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CARBON REPLICA PREPARATION FOR ELECTRON MICROSCOPY

Walter T. Giba and Rolf H. Muller

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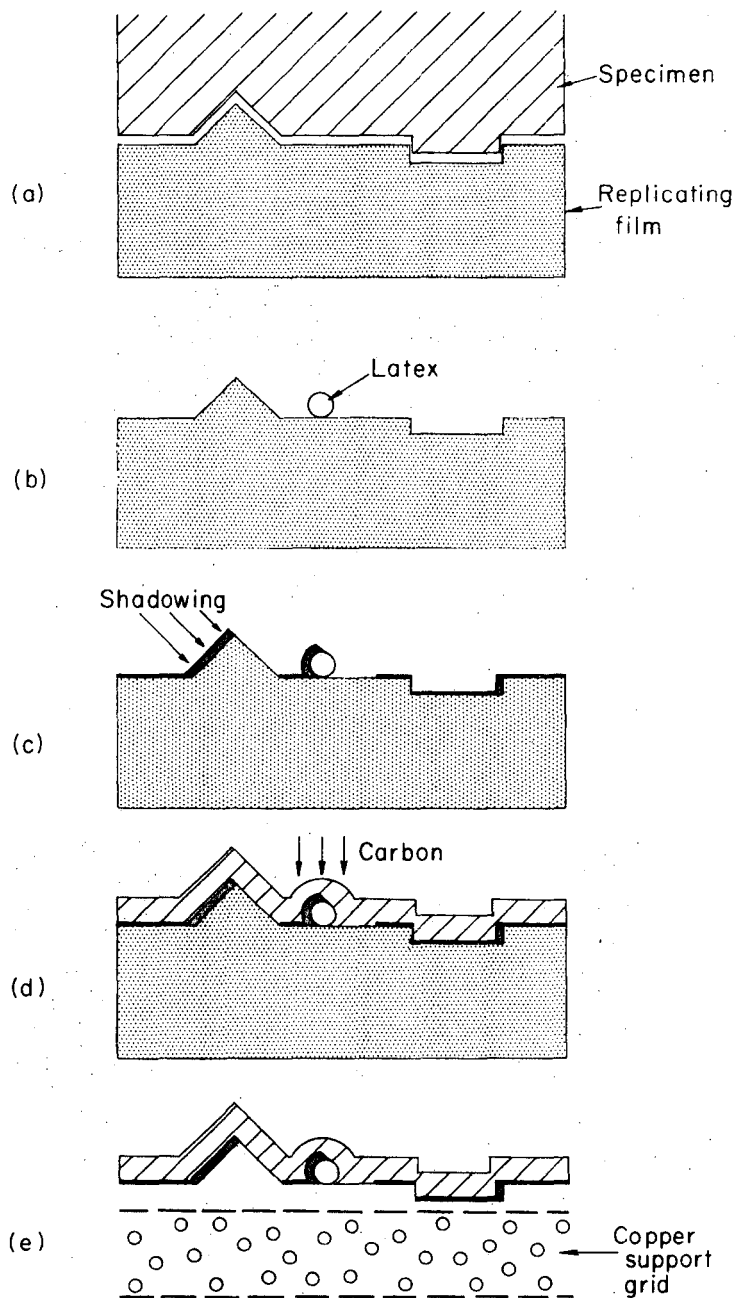
ABSTRACT

Detailed procedures are given for the preparation of two-step carbon replicas of metal surfaces for examination by transmission electron microscopy. The technique employs a plastic replicating film and is particularly suited for metal surfaces resulting from electrochemical dissolution.

Electron microscopy of surface replicas allows the detailed study of the topography of solid surfaces.¹ If the specimen is not to be destroyed during replication, a two step procedure² has to be employed.* The process described here has been developed for the study of copper surfaces which result from anodic dissolution in aqueous electrolytes at high rates. It is based on the use of a softened replicating film on which an imprint of the specimen surface is produced (Fig. 1). After hardening, the replicating film is separated from the specimen. As an internal standard, latex particles of known dimension are placed on the imprint which is then shadowed with an electron-absorbing material. In the second replication step, a uniform carbon film, which is transparent to the electron beam and stable under prolonged exposure to it, is deposited on the plastic imprint. This carbon replica is then separated from the plastic for examination in the electron microscope. The approximate resolution of replicas produced by a two step plastic-carbon technique has been given as 140\AA .^{2**} The manufacturer of the replicating plastic film used here claims a resolution of 100\AA .

* A non-destructive one step technique has recently been reported.³

** The corresponding figure for a one-step replicating process is 45\AA .



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Fig. 1. Principle of two-step replication process with a plastic replicating film. (a) imprint of specimen surface in plastic replicating film; (b) deposition of latex particles on imprint; (c) shadowing of imprint and latex particles; (d) deposition of carbon film; (e) dissolution of plastic film leaves carbon replica with shadowing on microscope support grid.

The principal difficulty of this particular replicating process lies in the proper choice of the replicating film and the method to dissolve it at the end. The film should be softened in a reproducible manner and separate well from the specimen. Except for very rough surfaces, these requirements are most easily satisfied with a thick film. On the other hand, a thin film is best suited to avoid warping and tearing of the carbon replica due to swelling of the plastic film during its dissolution. Best results have been obtained with Bioden R.F.A. Acetylcellulose - Paraffin Replicating Film* of 0.034 mm thickness.

