Towards Automatic Electron Tomography for Rod-Shaped Specimen∗

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This paper describes a method for automatic acquisition of a high-resolution transmission electron microscope (TEM) tilt series over the full angular range from −90° to +90° for TEM tomography. The goniometer controller of a conventional TEM was modified to allow external computer control over specimen position along the three translational and one rotational axis. The TEM parameters, stage motion, and image acquisition were synchronously controlled by home-coded software. The shift in the specimen position with each step of rotation was determined at low magnification by the cross-correlation function and then compensated by moving the stage. The image shift coil and objective lens were only used for fine-tuning. As a result, a tilt series over a full angular range was automatically collected at a magnification of around ×200k. The specimen position remained within less than around ±100 nm in the three directions during image collection. [DOI: 10.1380/ejssnt.2010.178]

Keywords: Electron microscopy; Electron Tomography; Computer Control

I. INTRODUCTION

Transmission electron microscope (TEM) tomography is one of the most powerful methods available for three-dimensional (3D) imaging with a resolution of a few nanometers, allowing one to study the internal structure of materials and biological samples. In this process, called tomographic reconstruction, a 3D image is constructed from a tilt series, that is, a set of images collected at different angles over a large angular range. It is impossible to accurately set the rotational axis of the goniometer stage perpendicular to the optical axis of the TEM; furthermore, it is difficult to accurately set the specimen exactly on this rotational axis. Inevitably, the specimen will shift from the right position with every change in the rotational angle, and this shift must be compensated. For this reason, manual collection is very common, though it is slow and, as a result, leads to large electron dose of the specimen. Radiation-sensitive samples will be easily damaged during collection. To overcome these problems, some methods have been developed for the automatic collection of the tilt series. The basic instrumentation and methodology for this have been described by Koster et al. [1]. Their main idea is to determine the specimen shift along the x-, y-, and z-directions (image displacement and defocus) and compensate for this by changing the settings of the image shift coil, beam position, and objective lens current. A cross-correlation function was used to determine the displacement from the two images taken before and after each step. The defocus value was detected by using the beam-tilt-induced method [2]. Dierksen et al. developed a method to observe a radiation-sensitive sample under low-dose conditions [3, 4]. In this method, different specimen areas were used for tracking, focusing, and collecting images. A variation of their approach has been adopted in commercial tomography software. To reduce the difficulty with tracking and focusing, a predictive data collection scheme was developed with which the specimen shift due to stage rotation could be predicted dynamically and compensated for by changing the settings of the image shift coil, beam position, and objective lens current during image collection [5, 6]. This scheme achieves the best accuracy and efficiency achieved thus far. However, in this scheme, the specimen position changes by a larger extent during collection when the angular range of the collection is larger. In an extreme case, the image rotation and magnification can change because of the specimen is shifted along the z (optical) axis. These changes degrade the quality of the 3D reconstructed image. The large displacement of the specimen position along the x- and y-axes disrupts tilt series collection because the current that can be passed through the image shift coil is limited. To construct a 3D image, a complete tilt series over an angular range from −90° to +90° is required without any missing wedges [7, 8]. Therefore, it is important to compensate for specimen position over this large angular range to obtain a high-quality 3D reconstructed image.

We modified the goniometer controller of a conventional TEM so that the specimen could be moved along the x-, y-, and z-directions using an external computer. We developed software to automatically collect a tilt series of rod-shaped specimens over the range of angles from −90° to +90°. This software utilizes a digital low-pass filter and the Sobel filter for image enhancement prior to cross-correlation to determine the shifts. This approach could be adopted for the collection of the tilt series of rod-
II. EXPERIMENTAL SETUP

A. Computer control

A schematic of the experimental setup is shown in Fig. 1. It consists of a TEM (JEOL 3000F), a charge-coupled device (CCD) camera, and a control personal computer (PC). The acceleration voltage of the TEM was set to 300 kV. A side-entry-type specimen holder (Gatan, model 912J) with an interchangeable end tip designed for narrow-gap pole pieces was used. A specimen is mounted on the top of the tip shown in Fig. 2(a). We fabricated the tip (Fig. 2(b)) to allow a complete rotation of the tip at the pole pieces with a gap of 1.8 mm. The TEM is equipped with a computer that communicates with a microprocessor to control the lens currents (condenser, object, intermediate, and projector) and coil currents (beam deflector and image shift) through an RS-232C interface.

We developed software in Visual Basic and C++ to automatically collect the tilt series; the software was run in Windows XP on a computer with an Intel Xeon processor and 2 GB of random access memory. This software can read and correct all the settings of the TEM, such as lens currents and coil currents and motion of the fluorescence screen. The automatic procedure for the collection of a tilt series is described in section 4.1.

