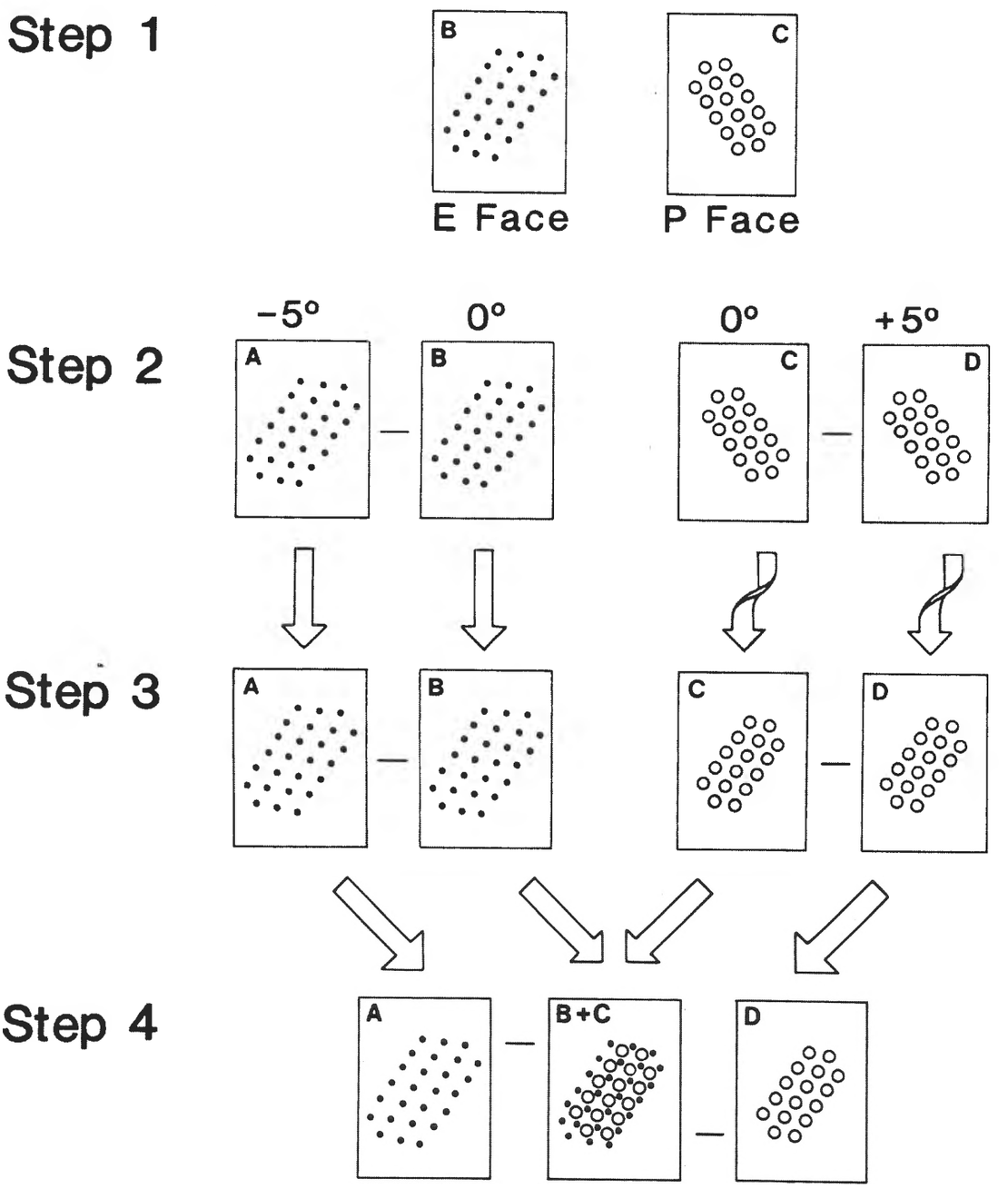

Fig. 1

Figure 1.

Diagram illustrating the four-step sequence for aligning and mounting stereo images of complementary replicas to form an array of three photographs containing two stereo images as in Figures 5,7,9, and 10. For details see text (technique 3).