Preparation of Imprints in Replicating Film (First Step Replica)

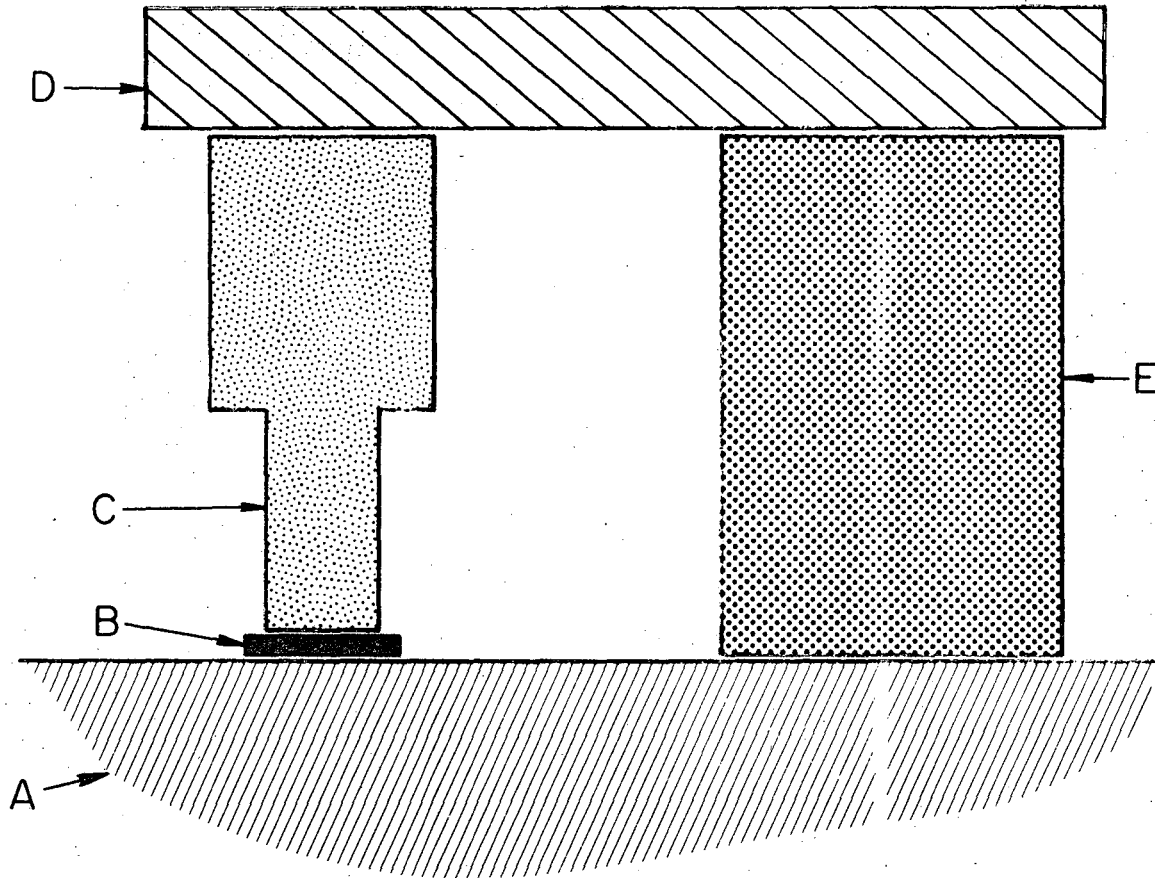
The following steps have been found to yield consistently good replicas of specimens which were 0.5x3 mm in size and embedded in cylindrical epoxy holders of 5 mm diameter.

1. Put a sheet of replicating film on a glass plate, partially cover with a clean microscope slide and cut into small squares (approximately 4x4 mm) with a scalpel. Avoid touching the film with fingers.

2. Put a drop of methyl acetate in the center of a square film piece with a small camel hair brush. The diameter of the drop should cover the area to be replicated. The amount of liquid has to be sufficient to soften the film surface, but should not completely penetrate the film.

3. Wait until the surface of the plastic film shows the characteristics of a glue, then press it on the specimen's surface (Fig. 2). If the film is too dry, no faithful imprint is obtained; if it is too soft, the film breaks.

* Oken-Shoji Co., Ltd., 5, 6-Chome Ginza Chuo-ku, Tokyo, Japan. (E.F. Fullam, Inc. P.O. Box 444, Schenectady, N. Y. 12301.)



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Fig. 2. Arrangement for making imprint in replicating film:
A - flat surface; B - replicating film (softened face up);
C - specimen (surface down); D - weight; E - gauge block of
same height as specimen.

4. Keep the film under pressure without motion for 5-10 minutes, then peel it off the specimen surface.

Mounting of Imprints

For the operation to follow, the plastic imprints must be firmly supported and held flat.

1. Prepare hollow, round edge punch slightly larger than area to be replicated (4 mm diam.).

2. Punch holes (up to 12) in a 3 in. section of 3/4 in. wide frosted cellophane tape stuck to a Teflon sheet.

3. Place a piece of replicating film with the imprint facing up on one end of a microscope slide (1x3 in.) and hold it down on its edges with the cellophane tape so that the imprint shows through the first hole in the tape.

