

The logo consists of the word "Leitz" in a white, elegant script font, with the word "WETZLAR" in a smaller, white, sans-serif font directly below it. Both are centered within a solid black rectangular background.

INSTRUCTIONS

**Ultra-Microtome
Type Fernández-Moran**

ERNST LEITZ GMBH WETZLAR

53-11 a/Engl. R

Instructions

Ultra-Microtome, Type Fernández-Morán
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Ultra-Microtome, Type Fernández-Morán

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1. General information

The instructions for use describe the assembly and operation of the Fernández-Morán Type Ultra-Microtome, but it is not intended as a text book for ultra-microtomy. It is therefore advisable first to study the relevant technical literature thoroughly, since satisfactory sections can only be obtained with exact knowledge of the preparation technique. Those who already possess experience in ultra-microtomy should remember that certain factors such as the hardness of the object block, the cut of the specimens, shape and size of the glass knife etc. have to be adapted to the operating principle of the ultra-microtome to obtain successful working conditions.

Our microtome laboratory will be glad to supply information at all times regarding matters connected with preparation and cutting technique. If required, instruction on the instrument can also be given there.

2. Assembly and operation of the ultra-microtome

2.1 Choice of site (Fig. 5)

The ultra-microtome should be set up in a room where the temperature varies as little as possible throughout the year. It will therefore be appreciated that conditions under which direct sunlight strikes the instrument or where there is considerable circulation of air are unsuitable. The ideal room is one which is free from vibration, is centrally heated in winter and has windows facing north. The best place in such a room is one where the necessary electrical sockets for the power supply are available (earthed socket, 110, 220 or 240 volts a.c., 10 amps., 50 cycles). The instrument should not be placed near a window or radiator and should be as far as possible from the door. Fig. 5 gives an example of the correct site for the ultra-microtome. A laboratory in which several persons are working or a connecting room should be ruled out as unsuitable from the start. Nor should the workroom be situated in a part of a building which is subject to heavy vibrations.

2.2 Microtome table and swivel arm (Fig. 6)

First screw the swivel arm (4) to the microtome table. This is used to hold the stereomicroscope and the lamp unit.

Screwed to the table surface is the mounting ring (43) with its holes (44) and (45). These correspond to the holes or threads (44') and (45') in the foot of the swivel arm. Place the swivel arm on the mounting ring as shown in Fig. 6 and screw it firmly in position by means of the socket head hexagonal bolts and wrench (33, Fig. 4a). Make certain that the pins (46) and (46') fit well and that the two long socket head hexagonal bolts are inserted through the holes (45) and (45'). The two short socket head bolts connect (44) with (44').

The swivel arm will now be in its proper position in relation to the subsequent position of the ultra-microtome. The locking mechanism of the

swivel arm should then be tested by moving it backwards and forwards. It should engage correctly in the position shown from Fig. 2.

The special table for the ultra-microtome (2, Fig.1) stands on rubber shock-absorbing feet and should be placed on a firm level surface. Any slight irregularities on the surface of the floor can be corrected by placing pieces of wood underneath the feet. There is a risk that the rubber feet will be torn off when moving the microtome table and care should therefore be exercised when setting up and aligning the table. After placing the table in position, test the lock to see if it works properly. The key for this lock will be found in the accessory compartment in the upper part of the packing case (47, Fig.8). If the upper drawer sticks, the table has not been properly set up. (The table is tilting forwards and must be corrected by placing pieces of wood beneath the feet).

2.3 Microscope and lamp unit (Fig. 7)

Figs. 7a and 7b

The compartment for accessories lies in the upper part (47, Fig.8) of the packing case and is rendered accessible by opening the lid marked with red borders. The lamp unit (10) and the stereoscopic binocular microscope (9) can then be removed. The lamp unit (Fig. 7a) consists of the fluorescent tube (10.a) with mount, the attached supply cable (10.b) and the dove-tail guide (10.c) with tilting device (10.d) for the microscope.

The stereoscopic binocular microscope (7b) consists of the prism head (9a), focusing knob (9d) the 18.5x paired eyepieces (9c) and the pair of objectives in slip-on mount (9b) with an initial magnification of 4x.

To facilitate alignment of the object block tip under the microscope in the preparations for cutting and to help in finding and recovering the sections which may float away to one side from the knife, the microscope can be tilted to one side with the swivel arm locked in position. The best way of doing this is to hold the focusing knobs (9.d) of the microscope tube in both hands and then tilt the microscope to the left or right, as required.

The lamp unit and microscope are secured to the swivel arm as shown in Fig. 7b. The supply cable is secured by means of the attached wooden sleeve in the hole (2.a, Fig.6) of the microtome table and is connected at (10.b) to the bridge (8.a) of the control unit (Fig.11). The free piece of cable between the hole in the table (2.a, Fig.6) and the control unit connection (8.a, Fig.11) should be laid in the groove (2.g, Fig.10) provided for this purpose beneath the table surface.

2.4 Unpacking and assembly (Figs. 8, 9, 10, 11)

- a) Remove rotor (11), heating rod (17), support drive (14) and the other accessories (see Fig.8) and the enclosed wrenches (17 mm and 10 mm) from the packing case (Fig.8).

NOTE: THE SIDE JOURNALS OF THE ROTOR ARE ALREADY PROVIDED WITH A SPECIAL GREASE FOR THE ROTOR BEARING AND ARE MERELY COVERED BY TWO STRIPS OF OIL PAPER. SIMPLY REMOVE THIS OIL PAPER BUT DO NOT CLEAN THE ROTOR JOURNALS.

IN EXCEPTIONAL CASES STRIPPING OF THE OIL PAPER FROM THE JOURNALS MAY RESULT IN A CONSIDERABLE AMOUNT OF THE GREASE BEING REMOVED AND THIS SHOULD BE RENEWED BY APPLYING A THIN FILM OF GREASE. SEE ALSO SECTION 4.2.

- b) Lift off the upper part of the packing case after loosening the side retaining screws (Fig. 8).
- c) First free the microtome base by removing the retaining strips (47.a) (17 mm bolt) and (47.b) (10 mm bolt). Place the microtome carefully on its table as shown in Fig. 2, and draw the connecting cables (1.a, Fig. 9) through the holes (2.a, Fig. 6) of the microtome table the left cable through the left hole, the right cable through the right hole. Now draw the drive belt (16) through the longitudinal slit (2.b) of the table surface (Figs. 3 and 6), place the microtome on the retaining screws (2.d) and make certain that these screws engage exactly in the rubber buffers on the base plate of the microtome.
- d) Unscrew the rotor protective caps (12.a, Fig. 2 and 9a), using wrench (32, Fig. 4a), and draw the drive belt outwards over the left rotor bearing as shown in Fig. 9b. Place the rotor carefully in its bearing as shown in Fig. 9b.

Caution: Place the rotor gently in position so as not to damage the sapphire bearings!
(11.b)

Place the drive belt loosely in position and screw up the rotor protective caps again.

When screwing on the rotor protective caps make certain that these are not too loose nor too firmly in position.

UNDER NO CIRCUMSTANCES SHOULD THE MICROTOME BE LIFTED OR PUSHED SUBSEQUENTLY. NOTE THE FOLLOWING POINTS IF REPACKING IN THE CASE: PLACE THE ROTOR (11) CORRECTLY IN ITS RELEVANT COMPARTMENT OF THE PACKING CASE, PACK THE SUPPORT DRIVE (14) SEPARATELY. SEE THAT THE BELT (16) AND SUPPLY CABLE (1.a) ARE IN THE SAME POSITION AS SHOWN IN FIG. 9a.

- e) Connect the supply cable for heating and alternate drive (1.a, Fig. 9a) as described in section 2.3 to the bridge (8.a) of the control unit (Figs. 10 and 11), screw in position with attached milled ring and place beneath the table surface (2.g, Fig. 10). The plugs of (1.a, Figs. 9 and 11) are not homopolar, and it is therefore necessary to see that the plugs and socket are poled correctly when making the connection.
- f) Secure the coarse and fine control for the support drive (14, Figs. 2 and 8) (27 and 28, Fig. 3) and heating rod (17, Fig. 3 and 8) to the microtome in the following manner: insert the support drive in the hole

provided (see Fig. 3) as far as it will go and secure with hexagonal bolt (30). If the heating rod was packed separately, unscrew the milled nut at the rear end of the rod and insert the heating rod in the diametral opening of the microtome rotor so that the socket head hexagonal bolt (17, Fig. 3) points upwards at the front end of the rod: the contact pins at the rear end of the heating rod will then engage in the corresponding contact slits of the rotor.

WHEN INSERTING THE HEATING ROD THE LEFT HAND IS USED TO HOLD ITS FRONT END AND TO PUSH THE ROD IN AS FAR AS IT WILL GO. THE BEST POSITION FOR THE ROTOR IS SO THAT THE HEATING ROD ENTERS IT OBLIQUELY FROM THE FRONT AND TOP. THE ROD SHOULD THEN BE TIGHTENED UP BY MEANS OF THE MILLED NUT. THIS MUST BE CARRIED OUT CAREFULLY TO AVOID LIFTING THE ROTOR FROM ITS BEARINGS AND POSSIBLY DAMAGING THE SAPPHIRE BEARINGS.

- g) Take a pair of carbon brushes (39, Fig. 4b) from the accessories, unscrew the black retaining screws on the left protective cap of the rotor (12.a, Fig. 2) and insert the brushes in the holes provided. This ensures current supply to the heating rod.

2.5 Motor drive (Fig. 12)

Assemble the motor drive (3) as shown in Fig. 1 beneath the microtome table. Place the drive belt (16) over the plastic-covered pulley wheels of the microtome and motor and carry out coarse alignment of the motor drive by moving the base. The drive belt and the drive pulley should be in line.

Adjust the belt tension as loosely as possible by setting (3.a, Fig. 12). Connect supply cable (3.b) with bridge (8.a, Fig. 11) of the control unit (see also sections 2.3 and 2.4).

This completes all the connections at the instrument end.

The opening in the table should now be closed with the cover (2.f) (Fig. 10), guiding two cables each through the open grooves at the side.

2.6 Working hood (Fig. 4a)

The lower part of the plexiglass hood (5, Figs. 1, 2 and 4a) is placed over the microtome after first swinging out the swivel arm to one side. The metal angle (2.e, Fig. 6) attached to the surface of the table hold it in its prescribed position. The front part which was removed can now be placed in position.

The instrument is for alternating current 110/220 and 240. In general the electrical unit is adjusted for the existing Voltag as ordered (check packing note). For alterations loosen retaining screws (8h, Fig. 13) and remove control unit. On the rear of the unit Fig. 17 adjust red lacquered voltage setting screw with indicator for 110, 220 and 240 V in accordance with the existing mains supply.

The instrument should then be connected to the mains supply from the socket (6, Fig. 1) using the supply cable (40, Fig. 4a). The ultra-microtome is then ready for use.

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- b) Lift off the upper part of the packing case after loosening the side retaining screws (Fig. 8).
- c) First free the microtome base by removing the retaining strips (47.a) (17 mm bolt) and (47.b) (10 mm bolt). Place the microtome carefully on its table as shown in Fig. 2, and draw the connecting cables (1.a, Fig. 9) through the holes (2.a, Fig. 6) of the microtome table the left cable through the left hole, the right cable through the right hole. Now draw the drive belt (16) through the longitudinal slit (2.b) of the table surface (Figs. 3 and 6), place the microtome on the retaining screws (2.d) and make certain that these screws engage exactly in the rubber buffers on the base plate of the microtome.
- d) Unscrew the rotor protective caps (12.a, Fig. 2 and 9a), using wrench (32, Fig. 4a), and draw the drive belt outwards over the left rotor bearing as shown in Fig. 9b. Place the rotor carefully in its bearing as shown in Fig. 9b.

Caution: Place the rotor gently in position so as not to damage the sapphire bearings! (11.b)

Place the drive belt loosely in position and screw up the rotor protective caps again.

When screwing on the rotor protective caps make certain that these are not too loose nor too firmly in position.

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- e) Connect the supply cable for heating and alternate drive (1.a, Fig. 9a) as described in section 2.3 to the bridge (8.a) of the control unit (Figs. 10 and 11), screw in position with attached milled ring and place beneath the table surface (2.g, Fig. 10). The plugs of (1.a, Figs. 9 and 11) are not homopolar, and it is therefore necessary to see that the plugs and socket are poled correctly when making the connection.
- f) Secure the coarse and fine control for the support drive (14, Figs. 2 and 8) (27 and 28, Fig. 3) and heating rod (17, Fig. 3 and 8) to the microtome in the following manner: insert the support drive in the hole

3. Operation of the ultra-microtome

If the top drawer of the microtome table is drawn out the control unit for the motor, rod heating and illumination becomes accessible. This has the following controls (see Fig. 13):

- 8. b) Mains switch with pilot lamp and fuse; this turns the current on and off for the entire apparatus.
- 8. c) Switch for lamp unit
- 8. d) Switch and regulating knob for rod heating, also fuse and ammeter for the heating current
- 8. e) Switch (on/off) with pilot lamp and fuse for the entire motor drive
- 8. f) Selector switch for constant or variable speed drive
- 8. g) Regulating knob for high-speed motor on constant or variable speed drive
- 8. h) Retaining screws

Operation of the instrument is first checked by means of the controls (8. b - 8. d) to see that

the mains pilot lamp lights up,
the fluorescent tube lamp ignites,
the heating current is supplied, i. e. the ammeter deflects when (8. d) is operated.

SWITCH OFF THE HEATING CURRENT AFTER CARRYING OUT THIS TEST.

If the controls do not react in the manner described above, check the fuses, the fluorescent lamp, the electrical connections at the control unit (8. a, Fig. 11) and make sure that the heating rod and the contact carbons at the rotor are properly in position.

NOT UNTIL THIS INSPECTION HAS BEEN COMPLETED SHOULD THE MOTOR DRIVE AND RUNNING OF THE ROTOR BE CHECKED.

The front part of the plexiglass hood is always removed for this purpose and possibly the lower part as well.

The microtome rotor is then turned by hand to see that the heating rod can pass the microscope unhindered. If this is not the case, the position of the microscope should be altered accordingly by horizontal adjustment (4. a) of the swivel arm (Fig. 7 b).

Now switch on the motor drive by means of switch (8. e): If the selector switch (8. f) is pointing downwards, the rotor is running at a constant speed, i. e. the high-speed motor (3. f, Fig. 12) runs on its own and its speed is regulated by means of (8. g). With the selector switch pointing upwards, the motor is on the variable speed drive, i. e. the high-speed motor (3. f) and the slow speed motor (3. c, Fig. 12) work alternately. In this position a micro switch is operated and the high-speed motor is uncoupled electromagnetically for a given time by means of a trip cam on the right rotor journal (Fig. 14). It then free-wheels and the slow-speed motor causes the rotor to turn slowly through a given distance (cutting operation). The high-speed motor is then coupled up again and the microtome rotor revolves again at a higher speed (bringing the specimen round to the starting position). In this respect the

speed of the high-speed motor and that of the slow-speed motor can be regulated independently of each other by means of (8.g) and the regulating knob (3.d) respectively. The regulating range of the high-speed motor covers rotor speeds between 60 and 20 r.p.m. (see section 4.4). The slow-speed motor permits cutting speeds of between 3 and 50 mm/sec. The scale (3.e, Fig. 12) is graduated in the following manner: scale setting 0 = 3 mm/sec., every further scale division corresponds to an increase in speed of 3 mm/sec. Calibration of the scale (8.g, Fig. 13) in r.p.m. must be undertaken by the operator himself.

