



**MICROM**

**MICROTOME CRYOSTAT**

**HM 505 E**

**INSTRUCTION MANUAL**

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## CERTIFICATION

MICROM International GmbH certifies that this instrument has been tested and checked carefully. Its technical data was verified before shipment to be in accordance with the published specifications.

The instrument complies with applicable international safety regulations.

## WARRANTY

This MICROM product is warranted against defects in material and workmanship for a period of 1 year. Parts which prove to be defective during the warranty period will be repaired or replaced free of charge by MICROM International GmbH. No other warranty is expressed or implied. Unauthorized modification or repair by third party persons will void the warranty.

The warranty will expire in case of improper or wrong use of the instrument and in case the warning and precautionary messages are not observed. MICROM International GmbH is not liable for any occurring damage.

Errors and omissions excepted. Subject to amendment and improvement without further notice.

This instruction manual will be supplied together with each instrument. Further copies can be ordered at the nearest MICROM sales office by giving the serial number of the instrument, the number of the instruction manual and the date of issue.

This instruction manual is available in the following languages:

	Cat. No.
German:	386 230
English:	386 240
French:	387 290

## INTENDED USE

Dear Customer,

Before putting the instrument into operation, please read these operating instructions carefully to familiarize yourself with its proper operation and functions.

Only skilled or specially trained personnel must operate the microtome, i.e. clamping the specimen, trimming, sectioning and taking off the sections from the instrument. The listed and marked safety measures as well as the regulations of your respective lab must strictly be observed.

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MICROM Ser. No. : ..... 17434 .....

Please check the MICROM Ser. No. on the type plate of your instrument and enter this number here. This way, questions and service can be handled faster.

Instruction Manual No. 386240

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**ATTENTION!**

Please observe the following general precautions during operation of this instrument. Failure to comply with these precautions violates safety standards and the intended use of the instrument. MICROM International GmbH is not liable for misuse of the instruments and failure to comply with basic safety requirements.

**INSTRUMENT GROUNDING**

To avoid injury from electrical current, the instrument must be connected with the safety ground. The instrument is equipped with a three wire ground plug. The power outlet must be connected to the safety ground and must meet the International Electrotechnical Commission (IEC) regulations.

**CAUTION: MAINS VOLTAGE**

Never remove instrument covers during operation. Component replacements as well as adjustments must only be made by trained service personnel. Unplug the unit before removing or opening the covers.

**DANGER IN EXPLOSIVE ENVIRONMENT**

The instrument must not be operated in the presence of flammable gases.

**CARE IN USING MICROTOME KNIFE**



To diminish the danger of being injured by the knife or blade, use the knife guard when adjusting specimen and knife. If possible, the specimen should be clamped in before the knife is inserted into the knife holder. Before changing the knife holder, always remove blade or knife! Unused knives should always be kept in a knife case. Never place the knife with the cutting edge upwards. Never try to catch a dropping knife!! Never check the sharpness of the cutting edge with your fingers.

The cutting edge is extremely sharp!

## **SAFETY PRECAUTIONS**

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### **HAZARD OF FROSTBITE**

Avoid permanent touching of metal parts inside the cryostat microtome chamber. Frostbite at unprotected hands and arms may occur at working temperatures down to  $-35^{\circ}\text{C}$ .

### **HAZARD OF RADIOACTIVE RADIATION**

When working with radioactive specimens observe all applicable radiation safety procedures.

### **HAZARD OF INFECTION**

Use the appropriate safety and disinfection measures when working with infectious specimens.



## PART 1 INTRODUCTION

### 1-1 DESCRIPTION OF THE CRYOSTAT HM 505 E

The cryostat HM 505 E from MICROM International GmbH is a highly efficient microtome cryostat for a cutting temperature range down to - 35°C. Designed as an upright standing unit with an open-top working area, the instrument allows convenient and ergonomic handling in standing and sitting position.

The cryostat has a cooling system to control the temperature of the working area of the microtome and knife carrier as well as to cool the freezing stations for freezing-on specimens onto the specimen stages.

Further optional accessories for the HM 505 E is the heat extractor as well as the active deep freezing device.

The instrument is equipped with an automatic 24-h-defroster with interrupt key.

The rustproof microtome has a section thickness range from 1 µm to 500 µm, divided into section thicknesses and trimming thicknesses. For the protection of knife and specimen, the instrument retracts the specimen at the end of the cut. The motorized coarse feed system allows specimen forward and backward travel. This way specimen and knife edge distance can be adjusted quickly. Moreover, the automatic approach system guarantees a fast and exact adjustment between specimen and knife edge. An additional trimming function with values from 10 µm to 500 µm permits the fine adjustment up to the first cuts and results in larger section thicknesses when trimming.

