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OBJECT HOLDERS AND CAMERAS FOR CONTACT MICRORADIOGRAPHY

by

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INTRODUCTION

In contact microradiography thin specimens in contact with a photographic plate or film with an extremely fine-grain emulsion are exposed to low energy roentgen rays. The images, which are of natural size, can be examined under an ordinary light microscope. Quantitative determinations can be made of the components of the material studied by microphotometry of the radiograph.

The method, which is widely used in biologic research, has proved useful in the investigation of the course and degree of mineralization of hard tissues.

Most of the few commercially available roentgen apparatuses for microradiography have no specimen holder or camera accessories. Holders and cameras suitable for microradiography in biologic and technical fields have been described by *Sherwood* (1947), *Bergman* (1957), *Johnson & Andrews* (1958), *Longobucco* (1960), *Bhussry & Parikh* (1962), *Scott, Nylen & Pugh* (1962), *Oderr, Dauzat & Montamat* (1963), *Peterson & Kelly* (1964) and *Friberg* (1964). Three cameras with specimen holders are described below; two for use with Philips roentgen diffrac-

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tion tube (25293/52) and diffraction generator (PW 1010/30) and one for Philips CMR-5 apparatus. The holders are improvements on present constructions and are intended mainly for examination of mineralized tissues. If the images are to be satisfactory, the specimen must be in contact with the film during the exposure. The purpose of the holders described below is to enable a simplification and standardization of the technical performance of contact microradiography.

DESCRIPTION

Specimen holder and cameras for Philips roentgen diffraction tube

The specimen and the photographic plate are pressed together against a supporting surface by atmospheric pressure. The supporting surface forms the floor in a chamber, whose roof con-

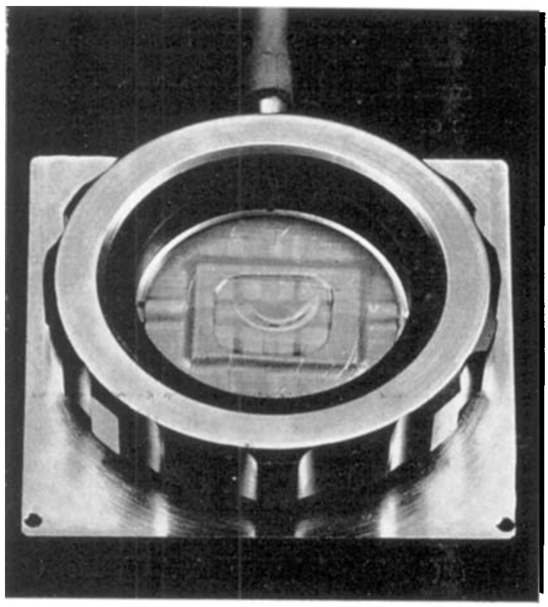


Fig. 1. Object holder for contact microradiography intended for camera constructions I and II.
The holder is loaded with a photographic plate and a ground section of a rat incisor embedded in bioplastic. The plate and the section are covered by a thin plastic sheeting, Du Pont Mylar 6.5 μm thick.
The corners and the edges of the photographic plate are covered by glazed cloth to prevent them from damaging the Mylar film.

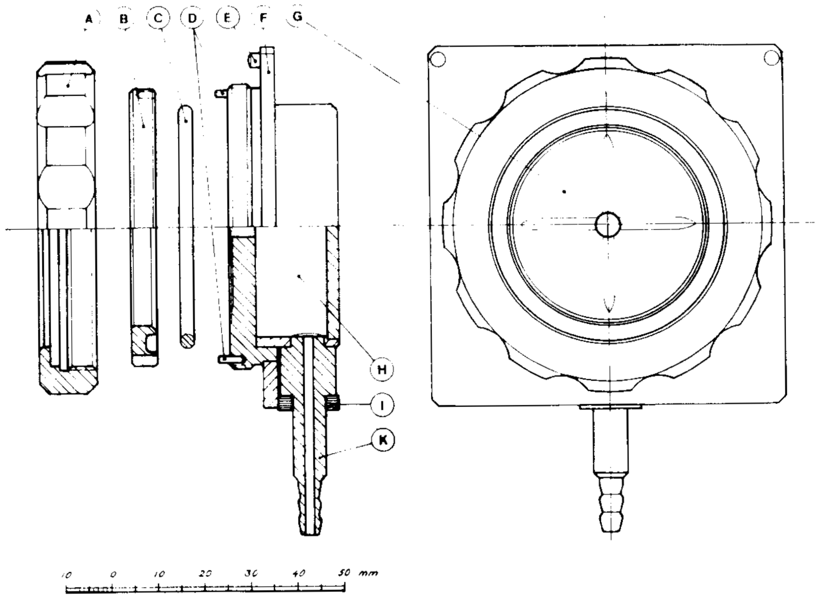


Fig. 2. Object holder for contact microradiography intended for camera constructions I and II. (For details, see text).

