

An Experimental Protocol Development of Three-Dimensional Transmission Electron Microscopy Methods for Ferrous Alloys: Towards Quantitative Microstructural Characterization in Three Dimensions

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The majority of engineering steels are ferromagnetic and structurally inhomogeneous on scales ranging from nanometers to micrometers, and their physical properties depend on the three-dimensional (3D) features in their microstructures. Thus, obtaining a 3D image with a large field of view is desirable for transmission electron microscopy (TEM) based microstructure characterization in order to establish the relationship between the microstructure and the physical properties with a reasonable statistical relevancy. Here, we use a conventional sample preparation process, *i.e.*, mechanical polishing followed by electropolishing, and optimizing experimental protocols for electron tomography (ET) of ferromagnetic materials, to carry out microstructural characterization of engineering steel. We determined that the sample thickness after the mechanical polishing step is a critical experimental parameter affecting the success rate of tilt-series image acquisitions. For example, for ferritic heat-resistant 9Cr steel, mechanical thinning down to 30 μm or less was necessary to acquire an adequate tilt-series image of the carbide precipitates in the annular dark-field scanning TEM (ADF-STEM) mode. However, acquiring tilt-series images of dislocation structures remains a challenge due to an unavoidable, significant electron beam deflection during specimen tilt, even with a thinned sample. To overcome the electron beam deflection problem, we evaluated several relatively accessible approaches including the “Low-Mag STEM and Lorentz TEM” modes. Although rarely used for ET, both modes reduce or even zero the objective lens current, likely weakening the magnetic interference between the ferromagnetic specimen and the objective lens magnetic field. The advantages and disadvantages of these experimental components are discussed.

KEY WORDS: electron tomography; transmission electron microscopy (TEM); scanning transmission electron microscopy (STEM); magnetism; precipitate; dislocation.

1. Introduction

In order to quantitatively analyze material microstructures, three-dimensional (3D) observation using various microscopy methods has been performed to rationalize relationships between the microstructure and material properties. The objects under 3D observation vary from atomic clusters at a sub nanometer scale to macroscopic bulk materials at a sub millimeter scale. This study focuses on 3D transmission electron microscopy (TEM) observation that covers a sub nanometer to micrometer length scale with the capability to clarify assorted microstructural information, including morphology, crystal structures, and chemi-

cal compositions, for a particular region of interest in a sample.

The stereoscopic imaging method using TEM has been used as a conventional 3D observation method. However, the electron tomography (ET) method has recently shown significant promise for providing better quality of quantitative 3D information than the stereoscopic imaging method. The basic concept of ET is the same as that of X-ray computed tomography (CT). In ET observation, a sample is intermittently tilted in an electron microscope with the object's image in the sample subsequently acquired at each specimen tilt angle (tilt-series acquisition), as illustrated in **Fig. 1**. After the tilt-series dataset acquisition, a 3D representation of the object is reconstructed and visualized using an algorithm such as the back-projection method. Recent advancements in ET technique enable a variety

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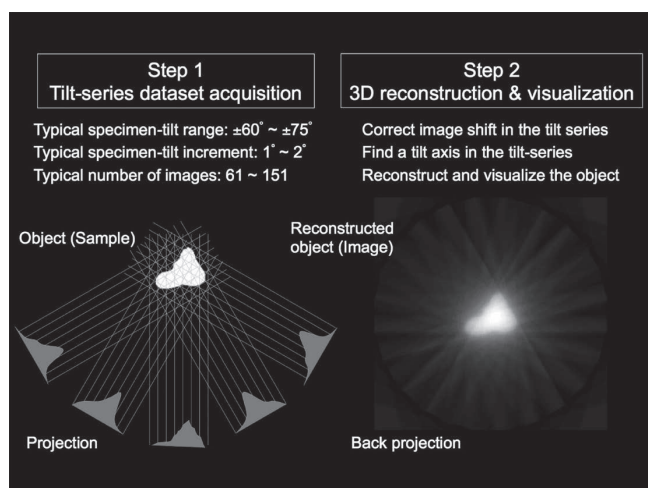


Fig. 1. Basic procedure of electron tomography. Step 1, tilt-series dataset acquisition, is carried out on a transmission electron microscope. A dedicated specimen holder which is capable of tilting the specimen up to more than 60° is necessary. Step 2, 3D volume reconstruction, is performed in a computer.

of 3D observations, such as 3D atomic arrangements and nanoparticle morphology,^{1–3)} morphology and spatial distribution of precipitates,^{4–6)} and dislocations^{7–10)} in crystalline materials.

However, ET observation of steel has some limitations. Steels, except for the austenite-based steels, are ferromagnetic at room temperature, and using TEM to observe a thin-foil steel sample results in image distortion, deflection of the electron beam, and temporary bending of the sample due to the magnetism of the ferrous iron phase. The magnetism influence of a thin-foil steel sample makes tilt-series acquisition difficult, and the resultant 3D reconstruction from the tilt-series dataset is usually not reliable. One solution for reducing the magnetism influence is the fabrication of a small thin-foil sample of size 10–20 μm using a focused ion beam (FIB). However, sample bending from the magnetism can occur even in these small FIB-produced samples during the tilt-series acquisition. Therefore, thus far, there is no fundamental solution to overcome the magnetism problems in the ET observation of steel samples.

