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Thin graphite support films for high resolution electron microscopy

SUMIO IIJIMA

Department of Physics, Arizona State University, Tempe, Arizona 85281, U.S.A.

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A method is described for preparing graphite films down to about 2nm in thickness. First, a suspension of small flakes in ethylene dichloride is prepared by repeated cleavage of a graphite crystal. This is then used to prepare a relatively thin film of graphite which is applied to grids previously coated with a holey carbon film. Fragments of tungsten oxide crystals are placed on the grid which is then exposed in the electron microscope to an electron beam of high intensity. As the result of an apparent reaction between the tungsten oxide and the carbon, the graphite film is etched, thereby producing a film which is both much thinner and cleaner than the original. The thickness of films prepared in this way has been estimated and their characteristics described. A brief account is given of the use of such a film using ferritin as a test object.

INTRODUCTION

Background 'noise' arising from amorphous carbon support films is a crucial problem for conventional high resolution transmission electron microscopy of non-crystalline objects such as single atoms, clusters of atoms and molecules. At a resolution of around 0.3nm carbon films show detail comparable with that sought from the specimen molecules even though they are prepared as thinly as possible, down to 2nm. Many people have been trying to overcome this problem, for example, by the use of sophisticated image processing by computers, but without convincingly successful results so far. Therefore in order to study the image formation of phase objects, the use of a 'noiseless' film is desirable.

For this purpose it is theoretically expected that if we use sufficiently thin single crystalline films of light atoms, the background noise can be eliminated. This has been attempted by a few workers (Hashimoto *et al.*, 1974; Mihama *et al.*, 1974; Moodie and Warble, 1974) who used single crystals of BeO, MgO or graphite for observing very fine metal particles, single atoms or gas molecules. Recently Johansen (1975) has reported a method to prepare thin graphite films which is different from our technique.

PREPARATION OF THIN GRAPHITE FILMS

First, relatively thin graphite films (from Union Carbide Corporation) were prepared by repeated cleavage of a graphite crystal between adhesive tape. Small flakes of cleaved graphite crystal stuck on the tapes were separated in ethylene dichloride. The suspension of the graphite flakes was centrifuged, the solvent decanted and fresh solvent added. This procedure was repeated several times to clean the graphite surface. The final suspension can be stored in a bottle. The flakes of graphite were then collected onto a holey carbon film by dipping a specimen grid into the suspension. Generally films prepared directly from these suspensions were found to be neither sufficiently thin nor clean enough for use as support films.

The next step was thinning in the electron microscope of the graphite films after they had been collected on the holey carbon films on the specimen grids. On the same grid, small pieces of WO_3 crystallites sitting on the graphite film were irradiated and decomposed with an intense electron beam. During the decomposition, the surface of the graphite film was etched, probably by the following chemical reaction: $WO_3 + C \rightarrow W$ (or WC) $+ CO_2 \uparrow$. This reaction reduces the thickness of the graphite films down to about 2nm, depending on the thickness of the initial film, and also cleans the surface of the film. The areas thinned are large enough for observing molecules or small particles up to about 20nm diameter. A disadvantage of this method is that small crystalline particles of W or WC are left on the films.

ESTIMATION OF THE THICKNESS OF THE FILM

Because of easy cleavage of the graphite crystal along the basal plane, it is rather difficult

to estimate the film thickness by the standard method of observing the thickness contour for a particular reflection appearing in a wedge-shaped edge of the film. The intensities of the diffracted beams are so sensitively dependent on crystal orientation that they may not be used to estimate the thickness unless the illuminating conditions are accurately known. Such an experiment is difficult because the films prepared by the present method are often curled and, furthermore, etched areas are not large enough to give reliable selected area diffraction patterns.