When checking the motor and operation of the rotor for the first time, it is usually found that the drive belt comes off the pulley wheel of the microtome rotor or of the motor. This is an indication that the drive is not exactly aligned with the microtome. The base of the motor should therefore be adjusted until the belt runs in line with the belt pulley. Care should be taken to see that the motor base does not touch the frame of the table and that the drive belt does not come in contact with the surface of the table or the base of the microtome. In some cases it may be necessary to place pieces of wood or, better still, fairly firm rubber washers underneath the base plate of the motor. If the floor is smooth it is not normally necessary to secure the motor base in any other manner, since it rests firmly on the ground as a result of its weight and rubber support. It can, however, also be secured firmly to the floor by means of a suitable adhesive.

If, when the ultra-microtome is operating on variable speed drive, the prescribed sector of the circular path to be described slowly by the specimen does not lie exactly adjacent to the knife, or the prescribed course of the cutting operation is disturbed in some way, the instrument should be readjusted to the normal position by removing the right rotor protective cap and carrying out the following corrections in accordance with Fig. 14:

- a) Loosen the locking screw (11.g) with a suitable screwdriver or piece of metal
- b) Adjust the rotor with the heating rod and specimen in position to the required distance from the blade of the knife (approx. 40 mm) and block the trip cam (11.h) in the position shown in Fig. 14 by means of (11.g)
- c) Replace rotor protective cap as described in section 4.1.

Check motion of rotor: The noise of the micro switch (11.i) indicating that the instrument has been changed over electromagnetically from the slow to the quick speed should not be heard until the specimen has passed the knife; otherwise the sections may be cut irregularly.

Afterwards the function of the rod heating should be checked again when the motor is running satisfactorily (on constant and variable speed drive) by setting the heating current to 0.5 amps. on the ammeter and watching the behaviour of the needle: If this flickers heavily, particularly when changing over from the variable speed drive, the contact carbons and collecting rings on the rotor should be cleaned as in section 4.

If it goes back to 0, pressure of the carbons on the rotor slipring is too weak and there is not enough contact. This can often be put right by

polishing the contact surfaces of the carbons on a piece of ground glass. In extreme cases the trouble can be remedied by breaking the solder connection between the carbon and end of the spring and then lengthening the steel spring by simply pulling the coils apart.

When the ultra-microtome is found to be operating satisfactorily, it should be run for a total of one hour (with occasional checks), first on constant and then on variable speed drive. It is also advisable to allow the instrument to run for five minutes on the drive required before beginning the daily work.

4. Maintenance of the ultra-microtome

- 4.1 After some time in operation it may happen that the needle of the ammeter varies considerably from the scale value set when the instrument is running with the rod heating switched on. This can be attributed to soiling of the collecting rings on the left rotor journal due to contact with the carbons. The collecting rings and the carbons should then be cleaned immediately. (See Figs. 15 and 16).

Remove the retaining screws (12.b) and the contact carbons (39) from the left rotor protective cap, loosen the two socket head hexagonal bolts (12.d) with the wrench (32, Fig. 4a) and remove the protective cap carefully, taking care not to damage the pins (12.e). The collecting rings (11.e) and the contact surfaces (39) thus exposed should then be cleaned with a rag soaked in gasoline. The protective cap is then replaced by adopting the following procedure: Slide the protective cap without contact carbons on to the bearing (so that the pins engage) and tighten up the socket head hexagonal bolts evenly so that there is no lateral play in the rotor bearing. Only then should the contact carbons be replaced. Note: The screw (12.f₁, Fig. 15) is used for lateral adjustment of the microtome rotor; the plate (12.f₂, Fig. 16) presses against the steel ball (11.f) of the rotor. In the same way the relief bearings (11.c) are adjusted and the arresting screws (12.g) are covered by sealing varnish.

UNDER NO CIRCUMSTANCES SHOULD THE ABOVE-MENTIONED PARTS BE ADJUSTED BY THE OPERATOR AT THE RIGHT OR LEFT BEARING, OTHERWISE NO GUARANTEE CAN BE GIVEN FOR THE CORRECT SETTING OF THE ULTRA-MICROTOME.

- 4.2 After long periods of idleness or under unfavourable climatic conditions it may happen that the microtome rotor turns jerkily when running slowly. In such cases it is necessary to lubricate the rotor journals with the special grease (41) supplied (Fig. 4a). This is done by removing the rotor protective caps (12.a, Fig. 3) and rubbing the running surfaces of the journals (11.d, Fig. 16) of the rotor firmly and evenly (preferably with your forefinger) with a little fat so that the coating is not too thick.

Note: It is advisable to remove the rotor from its bearings for this purpose. At the same time the sapphire bearings can be cleaned of old residues of grease with a soft rag soaked in gasoline.

EXTREME CARE SHOULD BE EXERCISED WHEN REMOVING AND REPLACING THE ROTOR, SO THAT THE SAPPHIRE BEARINGS AND THE RELIEF BEARINGS ARE NOT DAMAGED.

The new film of grease should be applied by turning the rotor by hand, bit by bit.

PLEASE NOTE THAT OIL OR ANY OTHER GREASE APART FROM THAT SUPPLIED BY US SHOULD NOT BE USED FOR LUBRICATING THE ROTOR BEARING UNDER ANY CIRCUMSTANCES. THIS WILL NOT ONLY DISTURB EVEN RUNNING OF THE ROTOR BUT MAY BLOCK IT COMPLETELY.

The rotor protective caps should be replaced as described in section 4.1.

- 4.4 The control unit does not contain any complicated fittings and repairs such as replacement of a resistance or fuse etc. can either be carried out by a technician from our agency or by a skilled mechanic. The control unit is removed from its drawer by undoing the screws (8.h, Fig.13), lifting the drawer from its arresting mechanism in the running rail and disconnecting the cable connection (8.i) as shown in Fig.17. When replacing the control unit care should be taken to see that the cable connection is secured by pushing out the bow-shaped spring (8.k). The wiring diagram shown in Fig.19 can be used as a guide. The adjustable resistance (8.l, Fig. 17) determines the regulating range for the constant speed drive motor (3.f, Fig. 12), knob (8.g, Fig. 13). If the existing current supply should make a correction of the regulating range necessary in some cases, this can be effected by adjusting the resistance after dismantling the control unit.

To replace the fluorescent tube lamp (10.a) it is advisable to dismantle the lamp unit as shown in Fig.7a. When changing the fuses in the control unit (see Fig.13) make sure that they are of the same type.

5. The production of ultra-thin sections with the Leitz Ultra-Microtome, Fernández-Morán Type
- 5.1 Preparation of the specimens; general instructions
- 5.11 Biological specimens should be embedded in methacrylate, Vestopal or Araldite (see section 7). The gelatine capsules used as embedding moulds should not exceed a capacity of 0.3 g (American No.2), as the specimen shells and the specimen clamp (35 and 34, Figs.4b and 4a) are suitable up to this size. Before tapering the specimen blocks they are secured in the specimen shell as shown in Fig.18 with "Technovit" cement (in solution and powder form, supplied by Messrs. Kulzer and Co./ Bad Homburg) in the following manner:
Before preparing the cement, the specimen block is shortened so that it projects approximately 3-5 mm out of the shell. Then stir the cement compound well (stir the powder into the solution until a glue-like consistency - not too thick - is obtained), pour it into the specimen shell until it is half full and insert the specimen block immediately. Wipe

off any surplus cement, holding the shell by means of pincers. After about 20 minutes the plastic cement will have hardened. If, however, the block is to be held at a given height with the pincers, the surplus cement is not wiped off but removed later when it has hardened with a metal knife. Following this the specimen shell with the specimen block cemented in position is placed in the specimen clamp and pointed. When placing the specimen in position make certain that it is at the correct angle. It should always pass through the knife at right angles, and the shell should therefore be adjusted accordingly.

ALTHOUGH THE PROCESS OF CEMENTING THE SPECIMEN INTO PLASTIC BLOCKS OF THIS KIND REQUIRES SOME TIME, WE RECOMMEND IT BECAUSE DIRECT CONTACT WITH THE BLOCK IN THE CLAMP IS THEN AVOIDED, WHICH COULD LEAD TO UNCONTROLLABLE ERRORS DUE TO PLASTIC AND ELASTIC DEFORMATION WHEN THE SPECIMEN IS ADVANCED (SEE SECTION 7).

The best method of removing the plastic blocks from the specimen shells is by knocking them out with a suitable object or by dissolving them out with acetone; the latter procedure should, however, only be adopted in the case of blocks which have been cut up. On the other hand, it is advisable to keep the specimen blocks in their cemented state if it is intended to make a collection of blocks.

The pointing or tapering operation is carried out under a binocular prism magnifier (approx. 30 x magnification) or under a stereoscopic binocular microscope, if greater magnifications are desired (e.g. for precise work). The pointing is carried out either from above (a procedure very frequently adopted) or from the side. The latter technique (see Fig. 20) has the advantage that a good pyramid is obtained in a simple manner and that, when subsequently placing the specimen clamp in position in the microtome, one surface of the pyramid is always facing the knife blade. This is important for successful ribbon cutting.

When pointing the specimen from the side, **one** hand is used to hold the specimen clamp horizontally under the binocular microscope whilst the other hand guides the injector type of razor blade used for cutting purposes vertically from the tip of the block through the plastic. In this way a "roof" is first cut by turning the specimen clamp by **stages** (Fig. 20, stages 2 and 3), finally converting this to a point (Fig. 20, stages 4 and 5). Extra hard blocks are given their basic pyramid form by careful filing.

NOTE: THE EDGES OF THE BLOCK SURFACE MUST BE CUT SMOOTH IN THE SAME WAY AS THE SURFACES OF THE PYRAMID.

Glass knives or diamond knives are suitable for biological specimens. Further particulars can be obtained from our list No. 53-12a/Engl. "Ultra-Microtome, Type Fernández-Morán". Attention is drawn, however, to the following important fact: Only specimens in relatively soft embeddings can be cut with a glass knife. On the other hand, both soft and hard embedded specimens can be cut with a diamond knife, and the advantage of good embedding hardness such as slight deforma-

tion during cutting is only obtained with a diamond knife. This is particularly apparent on polyester materials and on methacrylate embeddings, since these can be produced in very varying consistencies.

In general we recommend the following embedding hardness for biological specimens:

<u>Glass knife</u>	<u>Diamond knife</u>
Butyl/methyl-methacrylate 7:3 to 6:4 parts	Butyl/methyl-methacrylate 5:5 to 2:8 parts
Vestopal W (Kellenberger type)	Vestopal W with increased proportion of hardener or Vestopal H
Araldit (Glauert type et al.)	Araldit (Glauert type et al.)

- 5.12 Inorganic specimens are either cut direct or after previous embedding. The general rule is not to embed metals unless they are thin foils or wires. More compact specimens are either cemented into the specimen shells (see section 5.11) or they are shaped to suit the specimen clamp. Metal sections are produced from small block surfaces (max. 200 square micron). For this reason compact specimens should be turned to a pointed cone or embedded pieces of metal should be pointed in a suitable manner.

All other materials must be embedded. Methacrylate is best used for this purpose, attention being paid to suitable hardness of the block. The blocks are prepared in accordance with the instructions given in section 5.11 for biological specimens.

Satisfactory ultra-thin sections of inorganic materials can only be obtained with a diamond knife. The routine attainable cutting thickness for series of sections naturally depends on the nature of the specimen, the embedding and the size of the cutting area. On an average it lies between 300 and 500 A.V. with a maximum cutting area of 100 x 100 micron for metals and 200 x 200 micron for other materials.

5.2 Production and preparation of the glass knives

New glass knives must always be prepared shortly before the cutting operation, as much of their sharpness is lost when they are stored for long periods. Only strain-free surface polished plate glass of 5 mm in thickness should be used. This should either be purchased in strips 50 mm wide or suitable stocks can be prepared from a sheet of glass. The following tools are required for preparing glass knives:

- 1 pair of tile breaking pliers (Fig. 21a)
- 2 pairs of flat nose pliers, the jaws are covered with a lead foil (Fig. 21b)
- 1 steel wheel glass cutter (Fig. 21c)
- 1 ruler

First of all a strip of glass 40 mm in width is separated from the main sheet. This is performed by making a uniform and continuous score on the glass with the glass cutter, placing the glass with this score facing downwards between the jaws of the tile breaking pliers (so that the scored side rests on the protective rubber of the pliers) and breaking off the glass strip by exerting slight pressure with the pliers. Before

breaking off a knife from this strip of glass, it is scored with the glass cutter as shown in Fig. 22 at intervals of about 15 mm to a width of approx. 5 mm so that a piece of glass can be broken off with the flat-nose pliers as shown in Fig. 23. This gives a natural and clean edge from which the subsequent cutting blade can be formed.

A suitable template is then made from thick white cardboard as shown in Fig. 24, and the required dimensions of the glass knife to be broken off are noted on it. The piece of glass obtained as shown in Fig. 23 is then laid on this template so that, from the 1 mm mark above the point of intersection 1-1/2-2 in Fig. 24, the edge 1' in Fig. 25 runs downwards along the line 2-2. A line is then marked with the glass cutter in the direction of the arrows (3 and 4, Fig. 24), so that the subsequent knife (1) with the cutting edge (1') and the two pieces of waste glass (2) are obtained as shown in Fig. 25. These waste pieces of glass are now separated by means of the flat-nose pliers from the knife (1). The force exerted by the pliers for this purpose should be directed downwards at an oblique angle as shown in Fig. 23.

The blade of the knife is very important in obtaining satisfactory ultra-thin sections. Each glass knife should therefore be examined under the microscope to ensure that it is free from fissures. For this purpose a microscope with the ULTROPAK Incident Light Illuminator and a UO 22 objective and 10x eyepiece can be used. The knife is placed in plasticine on a metal specimen holder so that it is in the position shown in Fig. 26 in relation to the objective of the microscope. With the dark field observation obtained with the ULTROPAK Incident Light Illuminator slight adjustments can then be made to the position of the knife so that the cutting blade is well illuminated. It will then be possible to detect even very small fissures in the blade of the knife Fig. 27. In general, all glass knives will be discarded in which more than one third of the blade has become unusable due to fissures or small cracks in the glass. By making suitable marks on the side of the knife (with a crayon etc.) the position of the fissures can be indicated. This later serves as a guide in aligning the specimen to the part of the knife which is free of fissures.

For sectioning on the ultra-microtome, the glass knife is fitted with a collecting tray which can easily be made by attaching adhesive tape or Scotch tape round the glass knife as shown in Fig. 28. The tray need only be sealed with some paraffin at the place marked c in Fig. 28.

Before inserting the glass knife in the support of the ultra-microtome, the angle should be set to 0 on the scale (20.a, Fig. 29) and fixed with the lever (22). The glass knife is then placed in position so that the lower edge marked a in Fig. 28 is flush with the lower frontal edge of the knife clamp in the support (20).

The knife is then viewed from the side and aligned by means of the adjusting gage for glass knives (36, Fig. 4a and Fig. 30) in such a way that the surface marked (b, Fig. 28) is vertical and the blade of the knife is at approximately the height shown in Fig. 30 by the edge (36.a) of the adjusting gage. Then clamp the knife firmly on position by means of (21, Fig. 3). After carrying out this preparatory work, the glass knife holder is adjusted to a cutting angle of approximately 6-10° by means of the angle setting lever (22, Fig. 29).

5.3 Use and care of diamond knives *)

The production of diamond knives from naturally occurring diamond crystals requires great care and skill and involves a tedious grinding process. A diamond knife is therefore a product of very high quality and only skilled operators with suitable experience of diamond knives should use it.

NOTE : DIAMOND KNIVES ARE RELATIVELY STABLE TO THE FORCES EXERTED VERTICALLY TO THE BLADE DURING THE CUTTING PROCESS BUT POSSESS VERY LITTLE RESISTANCE TO FORCES EXERTED Laterally TO THE BLADE. EXTREME CARE SHOULD THEREFORE BE TAKEN WHEN CLEANING THE KNIFE, WHEN CUTTING THE SPECIMEN BLOCK (SECTIONS SHOULD NOT BE TOO THICK, UPPER LIMIT 0.5 micron) AND PARTICULARLY WHEN COLLECTING THE SECTIONS WITH THE COPPER NET.

