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A Technique of Sample Preparation for Petrographic Investigation by Electron Microscopy

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Abstract

A technique is described for the application of transmission electron microscopy to petrographic investigations. Polished and etched thin sections are the basis for acetate peels which are replicated for electron microscopy by the two-stage (acetate peel—carbon film) method. Photomicrographs are made of the acetate peel and of the mounted carbon film replica. In addition, lettered 'finder' grids (specimen holders) are used as replica mounts. This system makes possible nearly continuous visual correlation between observations of the thin section by polarizing microscopy and observations of the carbon film replica by electron microscopy. The observer may thus bring both optical criteria and high resolving power to bear on the same object (*e.g.*, a single crystal or a textural pattern). The stages of increased resolution and detail obtained with this technique are illustrated for a vitric clast in lunar sample 12073 and for a zeolitized glass shard from an ash-flow tuff.

Introduction

The transmission electron microscope (TEM) offers unexcelled ability to resolve petrographic and mineralogic textures. The image in the TEM is produced by a focused electron beam which passes through the specimen and onto a fluorescent screen. Because the penetrative power of the electron beam is low, it is necessary to prepare ultrathin sections of rock or to make thin metal film replicas of the rock surface.

Ultrathin sections are commonly prepared in two ways. Either a mineral is crushed and cleavage flakes mounted for observation, or else a small, thin core (2–3 mm across) is further thinned by ion bombardment. In both these methods, only a small area of a mineral or rock is prepared. Ultrathin sections have an advantage over replicas in that, since the actual rock or mineral is observed, an electron diffraction photograph may be obtained as well as an electron micrograph. Most geological applications of the TEM have used ultrathin sections and have been confined to the field of mineralogy-crystallography.

Metal film replicas can be made directly from a rock, in which case the rock must be dissolved away to free the replica, or else from an acetate peel of the rock surface, in which case the acetate peel must be dissolved away. Since the rock or mineral specimen itself is not observed in the TEM in this method, mineral identification must be made from visual clues such as morphology of grains, etch characteristics, *etc.* In spite of the possible difficulty of mineral identification, metal-film replicas yield a wealth of textural information which is not revealed by other means. Additionally, a large area of the rock surface is replicated in one step. We believe that metal film replicas, based on acetate peels, offer the best and most convenient method for petrographic investigations by TEM.

Fischer, Honjo, and Garrison (1967), using replicas, demonstrated the great potential of the TEM as a petrographic tool in their study of homogeneous limestones. We introduce here a technique of procedure and sample preparation that is similar to the one used by Fischer *et al*, but is more flexible and may be adapted to rocks of heterogeneous compositions and textures. We hope that this new technique will lead to more widespread application of the TEM in petrographic studies.

Technique

The technique is based on the two-stage replication procedure (Bradley, 1961) as adapted by Honjo and Fischer (1965) and applied by Fischer *et al* (1967). In outline, their steps are:

1. Embedding a rock chip in an epoxide plug.
2. Grinding and polishing a surface on the embedded rock chip.
3. Light etching in acid of the polished surface.

4. Making an acetate peel of the etched surface.
5. Shadowing the acetate peel in a vacuum evaporator with chromium.
6. Backing the shadowed peel in a vacuum evaporator with a layer of carbon.
7. Cutting the replica into squares (about 2 × 2 mm) of a size to fit on TEM specimen holders.
8. Dissolving the acetate peel backing with acetone.
9. Capturing the carbon film replicas on TEM specimen holders.

The advantage of their technique is that very fine textural detail is brought out by the polishing and etching process and is faithfully reproduced by the acetate peel and in turn by the chromium-carbon film. Additionally, several peels can be made from the same rock surface, and the rock itself is not lost. A drawback of the technique is that it uses rock chips embedded in plugs. The rock is therefore removed from the context of a thin section upon which standard mineralogical determinations can be made with the polarizing microscope. The problem is compounded by the limited field of view of the TEM which is on the order of 50 μm at lowest magnification. Because of these limitations, it may be extremely difficult to find a particular object, to identify it once found, or to apprehend textural patterns larger than a few hundreds of microns.

