Method to separate markers’ area from observing area for rod-shaped specimen

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Transmission electron microscope (TEM) tomography is one of the most powerful techniques available for the visualization of the 3D structures of materials. A recent modification to the TEM specimen holder and the fabrication of rod-shaped specimens using a focused ion beam have made it possible to obtain a tilt series with an angular range of 180°. Therefore, it has become possible to reconstruct 3D images without missing wedge. Moreover, it is important to accurately alignment of the tilt series before computing the 3D reconstructions. The fiducial marker method is a typical, accurate alignment method that uses gold particles as markers. Usually, markers are placed by putting a solution containing the markers on the specimen. However, it is difficult to disperse markers suitably on the specimen. In an extreme case, no markers might be placed on the observing area. If markers can be placed on the area, they often cannot be identified because of the contrast of the specimen, and this makes it difficult to select the position of the markers. To overcome these problems, we developed an efficient method for placing markers on a rod-shaped specimen in a vacuum vessel. In the developed method, markers can only be placed on the carbon layer that is deposited to protect against ion beam irradiation. This method was demonstrated by using a high-density Au/SiO₂ nanocomposite material. The outline of this method is as follows:

1. A carbon layer is deposited on the surface of the bulk specimen in a focused ion beam system.
2. A cubic specimen is trimmed from the bulk.
3. The cubic specimen is fixed on the top of an interchangeable end tip of a specimen holder (Gatan, model 912J).
4. The cubic specimen is fabricated into a rod-shaped specimen and a mask, as shown in fig. 1.
5. The end tip is placed in a vacuum vessel, as shown in fig. 2. The relative positions of the end tip and the gold source in the vessel are determined from the relative positions of the mask and the rod-shaped specimen.
6. The gold particles (markers) are placed on the carbon layer of the rod-shaped specimen by heating the tungsten wire.

Selected TEM images of the collected tilt series are shown in fig. 3. The images indicate that almost all the markers were placed on the carbon layer. The reconstructed volume after the tilt series was aligned using the markers is shown in fig. 4. The result indicates that our method makes it possible to apply the fiducial marker method to a sample on which marker identification is difficult, such as the high-density nanocomposite material used in this study.
FIG. 1. Schematic illustration of fabrication of rod-shaped specimen and mask from cubic specimen.

FIG. 2. Experimental setup in vacuum vessel.

FIG. 3. Selected TEM images of the collected tilt series. θ is the rotational angle of the TEM specimen. (a) θ = -90°, (b) θ = 0°.

FIG. 4. Reconstructed volume after the tilt series was aligned using the makers on the carbon layer.