Special Contributions

Microstructure Analysis by Means of the Orthogonally-arranged FIB-SEM

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OVERVIEW: Serial sectioning using a combined FIB and SEM is a method for three-dimensional imaging of material microstructures. Hitachi set out to achieve precise three-dimensional imaging of material microstructures with higher spatial resolution and contrast than previous instruments by utilizing a configuration in which the FIB and SEM are positioned orthogonally (perpendicularly), this being the ideal orientation for three-dimensional imaging. This manuscript gives an overview of an FIB-SEM with an orthogonal configuration intended for three-dimensional imaging, describes the multi-functionality and high-performance imaging that are features of the instrument, presents examples of its use, and considers future developments.

INTRODUCTION

THE basis of structural observation in either light microscopy or electron microscopy is essentially recording of two-dimensional images, either through surface observations produced by reflection, or through projected observations produced by transmission. However, apart from a very small category of exceptions, the structure of materials and biological object is by nature three-dimensional. Consequently, innovative means have long been employed to allow three-dimensional observations.

For specimens such as the metals and ceramics that we study, microstructural observation is required over a wide range of scales from the subnanometer level to the micrometer and millimeter level. In this context, three-dimensional reconstructed image observation with serial sectioning using a hybrid "focused ion beam-scanning electron microscope" (FIB-SEM) is of interest for the comparatively broad coverage that it provides.

Here we provide an overview of a perpendicularly-oriented FIB-SEM (SMF-1000, Hitachi High-Tech Science Corporation) that we adopted to provide highly precise three-dimensional observations of materials structures. We also describe practical examples of its use in observation. We adopted this instrument in February 2011, and since then, the equipment and related observational technologies have proliferated widely as "Multi-Scale Analytical Equipment for Three-Dimensional Microstructures" under the Nanotechnology Platform initiative of the Ministry of Education, Culture, Sports, Science and Technology (MEXT). We also present some practical examples of observation under these initiatives in various research fields.

INSTRUMENTATION AND ITS TECHNIQUES[1]

Serial sectioning by an FIB-SEM unit combining FIB and SEM functions in one unit has been used widely to date. In this technique, the surface layer of a specimen is ground by FIB, the ground layer is observed repeatedly by SEM, and the series of SEM images acquired is integrated by computer to reconstruct a three-dimensional image. As a result, the quality of the SEM image depends greatly on the quality of observation and analytical results. One issue to note during serial sectioning observation by FIB-SEM is the fact that even though this is nominally an SEM observation, it is observation of a surface which has been cut by FIB, not a morphological observation revealing surface irregularities. In other words, the external form of the specimen is not observed. An almost perfectly flat surface is created, and contrast within its internal structure is observed by secondary electrons or reflected electrons. Consequently, the contrast observed results from the type and density of constituent elements of the specimen, and in the case of crystalline specimens, its structure or channeling (orientation), and the technique is essentially inapplicable for topographic contrast. The type of contrast to be used for a desired observation is a parameter (detector and detection parameters) which must be investigated.

