

Simple Method of Obtaining Replicas of Selected and Rather Wide Areas in Electron Microscopic Study

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Abstract

A simple method of obtaining replicas of selected areas of specimen surface is described. In the method, no specific apparatus other than an usual optical microscope is required as in Fourier's method, in addition, handlings involved are very simple. The method is based on an usual two-stage filmy replica technique, and to observe rather wide area the slit type specimen carrier is employed. The correct positioning of the replica on the specimen carrier is done with ease in a short time. The advantages, disadvantages and applications of the method are also discussed.

1. Introduction

In the investigation of surface patterns of specimens by electron microscope, it is desired to obtain the replicas of the same area previously observed by optical microscope and to obtain the successive replicas of the selected area. Therefore, several workers¹⁾ have developed various methods for these purposes. However, there exists some specific apparatus and needs some troublesome and skillful handling in order to position the replica film on the specimen carrier so as to fit the desired area in the replica film to the hole of the specimen carrier. Fourier²⁾ has developed a method, in which no apparatus other than an optical microscope is required and slit type specimen carrier is employed instead of the mesh type one. In his method, however, it is necessary to acquire skill in positioning the specimen carrier so that the desired area in

* A part of the work was presented in Japanese J. Appl. Phys. 1 (1962) 239,

the replica is centered in the slit in the specimen carrier, and in applying a drop of collodion between the specimen carrier and replica without deflecting the carrier.

The present technique, mainly based on a two-stage filmy replica technique and a technique pasting the two-stage replica on the specimen carrier with cement, both developed by Fukami,³⁾ was applied to an investigation of growth patterns of zinc-oxide crystal grown by chemical reaction of zinc-fluoride with air at elevated temperature.⁴⁾ In order to observe rather wide area and to investigate changes in pattern, the slit type specimen carrier was employed.

2. Procedures

The procedures are as follows.

(1) The first-stage replica of the specimen surface is prepared by acetylcellulose filmy replica system (in our case Borden R.F.A. film which is 0.034 mm thick was used), then, chrome oblique reinforced by carbon two-stage replica is made. A square sheet of about 2 mm is cut from this filmy replica so that the desired area is located at about the center of the sheet. Fig. 2 (a) illustrates a micrograph of replica of zinc-oxide crystal. Then, the sheet laid between two sheets of slide glass is heated at about 60°C for an hour to flatten it. Referring to Fig. 1, the objective of the lowest magnification B, which is covered with a metal sheath is available for positioning the replica on the specimen carrier. To the top surface of the metal sheath of the objective B, a small glass sheet D is fixed by cement, to which a square sheet of acetylcellulose film F steeped in methyl acetate, larger than the specimen carrier in size and 0.08 mm in thickness, is attached. The film F is fastened to the glass sheet by hardening process of acetylcellulose film. On the other hand, on a glass block P settled on the stage of the optical microscope, the two-stage replica prepared as above is placed as the carbon evaporated surface is faced upward. Also, on the glass block P an electron microscope specimen carrier G, which has four slits whose size are about 2×0.1 mm is placed with its reverse surface upwards. On the surface of the specimen carrier a very thin layer of Epoxy cement is spread previously. The objective A mounted on the revolver C is $\times 10$, and the overall magnification of the microscope is $\times 100$ in this case.