The diamond itself is always held in a metal mount and the complete unit is generally referred to as a diamond knife. These knives are supplied cemented into a cutting trough (see Fig. 31) and they are packed in a special container for transportation.

The service life of a diamond knife blade depends on the properties of the material being cut. Other influencing factors are the cutting thickness and surface of the specimen. The latter is of importance in the case of biological materials, since to all intents it is possible to cut sections over the entire length of the diamond blade, which means that the whole of the blade is subjected to wear. In some cases, therefore, it will be necessary to decide on the size of the knife section to be used for cutting. For metal sections the maximum cutting size is fixed at 100 x 100 micron, as larger sections could not be produced in a rational manner due to the compression which occurs. Only when producing surface sections can the knife blade be used over its entire length.

Generally speaking, however, the diamond knives are not used over their entire width for cutting. With a new diamond knife it is therefore advisable to start at one side of the blade and to continue using it until signs of wear are apparent. As soon as this is seen, it is advisable to start using a new cutting section. Maximum wear on a cutting section of the knife can be recognized in the fact that the section is rendered useless by the serrations caused by the fine tooth effect of the blade. If the hole of the diamond knife shows signs of wear, it is necessary to regrind it.

Cleaning the diamond knife blade

The diamond knife blade should be carefully cleaned before each cutting operation. The best way to do this is first to clamp the section collecting tray or trough in the support of the microtome (see Figs. 3 and 29).

*) Further information on diamond knives can be obtained from our lists No. 53-12a/Engl. Ultra-Microtome, Type Fernández-Morán, and No. 53-14R/Engl. "Use and care of diamond knives".

A small pith rod should then be taken, tapered at one end, and moistened with absolute alcohol or, better still, with distilled water. This is then applied at right angles to one end of the cutting blade and drawn gently across the entire knife. After cleaning the blade of the diamond knife, it should be examined by means of the stereoscopic microscope attached over the microtome (switch on the illumination system). Under no circumstances should acetone, chloroform or similar solvents be used as a wetting agent, since this will affect the cementing substance used to seal the knife against the collecting trough.

The space at the side between the knife and the knife mount or section collecting trough is sealed with plastic cement so that no liquid can emerge from the trough. This sealing may become defective after long periods in use, in which case new cement can be placed in position by means of a pointed piece of wood to restore the sealing. See section 5.11 regarding preparation of the plastic cement "Technovit".

The collecting trough and diamond knife are fixed in the microtome support as shown in Figs. 3 and 29. When using diamond knives it is very important to choose the best cutting angle in order to produce successful work, and normally this lies between 2° and 5° . It is necessary to carry out trial cutting operations to find the best angle, and for this purpose the cutting angle should be set between 1° and 6° and a series of test sections cut (approximately 10 - 15 sections of equal thickness, thick sections not being included in the calculations. The collecting trough is fixed in position and the angle varied in practically the same manner as described for glass knives in sections 5.2 and 5.3 (parts 20, 20.a, 22, Figs. 3 and 29 respectively).

NOTE : WHEN THE DIAMOND KNIFE IS SUPPLIED ITS CUTTING ANGLE IS STATED. IF THE DIAMOND KNIFE SHOULD HAVE AN ANGLE OF 45° , THE FIGURES ON THE SCALE WILL GIVE THE DIRECT ANGLE OF SETTING WITH THE COLLECTING TROUGH IN POSITION! FOR LARGER CUTTING ANGLES A CORRECTION OF + 0.5 DIVISIONS ON THE SCALE WILL BE NECESSARY PER DEGREE.

SETTING and CUTTING ANGLES

Cutting angle	Scale value
45°	0.0 $^{\circ}$
46°	0.5 $^{\circ}$
50°	2.5 $^{\circ}$ etc.

When cutting is finished the diamond knife must again be cleaned carefully. The collecting trough can be left in the microtome support for this purpose, but after cleaning the diamond knife it should be kept in its carrying case until the next time it is used.

CARE SHOULD BE TAKEN TO SEE THAT NO UNAUTHORIZED PERSONS TOUCH THE DIAMOND KNIFE, PARTICULARLY ITS CUTTING EDGE. OTHERWISE THERE IS A RISK OF THE FINE BLADE BEING DAMAGED IN THIS MANNER.

5.4 Operation when cutting thin sections

Before commencing cutting, a brief check should be made on the instrument: Test the rod heating by operating the appropriate regulating knob. Switch on the illuminating system, make sure that the motor is running correctly and that the rotor turns properly:

- a) biological material and embedded, non-biological material which is not too hard:

The total time taken for the rotor to make one revolution should first be fixed to approximately 15 sec.; then start off by setting a cutting speed of about 5 mm/sec. by means of regulating knob (3.d) on scale (3.e) of the slow-running motor in Fig. 12 (setting of approx. 0.5 on the scale) and then adjust revolution as a whole by regulating the quick-running motor (3.f, Fig. 12) by means of potentiometer (8.g) on the control unit Fig. 13.

NOTE: THE REGULATING KNOB ON THE SLOW-RUNNING MOTOR MAY ONLY BE OPERATED WHEN THE MOTOR IS RUNNING.

- b) compact metal specimens or similar material which can only be processed at high cutting speeds:

In this case empirical regulation of the constant-speed drive is effected by means of the potentiometer (8.g) on the control unit Fig. 13.

After testing all these operations the motor drive and heating should be switched off again.

After first removing the front part of the plexiglass cover (5, Fig. 2), the pointed specimen block should be fixed in the clamping device of the heating rod (17, Fig. 3), using the socket head hexagonal wrench (32, Fig. 4).

After concluding these preparations, the following instructions should be followed carefully (see also Figs. 3, 7b, 29):

- 5.41 The microtome support without knife should first be pushed back as far as possible by means of the coarse adjustment (28) and the rotor set by hand so that the heating rod with the specimen pointing towards the support is approximately horizontal.
- 5.42 After swinging the observation microscope into position, align it by means of the horizontal control (4.a, Fig. 7b) so that the tip of the specimen block appears in the top third of the field of view in the microscope (see Fig. 32a): The microscope is focused by means of the control knob (9.d, Fig. 7b). If the tip of the object should be slightly to one side in the field of view, the position can be adjusted by undoing the locking screw (17) of the heating rod and adjusting the specimen holder accordingly, or by tilting the microscope tube slightly to one side (see section 2.3).
- 5.43 Secure the knife in the knife holder (20) of the support: Loosen socket head bolt (21, Fig. 3) with wrench (32, Fig. 4) and secure knife firmly in position; set the cutting angle of the knife with lever (22). See sec-

tions 5.2 and 5.3 for details of how to insert glass or diamond knives. Clean the blade of the knife.

5.44 Introduce the cutting blade carefully by means of the coarse adjustment (28) of the support device, at the same time watching it carefully in the field of view of the microscope, so that it enters the lower third of the field of view (see Fig. 32 b). Generally speaking, the blade of the knife is not in the focal plane set on the microscope and therefore appears as a blurred, light outline when entering the field of view: Bring the knife blade sharply into focus by means of the control knob (9.d, Fig. 7b) and then adjust the tip of the specimen to the same focal plane by turning the rotor by hand. Align the cutting blade of the knife to the specimen by operating the lateral adjustment (25, Fig. 3) of the support after first loosening lever (26). Arrange the knife blade and the specimen surface parallel to each other by loosening lever (24) on the swivelling support section (23).

5.45 Remove the specimen downwards away from the knife. Then fill the collecting trough with distilled water (finely graduated pipette) until the liquid is on a level with the knife blade. By careful addition or removal of distilled water set the level of the liquid so that the surface of the water near the blade reflects brightly when the illuminator is switched on and it is viewed through the microscope. It is important to set the reflection in this manner to produce the interference colours of plastic sections; the thickness of the section can then be estimated from the colours.

During this preparatory work the horizontal adjustment control (4.a, Fig. 7b) of the microscope must not be moved to any appreciable degree, otherwise the revolving specimen may subsequently be damaged.

5.46 Introduce the tip of the specimen to the field of view of the microscope again (by turning the rotor by hand). The tip of the specimen and the knife blade should then still be in the upper and lower third of the field of view respectively. Now exercise constant microscopic control and advance the knife carefully by means of the coarse adjustment (28, Fig. 3) of the support device until it is directly in front of the tip of the specimen (make certain that the microscopic image is properly focused). The tip of the specimen should not touch the knife (see Fig. 32).

5.47 Make certain that the arresting levers (22, 24 and 26, Fig. 29) are tightened up. The arresting lever (15) for the coarse and fine adjustment of the support device should, however, not be arrested during cutting or only slightly.

Switch on the motor drive and heating (see section 3 and Fig. 13), look through the microscope and advance the knife slowly into the path of the specimen by operating the support fine adjustment (27) until the first sections reach the surface of the water: If a complete surface has to be cut from a specimen block, it is advisable to disconnect the coupling for the variable-speed drive switch (8.f, Fig. 13) and to cut with the high-speed motor. Great care should however be exercised in order

to protect the blade of the knife. Do not cut thick sections and, if necessary, alter the cutting position of the knife later.

- 5.48 After cutting the specimen by hand as described in section 5.47, allow the thermal advance system of the specimen to operate. Place the front part of the plexiglass cover in position. The sections will then appear one after the other in a ribbon on the water and will show interference colours.
- 5.49 The automatic cutting operation should now be observed to check the uniformity of the cutting sequence and the smoothness of the individual sections. When using glass knives, important points are the condition of the knife blade, or for diamond knives the correct choice of the blade angle. If any lack of uniformity is present, switch off the motor and push back the support device, then change the section of the knife used for cutting or the angle of the knife.

Another important point is the speed of the specimen: This should therefore always be adjusted by means of the regulating knob (3.d, Fig. 12) on the slow-running motor (biological specimen) or regulated by means of the potentiometer (8.g, Fig. 13) on the control unit (metals etc.), and then the effect should be observed. Each specimen has its best cutting speed.

It is often a help to remove the first rather thick sections by means of a small pointed stick such as a toothpick, but without damaging the knife.

Uneven sections may result from the embedding material being too soft or of varying softness or even defective; the specimen should therefore always be checked (see section 6).

If the sections are uniform but too thick, either the cutting speed or, better still, the rod heating should be varied, if permissible (see above).

It is necessary for the operator of the ultra-microtome to establish the best conditions as regards section thickness in relation to the circumstances resulting from embedding and the specimen itself. In the case of embedded specimens it is therefore necessary to use what is known as an empty specimen block, i.e. only the actual embedding material is cut. This eliminates all errors caused by the specimen and helps to familiarize the operator with the ultra-microtome and its reactions to variation in the heating, cutting speed, quicker operating speed etc.

To determine the rod heating required for a given section thickness, it is necessary to cut a series of sections at different heating stages and a constant rotor speed, and then compare the sections obtained.

THE ROD SHOULD BE ALLOWED TO COOL DOWN AFTER CUTTING EACH SERIES OF SECTIONS, THE COOLING TIME ALWAYS BEING EQUAL TO THE HEATING UP TIME. ON THE OTHER HAND, THE HEATING RODS CAN ALSO BE EXCHANGED TO RENDER THE OPERATOR INDEPENDENT OF COOLING TIMES.






The section thickness is dependent not only on the rod heating but also on the speed of the rotor. After finding out the best specimen speed, the r.p.m. of the rotor should be adjusted by regulating the quick-running motor.

The following figures are provided as a guide for regulating the microtome:






Biological and non-biological embedded materials		Compact materials such as metals etc.	
Rotor r.p.m. (quick + slow)	Initial heating	Rotor r.p.m. (quick only)	Initial heating
6 r.p.m./min	0.4 amps.	30 r.p.m./min	0.8 - 1 amps.
3 r.p.m./min	0.4 amps.	40 r.p.m./min	1.5 amps.

The cutting thickness can then be estimated easily by means of the interference colours which occur *) (for correct illumination see section 5.45).

Methacrylate

dull grey		300 Å
grey		300 - 500 Å
silver grey		500 - 700 Å
white		700 - 900 Å
yellow		900 Å

Polyester resin (e.g. Vestopal)

grey		300 Å
silver grey		300 - 500 Å
white		500 - 700 Å
yellow		700 - 1400 Å
copper		1400 Å

Metal sections which do not show colours of thin ribbons can only be judged for their thickness after viewing them through a microscope. If the section appears to be brown in transmitted light at a magnification of 200x, it will be suitable for examination under an electron microscope.

Before judging the section thickness, sections of specimens embedded in plastic have to be stretched. Chloroform vapour is used for stretching the methacrylate or polyester sections: A piece of filter paper soaked in chloroform or a camel hair brush saturated with chloroform is applied to the sections floating on the water, whilst observing them through the stereoscopic microscope, and the chloroform vapour is allowed to take effect, only briefly on methacrylate and considerably longer on polyester.

Like ultra-thin sections, metal sections are also compressed. Until now, however, no suitable method of stretching them has been discovered.

5.5 Removal of the sections

When transferring the sections to the normal type of coppergrid-specimen holder *) used today in electron microscope technique, the ultra-thin sections are manipulated on to it in such a way as to protect the blade of the knife. Using fine watchmaker's pincers the grid with

*) Supplied by Messrs. Hölscher KG., Hamburg-Wellingsbüttel

its filmed side (formvar or collodium film, see REIMER, 1959) is brought close to the blade of the knife and over the sections so that these can be seen through the mesh of the grid. Then approach the surface of the water closely until the sections and grid touch: Forces of adhesion press the sections to the surface film of the grid and prevent them from floating off; they can then be removed without damaging them.

TAKE GREAT CARE TO SEE THAT THE BLADE OF THE KNIFE IS NOT TOUCHED. THIS IS PARTICULARLY RISKY IN THE CASE OF DIAMOND KNIVES AND CAN EASILY CAUSE FISSURES.

6. Sources of error when cutting

- 6.1 The possibility of error is least with the ultra-microtome itself, as all instruments are carefully tested before leaving our factory for satisfactory operation and reproducible uniformity of the specimen feed when cutting. In spite of this, it is advisable to take the precaution of testing the instrument with the following specimens in the event of faulty results being obtained:

<u>Biological specimens</u>		<u>Non-biological specimens</u>	
Glass knife	Diamond knife	Diamond knife	
1) Methacrylate butyl/methyl 7:3	1) Methacrylate butyl/methyl 2:8	1) Specimens embedded in methacrylate butyl/methyl 5:5 to 2:8	
empty block! cutting area approx. 0.2 x 0.2 mm	empty block! cutting area approx. 0.2 x 0.2 mm	empty block! area approx. 0.2 x 0.2 mm	
2) Polyester empty block area approx. 0.2 x 0.2 mm	2) Polyester empty block area approx. 0.2 x 0.2 mm	2) Metals Pure aluminium area approx. 50 x 50 micron	

Characteristic features of errors which occur in spite of satisfactory operation of the ultra-microtome are as follows:

- 6.2 Compression of individual sections or folds in them: These result from setting the blade at an incorrect angle, from embedding errors, an excessive specimen area, from securing the collecting trough incorrectly and from not arresting the controls for the support sufficiently: They may also be due to excessive cutting speeds (e.g. in the case of embedded biological specimens).
- 6.3 Visible oscillation marks in electron microscopy (so-called "chatter" = strips vertical to the direction of cutting), visible either only in the specimen part of the section or also in the specimen and surrounding methacrylate. The first error is due to excessive differences in hardness between methacrylate and specimen on the one hand (vary the embedding) and to varying regions of hardness in the specimen itself on the other hand (including embedding errors). If, however, these chatter marks

run across the specimen and surrounding embedding medium, this can be due to one of several causes: either the specimen block has been incorrectly secured to the microtome, the support members have been insufficiently arrested, the cutting angle of the knife is wrong or the pyramid of the block has been incorrectly prepared (too slender). Incorrect cutting speeds or excessive cutting areas on embedded biological specimens can also be a reason.

