Special Contributions

Development of SEM for Realtime 3D Imaging and Its Applications in Biology

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OVERVIEW: We have developed a device for displaying TV-rate 3D images on a monitor by tilting the electron beam of an SEM to the left and right for each scan line to acquire dual (stereoscopic) images simultaneously. It has also improved the resolution of stereoscopic imaging by developing optics that minimize the off-axial aberrations that are associated with this electron beam tilting. Among the benefits of this realtime stereo SEM is that 3D imaging makes it possible to manipulate the sample under observation. Its effectiveness for this purpose was demonstrated by installing a prototype micro-manipulator in an SEM sample chamber and using it in a realtime stereo SEM to perform microanatomical dissections on biological samples.

INTRODUCTION

THE ability of scanning electron microscopes (SEMs) to image the three-dimensional (3D) surface structure of samples has numerous applications in the life sciences as well as in materials science. They are particularly valuable in medical biology where they are used to study the fine 3D structures of cells or tissues, which are difficult to analyze under a transmission electron microscope[1].

Unfortunately, conventional SEMs are only able to view or capture monocular images in the manner of a camera that takes a single photograph. Accordingly, attempts have been made since SEMs first entered practical use to achieve binocular vision by taking two separate images of the same field of view from different angles (stereoscopic images) and then viewing them either with a parallel or cross-eyed method to achieve 3D imaging. Unfortunately, the complexities of obtaining stereoscopic images and the difficulties of stereoscopic display have meant that applications for 3D imaging have been limited up to now. Furthermore, conventional SEMs are not capable of 3D imaging in realtime. Although some attempts at realtime 3D viewing by SEM have been made, they have not been considered to be of much practical use due to reasons of resolution or ease-of-operation.

Recently, however, advances in SEM technology together with progress in computing and display techniques have ushered in a new era in 3D imaging for SEMs. Accordingly, we decided to embark on development work aimed at achieving a genuinely practical implementation of a realtime stereo SEM, and along with pursuing this development, have commercialized some of the results of this work. This article explains the principles of 3D imaging on an SEM, presents an overview of the newly developed realtime stereo SEM, and introduces a demonstration utilizing the capabilities of an SEM for in-place sample manipulation.

STEREOSCOPIC 3D IMAGING BY SEM

As noted above, 3D imaging on an SEM requires two images, corresponding to the parallax of the human eyes. While there are some different ways in which these stereoscopic images can be obtained, the most common are: (1) tilting the sample to acquire images from different angles (stage tilting), and (2) changing the direction of the incident electron beam to acquire images from different angles (electron beam tilting)[2].

Stage Tilting

This technique involves tilting the sample stage to obtain images from two different angles corresponding to the angle of parallax [see Fig. 1 (a)]. Because any SEM capable of tilting the sample can obtain stereoscopic images, this technique has been used for a long time and can obtain high-resolution images[3]. However, obtaining images with the same field of view after tilting the sample is actually quite complicated and time-consuming, and the nature of this technique means it cannot be used for 3D imaging of the sample shape in realtime.

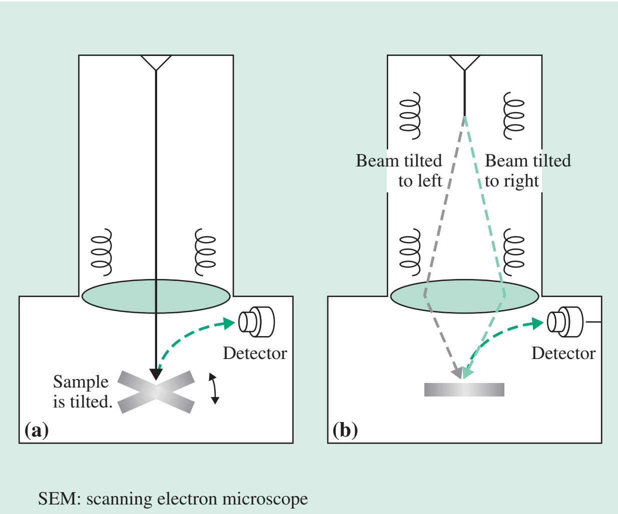


Fig. 1—Stereoscopic Imaging by SEM.
The figure shows how imaging is performed (a), by tilting the stage, and (b), by tilting the electron beam.