sists of a thin plastic sheeting (DuPont Mylar 6.5 μm thick). The air is withdrawn from the chamber through a hole in the supporting surface. Atmospheric pressure then presses the plastic film, the specimen and the photographic plate against the supporting surface. The plastic sheet is held in position by an O-ring, which prevents the entry of air into the evacuated chamber. To prevent the plastic film from being damaged by the edges of the photographic plate the edges are covered by a frame of glazed cloth. The construction is illustrated in Figs. 1 and 2. The O-ring (C), Fig. 2, is placed in the holder (B) and by means of a threaded ring (A) it is forced against the supporting surface (G). Two pins (D) prevent the holder (B) from rotating and wrinkling the plastic film when the holder is screwed tight. Behind the supporting surface (G) is a chamber (H), which increases the volume of the space to be evacuated. This diminishes the effect of any leakage, which might occur if the plastic film is wrinkled or damaged. The air is aspirated through the nozzle (K). If the exposure time is very long, the volume of the chamber (H)

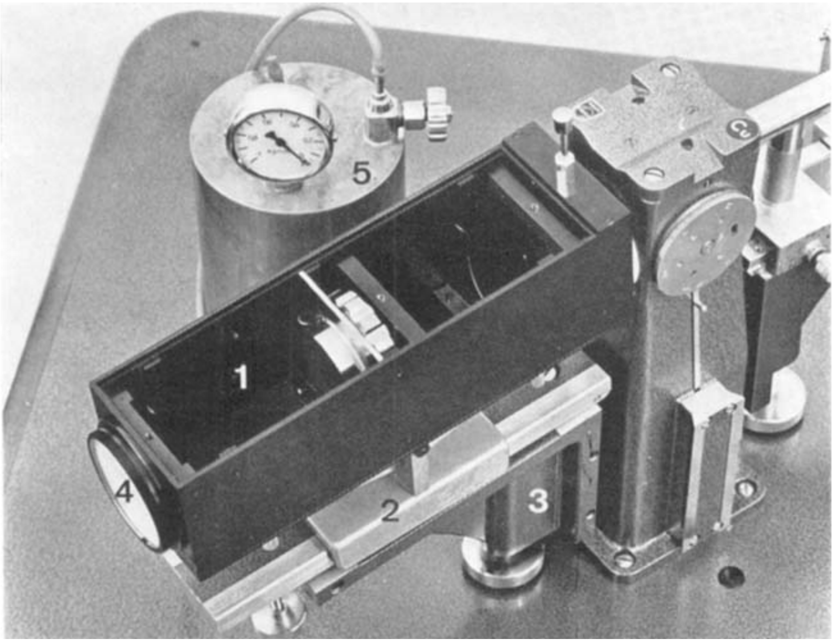


Fig. 3. Camera I with its lid removed is ready for use on Philips bracket before a vertical roentgen tube. The light-tight lid of the gable of the camera has been removed and the lead glass plate and the fluorescent screen are visible. The object holder is placed in the mid-position of the camera and is connected with a vacuum container.

1. Light-tight camera.
2. Rider
3. Bracket
4. Lead glass and fluorescent screen.
5. Vacuum container.

in the holder may not be large enough to maintain sufficient negative pressure. The holder can therefore be connected with a larger vacuum chamber through the nozzle (K). The purpose of the felt washer (I) is described later. The hole in the supporting surface is also seen in Fig. 2. The holder has a square plate (F) which guides it into correct position in the cameras. The pins (E), which are detachable, can be used to allow tilting of the holder for stereoscopic microradiography.

In one of the cameras the target-film distance can be varied. The holder fits both cameras. Camera I (Figs. 3 and 4), which is used with a vertical tube, consists of a light-proof box mounted on a rider running on Philips PW 1012/10 bracket with adjust-

