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WARNING

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WHEN WORKING WITH LIQUID NITROGEN (LN2)
PLEASE READ IN ANY CASE THE CHAPTER
"PRECAUTIONS TO BE OBSERVED DURING CRYOPREPARATION"
ON P. 5.

We are constantly endeavouring to still further improve our instruments and to adapt them to the requirements of modern test and research methods. This involves, in certain cases, modifications in the mechanical and optical structure of our instruments. All descriptions and illustrations in catalogues and instruction manuals as well as specifications relating to the mechanical features and optical data must not be regarded as binding.
I. PRECAUTIONS TO BE OBSERVED DURING CRYOPREPARATION

Provided the main safety precautions are observed, and the usual modern instrumentation is used, there is no real risk of accidents occurring during cryopreparation work. However please ensure that the few critical rules summarized below are observed by all who work with the FC4 system or the cryopreparation work involved with it.

WHEN WORKING WITH LIQUID NITROGEN (LN2) THE FOLLOWING POINTS SHOULD BE CAREFULLY NOTED

1. LN2 is extremely cold. It boils at -196°C. Nitrogen gas (GN2) escapes therefore also with very low temperature from the boiling LN2. Always consider that both LN2 and GN2 as well as elements cooled by LN2 or cold GN2 (e.g. pipes, valves, hoses, containers or stoppers) can cause severe affects, e.g. frost bits on the skin or combustions of the eye.

2. When liquid nitrogen evaporates it expands in a ratio of 1:700. Accordingly 1 litre of liquid nitrogen produces almost 1 m³ of nitrogen gas (GN2). Care should therefore be taken to ensure that when large quantities of nitrogen are evaporated (e.g. when transferring LN2), the room should always be well ventilated. If necessary to dispose of waste LN2 use caution: It should be dumped into an outdoor pit or container filled with gravel, where it will evaporate rapidly and safely.

3. Nitrogen gas (GN2) is odourless and tasteless and will be inhaled like air. GN2 is non-toxic, but a high GN2 content in the air (normal percentage about 78 % to about 21 % of oxygen) reduces the oxygen-content and produces immediate fainting and deep unconsciousness without any previous symptoms (such as dizziness and numbness). When there is doubt about the adequacy of ventilation, use an oxygen analyzer with a 0 to 25 % scale to check for oxygen. It becomes dangerous when the percentage of oxygen drops below 18 %. If an unconscious person stays in the low-oxygen environment then death may occur.

IN SERIOUS CASES NOTIFY DOCTOR AND AMBULANCE IMMEDIATELY.

IF BREATHING STOPS, APPLY ARTIFICIAL RESPIRATION AT ONCE.

4. For the reasons given above, never put LN2 bottles in a closed storage room or chamber. The evaporation rate from dewar vessels can rise to several litres a day if they have become defective due to improper handling (hanging or mechanical damage) or to natural wear over many years of use. In closed rooms this can lead to a dangerous drop in the oxygen level in the air.

5. Remember that 3.5 to 7.0 litres of LN2/h are evaporated during normal operation of the FC4-system. This quantity is the equivalent of 2.5 to 5 m³ of GN2/h. So always keep the working premises well aired and regard advice given above (point 2 and 3).

6. Take great care if you bring objects at room temperature into contact with LN2. First an insulating gas layer is formed, which prevents any large transfer of heat. During this initial period comparatively little LN2 evaporates. However, once the object has cooled down there often occurs unexpected strong boiling and spurting of LN2.
7. When working with LN2 avoid protective glasses (a), boots (c), walking shoes (e) and protective gloves (g) out of which the LN2 cannot easily escape if entered. LN2 which has splashed into the closed protective glasses (a) or into the open boots (c) or into the shoes (e) or the protective gloves (g) evaporates suddenly and causes serious burns before there is time to take or pull them off. So always USE PROTECTIVE GLASSES (b) WITH SIDE PROTECTION AND WHICH ARE OPEN AT THE TOP AND ESPECIALLY AT THE BOTTOM. Only use boots if you have loose (NOT NARROW) trousers coming OUTSIDE the boots (d) and completely covering the gap. Wear only open slip-on sandals (f) in the lab, no walking shoes or court shoes. Always use CUFFLESS TROUSERS if you wear slip-on sandals. Never wear protective gloves when pouring LN2 or when putting the dewar head on the dewar vessel. The most you can use is an open flannel cloth (h) to protect your hands from the cold. Asbestos gloves should only be used to grasp dry cold parts. They are unsuitable for LN2 work.

8. In the case of burns from LN2 splashes, rinse the affected skin immediately with copious amounts of water at hand temperature. For serious burns arrange for a skin specialist to see them at once.

IN THE CASE OF LN2 AFFECTING THE EYE ARRANGE FOR AN EYE SPECIALIST TO SEE IT AT ONCE. RINSE THE AFFECTED EYE IMMEDIATELY WITH WATER AT HAND TEMPERATURE.

9. If possible never use glass dewar vessels in the lab, and especially no glass dewars larger than the 2 litres capacity without complete metal envelope. Glass dewars often burst for no obvious reason or due to unintentional mishandling which cannot always be avoided (e.g. contact with metal instruments etc.). Never work without open protective glasses when using LN2 in a glass dewar. Whenever possible only use metal dewars specifically designated for storage of LN2, since only containers of this kind exclude risks during storage of LN2. For routine cryopreparation metal troughs (1 cm styrofoam insulation), styrofoam containers or plastic troughs 7) are eminently suitable and ensure completely riskfree cryopreparation.

10. Check the evaporation rate of your metal dewar regularly every three months and compare these rates with the rate given by the manufacturer. The evaporation rate of an undamaged metal dewar, if in good condition, should come well under 1 litre of LN2 per day. Defective dewar vessels with higher evaporation rates are a safety risk, and should be taken out of work or repaired.

7) Plastic troughs for LN2 cryopreparation can be obtained in various sizes and designs and also made to customers requirements by the Kurt MIGGE GmbH Company, Am Taubenfeld 31, D-6900 Heidelberg-Wielblingen.
11. Standard dewar vessels such as the VR 16 metal dewar of the FC4 system, are not pressure vessels, and so should only be closed with the stoppers provided (sketch A) which leave a sufficiently large gap for any sudden evaporation of GN2 and which sit loosely on top of the dewar neck. Make sure, by frequent checks that the stoppers are not stuck on hard through ice deposits (sketch B). Never close a dewar with a home-made closure (sketch C). A gas-tight closed dewar vessel can liberate GN2 with an explosion and burst, after being subjected to shaking or movements (e.g. in transport). When this happens, perhaps in a closed vehicle, all the LN2 may evaporate in one go (35 litres LN2 = 25 m³ GN2!). A proper stopper "rises" when movements cause sudden evaporation in the dewar of large amounts of LN2 in the neck, which push up the stopper and let some of the GN2 out (sketch D). The small quantity of LN2 which sometimes escapes with it, does not represent any great danger to safety.

PLEASE NOTE: The dewar vessel closure with the dewar head of the automatic refill system of the FC4 system is officially equipped and doubly protected by means of its ascending pipe with current-free open solenoid valve and its excess pressure valve set to a pressure of 0.35 bar. Any potentially damaging build-up of pressure in the dewar is practically eliminated as a result of these two safety measures.
12. Do not leave LN2 standing in open vessels where it can exchange with the room atmosphere. Liquid nitrogen's boiling point (-196°) is lower than liquid oxygen's boiling point (-183°C). In cases where the exchange surfaces are extensive enough, oxygen from the air will be taken up in exchange for nitrogen. LN2 with a high liquid oxygen content has a faintly bluish colour. Caution is advised in a case like this, since concentrated liquid oxygen promotes vigorous burning.