An alternative method of estimating the film thickness is to calculate the intensity of the transmitted beam against crystal thickness and to compare differences in contrast between a region of the crystal and that of the vacuum. Figure 1 shows the intensities for the (000) beam and some lower order reflections (solid lines) plotted against the crystal thickness for the case where the incident electron beam is exactly parallel to the (001) zone axis of the graphite crystal.* The calculations were also made for the cases where the incident beam is tilted by 1.5, 2.0 and 3.0° about the b axis. In Fig. 1 the intensities of the (000) beams for these tilts are shown by broken lines. For this calculation the multi-slice method for dynamical electron diffraction was used by taking into account 13 beams (Skarnulis, 1976). For the tilt angles which are less than 2.0° the (000) beam intensities do not differ appreciably up to a crystal thickness of about 4.5nm. This is the region of thickness in which we are interested. For crystals with a thickness of over 4.5nm it will be necessary to have precise knowledge of the crystal orientation. In this calculation the effect of the absorption of the electron wave was ignored. If there is some absorption resulting from thermal motion of the atoms, the intensity of the (000) beam will decrease. Some of the thermally scattered electron waves, however, can pass through the objective aperture, increasing the image intensity by an amount depending on the size of the aperture. In other words, the error in this method due to the absorption effect can be reduced to some extent. While keeping in mind the possible errors mentioned above, the calculated intensity for the (000) beam can be directly compared with that of the experimental image.

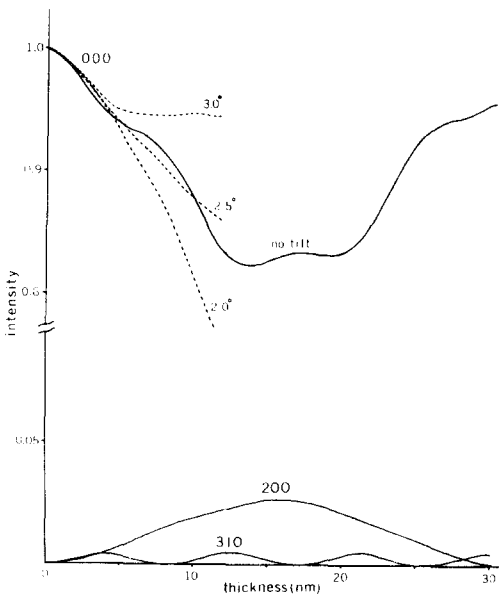

Fig. 1. Calculated intensities for the (000) beam and some reflections through a graphite crystal against crystal thickness. Dotted lines show intensities for the (000) beam in cases where the incident beam is slightly tilted from the (001) zone axis. Tilt angles are indicated on each line. For a crystal with a thickness of less than 5nm, crystal thickness can be estimated by measuring the decrease in contrast of the image of the film.

*We take an orthorhombic cell for the crystal structure of graphite instead of a hexagonal cell.

OBSERVATIONS OF THE GRAPHITE FILM

Figure 2 shows through-focus images of an edge region of a single crystal film of graphite. The images were recorded at 100kV by using only the transmitted beam selected by an objective aperture with a radius of 4.4nm^{-1}. Amounts of defocus are (a) 42.5nm, (b) −25nm, (c) −92.5nm and (d) −160nm. The edge of the film is seen near the bottom of each image (running horizontally), giving characteristic edge images due to defocusing of the objective lens.

First of all it is quite obvious, as we expected, that there is essentially no noise in any of the images compared with that shown by thin carbon films prepared by the usual process of evaporation. The direct magnification of the images was 400,000. At this magnification the