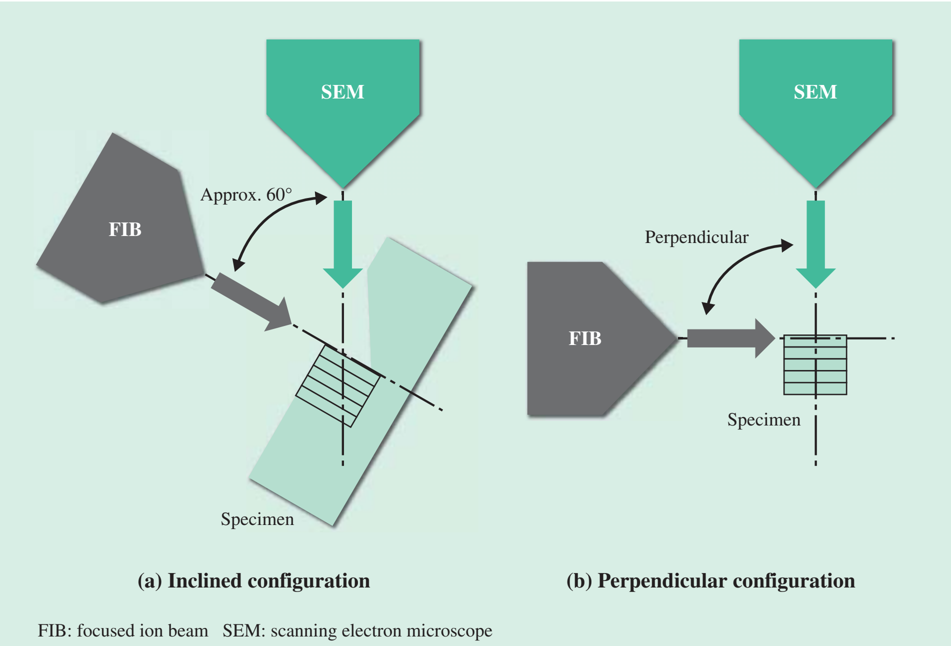

Fig. 1—Configuration of FIB and SEM in FIB-SEM.
(a) shows how the FIB and FEM are oriented at an angle of about 60° to each other on a conventional FIB-SEM. This orientation is not ideal for three-dimensional imaging because the FIB cutting surface is not perpendicular to the optical axis of the SEM. (b) shows how, on the orthogonally-arranged FIB-SEM, the FIB cutting surface is perpendicular to the optical axis of the SEM, thereby enabling undistorted observation of the surface and eliminating field of view misalignment.

To date, general FIB-SEM instrument has been employed for this purpose; specifically, as shown in Fig. 1 (a), instrument in which the optical axes of FIB and SEM units intersect each other at a roughly 60° angle. This is the best configuration for FIB and SEM to visualize the same point and is ideally suited, for example, for SEM location of a site and FIB preparation and removal of a sample for TEM observation. However, if the objective is limited to serial sectioning observation, this configuration is not necessarily ideal for the following two reasons:

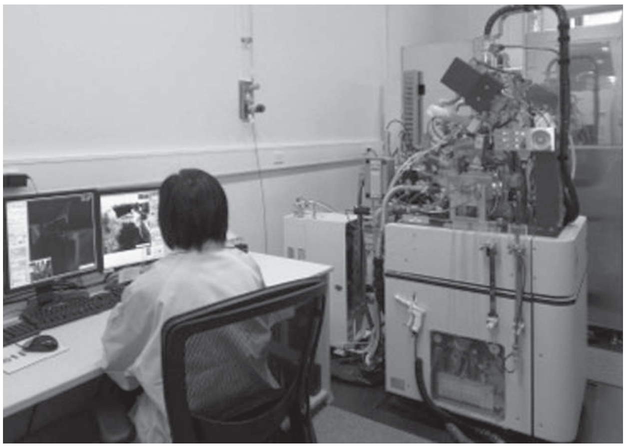

Fig. 2—External Appearance of the Orthogonally-arranged FIB-SEM Apparatus.
The FIB is located on the left side of the SEM, which is positioned above the instrument on the right of the photograph. The specimen is inserted from the front side, as viewed in the photograph.

(1) As shown in Fig. 1 (a), repeated slicing by FIB causes field of view misalignment in an SEM image. Because the area cut is offset from the center of the SEM image (dotted line in the figure), correction is required.
(2) The SEM optical axis is not perpendicular to the FIB sliced plane. This results in SEM observation of an inclined surface, and for example, when attempting observation using a secondary electron image, most of the dynamic range in contrast is consumed by phenomena arising from high/low differences. Additionally, because an inclined plane is observed, the vertical and horizontal scales even within the same SEM image differ and require correction.

For these reasons, an ideal equipment configuration for the purpose of serial sectioning has the FIB cutting surface perpendicular to the SEM optical axis, as shown in Fig. 1 (b).