13. Make sure that your dewar vessel is filled exclusively with LN2. Put up a notice in the central distribution place where the filling takes place, stating clearly "ONLY LIQUID NITROGEN" or similar if different liquefied gases are delivered from there. Check the colour of the cryogen. BLUISH COLOUR INDICATES THE PRESENCE OF A HIGH PERCENTAGE OF OXYGEN (cf. previous point 12). Liquid oxygen becomes concentrated during long periods of storage, as its boiling point (-183°C) is higher than the LN2 boiling point (-196°C).

14. Never fill a dewar vessel right up its neck with LN2 (sketch A). If there is a delay in the boiling there is the danger that the gas bubbles which keep growing in size rapidly may force out the LN2 through the neck (sketch B). So make sure that the LN2 level is always below the neck part (sketch C). Remember too that length L of the casing is usually different from the dewar neck length L.
II. MOUNTING THE FC4E ON THE ULTRACUT

This FC4E system is suitable for use with both the ULTRACUT (1977 to 1982 model) and the ULTRACUT E (1983 model). The ULTRACUT E can be recognized by its blue base (1-E) and by the name ULTRACUT E written on the cover (25-E). The ULTRACUT, on the other hand, has a dark grey base (1) and the word ULTRACUT written on the cover (25). The two models differ on three points which are crucial for cryo-work:

- In the ULTRACUT (1977/82) the specimen moves by a stroke height (H1) of 25 mm during the cutting movement, whereas in the ULTRACUT E (1983) the stroke height (H2) is 15 mm. The different stroke calls for different height of the heat exchangers (197/198/199) respectively (197E/198E/199E) on the specimen bridge (193) of the FC4E.

- All ULTRACUT E instruments are equipped with a microscope carrier (29-E) to take a stereomicroscope with a working distance (A2) of 10 cm, but most of the ULTRACUT models from 1977 to 1982 are fitted with a microscope carrier (29) to take stereomicroscopes with a working distance (A1) of 7 cm. The greater working distance is imperative as it provides sufficient clearance to remove the section. So if your present ULTRACUT has of the old microscope carriers (29), which can easily be recognized by the straight line at the lower edge (see arrow on sketch), it will have to be exchanged by your REICHERT agent for one of the new carriers. You can continue to use your existing stereomicroscope (30) after removing the auxiliary lens (e) and mounting a dust shield glass (f) if the new carrier (29-E) is used.
Basically the FC4E SYSTEM consists of the following main parts:

- Dewar vessel (101) for liquid nitrogen
- Dewar support (104) with casters
- Dewar head (105) for filling chamber automatically with liquid nitrogen
- Control unit (118)
- Cryochamber (147) with knife holder
- Specimen bridge (193) with specimen holder

In addition there are the necessary cables and tubes for connecting and operating the instrument, as well as the cryo-tools, which are supplied as separate accessories.

Before the FC4E is mounted the STANDARD SPECIMEN AND KNIFE HOLDERS AND THE REFLEXOMAT FEED PIPE HAVE TO BE REMOVED FROM THE ULTRAMICROTOME. Proceed as follows (cf. ULTRACUT or ULTRACUT E manuals):

- Completely remove the thumb screw (23)
- Pull the specimen holder (segment arc 35-E or rotary head 35) off the specimen arm (24) in a forward direction.
- After loosening the lever (6) lift the knife holder (9/10) upwards off the guide (7) on the knife support (8) and move lever (6) in middle position
- Completely empty the REFLEXOMAT via the feed pipe (17) by pressing the knob (5) several times. Turn knob (5) anticlockwise to the stop. Pull the feed pipe (17) out of the silicon tube (i) and then push the silicon tube into the slit between the support (8) and the cover (4)
The FC4E CRYOCHAMBER IS MOUNTED ON TO THE KNIFE SUPPORT OF THE ULTRAMICROTOME with the help of a robust guideplate which is fixed in place as follows:

1. Remove hexagonal keys (42 resp. 192) from left and/or right hand side holes in the cover (4).

2. The guideplate (169) laid on top of the surfaces (x) and (y) of the knife support (8). The stop (168) must be in the rear left hand corner. Tighten the guideplate (169) by means of the screws (166).

THE FIRST MOUNTING OF THE FC4E SYSTEM ON THE ULTRAMICROTOME includes a one-off adjustment to the cryochamber (147), which ensures that for all subsequent mountings of the cryo-device there will be a reliable and rapid change-over from normal operation to cryo-operation, for which the position of the bridge (193) is determined by the stop on the rear leg of the bridge and the chamber position is determined by the adjustable stop (168) on the left guide rail (167).
Before the final adjustment, a check is made to ensure that the bridge has the right heat exchangers (197/198/199) respective (197E/198E/199E) to correspond with the particular ULTRACUT model in use (For differences between ULTRACUT and ULTRACUT E compare text and drawing at the begin of the manual measure the distance C1/C2 respectively B1/B2 according the sketch.

Contact your agent or REICHERT-JUNG, A-1170 Vienna, Austria, if the bridge you have is not the correct one. Once it has been established that it is the right bridge for your present ultramicrotome model, the procedure is as follows:

- Remove plastic cap from knob (47/48E). Insert extension (228) and tighten with Allen key (191). Put back the plastic cap.

- Before mounting check the chamber from underneath to make sure that the clamp is open. The clamp can be opened and locked by using the thumb screw (149).

- Move the knife support (8), with its guide rails (167) mounted in position, by turning the coarse feed knob (3) anticlockwise, as shown by arrow to the feed limit. Now use the cross feed (47 resp. 48E) to centre it accurately. Move the clamp lever (6) to the middle position.

- Place the bridge (193) on the rear wall of the cryochamber (147), as shown in the drawing. Now put the chamber (147) with the bridge mounted on it, onto the knife support (8), positioning it so that the chamber is located centrally and its sides align with the sides of the ULTRACUT, and so that the bridge (193) is just touching the specimen arm (24).
Adjust the height of the specimen arm (24) by the hand wheel (15/16), with its lever (12) raised, to match the height of the receiving aperture in the rear leg of the bridge (193). Then slide in the chamber with bridge (193) attached, until it reaches the stop and tighten with clamping screw (149). Clamb Objectbridge (193) with clambingscrew (194) onto the specimen arm (24).

Finally check by moving the mounted chamber (147) with the drive knob (3) within the motion limits of this knob, if the bridge (193) will still move freely when the hand wheel (15/16) is turned. If it does not move freely adjust the position of the chamber (147) by means of the stop screw (168).

ALL SUBSEQUENT MOUNTINGS OF THE CHAMBER (147) and of the bridge (193) are simply a matter of inserting them up to the preset stops. All that is required to DISMANTLE THE CHAMBER (147) AND THE BRIDGE (193) is to loosen the screws (149) and (194) and take off the two parts (147) and (193). However, the quideplate (166/167/169) must be taken off before inserting the standard knife holder and then they have to be put back again. WHEN CHANGING OVER FROM CRYOWORK TO WORK AT NORMAL TEMPERATURES a standard specimen can be inserted STRAIGHT AWAY, since the heating of the chamber wall and bridge prevents any sudden cooling of the corresponding elements and the alloys used in the construction of the instrument are not affected by minor temperature variations.
ELECTRICAL CONNECTIONS TO THE CHAMBER (147) AND BRIDGE (193) are made after assembly in the following way:

- Connection of the multiplug (196) attached to the bridge (193) with the corresponding socket (165) on the left side of the chamber at the level of the cover (164).