- 6.4 Tearing of the sections. Cause: faulty embedding (bubbles in the specimen block or inside the specimen are often an important factor in such cases), block not cut correctly (roughened edges at the surface of the block), marked difference in hardness between specimen and embedding material, hard places in the actual specimen, residues of specimen on the knife blade, fissures on the knife blade.
- 6.5 Irregularities in section thickness. These result from embedding material which is too soft, an excessive cutting area, faulty clamping (see 6.1 and 6.2), excessive rotor speed (e.g. for metal sections) and too much variation from the room temperature (draughts).
- 6.6 When cutting methacrylate or polyester, the biggest source of error lies in the embedding material itself (see WALTER 59 and 61). Since the plastics are elastic and, for example, methacrylate also exhibits certain flow tendencies even at room temperature due to its thermoplastic properties, deformation of the specimen block occurs during every cutting operation. In the case of very large cutting areas, this may become apparent by occasional omission of a section or by irregular section thickness. The property of these embedding materials to swell slightly on contact with water is also responsible for the phenomena mentioned. If errors of this kind should become too pronounced, it is advisable to reduce the cutting area of the block as far as possible under the prevailing conditions of examination.

7. Bibliography

Borsysko, E., Phasecontrast microscope study of gross structural changes occurring the preparation of cells for thin sectioning by means of the methacrylate embedding technique. *J. Appl. Phys.* 26, 1394 (1955)

Fernández-Morán, H., A new microtome with diamond knife. *Industr. Diamond Rev.* 16, 128 (1956) und *Mikroskopie* 12, 81 (1957)

Borysko, E., Applications of a diamond knife for ultra-thin sectioning to the study of the fine structure of biological tissues and metals. *The Journal of Biophys. and Biochem. Cytol.* 2, 29 (1956)

Fernández-Morán, H. and A. Engström, Ultrastructural organization of bone, *Nature* 178, 494 (1956)

Glauert, A.M. and R.H. Glauert, Araldite as an embedding medium for electron microscopy. *J. Biophys. Biochem. Cytol.* 4, 191 (1958)

Kellenberger, E., L'utilisation d'un copolymère du groupe des polyesters comme matériel d'inclusion en ultramicrotomie. *Experientia* XII, 421 (1956)

Luft, J.H., Permanganate - A new fixative for electron microscopy. *Biophys. Biochem. Cytol.* 2, 799 (1956)

Palade, G.E., A study of fixation for electron microscopy, *J. Exper. Med.* 95, 285 (1952)

Reimer, L., Elektronenmikroskopische Untersuchungs- und Präparationsmethoden. Springer Verlag, Berlin, Göttingen, Heidelberg. 1959 (further literature obtainable from the publishers)

Ryter, A. et E. Kellenberger, L'inclusion au polyester pour l'ultramicrotomie. *J. Ultrastruct. Res.* 2, 200 (1958)

Walter, F., Studien zur Präparation pflanzlicher Objekte für die Elektronenmikroskopie in Verbindung mit einer einfachen Methode zur Herstellung von Dünnschnitten. *Z. wiss. Mikrosk.* 63, 227 (1957)

Walter, F., Die Wirkungsweise der Fließbewegung von Polymethacrylat bei der Herstellung von Ultradünnschnitten. *Z. wiss. Mikrosk.* 64, 106 - 110 (1959)

Walter, F., Ultramikrotomie, I Das Ultramikrotome nach Fernández-Morán. - *Leitz Information Sheets* vol. I/8, 236 - 243 (1961)

Wohlfahrt-Bottermann, K-E., Die Eignung und Anwendung von Phosphorwolframsäure und Thalliumnitrat als Kontrastmittel zur Darstellung cytoplasmatischer Strukturen. *Proc. Stockholm Conf. Electr. micr. S*, 124 (1956)

Wohlfahrt-Bottermann, K.E., Die Kontrastierung tierischer Zellen und Gewebe im Rahmen ihrer elektronenmikroskopischen Untersuchungen an ultradünnen Schnitten. - *Naturwissenschaften* 44, 287 (1957)

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Fig. 1 General view of the ultra-microtome, Fernández-Morán type

1. Ultra-microtome
2. Microtome table
3. Microtome drive
4. Swivel arm with microscope and illuminator
5. Plexiglass cover
6. Socket for mains connecting cable
7. Lock for microtome table drawer
16. Drive belt

Fig. 2 Ultra-microtome (part view)

4. Swivel arm with microscope and illuminator
5. Plexiglass cover
7. Lock for microtome table drawer
8. **Control unit**
9. Stereoscopic binocular microscope
10. Fluorescent tube lamp
11. Microtome rotor
- 12.a Rotor protective cap
13. Support device
14. Coarse and fine adjustment screw of the support
15. Arresting lever for 14

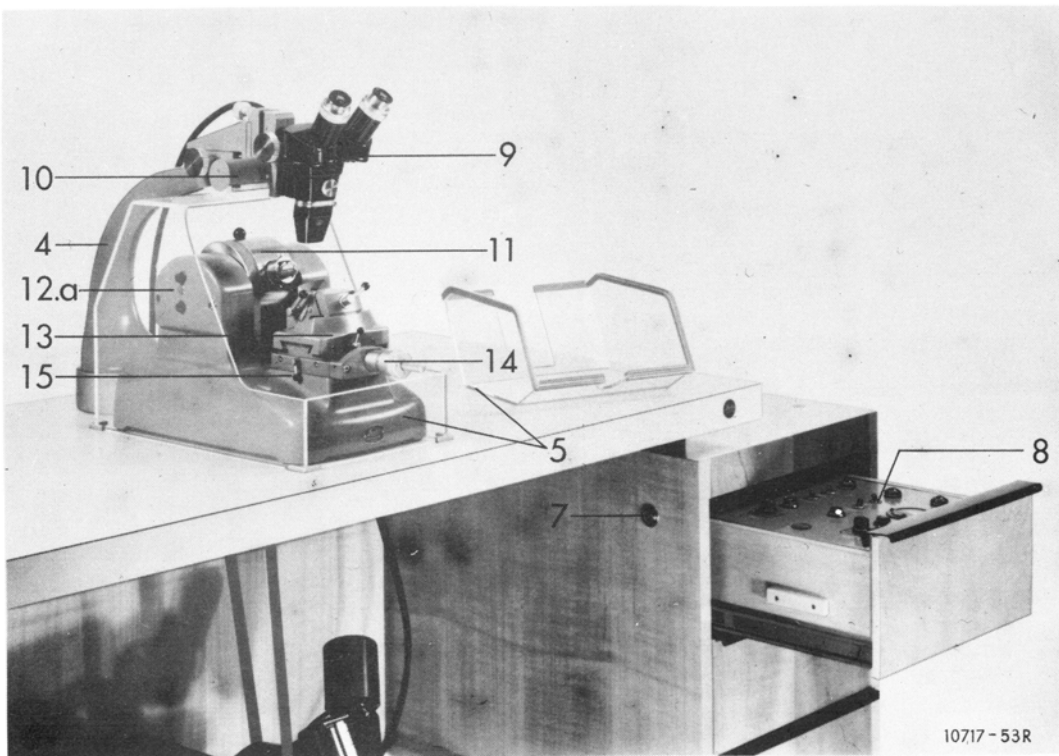
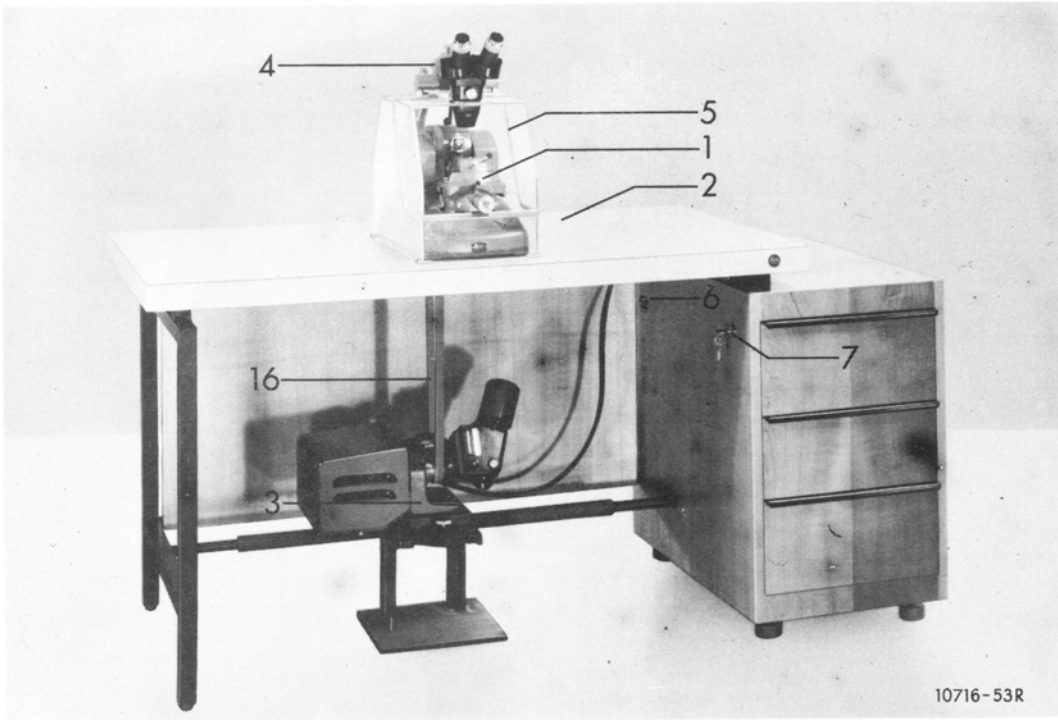
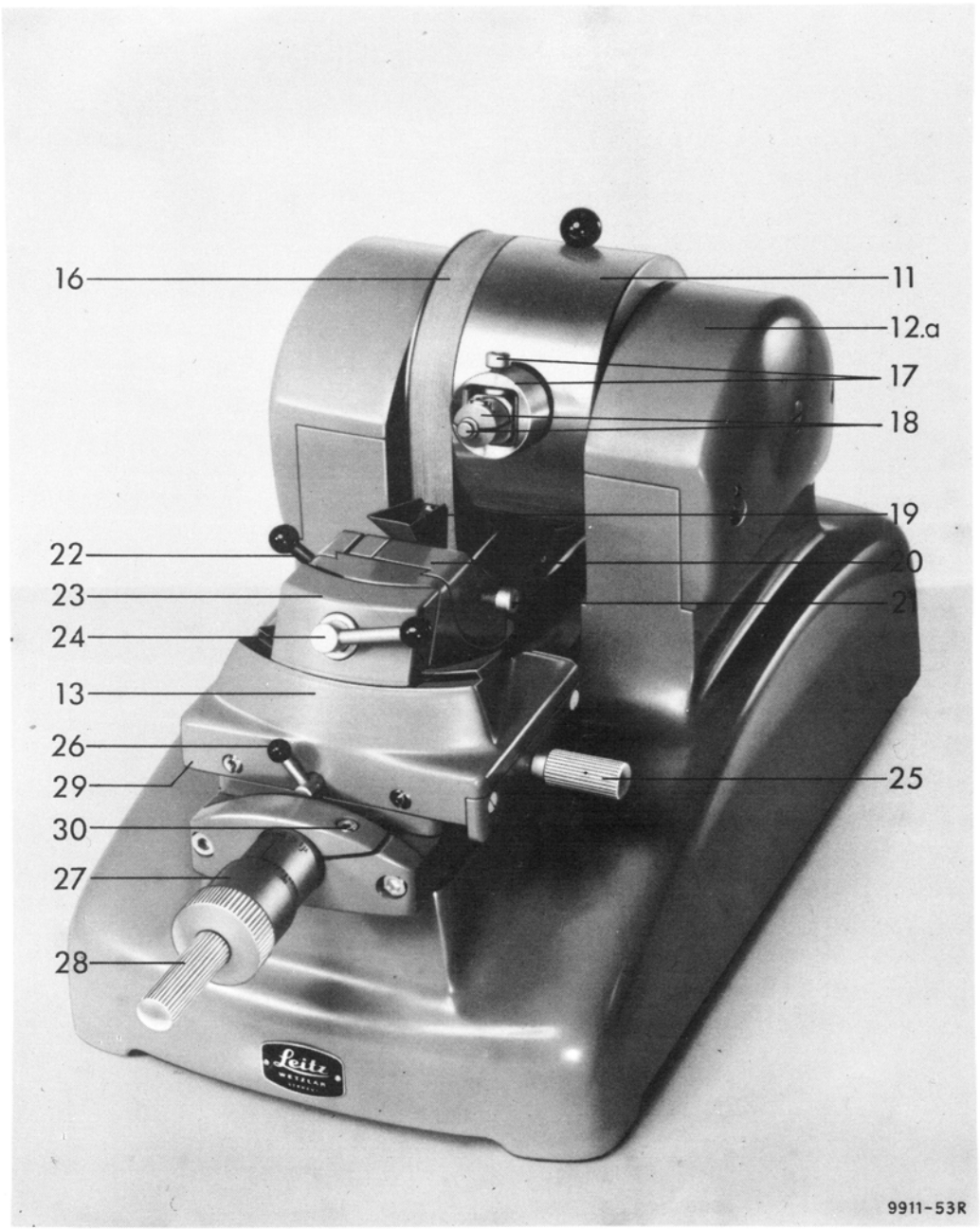


Fig. 3 Ultra-microtome (part view)

11. Microtome rotor
- 12.a Rotor protective cap
13. Support device
16. Drive belt
17. Heating rod with clamping screw
18. Specimen holder with specimen
19. Microtome knife (glass or diamond type)
20. Holding device for microtome knife
21. Clamping screw for microtome knife
22. Arresting lever for setting angle of knife
23. Laterally adjustable support unit
24. Arresting lever for 23
25. Milled knob for lateral adjustment of support
26. Arresting lever for 25
27. Fine adjustment screw of support drive
28. Coarse adjustment for support drive
30. Retaining screw for support adjustment 27 and 28



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12.a

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Fig. 4a Ultra-microtome, accessories

- 31. Wrench for hexagonal socket bolts 18 and 30
- 32. Wrench for hexagonal socket bolts 17 and 21
- 33. Wrench for securing the swivel arm on the microtome table
- 34. Specimen clamp
- 36. Glass knife adjusting gauge
- 40. Connecting cable
- 41. Container with special grease for microtome rotor
- 42. Plexiglass cover

Fig. 4b Ultra-microtome, accessories

- 35. Specimen shells
- 37. Fluorescent tube lamp
- 38. Spare fuses
- 39. Contact carbons for rotor