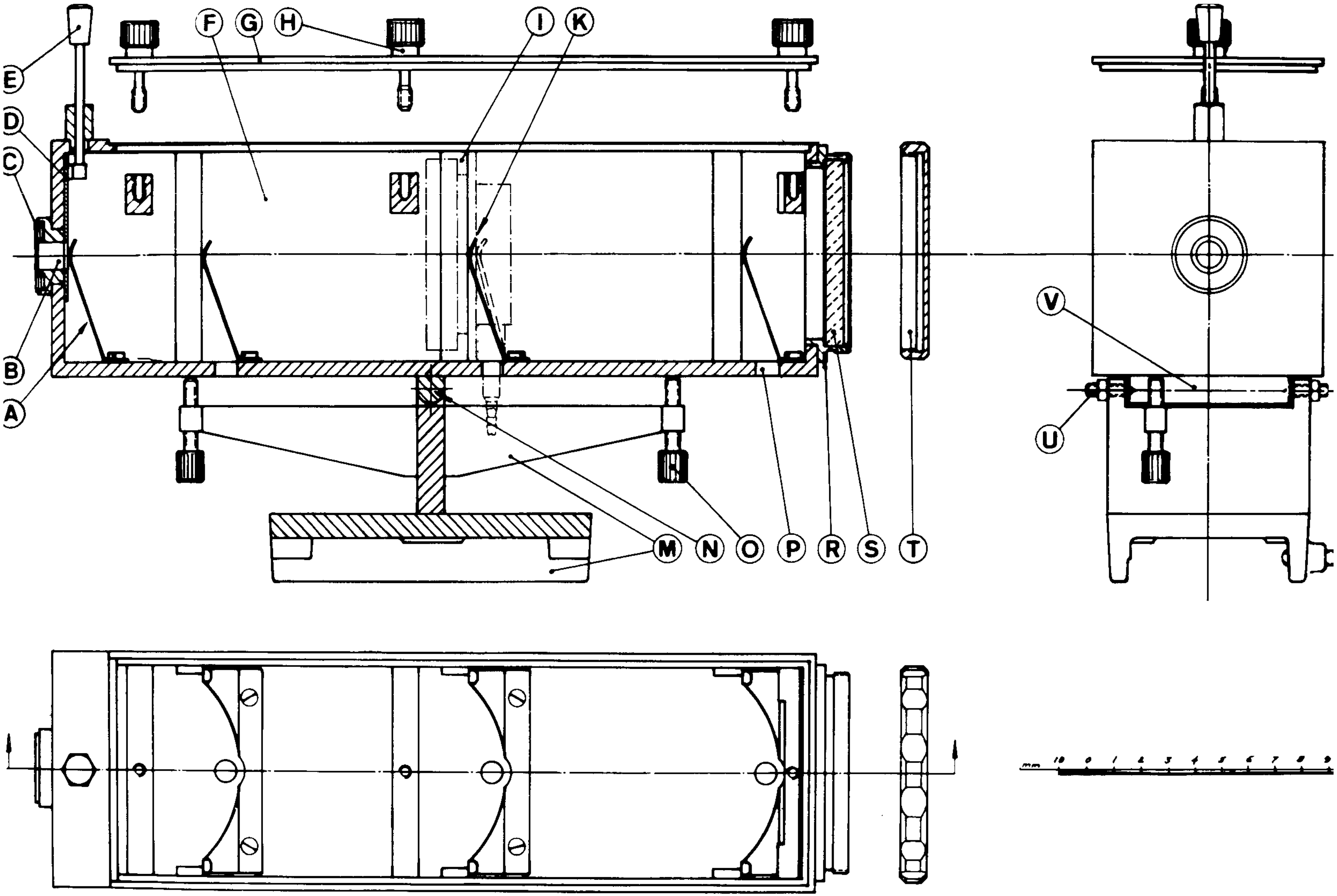


Fig. 4. Camera I for contact microradiography. For details, see text.

able support track. The box (F), Fig. 4, is made of duralumin plates fastened together by screws. The lid (G) can be readily removed when the three screws (H) with knurled heads are loosened. The inner surface of the longitudinal walls is provided with tracks along which the guide plate (F), Fig. 2, can be adjusted and held in position by springs (K), Fig. 4. In Fig. 4 the holder is delineated by the dot-dash sequence in the middle of the three possible positions corresponding to target-film distances of 100, 200 and 300 mm. The diameters of the corresponding field sizes are 12, 24 and 36 mm. The nozzle (K), Fig. 2, is placed into one of the holes in the floor of the camera. The felt washer (I), Fig. 2, is gripped between the floor of the camera and the fitting of the nozzle, thereby preventing the entry of light. The two remaining holes (P), Fig. 4, are sealed with rubber stoppers. The camera is placed on the bracket before the window of the vertical tube, the rider (M) being screwed tight to the support track. The position of the camera relative to the beam can be adjusted, the bracket and its support track allowing a certain degree of two dimensional movement of the camera. Screws, (U) and (O), on the rider (M) allow adjustment of the camera to either side and downward tilting about an axis (N). The camera is fixed to the rider by means of a transverse bar (V) screwed to the underneath surface of the former. Each end of the bar is provided with a conical hollow, shaped to receive the tip of a screw (U). If the camera is to be adjusted transversely, it can be done by moving the screws (U) simultaneously in one direction or other and afterwards locking them in position by tightening the bolts. When the camera is tilted about the axis (N) the adjustment screws (O) are used.