4. Proceed with other pieces of plastic film centered under successive holes in the tape. (Fig. 3.)

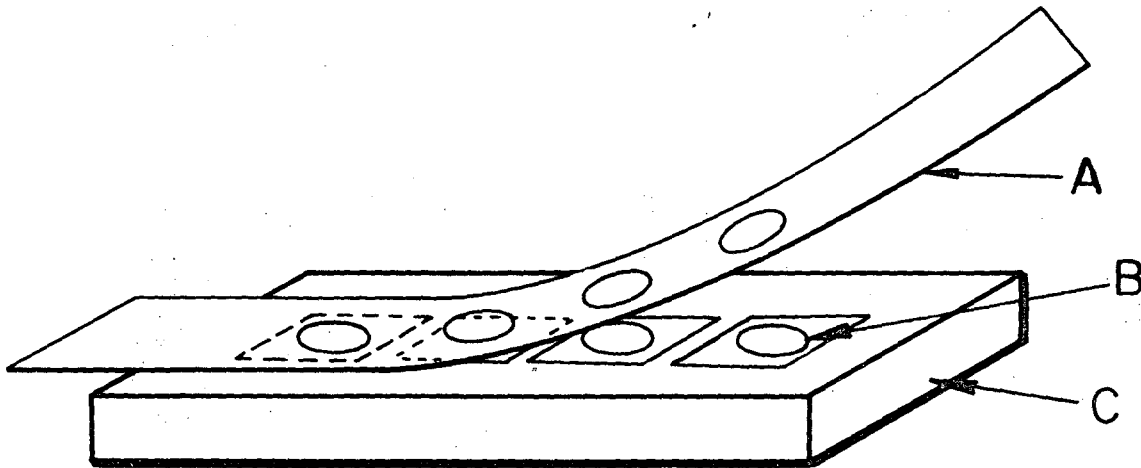
5. Shave off raised edges of imprints with scalpel blade.

Latex Deposition

As an internal standard of magnification and shadowing angle, polystyrene latex particles of known diameter* are deposited on the mounted imprints.

1. Dilute latex suspension with distilled water to 0.2% solid contents.

*Dow LS-063-A, $0.557 \pm 0.0108\mu$, 10% solids - other diameters available are: 1.86μ and 6 to 14μ .



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Fig. 3. Mounting of plastic imprints.
A - frosted cellophane tape with holes being lowered on
microscope slide with imprints
B - pieces of plastic replicating film with imprints
C - microscope glass slide

2. Fill perfume sprayer* with suspension and connect to dichloro-fluoromethane micro duster.**
3. Place mounted imprints vertically 15 in. from sprayer nozzle.
4. Operate sprayer for 30 sec. directed toward mounted imprints.

Tungsten Oxide Preparation

Tungsten oxide has been chosen as a shadowing material because of the low granularity of the deposit⁴ and the easy availability of the material. The tungsten oxide is prepared for each deposition by the oxidation of a tungsten wire in the following way.

1. Cut 0.13 in. diam. tungsten wire to length required (4-1/2 in.) for connection to binding posts in vacuum evaporator.***
2. Bend wire in the center to form a V-shape with sides approximately 1/4 in. long.
3. Connect one tungsten wire each to both sets of binding posts. Adjust copper connectors so that distance between them is 5 cm. Place V-shape in the center.
4. Evacuate evaporator chamber to 0.1 micron and clean each wire successively by heating with 40 amps. for 10 sec.
5. Fill evaporator chamber with air and oxidize tungsten wires by heating with 30 amps for 120 sec. each.

* An inhaler spray can also be used.

** Texwipe Co., Hilldale, New Jersey; nitrogen of 4 atm pressure can also be used.

*** Varian MIKROS model VE-10.

6. Remove oxide on straight part of tungsten wire with scalpel or knife, leave it in place at V-shaped part.

Shadowing and Carbon Film Preparation (Second Step Replica)

Deposition of the shadowing material (WO_2) at an oblique angle is primarily responsible for making the topography of the replica surface visible. Without breaking the vacuum in the evaporation chamber, a carbon film is deposited on the plastic imprint immediately after deposition of the shadowing material.

1. Prepare two 18 in. diam. carbon electrodes* with tips of 1 mm diam. and 5 mm length** each. Insert in spring-loaded electrode holder.*** Connect to one pair of binding posts in evaporator.

2. Install one tungsten wire with oxide coating on other pair of binding posts.

3. Place glass slide with mounted imprints in evaporator chamber so that it is 13 cm straight under the tips of the carbon electrodes, 10 cm and 45° from tungsten oxide source.**** (Fig. 4.)