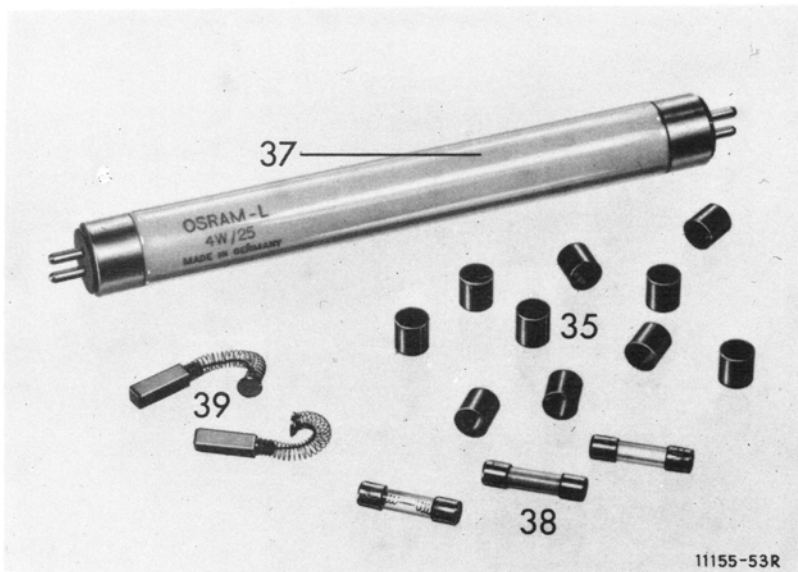
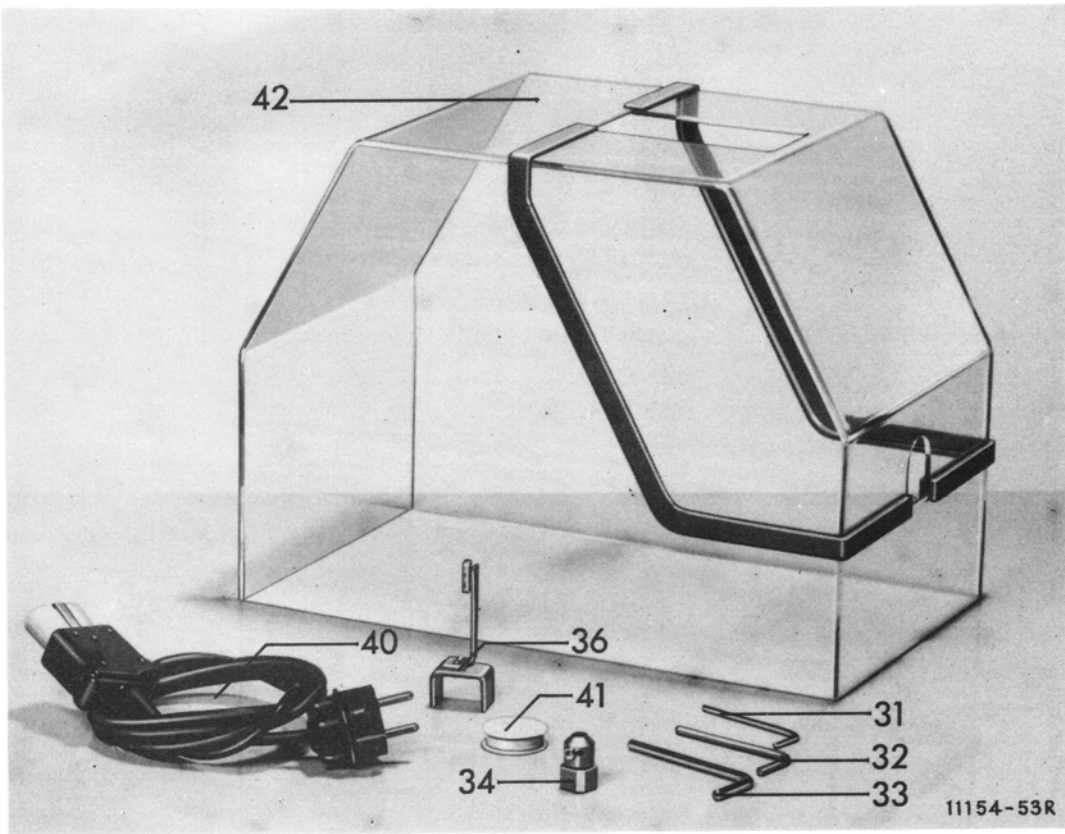


Fig. 5

Example of suitable site for the ultra-microtome

Door

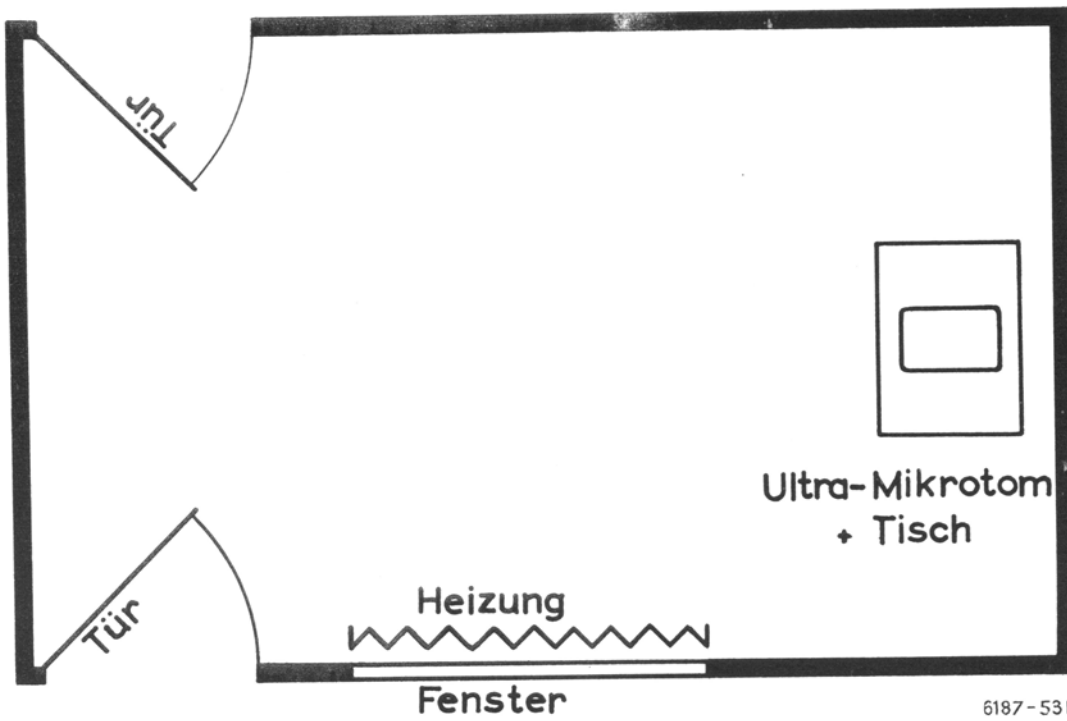
Ultra-microtome
+ table

Door

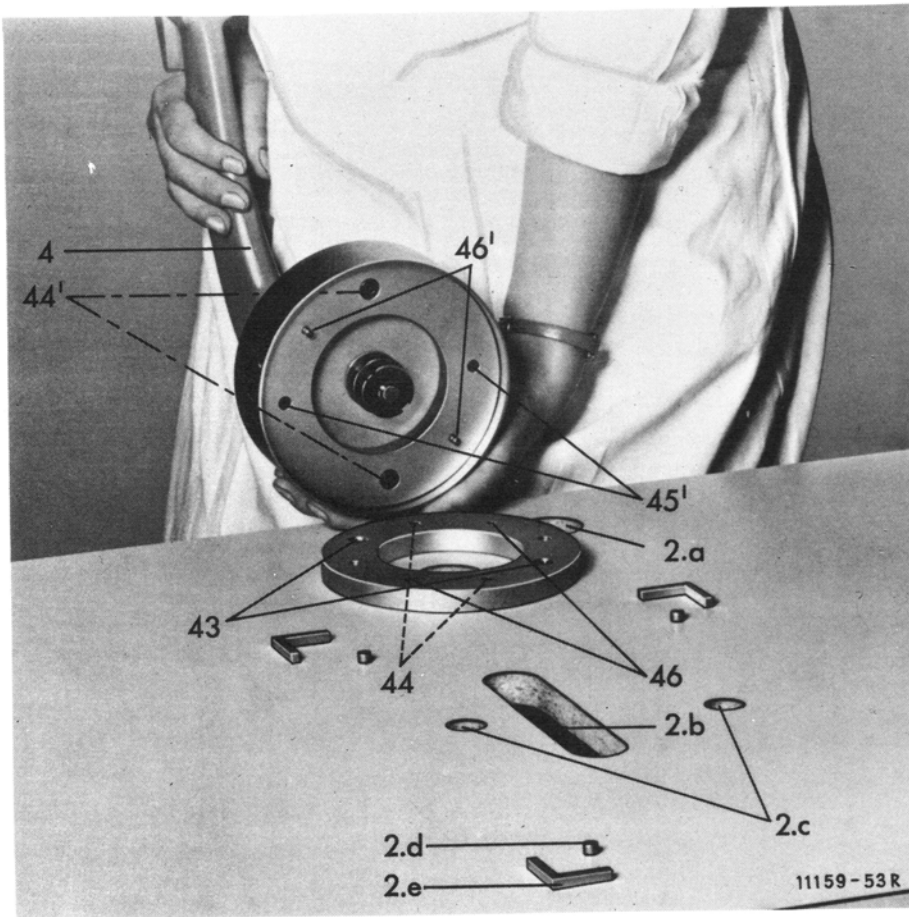
Heating
Window

Fig. 6

Assembling the swivel arm on the microtome
table (explanations in text)



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Fig. 7a Lamp unit

- 10.a Fluorescent tube lamp
- 10.b Electric supply cable
- 10.c Dove-tail guide
- 10.d Tilt device for the microscope

Fig. 7b Stereoscopic binocular microscope

- 4.a Horizontal adjustment of swivel arm
- 9.a Prism head
- 9.b Pair of objectives
- 9.c Pair of eyepieces
- 9.d Focusing control
- 10.d Tilt adjustment for the microscope

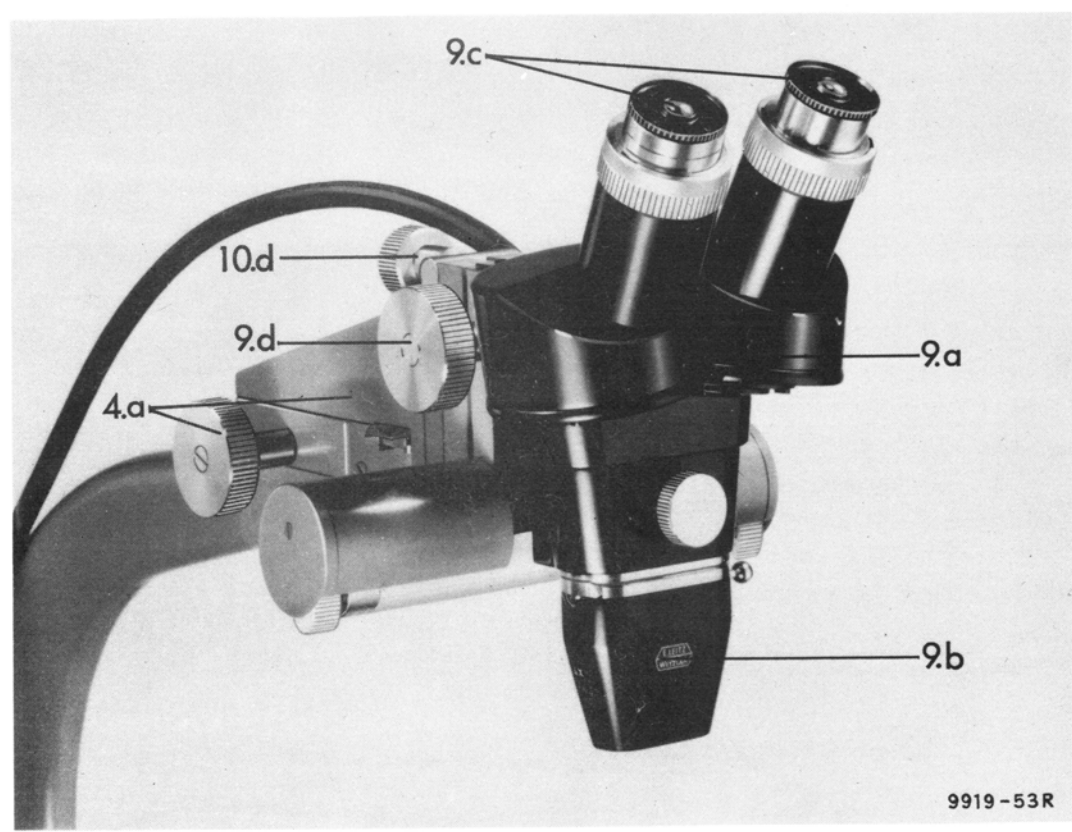
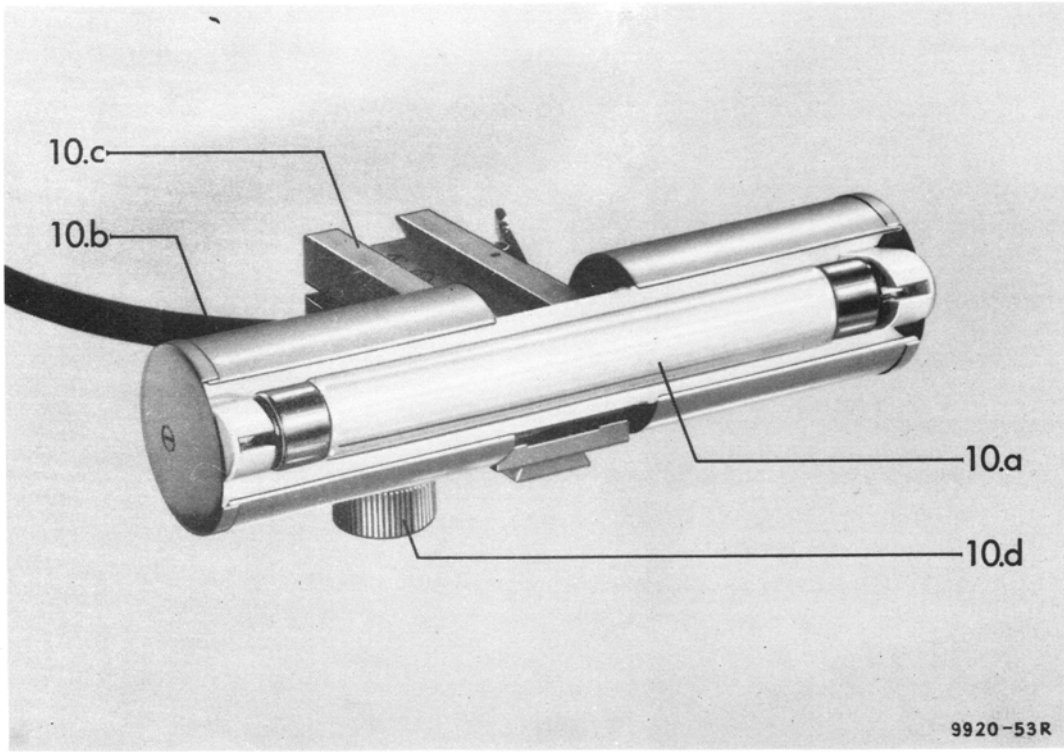


Fig. 8 Packing crate for the ultra-microtome, upper part visible

- 9. Stereoscopic binocular microscope
- 10. Fluorescent tube lamp
- 11. Microtome rotor
- 14. Coarse and fine adjustment screw of the support (packed)
- 17. Heating rod with clamping device
- 47. Packing crate (upper part)