To achieve this end, we introduced an observational instrument configured with the FIB and SEM elements perpendicular to each other (SMF-1000, Hitachi High-Tech Science Corporation). Fig. 2 shows a photograph of the instrument as it appears. This instrument creates no changes in contrast due to high/low differences on the cutting surface, a configuration ideally suited to internal structural observation. Additionally, to collect as much information as possible in a single observation, the instrument is provided with an energy dispersive X-ray spectrometer (EDS), an electron backscatter diffraction (EBSD) detector, and specimen preparation equipment such as a plasma cleaner, argon ion gun, and gas deposition gun. The image detectors provided are an SEM in-lens type secondary electron detector and an in-lens reflected electron detector (i.e., one located coaxially on the optical axis of SEM microscope tube), and an Everhart-Thornley (E-T) detector, as well as a scanning transmission electron microscope image (STEM) detector immediately below the specimen.

APPLICATION EXAMPLES FOR MICROSTRUCTURE OBSERVATION

Two-dimensional Image Observation of Clean Surface

Before discussing an example of three-dimensional reconstructed image observation, we touch on the fact that the quality of ordinary two-dimensional images (SEM) per se is high. While the internal microstructure sometimes cannot be seen from the specimen surface due to issues such as surface layers or oxide layer, the cutting in FIB allows observation of a fresh surface without such impediments. For example, Fig. 3 shows a specimen of a steel material with a surface finished to smoothness by electropolishing. A surface oxide layer has been formed during electropolishing, and rather than contrast in the internal structure, all that can be observed is irregularities in the surface oxide layer. This is shown on the left half of Fig. 3. The right half of the figure shows the specimen with its surface oxide layer removed by FIB (located at the top of the page), and we see that in this portion, the contrast in sample structure is revealed. For metallic materials which undergo surface oxidation simply with exposure to the air, the ability to produce a fresh surface in the SEM chamber is a great advantage.

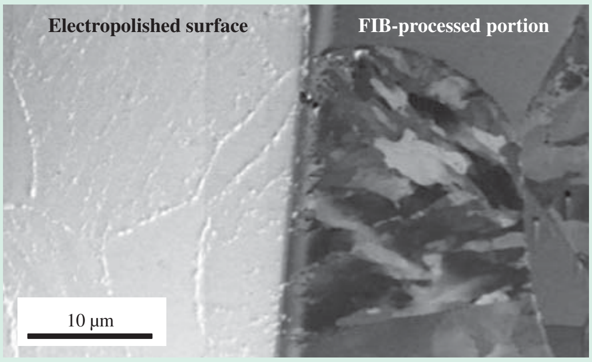

Fig. 3—Steel Material Surface-finished by Electropolishing.
The figure shows an electropolished surface (left), and the same surface cut by FIB (right).

SEM Image “Depth” Resolution

When considering the spatial resolution of three-dimensional reconstructed images obtained by FIB-SEM serial sectioning, it is necessary to take account of the slice thickness (z direction) as well as the probe diameter. The z-direction resolution is dependent on the performance of the FIB, being determined by the pitch of FIB slices. However, because the electron beam penetrates a long way into the specimen when using a high SEM accelerating voltage, the image also contains information from the depth direction, which defeats the purpose of having a narrow slice pitch. Accordingly, keeping the accelerating voltage as low as possible to minimize how far the electron beam spreads into the specimen is important for improving the spatial resolution in practice. The instrument is able to produce three-dimensional reconstructed images with an effective spatial resolution of 2 nm by performing SEM observations with a low accelerating voltage and using an FIB capable of precise milling.

Example of Three-dimensional Reconstructed Image Observation

As discussed above, the cut surface in FIB eliminates topographic contrast and enhances the internal microstructure of the specimen. To date, there are many examples of observation on this basis.