Two knife carriers are available. The standard knife carrier is designed so the knives can be easily clamped in place and adjusted. The disposable blade holder EC takes up high and low profile blades. The corresponding anti-roll guides facilitate taking off sections.

Section counter, sum of section thicknesses and remaining travel to the front end position are indicated on the display of the operating panel.

In addition the instrument is equipped with a handwheel brake.

# INTRODUCTION

1 - 2

## TECHNICAL SPECIFICATIONS HM 505 E

Cooling of microtome chamber (at +20°C ambient temp.)	temperature range .....	+5°C - -35°C
	resolution .....	1°C
	automatic defrosting .....	1 each 24 h
Microtome:	Section thickness range .....	1 - 500 µm
	Fine section thickness range .....	1 - 20 µm
	Resolution .....	1 µm for 1 - 10 µm 2 µm for 10 - 20 µm
	Trimming thickness range: .....	10 - 500 µm
	Resolution: .....	10 µm for 10 - 80 µm 20 µm for 80 - 100 µm 50 µm for 100 - 300 µm 100 µm for 300 - 500 µm
	Specimen retraction during return travel .....	40 µm
	Horiz. feed range .....	28 mm
	Vertical specimen stroke .....	64 mm
Section counter:	Five-digit LED display with reset	
Sum of section thicknesses:	Five-digit LED display with reset	
Remaining travel to front end position:	Five-digit LED display	
Size of specimen stages:	.....	30, 40, 50, 55, 60, 70 mm special sizes upon request
Specimen orientation:	x - and y - axes: .....	universal 8°
	z - axis: .....	up to 360°
Cutting drive:	.....	manual by means of handwheel
Coarse feed:	.....	motorized, graduated and continuous
Microtome chamber illumination, heated sliding window		
Storage temperature range:	.....	-20°C - +50°C
Operating conditions:	.....	+5°C - +40°C (at a max. rel. humidity of 60%) altitude up to 2000 m M.S.L. for indoor use only
Power requirements:	.....	100V/12 A +/-10% 50Hz 100V/12 A +/-10% 60Hz 115V/12 A +/-10% 60Hz 220V/8 A +/-10% 60Hz 230V/8 A +/-10% 50Hz 240V/8 A +/-10% 50Hz
Pollution degree	.....	2
Overvoltage category:	.....	II
Dimensions:	.....	775x800x1200 mm (wide/deep/high)
Weight:	.....	135 kg

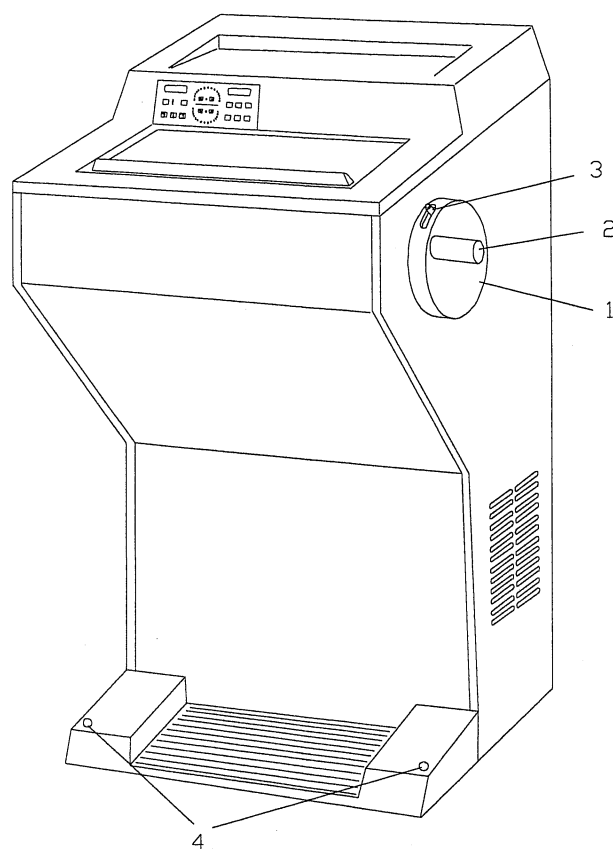


Fig. 1

**PART 2 OPERATING INSTRUCTIONS**

**2-1 SETTING UP THE CRYOSTAT**

Unpack the instrument and remove packing as follows:

Loosen and remove the cover of the case, remove the packing material. Loosen the side parts at the bottom and remove them by lifting them over the instrument. To remove the pallet from the instrument, cut off the packing material in the foot area, if necessary. The pedestal of the instrument is equipped with rollers so it can be easily moved to the installation site.

