Reichert-Jung

MM 80

Operating Instructions

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MM 80/GA/E – 87/12

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METAL MIRROR CRYOFIXATION (impact freezing)

Installation and Assembly

Before connecting the main unit (1) to the electric power supply (4), check the voltage setting of the voltage selector (3) on the rear panel (2).

Metal Mirror Support

The metal mirror support (133) is plugged into the liquefier (70) and secured with the locking screw (134).

Impact Freezer

First load the spring of the impact freezer (136) by pulling up on the knurled knob (138) of the rod (137) until it clicks into position. Rotate mounting plate (27) to working position i.e. spring loaded release knob (28) to the left, clamp (29) to the right.

Slide impact freezer (136) onto mounting plate from front to rear up to back stop and fix by rotating clamp (29) clockwise.

Connect impact freezer plug (9) to socket (14) on the rear panel (2) of the main unit (1).

Press the LIGHT BUTTON "L" (72) and adjust illumination with the light control knob (139).

How to become familiar with the Impact Freezer

For best results it is recommended that the user familiarizes himself with the instrument as follows:

Hold the knurled knob (138) with one hand and press the release button (156) with the thumb of the other hand. With the release button (156) pressed down, guide the rod (137) slowly downwards and then gently try to lift it up again. You will notice that while the release button (156) is pressed a built-in reverse motion stop lock inhibits any upward movement. Therefore always keep the release button (156) pressed down until the rod has definitely completed its travel. This guarantees bounce-free delivery of the specimen onto the metal mirror.

Now set the PRESSURE selector (155) to "10", hold the knurled knob (138) with one hand as described above, briefly press the release button (156) and guide the rod (137) as far as it will go. At a certain point you will feel that the force driving the rod (137) downwards increases considerably. This additional secondary pressure builds up after the first layers of the specimen are optimally frozen and ensures firm contact with the metal mirror until the entire specimen is completely frozen. The point at which this secondary pressure is activated is determined by the setting of the THICKNESS selector. Thickness in this case is the height of the compressed specimen carrier. Although the thickness of the carrier with the foam cushion and the specimen is about 12 mm, the selector must be set to 3 mm, because this is the resulting thickness after the impact of the freezer.

The point at which the secondary pressure is generated on the path of the rod (137) can be felt as it is guided downwards. Vary the THICKNESS setting between "0" and "12" and notice the difference.

With the "SPEED" selector (154), a spring accelerating the rod (137) after pressing the RELEASE-button (156) is preloaded. The higher the setting of the selector the higher the speed of the rod. The optimum value for different specimens has to be empirically determined with a series of tests applying different speeds. As a rule of thumb: the harder the specimen, the higher the speed may be.

Cooling Down the Instrument

Automatic LN_2 Refilling

An optional automatic refilling unit (10) is available for use with the main unit (1). If an already existing refilling unit for the REICHERT-JUNG FC4 or FC4D cryosectioning system is to be used, an additional PCB (printed circuit board) may have to be built into the main KF 80 unit (1) by an authorized service engineer.

If the automatic refilling unit (10) is to be used, the main unit (1) is best positioned on the left hand side of a working table with sufficient room to the left of the table for the Dewar vessel (54). The Dewar vessel may also be placed in front of the table close to the left-hand side of the main unit (1). Stand Dewar vessel (54) on castor support (55) and open cap (56). Fill with liquid nitrogen (LN_2) up to about 15 cm below the opening of the vessel (54). Before inserting the refilling unit (10) into the Dewar vessel (54) ensure that the sealing ring (57) is correctly positioned on the underside of the flange (58) and that the sealing cap (59), with the sealing ring (60), is completely closed. Connect multiplug (11) to socket (12) of the main unit rear panel (2). Connect stiff polyamide tubing (34) with ARMAFLEX insulating hose (35) to LN_2 outlet (36) of refilling unit (10) and to LN_2 inlet (37) of main unit (1).

Carefully introduce the refilling unit (10) into the full Dewar vessel (54). This should be done slowly to prevent the LN_2 from boiling over. If there are any signs of boiling immediately raise or remove the refilling unit, thus interrupting contact with the LN_2.