Electron Beam Tilting

This technique involves tilting the electron beam to obtain the stereoscopic images [see Fig. 1 (b)]. The technique first appeared in the 1970s in the form of an SEM capable of producing 3D images with a scan rate comparable to television (TV) images(4). Unfortunately, a number of problems needed to be resolved before the technique could enter practical use, including the loss of resolution due to the off-axial aberrations induced by electron beam tilting and the problem of how to display the images in realtime.

DEVELOPMENT OF REALTIME STEREO SEM

Having chosen to use the electron beam tilting technique described above when it set out to develop a practical realtime stereo SEM capable of 3D imaging in realtime, we needed to find a way to obtain stereoscopic images with high resolution while minimizing the aberrations associated with electron beam tilting. It was also considered desirable that the images obtained be viewable with a TV scan rate. A way to display the 3D images was also required. Accordingly, we started by studying ways to obtain high-resolution stereoscopic images at high speed using electron beam tilting.

Development of Electron Beam Tilting Technique Using Focusing Action of Lens

To achieve high-speed acquisition of stereoscopic images, it is desirable that the images from the two different angles be obtained simultaneously by performing electron beam tilting for each line or frame. To achieve this, the electron beam is tilted at an angle of $t0$ by the tilt angle control coil and then focused by the objective lens so that it reaches the sample at an angle of $t1$, as shown in Fig. 2. Because the stereoscopic images can be obtained by this technique at a rate determined by the scanning speed, 3D imaging can be performed with a TV scan rate. Furthermore, because the electron beam can be tilted in any direction or at any angle, the image can be rotated for flexible 3D viewing without any need to rotate the sample mechanically. The practical implementation of an SEM that uses this technique makes possible realtime 3D imaging of biological tissue with complex structure, and enables the two stereoscopic images to be obtained simultaneously in a single operation (see Fig. 3).

In conventional SEM imaging, the electron beam is controlled so that it passes through the center (axis) of the objective lens. However, because electron beam tilting relies on the focusing action of the objective

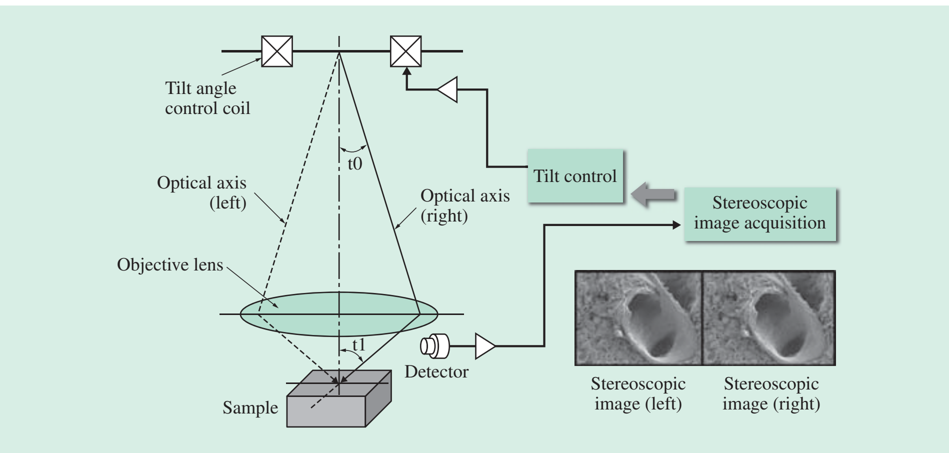
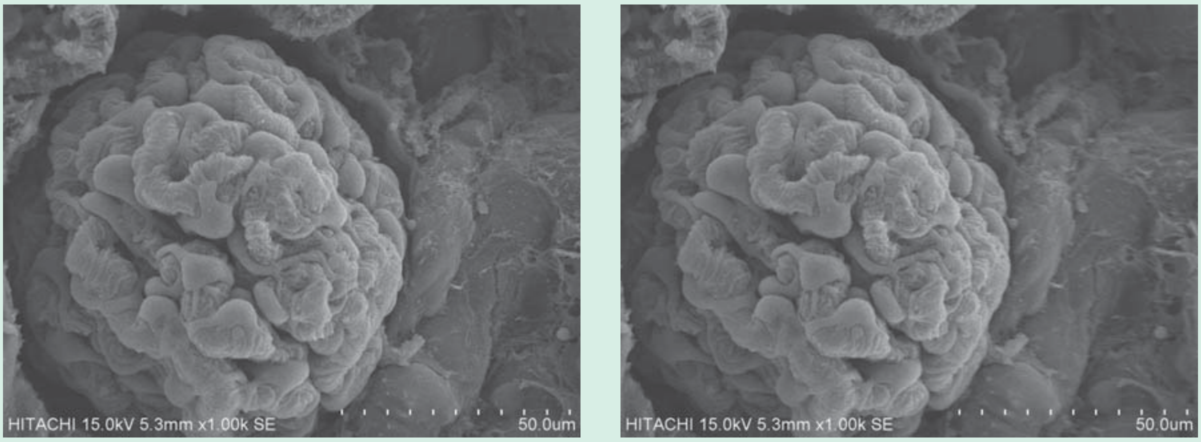