Fig. 9a Lower part of packing crate with microtome body

- 1.a Connecting cable
- 12.a,b Rotor protective caps
- 47.a,b Retaining strips

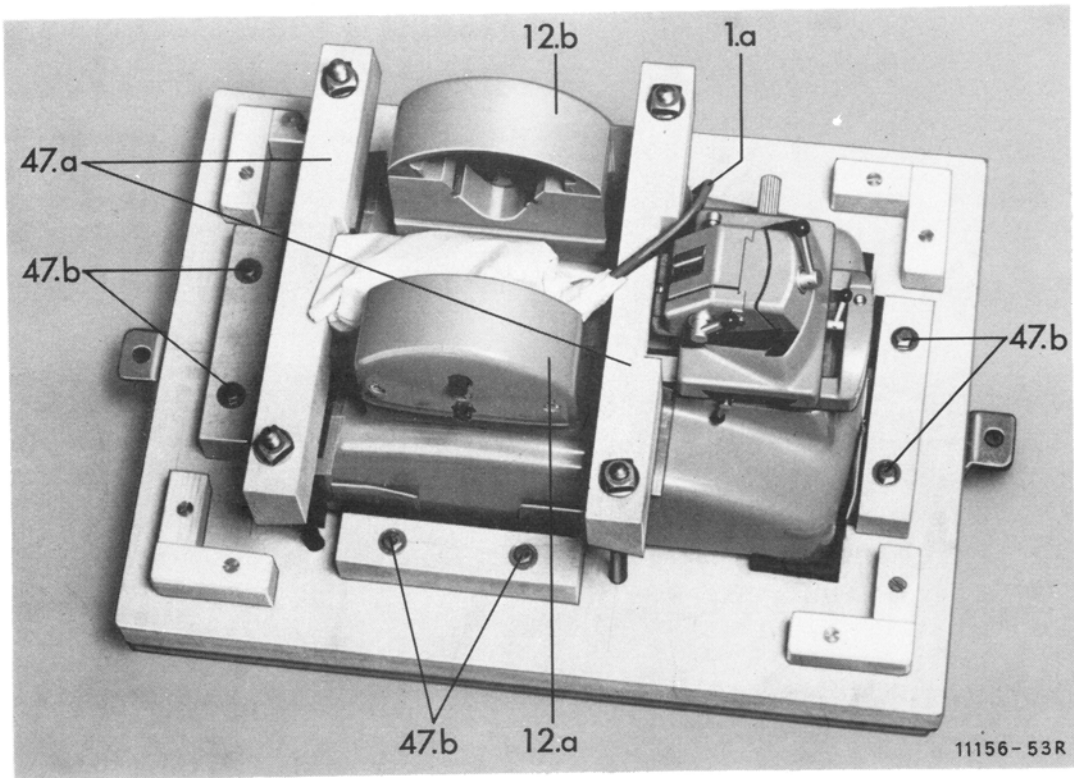
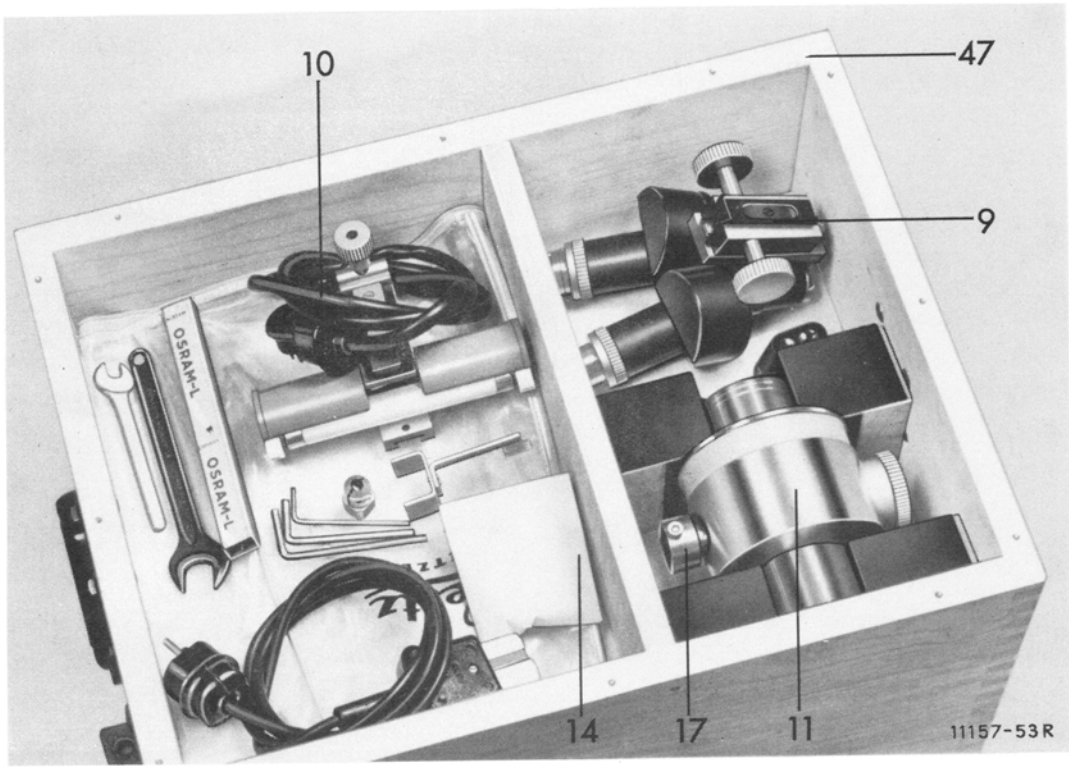


Fig. 9b Inserting the microtome rotor in the sapphire bearings

11.b Sapphire bearings

Fig. 10 Rear view of the special table for the ultra-microtome

2.f Cover for closing the table

2.g Recess for cable

Fig. 11 Arrangement of electrical connections for ultra-microtome

1.a Cable for rod heating and electro-magnetic coupling

3.b Cable for motor unit

8.a Bridge

10.b Cable for lamp unit

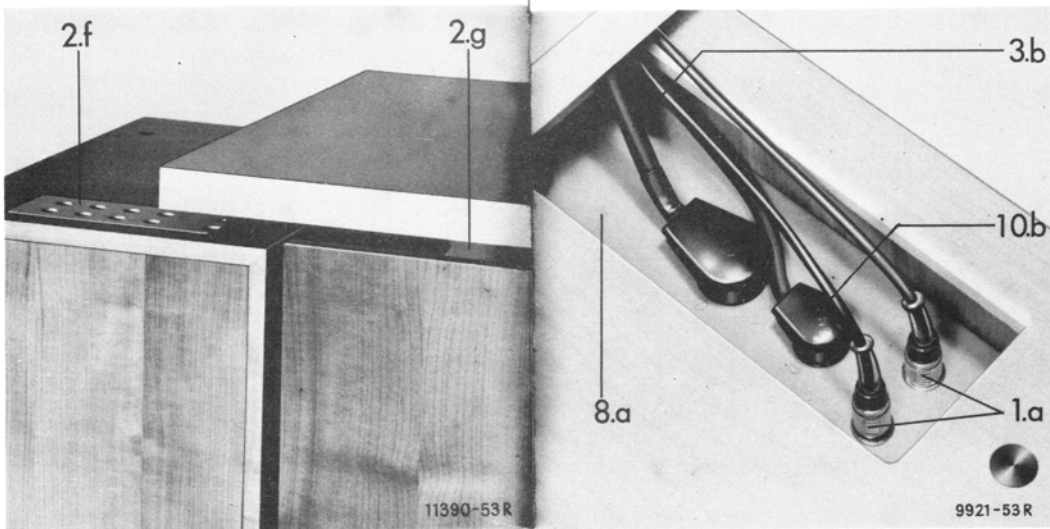
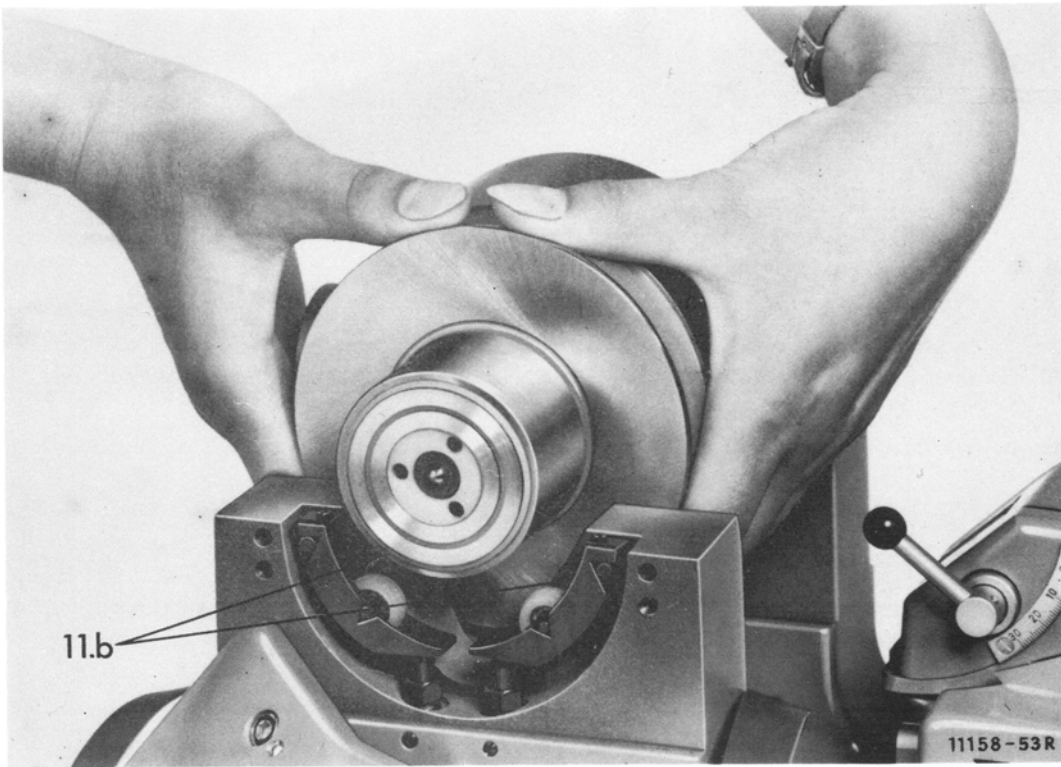


Fig. 12 Motor drive for the ultra-microtome

- 3.a Setting screw for regulating belt tension
- 3.b Supply cable
- 3.c Slow-running motor
- 3.d Regulating knob for speed of slow-running motor
- 3.e Speed setting scale
- 3.f Quick-running motor

Fig. 13 Control unit

- 8.b Mains switch with pilot lamp and fuse
- 8.c Switch for lamp unit
- 8.d Switch and regulating knob for rod heating,
also fuse and ammeter for the heating current
- 8.e Switch with pilot lamp and fuse for the entire
motor drive
- 8.f Coupling switch for changing to constant and
variable speed drive
- 8.g Regulating knob for quick-running motor on
constant or variable speed drive
- 8.h Retaining screws

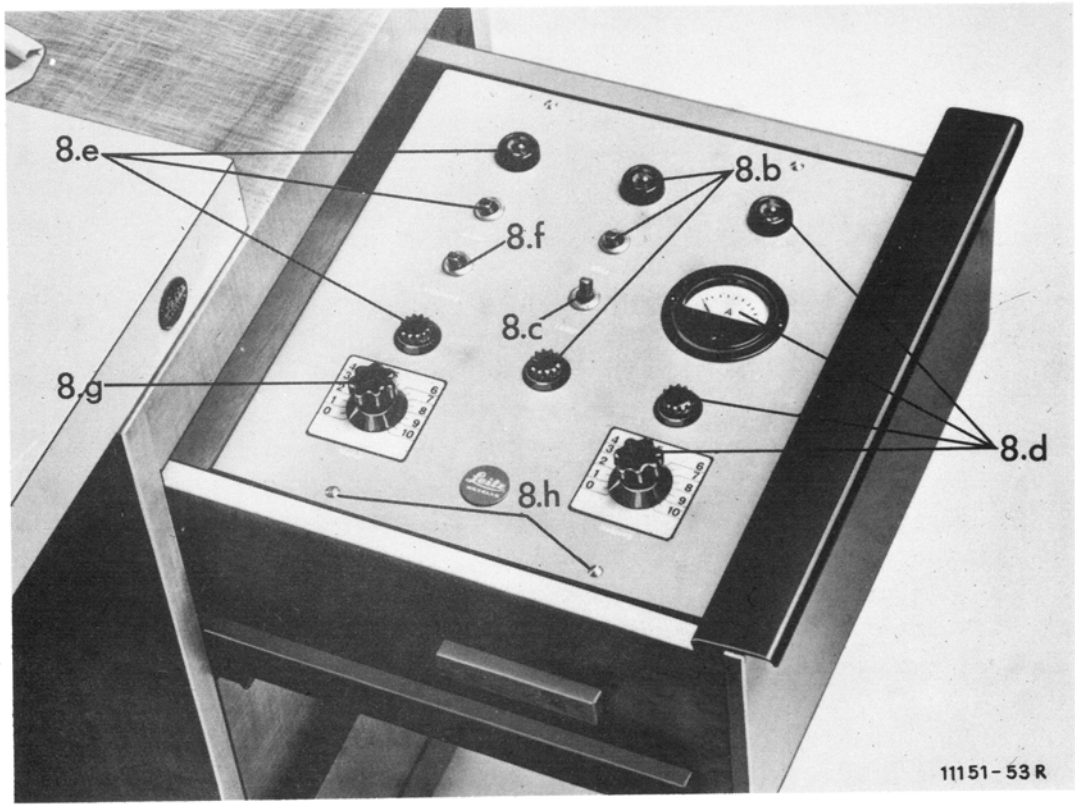
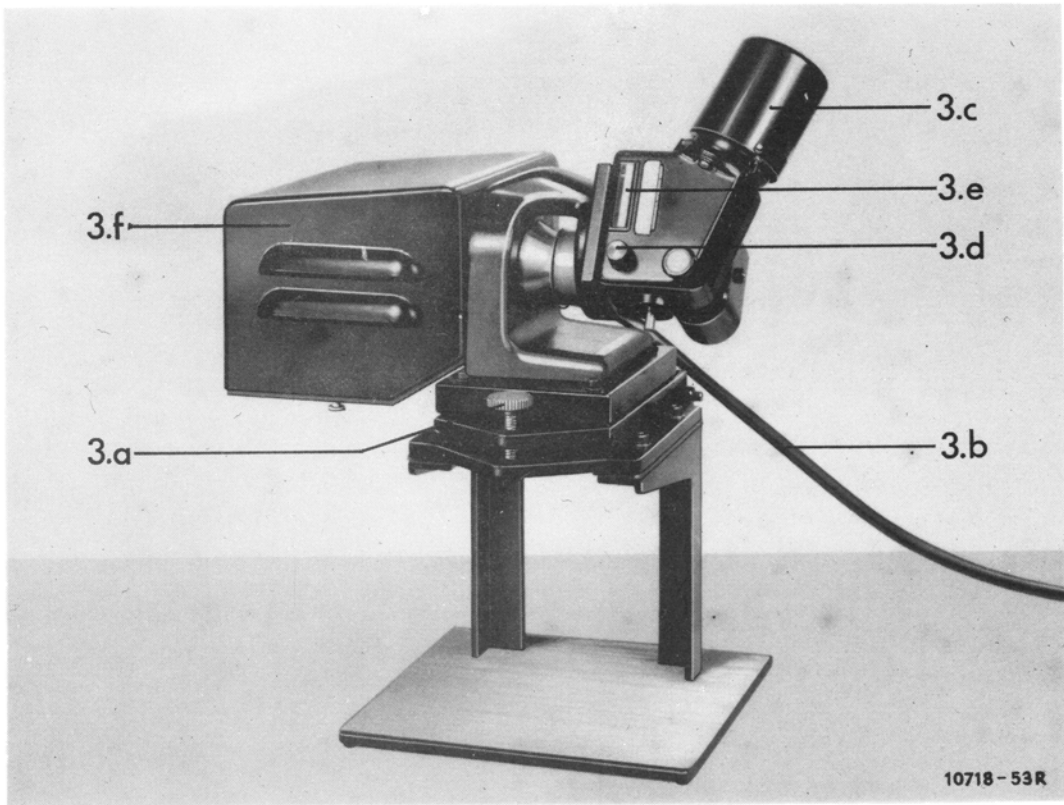


Fig. 14 Ultra-microtome, setting of the switching
cam for variable speed drive

- 11.g Setting screw
- 11.h Switching cam
- 11.i Micro switch

Fig. 15 Ultra-microtome, side view

- 12.b Retaining screws for the contact carbons
- 12.d Socket head hexagonal bolts of the protective cap
- 12.e Pins of the protective cap
- 12.f₁ Cover screw for rotor adjustment
- 12.g Arresting screw