**CAUTION!** The instrument must only be moved in an upright or slightly tilted (approx. 30°) position.

The installation site must be chosen so that enough ventilation for the cooling system is guaranteed. The distance between wall and rear panel should be approx. 10 - 15 cm. Moreover, the suction areas on either side must be kept free.

Please avoid draught by open doors or by air conditioning systems at the installation site. In addition, the microtome chamber should not be exposed to sunlight.

Both measures reduce the formation of frost and therefore results in a more favourable working condition. A high air moisture as well as high ambient temperatures reduce the maximum performance of the instrument.

Turn the screws (fig. 1.4) on the front foot ends to fix the instrument. Then install the handwheel handle (fig. 1.2), which is separately packed, on the handwheel (fig. 1.1).

## OPERATING INSTRUCTIONS

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### 2-2 INITIAL TURN-ON

**CAUTION!** Before switching on the instrument for the first time, please check if the power requirements indicated on the type plate correspond to the power supply voltage being used.

No other instruments should be connected to the circuit used for the cryostat, as the compressor needs high surge currents when started. Do not use multi-socket power outlets with small wire sizes for the supply of the instrument.

Now connect the plug of the instrument to the power outlet. Then turn on the main switch on the backside of the instrument.

Then insert the section waste tray between knife carrier and base plate in way that the side with the lower rim of the section waste tray faces the frontside. Then install the brush shelf on the frontside of the base plate.

**NOTE!** After having turned on the instrument, the right display (fig. 10.2) on the operating panel shows the code E-03. Press button (fig. 10.3) to turn off that code. Now the instrument can be operated. (See 2-11, error code indication).

Then close the heated sliding window and cool down the instrument, which will take approx. 2 - 4 hours according to the set chamber temperature.