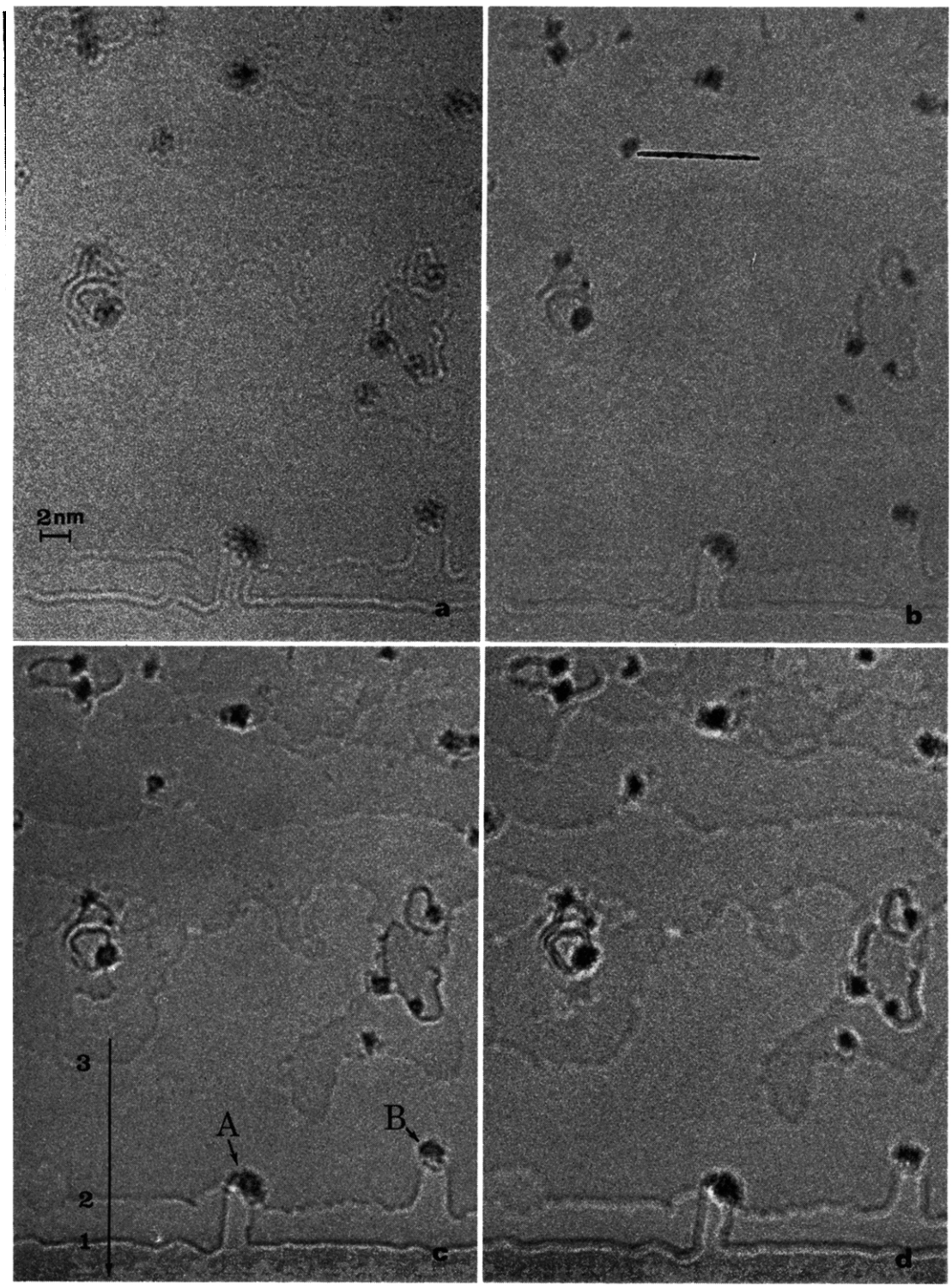

Fig. 2. Through-focus images of an edge region of a graphite film. Amounts of defocus are (a) +4.25nm, (b) −25nm, (d) −92.5nm and (e) −160nm. The images were recorded by using (000) beam. The edge of the film (shown by 1) is seen near the bottom of the images. Contour lines running nearly horizontally are surface steps with a few atomic layers of carbon atoms. The dark particles are probably metallic tungsten. ×2,500,000.

granularity of the photographic emulsion, which was measured to be 2–5μm dia. for our photographic plate (Kodak Electron Image Plate), corresponds to a resolution 0.02–0.05nm, so that the background noise arising from the photographic emulsion becomes negligible for work with a practical resolution of 0.3–0.4nm.