The present study proposes an experimental procedure for ET observation of a steel sample with a field of view larger than several hundreds of square micrometers. Obtaining a 3D representation across a large field of view is desirable in TEM microstructural characterization to determine the relationship between the microstructure and the physical properties with a reasonable statistical relevancy. First, for preparation of the large field of view samples described above, millimeter-sized ferromagnetic iron alloy samples are thinned using a conventional twin-jet electropolishing method. Second, in the tilt-series acquisition, the feasibility of several imaging modes in both TEM and scanning transmission electron microscopy (STEM) are examined. In addition to the conventional TEM/STEM imaging modes, the Low-Mag (LM) and LM Lorentz modes are employed, with the objective lens current tuned to small values or zero in order to minimize the magnetic field applied to a sample. Third, the 3D reconstruction algorithms suitable

for tilt-series data sets of ferromagnetic samples are discussed, and finally, the optimum conditions for ET observation of precipitates and dislocations in steel samples are proposed.

2. Experimental

2.1. Sample Preparation

This study utilized three kinds of iron alloys that are ferromagnetic at a room temperature. The first sample is 9Cr ferritic heat-resistant steel in which $M_{23}C_6$ metal-carbide particles were precipitated at the martensite lath boundaries. The second and third samples are alloys of Fe with 2 mass% Cu that forms ϵ -Cu phase precipitates and Fe with 0.94 mass% V, 0.19 mass% C that forms V-C compounds in the ferrous α -Fe matrix.

These alloy samples were cut into 3-mm-diameter disks or smaller rectangular plates about 1 mm $\times$ 2 mm, and then mechanically polished to 30–100 μm thickness by following a standard polishing method.^{11–13)} SiC powder coated polishing papers No. 1 000–4 000 and alumina powders approximately 0.3 μm in diameter were used for the mechanical polishing. The rectangular-plate sample was fixed on a 3-mm-diameter Mo single-hole grid using a thermosetting resin to ensure deformation-free mechanical polishing. After the mechanical polishing, an electron transparent region was obtained using twin-jet electropolishing. The electropolished surface was cleaned by low-energy argon ion milling at an acceleration voltage of 300 V.

Square thin-foil specimens smaller than 20 μm were also prepared using FIB milling in order to examine the influence of sample shape on the tilt-series observation. These FIB milled specimens were finished by low-energy argon ion milling to remove the damaged surface layer created by the FIB milling.

2.2. Electron Microscopy Observation

In order to study the feasibility of various measurement conditions for 3D imaging of ferrous alloys, we used the following TEMs: the FEI Tecnai F20 Super Twin (ST) and the FEI Titan 80–300 ST equipped with and without a CEOS TEM image corrector for spherical aberrations. The Tecnai F20 microscope is equipped with a double-condenser lens system operated at a 200-kV accelerating voltage, and the Titan 80–300 microscopes were operated at 300 kV. The three-stage condenser-lens system of the Titan 80–300 enables different illumination modes such as parallel and condensing illumination. These FEI microscopes can operate in conventional TEM/STEM modes as well as the LM-TEM, LM-STEM, and LM-TEM Lorentz modes. The present study investigated the feasibility of these LM imaging modes for observing precipitates and dislocations in ferromagnetic steels. **Table 1** summarizes the lens settings for the different imaging modes. The lens currents in Table 1 are represented as percentages of the maximum current. Here, we focus on the objective-lens currents. In the case of the Lorentz TEM mode, where the main objective is observation of the magnetic image contrast, the objective-lens current can be set to 0% for visualizing the sample's original magnetic microstructures such as magnetic domains and walls. Furthermore, the Lorentz TEM mode can alter

Table 1. Lens configurations of electron microscopes used in the present study under various imaging modes.

Apparatus	FEI Tecnai F20 Super Twin 200 kV					FEI Titan 80–300 Super Twin 300 kV	
Observation mode	TEM	LM-TEM	Lorentz TEM	STEM	LM-STEM	LM-STEM	μ -probe STEM
Condenser lens 1 (CL1) current (%)	13.9%	13.9%	13.9% (Valuable)	21.2%	21.2%	36.6%	36.6%
Condenser lens 2 (CL2) current (%)	Variable	Variable	43.0%	32.0%	37.2%	29.1%	38.0%
Condenser mini-lens current (%)	83.9%	83.9%	0% (Variable for magnetizing sample)	–83.9%	83.9%	97.9%	66.1%
Objective lens current (%)	~91.3%	~6.0%	0% (Variable for magnetizing sample)	91.9%	6.0%	4.2%	88.8%
Objective mini-lens current (%)	0%	0%	0–100% (Change with focusing)	0%	0%	0%	0%
Magnification (times)	6 200–910 000	19–1 850	110–44 000	10 000–330 000 000	150–155 000	140 000 (Max.)	10 000 (Min.)
Camera length	–	–	–	30 mm (Min.)	5.13 m (Min.)	9.1 m (Min.)	–
Incident beam convergence angle	–	–	–	–	–	0.2 mrad (70 μ m CL2 aperture)	1.6 mrad (70 μ m CL2 aperture)

both the objective-lens currents and the condenser mini-lens currents to observe the magnetization processes in a sample. In comparison, the objective-lens currents for the LM-TEM and LM-STEM modes were set close to zero, at approximately 5% (1 000 Oe magnetic field). Although this 5% objective-lens current is much smaller than that (~90%) used for conventional TEM/STEM modes (including the “ μ -probe STEM” mode), it should be noted that this 5% objective-lens current is sufficient to magnetize the α -Fe ferromagnetic phase.