2-3 COOLING OF MICROTOME CHAMBER

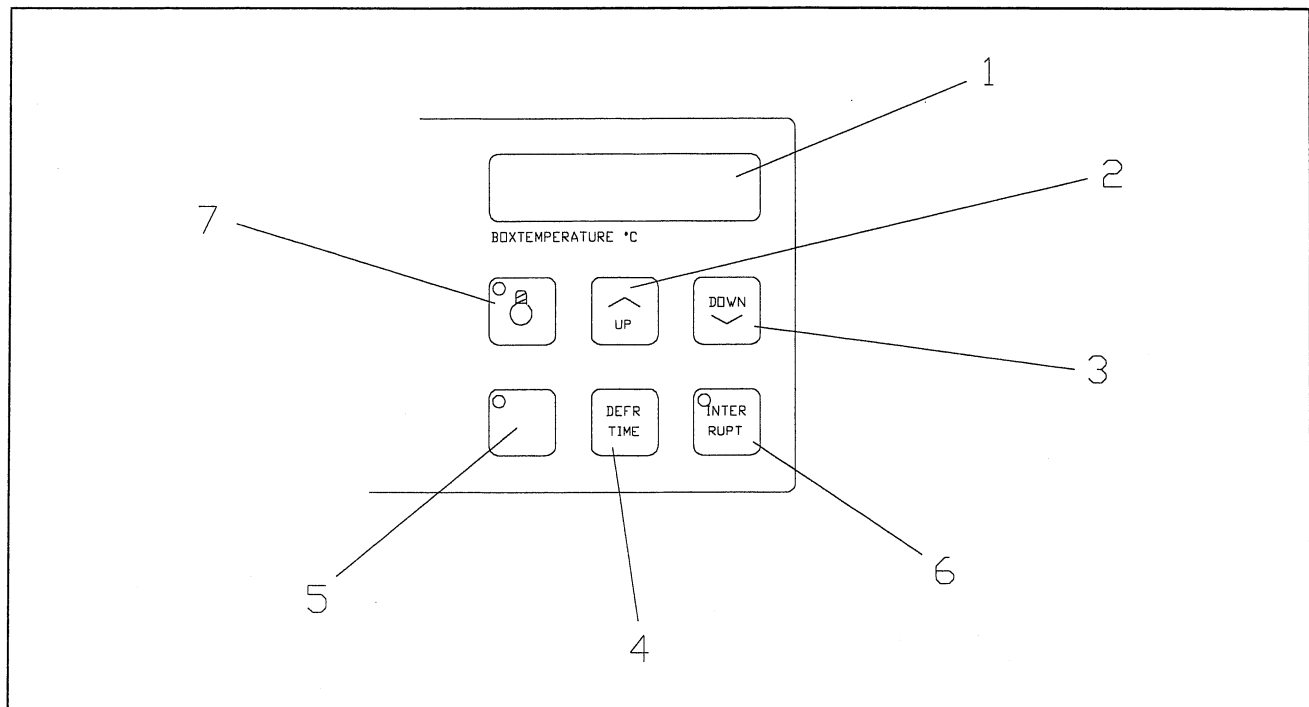


Fig. 2

2-3-1 ACTUAL AND SET VALUE OF TEMPERATURE

The cooling of the microtome chamber can be controlled by the corresponding part of the operating controls. The actual temperature of the microtome chamber is shown in °C on the digital display (fig. 2.1). Briefly press the UP- or DOWN-button (fig. 2.2 or 2.3), the set value of the temperature in the microtome chamber is shown for approx. 2 sec. Afterwards, the display shows again the actual value of the temperature of the microtome chamber. To change the set value, press permanently the UP-button (fig. 2.2) or DOWN-button (fig. 2.3). The UP-button leads to higher, the DOWN-button to lower temperatures.

The valid range of the set value goes from +5°C down to -35°C. If the UP-button (fig. 2.2) is used beyond the limit of +5°C, the display shows - - - and the temperature control is turned off. Using the DOWN-button (fig. 2.3) the cooling system is turned on again.

2-3-2 DEFROSTING THE EVAPORATOR

Every 24 hours the evaporator in the rear part of the microtome chamber is defrosted automatically.

During a defrosting process, the display (fig. 2.1) shows a temperature with the letter H. This temperature refers to the heating of the evaporator and not to the temperature of the cooling chamber, which is normally shown. This value, however, is of no importance for the operation of the instrument.

Push the button DEFR TIME (fig. 2.4) and the set defrosting time is shown for approx. 2 sec. on the display (fig. 2.1). It is advisable to set the time of the defrosting process not during routine working time. To change the starting time of defrosting at intervals of 15 minutes, simultaneously press the button DEFR TIME (fig. 2.4) and the button UP (fig. 2.2) or DOWN (fig. 2.3). Defrosting can only be carried out at the set defrosting time, if the real time is set correctly.

**2-4 CUTTING MOVEMENT AND RETRACTION**

The real time can be read on the display (fig. 2.1) if the button (fig. 2.5) is pressed in addition to the already pressed button DEFR TIME (fig. 2.4). To correct the real time at intervals of one minute, additionally press the UP- or DOWN-button. After finishing the adjusting process, the display (fig. 2.1) shows the set temperature of the microtome chamber after two more seconds.

If needed, the daily defrosting can be interrupted or cancelled. Press the button INTERRUPT (fig. 2.6), the LED in this button lights up. If the function INTERRUPT is chosen during the defrosting period, the LED blinks for the rest of the suppressed defrosting process. A cancelled or interrupted defrosting process must be repeated later on, as otherwise the evaporator will cover completely with frost and cannot cool any more.

For this, press the button (fig. 2.6) again to turn off the function INTERRUPT (LED is off). The next defrosting process will then be started when the next pre-selected defrosting time is reached.

If the function INTERRUPT is not deleted manually, this function will be automatically deleted when reaching the next defrost time.

Immediate defrosting can be carried out at any time by pressing the button DEFR TIME (fig. 2.4) simultaneously with the button RESET (fig. 4.11).

**2-3-3 ILLUMINATION OF THE COOLING CHAMBER**

To illuminate the microtome chamber, a fluorescent lamp is above the chamber, which can be turned on and off by means of the button (fig. 2.7).

At low temperatures and after long periods of being turned off, the lamp might flare when turning it on. Used lamps can be changed by the user himself. (See part 5.3).

To start the cutting movement of the microtome, turn the handwheel. As the specimen moves down, sectioning is carried out (fig. 3.2). Continue turning the handwheel to bring the specimen back up (fig. 3.4). To protect the knife and specimen during return travel, the specimen is retracted (R).

Then the LED RETRACT (fig. 4.1) on the operating controls lights up.