- Connection of the multiplug (148) attached to the chamber with the corresponding socket (122) on the rear panel of the control unit (118).

- Before connecting the control unit (118) to the mains, check the voltage specifications on the rear panel of the control unit. If the mains voltage is the correct one, connect it up. Otherwise adjust the voltage via an intermediary mains connection unit. Adjust voltage properly to indicated value.

The cryo system will now be ready and can be put into operation as soon as the twin tanks in the cryochamber have been filled with liquid nitrogen (LN2). The filling with LN2 can be achieved with the automatic refill unit, especially developed and recommended for use with this instrument. The operator must previously have familiarized himself with all the controls needed for cryosectoning in the cryochamber (see following chapters).
III. KNIFE HOLDER AND SPECIMEN HOLDERS

The system which has now been installed and connected in accordance with the foregoing instructions, is now ready for use and could be refrigerated with LN2 straightaway. However, it is strongly recommended that you start by acquainting yourself with all the manipulations and controls needed for sectioning at low temperature, by using them first of all at room temperature with ordinary plastic embedded specimens, so as to make sure that the knife holder in the cryochamber (147) and the specimen holder on the bridge (193) are working properly. This testing of the system with ordinary plastic blocks should always be repeated whenever doubts arise about the satisfactory working of the FC4E system.

In view of the time necessary - at least 10 minutes - to equilibrate the temperature of a newly inserted knife to match the chamber temperature, two KNIFE-CARRIERS (171) for alternate use are provided as standard equipment in the FC4E. Each of these knife-carriers can take three knives: two triangular knives (k) and one trimming knife ("cylindrical trimming rod" 173) with an elliptical cutting edge. As a result of this arrangement the knife does not have to be changed so often. There is also a knife carrier always in reserve for quick replacements. If necessary this replacement holder can be equipped differently to the one in use in the chamber, perhaps with diamond knives.
The insertion of new knives into the carrier (171) always takes place outside the chamber. Since the holder with the used knife is at a temperature of \(-50^\circ\text{C}\), a manipulator (190) is needed. This is screwed onto the aluminium top (177) into a metric 4 mm screw thread. The clamping screws (151) are then loosened, and the knife carrier is taken out of the cryochamber and heated to room temperature. The quickest and simplest way to do this is by using a thermostatic heating plate at about +100°C. THE REICHERT HL 138 HEATING AND ILLUMINATING PLATE has proved excellent for cryo work. The left plate (d) can be set at any temperature between 40 and 120°C by turning the knob (c). The knife carrier (171) with the used knives (k) is taken out of the cryochamber by using the manipulator (190), and is immediately placed on the cool right hand plate, where the layer of frost, which forms whenever the carrier meets the moist room atmosphere, first of all melts and then evaporates. After this the warm knife-carrier (171) with the manipulator (190) still attached to it, is transferred to the aluminium block (e) that rapidly cools it to a temperature which makes it possible to exchange the knives.

To insert or replace the knives, the manipulator (190) has to be removed, and the aluminium top (177) is also taken off the carrier (171) after first loosening the screw (178) with the hexagonal key (192). To remove the triangular knives in order to replace them, screws (175/176) are loosened. For replacement of the cylindrical trimming tool (173), screw (174) is loosened. Before fitting the new knives into the knife carrier, all surfaces are carefully cleaned to remove any glass splinters, specimen remains or other dirt, and the adjustment blocks (172) are checked. These engraved blocks are used to preselect the CLEARANCE ANGLE for the right and left knives. The clearance angle can be 0°
- without any block (172) - or values of 3°...6°...9°... which are engraved on the block (172). If other values which come between the above engraved clearance angles are absolutely necessary, then extra layers can easily be added to the blocks (172) to obtain the required value. Care must be taken when CLAMPING IN GLASS GLASS KNIVES (k). The clamping pressure must never be increased to such an extent that the glass splinters. The cutting edge of the CYLINDRICAL TRIMMING TOOL (173), and also the cutting edges of diamond and glass knives, must never be allowed to come into contact with a hard object, as this immediately produces chipping. With proper use the trimming tool will have a sufficient life. The trimming tool should project about 1 mm above the cutting edges of the triangular knives. Once the knives have been inserted, the top (177) is replaced and secured to the knife carrier (171) with the screw (178).

For the TRIAL RUN AT ROOM TEMPERATURE one of the two glass knives, set with a clearance angle of 6°, is fitted with a trough (b) made of plastic adhesive tape, and sealed in the usual manner with a drop of wax, so that this knife (k/b) permits normal floating off of the sections onto a water surface. As specimen, a flat embedding has to be clamped into the appropriate SPECIMEN HOLDER E (207) or R (208) and the clamping jaws are tightened with the key (191) or (192), as in standard ultramicrotomy. The embedded specimen should not project out of the holder further than is absolutely necessary (max. 2 mm). The actual trimming takes place in the chamber under conditions which simulate frozen section preparation. If the jaw-holders (207) and (208) are not available then a spe-
cimen carrier (204) can be used: The cap (b) of a cylindrical plastic block or the tip of a flat embedding are sawn off and glued onto the disc head of the SPECIMEN CARRIER (204). In this case, too, the specimen should not protrude more than 2 mm beyond the specimen carrier. It is advisable to harden the glue in a thermostat to ensure that the block is securely fastened. The INSERTION OF THE SPECIMEN CARRIER (204) INTO THE STANDARD SPECIMEN HOLDER (200) provided for the purpose takes place in the chamber, once again under conditions used for frozen sectioning. This involves lifting up the holder (207), (208) or (200) with the manipulator (190), and inserting it in the appropriate hole of the bridge (193) which is already mounted, at the same time opening the clamping screw sufficiently with the hexagonal key (191) to allow this insertion. After insertion the holder is turned through its longitudinal axis into the required position using the thick rod of the tool (202) which is inserted into the holes. Finally screw (195) is tightened to fix it in position. Before inserting the specimen carrier, care is taken to ensure that the clamping ring (201) has been unscrewed sufficiently by the manipulator (202) to allow the carrier to pass in completely up to its head. In frozen sectioning this insertion is carried out with the help of TWEEZERS (206), which are colled to the appropriate temperature for work with frozen specimens beforehand by means of LN2. To obtain good sectioning results it is essential for the specimen carrier to be pushed in right up to their necks, so that the head is supported by the side shoulders of the holder. Sketch A shows the specimen carrier pushed in the direction of the arrow up to the end stop, and then fixed in position by turning the clamping ring (201) with the manipulator (202).

