Review

Development of an environmental high-voltage electron microscope for reaction science

Nobuo Tanaka¹,* Jiro Usukura¹, Michiko Kusunoki¹, Yahachi Saito², Katuhiro Sasaki², Takayoshi Tanji¹, Shunsuke Muto² and Shigeo Arai¹

¹Ecotopia Science Institute and Graduate School of Engineering, Nagoya University, Chikusa-ku, Nagoya 464-8603, Japan and ²Graduate School of Engineering, Nagoya University, Chikusa-ku, Nagoya 464-8603, Japan

*To whom correspondence should be addressed. E-mail: a41263a@nucc.cc.nagoya-u.ac.jp

Abstract

Environmental transmission electron microscopy and ultra-high resolution electron microscopic observation using aberration correctors have recently emerged as topics of great interest. The former method is an extension of the so-called in situ electron microscopy that has been performed since the 1970s. Current research in this area has been focusing on dynamic observation with atomic resolution under gaseous atmospheres and in liquids. Since 2007, Nagoya University has been developing a new 1-MV high voltage (scanning) transmission electron microscope that can be used to observe nanomaterials under conditions that include the presence of gases, liquids and illuminating lights, and it can be also used to perform mechanical operations to nanometre-sized areas as well as electron tomography and elemental analysis by electron energy loss spectroscopy. The new instrument has been used to image and analyse various types of samples including biological ones.

Keywords

environmental high-voltage electron microscope, reaction science, in situ observation, 3D tomographic observation, energy-filtered images in TEM/STEM

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Introduction

Recently, energy and environmental problems have become important concerns, and these problems can be resolved through ‘green technology’ or ‘green manufacturing’ [1]. Attention must be paid to these concerns from the first step of the development of materials and device because the cost of the previous methods for estimations and treatments after production is high and they may not be environmentally friendly. To properly assess the development of materials and devices, samples need to be analysed under actual usage/reaction conditions. For this purpose, the necessity of environmental and in situ observation is increased for structural and elemental analysis.

Transmission electron microscopy (TEM) is one of the most useful methods for the analysis of nanomaterials and nanodevices with atomic dimensions. However, there are several limitations in TEM such as (i) the requirement that the thickness of samples should be less than less than 0.1 μm, (ii) the requirement that samples should be placed under vacuum and (iii) the generation of projected images of samples instead of three-dimensional (3D) structural information. Various techniques to resolve or minimize these limitations have been developed since the 1970s. Since 2007, a new high-voltage scanning transmission electron microscope (HV-STEM) equipped with an open-type environmental cell, large tilting holders, high-sensitivity TV
cameras and an imaging filter for electron energy loss spectroscopy (EELS) and energy-filtered imaging has been developed at Nagoya University; this instrument has been named the reaction science high-voltage electron microscope (RSHVEM) [2]. In this paper, we describe the details and some applications of this instrument.

Details of the instrument

Figure 1 shows an illustration of a general view of the RSHVEM (JEOL; JEM-1000K RS). The height of the tank that houses the accelerating tube is 6.7 m, and the length of the microscope column is 3.6 m. The depth of the base that houses an anti-vibration stage is 3.7 m. The electron gun uses a LaB₆ thermionic cathode, whose chamber is evacuated by two dedicated ion pumps with pumping speed of 20 L/s to improve the vacuum and increase the life time of the cathode. At 1 MV, we can observe aluminum samples with thickness of approximately 5 μm. The three-stage condenser lens system focuses an electron beam to a diameter of less than 1 nm, which enables bright-field (BF) and dark-field STEM and elemental-mapping STEM using EELS.

The most important specification of the present RSHVEM is high-resolution observation at 1 MV under gas pressures to a few tenths of an atmosphere, for example, 13 300 Pa (=100 Torr). The other specifications are a 1-MV maximum accelerating voltage, a point-to-point resolution in TEM mode of less than 0.15 nm, STEM resolution less than 1 nm, an energy resolution in EELS less than 1.5 eV and the use of ordinary sample holders with ±70° tilting angles and a dedicated holder for electron tomography capable of tilting ±180° tilting. Particular emphasis is placed on the development of a new environmental cell for high-voltage electron microscopy (HVEM) that maintains the lattice resolution of less than 0.20 nm under gaseous conditions.