Nevertheless, we examined the feasibility of using an objective-lens-less imaging method (LM-TEM/STEM) for ET observation of ferromagnetic steel samples based on their promising aspects. First, the magnetic interference between the sample and objective lens would be weak to negligible for objective-lens-less imaging modes. Therefore, it is expected that the volume reduction process can be omitted for a magnetic sample. Second, the low spatial resolution in the objective-lens-less imaging modes is not necessarily a serious problem. For the electron microscopes used in this study (Tecnai F20 and Titan 80–300 equipped with and without a TEM image corrector), the nominal spatial resolution for the objective-lens-less mode is approximately 2 nm for TEM and 10 nm for STEM. These values of the spatial resolution are suitable for quantitative analyses of the spatial distribution and the quantities of precipitates and dislocations in some steel samples, as these microstructural analyses do not require an ultrahigh (atomic-scale) resolution. Furthermore, if the electron microscope is equipped with a TEM spherical aberration corrector, the spatial resolution of the objective-lens-less TEM modes is improved.

2.3. Electron Tomography (ET) Observation

The ET observation consists of two steps: (1) acquisition of a tilt-series dataset and (2) reconstruction of the 3D volume, as shown in Fig. 1. Since the samples in this study are ferromagnetic, the projection requirement⁴⁾ could have serious violations affecting the collected TEM/STEM tilt-

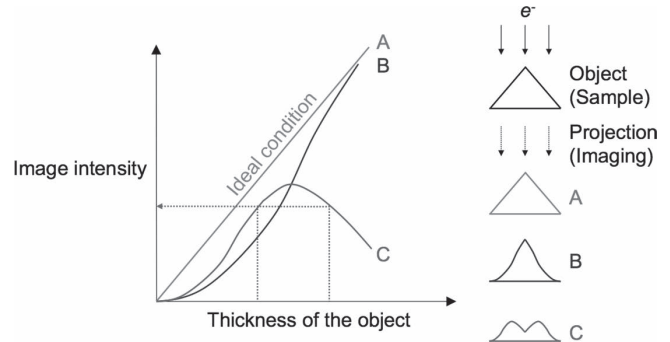


Fig. 2. Schematic explanation of the projection requirement. For accurate tomographic 3D reconstruction, the image intensity should be monotonic functions of physical properties of the specimen such as density, thickness, and degree of order.

series datasets. The projection requirement is an essential imaging condition for reconstructing a reliable 3D image. **Figure 2** illustrates the relationships between the image intensity and the specimen thickness; the projection requirement is satisfied for cases A and B, but is violated for case C. The violation of the projection requirement is mainly due to diffraction contrast such as thickness fringe, bend contour, and strain contrast. Because these changes in the diffraction contrast are sensitive to the diffraction conditions, we minimized these changes by using an incident beam-tilt function while collecting the tilt-series acquisition. The total acquisition time for a tilt-series dataset was 1–2 hours depending on the number of images acquired. Several images for which the projection requirement was seriously violated were excluded from the tilt-series dataset, and a 3D image was reconstructed using the weighted back projection (WBP)^{3,4,14,15)} or the simultaneous iterative reconstruction technique (SIRT).^{3,15,16)}

3. Results and Discussion

First, we introduce the optimized conditions for ET

observation of ferromagnetic samples. Second, we present the results of preliminary 3D observation of precipitates and dislocations under the optimum observation conditions.

3.1. Effect of Sample Shape on ET Observation

In order to fabricate a thin-foil sample using the FIB microsampling technique, a rectangular-plate sample 10–20 μm in size was cut from the bulk sample, and then thinned by a Ga^{2+} ion beam to a sample thickness that was transparent to the electron beam. The thin-foil specimens obtained by FIB milling were wedge-shaped to some extent, resulting in a varying thickness in the electron transparent region from several micrometers to several tens of micrometers. In other words, the specimen thickness was not uniform in a wide field of view, even though the FIB milled sample had a small total volume.

In contrast, for the thin-foil specimens prepared using electropolishing as the final thinning process, in general 3 mm disks of thickness 50–100 μm were collected from the bulk sample by mechanical processes, and then, the central portion of the disks were thinned by twin-jet electropolishing or argon ion milling. These thin-foil samples were considerably larger in volume than the FIB milled samples, which is believed to be disadvantageous in ferromagnetic sample observation because the magnitude of magnetization is proportional to the sample volume. However, if appropriate mechanical polishing was performed for the flat sample preparation and optimized electropolishing conditions such as optimized voltage, current, temperature, and flow-rate of the solution were used, we could obtain a wider field of view in the electropolished sample than a sample prepared using the FIB microsampling technique. The electropolished ferromagnetic material sample with a wide field of view and uniform thickness was advantageous for low-magnification imaging of the microstructure. Furthermore, we found that the magnetic interactions between the electron beam and the electropolished sample prepared under optimized conditions remained constant during the tilt-series acquisition. Actually for the 3 mm disc sample thinned down to as thin as 30 μm by mechanical polishing prior to electropolishing, STEM

tilt-series acquisition of the dispersed precipitate microstructure in a ferritic steel sample was possible even in the strong objective-lens field of the normal STEM mode. **Figure 3** shows an LM-STEM image of the 9Cr ferritic heat-resistant steel for which the STEM tilt-series dataset was acquired, as demonstrated in **Fig. 9**. Martensite laths of various widths are visible in a field of view exceeding 100 $\mu\text{m} \times 100 \mu\text{m}$, indicating that the electropolished sample is beneficial for quantitative analysis of the ferritic steel sample microstructure to show the micron scale inhomogeneity.

As for sample preparation using a FIB, Kacher *et al.*¹⁷⁾ succeeded in collecting tilt-series of dislocation substructures in nonmagnetic austenitic steel using a TEM sample preparation procedure, where the FIB microsampling and thinning in the thin-foil portion of an electropolished sample produced a micrometer-sized thin foil normal to the desired crystallographic orientation. This method is effective for tilt-series acquisition under a constant diffraction condition, although its feasibility for similar tilt-series observation of dislocation substructures in ferromagnetic samples has not been reported. This final treatment using the FIB microsampling technique is capable of reducing the final sample volume drastically, although an FIB mill may not be easily accessible for everyone.