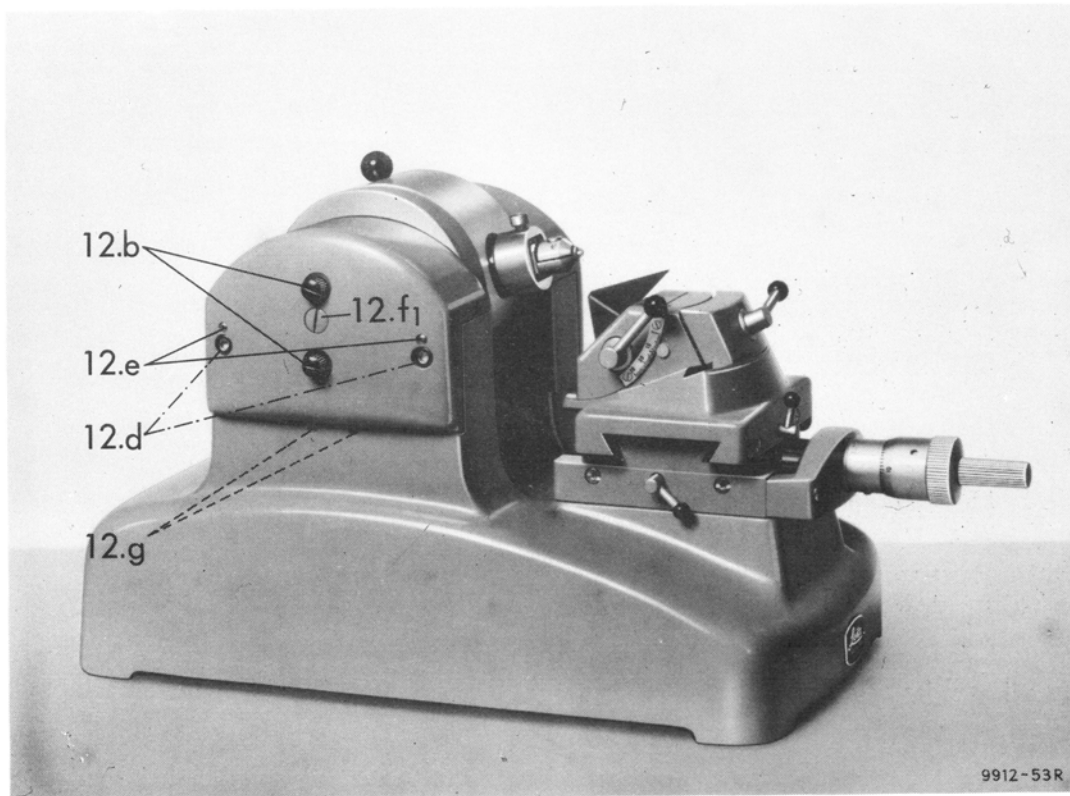
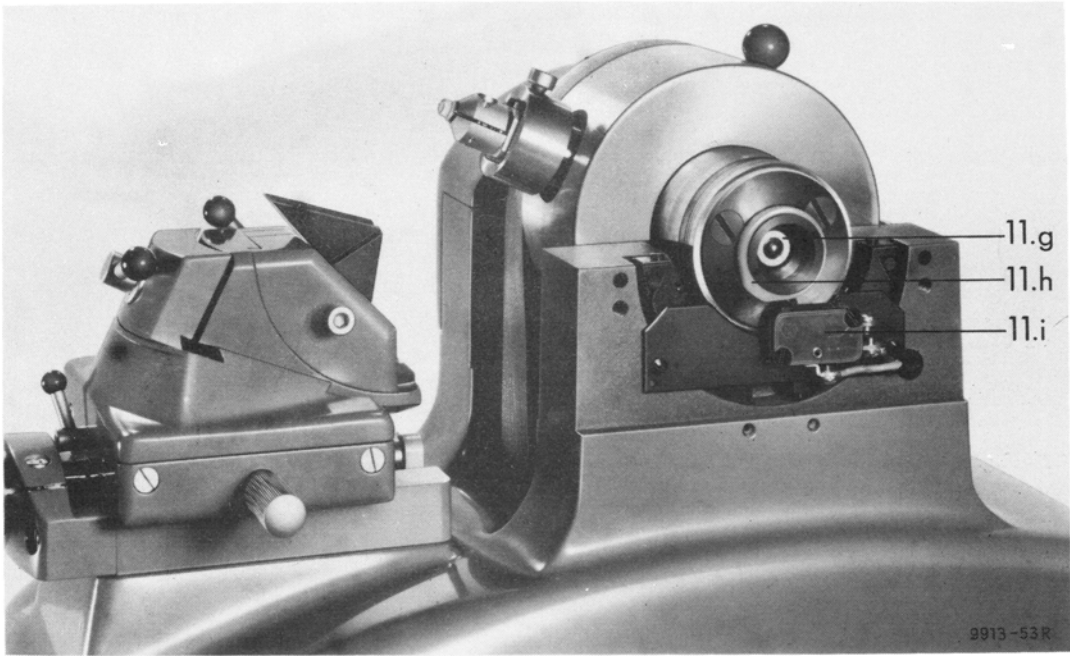


Fig. 16 Ultra-microtome, left rotor protective cap removed

- 11.c Relief bearings
- 11.d Running surfaces
- 11.e Contact rings
- 11.f Rotor steel ball
- 11.e Pins of the protective cap
- 12.f₂ Plate for lateral rotor adjustment
- 12.g Arresting screws for relief bearings
- 39. Contact carbons for rod heating

Fig. 17 Position of main connection at control unit

- 8.i Cable connection from connecting bridge to control unit
- 8.k Arresting strap for 8.i
- 8.l Adjustable resistance

Fig. 18

Materials and equipment for embedding the specimen blocks

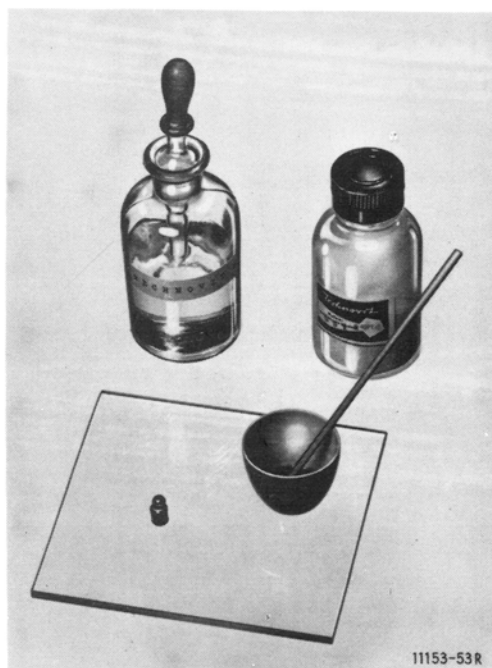
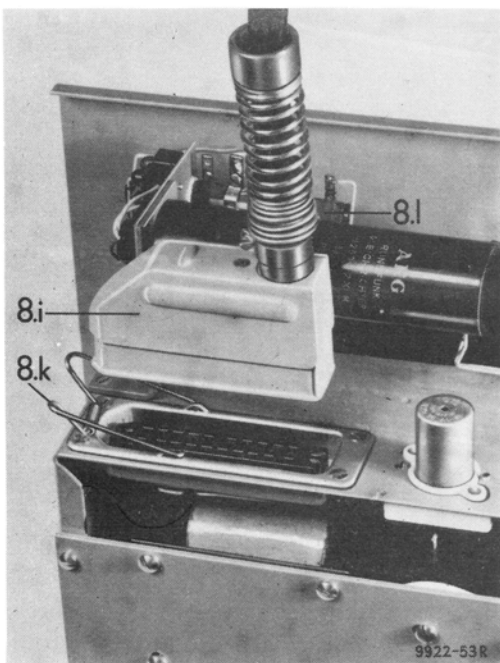
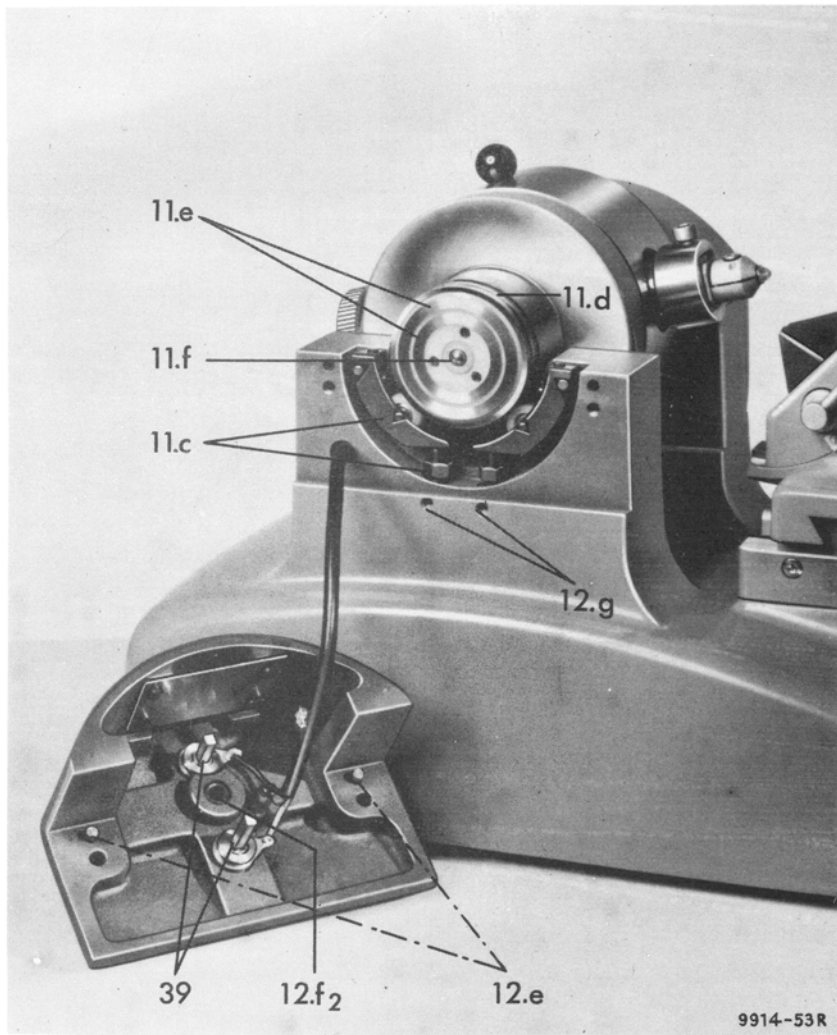
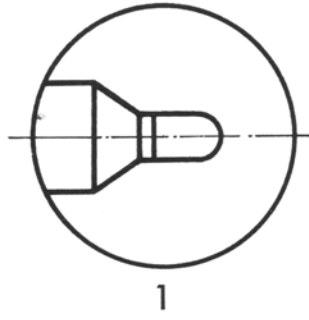
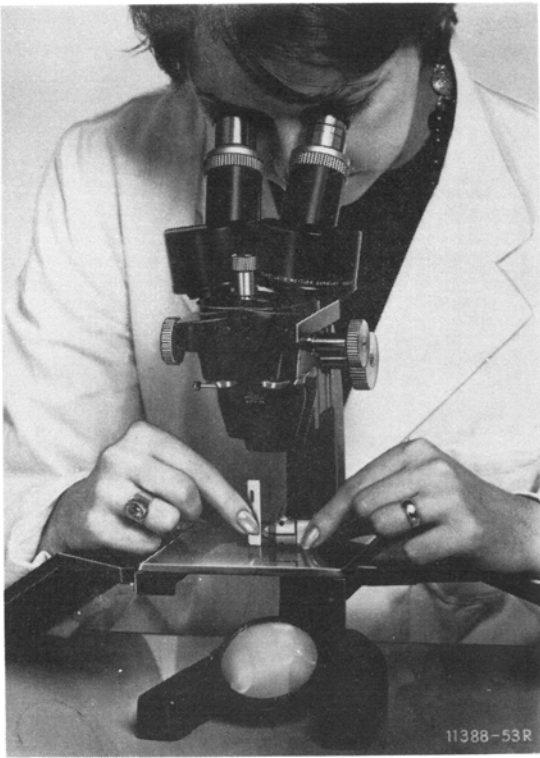
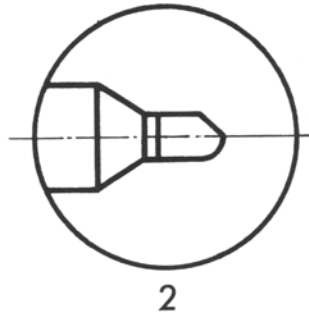


Fig. 20

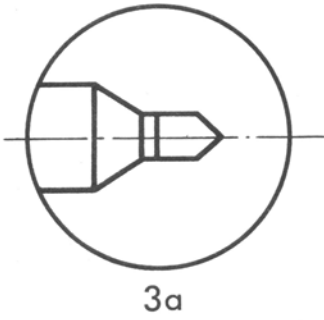
Pointing the specimen block (further explanations
given in the text)



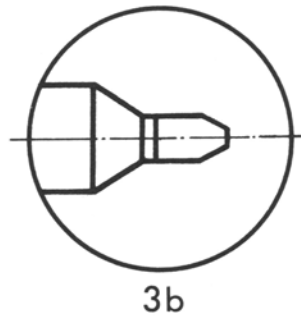
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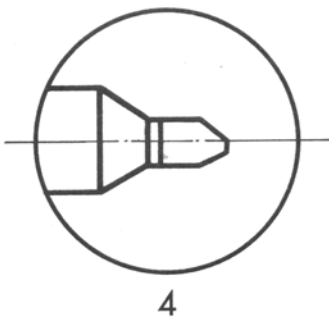
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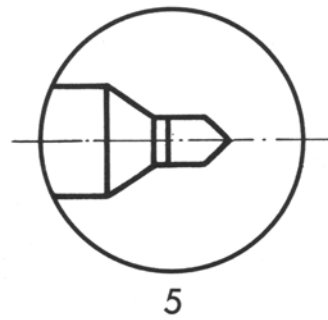
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Fig. 21 Tools for making glass knives
a. tile-breaking pliers, b. flat nosed pliers,
c. steel wheel glass cutter

Fig. 22 Indication of how the glass is marked for
making glass knives

Fig. 23 Breaking off a piece of glass
Arrow a: this part of the glass should be
held firmly on the table
Arrow b: marking line should be upwards
and directly over the table edge
Arrow c: direction of break with pliers

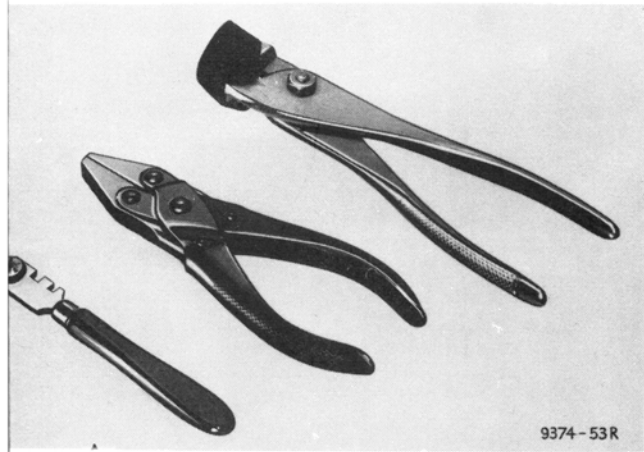
Fig. 24 Pattern for marking glass knives

Fig. 25 A correctly marked piece of glass

Fig. 26 Aligning the knife blade to the microscope
objective for examination purposes

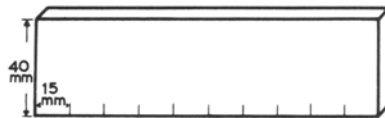
Fig. 27 Photomicrograph of a glass knife blade
in incident light (Ultropak Illuminator).
Magnification 200x. The upper part of the
picture shows a satisfactory portion of the
glass knife blade, the lower half shows a
fissured, unusable section

Fig. 28 Glass knife with collecting trough cemented
in position (further explanations in the text).