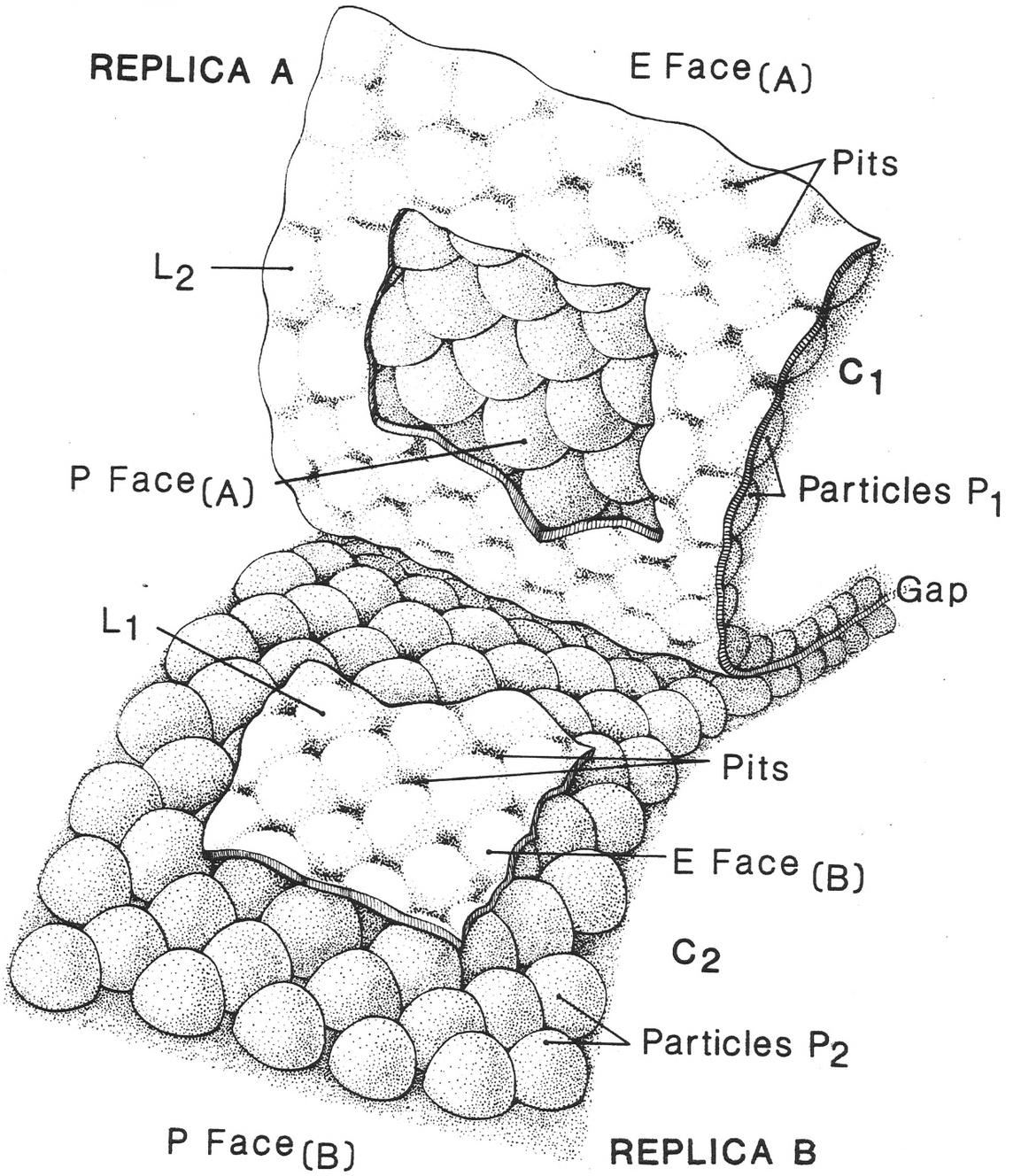

Figure 2.

Artist's drawing depicting a model of a gap junction that

has been split into two complementary replicas (A and B) in the process of freeze-fracturing the two cells (C_1 and C_2) whose plasma membranes come together to form the junction. The cytoplasm of cell C_1 may be imagined to lie behind the upper part of the split junction (A); the cytoplasm of cell C_2 lies below the lower part of the junction (B). The lower part of the model shows predominantly the particulate P-face, the upper part the corresponding pitted E-face. For simplicity, the central depressions (which are not always seen on tops of the replicated particles, but are thought to be the central channels) are not indicated. The intercellular gap is shown in a cross-fracture of the junction on the right. Since the fracture plane was never seen to follow the gap, the gap is not shown where the junction is fractured en face. The gap is probably contained with the layer L, while the fracture plane steps from one side of the layer (L_1) to the other side (L_2). The presence of a common layer (L) separating the two sets of particles (P_1 and P_2) from the two cells (C_1 and C_2) becomes evident by three-dimensional visualization of the complementary replicas using stereo imaging as in Figures 4 and 6. The E-face always appears pitted after unidirectional shadowing, whether (as L_1) it belongs to cell 1 or (as L_2) it belongs to cell 2. The diagram illustrates a patch of the pitted layer L1 which has become detached during fracture from the upper half (A) and remain with the particulate surface (P_2), thereby leaving the central opening (window) in the pitted area of the upper half (A). Through this window a set of particles (P_1 of cell C_1) can be seen by examining the E-face of replica A. The detached patch overlies the particles (P_2 of cell C_2) that show up as the

particulate P-face on replica B. The particles P_i originate in the membrane of cell C_1, and the particles P_2 originate in the membrane of cell C_2; i.e., during freeze-fracture, the membranes separate so that P_i travels with C_1 and P_2 travels with C_2. By contrast, during freeze-fracture the membranes separate so that the E-faces are exchanged between the cells: Viewed from above, the patch of pitted E-face labeled L_i (which forms the top of a composite layer into which the particle of replica B can be seen to bulge) originated from the membrane of the upper cell (C_1). The pitted surface L_i is the external leaflet (E-face) of the membrane of cell C_1. The area (L_2), which remains with the upper cell (C_1) after the "window" (L_1) has been removed, originates from the E-face of the membrane of the lower cell, C_2. See text for additional discussion.