4. Evacuate evaporator chamber to 0.02 microns.

5. Evaporate tungsten oxide by passing 35 amps for 10 seconds.

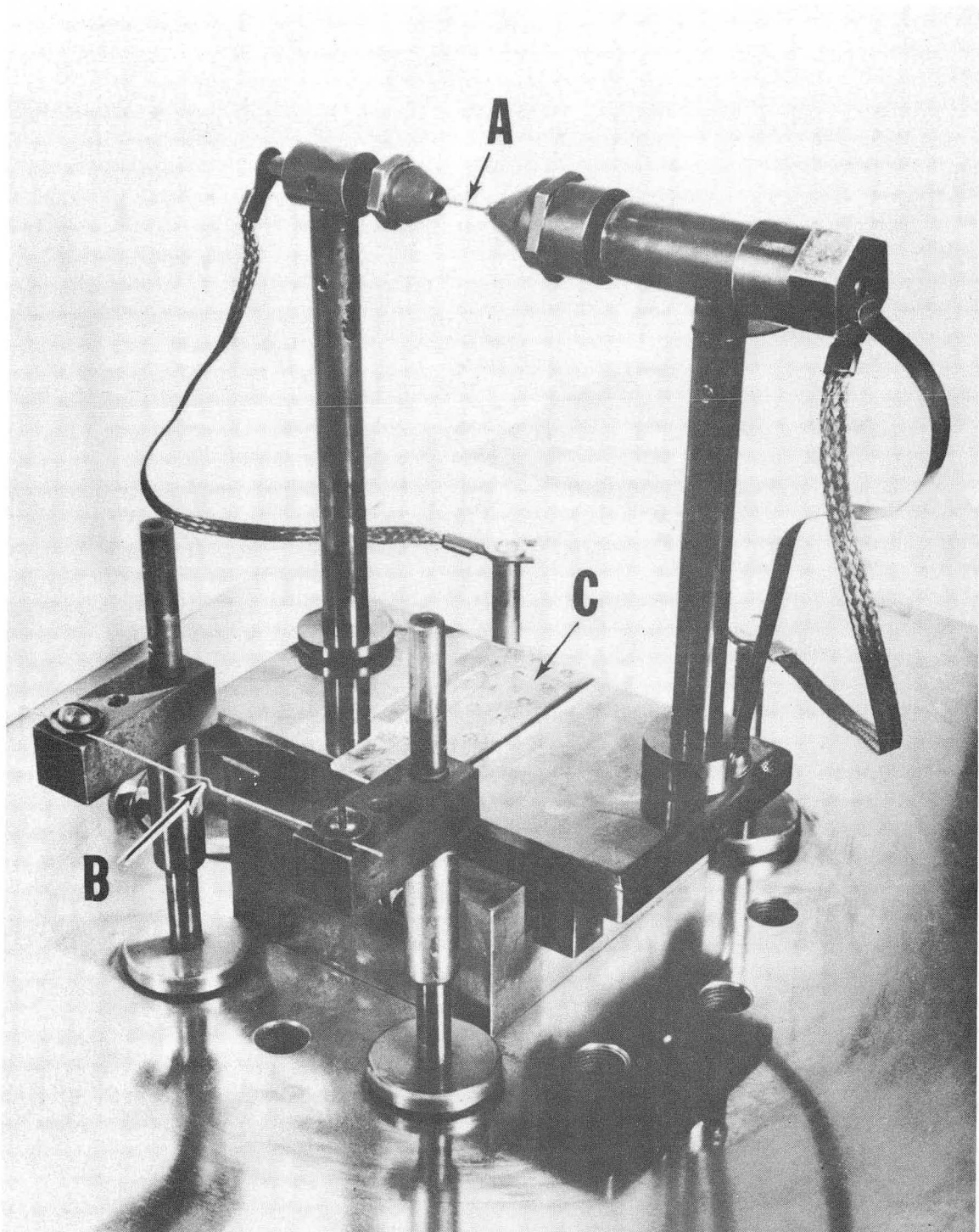
6. Evaporate carbon with full current (approximately 80A) until tips have disappeared (approximately 5 sec).

* No. 1203 Carbon Rods, E. F. Fullam, Inc. Box 444, Schenectady, New York, 12301.

** EFFA 1204 Carbon Rod Sharpener, supplier as above.

*** LADD 636 Carbon Rod Evaporation Unit, LADD Research Industries, Box 901, Burlington, Vt., 05402

**** For smooth specimens, the relief is accentuated by a shadowing angle of 30° or less.



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Fig. 4. Vacuum evaporator prepared for shadowing and carbon deposition.

- A - carbon electrodes in spring loaded holder
- B - tungsten wire with oxide coating on V-shaped part
- C - glass slide with mounted imprints

Dissolving Cellulose Acetate Contents of Replicating Film

In order to make the carbon replica available for use in the electron microscope, the plastic replicating film in which the original imprint was made has to be dissolved. At the same time, the carbon replica is deposited on the final support; a copper specimen grid which fits into the electron microscope specimen chamber. This is the most delicate step in the replicating process.

First, the replicating film is affixed to the specimen grid to avoid warping. Then the cellulose acetate contents in the replicating film is slowly and uniformly dissolved to avoid wrinkling and tearing of the carbon replica. Finally, the paraffin contents of the replicating film are dissolved. Characteristic for the procedure given is that the carbon replica remains in contact with the copper specimen grid during the entire dissolution process. Thus, many problems of free floating carbon films are avoided.

1. Cut approximately 2x2 cm piece from fine (30 mesh) stainless steel wire mesh and put it on a microscope slide. This wire mesh, throughout the processing steps to follow, serves the purpose of handling the replica without touching the delicate carbon film on the copper specimen grid.
2. Place copper specimen grid with etched (dull) side up on top of stainless steel wire mesh.
3. Cut square shape out of carbon coated imprint from frosted tape on glass slide and place it, carbon side up, on copper specimen grid. Center the imprint on the copper specimen grid and let the four corners overhang.

4. Place the microscope slide with specimen grid and imprint under a stereo microscope and deposit one or two drops of methyl acetate from a hypodermic syringe (with #27 needle, approximately) on the wire mesh next to the specimen on grid. The softening of the replicating tape by the methyl acetate vapors causes the corners of the tape to lift up. Immediately press them down with the tips of a tweezers so that they stick to edge of wire mesh.