With texturally or compositionally heterogeneous rocks, it is desirable to have complete and continuous visual correlation between what can be seen in the polarizing microscope and what can be seen in the TEM. In the technique being introduced, polished thin sections are used instead of plugs as a basis for acetate peels. In addition, special lettered "finder" grids (Fig. 3a) are used as specimen holders. Finally, photographs are made at several stages in the replication. It is then possible for the observer to study by TEM a specific object that he may have noticed in a thin section. Conversely, he can refer to more familiar optical means to identify an object that he may have noticed in the TEM image.

Figures 1–5 show several stages in the replication for electron microscopy of a vitric clast from Lunar sample 12073, Apollo 12. Note how a single object, a vitric clast in this case, can be studied both by polarizing and electron microscopy.

The necessary steps of the technique are given in the following outline.

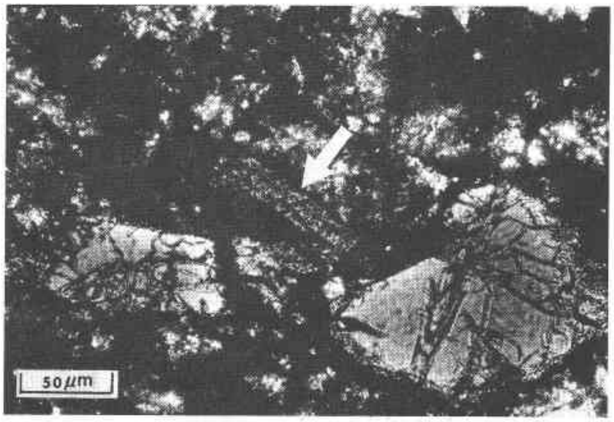


FIG. 1. Optical photomicrograph of polished thin section of a vitric clast (arrow) in lunar breccia 12073.

Outline of Technique

1. Preparation of uncovered thin section.
2. Polishing of uncovered thin section.
3. Light etching of polished thin section by acid.
4. Preparation of acetate peel of the etched surface.
5. Chromium shadowing and carbon backing of the acetate peel in a vacuum evaporator.
6. Cutting of shadowed and carbon-backed peel into strips about 2.5 mm wide.
7. Photomicroscopy at low power of acetate strips.
8. Cutting the strips into 2.5 × 2 mm rectangles.
9. Dissolving acetate backing from rectangles and mounting metal film replicas on TEM, lettered copper "finder" grids (specimen holders).
10. Cursory examination of mounted replicas in the TEM.

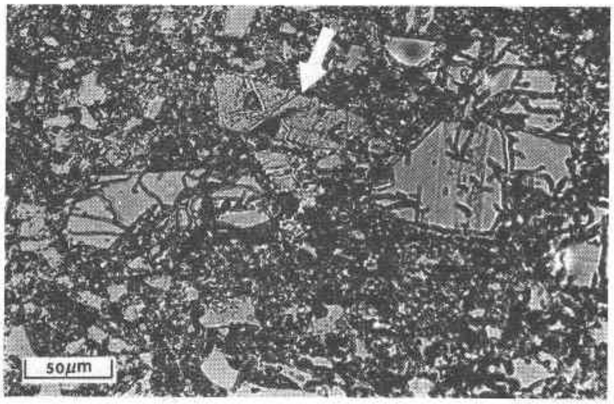
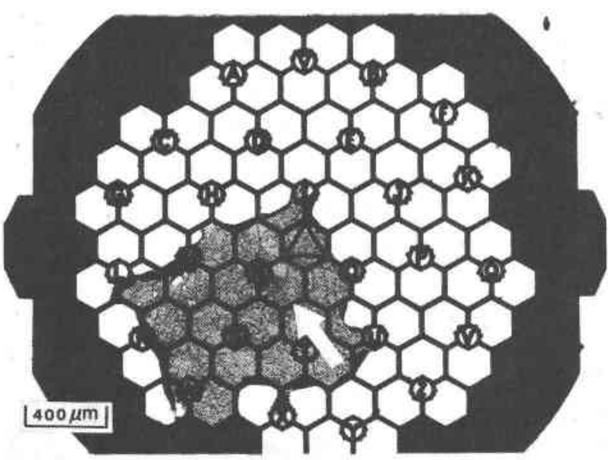


FIG. 2. Optical photomicrograph of an acetate peel of the thin section in Figure 1. Note the increased detail shown in the clast (arrow) at approximately the same magnification.