Then again insert the refilling unit, proceeding very slowly. Use pliers (61) to clamp the flanges (58, 62) tightly together to ensure that the Dewar is hermetically sealed. Evaporating nitrogen gas (GN2) will escape through the open solenoid valve in the refilling unit until the START button (63) is pressed and/or the multipin plug (11) is plugged into the socket (12) on the rear panel (2) of the main unit (1). The experienced user is able to shorten the first filling by rapid consecution of these steps.

The schematic diagram on the front panel (52) will now indicate the filling level of both the Dewar vessel (80) and the liquefier (81). Two intermittent acoustical and optical signals, one for the Dewar vessel (82) and one for the liquefier (83) will alert the user once LN_2 is exhausted. The acoustical warning signal can be switched off with switch (82) or (83). The optical warning signal will continue until LN_2 has been refilled.

If the instrument has already been cooled down and the Dewar vessel (54) requires refilling press the red STOP button (131) and open sealing cap (59). Once the Dewar vessel (54) has been filled through the open cap it must again be closed and the START button (63) pressed.

To commence rapid cooling press RC button (64) and START button (63). Wait until the desired temperature (-190°C) is reached. The metal mirror temperature is indicated on the digital display (76) if the button CRYOGEN (77) is pressed and lit up. The button GAS (78) switches the temperature measurement to a sensor (79) in the gaseous N_2 above the metal mirror.

Important: for best results when impact freezing the KF 80 must be operated in the RC mode, otherwise the metal mirror will not each the lowest possible temperature.

Manual LN_2 Refilling

The KF 80 may be refilled manually without the optional automatic refilling unit (10) using a 4 l to 5 l Dewar flask (85) as follows:

Slowly pour LN_2 from the flask (85) onto the metal plate (86) until it is covered with LN_2. Repeat until the desired temperature (-190°C) is reached. The temperature of the metal mirror support (133) is indicated on the digital display (76) if the button CRYOGEN (77) is pressed and lit up. The button GAS (78) switches the temperature measurement to the sensor (79) in the gaseous N_2 above the liquefier.

Refill LN_2 using this procedure before each metal mirror cryofixation until the top indicator lamp (80) lights up.

Preparing Metal Mirrors

The shiny side of the metal mirror 140 (~~139~~) is used for cryofixation. This side should be first cleaned with WENOL (or similar polishing paste as used for polishing the Wenelt-cylinder) and then washed with alcohol. Introduce the tips of the forceps into the holes at the rim of the cylinder (~~139~~ 140) and place it on the metal plate (86).

CRYOFIXATION

CRYOFIXATION OF TISSUE SLICES

Affix a piece of transparent Tesafilm office tape to a specimen carrier (151). This piece of tape must be smaller than the specimen carrier itself. After cryofixation the frozen specimen can be easier removed from the tape than from the carrier without tape. If cryosubstitution or freeze-drying is to follow cryofixation, it is useful to minimize one dimension of the specimen. This is achieved by cutting a thin slice (0.3 mm to 1.5 mm) with the MACROTOME tissue slicer (141).

The cover (142) is removed by turning the two locking knobs (143) 90°. Place the plastic plate (144) onto the spring-loaded table (145) rough side up. Slide a cutting blade (147) out of the dispenser (148) and clean it with alcohol or xylene.

Insert the cutting blade (147) into the opening of the blade handle (149) and tighten the Allen screw with a 2 mm Allen key. Prepare a specimen slice (146) of less than 25 mm diameter and place it on the table (145). It is advantageous if final slice has a thickness of between 0.5 and 1.5 mm. Using spacer disc (150) placed in the center of the cover (142) the thickness of the resulting slice is defined. If no spacer disc (150) is inserted the slice will be approx. 1.5 mm thick. With a disc a slice of approx. 0.5 mm is produced. The thinner the slice, the faster and more effective the subsequent freeze-substitution and low temperature embedding! Place cover (142) on top of the specimen and position the two knurled screws (143) above the corresponding openings of the MACROTOME tissue slicer. Press the knurled screws (143) down and rotate them 90°.
Insert the cutting blade (147) between MACROTOME (141) and cover (142) so that the sharp edge points towards the specimen.

Move the blade towards the specimen with a sawing movement.

In order to avoid drying artifacts, place the MACROTOME in a humid chamber (e.g. glove box), open the cover (142) and use forceps to place the slice on a specimen carrier (151) fresh cut surface upwards.