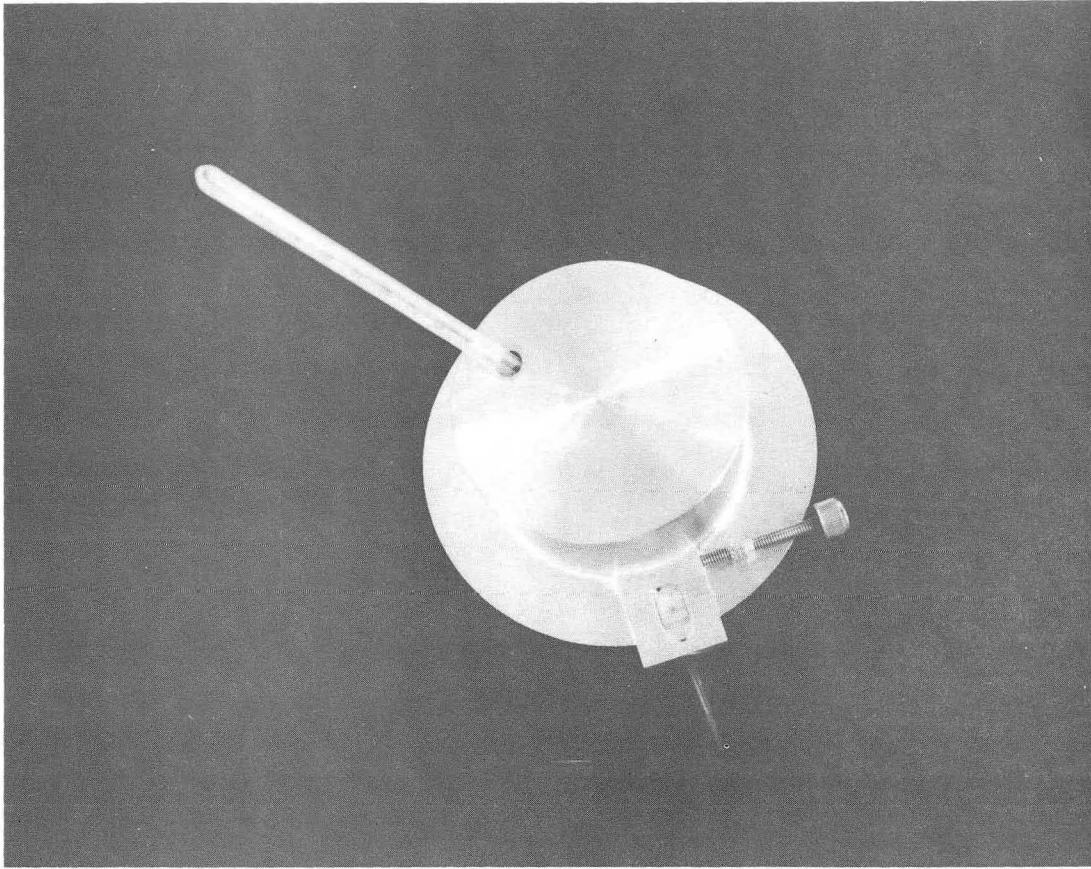
5. Remove the wire mesh with the specimen grid on it and place it on methyl acetate soaked filter paper in an open petri dish.

6. Place petri dish in a desiccator which contains a methyl acetate pool in the bottom. Close desiccator and wait over-night for dissolution of cellulose acetate contents of the replicating tape. Make sure there is enough methyl acetate in the desiccator. The filter paper in the petri dish should be moderately moist.

Replica Washing Device

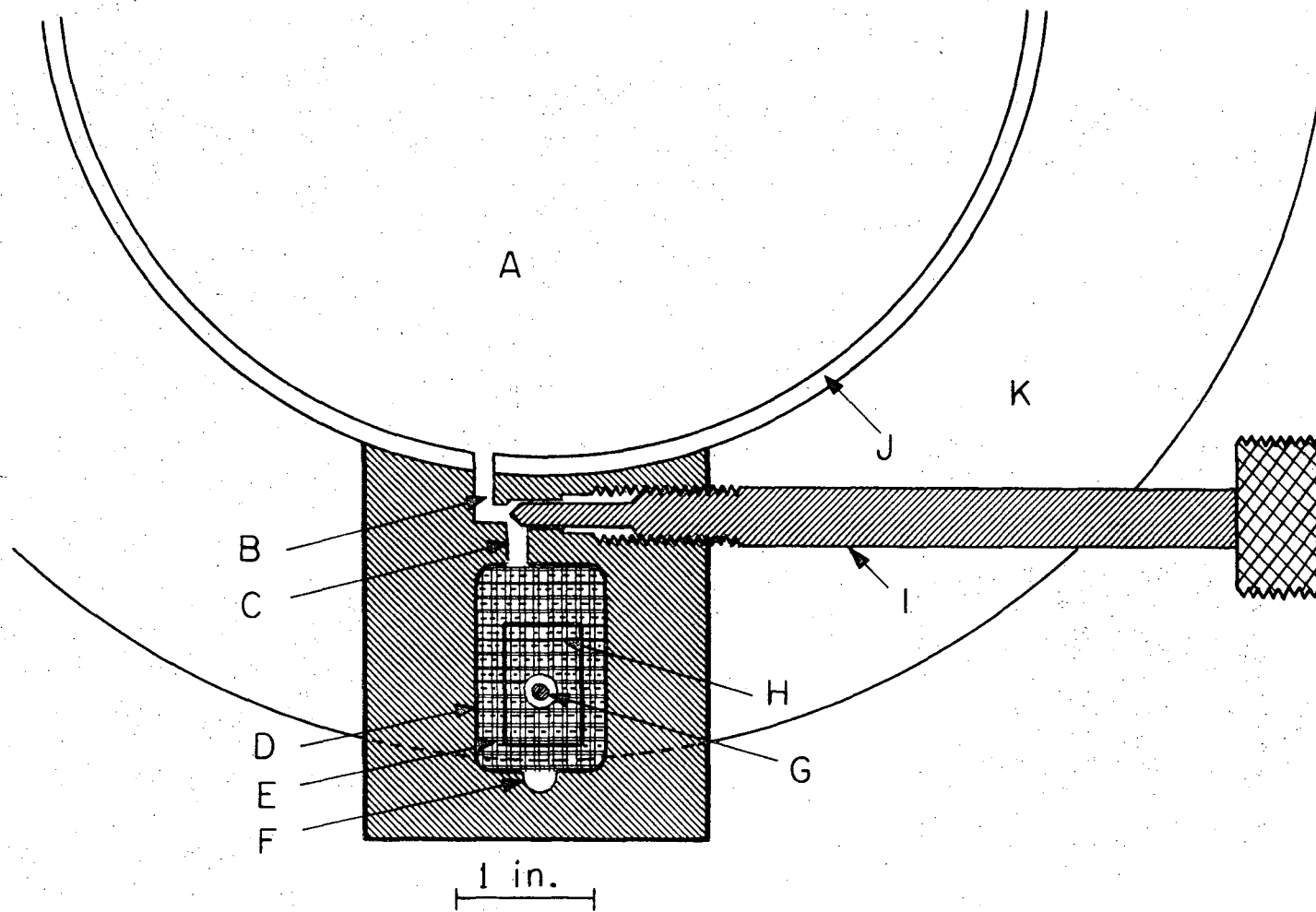
In the final processing steps of the replica, the paraffin contents of the replicating film are dissolved, together with any cellulose acetate residues. For this operation, a replica washing device shown in Figs. 5 to 7 has been developed. It consists of a solvent reservoir, which can be placed on a hot plate, an extraction basin where replicating film is dissolved by a flow of hot solvent, and valves for controlling the flow. The bottom of the extraction basin is lined with a stainless wire mesh (30 mesh) which serves to evenly distribute the flow of solvent.