Fig. 2—Stereoscopic Imaging Using Electron Beam Tilting.
The figure shows how the tilt angle control coil (which is different from the deflecting coil used for scanning) is incorporated above the objective lens. This tilt angle control coil is used to tilt the angle of the electron beam left and right for each line.



3D: three dimensional

Fig. 3—Stereoscopic Images Obtained by Electron Beam Tilting (Glomerulus from Rat Kidney).
These images can provide a 3D image when viewed using the cross-eyed method. Electron Beam Tilting is a practical technique for viewing at low magnification.

lens to obtain the two stereoscopic images, the electron beam needs to be directed through the lens at a point away from the center (off-axis). This tends to result in a loss of resolution due to the aberrations associated with electron beam tilting.

The resolution, R_{eso}, when using electron beam tilting can be calculated using the following formula (mean squares method).

$$R_{eso} = \sqrt{\Delta W_{S0}^2 + \Delta W_{RL}^2 + \Delta W_{C1}^2 + \Delta W_{C0}^2 + r_d^2 + (r_{SS})^2} \quad (1)$$

Here, ΔW_{S0} = spherical aberration, ΔW_{RL} = comatic aberration, ΔW_{C1} = off-axial chromatic aberration (sum of chromatic aberration due to magnification and rotation), ΔW_{C0} = axial chromatic aberration, r_d = diffraction aberration, and r_{SS} = light source diameter on sample.

Using this formula to analyze the relationship between the electron beam tilting angle and the resolution on a general-purpose SEM, consisting of a thermal-electron gun electron source and out-lens, indicates that the fall in resolution when electron beam tilting is used is due to the off-axial aberrations (comatic aberration and off-axial chromatic aberration) (see Fig. 4). Spherical aberration, axial chromatic aberration, and diffraction aberration are all independent of the electron beam tilting angle and are present even when $t1 = 0°$. Accordingly, they are omitted from the diagram because they have little influence on the loss of resolution when using electron beam tilting. Furthermore, because astigmatism can be reduced using the stigmator that is typically provided on general-purpose SEMs, it is not included when considering resolution for electron beam tilting.

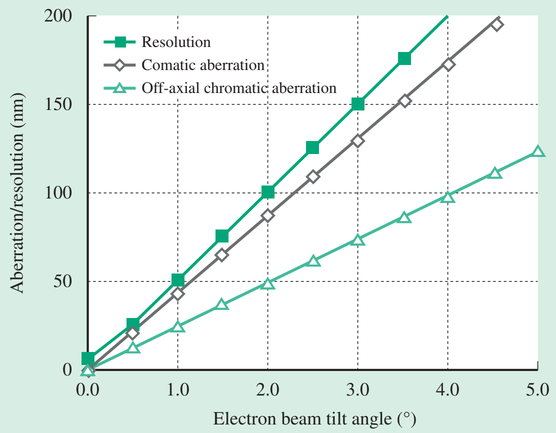


Fig. 4—Relationship between Electron Beam Tilting Angle and Resolution.
Tilting the electron beam degrades the resolution due to comatic aberration and off-axial chromatic aberration.

This indicates that the resolution for electron beam tilting at an angle of 3°, for example, will fall to approximately 150 nm. This is equivalent to a magnification of about ×2,000, indicating that blurring can be anticipated when the technique is used at any higher magnification. Despite this, an instrument capable of 3D imaging with realtime stereo display at low magnification and conventional SEM operation at high magnification would still be useful. This was the thinking behind the development of SEM SU3500 (see Fig. 5).