Two kinds of methods exist for environmental observations under gaseous conditions. One method involves the use of a closed-type cell, where amorphous carbon (C) or silicon nitride (SiN) membrane films are used to achieve separation between atmospheric gases around samples and the vacuum in the other part of the column [3]. The other method uses an open-type cell without membrane films and with differential pumping [4–8]. We adopted the latter method to maintain the image resolution and to avoid contamination and damage of the membranes.

To achieve the desired environmental specifications, we developed a new side-entry sample holder without a stopper at the opposite side of a goniometer and enabled the environmental cell to be inserted in the same side. In this case, ordinary sample holders can be used. When the cell is extracted, the instrument can be used as an ordinary HVEM under vacuum conditions. The time required for the insertion/extraction of the cell is of the order of a few minutes. To enable the use of the open-cell system, a new three-stage differential pumping system comprising five turbo molecular pumps and a scrolled pump was developed around the objective lens. Figure 2 shows the vacuum characteristics from the gas introduction stage to the re-evacuation stage. The time to evacuate the cell under gas environment to less than 10⁻³ Pa, the so-called rumpling time, is less than 5 min.

The image recording system for the RSHVEM consists of three CCD cameras for recording low magnification images and electron diffraction patterns (Hamamatsu; 2 k × 2 k pixels), for recording high-resolution images (Gatan; ORIUS; 2 k × 2 k pixels) and for obtaining EEL spectra and energy-filtered images (Gatan imaging filter (GIF); 2 k × 2 k pixels).

**Fig. 1.** Illustration of a general view of a reaction-science high-voltage electron microscope (RSHVEM).
Observation for confirming the basic specifications

Figure 3 shows a high-resolution TEM image of a [110]-oriented cubic silicon carbide (SiC) crystal recorded at 1 MV, where the dumbbell structure spacing of 0.109 nm is clearly discriminated. The separation of the dumbbell is 0.109 nm. This figure proves that the lattice resolution of the RSHVEM at 1 MV is approximately 0.1 nm.

For EELS at 1 MV, the zero-loss peak (ZLP) of a width of 0.87 eV was recorded, which proved a good performance of the GIF for spectrometry and a high stability of the total electric system of the RSHVEM. Fig. 4a and b shows a BF image and an energy filtered (EF) image of a particle of Ce₂O₃, respectively. Using the M-edge of cerium (ΔE = 883 eV), sufficient image contrast is achieved in Fig. 4b that shows the distribution of cerium (Ce) in the particle.

Figure 5a and b shows high-resolution lattice images of a [001]-oriented gold (Au) film under vacuum conditions and under gaseous conditions, respectively. Even at 11000 Pa of nitrogen gas, (200) lattice fringes of gold are clearly observed. This resolution represents the best reported image resolution under gaseous conditions using the open-type environmental cell for environmental TEMs (ETEM). This high-resolution was achieved...
because (i) we used high-energy incident electrons that pass through the gas layers with minimal inelastic scattering and (ii) thinner gas layers were present because of the new design of the environmental cell, which is different from previous design [4–8].

Examples of application of the RSHVEM

Observations under gaseous conditions

Copper particles on silica particles

Copper (Cu) particles were prepared by the vacuum deposition of Cu onto commercial silica (SiO₂) powders inside the RSHVEM. A specially designed sample holder with two filaments, one for sample support and heating and the other for metal deposition, was used [9]. Figure 6 shows a low-magnification BF-TEM image of the sample containing deposited Cu particles. First, in situ oxidation was performed at 700°C through the introduction of oxygen gas at 1 Pa. Electron microscope images and EELS spectra were recorded simultaneously. In the micrographs and the corresponding diffraction patterns, copper monoxide and copper were detected. In the micrographs, a change in the image contrast from multiply twinned Cu particles to flat-contrast particles was observed. Figure 7a and b shows a series of EELS during the oxidation process. Within the duration of the measurement, the K-edges of oxygen evolved. The comparison with the standard spectra of Cu, Cu₂O and CuO revealed that the final state of the particles obtained in Fig. 7b was CuO. Figure 7c shows EELS of the reduction process of the oxidized particles induced through the introduction of a 30 Pa gas comprising 5% H₂ and 95% N₂ at 700°C. The K-edges of oxygen gradually decreased, and finally we obtained pure Cu particles. During the oxidation and reduction processes, the particle shape was similar.