Different procedures are required for CLAMPING THE SPECIMENS IN THE VARIOUS HOLDERS during cryowork. The STANDARD SPECIMEN HOLDER (200) always stays in the specimen bridge (193). The specimen carriers (203/204/205) with their specimens are inserted into the cold holder (200) by using the SPECIAL TWEEZERS (206). Similar conditions apply to SPECIMEN HOLDER R (208) which is used for holding rigid, brittle,
hard specimens. The teeth of the two clamping jaws of this holder give good reliable clamping even with relatively low pressing power, but there is insufficient thermal contact between the holder and the specimen, so that the specimen finally reaches the GN2 temperature. Conditions are quite different with SPECIMEN HOLDER E (207) USED FOR ELASTIC SPECIMENS into which the specimens are clamped in their elastic state at room temperature, thus forming good thermal contact between the specimen and the clamping jaws with their relatively flat profile. The manipulator (190) is then screwed into the holder containing the clamped specimen. When this warm holder has been inserted into the chamber a short time must be allowed - at least ten minutes - for thermal equilibrium to be established. For temperature settings below -120°C, this time allowance must be increased to 15 minutes.
Once the specimen is mounted, the knife-carrier (171) is inserted in the cryochamber (147). Simulating frozen section routine, the knife-carrier (171) is placed in the chamber by using the manipulator (190). It is inserted behind the two clamps (152) on the base (153) of the knife-holder and pushed in the direction against the specimen and the blacklighting tube (154). When this is done the two clamps (152) grip into grooves on the two sides of the knife-carrier (171). The two screws (151) are then tightened with the hexagonal key (191) and the clamps (152) are pulled downwards pressing the knife-carrier (171) against the upper surface of the knife-carrier base (153). The two screws (151) have to be tightened evenly to maintain the position of the knife-carrier. This is done by tightening each screw in turn by a small amount. Care must be taken to avoid completely tightening one screw while the other is still loose. The knife carrier will maintain its position provided the two corresponding surfaces (underside of the knife-carrier and top side of the knife carrier base) are absolutely clean and plane. If a lateral deflection occurs, both surfaces must be checked. If they are both found to be clean, it might be due to the formation of a ridge on one of the edges of the underside of the knife-carrier (171) caused by contact with a hard object. Any such ridge can best be removed by abrasion with fine emery paper on a plane surface (marble or glass sheet).
To ensure accurate advance and adjustment there is an ADJUSTMENT AND SWIVELLING DEVICE FOR THE KNIFE-CARRIER (171) consisting of connection lever (179), a pincer clamp (181) and a stainless steel ruler (184) with adjustment wheel (188). After tightening both screws (151) and taking off the manipulator (190), the connection lever (179) is screwed onto the aluminium top (177) of the knife-carrier (171) with the screw (180) as shown in the sketch. The screw (180) is tightened with the hexagonal key (191). After this the free end of the connection lever is clamped in the pincer clamp (181) which slides with its magnetic base (183) on the ruler (184) and whose spring-mounted jaw (182) is opened for the slipping-on process. When the double jaws of this clamp (181, 182, 183) are closed, it matches the position of the knife-carrier (171) and the connection lever (179). The two screws (151) can then be loosened to allow coarse adjustment by sliding the base (183) in a longitudinal and crosswise direction. The crosswise direction produces a swivelling movement round a bolt, which is mounted in the knife-carrier base (153) and which meshes into a groove on the underside of the knife-carrier (171). In addition to this manual coarse adjustment, there is the adjustment wheel (188) to provide precision pivoting of the knife carrier round the vertical bolt axis. The extent of the pivoting can be read off the ruler (coarse and off the wheel (fine) scale.
The eccentric arrangement of the two triangular knives causes the pivoting to be non-eucentric round the middle of the cutting edge, which involves either a forward or backward movement and has to be compensated by appropriate sliding of the clamp (183) on the ruler (184) or by the drive (3) of the knife support. The knife is then accurately set by longitudinal drive (3) and transversal drive (47 resp. 48E) which is located under the connection box (cover 164). As with work at room temperature, the setting is greatly facilitated by the reflection provided by the backlighting. Owing to the eccentric arrangement of the two triangular knives, the reflection in a precut plane surface appears in only one of the eye-pieces of the stereo-microscope, even if a scattering layer (such as parchment or a scattering foil) is inserted between the chamber floor and the outlet hole on the knife-support. After completing the setting for knife/specimen adjustment, the knife-carrier (171) is screwed tightly onto the base (153) with the screws (151) in the manner already described (i.e. alternately). Then the screw (180) is loosened and the connection lever (179) is taken off the aluminium top (177) together with the clamp (181/182/183) which is thus lifted off the ruler (184).
SECTIONING IN THE CRYOCHAMBER is very similar to standard sectioning with the ordinary ULTRACUT. In the present case (i.e., with chamber at room temperature) the left hand glass knife (k: see sketch A) is used to prepare a smooth cutting surface by advancing the knife (k) towards specimen, first with the coarse drive of the knifesupport, then with the knife fine drive (click-stop on the left side of the ULTRACUT) whilst keeping it under constant microscopic supervision. When the SMOOTH CUTTING SURFACE HAS BEEN PREPARED, it is TRIMMED INTO SHAPE WITH THE CYLINDRICAL TRIMMING TOOL (173) as shown in sketch B after the chamber has been retracted with the coarse drive, by transverse adjustment of the chamber, in the direction of the arrow. After the right side edge has been trimmed the chamber is once again moved back with the coarse feed and then the left hand side is similarly trimmed with the other side of the trimming tool (173) as shown in sketch C. After completing the trimming, which can be kept under observation with the structure viewer mirror (217), the specimen is turned through 90° with the holder, as in sketch D. This involves moving the chamber back with the coarse feed, loosening the clamping screw (195) and turning the specimen holder (200, 207 or 208) with the manipulator (202), which has to be inserted into the holes provided around the holder, as in sketch D. The screw (195) needs only to be turned a little (about 180°) for this purpose. Whilst this is happening the bolt remains in spring-loaded contact with the corresponding notch in the specimen-holder, which thus maintains its position during the rotation. It is only when the holder has to be replaced that the screw (195) needs to be unscrewed sufficiently to make the spring-bolt release the track in order to allow the holder to be removed. When the 90° turn has been completed the clamping screw (195) is retightened and the cutting surface can then be trimmed on the two remaining sides. As in ordinary ultramicrotomy, good sectioning results are very dependent on precise, clean trimming of the cutting face, which should normally not exceed 0.2 x 0.2 mm and should contain no large...
ice-crystals (milky-cloudy appearance of specimen when backlit). When sectioning plastic embedded specimens in the initial learning process and also when cheking later on in cases of unsatisfactory sectioning, the cutting face should be limited to an area of \( \leq 0.5 \times 0.5 \text{ mm}^2 \) in a square or rectangular shape (maximum width of the rectangle could be 0.5 mm but 0.3 mm would be better). The lengths of the sides can be estimated with the help of the graticule available for the microscope. When working at low temperatures in the extreme limits (cutting surfaces of less than \( 0.2 \times 0.2 \text{ mm}^2 \) with native biological specimens) the lengths can be best checked with a micrometer eyepiece which is obtainable as an accessory to the STEREOSTAR stereomicroscope and which can be calibrated in the usual way with a mm-ruler. In such cases an additional high intensity halogen lamp with fibre optics is advantageous. After the specimen has been trimmed, the trough (b) on the right hand knife is filled with clean water using a pipette or syringe and the reflection is adjusted in the usual way. Now it is time to start cutting with sections in the usual manner. Provided all parts have been securely clamped and that the plastic embedded specimen is held properly in its holder and is correctly trimmed good uniform sections should emerge in the silver to matt gold interference range, which when examined in the stereomicroscope or the TEM should show no vibration or chatter.

The whole of the preparatory process described in this chapter should be repeated and practised until it can be carried out reliably and quickly, and all the manipulations have become second nature.
IV. CRYO TOOLS FOR PREPARING SECTIONS

For work at low temperatures and in particular when preparing frozen sections of native material, the grids for supporting the sections together with their special holders are positioned immediately behind the knife cutting edge. The risk of the grid and specimen warming up and consequent recrystallisation of an amorphous frozen native section is largely eliminated if pre-cooled instruments (e.g. TEFLON needles) are used to transfer the sections.