B. Goniometer stage

The specimen can be moved along three axes ($x_G$, $y_G$, and $z_G$-axes, as shown in Fig. 3) by controlling the motor-driven goniometer in our TEM. Here, $y_G$ is the rotational axis, and $\theta$ is the angle of rotation. The $x_G$- and $z_G$-axes rotate with $\theta$ in our TEM. That is, the $x_G$- and $z_G$-axes are perpendicular and parallel to the $z$ (optical) axis at $\theta = 0^\circ$, but perpendicular and parallel to the optical axis at $\theta = 90^\circ$, respectively, as shown in Fig. 3. The specimen position ($x$, $y$) and the specimen height ($z$) are compensated by a combination of the changes along the $x_G$, $y_G$, and $z_G$-axes during image collection.

The goniometer of our TEM was designed for only manual operation. Signals to change the position along the $x_G$, $y_G$, $z_G$, and $\theta$-axes of the goniometer were outputted from the trackball, button, and foot switch into the control circuit of the goniometer, respectively. The goniometer was modified to be controlled by the output pulse from the control PC. The rotational angle can be set and read at a resolution of 0.1°. The resolution of the movement in the $x_G$, $y_G$, and $z_G$-directions is on the order of a few dozen nanometers, but is not constant because of the mechanical play of the goniometer.

C. Image processing

The displacement between two images can generally be measured using the cross-correlation function (CCF). The position of the correlation peak indicates the displacement in terms of the distance and direction. However, the displacement cannot be measured accurately if the image includes fixed-pattern noise from the CCD camera. An effective solution is to enhance the edges using a digital contour filter before using the CCF. A Sobel filter is a widely used digital contour filter. This filter is not suit-
able for images with a small signal-to-noise ratio (SNR), because it is highly sensitive to image noise. To overcome this drawback, it is effective to run the images through a digital low-pass filter that replaces each pixel value by the mean value of its neighboring pixels prior to using the Sobel filter [9].

We used the digital low-pass filter and the Sobel filter prior to the CCF to measure the displacement between two images. The optimal window size of the digital low-pass filter for smoothing depends on the image size, sample, and SNR of the image. In this experiment, a window of $15 \times 15$ pixels was used for a $1024 \times 1024$ pixel image. Figure 4 shows examples of digital low-pass filtered and Sobel-filtered images.

III. PRE-CALIBRATION

In this section, the pre-calibration of the microscope controls needed for automatic collection is described.

A. Image shift coils

The relationship between an induced change in the activation of the image shift coils and the resulting image shift is given by Eq. (1). This must be calculated to compensate for the shift in the specimen position caused by the use of the image shift coils.

$$I_s = S \begin{pmatrix} \Delta s_x \\ \Delta s_y \end{pmatrix},$$

where $I_s$ is the displacement along the $x_I$- and $y_I$-directions, and $S$ is a $2 \times 2$ matrix describing the orientation and proportionality between the image displacement $I_s$ and the induced change in the image shift coils $(\Delta s_x, \Delta s_y)$. Details of the method for measuring the matrix $S$ have been described by Koster et al. [1]. The matrix should be measured at all magnifications used for automatic collection.

B. Goniometer

The specimen position $(x)$ and specimen height $(z)$ must be compensated by a combination of changes along the $x_G$- and $z_G$-directions. The $x_G$- and $y_G$-axes are projected parallel to the $x_I'$- and $y_I'$-axes that are rotated by $\beta$ against the $x_I$- and $y_I$-axes at $\theta = 0^\circ$, if the rotational axis ($y_G$) is not projected parallel to the $y_I$-axis in the image at $\theta = 0^\circ$, as shown in Fig. 5, as is the case with our TEM. The orientation and proportionality between the change in the goniometer $(\Delta g_x, \Delta g_y, \Delta g_z)$ and the resulting image displacement and defocus $I_G$ is given by Eqs. (2), (3), (4), (5), and (6):

$$\begin{pmatrix} \Delta g_x \\ \Delta g_y \\ \Delta g_z \end{pmatrix} = G I_G,$$

$$G = \begin{pmatrix} A \cos \theta & 0 & B \sin \theta \\ 0 & C & 0 \\ -D \sin \theta & 0 & E \cos \theta \end{pmatrix},$$

$$I_G = \begin{pmatrix} \Delta I_x' \\ \Delta I_y' \\ \Delta I_z' \end{pmatrix},$$