For tin (Sn) particles also, we observed a transition from particles in a liquid state to solid particles of tin oxide (SnO) through the introduction of oxygen gas at 5 × 10⁻⁵ and 250°C [2].
Catalytic reaction of platinum particles with carbon nanotube (CNT) layers

Platinum (Pt) is a typical catalytic metal, and a reduction of the size of its particles enhances the catalytic activity. Currently, maximization of the activities of such catalyst is important for its applications to electrodes in fuel cells. The microstructure and interfaces with various types of supports need to be elucidated at an atomic resolution. The present study focuses on the reaction of Pt particles with a layer of carbon nanotubes (CNTs). The CNT layer was prepared using the method developed by one of the present authors [10], where hexagonal SiC particles were heated at 1700°C using a vacuum furnace to form CNT layers around each SiC particle. Platinum was then deposited onto the layers using direct current sputtering apparatus outside the RSHVEM as shown in Fig. 8a. The composite particles were dispersed onto a tungsten spiral wire used as an electrical resistance heater in a sample holder [9]. The sample was inserted into the RSHVEM and observed in situ at 1000°C in the presence of oxygen gas at $1.8 \times 10^{-4}$ Pa.

During the in situ HVEM observation, the Pt particles reacted with the surface of the CNT layers. The surface was gradually etched by the desorption of the carbon atoms, and finally the Pt particles sank into the lower side of the layers as shown in Fig. 8b and c [11]. We also succeeded in the in situ observation of the oxidation process of the surface of CNT layers at an atomic resolution.

Fracture process of Cu/Si interfaces

Large-scale integrated devices contain various nanometer-sized components and their interfaces. The mechanical strength of such interfaces, because of their extremely small dimensions, is believed to be sensitive to foreign gas atoms that are diffusively supplied from an external environment. In particular, the effect of hydrogen on the interfacial strength is an important issue because hydrogen behaves as a typical embrittling element. Using the RSHVEM, we attempted in situ observation of the fracture process of a semiconductor/metal interface under various gaseous atmospheres, including hydrogen gas.

The experimental TEM sample including a microcantilever was a sputter-deposited multilayer prepared via a standard focused ion beam (FIB) instrument, as shown in Fig. 9a. The cantilever sample comprises carbon (C), silicon nitride (SiN), copper (Cu) and silicon (Si) layers. The sample was mounted on a gold wire and attached to a piezo-driven nanoindenter holder (nanofactory). The hardest SiN layer was pressed with a diamond indenter tip to avoid plastic deformation at the loading point. During the application of the load, the fracture initiation from the Cu/Si interface edge was particularly focused.
The atmospheric gas was a mixture of N₂/H₂ gases (95:5), and the pressure was 300 Pa [12]. The present *in situ* observation revealed that the fracture was initiated at the upper edge of the Cu/Si interface as shown in Fig. 9b and that it propagated to the bottom edge of the interface within a frame rate of 1/30 s. Simultaneously, we successfully measured the applied load as shown in Fig. 10; the measured fracture load of the Cu/Si interface under the gaseous atmosphere $P_c$ was 75.3 μN. Such quantitative data allowed us to calculate the local fracture stress at the Cu/Si interface via numerical analysis, e.g. the finite element method, which is necessary to evaluate the local strength.

The present example illustrates the dependence of...

**Fig. 9.** *In situ* fracture process initiated by a piezo-driven tip (upper right) under hydrogen atmosphere. Low-magnification image of a cross-sectional sample of a vacuum-deposited C/SiN/Cu/Si multilayer before loading (a) and after loading in a hydrogen atmosphere (b).