Fig. 4 is an example in a titanium molybdenum (Ti-Mo) alloy showing determination of the shape, distribution, volumetric ratio, and other aspects of precipitate, also known as “omega phase[2].” Omega phase greatly affects the mechanical characteristics of a β-titanium alloy but precipitates very finely and at high density, complicating quantitative evaluation. The SEM image shown in Fig. 4 (a) is an observation by an in-lens type secondary electron detector made at an accelerating voltage of 2 kV. Because the molybdenum content of omega phase is low with respect to the matrix, at approximately 5%, its density difference is observable as dark contrast. Fig. 4 (b) is a three-dimensional reconstruction of 260 SEM images produced with a slice thickness set at 2 nm. The result is that the volumetric ratio, size, shape, and other aspects of omega phase particles can be evaluated quantitatively. Consequently, because the FIB cut surface is perpendicular to the SEM optical axis, baseline contrast is uniform, and even slight differences in composition can be enhanced to allow observation. Fig. 5 (a) is an example of observation of the distribution of precipitate particles observed near the interphase interface of δ-ferrite and tempered

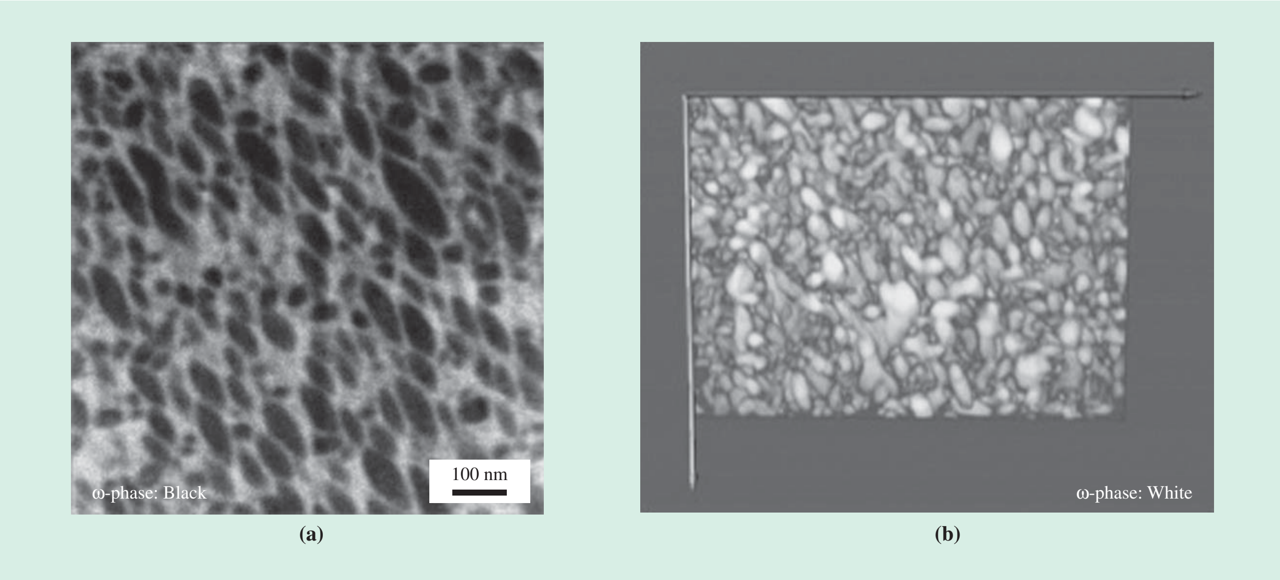

Fig. 4—Observation of ω-phase in Ti-Mo Alloy.
(a) shows a two-dimensional image used in serial sectioning, and (b) shows the three-dimensional reconstructed image.

martensite in iron-chromium (Fe-Cr) based heat-resistant steel(3). Because the contrast in the image is high, once a type of precipitate is identified by EDS, many types of precipitates can be distinguished by contrast alone. Fig. 5 (b) is an observation made to determine how a vanadium-based carbonitride (black) and a gray chrome-based carbide (gray) are distributed at their phase interface. Because precipitate distribution at the interface cannot be determined accurately either through projected image observation by TEM or through surface observation by SEM, high-contrast, three-dimensional image observation is an extremely effective means for such determination. Such observation is also progressing from material specimens to biological specimens. Fig. 6 is an example of observation during serial sectioning of