Place a piece of wet filter paper on the bottom of the humidity chamber (152) and put the carrier (151) with the specimen facing downwards onto the humidity chamber (152). Transfer one of the precooled copper cylinders into the recess of the metal mirror support (133) and open flap (67).

Select the appropriate parameters for impact freezing: set the THICKNESS selector (153) to "1". The acceleration for the impact is adjusted with the SPEED selector (154). For mechanically sensitive specimen a low speed "1" is recommended, for more robust specimens the speed may be increased up to "11". The PRESSURE selector (155) does not influence the primary pressure of the first contact between specimen and metal mirror. This selector (155) determines the secondary pressure which the specimen is held in contact with the metal mirror after the first layer is completely quickfrozen.

Attach the specimencarrier (151) to the magnetic end of the slamming rod (137), remove the humidity chamber (152) and use the thumb to press the red RELEASE button (156), while the fingers rest at the finger grip (157). The specimen should remain in contact with the cold metal mirror for at least 1 min. Place a container (158) filled with liquid nitrogen from a Dewar flask on the metal plate (86). Press the ejector rod (159) down while lifting the rod (137) up with the knurled knob (138), until it locks in the starting position.

Use pre cooled forceps to transfer the specimen carrier (151) together with the frozen specimen (146) upside down onto the metal plate (86). Separate the frozen specimen from the carrier with precooled fine forceps and place the specimen into the liquid nitrogen cooled container (158). This container is used to transfer the specimen to the next preparation step (e.g. cryo-substitution or freeze drying).

If it is necessary to divide the frozen specimen slice into smaller parts, remove the metal mirror (140) from the metal mirror support. Close flap (67). Place the frozen specimen in the recess of the metal mirror support (133). Holding the specimen in precooled forceps it can be divided into smaller parts with a precooled scalpel. Collect the specimen pieces for further processing into the container (158) filled with liquid nitrogen.

<u>Cryofixation of specimens on specimen pins</u>

Select THICKNESS (153) setting "6", SPEED setting "1" and PRESSURE setting "5". Place a polyethylene foam cushion (160) in the pin carrier (161) and insert a pin (16, 17) in the center. Place a piece of wet filter paper on the bottom of the humidity chamber (152). Prepare a tissue slice of 1 or 1.5 mm thickness with the MACROTOME (141) as set out in section Automatic LN_2 Refilling. Cut a piece of tissue from the slice in such a way that it fits onto the pin (16). The small tissue piece is then placed on the pin (16) with the freshly cut surface facing up and the whole pin carrier (161) is then put on the humidity chamber (152) with the specimen facing downwards. Transfer one of the precooled metal mirrors (140) into the recess of the metal mirror support (133) and open flap (67).

Attach the pin carrier (161) to the magnetic end of the rod (137), remove the humidity chamber and use the thumb to press the red RELEASE button (156), while the fingers rest at the finger grip (157). The specimen should remain in contact with the cold metal mirror for at least 2 min. Insert the LN_2 transfer container (113) into the opening (115) and fill with LN_2 by pushing it down as far as possible with the wooden applicator stick (95). Press the ejector rod (159) down while lifting the rod (137) up with the knurled knob (138) until it locks in the starting position. Use precooled forceps to place the pin carrier (160) onto the LN_2 transfer container (113), metal plate up. Remove the specimen pin (16) from the carrier (160).

The specimen (146) frozen onto the pin (16) can now be transferred into a cryo-ultramicrotome in the container (113). Warm the pin carrier (160) to room temperature and dry before reuse. A clean precooled metal mirror (140) should be taken for the next freezing procedure.

Cryofixation of Suspensions

Transfer one of the precooled metal mirrors (140) into the recess of the metal mirror support (133) and open flap (67).