**Fig. 10.** Diagram of the load as a function of time which was used to determine the maximum stress of fracture of the sample is 75 μN.

**Fig. 11.** Energy-filtered TEM images of a multilayered oxide, which provide insights into the image delocalization effects. Image acquired at 200 kV (a), that acquired at 1 MV with the RSHVEM (b) and corresponding ADF-STEM image at 200 kV (c).

Energy-filtered TEM of an oxide multilayer

Elemental distribution images were obtained using a post-column type energy filter in the present RSHVEM. Figure 11a and b shows energy-filtered cross-sectional TEM (EF-XTEM) images of a multilayer of PrMnO₃, SrTiO₂ and LaMnO₃ using the Ti-M₂,₃ core-loss spectrum (edge onset of ~47 eV) taken using a high-resolution TEM at 200 kV and taken using the present RSHVEM at 1 MV, respectively. The energy slit width was 5 eV, and the recording time was 2 s for both images, applying the edge jump ratio (two-window) method. The thickness of the sample was approximately 0.05 μm. The present example illustrates the dependence of...

Elemental and chemical mapping using a post-column energy filter...
accelerating voltage on the image delocalization effect of inelastically scattered electrons. Egerton’s formula [13] predicts a similar spatial resolution of 0.4–0.5 nm both at 1 MV and 200 kV using an energy loss of \( \sim 50 \text{ eV} \) because the advantage of a smaller wavelength for the higher energy is compensated by the smaller characteristic angle of inelastic scattering. However, the present EF-XTEM image at 1 MV exhibits sharp interfaces between Ti-containing and non-Ti containing layers, whereas the SrTiO\(_3\) layer shows a significant blunt feature in the image collected at 200 kV in Fig. 11a, as predicted by Egerton’s formula. This clear difference can be attributed to less beam broadening in the sample at higher voltages. It should be noted that the lattice-like fringes in the upper part of Fig. 11b do not indicate atomic column resolution of the method, but rather result from interference effects of inelastic scattering, as judged from the corresponding annular dark-field (ADF)-STEM image in Fig. 11c. The present example demonstrates another advantage of HVEM: EF-XTEM images with better spatial resolution can be obtained with a shorter recording time using core-loss edges of a lower-energy region compared with those obtained by medium-voltage instruments. Higher voltages increase the mean free path (MFP) for the inelastically scattered electrons and facilitate the low-loss region to utilize for EF imaging. In Fig. 11b, the filtered image using the Ti-M\(_{2,3}\) edge gives a 0.5-nm resolution, which is the best resolution ever obtained using the core-loss edge at less than 50 eV and could be substantially better than the record obtained by a 300-kV instrument equipped with an in-column-type energy filter using the oxygen K-edge (\( \sim 530 \text{ eV} \)) [14].

Chemical-state mapping of a semiconductor device by STEM-EELS

Another demonstration of the capability of high-voltage EELS imaging using the STEM mode was attempted for a thick semiconductor sample because there is demand of high-through-put analysis of devices for real-world use, and the typical thickness of samples prepared by FIB can be \( \sim 100 \) nm, which exceeds the thickness usually appropriate for EELS by a factor two. As previously discussed, the MFP for inelastic scattering is 1.5 times greater than that of medium-voltage instruments, which allows the examination of thicker samples.