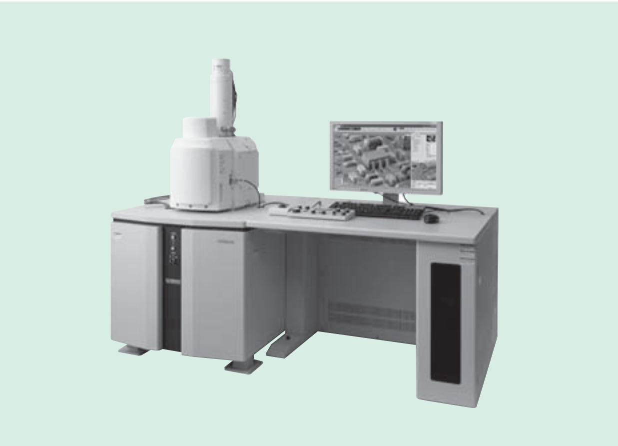


Fig. 5—SEM SU3500.
This SEM uses electron beam tilting to provide realtime stereoscopic imaging.

Development of Optics with Low Aberration

Nevertheless, achieving high resolution on a realtime stereo SEM is an essential step in the development and practicality of the instrument. Accordingly, to enable 3D imaging at high magnifications, we investigated ways of reducing aberrations due to the use of electron beam tilting[(5), (6)]. Specifically, a lens that reduces aberration due to electron beam tilting (aberration reduction lens) was added on the electron source side (relative to the objective lens), and steps were taken to reduce aberration due to the objective lens (see Fig. 6).

Incorporation of this aberration reduction lens into the optics of the realtime stereo SEM was shown to provide a marked improvement in the resolution of the two stereoscopic images. For example, the modified optics can achieve a resolution of 15 nm at a tilt of 3°, which means that observations can be made with magnifications of ×20,000.

ADVANCES AND INNOVATIONS IN 3D IMAGING TECHNIQUES

The ability of the realtime stereo SEM to obtain the two stereoscopic images simultaneously meant that there was a need to investigate techniques that would enable it to be used for simple on-screen 3D imaging. Numerous techniques exist for 3D imaging and the following two were identified as being suitable for the realtime stereo SEM and for a wide range of applications. The commercial models of the realtime stereo SEM can use either of these techniques.

Viewing through 3D Glasses

The easiest and most widely applicable technique is to display the left and right stereoscopic images in red and blue, overlaid on each other, and to view them through 3D glasses with red and blue color filters (cellophane) covering the respective lenses. This technique is called anaglyph 3D. Because the