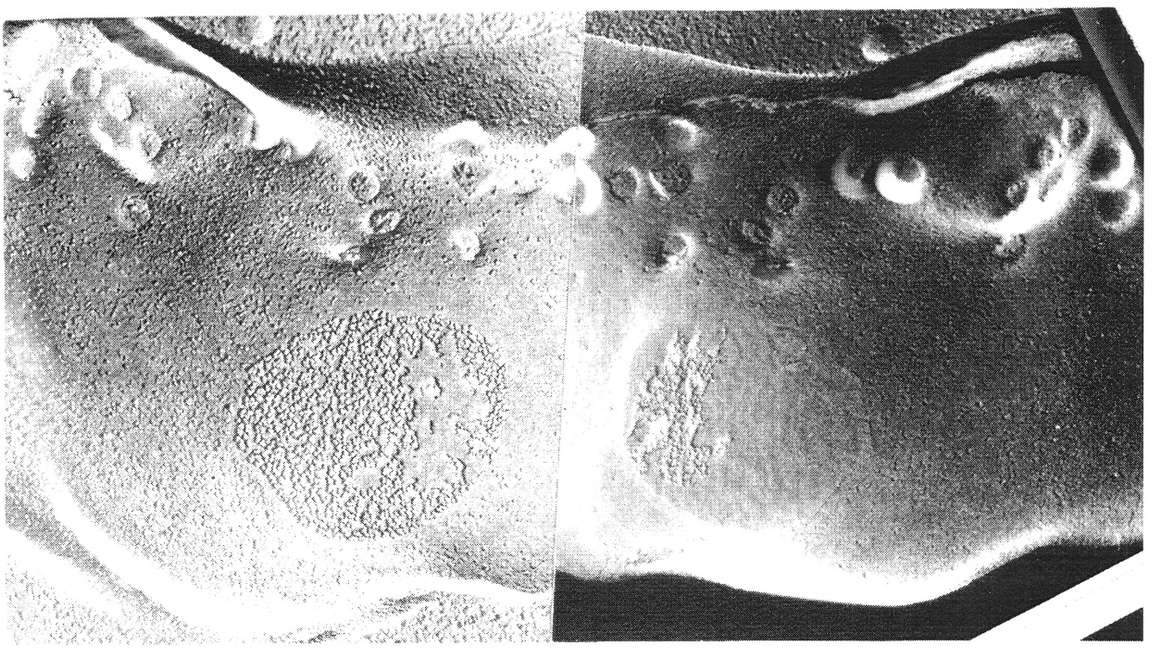

Figure 3.

Electron micrograph of a complementary replica of a freeze-fractured gap junction in a sheep cardiac Purkinje fiber. The pictures were mounted to display the symmetry of details of the replicas. The left panel is made up predominantly of the particulate P-face of the membrane; the right panel shows the pitted complementary E-face. Numerous caveolae present in the upper part of the micrographs also display symmetrical features. The dark bottom of the right panel is an artifact of fracturing the right replica. This is a non-stereo image. This and all subsequent electron micrographs have been double-printed so that shadows appear black. X 50,000

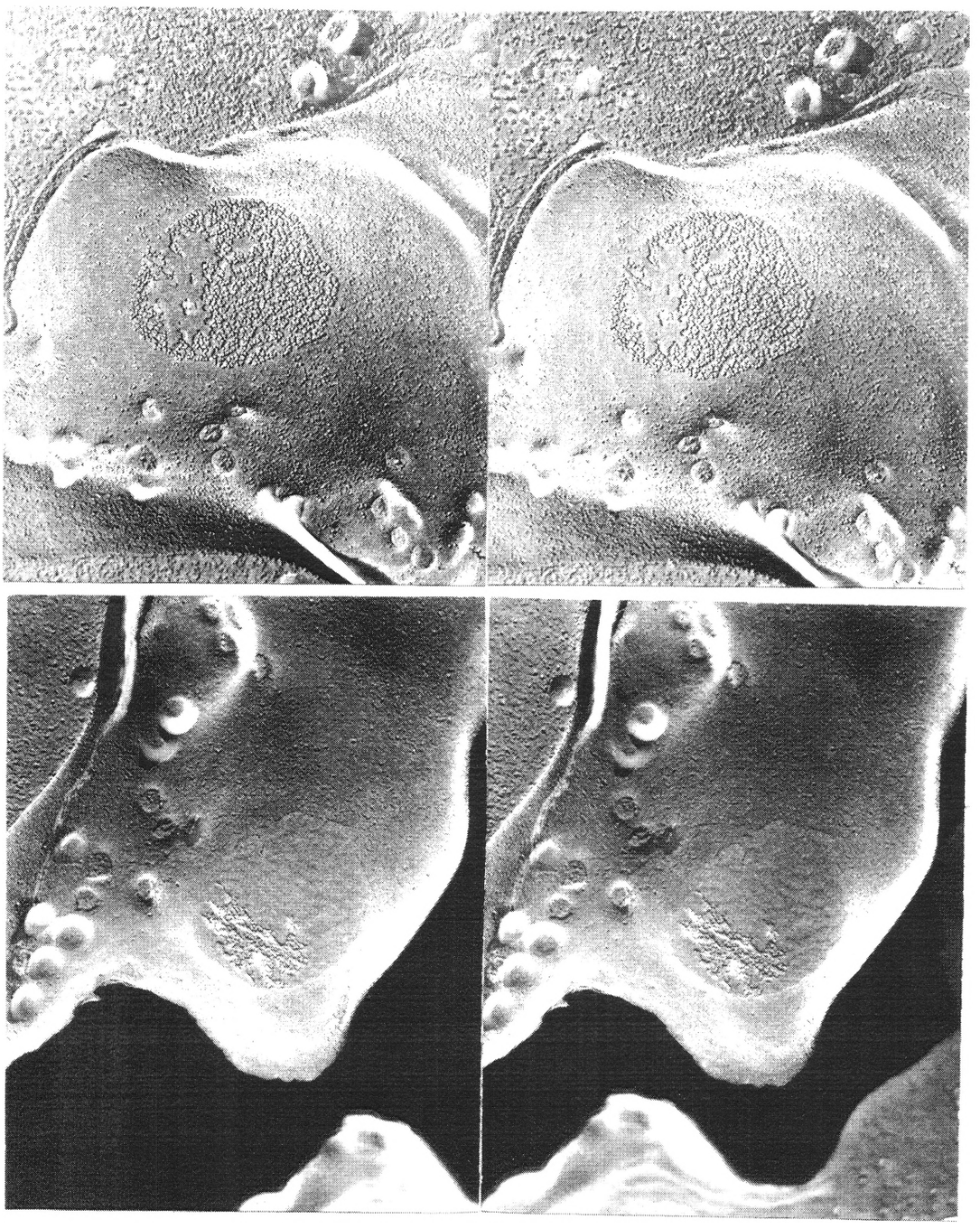

Figure 4.