The cross-sectional sample of a gate area in CMOS-FET of a thickness of 100 nm was scanned using a probe with a diameter of \( \sim 2 \text{ nm} \) and a step interval of 5 nm, and a ‘datacube’ of \( 128 \times 93 \text{ pixels}^2 \times 2048 \text{ channels} \) with an energy dispersion of \( 0.1 \text{ eV/channel} \) (including the ZLP) was obtained. The parent datacube was first processed by aligning the spectra using the ZLP, and then Fourier-log deconvolution was used to remove the plural scattering. The Si-L\(_{2,3}\) edge was isolated by subtracting the pre-edge background using the power-law modelling. The region of interest includes different silicon compounds. The multivariate curve resolution (MCR) technique was applied to the dataset to simultaneously separate the mixed spectra into their pure components and their spatial distributions [15]. Figure 12 shows the spatial distributions of compounds (a, b and c), the residual noise (d) and the resolved spectral components (e) resulting from the three-component MCR processing. It is apparent from the resolved spectral profiles that the first three figures in the top row correspond to SiO\(_2\), crystalline Si and Si\(_3\)N\(_4\) respectively. It should be noted that this chemical mapping was performed without the use of any reference spectra or \textit{a priori} information about the data. Furthermore, such an analysis is not feasible with 200 kV instruments because the Si-L\(_{2,3}\) edge is obscured by the tail of higher order plasmon peaks at this thickness.

**3D reconstruction in RSHVEM**

Carbon microcoils (CMCs) in TEM

A carbon microcoil (CMC) is a potential material for the absorbance of electromagnetic waves for various types of shielding, and it differs from the CNT discovered by Iijima [16]. A holographic observation of the electrostatic field caused by current along the coil was performed by Yamamoto \textit{et al.} [17]. However, the growth process has still not been clarified because the details of the 3D structure are unknown. The present sample was prepared by a chemical vapour deposition method using acetylene gas and a catalyst of nickel alloy particles [18]. The CMC was mounted onto a thin metal wire by deposition of tungsten as a glue in a FIB instrument. The
sample holder was a newly developed holder capable of full 360° rotation in the RSHVEM. The tomographic images were recorded in a step of 1° from 1 to 180° on one rotation axis.

Figure 13a and b shows tomographic images reconstructed from 180 BF-TEM images, which show clearly a 3D structure and the cross-section of the coil observed in different directions. One of the reasons for the precise reconstruction is that the linearity between the image contrast and projected potential (thickness) is maintained better in thicker areas by 1-MV HVEM than that by ordinary 200-kV TEM [J. Yamasaki, M. Mutoh, S. Ohta, S. Arai, N. Tanaka, submitted for publication]. A series of such observations can clarify the growth process of the coil.

Chromosome in TEM

In ordinary TEM of biological cells at an accelerating voltage of 100 kV, where the maximum thickness is approximately 0.1 μm, the whole cell with a thickness of approximately 5–10 μm is difficult to observe clearly. It must be sliced with a microtome. In HVEM, the large transmittance of electrons enables the observation of biological samples with thickness greater than 5 μm. The observation of the whole cell is valuable in biological science. The chromosome sample in mitotic phase was prepared via a specially developed method that allows the observation of the whole cell, where soluble proteins and cell membranes were removed and the
remaining cytoskeletal filaments and chromosome were fixed with 1% glutaraldehyde and MoOsO₄ and then subsequently freeze-dried. Figure 14 shows one of tomographic BF-TEM images of the chromosome in the NRK cell of a rat in M-phase, where the spindles appear to separate a pair of the chromosome. The tomographic reconstruction reveals the whole 3D structure of the chromosome and the connection of the surrounding spindles, which was discovered to be comprising actin filaments.

Yeast cells in STEM

HVEM is valuable for the observation of thicker samples, as already noted. However, inelastic-scattering electrons make images blurry, and it is still not easy to obtain high-contrast images in thicker biological samples. The RSHVEM incorporates energy filtering and scanning-image functions. The energy filter allows the acquisition of sharp images by filtering out the inelastic-scattering electrons from thicker samples. On the other hand, ADF-STEM images directly give an integrated amplitude contrast along the specimen depth in each pixel of the image. In the present experiment, the thicker biological sample was observed through the use of energy filtering and the STEM function at 1 MV. We used the budding yeast (Saccharomyces cerevisiae) cell with a diameter of approximately 3 μm as a sample to observe the structure of a whole cell. The sample was embedded in epoxy resin after being chemically fixed and sectioned at a thickness of ~3 μm. Figure 15a is the ADF-STEM image, showing a higher contrast and a sharper image when compared with energy filtering TEM images. Tomography using these STEM images gave clear 3D structures of the whole yeast cell, in which the shape and the distribution of the major organelles of the nucleus, vacuoles and...
mitochondria were identified inside the cell. We also obtained clear-cut BF-STEM images of the sample as shown in Fig. 15b. Using these images, the 3D reconstruction was successfully realized. HV-STEM tomography has shown the potential to visualize a whole cell structure in a single observation. The techniques are based on the results obtained using 300 kV STEM tomography [19].

