expected to become a powerful tool for obtaining AFM images of the restricted regions of bulky samples because the accurate approach of the AFM probe to the bulky samples is very difficult under optical microscopic observation.

In the present study, we also demonstrated that our AFM system is useful for the manipulation of samples under the SEM. We especially showed that the haptic device is effective for AFM manipulation because the operator can move the AFM probe rather freely to any position on the surface while feeling the interaction force detected by the cantilever on the sample surface.

As for the cutting performance of our system, we tested two type of cutting methods: simple cutting and vibration cutting. Our results showed that the vibration cutting with probe oscillation is very useful for the dissection of biological samples prepared and dried for SEM observation. We previously used the vibration cutting method for dissecting different samples including the soft polymers and dried collagen fibrils (Iwata et al., 1999, 2008). These previous studies also revealed the usefulness of vibration cutting for dissecting both hard samples and elastic soft samples. In the present study, we also showed how vibration cutting is further useful for the dissection of dried, fragile biological samples. On the other hand, simple cutting without probe oscillation is not suitable for the precise dissection of our samples, probably because it produces a sliding interaction between a probe of the AFM and the sample surface (Meyers et al., 1992; Leung and Goh, 1992), making it difficult to cut the sample smoothly for micro or nanometer-scale fabrication.

For dissecting samples in an SEM, sensing the distance between the AFM probe and the sample is very important to avoid any bumping of the probe onto the sample. Although the SEM provides three-dimensional images, a single micrograph from conventional SEM presents a view for only one eye, and it is difficult to realize correctly the depth of the observation field. Thus, an SEM with real-time stereo imaging by the simultaneous collection of stereo-pair images would be useful for manipulation in an SEM. We are now interested in the manipulation in this type of SEM, and the details will be described later in a separate paper.

In conclusion, we developed a novel nano manipulator based on an AFM which can be operated inside the sample chamber of the SEM. This system enabled us to observe the manipulation in real time observations by using the SEM. Through the coupling of the AFM manipulator with a haptic device for human interface, the operator can move the AFM probe to any position on the surface while feeling the loading force against the sample with the his/her hand. This system is expected to become a powerful tool for dissecting biological samples at various micro and nanometer-scales under SEM observation.

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