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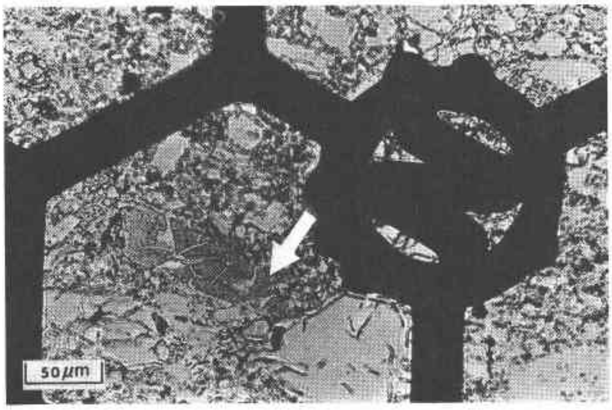

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FIG. 3. The carbon film obtained from the acetate peel is here mounted on the lettered copper "finder" grid (specimen holder). In 3a the clast is in the compartment indicated by the arrow but cannot be identified in this picture. Figure 3b shows a view of the clast at higher magnification. The area indicated by an arrow is enlarged by electron microscopy in Figures 4 and 5.

11. Photography of mounted replicas in the light microscope. These photographs (steps 7 & 11) are kept with the operator to refer to as he works.

Discussion of Steps

1. *Preparation of uncovered thin section*

The section must be somewhat thicker than normal (about 50 microns) to withstand the rigors of polishing, etching, and the preparation of an acetate peel (Fig. 1). Epoxy (*e.g.,* Scotchcast #3 resin) is used as both a mounting and impregnating medium since it withstands the effects of acids and acetone

FIG. 4. The electron microscope reveals details of the fractures in the clast and of the matrix surrounding it. The area indicated by the arrow is shown in Figure 5.

better than does Lakeside. Preparation of uncovered thin sections, mounted and impregnated with epoxy, is a routine matter for most commercial lapidaries.

2. *Polishing of uncovered thin section*

We polish sections on a Buehler lap with Petrothin polishing attachment.[1] This affords close control over the polishing process and enables repolishing of sections as desired. For a description of polishing technique and equipment, see Woodbury and Vogel

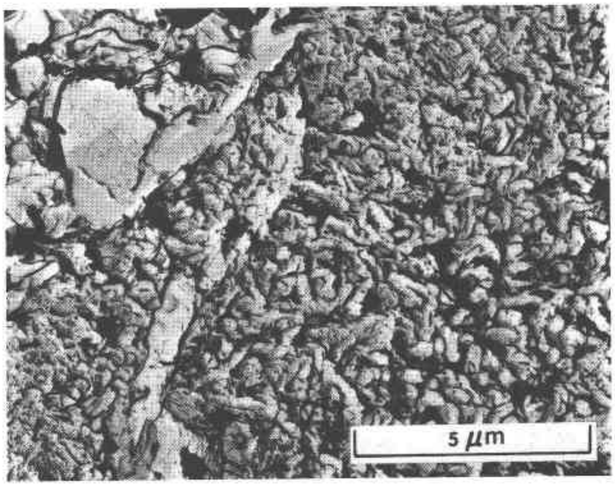


FIG. 5. Enlargement of area in Figure 4 reveals closely fitted glass shards as the major constituents of the clast. Compare with Figure 1 and note the increase in detail.

[1] Buehler Ltd., 2120 Greenwood St., Evanston, Illinois.

(1971). Final polishing with 0.3 micron alpha Alumina (Buehler Ltd.) is adequate for most purposes.

3. *Etching of the polished thin section*

Depending on its composition, the polished thin section is etched with either HCl or HF. It is impossible to give a formula of acid concentrations and etch times that will cover every situation of mineralogy, grain size, and objectives. For fine-grained carbonates, etching in 0.05 N HCl for about 45 seconds is average. For siliceous rocks, the section is held in fumes 6 to 8 mm above concentrated (50 percent) HF solution for 10 to 30 seconds, then rinsed. Only a portion of the section is etched—usually an area about 1 × 1.5 cm. This is accomplished in the case of HCl by swabbing only that area, or in the case of HF by making a template out of plastic.

Too much etching will spoil fine textural details and cause too much relief of the sample surface. The excess relief results in poor acetate peels and rupturing of the carbon film replica. Too much etching also may cause the thin section to become unglued during removal of the acetate peel.