A set of two stereo pairs of the gap junctions shown in Figure 3. This set, as well as those in Figures 6 and 8, should be looked at with a stereo viewer to bring out the layered structure of the junction and to see how the junction splits to produce complementary replicas. The upper stereo pair shows mainly the particulate P-face of the membrane; the lower stereo pair shows mainly the E-face. The large, branched, particle-free area in the upper stereo pair is a patch of the E-face (layer L in Figure 2) that covers the particles underneath it. A similarly shaped opening ("window") through which P-face particles are visible in the E-face can be seen in the complementary replica (the stereo pair in the lower panel). Such stereo views show that these particles lie in a different plane than that of the P-face particles in the upper panel. X 50,000

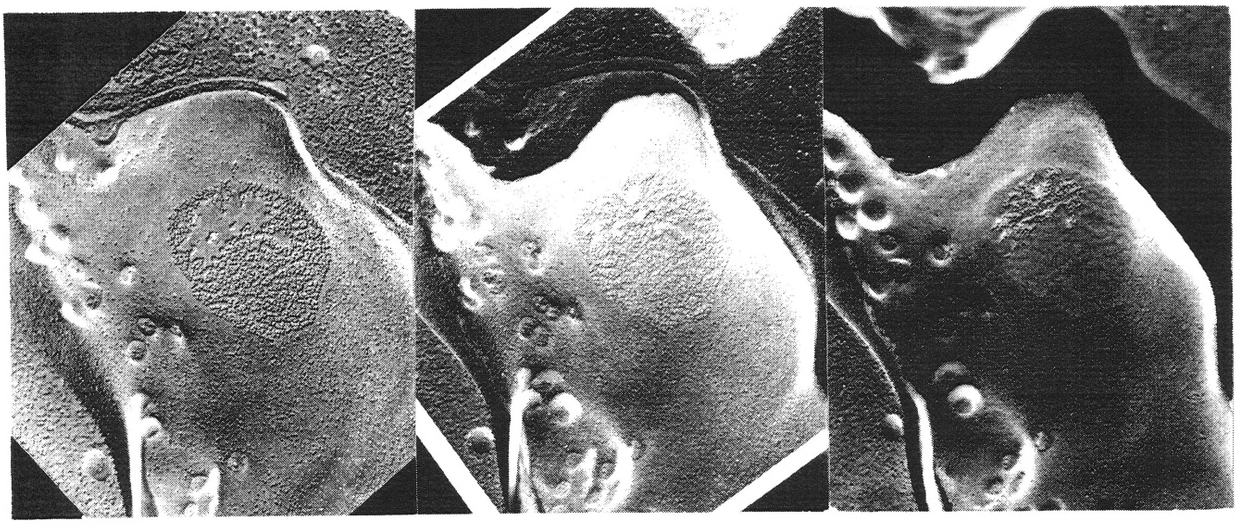

Figure 5.

The complementary gap junctional replicas shown in Figures 3 and 4, displayed as a sequence of three photographs containing two stereo images arrayed as in step 4 of Figure 1. This figure and Figures 7, 9, and 10 should be viewed with a stereo viewer in the manner explained in technique 3 in Methods and shown in Figure 1 in order to demonstrate that superimposition causes the E-face pits to fall in between the complementary P-face particles. Note that the particle pattern on the P-face is traceable on the E-face; for example, the small, particle-free oval area is also detectable under the E-face. Alternate stereo viewing of the right and left stereo pairs shows that, in favorable areas as on the right, the branched pattern of spaces between E-face pits is exactly filled with particles. The patterns of particle clusters can also be identified on the pitted areas. X 50,000