In contrast to the previous lengthy, tedious and erratic process, this device easily produces clean, unbroken carbon replicas, which remain



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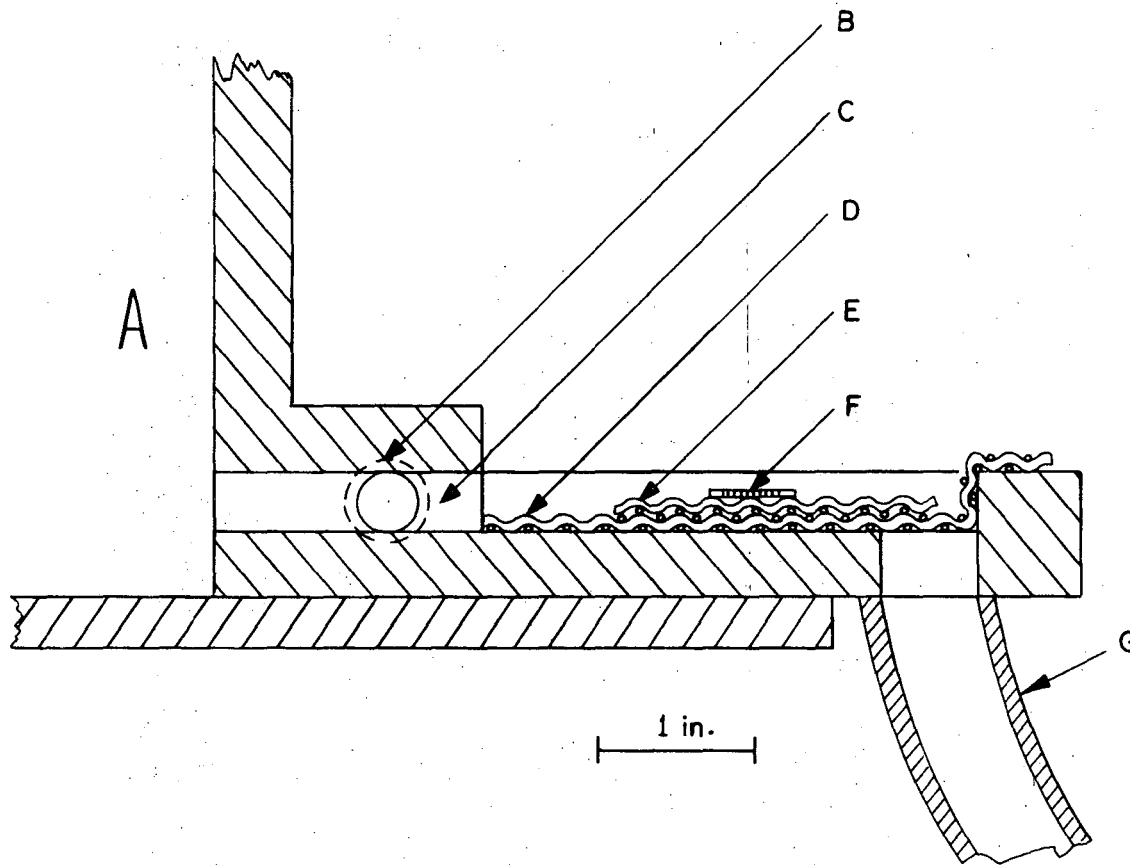
Fig. 5. Replica washing device with lid and thermometer.