A fluorescent screen (R) covered with lead glass and placed in the posterior gable of the camera allows direct observation of the irradiated field during actual adjustment of the camera. An opaque lid (T) is screwed tight after adjustment of the camera over the lead glass (S). Once the position of the camera has been adjusted, the camera may be removed and remounted on the bracket without readjustment being necessary. In the front gable of the camera, which faces the roentgen tube is an opening (B) that can be closed with a sliding lid (D) manipulated by a rod (E). The lid is pressed against the gable by a spring (A). If the

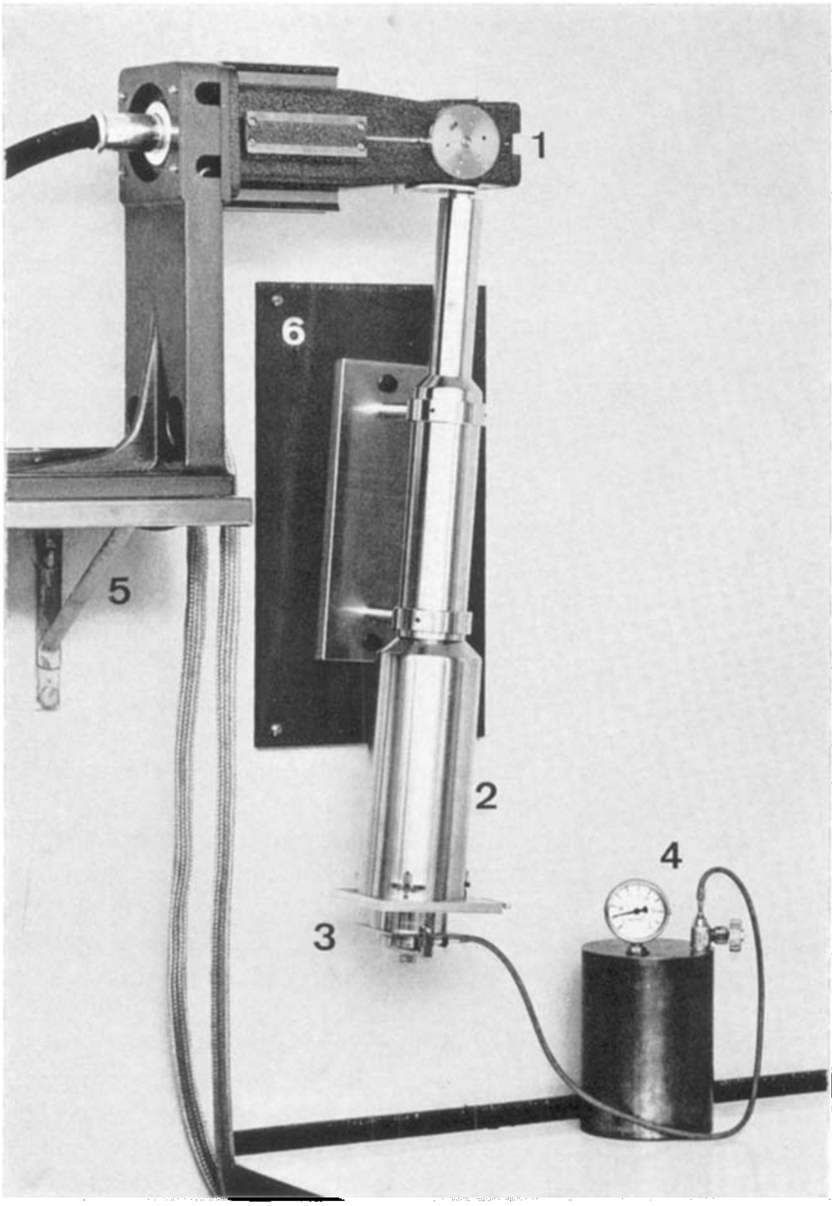


Fig. 5. Camera II for contact microradiography ready for use before a horizontal roentgen tube. Both the roentgen tube (1) and the nickel-plated brass tube (2) are fastened to the wall. The cassette with the object holder (3) are placed in position against the lower end of the brass tube and the object holder is connected with a vacuum container (4). The roentgen tube and the brass tube can be moved in relation to one another by adjusting the attachment of the former to the separate bracket (5) and/or the anchorage of the latter in the bakelite plate on the wall (6).

camera is pressed against the filter disc of the tube house the felt washer (C) around the opening (B) prevents the entry of light.