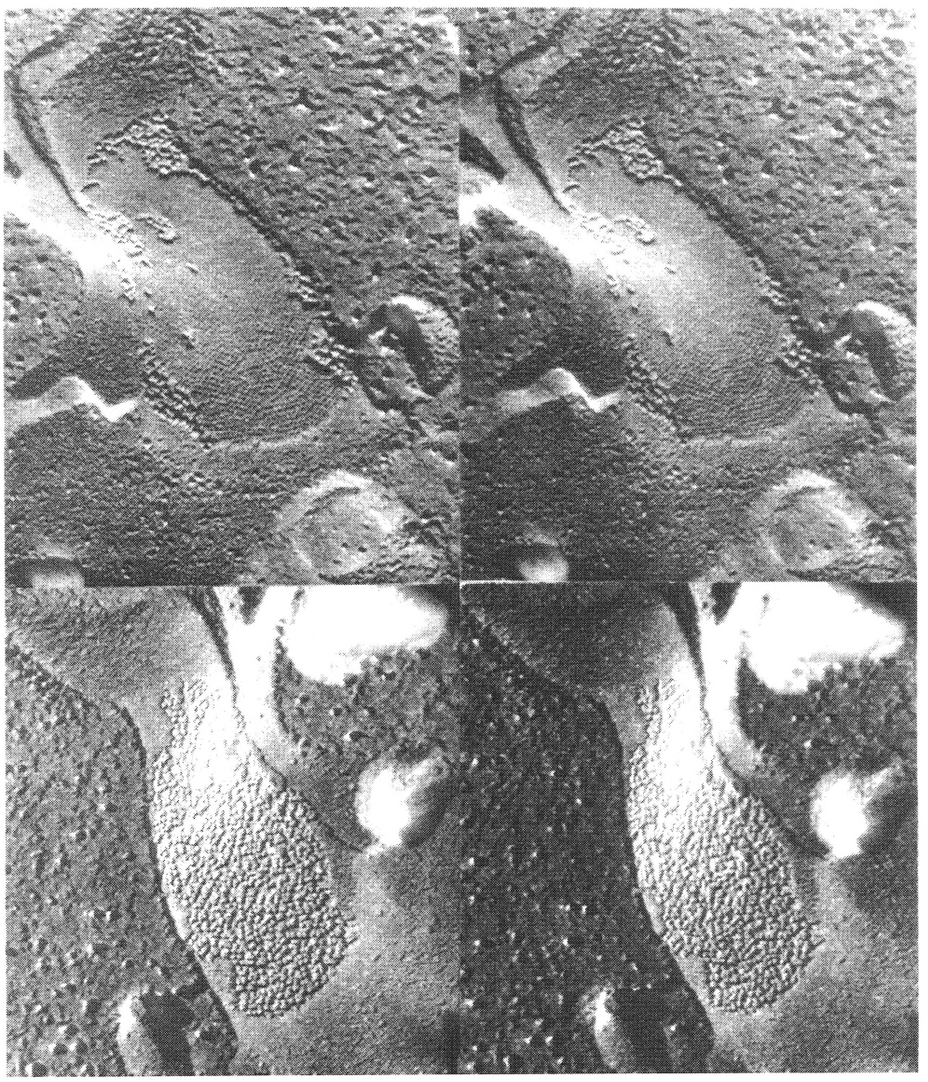

Figure 6.

A set of two stereo images of complementary faces of a gap junction which, on stereo viewing, displays marked curvature of its surface. A slight difference in slope produces a difference in shadowing angle that causes the pits to disappear in the upper region of the gap junction, whereas they are well visualized in the lower part of the patch of membrane. The clear outlines of the shapes of the patches of the E-face portions which appear on both complementary replicas facilitate identification of complementary areas on P- and E-faces. The most convenient areas for the purposes of such identification are those so small that they contain few particles or pits; such areas are suitable for matching at high magnification. The identifying landmarks are the structural details surrounding the areas to be matched, e.g., the edges of fractured membranes or the structures in the cytoplasms. X 100,000

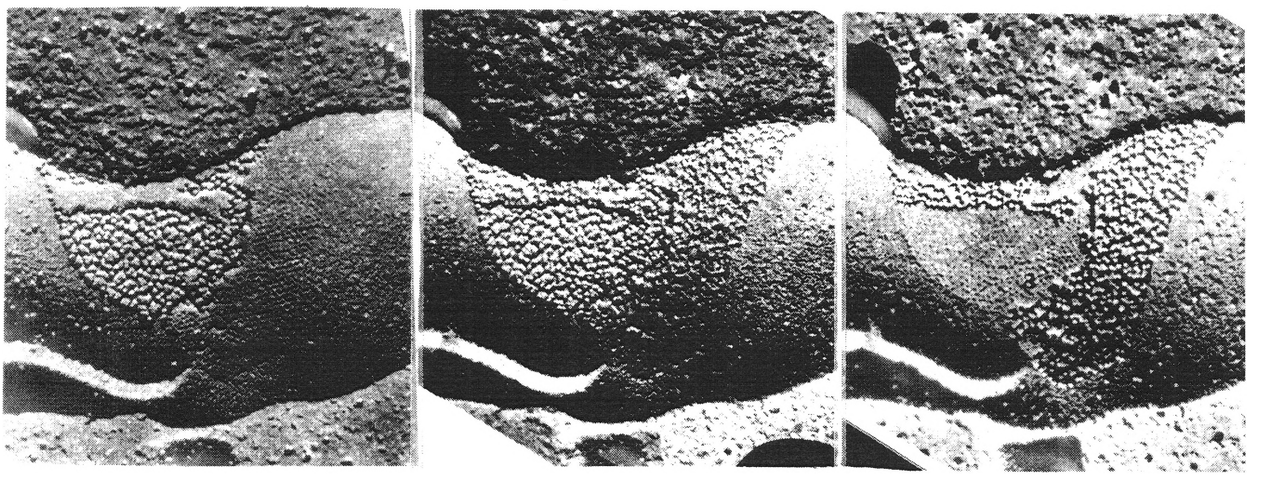

Figure 7.

Sequence of three panels for stereo viewing of complementary replicas mounted to be viewed as explained in Figure 1. At this higher magnification the location of pits in the spaces between particles is well demonstrated. The middle picture becomes filled with the particles from both faces, while at the same time it contains all the pits from both the: Left and right pictures. X 100,000