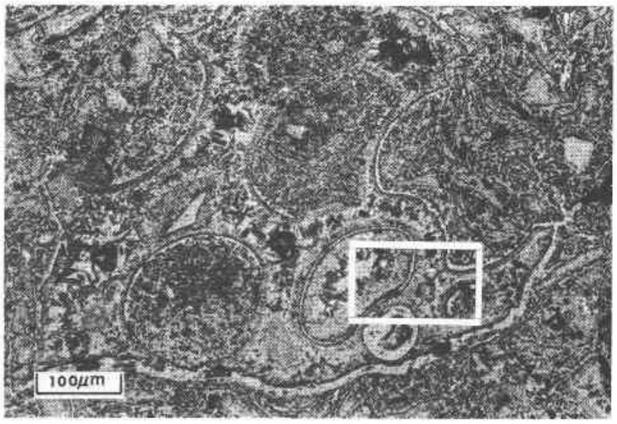
4. *Preparation of acetate peel of the polished and etched thin section*

Peels of the etched area are made with high quality acetate film available from suppliers of electron microscopy accessories.[2] Reagent grade acetone is used in preference to replicating fluid since washing of the replicas at a later stage is thereby easier.

Two or three drops of acetone are put on the etched area of the thin section, the acetate laid over and pressed down firmly with the thumb. The peel can be removed in twenty minutes. Several peels can be made from the same surface.

The finished peel is mounted on a glass slide by taping the edges. If the peel is for storage or study with the light microscope, it is mounted smooth side up; if for replication, it is mounted smooth side down. One peel should be left on the thin section to protect the etched surface and act as a cover glass to improve the image when the section is examined by optical microscope. These peels provide a wealth of textural information in their own right (*cf*, Figs. 2 and 6).

[2] E. F. Fullam, Inc., P.O. Box 444, Schenectedy, N.Y. 12301 or Ladd Research Industries, Inc., P.O. Box 901, Burlington, Vermont 05401.



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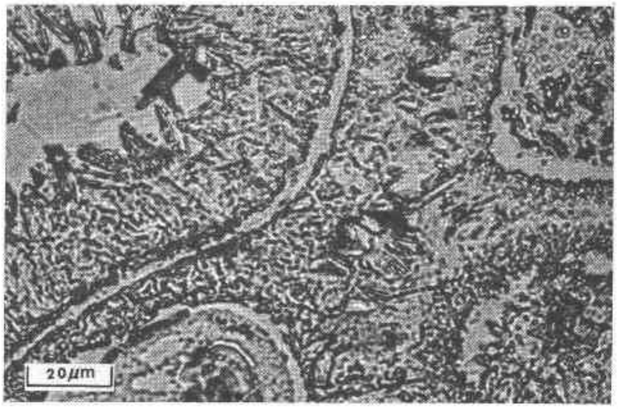

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FIG. 6. Optical micrographs of an acetate peel of a zeolitized shard from an ash-flow tuff, Palo Verde Mountains, California. Figure 6a shows a large shard which has been pseudomorphed by the zeolite, clinoptilolite. The kidney-shaped form, partly boxed, is a bubble within the shard. The boxed area in Figure 6a is enlarged in Figure 6b and is shown in a TEM mosaic micrograph in Figure 7.

5. *Replication of the acetate peel by chromium and carbon*

A peel is mounted on a glass slide and placed in a vacuum evaporator. A 3-nm thickness of chromium is evaporated at an angle of about 30 degrees. This provides a shadowing effect to help bring out relief. Carbon is evaporated straight down (90 degrees) to produce a film about 20 nm thick. See Bradley (1961) for details of the metal evaporation procedure.

6. *Cutting of replicated peel into strips*

The chromium-shadowed and carbon-backed peel is examined for quality and content under the light microscope. If the peel has no obvious defects, it is removed from the glass slide and all tape and excess acetate trimmed off with scissors. The peel is then cut into strips about 2.5 mm wide. If particular objects have been noted for study, the cuts are made so that these objects lie in the center of the width of the strips. The strips are examined for content under the light microscope. It is important at this stage to limit the number of strips to be processed since the next few operations are time-consuming.

7. *Photography of acetate strips*

Individual strips are numbered for reference and photographed in their entirety at low magnifications (Fig. 2). The object of photography at this stage is to forge a visual link between the final mounted metal film replicas and the original polished thin section. Entire strips are photographed since the larger the area represented, the easier it is to match it to the thin section. At a later stage, the mounted replicas will also be photographed (step 11). Thus a visual chain is forged to connect: thin section (step 1)—acetate peel (step 7)—mounted replica (step 11)—TEM image (step 12).