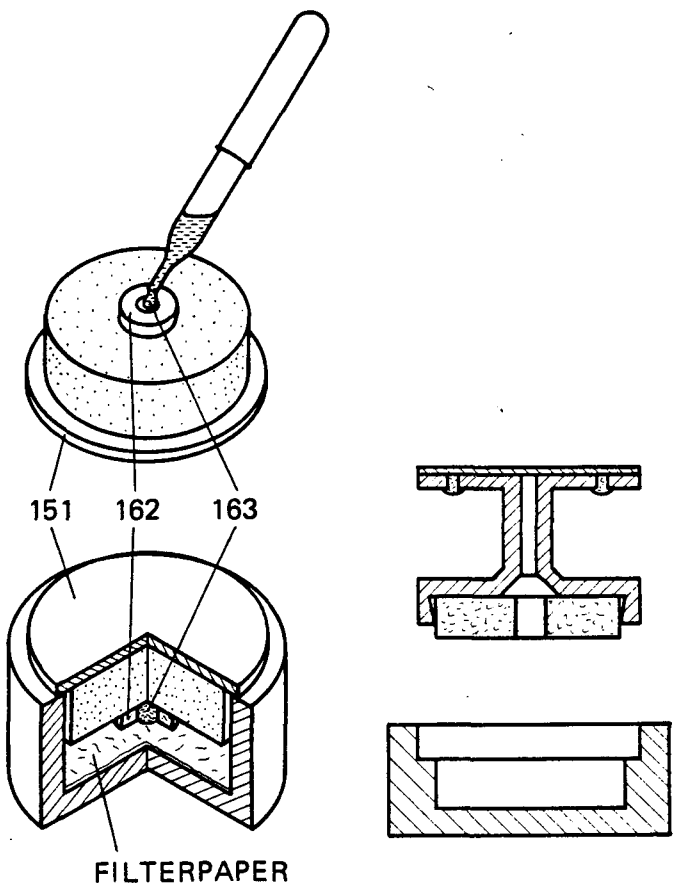
Set the THICKNESS selector to "1", SPEED to "1" and PRESSURE to "5". Place a piece of wet filter paper on the bottom of the humidity chamber (151). Affix a piece of transparent office tape to a specimen carrier (151), place a spacer ring (162) on it and apply a droplet of the suspension (163) into the spacer ring. Place the carrier (151) with the specimen facing downwards onto the humidity chamber (152).

Attach the specimen carrier (151) to the magnetic end of the rod (137), remove the humidity chamber and with the thumb press the red RELEASE button (156), while the fingers rest on the finger grip (157). The specimen should maintain contact with the cold metal mirror for at least 2 min. Insert the LN_2 container (113) into the opening (115) and fill with LN_2 by pushing down as far as possible with the wooden applicator stick (95). Press the ejector rod (159) down while lifting the rod (137) up with the knurled knob (138) until it locks in the starting position.

Place the specimen carrier (151) upside down on the metal plate (86) using precooled forceps. Remove the spacer ring (162) with the frozen suspension from the carrier (151) and place in the LN_2-filled container (113). The specimen can now be transferred to the next preparation step (e.g. cryosubstitution).