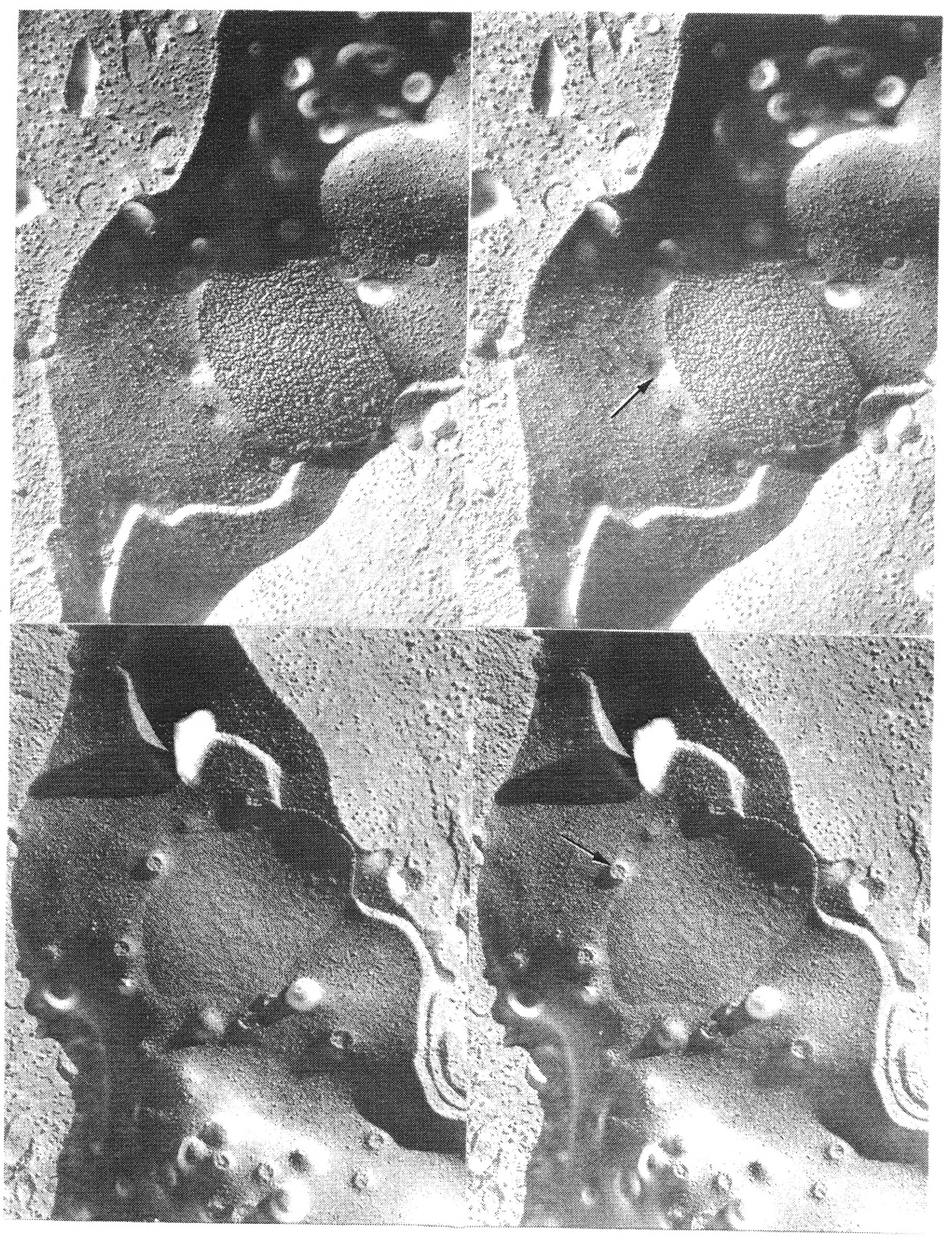

Figure 8.

Two stereo pairs of complementary replicas of a gap junction mounted so as to display symmetry of details on both surfaces. This junction split in such a way that only the pitted E-face appears on one replica, and only P-face particles appear on the other replica. Stereo viewing clearly shows a second layer of particles under the E-face of the lower stereo pair. This second layer of particles manifests its presence by bulges in the area between pits. Arrows point to the extra-junctional landmark, a caveolar neck, which is also present in Figures 9 to 13. X 50,000

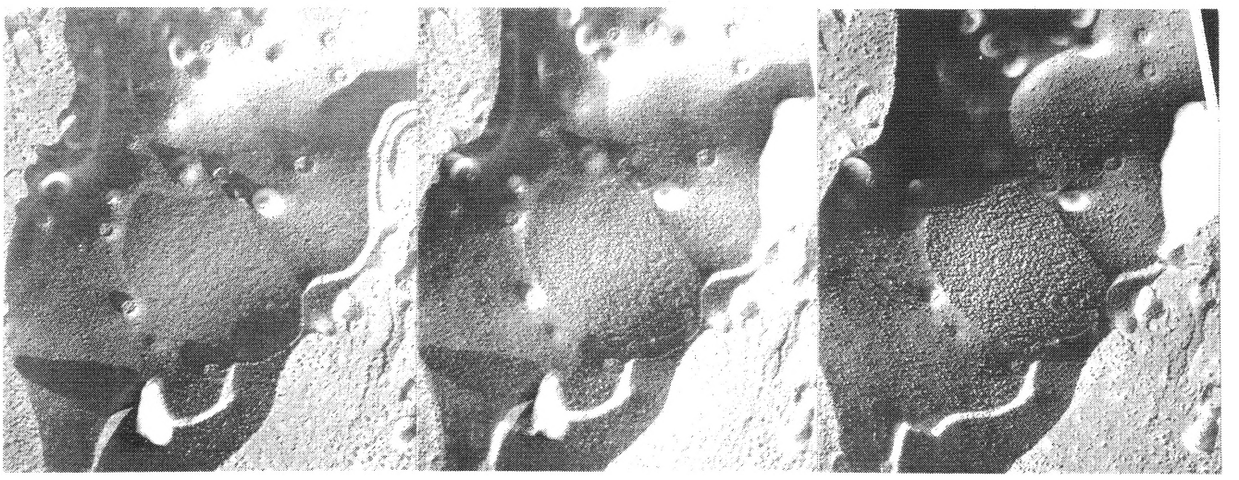

Figure 9.

Triple array of the gap junction shown in Figures 8 - 11, mounted for stereo viewing as in Figure 1. In the central area of the gap junction, where the alignment is good, it is evident that the pits fall into the spaces between particles, not on them. X 50,000