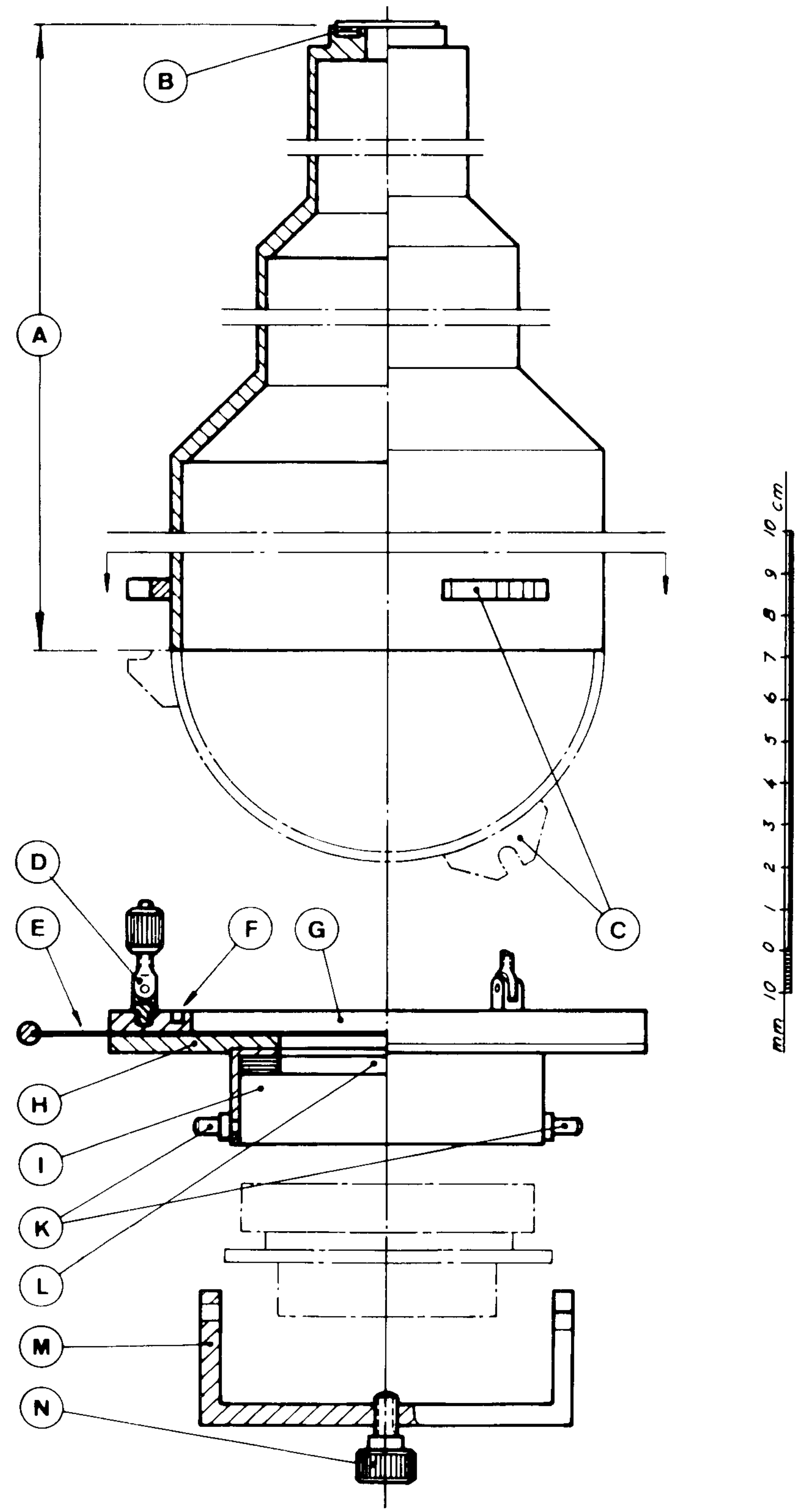
The camera is loaded in the dark room. After the specimen holder has been placed in desired position the lid of the camera is screwed tight and the opening (B) is closed with the sliding lid. The camera can then be exposed to daylight. When the camera has been mounted on the bracket and placed in position so that the felt washer lies pressed against the filter disc of the tube, the slide lid can be opened and the film exposed.

Camera II (Figs. 5 and 6) is used for a horizontal roentgen diffraction tube (1), Fig. 5, and is mounted on a wall. It consists of a 685 mm long nickel plated brass tube (2) and a cassette with a specimen holder (3). The holder, the same as for camera I, forms the bottom of a light-proof cassette, which is fastened to the lower end of the tube. Mounting of the tube on the wall allows adjustment of the positions of the tube relative to the window in the roentgen tube. In addition, the position of the roentgen tube can be changed because it is mounted on a separate bracket. The tube (A), Fig. 6, is in reality relatively longer than that shown in the figure. The upper (narrow) end of the tube (A) has a felt washer (B), which prevents the entry of light when the tube is pressed against the filter disc of the roentgen tube.