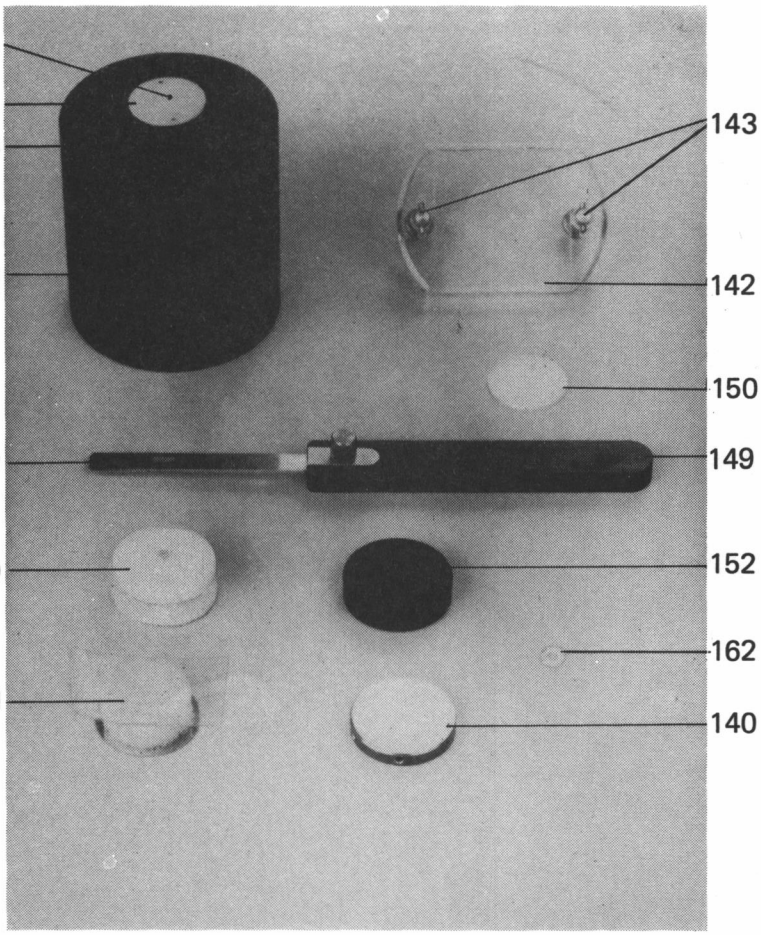
End of Cryowork

Proceed as outlined in KF 80 operating instruction chapter 2.4.



List of components for metal mirror cryofixation:

(133) Metal mirror support
(134) Locking screw
(136) Impact freezer
(137) Rod
(138) Knurled knob
(139) "LIGHT" control knob
(140) Metal mirror
(141) "MACROTOME"
(142) Cover
(143) Knurled locking knobs
(144) Plastic plate
(145) Table
(146) Specimen
(147) Cutting blade
(148) Cutting blade dispenser
(149) Cutting blade handle
(150) Spacer disc
(151) Specimen carrier
(152) Humidity chamber
(153) THICKNESS selector
(154) SPEED selector
(155) PRESSURE selector
(156) Release button
(157) Finger grip
(158) Transfer container
(159) Ejector rod
(160) Pin carrier
(161) Ejector needle
(162) spacer ring
(163) Suspension droplet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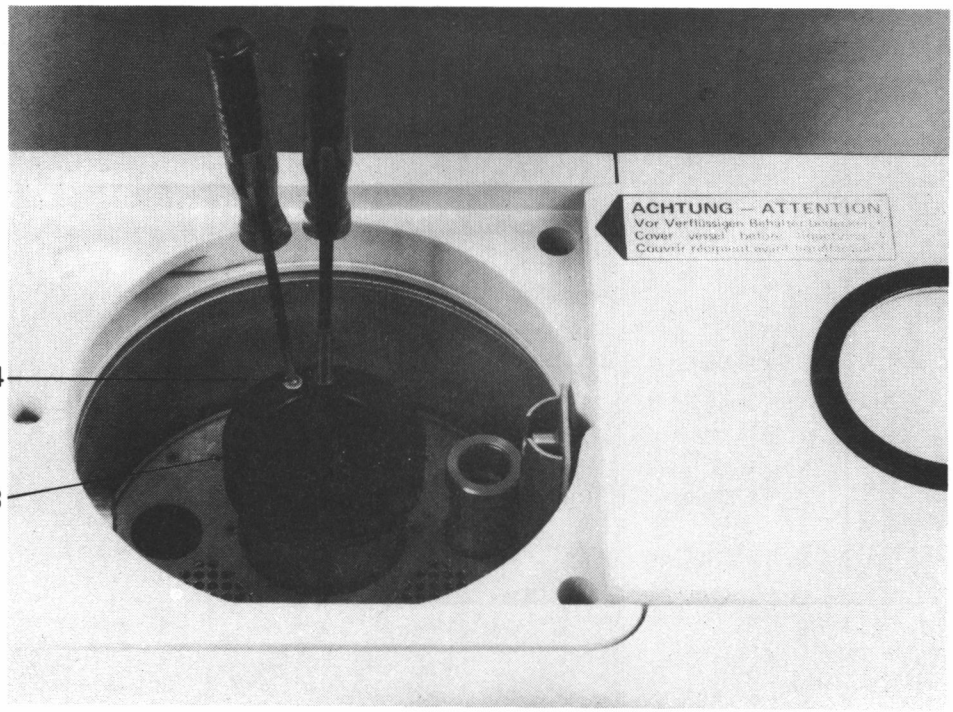
ACHTUNG – ATTENTION
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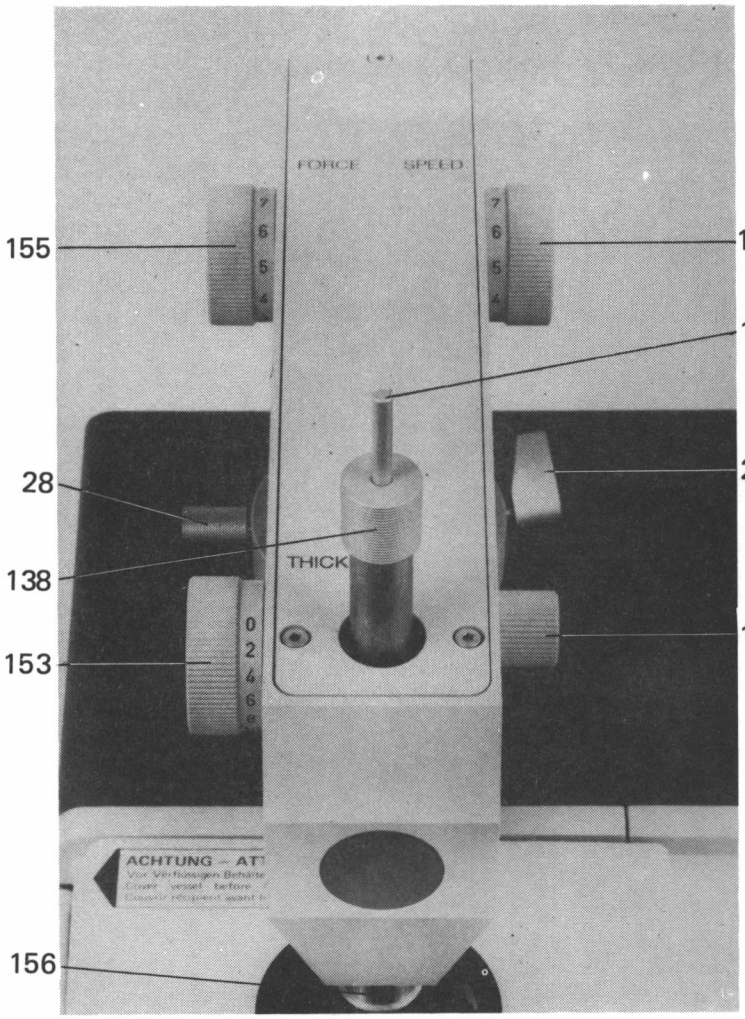
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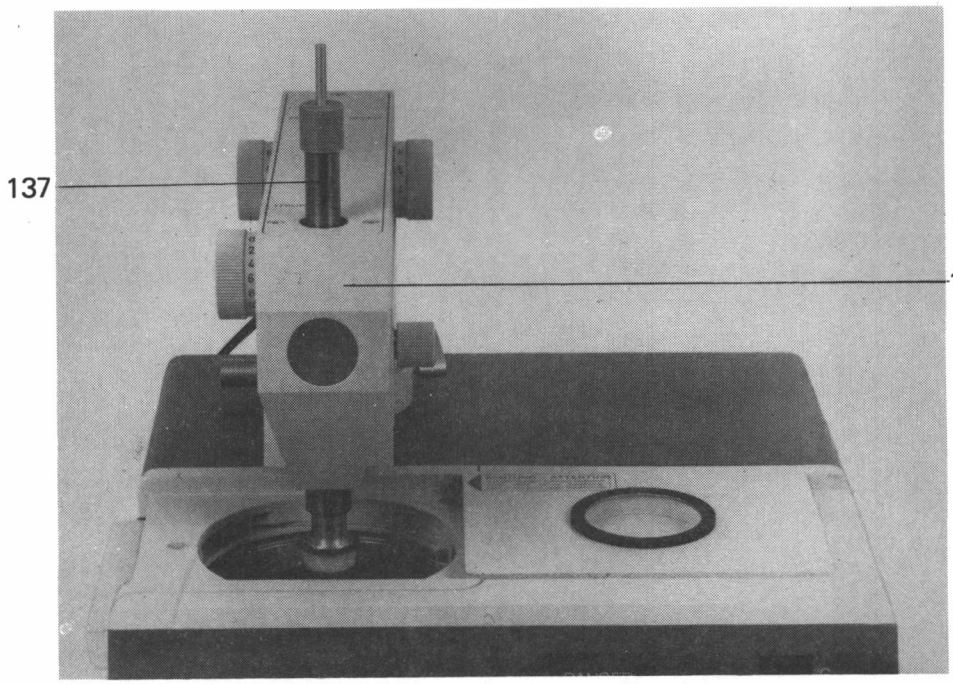
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