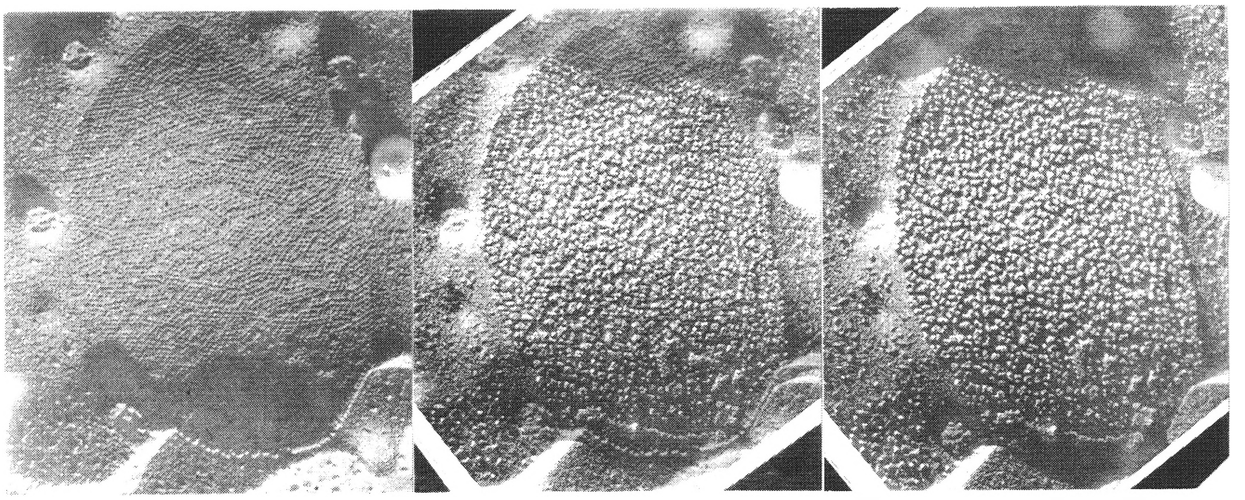

Figure 10.

A higher magnification of the array shown in Figure 9. X 100,000

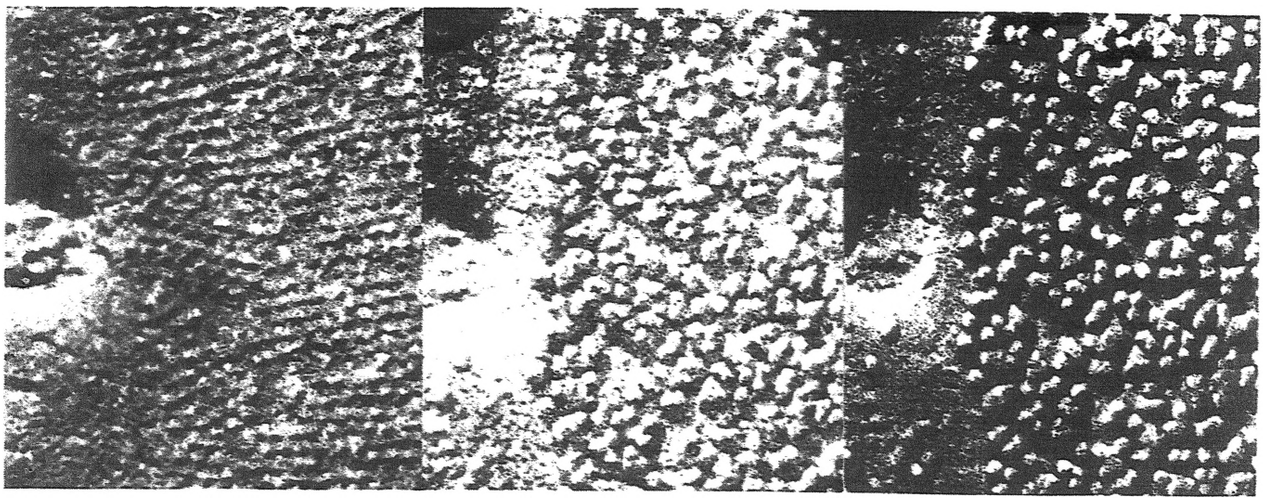

Figure 11.

A greatly magnified portion of the gap junction shown in Figures 8 -10. This is not a stereo set, although the two complementary faces (right and left panels) were combined to produce the central panel. Some of the particles on the particulate surface (right) are missing, while others are obscured by heavy shadowing. The array of pits on the pitted surface (left) is more regular than the particle pattern (right). The middle panel shows how well the features of the right and left panels can be matched. These images should be compared with the tracings and "maps" of Figures 12 and 13. Next to the left margin of the gap junction, a fragment of the caveola (indicated by an arrow in Figure 8) marks the level of an unusual triangular pattern that can be used to compare the locations of the corresponding particles and pits in all three panels of Figures 9 -10.This comparison of locations shows that the pits fall between the particles. Some of the particles are missing. X 400,000