- X=selected section thickness
- 1=specimen
- 2=cutting movement
- 3=knife
- 4=return travel
- R=retraction

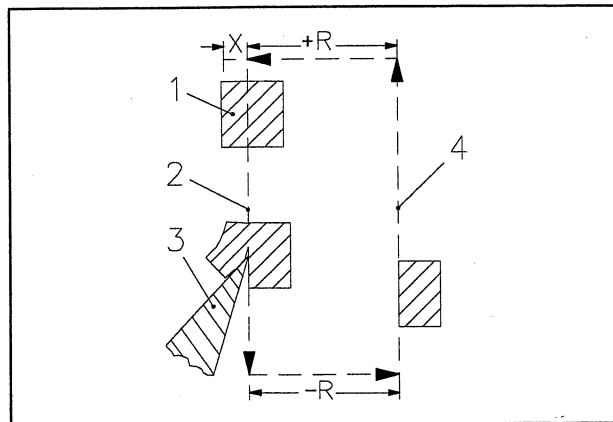


Fig. 3

**2-5 LOCKING AND UNLOCKING THE HANDWHEEL**

The function <handwheel brake> allows to lock the handwheel. A pin is situated on the rim of the handwheel, which can be pressed backwards. The handwheel is locked in the upper reversal point. The danger of being injured while adjusting specimen and knife is considerably reduced.

Continue working again, first press the pin to unlock the handwheel.

**CAUTION!** Lock in the handwheel brake, when working on the specimen holder and the knife carrier.

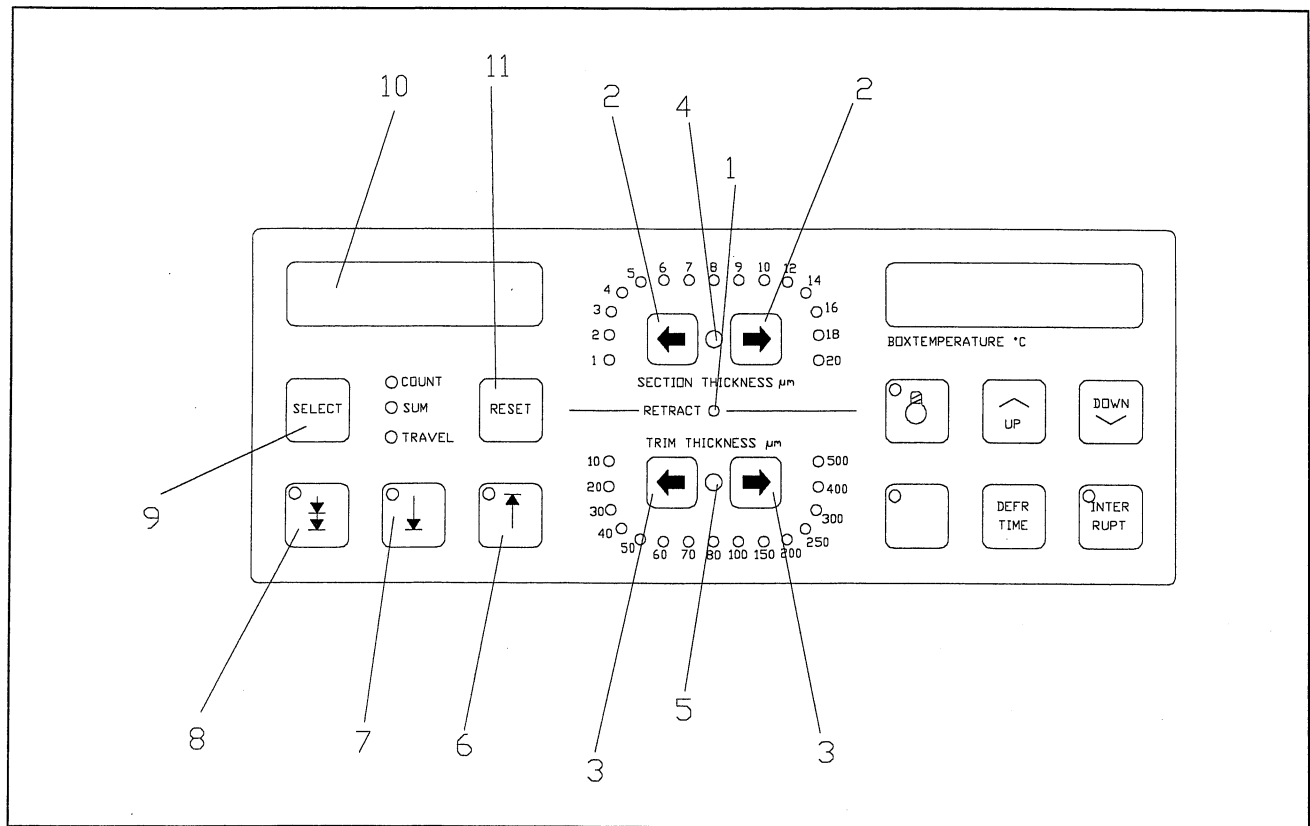


Fig. 4

**2-6 SETTING SECTION THICKNESS AND TRIMMING THICKNESS**

The desired section thickness can be selected by means of the buttons (fig. 4.2) on the operating controls and the trimming thickness by means of the buttons (fig. 4.3). The two left buttons lead to lower values and the two right buttons to higher values.