8. *Cutting acetate strips into rectangles*

The photographed acetate strips are cut with scissors into rectangles 2 × 2.5 mm (approximately). Cuts are made so that objects for study are near the center of the rectangles. The rectangles are examined under the light microscope for content and promising ones selected for mounting.

9. *Dissolving acetate backing from rectangles and mounting metal film replicas on TEM, lettered copper "finder" grids (specimen holders)*

The simplest method of mounting specimens is that described by Honjo and Fischer (1965). The acetate rectangle is immersed, metal-coated side up, in an acetone bath. In a few minutes, hastened by agitation, the metal film replica will come free of the acetate backing. Using a specimen holder held in tweezers, the replica is transferred to a series of two more acetone baths, sitting for 10 minutes in each.

From the last acetone bath, the replica is ready to be mounted on a special lettered copper "finder" grid. The grids measure 3.0 mm across and have built in letters so that each grid opening can be positively identified (see Fig. 3). They are available from E. F. Fullam, Inc. Mounting is a tricky operation and requires some skill and much patience. The problem is that the metal film replica usually curls up at the bottom of the acetone bath. The goal is to fish out the replica and have it lie flat near the center of the grid. Repeated manipulation of the replica in and out of the acetone with a plucking motion will usually cause the replica to uncurl and lie flat on the grid. If the rectangle is nearly equant and the carbon film relatively thick, the replica tends to uncurl more easily.

If all efforts fail to uncurl the replica, another technique may be resorted to. The replica is plunged into a bath consisting of 1:3 ratio of acetone and water. Differential surface tension between the two solutions will cause the replica to snap open and float on the surface. It can then be mounted on a lettered "finder" grid. A disadvantage of this second technique is the tendency of the replica to tear when it snaps open.

The mounted replica is dried by touching a Kimwipe tissue to the grid and also to the tweezers to soak up excess water or acetone. It can then be laid on a Kimwipe to finish drying.

10. *Cursory examination of mounted replicas in the TEM*

A replica is examined for quality in the TEM to reveal quickly whether the batch is worth processing further or discarding. The most common problems are poor quality metal film from faulty metal evaporation, and poor etch of the polished thin section. Grids with damaged or poor quality replicas can be recycled by cleaning with acetone in an ultrasonicator.

11. *Photography of replicas in the light microscope*

Mounted replicas are selected for quality and content and photographed at low magnification to include the entire grid/replica (Fig. 3a) and then at higher magnification to show the location of individual objects for study (Fig. 3b). These photographs represent the third link in the visual chain consisting of thin section—acetate peel—mounted replica—TEM image.

If these photographs show inadequate contrast, the mounted replicas should be examined under the light microscope in conjunction with photographs of the acetate strips. Study objects on the mounted replica are located by reference to their positions on the finder grid, and these locations are noted on the photographs of the strips. If this problem is anticipated, it is a good idea to photograph the 2 × 2.5 mm acetate rectangles in addition to, or instead of, the acetate strips. These photographs will show the actual segment to be mounted.

12. *Examination of replicas in the TEM*

The photographs of the metal film replica mounted on the finder grid are the TEM operator's roadmap. Photographs of the acetate strip or rectangle, with finder grid locations of study objects noted, may also serve as a roadmap (Fig. 6).

If the procedures outlined above have been followed successfully, the TEM operator should be able to relate objects on his viewing screen (Figs. 4, 5, and 7) to his photographs of the mounted replica, hence to his photographs of the acetate peel, and ultimately to the thin section.