Before insertion in the cryochamber the required number of grids (maximum of six grids with standard diameter 3 mm) are fixed into the grid holder (214/215/216) with the aid of a loading device (219 - 224, sketch A). For the loading the grid holder (214) is inserted into the clamping jaw (220) of the device. As shown in the sketch, it is then fixed into position with the screw (221). The spring-loaded clamping jaw (215) of the holder is then opened by loosening the screw (216), then the loading plate (223) is raised by loosening the screw (224) sufficiently to bring its upper surface between the edges of the two jaws of the grid holder (sketch B). The key (42 resp. 192) is used throughout to turn all these screws (216/221/224). Once the described position as shown in sketch B has been reached, the grids on the plate (223) can be pushed between the jaws of the open grid holder with the help of a needle and can be arranged in a row. Next the plate (223) is lowered by tightening the screw (224) as in sketch C. The grids are now lying on the edge of the lower grid holder jaw. When the spring loaded jaw (215) is tightened with screw (216) the grids will all go into a horizontal position as in sketch D.
The holder with its grids can now be taken out of the clamping jaw (220) by loosening screw (221) and be plugged to the cylindrical bolt (213) of the cryo holder (211/212). If the cryo holder is already in the cryochamber and pre-cooled then the manipulator (190) can be screwed onto the grid holder (214) which can then be transferred into the chamber.

The CRYO HOLDER (211/212/213: sketch A) is used to manipulate the grid holder (214/215/216) and also the STRUCTURE-VIEWER MIRROR (217) in the cryochamber. The cryoholder consists of a solid metal part (211) with rubber coating for putting on the lid (187) of the cryochamber (147). When its two spring-loaded limbs are pressed together, the slider (212) in the holder (211) provides precision height adjustment for the slit cylindrical bolt (213) which is used to plug in the grid holder and the structure viewer mirror (217). Height adjustment is done with one hand, by placing the hand on the holder (211) and squeezing the two limbs of the slider (212) between the thumb and forefinger and altering the height. The fine adjustment is done later by turning the grid holder (214) or the mirror (217) round the longitudinal axis of the cylindrical bolt (213) with the help of the hexagonal bar of the manipulator (202: see sketches B and C). The position of the grid is adjusted in such a way that the grid (n) comes immediately behind and a little below the cutting edge of the knife (k).

Like the MESACUT-structure viewer in the standard ultramicrotome, the mirror (217) provides simultaneous front and vertical viewing of the specimen and also its cutting face with the stereomicroscope (sketch C). If necessary two cryo-holders can be used at the same time as in sketch A.
After the sections are put onto the grids (n) the gridholder (214) is transferred with the cryoholder (211) either onto the top surface at the aluminium top (177) or to the "section press" (156) (see sketch D). If you want to use the aluminium top you have to work with manipulator (202) to align gridholder (214). After unlocking screw (216) with hexagonal key (191) the grids (n) are now placed onto the aluminium top (177).

With the pre-cooled stamping tool (218) you press now with the polished surface on your sections to flatten them. (see sketch E).

If you are using the section press (156) you put the grids onto the round plate. This plate is rotatable with tweezers (206) so you can put on a number of grids and rotate them underneath the pressing plate. Is the section press fully loaded you tighten screw by using the hexagonal key (191) so that the sections are pressed onto the grids.

The transfer to a freeze-drying apparatus then takes place preferably under LN2 or in a small container. The transfer of frozen-hydrated sections into a TEM or STEM requires a special system, which prevents moist room air from entering and also prevents the warming up to temperatures above -140°C both during the transfer and the entry into the EM.
V. RECOMMENDED ACCESSORIES FOR CRYO-ULTRAMICROTOMY

In addition to the accessories mentioned in the REICHERT-JUNG Catalogue for the FC4E system for use in cryo-ultramicrotomy, a very useful extra accessory is a commercially available HIGH POWER HALOGEN LIGHT SOURCE WITH FIBRE OPTICS. This light is especially applicable for critical sectioning in the cryochamber which will have to be viewed at high magnification in the stereomicroscope. The light from the fibre optics is very bright, but even so exhibits no disturbing infra-red components, which are liable to heat the surface of the specimen or the cryosections.

TRANSFER SYSTEMS FOR THE TRANSFER OF FROZEN HYDRATED SPECIMENS can be purchased from manufacturers of electron microscopes and accessories for electron microscopy. It is recommended to ask primarily the manufacturer of the EM used if there is experience with transfer systems offered for the special EM model. Besides EM-scope and HEXLAND in the UK, GATAN in the USA offers a transfer unit compatible with JEOL side entry systems.
VI. WORKING AT LOW TEMPERATURE

CONNECTING THE LN2 AUTOMATIC REFILLING DEVICE

In FC4E systems equipped with the automatic LN2 refilling device, the method of filling the dewar vessel (101) with LN2 and of connecting the cryochamber (147) is as follows (see also LIST OF COMPONENT PARTS and the chapter on "Precautions to be observed"):

➢ Stand dewar vessel (101) on castor support (104) and remove cap (103). The dewar vessel can now be filled with LN2. DO NOT OVERFILL THE DEWAR VESSEL: The LN2 level after filling should come to about 15 cm BELOW THE OPENING OF THE VESSEL.

➢ Before insertion in the dewar vessel, check the dewar head (105) with flange (107) and protective aluminium tube (110) to ensure that the sealing ring (108) is properly in position on the underside of the flange (107) and that the tube (115) with the sealing cap (112/113) is fully sealed (sealing ring 114).

THE CHAMBER IS FILLED WITH LN2 FROM THE DEWAR VESSEL (101) by means of a 6 mm thick polyamide tube (161) suitable for low temperature work. This tube is insulated by a 30 mm thick ARMAFLEX expanded rubber tube (162) pushed over it. The polyamide tube has been purposely kept short to minimize LN2 losses during the initial filling or later refills. This means that the dewar must be placed immediately next to the ULTRACUT. The length of the ARMAFLEX tube (162) is 75 cm, thus making it 5 cm longer than the polyamide tube (161). Please DO NOT
SHOTEN THE ARMAFLEX TUBE (162) but follow the instructions given below. In addition to this 75 cm long ARMAFLEX tube (162), a further ARMAFLEX tube in expanded rubber (163) is supplied as standard. This second tube which is only 30 cm long and has a larger bore, is used to carry off the cold GN2 from the phase separator (158/159) on the cryochamber (147). The chamber (147) is connected to the refill system, as shown in the sketch in the following manner:

» Stick the polyamide tube (161) onto the metal pipe (116) on the dewar head (105).

» The ARMAFLEX tube (162) is pushed over the polyamide filling tube (161) so as to completely cover it.

» DO NOT SHORTEN the ARMAFLEX tube (162) but push it into itself until the free end of polyamide tube (161) appears. The elasticity of the ARMAFLEX material enables this to be done. Now stick the polyamide tube (161) onto the metal pipe (160) on the phase separator (158). With regard to LN2 consumption of the system, it is important to ensure that the ARMAFLEX tube (162) not only covers the full length of the polyamide tube (161) but that its free ends also envelop the two metal pipes (116) on the dewar head (105) and (160) on the chamber (147). Not only does this avoid excessive use of LN2 but it also prevents frost formation at the tube connections and vigorous boiling of LN2 during start of the refill process.

» Before putting the system into service, the shorter ARMAFLEX tube (163) must be put on the plastic tube (159) on the phase separator (158). This tube is used to carry away the GN2 formed during filling or refilling of the chamber (147) with LN2. The gas is conducted away from the ultramicrotome area and passes over the rear edge of the table.
Then with great care introduce the dewar head (105) into the full dewar (101) slowly enough to prevent any boiling over of the LN2. IF THERE ARE ANY SIGNS OF BOILING OVER IMMEDIATELY LIFT OUT THE DEWAR HEAD, thus COMPLETELY breaking any contact between the LN2 phase and the protective tube (110). Then start to put in the head again, proceeding very slowly.