3.2. Observation Mode

Commercially available TEM/STEM instruments provide the following imaging modes: (1) normal TEM/STEM modes capable of high-magnification imaging; (2) LM-TEM/STEM modes for which the objective-lens current is set to zero or near zero; and (3) LM-TEM Lorentz modes for which the objective lens current is definitively at zero. Although optional functions, such as the free lens control, enable setting various imaging modes by changing the individually applied currents for all lenses, these customized lens settings are not generally applicable for microstructural material observation using conventional electron microscopes. In this study, we attempted to use the LM-STEM and LM-TEM Lorentz modes for ET observation of ferromagnetic samples, and we examined whether the use of these imaging modes coupled with no or weak magnetic interference between the sample and the objective lens are applicable to 3D microstructural observation in ferritic steels with a limited resolution.

3.2.1. LM-STEM Mode

First, we describe a notable characteristic of the LM-STEM mode. The electron beam-convergence angle (α) for a normal STEM mode using the objective-lens field is $\alpha = 5\text{--}20$ mrad, optimized to a reasonable spatial resolution and contrast. Conversely, the LM-STEM mode without the objective-lens field can reduce α to ~ 0.2 mrad, enabling a parallel-beam STEM observation. As a result, the parallel-beam LM-STEM mode gives approximately 10 times the focus depth of the normal STEM modes. This is advantageous for tilt-series acquisition because one can avoid the out-of-focus conditions for high tilt angles that arise from long beam penetration distances in a specimen.

Second, we describe the effects of observation conditions on the LM-STEM images. In this study, we operated electron microscopes at acceleration voltages of 200 kV and 300

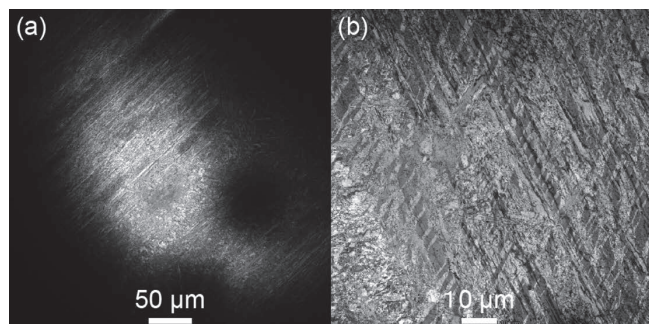


Fig. 3. LM-STEM dark-field images of a 9Cr ferritic heat-resistant steel sample acquired at 300 kV (Titan 80–300 equipped with a TEM image corrector). The sample was mechanically polished down to 30 μm and subsequently electropolished. A low-magnification image (a) visualizes a wide electron transparent field of view and its magnified view (b) shows various sizes of lath martensites. This specimen was capable of acquiring annular dark-field-STEM tilt-series datasets, as shown in Fig. 9.

kV. As the electron penetration depth is proportional to the root of the acceleration voltage, the penetration depth at 300 kV is 20% greater than that at 200 kV. From the viewpoint of the electron wavelength, the spatial resolution of the LM-STEM image at 300 kV is expected to also be approximately 20% higher than that at 200 kV, under a constant spherical-aberration coefficient of the probe-forming lens system. Furthermore, the condenser-lens systems should be discussed; the Tecnai F20 microscope has a two-stage condenser lens system that focuses the electron beam using the second condenser lens. On the other hand, the Titan 80–300 microscope has a three-stage condenser-lens system, where the third condenser lens focuses the electron beam, allowing the minimum size of the focused beam on the specimen to be smaller than that for the two-stage condenser-lens system. In contrast to TEM observation, for STEM, the size of the electron beam scanning the specimen strongly influences the spatial resolution. Indeed, the LM-STEM image in Fig. 4(a) acquired using the Titan 80–300 at 300 kV shows a higher spatial resolution than the LM-STEM image in Fig. 4(b) acquired using the Tecnai F20 at 200 kV. In addition, the size of the condenser-lens aperture is also an important parameter that strongly influences the spatial resolution of the LM-STEM image. When we selected a smaller condenser-lens aperture, the focused electron beam size decreased, also decreasing the electron dose and resultant image contrast. Therefore, the size of the condenser-lens

aperture needs to be optimized for LM-STEM. In this study, the optimized condenser-lens aperture size was 50–70 μm . Bright- and dark-field LM-STEM images show different characteristic image contrasts compared to normal STEM images. Figure 4 indicates that dislocations are recognized as dark lines in both the dark-field (a) and bright-field (b) LM-STEM images. This distinctive dislocation image contrast in LM-STEM is different from that in normal STEM/

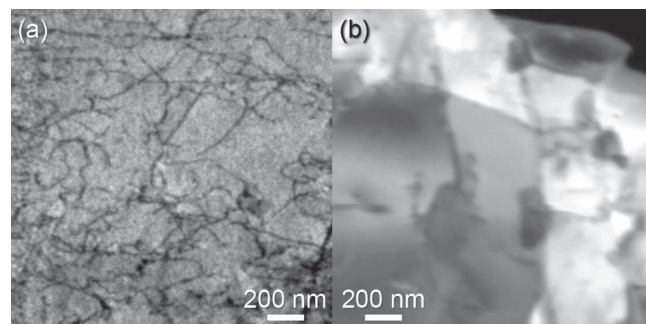


Fig. 4. LM-STEM bright-field image of an Fe–V–C alloy sample acquired at 300 kV (Titan 80–300 equipped with a TEM image corrector) (a) and LM-STEM dark-field image of a 9Cr ferritic heat-resistant steel acquired at 200 kV (Tecnai F20) (b). Both images visualize interactions between dislocations and precipitates. The spatial resolution of the 300 kV image appears to be higher than that of the 200 kV image, regardless of the imaging modes.