Conclusion

The preceding is not a recipe in that it is not necessary to follow it rigidly to obtain results; it can be modified to fit specific needs. We wish to emphasize more what *can* rather than what *must* be done. The procedure described should satisfy the most rigorous requirements of the petrographer, *i.e.*, to examine by TEM any object that he may have noticed in a (polished) thin section. If objects are numerous and easily recognizable in the TEM, the worker may dispense with photography. The advant-

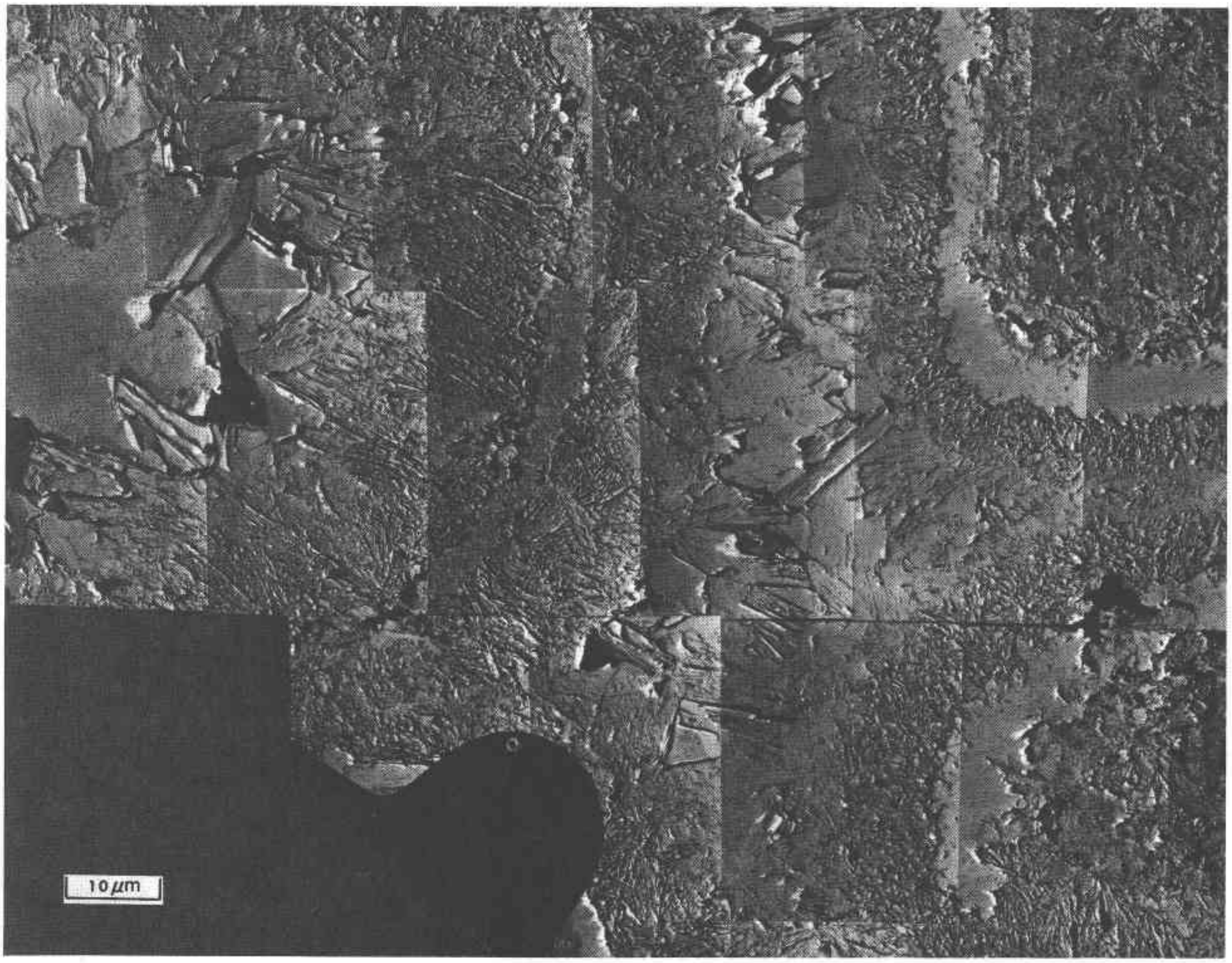

FIG. 7. A TEM mosaic micrograph of the area seen in Figure 6b. The tremendous increase in resolution reveals textural characteristics of the zeolite which has replaced the glass shard from the external margin (upper right) and from an interior bubble (center), and which has partially filled void space within a bubble (left center). The fibrous clinoptilolite coarsens to euhedral crystals which fill the interior of the shard (following solution of remaining glass) and which partially fill bubble void space. The flat material within the bubble and at the shard margin is impregnating epoxy; the band curving down to the left, outlining the bubble, is clay.

age of working from a thin section, as opposed to a rock chip, remains.

Acknowledgments

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