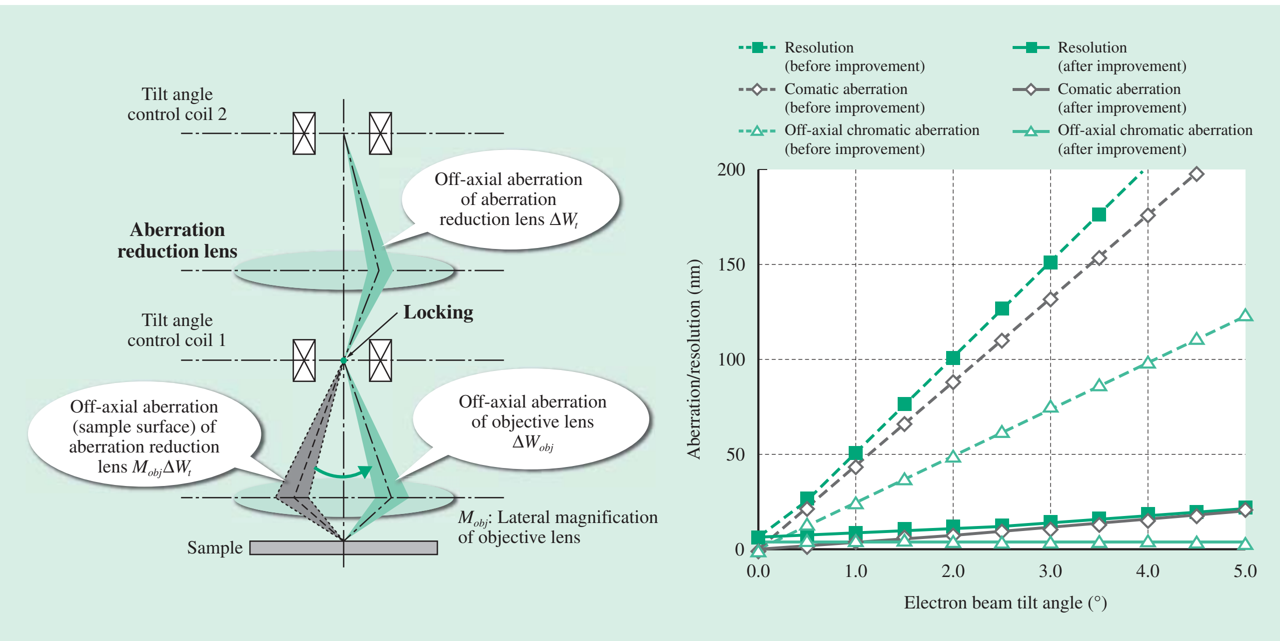


Fig. 6—Conceptual Diagram of Optics with Low Aberration.
The aberration in the objective lens when using electron beam tilting is canceled out by using the aberration reduction lens to induce an aberration that is symmetrical with respect to the optical axis.

effect is easy to produce on a PC screen, a realtime stereo SEM display screen can use anaglyph images to enable realtime TV-rate 3D viewing. Another advantage is that, if the two stereoscopic images are stored as anaglyph images, they can be viewed on a conventional projector or monitor by viewers wearing the 3D glasses. Accordingly, this is provided as a standard function on the commercial models of the realtime stereo SEM.

Another technique is to project the left and right images using light of different polarization and to view them through glasses with polarized filters. This can be done using either circularly or linearly polarized light. With this technique, 3D images from the SEM can be viewed in realtime by displaying the two stereoscopic images on a special-purpose monitor.

Naked-eye Viewing on Special-purpose 3D Liquid Crystal Display Monitor

Naked-eye 3D displays are a recent development that allow people to view 3D images without the need for the 3D glasses referred to above. This is frequently achieved either with a parallax barrier method or a lenticular method. The former separates the images delivered to the left and right eyes by placing a filter in the form of vertical or horizontal stripes on top of the image whereas the latter achieves this using a sheet of small lenses. However, because both methods require the light to pass through a filter, they still suffer from problems such as moiré patterns and loss of brightness. Furthermore, because the left and right images are displayed on the same monitor, the effect is to halve the monitor resolution.

In response, naked-eye 3D monitors with high resolution have been developed that are suitable for displaying images from the realtime stereo SEM.

MANIPULATION USING REALTIME STEREO SEM

We believe that this ability to perform realtime 3D imaging using an SEM is starting to bring major changes to the field of scanning electron microscopy. While there have been attempts in the past to manipulate samples inside an SEM, for example, because it has only been possible to work with monocular images on previous SEMs, these have struck difficulties when performing delicate operations because of the lack of depth perception provided by the image. The task can be likened to trying to thread a needle with only one eye open. When manipulating a sample in a realtime stereo SEM, by contrast, it is possible to obtain an accurate 3D view of the positional relationship between the sample and a tool (such as a dissection needle).

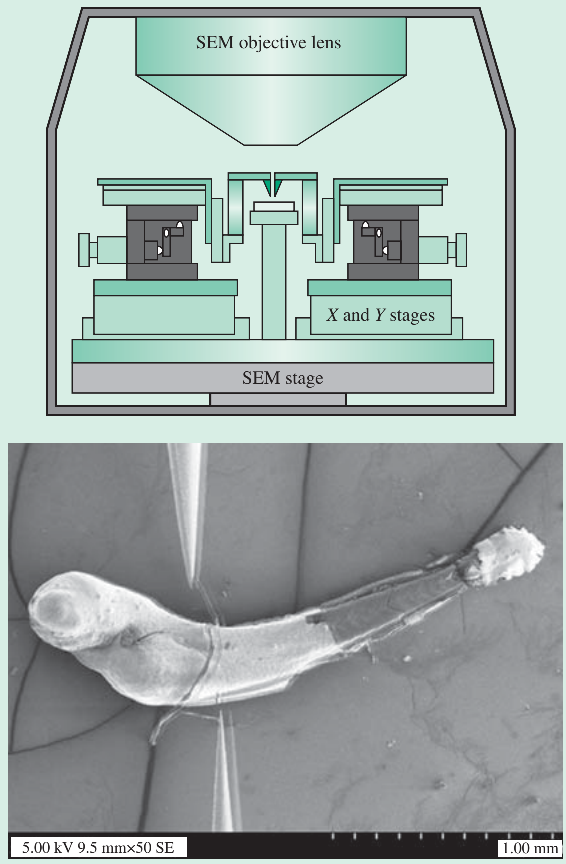


Fig. 7—Conceptual Diagram of Micro-manipulator in SEM Sample Chamber (top) and Image of Its Use for Microanatomical Dissection of Zebra Fish Embryo (bottom). The image at the bottom is the anaglyph overlay of two stereoscopic images.