The dark particles with sizes of less than 2nm are probably metallic tungsten which have been produced by reduction of the WO_3 crystals during etching the surface of the graphite. The appearance of the particle indicated by A in Fig. 2c, which corresponds to an optimum focus image, suggests that it has reacted with the graphite to erode a part of the film. A similar particle indicated by B has eroded only a part of the layers. A schematic drawing of the surface morphology of the edge of this film is shown in Fig. 3 where the number of layers does not represent an actual thickness. From the sizes of these particles (2nm dia.) we can estimate the film thickness near the edge of the crystal to be approximately 2nm. Other black particles are also found at some boundaries.

The boundaries become more distinct in the images taken at large underfocus of the objective lens, while in the image at −25nm defocus they can hardly be distinguished, suggesting that the image contrast of the boundaries arises from

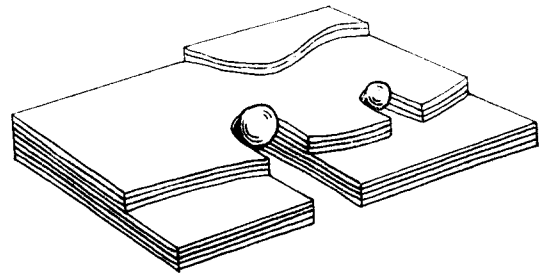

Fig. 3. A schematic drawing of the surface morphology of the film shown in Fig. 3.

phase contrast. It is a reasonable assumption, therefore, that these boundaries represent steps possibly of only a few atomic layers on the surface of the graphite crystal. The visibility of the fringe images at the boundaries differs at the positions indicated by 1, 2 and 3 in Fig. 2c, indicating that the heights of the steps differ (see Fig. 4). It is of interest to know whether the heights of some steps may correspond to a monoatomic layer of carbon atoms. The analysis of such image contrast can be made in terms of the imaging theory for thin objects and will serve to provide estimates of the heights of the steps. This will be discussed elsewhere (Iijima, 1977).

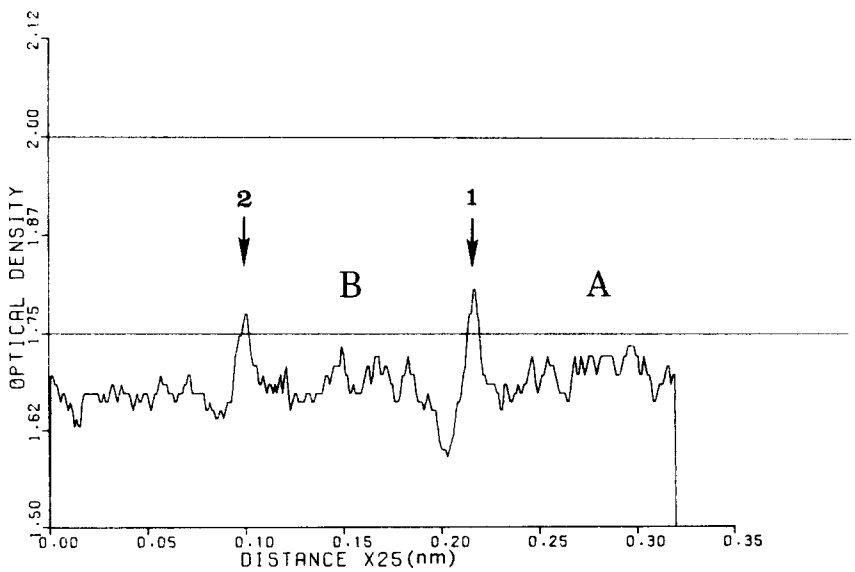

Fig. 4. Densitometer trace along the numbered line shown in Fig. 2c. Arrows 1 and 2 correspond to the positions of the edge of the film and a step respectively. 2% of change in contrast between regions A and B suggests that the thickness of the film is approximately 2nm according to the calculated intensity curves in Fig. 1.