When the tube (110) has been fully inserted, use pliers (117) to press the two corresponding flanges (102) and (107) tightly together, so that the sealing (108) of the dewar is hermetically tight. Evaporating nitrogen gas (GN2) will escape through the open solenoid valve in the dewar head. Then connect the multipinplug (109) into the socket (121) of the control unit (118).
VII. CRYOWORK BELOW -120° C

The system having been assembled, the operator having familiarized himself with all the manipulations at room temperature and the automatic refill system having been connected, it is now time to put the system into operation for the definite calibration of the temperature settings:

- First check that the two plastic baffle plates (189) have been taken out of the chamber.

- Insert the knife-carrier (171) with new knives either attached to the connection lever (179) of the adjustment and swivelling device (179/180) or to the manipulator (190).

- Insert the specimen holder (200), (207) or (208) into the cryochamber (147).

- Insert the cryoholder (211/213) with a loaded grid holder (214) or/and structure mirror (217) into the cryochamber.

- Insert the required accessories for precooling into the chamber.

- Connect the connection lever (179) to the pincer clamp (181/183).

- Adjust the control lever (12) on the hand wheel (15/16) of the ULTRACUT into a horizontal position and use the hand wheel to lower the bridge (193) to its bottom setting.

- Set the push buttons on the panel area (128) on the control unit (118) as follows (watch the LEDs):
  
  mainbutton (129, lights up)
  release H button (132) if it is pressed down (indicator light goes out)
  release FD button (133) if it has been left pressed down by mistake. *) (indicator goes off)
  press RC button (137) (lights up.)

*) The FD button is used to stop the automatic refilling process or to prevent any filling with LN2 of the twin tank.
The required temperature is set by pushing and rotating the knob (123/124). The temperature will be displayed in the windows (126/127). If the knobs (123/124) are not pushed the actual temperature is shown in the windows (126/127). Set knob (125) to "0".

When the main button (129) is pressed the LEDs (135-1) to (135-5) in the operational diagram (134) show the level of the contents of the storage dewar. The LEDs (136-1) to (136-5) show the level of filling in the chamber (147). The successive lighting of the chamber diodes one after the other (136-1) to (136-5) can be seen during the slow filling of the chamber. When the twin tank is completely full all the five LEDs are lit. With RC operation, when the twin tank is completely full, the LN2 overflows into the working chamber and comes into direct contact with the knife and specimen holders, for as long as the RC button (137) is pressed down. When the LN2 level in the working chamber has reached the level of the sensor, the LED (136-6) will light up on the operational diagram (134). Synchronously the filling process stops as indicated by the LEDs (141) and (140) coupled with the solenoid valve in the dewar head (105). The LED (141) lights up, if the valve is open. Contrary it goes out, if the valve is closed for pressurisation and refilling. LED (140) now indicates LN2 refilling. Subsequently there is a rapid succession of refills, always with a short pause in between, which can be observed on the diagram. Thus the "LN2 lake" in the working chamber is kept at an almost constant level. The temperatures of the knife holder base and the specimen bridge being in direct contact with the LN2 reach the lowest temperature value of approximately -190°C in a very short while as indicated by the instruments (126) and (127). For work at -190°C the RC button (137) remains depressed. For work at temperatures between -170°C and -120°C the procedure is as follows:
Release RC button (137). The diode (136-6) in the operational diagram (134) go out.

Adjust with knob (125) the required temperatur of the chamber.

Put the control lever (12) on the handwheel (15/16) of the ULTRACUT in its highest position and lock the bridge in the middle position by turning the handwheel. The specimen has now to be exactly at the level of the knife cutting edge.

As a rule sectioning is started independently of the displayed level IN THE TWIN TANK. The REFILL PROCESS operates so slowly that it hardly affects the practical work in the chamber, except that at the start of the refilling one or two sections may be skipped due to the development of GN2. Refilling the chamber before sectioning (pressing button 139) is only recommended for extremely thin sections in the range below 50 nm. In this case there is a period of about ten minutes for sectioning after the refilling process is completed and before the next refill starts.

Usually THE SPECIMEN CAN BE INSERTED IN THE SPECIMEN HOLDER during the cooling down is still in progress, perhaps even before the required sectioning temperature has been reached. Any time left in hand can be used for the SPECIMEN/KNIFE ADJUSTMENT.

The TEMPERATURE CAN BE ALTERED in a similar fashion to the method described above.

If there is going to be a BREAK IN SECTIONING the potentiometers 123/124 are returned to the "0°C" and (125) to "0".

If the break is going to be longer than two hours, it is advisable to completely close down the system and heat it up.
VIII. CRYOWORK ABOVE -120°C

The procedure differs from the one used for temperatures below -120°C, since for work in the temperature range above -120°C there is no rapid cooling (RC) with LN2 and instead the GN2 scavenging is conducted by the baffle plates (189) over a heating plate (155) which pre-warms it. The system is started as follows:

- First check that the two plastic baffle plates (189) have been inserted to the right and left of the heat exchangers (197) and (199). Please make sure that they have been pushed in right down to the floor of the chamber.
- Insert the knife carrier (171) with new knives either attached to the connection lever (179) of the setting and pivoting device or to the manipulator (190).
- If required, insert the cryo-holder (211/213) with a loaded grid holder (214) and/or structure viewer mirror (217) or other accessories for the cryochamber (147).
- Connect the connection lever (179) to the pincer clamp (181/183).
- Put the control lever (12) on the hand wheel (15/16) of the ULTRACUT into the highest position and turn the hand wheel to lock the specimen exactly in the height of the cutting edge of the knife.
- Check all electrical connections of the system FC4E.
- Set the push buttons on the panel area (128) on the control unit (118) as follows (watch the indicator lights):
  - Mainbutton (129) pressed (light comes on)
  - H button (132) released, if depressed (indicator goes out) cf. foot-note on p. 34
  - FD button (133) released, if depressed (indicator goes out)
  - Release RC button (137) if depressed
- Set potentiometer (125) immediately on maximum value turning the knob clockwise to the stop.

- The required temperature is set by pushing and rotating the knob (123/124). The temperature will be displayed in the windows (126/127). If the knobs (123/124) are not pushed, the aktual temperature is shown in the windows (126/127).

When the mainbutton (129) is pressed, the LEDs (135-1) to (135-5) in the operational diagram (134) will show the level of filling in the storage dewar (101). The LEDs (136-1) to (136-5) show the level of filling in the twintank of the cryochamber (147). When the twintank is completly full, all the five LEDs will be lit. The slow chamber filling can also be seen as the chamber diodes light up one after the other in order from (136-1) to (136-5). The specimen and knife carrier are cooled by the GN2 stream evaporating out of the twin tank. The fall in temperature can be watched at the indicators (126) and (127), until the required temperature is reached.

Sectioning is generally started without being dependent on the LEVEL OF FILLING IN THE TWIN TANK. During the final stage of cooling the SPECIMEN IS INSERTED INTO THE SPECIMEN HOLDER, and the period of waiting to reach the cutting-temperature can be used for SPECIMEN/KNIFE ADJUSTMENT.

Any ALTERATIONS OF THE TEMPERATURE are made in the way described as in adjusting cutting-temperature.