The selected values are also indicated by the corresponding illuminated LEDs.

The two orange LEDs (fig. 4.4 and 4.5) show which section thickness mode is chosen.

The graduation of the trimming thicknesses is divided into 4 ranges:

range	graduation
from 10μm to 80μm	10μm
from 80μm to 100μm	20μm
from 100μm to 300μm	50μm
from 300μm to 500μm	100μm

The graduation of the section thicknesses is divided into 2 ranges:

range	graduation
from 1μm to 10μm	1μm
from 10μm to 20μm	2μm

## OPERATING INSTRUCTIONS

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### 2-7 SPECIMEN FEED

#### 2-7-1 SPECIMEN COARSE FEED

For the fast forward and backward travel between specimen and knife edge, the cryostat microtome has a motorized coarse feed system.

To move the specimen holder backwards, press the button <coarse feed backwards> (fig. 4.6).

For short distances, press the button (fig. 4.6) only slightly. If this button is pressed for more than approx. 2 sec. the specimen holder moves automatically to the back end position and the motorized coarse feed shuts off. This function can be stopped at any time. For this, slightly press the button (fig. 4.6) again.

When the back end position is reached, the red LED in button (fig. 4.6) lights up.

**CAUTION!** For your personal safety, before activating the coarse feed forwards, move the anti-roll plate onto the blade against possible splintering of the blade. This might be caused by an incorrect coarse feed function.

The specimen holder is moved to the front as long as the button <coarse feed forwards> (fig. 4.7) is pressed.

**CAUTION!** With the function <coarse feed forwards> specimen and knife edge are adjusted very quickly. To avoid damages to specimen and knife, carefully observe this adjustment process.

Take note that the specimen and knife edge do not come in contact with each other. Carefully observe the narrowing gap between knife edge and specimen to stop the function <coarse feed forwards> in time before the specimen touches the knife edge.

When the front end position is reached, the motorized coarse feed is turned off. Then the LED in button (fig. 4.7) lights up.

**NOTE!** At the beginning of a sectioning series, it is advisable to use the coarse feed to move the specimen to the back third of the horizontal specimen movement, and, if necessary, to move the knife carrier towards the specimen.

#### 2-7-2 AUTOMATIC APPROACH SYSTEM

The automatic approach system performs the fast and exact approach of the specimen towards the knife edge and can be applied between  $-5^{\circ}\text{C}$  and  $-35^{\circ}\text{C}$ . Outside this temperature range, the automatic approach system is blocked (see part 2-11-1, error code E-06).

Before starting the automatic approach system, place the anti-roll plate onto the blade for your personal safety.

**CAUTION!** For your personal safety, before activating the automatic approach system, move the anti-roll plate onto the blade against possible splintering of the blade. This might be caused by an incorrect approach.

**DANGER!** When using different freezing techniques, for example by means of the fast freezing stations, liquid nitrogen,  $\text{CO}_2$  and the like, which generate a freezing temperature of below  $-35^{\circ}\text{C}$ , the automatic approach system can only be applied after the specimen will have adjusted itself to the respective chamber temperature.

As a safety measure, place the anti-roll plate onto the blade, otherwise an incorrect approach will be carried out and the blade might splinter.



Use the handwheel to make sure that the most protruding position of the specimen is opposite the knife edge.

Press button (fig. 2.5) to start the process of the automatic approach. The red LED in this button lights up. The specimen holder moves forwards until the specimen touches the knife edge.

Immediately afterwards this forward movement of the specimen holder is stopped and moved backwards by a safety distance of 200  $\mu\text{m}$ . The red LED in button (fig. 2.5) goes off.

When passing the next upper reversal point, the specimen holder automatically moves forwards again by 200  $\mu\text{m}$ . The specimen is now in a position to start sectioning.

If necessary, the process of the automatic approach can be cancelled by pressing the button (fig. 2.5) again. The automatic approach can also be stopped by pressing the button (fig. 4.6).

Passing the upper or lower reversal point by turning the handwheel, the automatic approach can also be interrupted.

When an automatic approach has been carried out, another approach can be started immediately, if needed. This might become necessary when the specimen has been oriented again.

### 2-7-3 TRIMMING AND FIRST CUTS

After the specimen and the knife are adjusted, further gradual feed for trimming can be carried out using the function <trimming>. For different sectioning series, deeper layers of the specimen can be reached with the function <trimming>.

The pre-selected trimming value is indicated by a corresponding LED which then lights up. To select another trimming value, press button (fig. 4.3).