Comparison with another ETEM method using module tips

As previously described, two methods exist for environmental observations in TEM and STEM; those using the closed-type cell and the open-type cell. Since the 1990s [4], high-resolution ETEM studies have been performed using the open-type cell. On the other hand, the membrane films in closed-type cell created a strong obstacle for clear observation, and inelastic scattering in the film degraded the resolution and contrast in TEM images. In the present RSHVEM, we used an open-type cell, and a resolution of 0.2 nm was successfully achieved under gaseous conditions, as shown in Fig. 5.

Recently, various modules for closed-type environmental cells have been developed for high-resolution E-TEM and E-STEM. The dark-field STEM observations of gold particles under gas or liquid conditions, where the (111) lattice planes of 0.235 nm in spacing were resolved, are particularly interesting [20]. This high-resolution is possible because STEM avoids the deterioration of images caused by inelastic scattering in membrane films and atmospheres, where no imaging lenses exist. However, the observation of samples in liquids such as water, even with use of the modules, may be risky for maintenance of instruments. Once the membranes are broken, the accelerating tubes and electron guns would be fatally damaged. For safety, even with use of the closed-type modules, we still consider that instruments should be equipped with at least a simplified differential pumping system. The closed-type modules for environmental observations, which are easy to use, have been realized by the recent development of microelectromechanical system (MEMS) technology that will also enable various types of manipulation of samples and in situ measurements of physical properties such as electric conductance in future.

Proposal of ‘in-place observation’ for reducing the effects of irradiation on samples

One of the most important problems to be considered in environmental electron microscopy is the effect of irradiation by ions of atmospheric gases produced by electron irradiation inside the cell. This effect increases with increasing gas pressure. Ordinary chemical reactions of interest in research proceedings occur under lower atmospheric pressures such as a few Pascal, and we may be able to study various types of the reactions without excessive heavy-ion irradiation. In the case of observing molecules adsorbed onto particle surfaces, however, the effects of irradiation are serious. Furthermore, a definite limitation exists with respect to decreasing the intensity of electrons incident on samples from the viewpoint of quantum noise in the images. Here, we propose ‘in place observation’ as one of the solutions to this problem, where electron beam is timely switched off during the chemical reaction between samples and gases. For the high-resolution in-place observations, the RSHVEM is equipped with a reliable beam blanking system [2]. The blanking is performed with electrostatic deflectors above the third condenser lens. The command of the blanking is computer-controlled and programmed with a link of the commands for gas injection and evacuation for in situ chemical reactions. We have succeeded in performing the ‘in place observation’ of (200) lattice fringes of a gold film on the TV screen of the present instrument at 1 MV without a large amount of sample drift. The technology should be useful for ordinary environmental TEM at accelerating voltages of 200–300 kV.

Conclusion

In the present paper, the development of a new HVEM for reaction science was described in detail, and some application data were presented to show advantages of HVEM for in situ and environmental observation of thicker samples. In addition, HV-TEM images using core-loss electrons with an imaging filter were shown to reveal the superiority of image
localization of interfaces of oxides. Effective signal processing methods for STEM-EELS mapping were demonstrated for elemental analysis of a semiconductor device. All the data were obtained using the new RSHVEM at Nagoya University at an accelerating voltage of 1 MV. The instrument has already been used for numerous other studies on metallurgical [21], catalytic [22] and chemical reactions. These data have refurbished previous HVEMs, whose main characteristic was their ability to allow observations of thicker samples. Finally, we described the importance of ‘in-place observation’, where the electron beam was smartly blanked during chemical reactions on various advanced materials to minimize the effects of irradiation of electrons and ions caused by incident electrons.

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