Potentiometer (125) for the GN2 heating plate stays nearly on the "max" setting throughtout. If SECTIONING IS GOING TO BE INTERRUPTED potentiometers (123/124) to "O°C" and (125) to "O". For breaks in sectioning which will last longer than two hours, it is advisable to turn the system off completely and to heat it up.
IX. REFILLING OR REPLACING DEWAR DURING CRYO-OPERATION

The length of time a standard-filled dewar vessel (101) will provide continuous, uninterrupted sectioning depends on the mode of operation. It can be from five hours (for temperatures of around -50°C) to ten hours (for temperatures around -170°C). If a longer uninterrupted period of sectioning is required, or if the dewar was only partly filled at the start of sectioning, then the procedure is as follows and should be implemented at the latest when the acoustic and visual warning N2D (130) are given. In this case you proceed as follows:

- Cancel the acoustic signal by pressing button N2D (130).
- Press the REFILL button (139): The twin tank will then be completely refilled with LN2.
- When the refill is completed, shown by LED (136-5) lightening up on the operational diagram (134).
- After a short wait and, if necessary, after warming up the end of the polyamide tube (161) with the fingers, pull this tube end off the metal pipe (160) on the phase separator (158).
- Open the pliers (117) and take out the dewar head (105) with the aluminium protective tube (110) of the dewar vessel (101).
There will now be at least five minutes to REFILL THE DEWAR (101). It is very helpful to have a RESERVE DEWAR (101) READY FOR USE ON ITS OWN CASTOR SUPPORT (104). This can be ordered as a separate accessory. This second reserve dewar is always kept ready filled with LN2 and when the dewar in use on the system is nearly empty, the reserve dewar is placed next to it. The polyamide tube (161) is now pushed onto the metal pipe (160) on the phase separator (158), and the aluminium protective tube (110) which is still deeply cooled is inserted into the filled reserve dewar immediately after having been taken out of the empty dewar. The pliers (117) are now closed again and within a few minutes the system is operating as usual again. If, however, there is no reserve dewar available and the aluminium protective tube (110) has become warm while the dewar was being refilled, the procedure like in chapter connecting the LN2 automatic refilling device.
X. END OF CRYOWORK - AUTOMATIC

"BAKING-OUT" OF THE CHAMBER

When the sectioning is completed the cryochamber (147) can be automatically "baked-out" by pressing H button on the panel area (128). IT IS IMPORTANT NOT TO RELEASE MAINBUTTON (129) at this moment as this would also cancel the baking-out. All the other functions in the system are automatically stopped from functioning by the pressing of the H button (132).

The baking-out is completed after about two hours. However, it is advisable to leave the H system on overnight or over the weekend, as this ensures that all the remains of water are evaporated away from the inside of the system (twin tank, LN2 connection system, GN2 outlet system) where water may have collected as the result of cryosorption of the cold inside surfaces. Overheating of the system during this continuous operation is prevented by the use of thermostitches. Maximum heat output during the continuous operation is automatically limited to 100 Watts. It is only during the initial heating of the chamber that greater heating powers are used.

If the amount of LN2 left in the dewar (101) is worth keeping for the next cryosectioning, in order to save costs, then proceed as follows:

- Pull the multiplug (109) out of the socket (121) on the rear panel of the control unit (118).
- Open and remove pliers (117).
- Take off dewar head (105) with aluminium tube (110) and store it in a safe place. It is advisable to put it on a tray of some sort to catch the frost layer which will thaw off.
CAUTION: Avoid the effects of force on the aluminium tube (110) as these could lead to defects in the system of slits (111).

Close the dewar opening with the protective cap (103).

The method described above for the REMOVAL OF THE DEWAR HEAD (105) reduces the evaporation rate considerably and ensures that the remaining LN2 can be kept over long periods of time for future use. If the amount of LN2 left in the dewar (101) is not worth bothering about, then the dewar head is left in place and in electrical contact with the control unit.

FAULTS IN CLOSING DOWN THE SYSTEM AFTER WORK IS CONCLUDED (e.g. interruption the cryogen feed through complete consumption of all the LN2 or pressing the FD button, comp. foot-note on p. 34, by mistake, stop of work without the H automatic system or current failure etc.) can result in complete ICING UP OF THE SPACES IN THE CHAMBER THROUGH CRYOSORPTION. When this layer of ice thaws, part of the water thus formed penetrates by surface tension into all the capillaries and slits, so that when the system is cooled down again every movement is blocked. If such a fault has occurred then the knife carrier (171) and specimen holder (bridge 193, and whatever specimen holders were being used) must be taken out of the chamber and be fully cleared of all water traces. After initial warming this is done on a hot plate or in a laboratory thermostat at about +80°C and it is best to dismantle the holders into their component parts, from which the remains of water will quickly evaporate at +80°C.
XI. LIST OF COMPONENT PARTS (numbered)

No. Description

ULTRACUT AND ULTRACUT E ULTRAMICROTOMES

1 to 100 = Component parts of the REICHERT-JUNG-ultramicrotomes ULTRACUT and ULTRACUT E. The numbers correspond with the manuals of these ultramicrotomes. If needed, a discrimination of the partially different ULTRACUT E is realized by adding the letter "E" to the number (e.g. microscop carrier 29 on ULTRACUT model 1977/82 versus different carrier 29-E on ULTRACUT E since 1983). Compare for all numbers up to 100 the mentioned ultramicrotome manuals.

AUTOMATIC LN2 REFILL DEVICE

101 Dewar vessel for the automatic LN2 refill of the chamber
102 Flange to secure dewar head (105) by means of pliers (117)
103 Cap to cover dewar vessel after removal of dewar head (105), especially during transport
104 Castor support for dewar vessel (101)
105 Dewar head with filling valve and refill control under cover (106)
106 Cover for valves and controls for automatic LN2 refill
107 Flange on dewar head (105) to correspond with flange (102) on the dewar vessel (101) to attach the dewar head to the dewar vessel by means of pliers (117)
108 Sealing ring on flange (107) to provide airtight seal for the contents of the vessel for LN2 refill of the cryochamber (147)
109 Multipin plug with cable connection for connecting automatic refill to the control unit (118)
110 Aluminium protective tube covering measuring diodes and pressure generator of the refill device
111 System of slits ("heat barriers") to reduce heat exchange between dewar head and cryogen

112 Sealing cap with safety valve (113) on connection tube (115)

113 Safety valve (0.35 bar) on sealing cap (112)

114 Sealing ring

115 Connection tube for cap (112)

116 Metal pipe for polyamide filling tube (161) for LN2 chamber filling

117 Pliers for clamping the dewar head (105) to the dewar vessel (101)
ELECTRICAL CONNECTIONS AND CONTROL UNIT

118 Control Unit

119 Socket for electric supply 110/220 Volts according to specification on rear panel of control unit

120 Fine wire fuse as specified on rear panel of control unit

121 Socket for multipin plug (109) of the automatic refill on the rear panel of the control unit (118)

122 Socket for multipin plug (148) of the cryochamber (147) on the rear panel of the control unit (118)

123 Potentiometer knob for adjusting the knive temperature

124 Potentiometer knob for adjusting the specimen temperature

125 Potentiometer knob for adjusting the chamber temperature

126 Digital display showing temperature of knife carrier (153)

127 Digital display showing temperature of the specimen holder

128 Push button area with buttons (129) to (133).

129 Main button (general switch)

130 Optical warning indicator N2D for consumption of LN2 stored in dewar vessel (101) and push button to clear acoustical warning signal

131 Optical warning indicator N2C for consumption of LN2 stored in chamber tank and push button to clear acoustical warning signal
132 H button for automatic backing out (final heating of the working chamber)

133 FD-button for switching off the refill Comp. foot note on p. 34

134 Operational diagramm with LED's (135/136), RC button (137), REFILL button (139) and LED-displays (140/141) for function of automatic LN2 filling system

135/1-5 LED-display indicating LN2 level in dewar vessel (10f)

136/1-6 LED-display indicating LN2 level in chamber twin tank and working chamber

137 RC button for automatic chamber filling with LN2 for the initial cooling process and also for sectioning in the temperature range of about -190°C

139 REFILL button for a single filling of the chamber tank before the start of sectioning

140 LED-display for REFILL

141 LED-display for solenoid valve function in dewar head (105)

142 Digital display for chamber temperature

143 Tray

146 Ventilation slits in the lid covering the electric elements
CRYOCHAMBER WITH KNIFE HOLDER

147 Cryochamber with twin tank for LN2

148 Multipin plug with cable socket for connecting the cryochamber (147) to the control unit (118)

149 Thumb screw for securing the cryochamber (147) to the ULTRACUT knife support (8)

150 Entry for cryo-transfer system

151 Clamping screws for attaching the knife carrier (171) to the knife carrier base (153)

152 Clamps for attaching the knife carrier (171) to the knife carrier base (153)

153 Knife carrier base

154 Back-lighting tube with cover glass plate

155 Gas heating plate

156 Section press

157 Cover plate ("cold sandwich plate") for depositing cold components in the chamber area. Plate temperature independent of temperature of knife and specimen holders
158 Phase separator for separating gaseous nitrogen from liquid nitrogen during filling and refilling of the chamber tank.