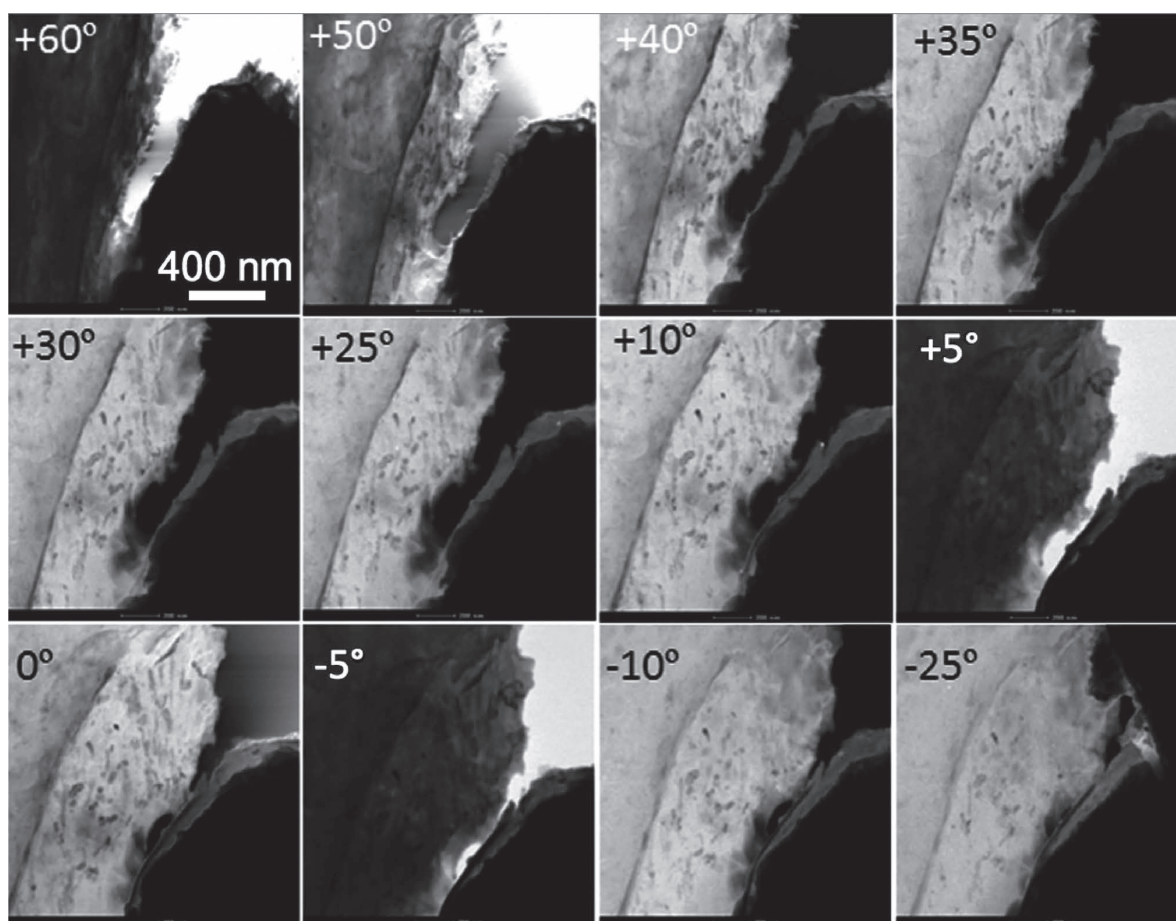


Fig. 5. A part of LM-STEM tilt-series dataset of an Fe–Cu alloy sample. ϵ -Cu precipitates are recognized in α -Fe matrix. Bright-field and dark-field images are intermixed due to the electron beam deflection as a function of the specimen tilt angle.

TEM because dislocations are observed as bright lines in normal STEM/TEM dark-field images.

3.2.2. ET Observation Using LM-STEM

In this study, we acquired LM-STEM tilt-series datasets of ferritic steel samples at a direct $2 \times 10^4 \times$ magnification. When the LM-STEM image exhibited a sudden focus or contrast change at a specific specimen tilt angle, we subsequently checked the imaging conditions before and after the specimen tilt.

Figure 5 shows a part of the LM-STEM tilt-series dataset of an electropolished Fe-Cu alloy sample. The acquisition conditions were as follows: the Titan 80–300 equipped with a TEM image corrector was operated at 300 kV; the second condenser-lens aperture was $50 \mu\text{m}$; the beam-convergence angle (α) was 0.35 mrad; the camera length was 9.1 m; and samples were measured in dark-field mode using an annular detector. The LM-STEM tilt-series shows several “contrast-reversals” with varying specimen tilt angles. For example, when the specimen tilt angle varied between 0° , -5° , and -10° , the image contrast appeared to be dark-field (DF), bright-field (BF) and DF again, respectively. This mixture of the BF and DF images was found to be caused by electron

beam deflection with the specimen tilt angle. **Figure 6** illustrates the locus of the direct beam ($hkl=000$) on the annular detector observed in the LM-STEM mode. When the ferromagnetic sample was tilted on the objective-lens pole piece, the magnetic field changed, deflecting the electron beam below the sample due to the Lorentz force and shifting it from the center of the annular detector, as illustrated in Fig. 6. The BF images appeared when the direct beam reached the annular detector, and the DF images reappeared when the direct beam moved further from the annular detector edge. We recognized that the magnitude of this beam shift depended on the specimen tilt angle and the locus of the beam shift was reproducible.