Since the roentgen beam used is divergent, the diameter of the tube cylinder has been given a longer diameter at the lower than at the upper end. The cassette consists of two rectangular plates, an attachment plate (G) and a back plate (H), with a cylinder (I) and a sliding lid (E). In the attachment plate are three hinged screws (D) with bolts.

The attachment plate has a circular perforation (90 mm in diameter) of the same diameter as the beam. Concentrically around this hole is a track (F) shaped to receive the end of the tube. The track guides the cassette into correct position when it is fastened to the tube and provides a light-proof connection. The cylinder of the back plate guides the specimen holder.

The holder delineated by the dot-dash sequence in the drawing, is loaded in the dark room and then inserted into the cylinder (I) and fastened with a stirrup (M). This stirrup is mounted


A
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on the pivots (K). With the screw (N) it presses the specimen holder so that the guide plate abuts on the edge of the cylinder. The holder is then in proper position.

In the slit between (G) and (H) is the slide lid (E). The lid closes the opening in the cassette over the specimen so that no light can enter. A felt washer (L) also prevents the entry of light. All further work can be done in daylight.

The cassette is now mounted on the tube in the way described. The three hinged screws are placed into slots in the three attachment flanges (C) and the bolts are tightened. The cassette is then firmly fastened in position with a target-film distance of 750 mm giving a diameter of the roentgen field of 90 mm. When the slide lid has been withdrawn to a stop position the camera is ready for exposure.

Camera for Philips CMR-5 apparatus

Camera III (Figs. 7 and 8) consists mainly of an upper part (A), Fig. 8, and a bottom plate of duralumin (F). The upper part has a flange (L) for attachment to the horizontal tube house of Philips CMR-5 apparatus. The camera can be mounted with or without O-ring. The taget-film distance can be varied by placing an intermediate tube of the desired length between the roentgen tube house and the camera. With the construction described the target film distance is 46 (without intermediate piece) and 66 mm respectively giving field size of 10 and 14 mm.

The upper part is equipped with a nozzle (N) for connection with a vacuum pump. An O-ring (E) is placed in a track in the bottom plate to secure air-tight connection between the bottom plate and the upper part.

The size of the irradiated field at different target-film distances is marked on the inner surface of the bottom plate. The photographic plate and the specimen are placed directly on the bottom plate in the outlined field.

The camera can be used in two ways. Air may be withdrawn from the path of the beam, i.e., in the camera and also in the continuous air space extending to the roentgen tube. The O-ring is placed between the camera and the roentgen tube house. Alternatively air may be withdrawn only from a part of the camera.

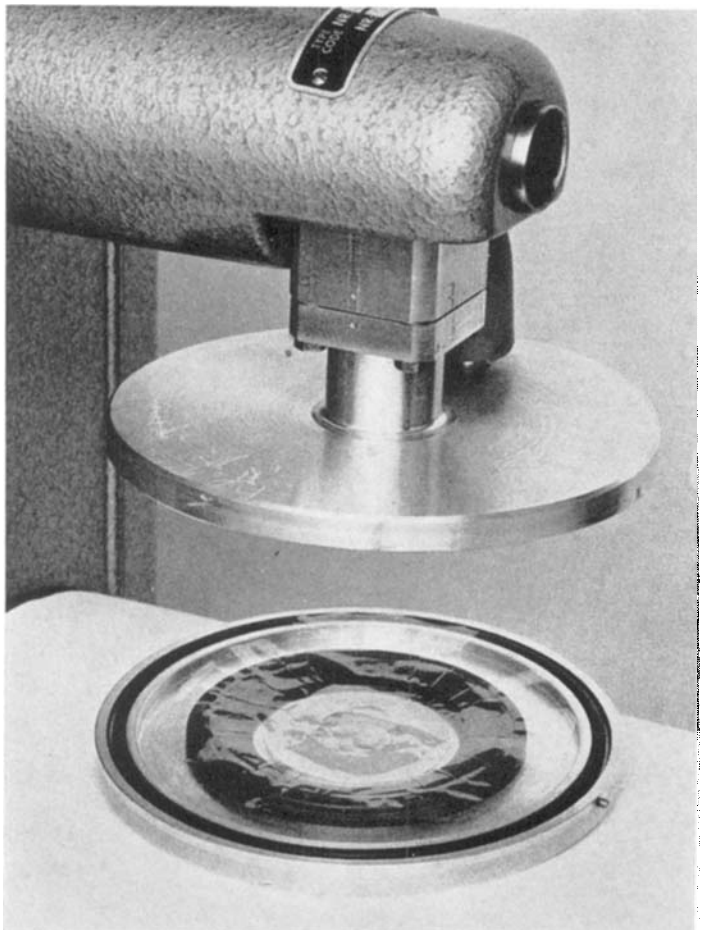

Fig. 7. Bottom plate of Camera III. Supporting ring and O-ring in position as well as film, object, protective ring of glazed cloth and Mylar film. Roentgen field size marked in centre.