Button (fig. 4.8) is used for trimming, available as pulse or permanent function.

The <pulse function> is used when there is still some distance between specimen and knife edge.

Press the button (fig. 4.8) (not longer than 2 sec.). Whenever the button (fig. 4.8) is pressed, the specimen holder together with the specimen moves forwards by the trimming value which was pre-selected.

To select the <permanent function> press the button (fig. 4.8) for more than approx. 2 sec. Then the red LED in this button lights up continuously and permanent trimming is selected. In addition, the yellow LED (fig. 4.5) lights up to show that the selected trimming value is activated. The feed is carried out at each handwheel rotation in the upper reversal point. Press the button (fig. 4.8) once more, to turn off the permanent trimming. The red LED in this button goes off.

The yellow LED (fig. 4.5) also goes off. However, the yellow LED (fig. 4.4) lights up to indicate that the function fine sectioning is activated.

The <permanent function> is used to get faster to the desired sectioning level.

## 2-7-4 FINE FEED

After having adjusted knife and specimen as well as having trimmed the specimen, sectioning can be started.

Turn the handwheel continuously in one direction to feed the specimen at the selected section thickness.

The pre-selected section thickness is indicated by the corresponding LED which then lights up.

To select another section value, press button (fig. 4.2).

## 2-8 INDICATION OF CUTTING PROCESSES

Information about the sectioning status can be seen on the display (fig. 4.10). Press the button SELECT (fig. 4.9) to show the various functions one after the other.

The following information about the present sectioning position of the instrument can alternatively be seen on the display (fig. 4.10):

- number of sections  
(the red LED COUNT lights up)
- sum of section thicknesses  
(the red LED SUM lights up)
- remaining travel to front end position  
(the red LED TRAVEL lights up)

For this, press the button SELECT (fig. 4.9) until the corresponding red LED lights up and the desired information is shown on the display. (fig. 4.10).

## 2-8-1 SECTION COUNTER

The section counter adds up the sections produced. After each downward movement of the specimen holder, the number on the section counter increases by 1. The counter can be reset to zero by means of the RESET-button (fig. 4.11).

## 2-8-2 SUM OF SECTION THICKNESSES

This value shows the sum in microns of the sections already cut. Trimming values as well as sectioning values are added up.

This value can also be reset to zero by means of the RESET-button (fig. 4.11).

## 2-8-3 REMAINING TRAVEL TO FRONT END POSITION

This value shows the distance, which is left for sectioning.

If the specimen holder is in the back end position, the display shows 28 000  $\mu\text{m}$ . This number decreases, the closer the specimen comes towards the front. If no further feed is possible anymore, the display shows automatically the remaining travel to the front end position. Independently, which information about the sectioning position was chosen before.

## 2-9 SPECIMEN HOLDER AND SPECIMEN ORIENTATION

### 2-9-1 SPECIMEN HOLDER

There are several possibilities to freeze on specimens.

Various specimen stages are available. Round specimen stages can be supplied with a diameter of 30 mm and 40 mm and rectangular specimen stages with a size of 50, 55, 60 and 70 mm. Special sizes on request. (See 2-12-2, additional equipment, optional).

Put the specimen together with the freezing compound on the specimen stage at positive temperatures. Then put the specimen stage with the bolt together with the specimen into one of the six fast freezing stations (fig. 5a).

The upper side of the freezing station must not be covered by frost or ice. To avoid this, use acetone, ethanol or the like. To avoid the formation of frost or ice on the freezing stations, use the enclosed cover caps.

### 2-9-2 SPECIMEN HOLDER USING THE ACTIVE DEEP FREEZING DEVICE (OPTIONAL EQUIPMENT)

Put the specimen together with the freezing compound on the specimen stage at positive temperatures. Then put the specimen stage with the bolt together with the specimen into one of the four front freezing stations (fig. 5b). These four fast freezing stations are equipped with a Peltier cooling element.

The upper side of the freezing station must not be covered by frost or ice. To avoid this, use acetone, ethanol or the like. To avoid the formation of frost or ice on the freezing stations, use the enclosed cover caps.

Briefly press the DOWN button (fig. 2.3) twice to start the active deep freezing device with Peltier cooling element. The letter P is shown on the display (fig. 2.1), while the displayed temperature is still the box temperature.

#### NOTE!

The deep freezing device can only be activated if the temperature of the cooling chamber is below  $-10^{\circ}\text{C}$ .

The Peltier cooling element is active for 8 min. and then switches off automatically. To turn off this function within those 8 min., briefly press the DOWN button (fig. 2.3) twice again. The letter P on the display (fig. 2.1) goes off.