We attempted LM-STEM tomography observation for an electropolished Fe-V-C alloy sample. **Figure 7** shows the typical LM-STEM images acquired under the following conditions: the Titan 80–300 equipped with a TEM image corrector was operated at 300 kV; the condenser-lens aperture was $50 \mu\text{m}$; the beam-convergence angle (α) was 0.2 mrad; the camera length was 9.1 m; and measurements were obtained in the DF using an annular detector. V-C type precipitates in a size range of several tens of nanometers can be clearly recognized. An LM-STEM energy-dispersive X-ray spectroscopy (EDS) elemental map confirmed the existence of vanadium in the precipitates.

The acquired LM-STEM tilt-series dataset of the Fe-C-V alloy sample was again a mixture of BF and DF images, as shown in Fig. 5. We excluded the BF images from the

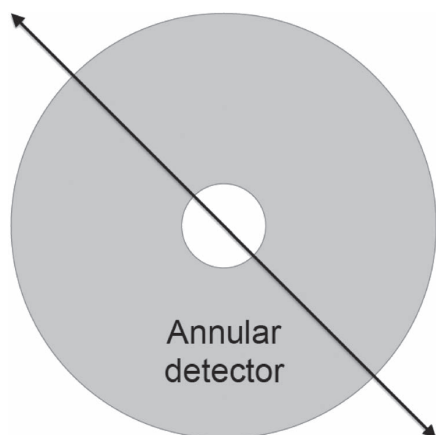


Fig. 6. A schematic illustration shows the direct beam translating across the annular STEM detector during the tilt-series acquisition in Fig. 5. Such a movement of the direct beam causes a mixture of bright-field and dark-field images in a tilt-series dataset, as shown in Fig. 5.

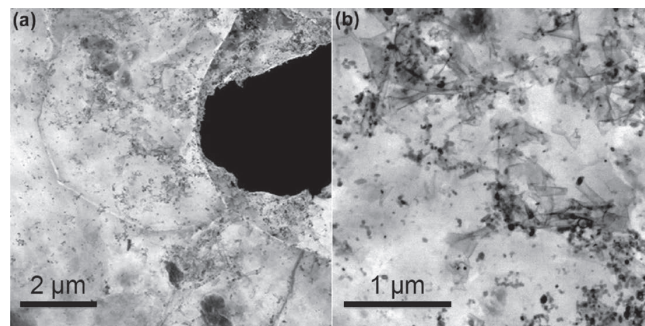


Fig. 7. LM-STEM dark-field images of an Fe-V-C alloy sample. The images were taken at (a) a low-magnification and (b) a medium-magnification. V-C precipitates are observed.

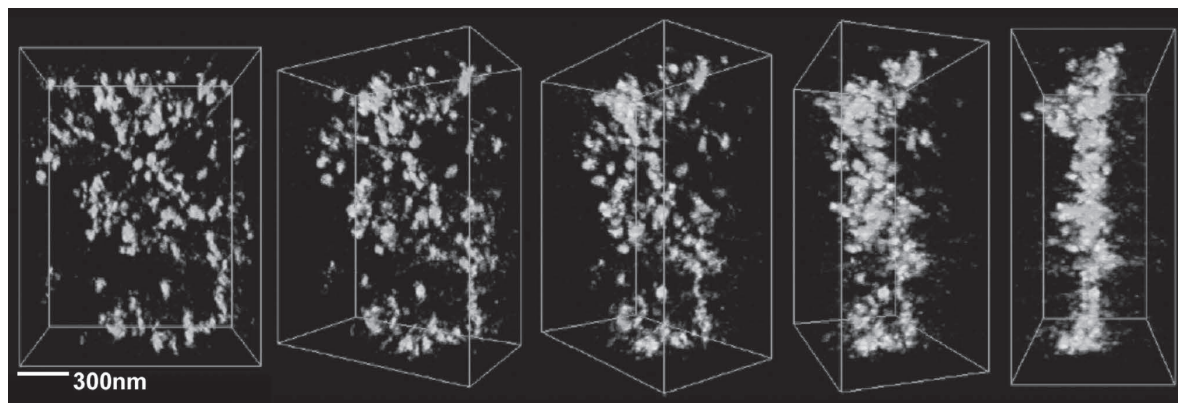


Fig. 8. Three-dimensional views of precipitates in an Fe-V-C alloy sample reconstructed from a LM-STEM tilt-series dataset. V-C precipitates are reconstructed in the 3D volume of $1407 \text{ nm} \times 1848 \text{ nm} \times 693 \text{ nm}$ and the pixel size of 5.25 nm/px .

tilt-series dataset and reconstructed a 3D image from the remaining 52 DF image frames in the angular specimen tilt angle range of -52° to $+58^{\circ}$, as shown in Fig. 8. Although the morphology of the V-C precipitates is not clear, it is possible to recognize their 3D spatial distribution. However, the scattered electron detection angle at the annular detector was not constant during the data acquisition due to the beam-deflection angle varied with the specimen tilt angle. This infers that the projection requirement (Fig. 2) is not satisfactory in the LM-STEM tilt-series dataset. Therefore, the 3D microstructure in Fig. 8 should be analyzed qualitatively.