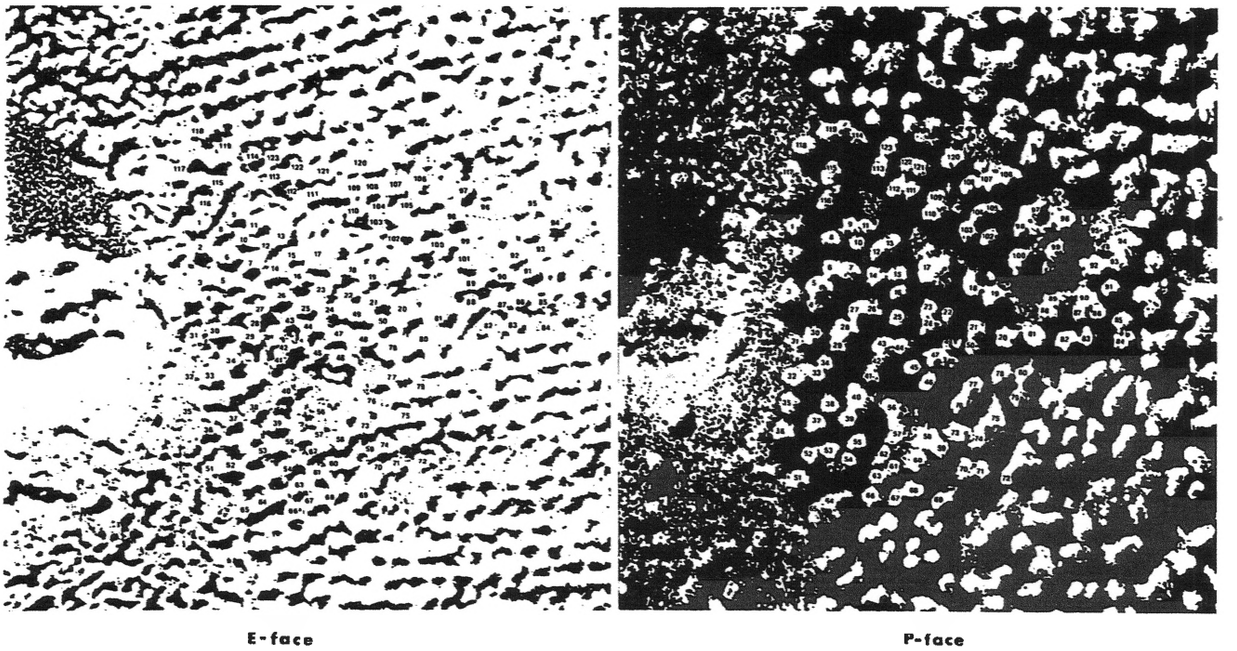

Fig. 12

Figure 12.

High contrast enlarged reproductions made with a copier and enlarger of the right and left panels in Figure 11. The right panel shows a "map" made by numbering the particles; the left panel shows the areas between pits corresponding to the numbered particles on the right (see text). The outline of a caveola (shown by arrow in Figure 8) is evident at the left margin of both panels.

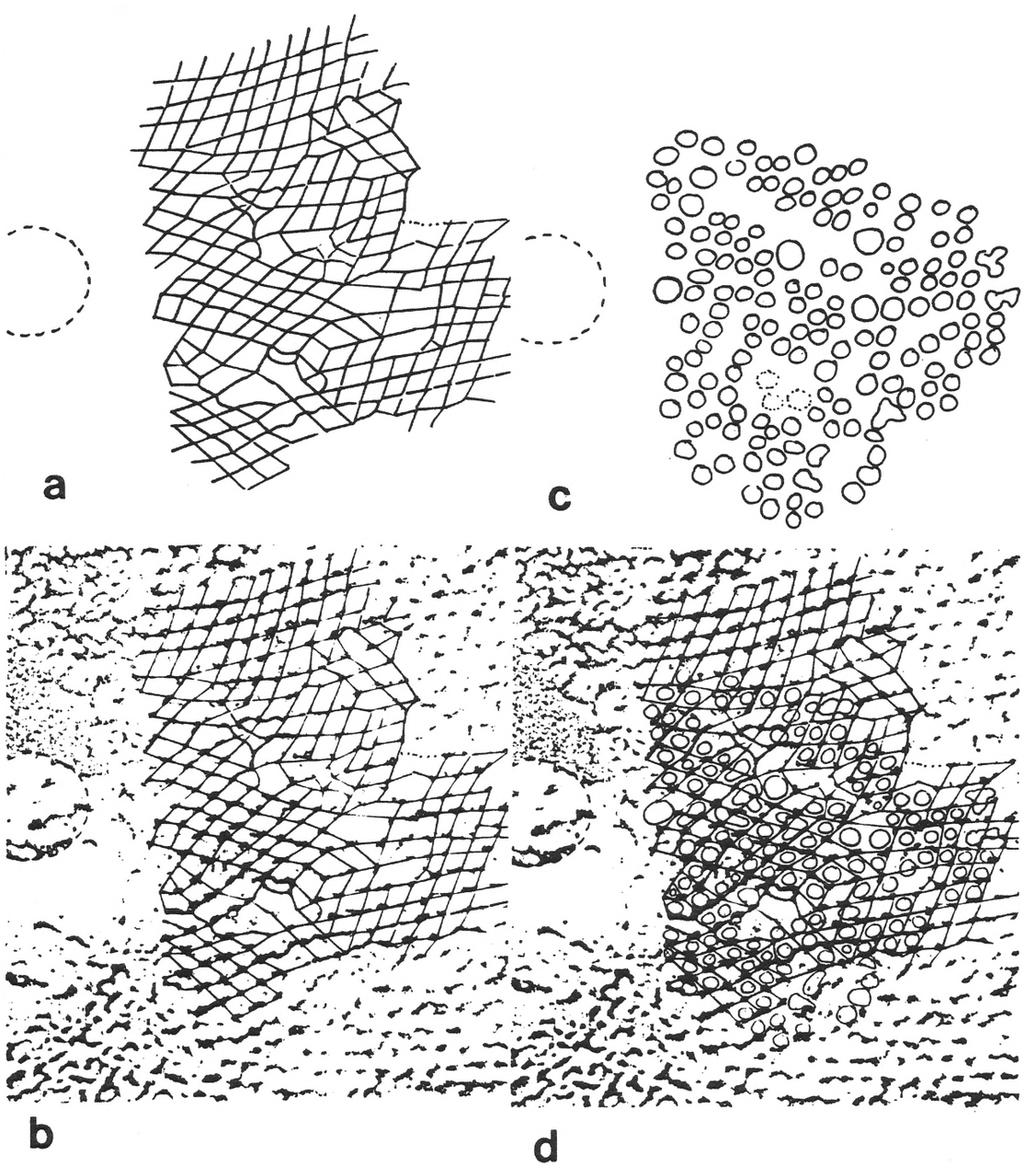

Figure 13. Diagrams showing the steps in the procedure for matching the structural features of the complementary gap junctional E-face and P-face shown in Figures 8-12. See text for description (technique 5).

Figures 15-19

A series of images of a small GJ mounted in the way explained in Fig. 1, to demonstrate the relations between pits and particles in its Face P and Face E. In the 3D images (Figs. 16-19) the regular pattern of pits can be matched with the positions between particles of Face P. The best fit of pits falling in the spaces between particles is shown in Figs. 17 and 18, after the stereo-images of both faces are superimposed as in step 4 of Fig. 1.