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Fig. 6. Extraction basin of replica washing device, top view cross section.

A = Solvent reservoir	E = Wire mesh lining	I = Valve plunger
B = Solvent inlet	F = Solvent drain	J = Reservoir wall
C = Valve seat	G = Copper specimen grid	K = Reservoir bottom
D = Extraction basin	H = Wire mesh grid support	



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Fig. 7. Extraction basin of replica washing device, side view cross-section.

A = Solvent reservoir
B = Valve
C = Solvent inlet
D = Wire mesh lining of extraction basin

E = Wire mesh grid support
F = Copper specimen grid with replica
G = Solvent drain

attached to the copper specimen grid, in about two minutes. Because the extraction process takes place outside the reservoir, light boiling of the solvent in the tank can be tolerated. The resulting fast evaporation and condensation of solvent in the extraction area even enhances the cleaning of the carbon film.

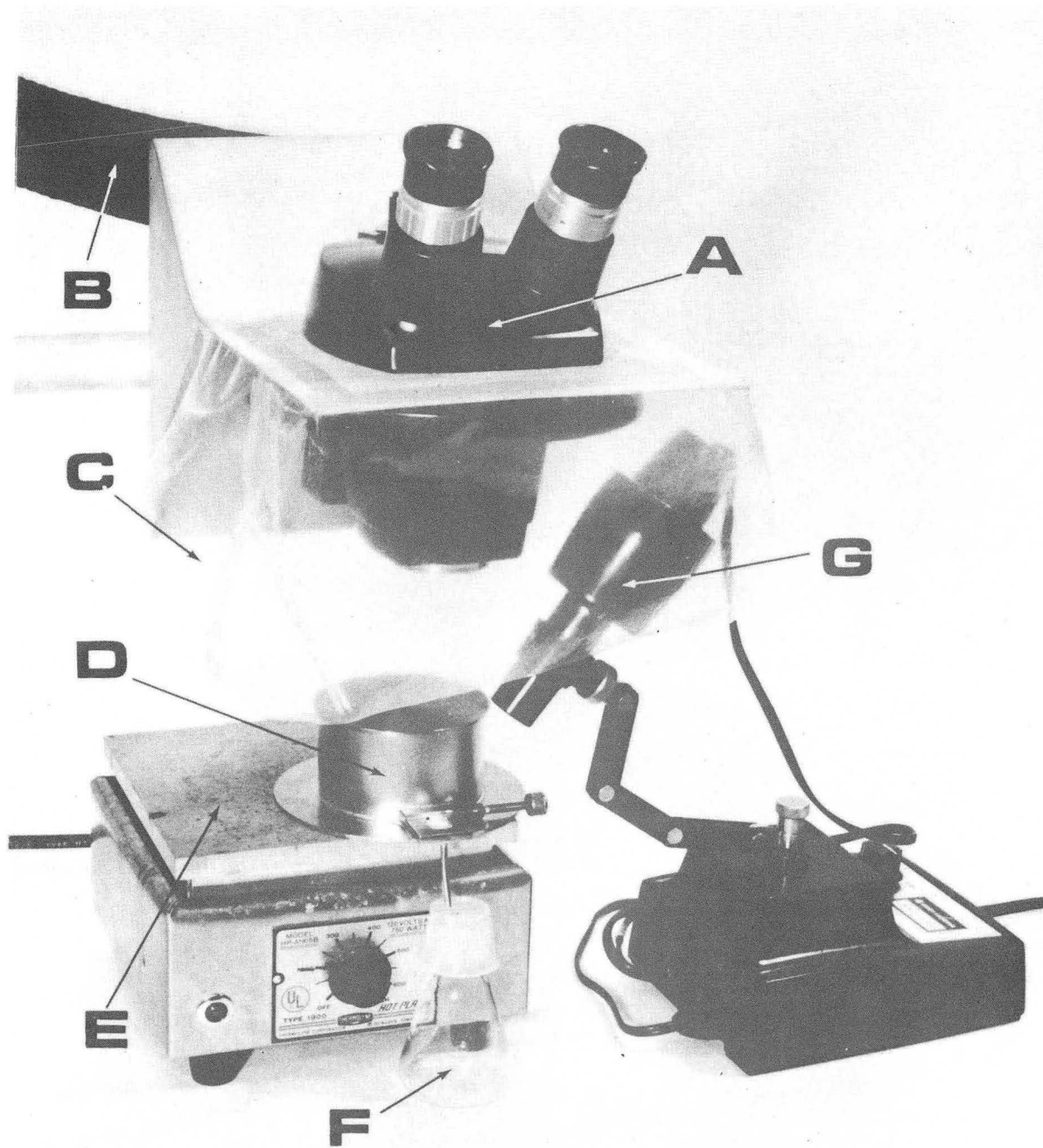
Dissolving Paraffin Contents of Replicating Film

1. Place a small hot plate, with temperature control, ($\pm 2^{\circ}\text{C}$) under a stereo microscope, equipped with hood and exhaust fan (Fig. 8). Put the replica washing device, filled with methyl acetate on top of the hot plate. Heat the methyl acetate close to boiling point (57°C) and check temperature with a thermometer. (The paraffin contents of Bioten Replicating Film melts at 40°C .)

2. After the methyl acetate temperature has stabilized, transfer a copper specimen grid (with replica on top) in the desiccator onto a fresh piece of stainless wire mesh (7x7 mm, 30 mesh). This step leaves a lot of dissolution products behind, provided that replica and substrate are moist.