3.2.3. LM-TEM and LM-TEM Lorentz Modes

The conventional LM-TEM mode without using the objective lens field provides a few thousands times the maximum direct magnification, and its spatial resolution power is insufficient for visualizing fine precipitates in steels. However, in the case of the LM-TEM Lorentz mode using a Lorentz lens (objective mini lens), the maximum direct magnification reaches several tens of thousands. Since the LM-TEM Lorentz mode was originally developed for observing magnetic microstructures, it is possible to avoid sample magnetization (zero the objective-lens field). Therefore, in a TEM spherical aberration corrector with an available LM-TEM Lorentz mode, one can expect to observe, for example, fine precipitates several nanometers in size. There are two kinds of Lorentz microscopy methods: the Fresnel method that visualizes magnetic domain boundaries using large defocus distances around 1 mm, and the Foucault method that visualizes magnetic domains as a DF image

contrast by selecting one of the separated diffracted beams from the electron beam deflection. The Foucault method is suitable for this study to visualize fine precipitates and dislocations under minimal influence of the objective-lens field, because it is operated near an in-focus condition, capable of selecting a desirable diffracted beam using a DF imaging. However, we were unable to perform LM-TEM Lorentz tilt-series acquisition for the ferromagnetic steel sample using the Foucault method. The main reason for this was that it was not possible to align the deflected electron beam in a constant ray path using the alignment coils.

3.2.4. Optimum Conditions for ET Observation of Precipitates and Dislocations

As demonstrated in Fig. 8, LM-STEM tomography is capable of 3D visualization of spatially dispersed precipitates several tens of nanometers in size. On the other hand, LM-STEM tomography was not appropriate for 3D imaging of dislocations in our experiments. At the beginning of this study, we expected LM-STEM to be a promising candidate for low-magnification ET observation of both precipitates and dislocations. This was because LM-STEM provides an enhanced image contrast with a large focus depth as a result of the high brightness and a parallel electron beam on a specimen, compared to the case of the normal STEM mode, which uses the objective-lens field for focusing the electron probe. For 200 kV imaging with the conventional two-stage condenser-lens system (Tecnai F20), the spatial resolution of LM-STEM was not satisfactory for 3D tomographic observation of precipitates and dislocations in steel

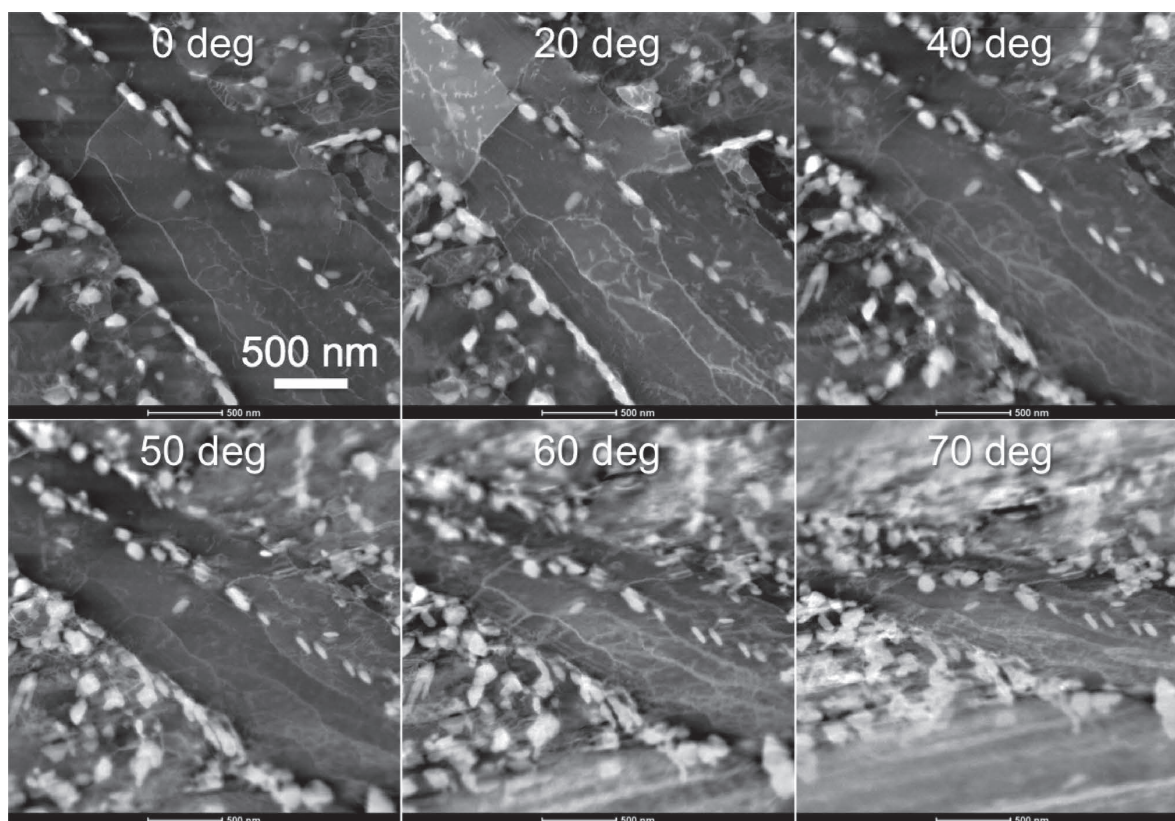


Fig. 9. A part of ADF-STEM tilt-series dataset of a 9Cr ferritic heat-resistant steel sample. Specimen tilt angles are denoted in the images.

samples. However, the LM-STEM mode operated at 300 kV with the three-stage condenser-lens system is promising for 3D tomographic observation, as demonstrated in Figs. 5(a), 7 and 8.