The O-ring between the camera and the roentgen tube should be removed to allow the entry of air. A Mylar film (B) is placed between the upper part of the camera and the O-ring (C) which is inserted in a supporting ring (D). The latter is pressed by the bottom plate of the camera against the O-ring. The underneath surface of the supporting ring has four radial gutters. When the camera is connected with a vacuum pump, the air between the O-ring (C) and (E) is withdrawn as well as that under the Mylar

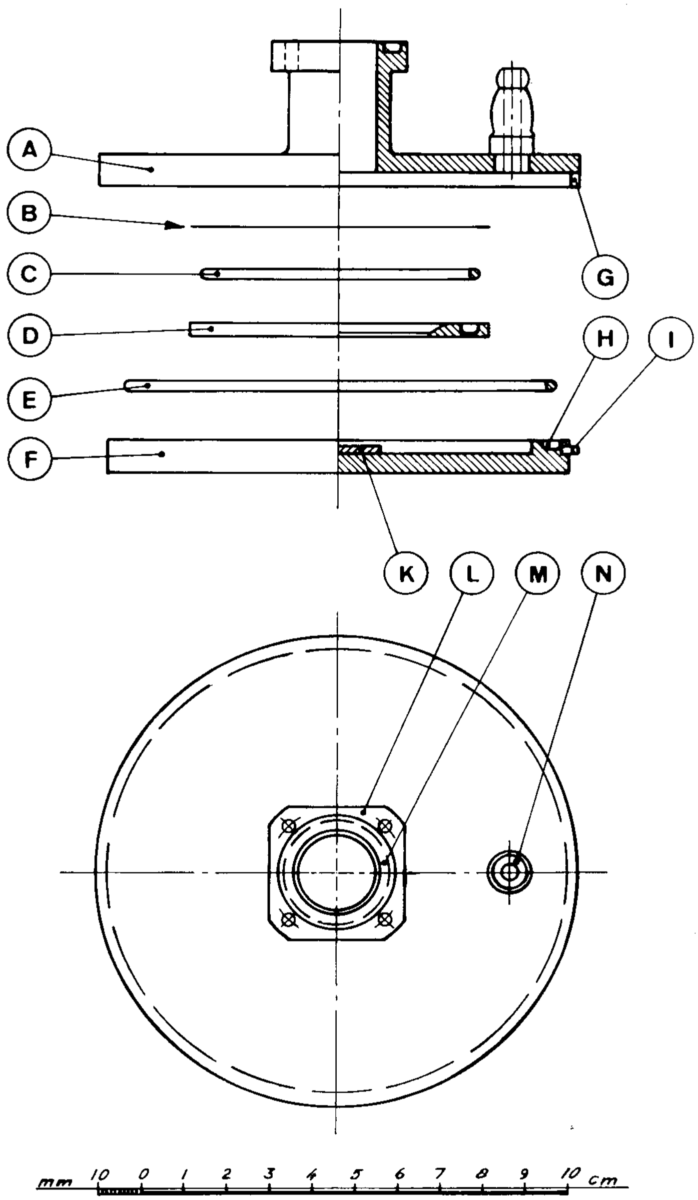

Fig. 8. Camera III for Philips CMR-5 apparatus. For details see text.

film the latter via the radial gutters in the supporting ring. This presses the Mylar film against the specimen and the photographic plate (K). Both when the camera is used with and without the ring (D), the negative pressure in the camera is sufficient to hold the bottom plate in position and exert the necessary pressure against the O-rings to prevent the entry of air.

The bottom plate has a guiding pin (I) shaped to fit a track (G) in the upper part. The CMR-5 apparatus has to be loaded in the dark, since its camera has no shutter.

DISCUSSION

The combination of specimen holder and camera should satisfy the following requirements:

1. There should be no leakage of roentgen radiation, or visible light.
2. The combination should be detachable and portable. (This requirement is less important if the roentgen apparatus is installed in a dark room).
3. The target-film distance should be variable.
4. If the position of the specimen holder relative to the source of radiation is not fixed the position of the camera should be adjustable under direct observation of the irradiated field.
5. The object holder should allow contact between the specimen and the photographic material during exposure.
6. Examination of specimens of varying sizes should be possible, as well as stereoscopic microradiography.