159 Plastic connection tube to be covered with ARMAFLEX tube (163) for taking away the GN2 from the phase separator (158).

160 Metal pipe on which polyamide filling tube (161) is stuck for LN2 filling of the twin tank.

161 Polyamide tube for LN2 filling of the twin tank in the cryochamber (147).

162 ARMAFLEX expanded rubber tube insulating the polyamide tube (161).

163 ARMAFLEX expanded rubber tube for conducting away cold GN2 from the phase separator (158/159).

164 Lid of electric connection box for cryochamber (147) with socket (165) for multipin plug (196).

165 Socket for multipin plug (196) of bridge (193) in the lid (164) covering the electric connection box of the cryochamber (147).

166 Screws for attaching the chamber guide rails (167/169) to the ULTRACUT knife support (8).

167 Guide rails for mounting the chamber (147) on the ULTRACUT knife support (8).

168 Adjustable stop on left guide rail (167) to adjust chamber position.

169 Guideplate for receiving chamber (147) on ULTRACUT knife support (8).
171 Knife carrier to take two triangular knives and one trimming tool (173)

172 Engraved adjustment blocks for pre-selecting the clearance angles (3°/6°/9°) in the knife carrier (171).

173 Trimming tool (cylindrical rod of special alloy) with elliptical cutting edge to shape the cutting face

174 Screw to attach the cylindrical trimming tool (173) to the knife carrier (171)

175 Screws to clamp the triangular knives to the knife carrier (171) with discs (176)

176 Discs for insertion into clamping screw (175)

177 Aluminium top for knife carrier (171)

178 Screw to attach the top (177) to the knife carrier (171)

179 Connection lever of knife pivoting device (181/184/188) adjustment and swivelling

180 Screw to mount the connection lever (179) on the top (177) of the knife carrier (171)

181 Pincer clamp with spring-loaded jaw (182) for holding the connection lever (179). Equipped with magnetic base (183) for mounting on the ruler (184)

182 Spring-loaded jaw for the pincer clamp (181)

183 Magnetic base for pincer clamp (181)
Stainless steel ruler for the knife adjustment and swivelling device to ensure precise movement of the pincer clamp (181) with adjustment wheel (188). Screwed to the chamber lid (187) by screws (185/186).

Mounting screws for attaching the stainless steel ruler (184) to the chamber lid (187).

Chamber lid

Adjustment wheel for precise pivoting of the knife carrier by means of the ruler (184).

Baffle plates 55 x 80 mm made of plastic for diverting the stream of GN2 over the heating plate (155) for sectioning in temperature range above -120°C.

Manipulator with insulated handle and metric 4 mm screw thread for mounting and movement of knife carrier (171/177), specimen holder (200/207/208) and grid holder (214/216) in the cold chamber.

Hexagonal key (Allen key), 3 mm, with insulated handle to unscrew and tighten the screws (151/174/178/180/194/195/216) in the cold cryochamber.

Hexagonal key (Allen key), 3 mm, with knurled handle for clamping of the knives in the knife carrier (171) and for use with the loading device (219) for the grid holder (214). This key is identical to Allen key (42) for the specimen block of the ULTRACUT and ULTRACUT-E. It is a part of the standard equipment of these instruments and does not form part of the FC4 system standard supply. It is available as accessory if needed.
SPECIMEN BRIDGE WITH SPECIMEN HOLDER

193 Specimen bridge

194 Clamping screw for attaching the specimen bridge (193) to the specimen arm (24) on the ULTRACUT. Comp. advice to part (191)

195 Clamping screw for mounting the specimen holders (200/207/208) on the specimen bridge (193)

196 Multipin plug with cable for connecting the specimen bridge to the socket (165)

197 Heat exchangers at the specimen bridge for increasing heat emission to the GN2 scavenging gas stream,

200 Standard specimen holder for holding the specimen carriers (203/204/205)

201 Clamping ring for attaching specimen carriers (203/204/205) in the holder (200) by means of hexagonal key (Allen key) on the manipulator (202)

202 Manipulator with insulated handle and hexagonal key (Allen key) 1.5 mm for unscrewing and tightening the screw (201) and with bolt for turning the specimen holders (200/207/208) in the bridge (193) round the longitudinal axis.
203 Specimen carrier for suspensions
204 Specimen carrier for tissues
205 Specimen carrier for freezing a specimen in a slit.
206 Special crossover tweezers for inserting or removing the specimen carriers (203/204/205) in or out of the standard specimen holder (200).
207 Specimen holder "E" with fine surface roughness on the clamping surfaces of the two jaws to take elastic specimens.
208 Specimen holder "R" with indented surfaces on both clamping jaws to hold brittle and hard specimens.
209 Clamping screws on specimen holders (207/208)
210 Clamping jaws on specimen holders (207/208).
CRYOTOOLS AND MISCELLANEOUS PARTS

211 Cryoholder for depositing on chamber lid (187) for grid holder (214) and cryostructure viewer mirror (217)

212 Slider for height adjustment on cryoholder (211)

213 Cylindrical bolt on slider (212) for sticking on grid holder (214) and cryostructure viewer mirror (217)

214 Grid holder for simultaneous clamping of six grids with 3 mm diameter with hole for sticking on bolt (213) of cryoholder (211/212). Grid holders are loaded by means of a special device (219/224).

215 Spring-loaded clamping jaw on grid holder (214) for clamping in grids by means of screw (216).

216 Clamping screw with hexagonal socket head for opening and closing the clamping jaw (215) of the grid holder (214).

217 Structure viewer mirror for front viewing of the cutting face of the specimen block in the cryochamber with hole for sticking it on to the cylindrical bolt (213) of the cryoholder (211/212).

218 Stamping tool with mirror finished pressing surfaces made of copper and teflon for manual pressing of the section on the grid.
219 Device for loading the grid holder (214) with grids.

220 Clamping jaw for fixing the grid holder (214) by means of a screw (221).

221 Clamping screw for tightening the jaw (220) with the key (42 resp. 192).

222 Adjusting screws for adjusting the loading plate (223).

223 Loading plate for depositing and pushing the grids into the open grid holder (214).

224 Levelling screw for lowering and raising the loading plate (223) by means of the key (42 resp. 192).

228 Extension knob for left/right movement (47/48E).

**ELECTRICAL DATA**

Type: 65 27 15

\[
\begin{array}{c|c|c}
V & 110 & 120 \\
\hline
VA & 550 & 110V \ 6.3 \text{ MT} \\
& & 220V \ 3.15 \text{ MT}
\end{array}
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