The most serious issue with LM-STEM tomography observation of ferromagnetic samples is that the deflected electron beam with specimen tilt cannot be aligned in a constant optical axis by the beam-alignment coils. This makes dislocation tilt-series acquisition under a constant diffraction condition difficult. We should also mention that the objective-lens field is not necessarily zero even in the LM-STEM mode, as seen in Table 1. For example, in the case of the Titan 80–300, the LM-STEM mode objective-lens current is 4%, corresponding to a magnetic field of 10^3 Oe applied to a sample on the objective lens pole piece. This magnetic field is sufficient to magnetize the α -Fe phase, resulting in the magnetic interference between the tilted ferromagnetic sample and the objective-lens field generating a large electron beam deflection in the LM-STEM mode.

In comparison, the normal STEM mode of the Titan 80–300 has a typical operation objective-lens current of 89%, producing a magnetic field of approximately 2.1×10^4 Oe (this value changes to some extent depending on the imaging and alignment conditions). This objective lens magnetic field is much stronger than that of the magnetized steel sample. Therefore, for the normal STEM mode, the electron beam-deflection angle with specimen tilt on the objective-lens pole piece is roughly one order of magnitude smaller than the beam-deflection angle for the LM-STEM mode. As a result, a mixture of BF and DF images in the tilt-series dataset, shown in Fig. 5, did not occur frequently in the normal STEM mode. In other words, the strong magnetic field of the objective lens system in the normal STEM mode is valuable from the viewpoint of suppressing the electron beam deflection during the tilt-series acquisition for a ferromagnetic sample.

Figure 9 shows a portion of an annular dark-field (ADF)-STEM tilt-series dataset of the 9Cr ferritic heat-resistant steel sample of thickness $30 \mu\text{m}$ after the mechanical polishing as shown in Fig. 3. In the tilt-series dataset acquisition, a high-angle triple-axis specimen holder¹⁰⁾ was used to align a diffracted beam of $g(hkl)=110_{\alpha\text{-Fe}}$ to the Bragg condition exactly on the specimen tilt axis. As a result, the ADF-STEM tilt-series images visualize both dislocations and carbides precipitated at the martensite lath boundaries. However, the influence of magnetic field interference between the sample and the objective-lens system was still present, including sudden changes in the image contrast of the dislocations and martensite matrix for specimen tilt angles higher than 20° , and the left/right edge areas of the STEM images becoming out of focus due to the ineffective dynamic focus alignments and electron beam astigmatism.

It is inferred that the ADF-STEM tilt-series dataset in Fig. 9 would be capable of 3D visualization of carbide particles. However, the dislocation line contrast would not be clearly resolved in a 3D reconstructed image because the dislocation contrast in the ADF-STEM tilt-series images was not constant (consistently visible as bright lines), caused by the sudden changes in the diffraction conditions with the specimen tilt angle. In order to realize high-resolution 3D

visualization of dislocations in such ferromagnetic samples, a method of tilt-series acquisition combined with correction of any deviation from a constant diffraction condition is required to eliminate the influence of the electron beam deflection. One possible method is to use an incident beam tilt function for precise diffraction alignments.¹⁸⁾ Another possible method is to use novel 3D reconstruction algorithms such as discrete tomography¹⁹⁾ and compressive sensing.^{20–22)} These new algorithms are promising since reducing the number of images in a tilt-series dataset does not notably degrade the accuracy of the reconstructed 3D object morphology. Therefore, acquisition of a set of dislocation images under a suitable diffraction condition from a tilt-series dataset along with 3D reconstruction using one of these new algorithms would enable high-resolution 3D visualization of the dislocation substructures in ferromagnetic steels. After overcoming the technical issues described above, ET observation of various microstructures in steels will be possible, allowing a paradigm shift from qualitative to quantitative understanding of the relationship between the mechanical properties and dislocation substructures in steels.

4. Conclusions

The present study focused on the development of an experimental protocol of ET observation techniques for ferrous alloys, and investigated the optimal conditions and methods for each ET process, from sample preparation to TEM/STEM mode settings and 3D reconstruction algorithms. The conclusions are described as follows:

(1) In the case of a sample with a wide field of view over several hundreds of square micrometers, the sample should be thinned to less than $30 \mu\text{m}$ by mechanical polishing before the final electropolishing. The thinning process effectively suppresses electron beam deflection and image quality degradation due to the magnetic field interference between the tilting ferromagnetic sample and the objective-lens system.

(2) For tilt-series dataset acquisition using a mass-thickness contrast of precipitates in an electropolished sample, the strong magnetic field of the objective-lens system in the normal STEM mode suppresses the electron beam deflection with specimen tilt, resulting in successful acquisition of the tilt-series dataset.

(3) For tilt-series dataset acquisition using diffraction contrast of dislocations in an electropolished sample, even for the normal STEM mode, the small angle of electron beam deflection results in significant changes in the diffraction conditions with specimen tilt. This degrades the resolution power of the resultant 3D reconstructed image of sample dislocations.

(4) We showed the possibility of objective-lens-less LM-STEM tomography observation in a wide field of view along with a preliminary 3D reconstruction result for V-C type particles of several tens of nanometers in size precipitated in the α -Fe matrix.

(5) Because the LM-STEM mode without use of the objective-lens field is strongly influenced by the electron beam deflection with a ferromagnetic sample tilt, the normal STEM mode is preferable for ET observation of fer-

romagnetic samples. Nevertheless, the LM-STEM mode requires further investigation as a promising 3D imaging mode, because the high brightness and highly parallel electron beams in the LM-STEM mode is advantageous for wide field-of-view tilt-series dataset acquisition for steel samples.

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