$$d = \begin{pmatrix} \Delta I_x' \\ \Delta I_y' \end{pmatrix} = \begin{pmatrix} \cos \beta & \sin \beta \\ -\sin \beta & \cos \beta \end{pmatrix} \begin{pmatrix} \Delta I_x \\ \Delta I_y \end{pmatrix},$$

$$\Delta I_z' = \Delta I_z,$$

where $\Delta I_x$ and $\Delta I_y$ are the image displacements along the $x_I$- and $y_I$-axes and $\Delta I_z$ is the defocus value along
the optical axis (\(z_l\)). \(\Delta I_x'\) and \(\Delta I_y'\) are the image displacements along the \(x_l\)- and \(y_l\)-axes. The \(x_l\)- and \(y_l\)-axes are rotated by \(\beta\) against the \(x_l\)- and \(y_l\)-axes. The constants \(A, B, C, D, E,\) and \(\beta\) are measured as follows. First, the displacement \(d_1 = (\Delta I_x, \Delta I_y)\), which is the value in the \(x_l\)- and \(y_l\)-directions, is measured between before and after changing the settings of the goniometer with \((g, 0, 0)\) at \(\theta = 0^\circ\) by using the collected images. Second, displacement \(d_2\) is measured with \((0, g, 0)\) at \(\theta = 0^\circ\). Third, displacement \(d_3\) is measured with \((0, 0, g)\) at \(\theta = 90^\circ\). Fourth, displacement \(d_4\) is measured with \((0, 0, 0)\) at \(\theta = 45^\circ\). Finally, these constant values can be calculated by solving the simultaneous equations that are obtained by substituting \(d_1, d_2, d_3,\) and \(d_4\) into these equations. Equations (2)–(6) are used to compensate for the shift in the specimen position by using the goniometer. Actually, these displacements change after every calibration due to the mechanical play of the goniometer. Therefore, we quickly measured \(d_1\) at \((10, 0, 0)\) after changing the setting of the goniometer to \((-10, 0, 0)\). The displacements \(d_2, d_3,\) and \(d_4\) were measured in the same manner and the constants \(A, B, C, D, E,\) and \(\beta\) were calculated. The specimen position cannot be compensated accurately by shifting it by \((\Delta g_x, \Delta g_y, \Delta g_z)\), as calculated by Eq. (2), due to the mechanical play. Hence, the determination of \(d\) and changing the specimen position by \((\Delta g_x, \Delta g_y, \Delta g_z)\) should be repeated a few times in the sequence of automatic collection (see section 4.2).

C. Objective lens

For focus adjustment, we use the beam-tilt-induced method [2] that utilizes the phenomenon of image shift induced by beam tilting. Details of the method for measuring the parameters required for focus adjustment are described in the article.

D. Condenser lens

Generally, a change in the magnification causes a change in the brightness of the image, because the electron beam spot size on the sample does not change whereas the spot size on the CCD camera does. Thus, the condenser lens power must be adjusted to maintain the image brightness in a range suitable for the CCD camera. The image brightness is determined by the spot size on the sample, the magnification, and the emission current [10]. We calculated the condenser lens current to adjust the image brightness at the observed magnification by using the relationship between the change in the image brightness and the condenser lens current, the magnification, and the emission current.

IV. AUTOMATIC TILT SERIES COLLECTION

A. Procedure

In this section, the procedure for the automatic collection of a tilt series using our software is described.

Before executing this procedure, the height of the specimen position should be set to a eucentric position by changing the direction of the \(x_G\)-axis to \(\theta = 90^\circ\) or \(\theta = -90^\circ\). The specimen is shifted before and after each tilt due to mechanical imperfection or specimen drift, even if its position is set to the eucentric position before the procedure. At the observed magnification, the shifts should be within the field of view of the CCD after every tilt. For example, a magnification of less than around \(\times 60\) was preferable to collect tilt series at every \(2^\circ\) over a range of rotational angles from \(-90^\circ\) to \(+90^\circ\) in our TEM. For tilt series collection at magnifications greater than \(\times 60\), the sample position compensation should be done at the lower magnification. Our proposed procedure is as follows. (Steps 2 to 15 are repeated until completion of the tilt series collection.)