Philips CMR-5 apparatus has many advantages but will not allow examination of specimens more than 3 mm in diameter. Larger specimens must therefore be cut into pieces. *Bhussry & Parikh* (1962) designed a specimen holder with a longer target film distance and thereby with a larger irradiated field. Their construction, which was used by *Scott, Nylen & Pugh* (1962), had no special arrangement for enabling fixation of the specimen against the photographic plate. In our construction for CMR-5 as well as for Philips diffraction tubes we have utilized *Sherwood*'s idea of holding the specimen against the plate by means of a vacuum holder. This does not allow evacuation of the air between the window of the tube and the Mylar film. We have not considered evacuation obligatory because the specimen is mineralized and the CMR-5 apparatus is operated near maximum voltage (5 kV). As a consequence the exposure time is, of course, prolonged. The exposure times used for mineralized objects — bone and dental tissue, 20—80 μm, have varied between 1 hour and 48 hours. The apparatus is air-cooled by means of an ordinary fan placed before a vent in the back of the apparatus.

The greater the target film distance the less the geometric penumbra. With preparations of the above mentioned thickness and such a long target film distance as that allowable by the CMR camera the geometric penumbra is negligible because it cannot be resolved by film emulsions or by the microscope.

In qualitative contact microradiography of hard tissue we make the first exposures with Ni-filtered radiation from a copper target using one of the three targets to film distances in Camera I. A series of exposures are made of each specimen so that even the most radiopaque parts of the specimen (e.g. fully mineralized dental enamel) will give an image where the photographic density is at least 1.

This is necessary if small differences in mineralization are to be discernible. When higher contrast is desired we make a series of exposures with the low energy radiation from the CMR-5 apparatus. With a high tension of 4.8 kV the maximum intensity is about 3 keV, i.e. on the high absorbing side of the K-absorption edge of calcium. This radiation gives not only better definition due to higher subject contrast but also sharper images than with the Ni-filtered copper radiation because of the decreased scatter of the photo-electrons in the photographic emulsion.

In quantitative microradiography it is important to keep the variation of the intensity in the irradiated field within reasonable limits. When using the three target film distances in Camera I the differences in intensity were found to be considerable even between adjacent parts of the field. To minimize this source of error Camera II was designed with a target film distance of 750 mm. This distance was chosen in order to keep the exposure times within reasonable limits. Fig. 9 exemplifies the variation of the blackening due to variation of the intensity of the radiation. The figures denote the photographic density.

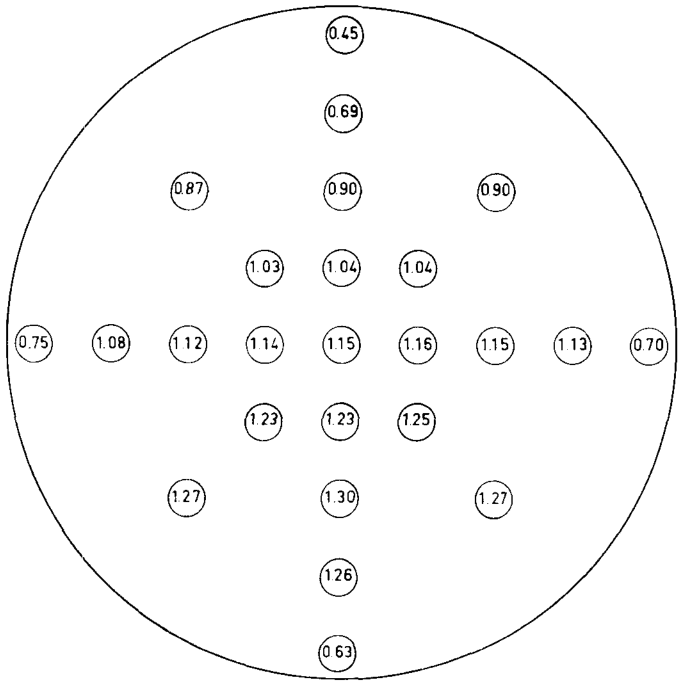

Fig. 9. Example of varations in radiation intensity within the irradiated field as illustrated by figures of photographic density. *Ilfex X-ray film* (Ilford).

SUMMARY

Detailed descriptions are given of three cameras with specimen holders for contact microradiography. The cameras, which are improvements on present constructions have been used in microradiographic studies on mineralized tissues.

RÉSUMÉ

PORTE-OBJEKT ET APPAREIL DE PRISE DE VUE POUR MICRORADIOGRAPHIE PAR CONTACT

Les auteurs donnent la description détaillée de trois appareils de prise de vue comportant des supports pour les préparations pour microradiographies par contact. Les appareils de prise de vue, perfectionnement des constructions actuelles, ont été utilisés au cours d'études microradiographiques de tissus calcifiés.

ZUSAMMENFASSUNG

OBJEKTHALTER UND KAMERAS FÜR DIE KONTAKTMIKRO-RADIOGRAPHIE

Drei Kameras mit Präparathaltern für die Kontaktmikroradiographie wurden detailliert beschrieben. Die Kameras, die Verbesserungen früherer Konstruktionen sind, wurden für mikroradiographische Studien mineralisierter Gewebe angewandt.

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