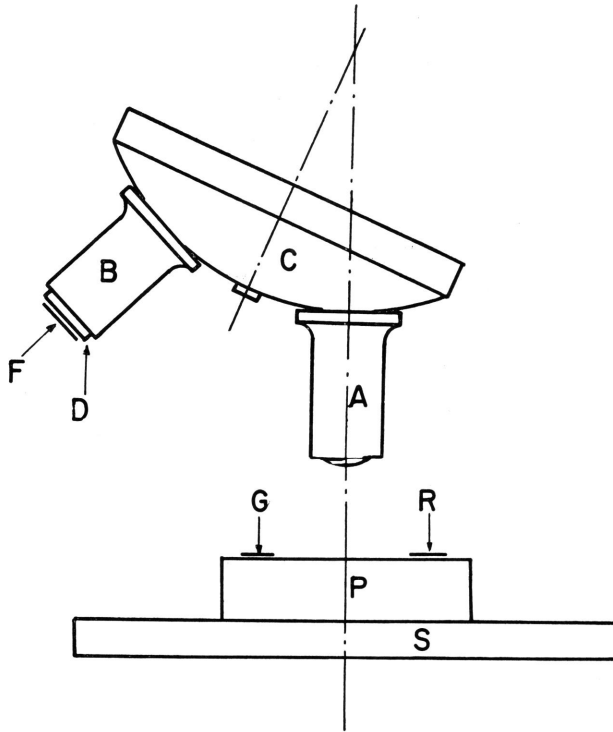


Fig. 1. Schematic arrangement of apparatus for positioning the replica on the specimen carrier. A, objective of $\times 10$; B, objective of $\times 6$; D, glass sheet; F, acetylcellulose film; G, specimen carrier; R, replica; P, glass block; S, microscope stage; C, revolver.

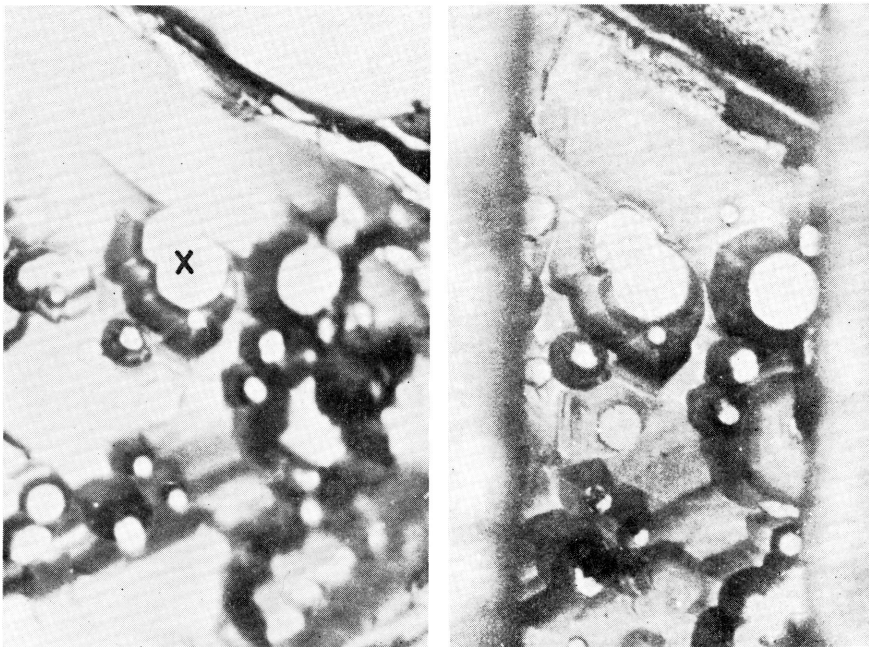


Fig. 2. Optical micrograph of the replica of the zinc-oxide crystal surface, (a) before and (b) after attaching to the specimen carrier.

(2) After these preparations, the microscope is focused on the specimen carrier using the objective A, and the stage is adjusted so that the central portion of one slit in the specimen carrier coincides with the cross-wire of the microscope. A small amount of methyl acetate is spread on the surface of the film F, the objective B is brought to the position of the optical axis of the microscope by turning the revolver. Next, the stage is raised until the carrier is attached to the film F, before methyl acetate has evaporated. After a few minutes, the stage is lowered. Then, the specimen carrier is left on the film F by adhesion due to small intrusion of the carrier into dissolved acetylcellulose film by methyl acetate. To do this, the portion of the upper surface of the glass block P where the specimen carrier is placed must be frosted. If it were not so, strong adhesion of the specimen carrier to the flat glass surface by cement spread on the surface of the carrier, will tend to prevent the carrier from separating from the glass surface, and this effect will tend to deflect the carrier from its initial location.

(3) The stage is adjusted so that the desired area in the replica film (for example, the mound on the surface of zinc-oxide crystal denoted by X in Fig.2 (a)) coincides with the cross-wire of the microscope using the objective A. The objective B is, then, brought to the position of the optical axis of the microscope. Again, the stage is raised until the replica is made slightly contact with the specimen carrier attached to the film F. After a few minutes, the stage is lowered. The objective B is dismantled from the revolver.