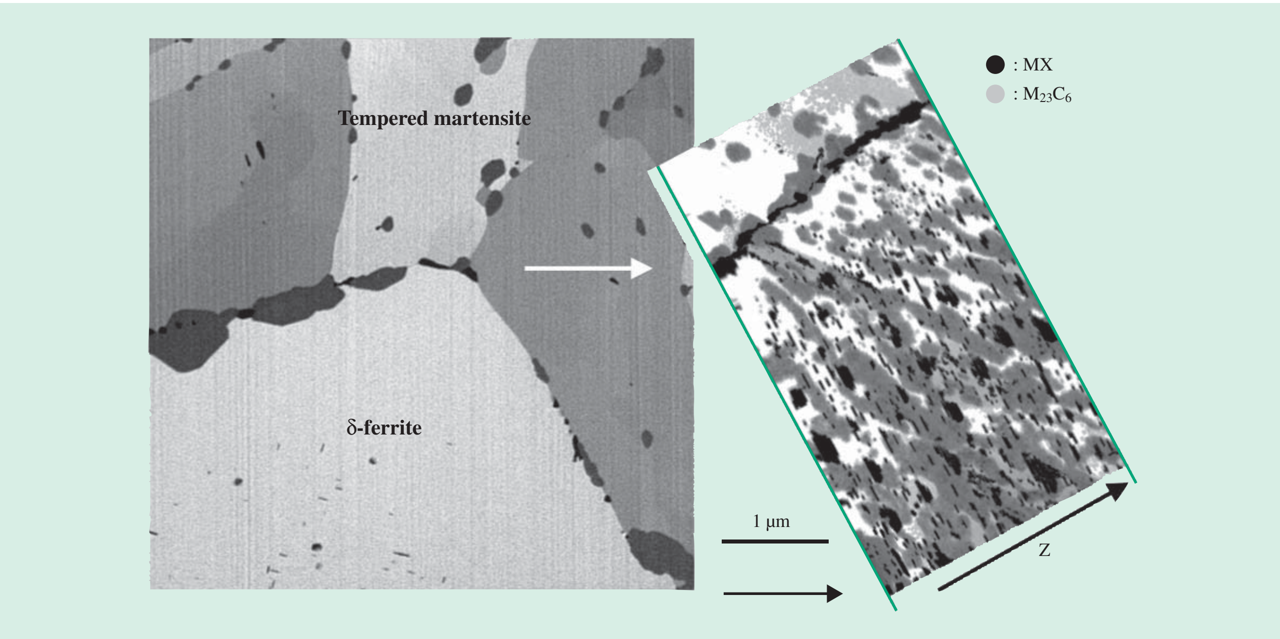

Fig. 5—Observation of Precipitate Distribution in Heat-resistant Steel.
(a) shows an SEM image during serial sectioning observation, and (b) shows the precipitate distribution at δ-ferrite - tempered martensite interface (oriented toward depth of printed sheet).