Figure 4 shows a densitometer trace for a scan along the line indicated in Fig. 2c. The region A represents the intensity of the incident beam without passing through the film. Region B represents the intensities of the (000) beam which has passed through films with different thicknesses. The mean intensity at B was compared with the one at A to give the ratio B/A as 2%. By referring to the intensity calculations of the (000) beam in Fig. 1, this value indicates that the thickness of the film at B is 2nm thus the height of the step at the position indicated by 2 in Fig. 2c should be less than 2nm. If it was 1nm, the height of the steps would correspond roughly to three graphite layers. These thicknesses agree roughly with what we estimated from the sizes of the tungsten particles. Here we do not intend to give an accurate measurement of the thickness of the film. The values obtained are sufficiently accurate to provide a general indication of the thickness range of the areas used as support films.

Finally, as an example of the application of thin graphite support films for biological work a bright field image of unstained ferritin molecules supported on such a film is shown in Fig. 5. The image was recorded at 100kV with a defocus of 92.5nm which is the optimum focus for our electron microscope. Apoferritin, that is the spherical shell of protein surrounding the iron-oxide core, is clearly visualized. The diameter of the spherical shell is approximately 5–8nm. Although a resolution of 0.3–0.4nm was obtained in this image, a discussion of the detailed structure of the apoferritin may not be meaningful because of the damage which may have been caused to the protein by the intense electron beam. In fact we noticed that the apoferritin disappeared after prolonged irradiation, leaving the iron-oxide cones. Apart from the problem of radiation damage, it is interesting to note that the contrast obtained in the region of the apoferritin was similar to that of carbon films formed by the usual process of evaporation so that if the latter were used, the background noise of the support film would probably obscure details of the image of the actual specimen. From this preliminary experiment we may conclude that the image contrast of the unstained protein seems to be sufficient even in a bright field image and the only problem is that of radiation damage. The latter may possibly be overcome by a development of the minimum exposure technique.

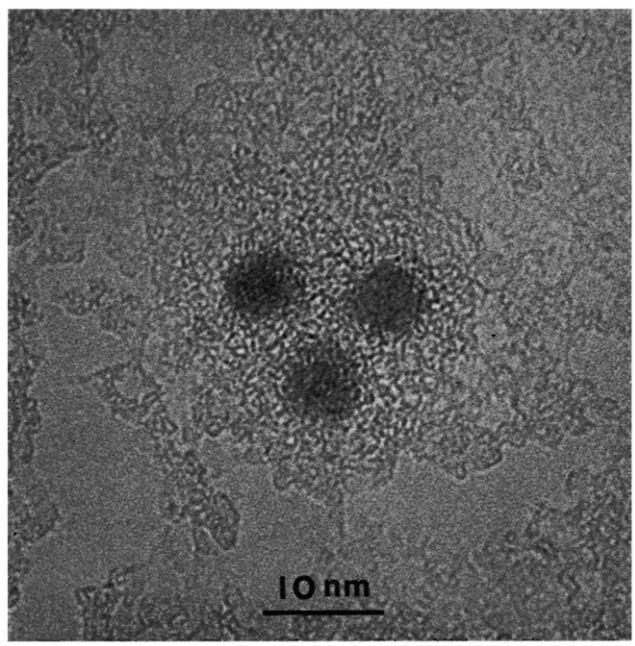

Fig. 5. A bright field image of unstained ferritin molecules supported by a thin graphite film. ×1,500,000.

The implications of utilizing the noiseless films described in this paper are important for high resolution electron microscopy and there may well be applications in many areas of materials science and biology.

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