(4) By inserting a razor blade between the film F and the glass sheet, the film F with the specimen carrier is separated from the glass sheet. Then, by curling effect of the film F, the specimen carrier on which the replica is fastened is unartificially separated from the film F. Fig. 2 (b) illustrates a micrograph of the replica mounted on the specimen carrier by above technique. It is clear from the photograph that the mound in the replica pointed out in Fig. 2 (a) is centered in the slit in the specimen carrier. After the cement has become hard, the specimen carrier with the two-stage replica is steeped in methyl acetate reinforcing the carbon film by paraffin, as usual method. The acetylcellulose film is dissolved and the carbon replica is left on the specimen carrier without deflecting from its initial position during the dissolution process.

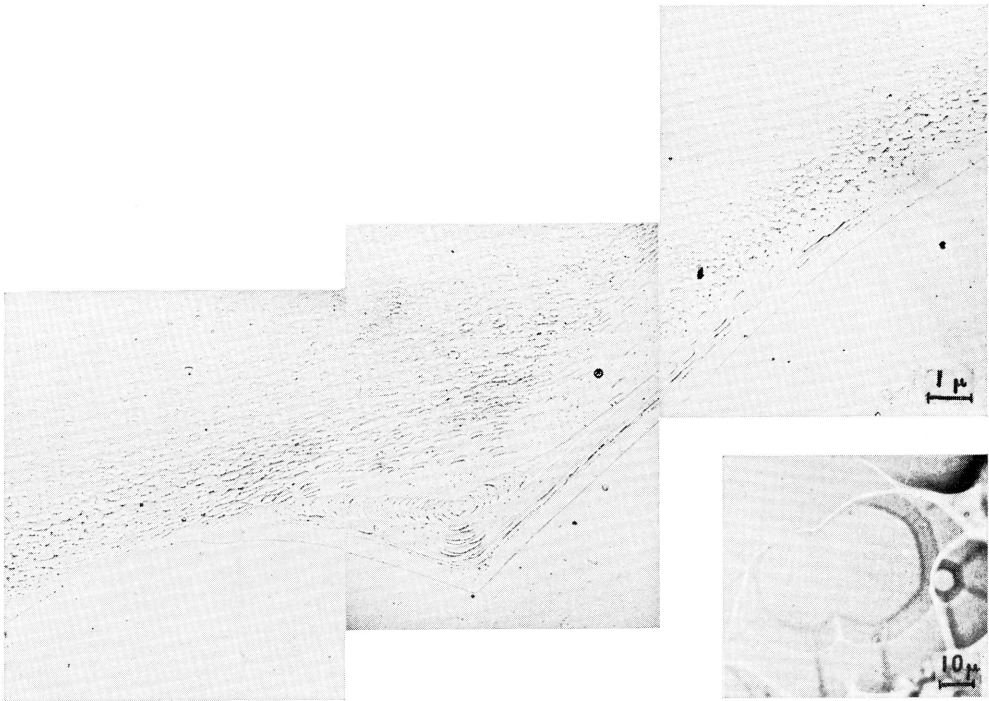


Fig. 3. Electron micrograph of the edge of the mound on the zinc-oxide crystal surface. The edge corresponds to the upper side of the mound in the optical micrograph shown on the lower right.

3. Discussions

The main advantages in the present technique are as follows. First, it requires no specific apparatus other than an usual optical microscope. Second, the handlings involved in the present technique are mainly rotating the screws of the microscope to translate the stage in horizontal and vertical directions, and hence, no skill is required. Moreover, the moving direction of the microscope stage in vertical direction is not necessarily parallel to the optical axis of the microscope, because attaching process of the replica to the specimen carrier is made using the same objective B at the same position as in the process of fastening the carrier to the film F.

The electron micrograph of the mound on the surface of zinc-oxide crystal denoted by X in Fig. 2 (a) is shown in Fig. 3. In the photograph changes in the growth pattern and correlations among them are well recognized. Details of the growth pattern of zinc-oxide crystal will be published in future. In this photograph, however, a few rumples in the replica film is recognized. This fact and the other that the carbon film must be thicker than in usual

case employing the mesh type specimen carrier in order to avoid tearing the carbon film, may be the two predominant defects in the case employing the slit type specimen carrier.

It was shown that the coincidence of the desired area in the replica with the central portion of the slit in the specimen carrier was very well, as seen from Fig. 2. Therefore, the present technique will be applicable to the case employing the mesh type specimen carrier, and this fact will relieve the weakness involved in our technique that the carbon film must be thicker. Finally, the method may be applicable to replicate successively the same area identified by fine scratches.

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