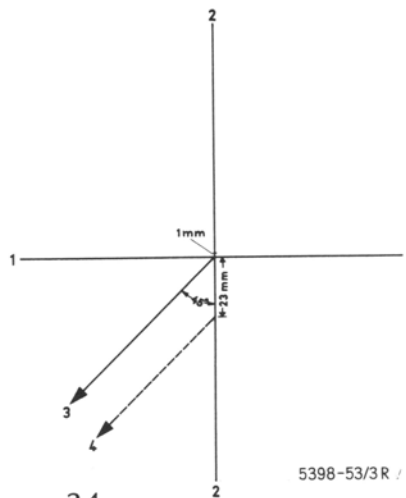
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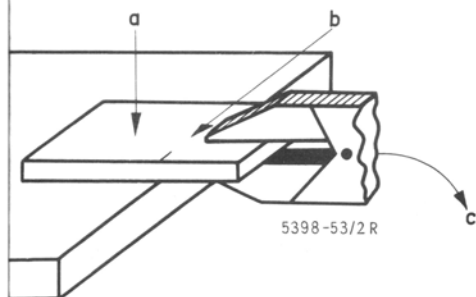
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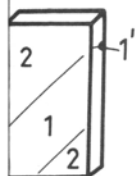
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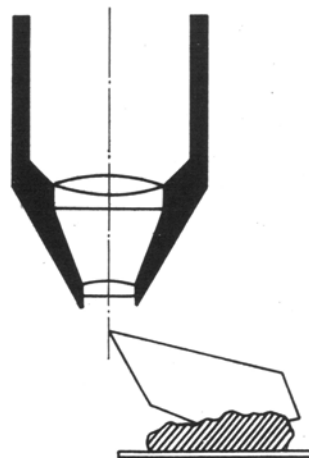
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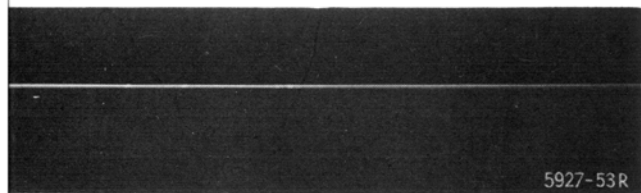
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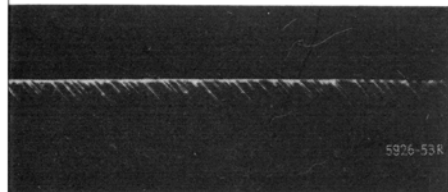


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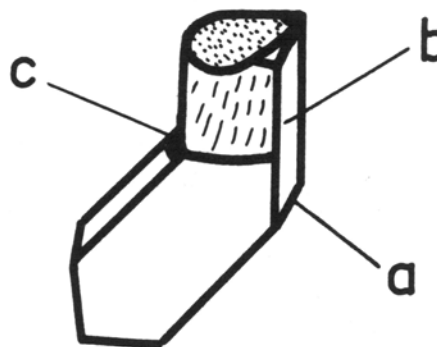


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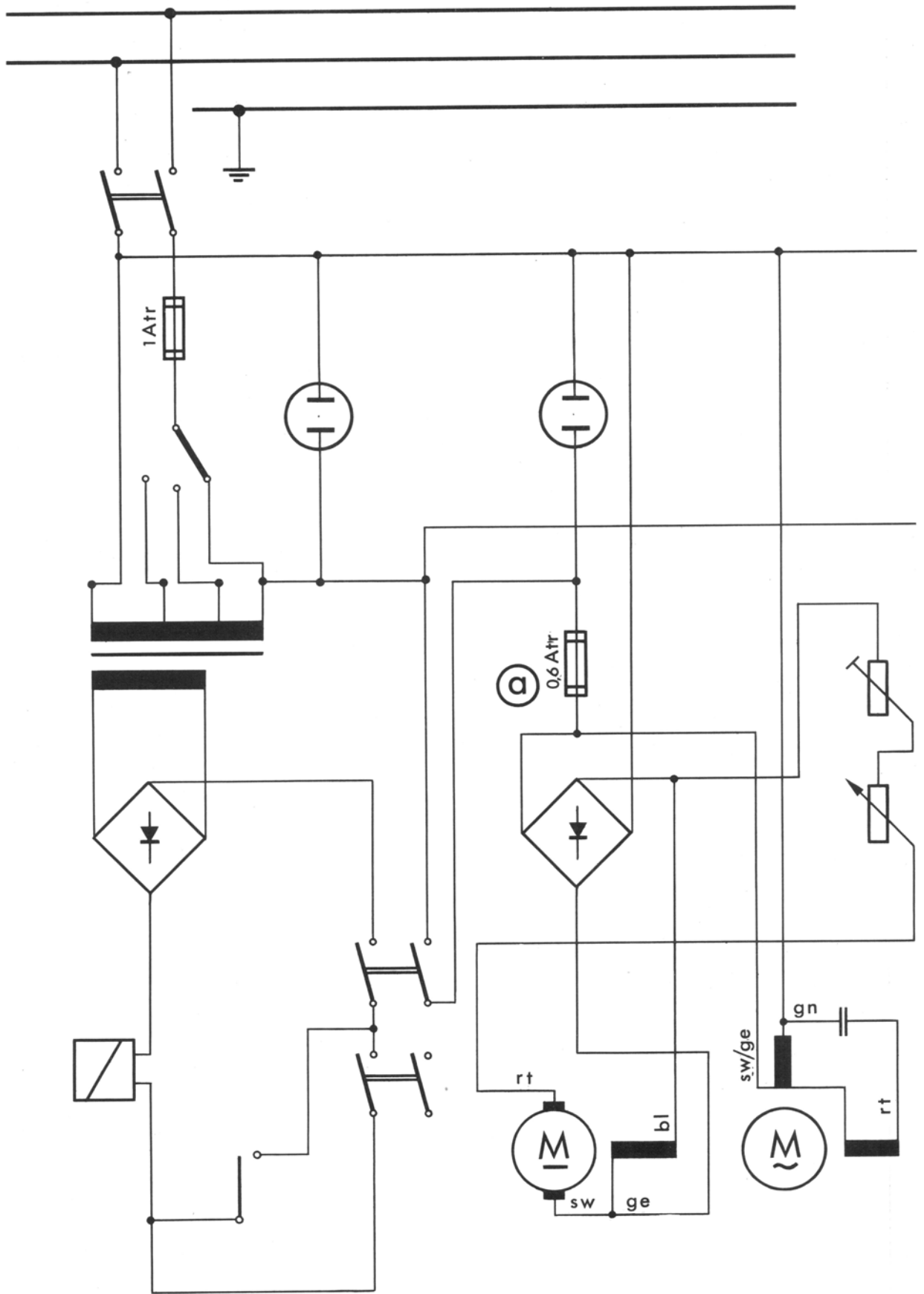
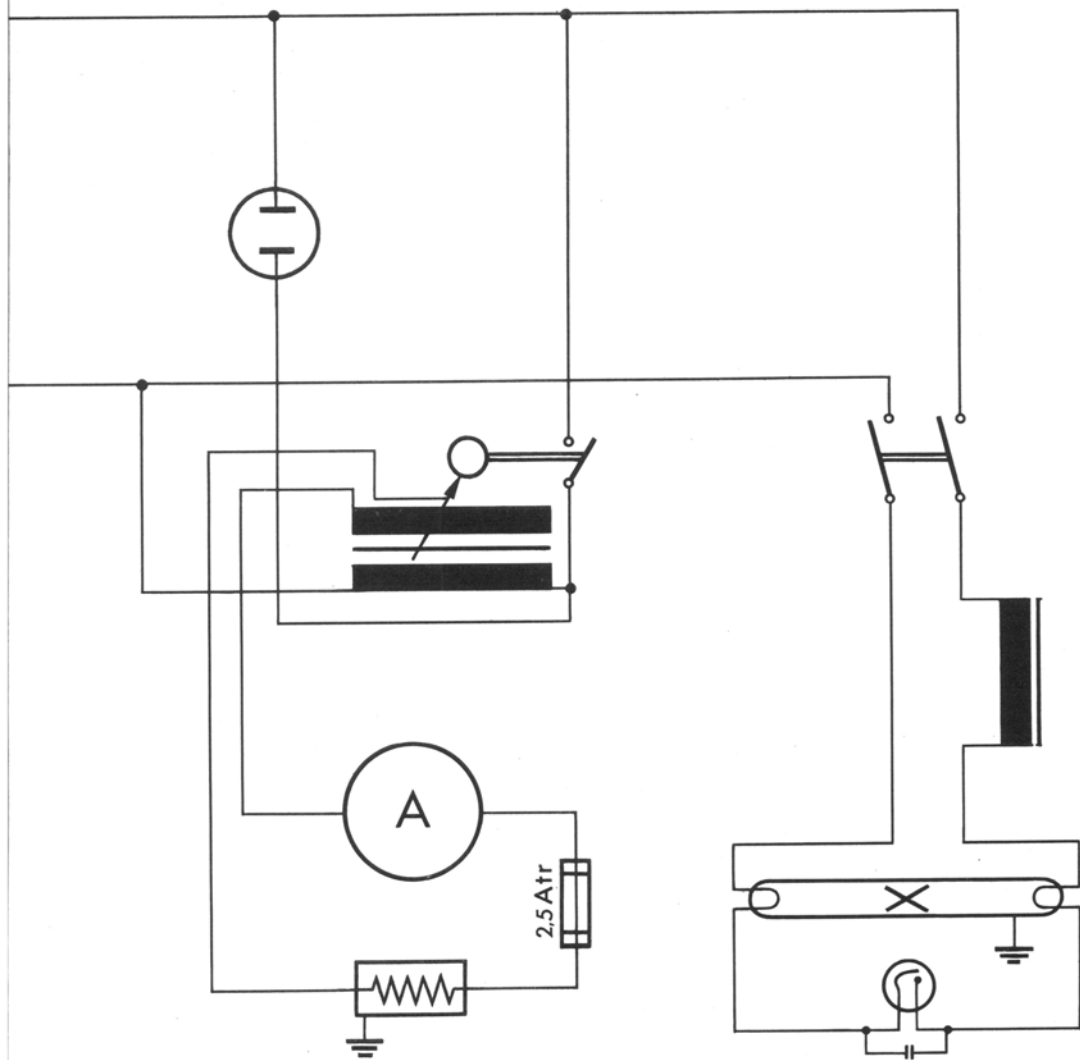


Fig. 19 Wiring diagram for ultra-microtome



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Fig. 29 Support device of the ultra-microtome

- 15. Arresting lever for support feed
- 20. Microtome knife holder
- 20.a Scale for setting the cutting angle
- 22. Arresting lever for knife angle setting mechanism
- 24. Arresting lever for lateral adjustment of support section
- 26. Arresting lever for lateral adjustment of support device
- 27. Fine adjustment screw of support feed
- 28. Coarse adjustment of support feed

Fig. 30

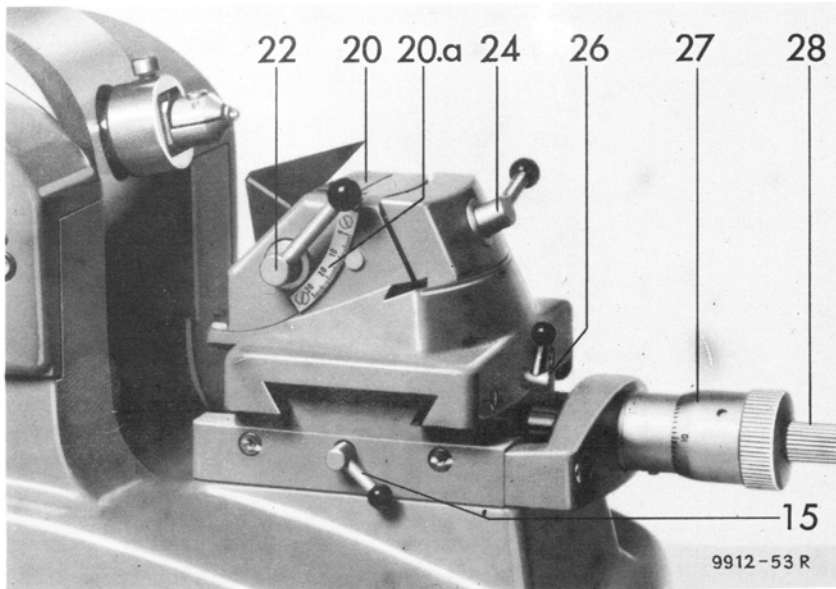
Inserting the glass knife with the aid of the
adjusting gauge(see further particulars in text)

Fig. 31

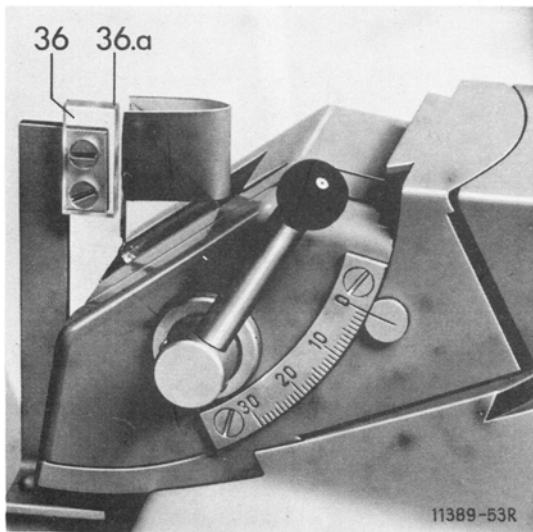
Diamond knife in collecting trough

Fig. 32

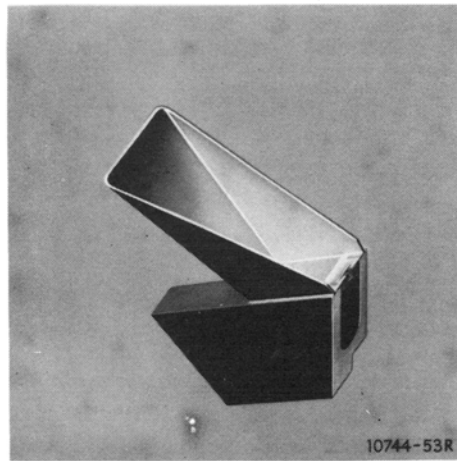
Alignment of knife and specimen under the
microscope



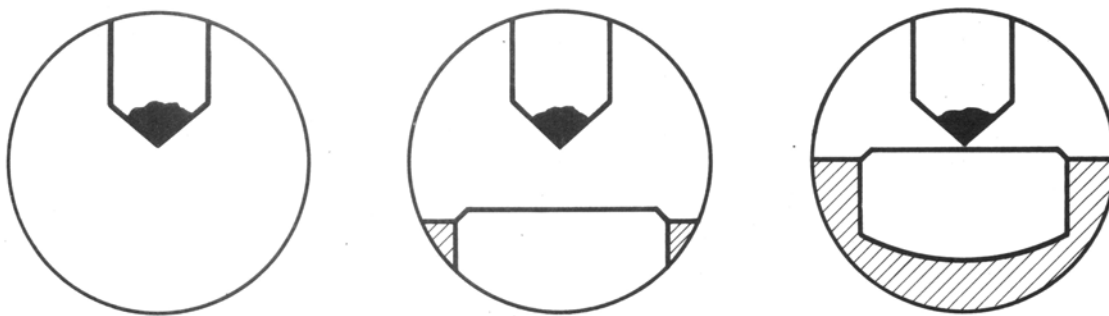
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