3. Place fresh stainless wire mesh with copper grid and replica in the extraction basin of the replica washing device.

4. While watching the replica through the stereo microscope, open the methyl acetate flow from the tank. Adjust the valve so that the replica is gently extracted from the bottom side. The copper specimen grid may be slightly lifted off the stainless steel wire mesh support by the methyl acetate. An occasional flow of liquid over the top of the replica removes any dust from its surface. Keep replica away from the



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Fig. 8. Stereo microscope with hood for dissolution of paraffin contents of replicating film.

- A - Stereo microscope
- B - Exhaust with fan
- C - Hood
- D - Replica washing device

- E - Hot plate with temperature control
- F - Waste solvent container
- G - Microscope light

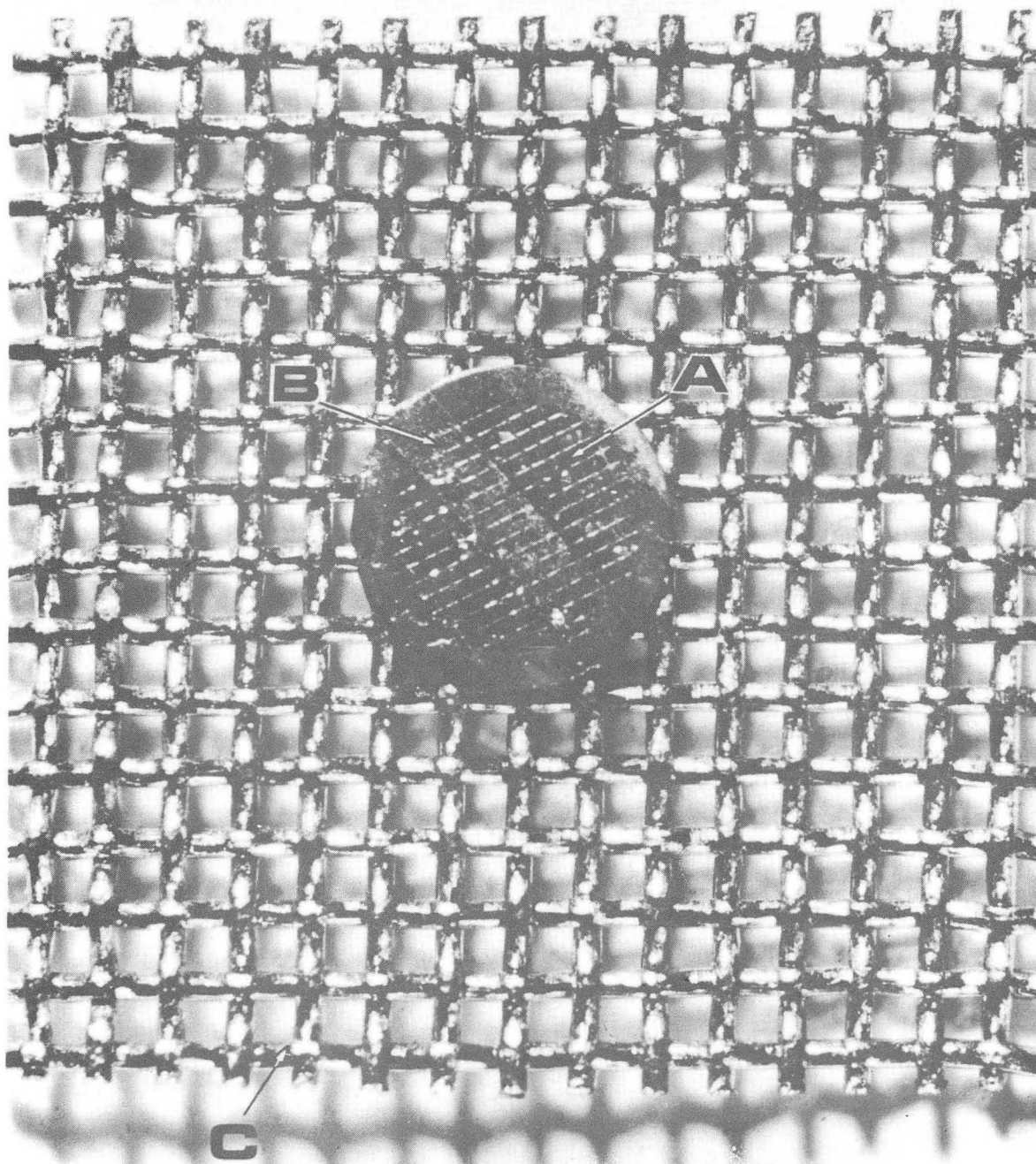
edges of the extraction basin, where surface tension may interfere.

5. After one or two minutes, stop the methyl acetate flow and remove the copper specimen grid with the replica from the extraction basin. At this stage, the replica should be clean and free of residues and dust. Store in a labeled covered container, on soft material (tissue). Care must be taken not to expose the final replicas to mechanical shock because they are very brittle and break easily.

An example of the degree of perfection obtainable by this technique is shown in Fig. 9, where only very few squares of the specimen grid, all outside the area of the replica, show breaks in the carbon film.

Acknowledgment

This work was conducted under the auspices of the United States Atomic Energy Commission.



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Fig. 9. Copper specimen grid (A) (94 mil. diam., 200 mesh) with replica of rectangular surface (B) on stainless wire mesh (C). Carbon film after final washing. Note absence of defects due to breaks, folds and plastic residues over entire area of imprint.

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