Accordingly, we have demonstrated the usefulness of the realtime stereo SEM by developing a micro-manipulator that can be fitted on the microscope and used to manipulate biological samples or perform microdissections[(7), (8)].

To achieve this, a prototype of the micro-manipulator was built that was able to be placed inside the SEM sample chamber and operated remotely. This micro-manipulator can be used to perform a variety of tasks or operations on the sample inside the SEM while using 3D imaging to view it in realtime. It is also possible to operate tools such as a dissection needle or tweezers with two hands by installing a number of these manipulators in the SEM sample chamber (see Fig. 7). Operations that can be performed in this way include using a dissection needle to remove a glomerulus (an agglomeration of blood vessels found in the kidney) from a sample and place it in a desired location, or to detach fibers from the lens of the eye.

By combining the micro-manipulator with a tactile feedback system using a haptic device, it is also possible for the operator to feel things like undulations on the surface of the sample or the force on the needle as they work. By incorporating additional techniques for sample preparation, there is also potential for providing the ability to perform actions under an SEM in much the same way as performing a dissection by hand under a stereoscopic microscope.

CONCLUSIONS

This article has explained the principles of operation and basic design of a realtime stereo SEM that was developed to take greater advantage of the characteristics that make SEMs suitable for viewing 3D structures, and has described how manipulation can be performed inside the SEM when it is used in biology.

Technical innovations in recent years have led to major advances in 3D imaging that are already making their presence felt in movies, television, and other areas of popular culture. Given the steady stream of new 3D electronic devices appearing on the market, such as digital cameras, TVs, personal computers, and mobile phones, the development of a realtime stereo SEM is very much in step with the times. Nevertheless, it will be extremely gratifying if this development is able to give a boost to the field of scanning electron microscopy and to be adopted in a wide range of applications. This development has the potential to open up the world of scanning electron microscopy that is different in so many ways from that of transmission electron microscopes.

ACKNOWLEDGMENTS

This research was partly conducted under a project for the development of a 3D nano-dissection biological microscope with haptic control (FY2006 to FY2008), which was included in the Leading Project of the Ministry of Education, Culture, Sports, Science and Technology, Japan under the title "development of elementary techniques for electron microscope in next generation." It was also conducted under a project for developing a realtime stereo SEM (FY2009 to FY2011), which was part of a program for "the development of advanced measurement and analysis system," undertaken under Japan Science and Technology Agency. We wish to express their gratitude for this assistance.

REFERENCES

(1) T. Fujita et al., "SEM Atlas of Cells and Tissues," IGAKU-SHOIN Medical Pub., Tokyo-New York (1981).

(2) S. Ito et al., "Stereo SEM Method," Japan Society of Microscopy Kanto Branch ed., New Scanning Electron Microscope, pp. 116–120, Kyoritsu Shuppan Co., Ltd. (2011) in Japanese.

(3) T. Ushiki, "Actioforma Stereo SEM Atlas of Mammalian Cells and Tissues 1," META Corporation Tokyo, Japan, ISBN978-4-904813-01-0 (2009).

(4) J. B. Pawley, "Design and Performance of Presently Available TV-rate Stereo SEM Systems," Scanning Electron Microscopy, 1978/I (SEM Inc., AFM O'Hare, IL 1978), p. 157 (1978).

(5) T. Ushiki et al., "Development of Biomicroscope for Hands-on 3D Nanodissection Using Force Control," Proceedings of the 65th Annual Meeting of The Japanese Society of Microscopy, p. 96 (2009) in Japanese.

(6) S. Ito et al., "Practical Development of a Real-time Stereo SEM," Proceedings of the 66th Annual Meeting of The Japanese Society of Microscopy, p. 179 (2010) in Japanese.

(7) F. Iwata et al., "Development of a Nano Manipulator Based on an Atomic Force Microscope Coupled with a Haptic Device: A Novel Manipulation Tool for Scanning Electron Microscopy," Arch Histol Cytol **72**, pp. 271–278 (2009).

(8) F. Iwata et al., "Use of AFM Manipulators in Electron Microscopes," O plus E **34**, pp. 229–234 (2012) in Japanese.

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