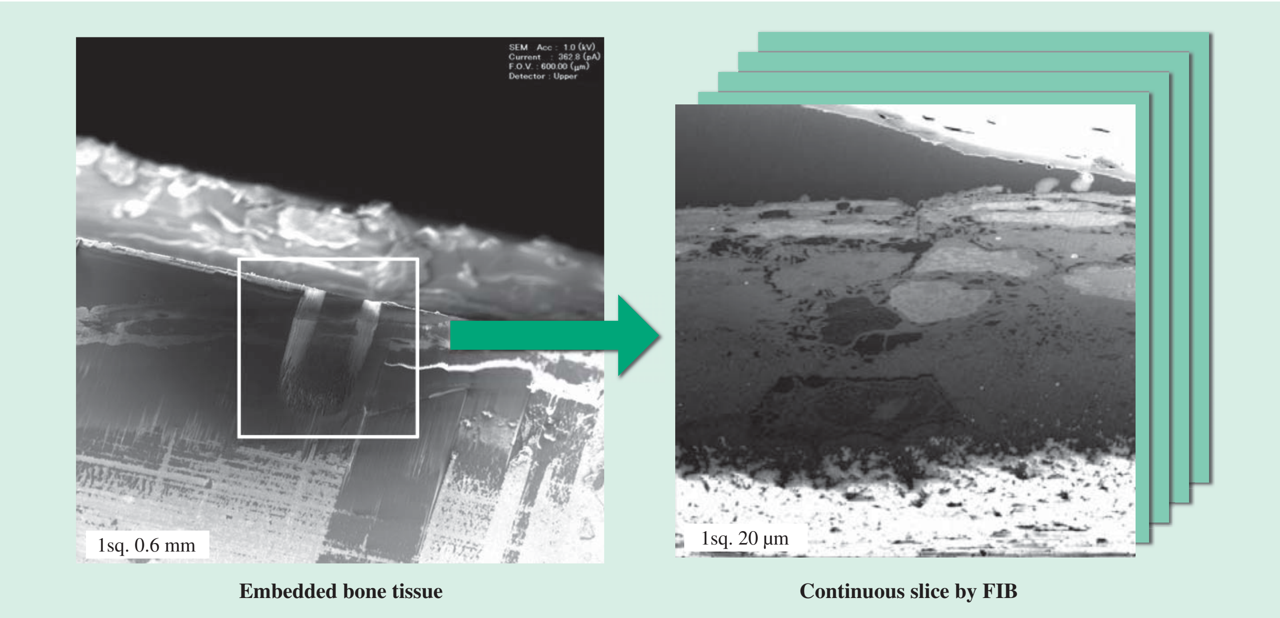

Fig. 6—Serial Sectioning Observation of Bone Tissue.
The location to be observed can be found using low magnification and a wide field of view, as in the image on the left. The edge in the middle of the image is the surface of a chick embryo skull, with the downward direction in the image corresponding to depth. The image on the right shows the field of view used to reconstruct the three-dimensional image.

bone tissue. Embedded chick embryo skull is observed from the surface layer to a deep region. In this instance, the site of desired observation is brought to the edge of the sample, which is sliced on a direct horizontal by FIB. Accelerating voltage of 1 kV allowed good imaging(4). In another example, when measuring the shape or volumetric ratio of spaces in a specimen with many spaces, such as a battery electrode material, the spaces should be filled with a resin or the like for observation, but in many cases, this cannot be done. Various innovations have therefore been devised. For example, serial sectioning is performed without resin filling, and with the specimen in its original shape or state; multiple images are acquired with different observational parameters for each slice; and the shape or ratio of spaces is determined using innovative observational parameters or analytical techniques, such as binarization of spaces and specimen material in image processing(5).

EXPECTATIONS FOR FUTURE DEVELOPMENT

As described, this instrument, with its perpendicular FIB and SEM configuration, is ideal not only for observation of three-dimensional reconstructed images produced by serial sectioning; it is also effective for observing the internal microstructure of a specimen through observation of a surface cut by FIB. To date, many diverse specimens have been observed successfully, but observation with greater precision and sensitivity will require further innovation in many respects. The commonality of these needs in many cases is a frequent request from users for a task that is difficult today. Rather than ultra-high-resolution, requests take the form of greater general utility or greater multifunctionality, for example, "observation on a larger scale," "expansion of the types of applicable specimens," and "delivery of a greater variety of information." Without compromising the characteristics imparted by the perpendicular configuration, we anticipate future development seeking more advanced levels of such structural analysis to proceed as follows.

(1) Additional FIB methods for slicing. Specifically, techniques for cutting over a greater range, for example, techniques for cutting a milli-unit range at a nanoscale pitch. These developments represent great prospects for expanding observational area and shortening observation times.

(2) Efforts to address a variety of specimen requirements. Many samples are prone to charging in SEM, damage in FIB, or are otherwise unsuited to FIB-SEM. Other samples require cooling, heating, or other atmospheric control.

(3) Techniques for effective collaboration through simultaneous acquisition of various information.

Specifically, augmentation with diverse image detectors, and installation of microcalorimeter EDS or other such detectors for high-precision analysis unavailable to date.
(4) Acquisition of previously missed information. For example, when various information is acquired simultaneously, better analytical techniques allowing extraction of required information alone.

As users in need of structural analysis, we look forward to realization of these developments.

ACKNOWLEDGMENTS

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