1. The first image of the tilt series is collected at a high magnification \(M_h\). The collected image size is \(1024\times1024\) pixels. The exposure time is \(T\) seconds.
2. The magnification is lowered to \(M_l\).
3. The condenser lens current is changed to maintain the image brightness in a range suitable for the CCD camera.
4. A reference image is obtained at \(M_l\).
5. The image-shift value is returned to the initial value.
6. The rotational angle is changed by one step.
7. The focus is adjusted by the beam-tilt-induced method and the shift along the \(z\)-axis is calculated.
8. An image is acquired and the shifts along the \(x\)- and \(y\)-axes are measured by using the reference image.
9. The specimen position change is compensated by moving the goniometer stage.
10. The setting values of image-shift coils are changed for fine alignment.
11. The magnification is set to \(M_h\).
12. The condenser lens current is changed to maintain the image brightness in a range suitable for the CCD camera; otherwise, the exposure time is changed to \(T\).
13. The focus is adjusted by the beam-tilt-induced method.

14. The setting values of the image-shift coils are changed for fine alignment.

15. An image of the tilt series is obtained.

Broadly, three steps—tilt the specimen, compensate for the sample position at lower magnification, and acquire the image at the required higher magnification—are repeated. The compensation for the sample position at lower magnification in the procedure is preferable because the electron dose is reduced, which is inverse proportion to the square of the magnification. At higher magnification, the shifts of sample position are compensated by changing the image shift coils and the objective lens.

In this procedure, the specimen is exposed to the electron beam only for the moment of the image collection in order to reduce the electron dose to the specimen. The beam is cut-off by controlling the beam deflector coils. By this means the electron dose can be reduced drastically, which increases the potential for collect a tilt series of electron-sensitive materials.

B. Compensation of sample position

Details of Step 8 in the procedure are described in this section. The displacement \((\Delta I_x, \Delta I_y)\) and defocus \((\Delta I_z)\) between the obtained image and the reference image are measured by the CCF and the beam-tilt-induced method. Figure 6 shows the relationship between the axes \((x_G, z_G)\) of the goniometer and those \((x_I', z_I')\) of the collected image. Point \(P_2\) is the compensated specimen position. To bring the specimen to point \(P_2\), the specimen must be first moved to point \(P_1\) along the \(x_G\)-axis and then to point \(P_1\) along the \(z_G\)-axis.

In the image, the specimen appears to move by and along the \(x_I'\) axis by these movements, as shown in Fig. 6. and are shown in Eqs. (7), (8), and (9).

\[
I_1 + I_2 = -\Delta I_x'
\]  

\[
I_1 = -(\cos \theta \cdot \Delta I_x' + \sin \theta \cdot \Delta I_z') \cos \theta
\]
\[ I_2 = -(\sin \theta \cdot \Delta I_x' - \cos \theta \cdot \Delta I_z') \sin \theta \]  

(9)

If the goniometer had no mechanical play, the specimen position can be adjusted once in the \( x_G \), \( y_G \), and \( z_G \)-directions by the changed value of the goniometer calculated by Eq. (2). However, the adjustment of the specimen to the right position need to repeat the sequence a few times (approximately 1–5 times) for all directions.

V. EXAMPLE OF COLLECTED TILT SERIES

A. Sample preparation

To validate our method, it was tested using a carbon rod on which gold particles had been deposited. The carbon rod was fabricated using a focused ion beam. The gold particles were deposited on only one side of the rod.

B. Tilt series

A tilt series was collected over a range of angles from \(+90^\circ\) to \(−90^\circ\) at approximately 2° steps. The magnification was approximately \(\times200k\). The tilt series was automatically collected by using the home-coded software. The compensation for the specimen position was carried out at a CCD magnification of \(\times60k\). Figure 7 shows selected images from the tilt series. The sizes of the images in Figs. 7(a) and (b) are 512×512 and 1024×1024 pixels, respectively.

The relative specimen position along the \( x_I \)- and \( y_I \)-axes calculated from the settings of the image shift coils during the collection of each image is shown in Figs. 8(a) and (b). The relative specimen position along the \( z_I \)-axis calculated from the settings of the objective lens is shown in Fig. 8(c). These results indicate that the specimen position remained within approximately ±100 nm in the \( x_I \), \( y_I \), and \( z_I \)-directions during collection.

VI. CONCLUSION AND FUTURE PROSPECTS

We have modified the goniometer controller of a conventional TEM and developed software to automatically collect the tilt series of rod-shaped specimens over an angular range from \(−90^\circ\) to \(+90^\circ\). With our method, the specimen position remained within approximately ±100 nm in the \( x_I \), \( y_I \), and \( z_I \) directions during the collection.

Because of the slow communication between the TEM and external computer, the collection of one whole tilt series takes approximately 2.5 h. However, the sequence of the collection procedure leads to achieve a collection of the tilt series.

Accurate image alignment before 3D reconstruction is important for TEM tomography. For fully automatic tomography, the alignment after collection must also be automated. This is now under development.

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