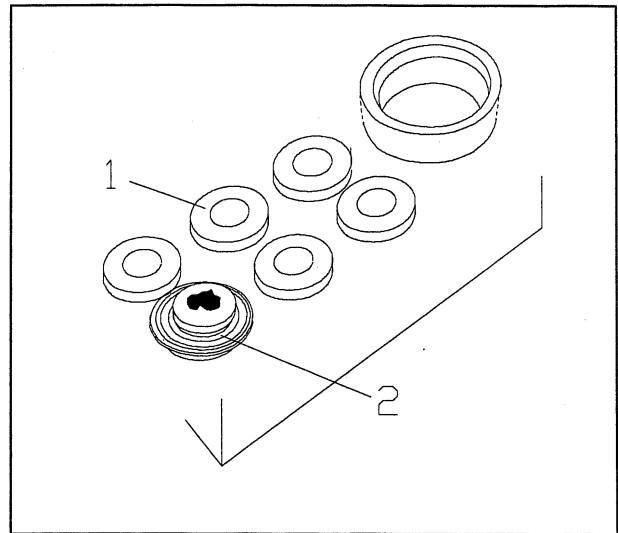


Fig. 5a

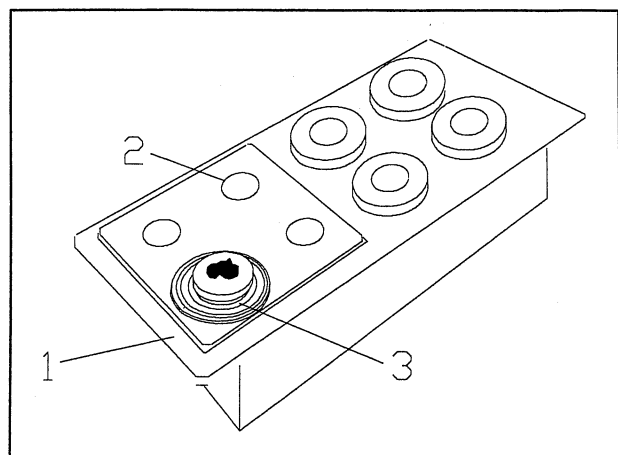


Bild 5b

## OPERATING INSTRUCTIONS

### 2-9-3 SPECIMEN HOLDER USING A HEAT EXTRACTOR (OPTIONAL EQUIPMENT)

Prepare and freeze the specimen as described in part 2-9-1 or 2-9-2.

To achieve faster and more even freezing of the specimen use a heat extractor. This way, the specimen is frozen at the same time from above and below, which reduces the formation of artefacts considerably.

The drop device (fig. 6.3) can be moved horizontally on a guide rail (fig. 6.1) and can be moved in a circle by means of a turning knob (fig. 6.2).

To pre-cool the heat extractor, move it backwards on the rail to the stop and lower it by turning the knob (fig. 6.2).

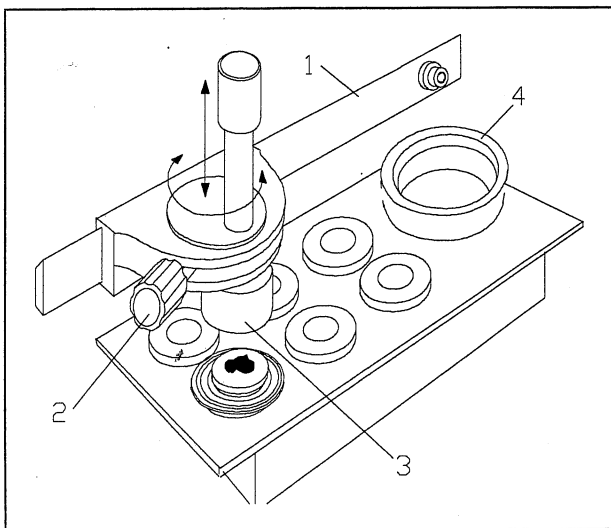


Fig. 6

When required, position the drop device (fig. 6.3) above the specimen and turn the knob (fig. 6.2) to put the stamp slightly on the specimen.

By further turning the knob, the pressure is increased, which might lead to a deformation of the specimen.

Should the drop device (fig. 6.3) stick to the specimen, loosen it by turning the handle of the drop device in a clockwise direction.

### 2-9-4 SPECIMEN ORIENTATION

In many cases, the orientation of the specimen in relation to the cutting edge would be advantageous.

This can easily be done by means of the orienting specimen holder on the microtome.

Loosen the clamping lever (fig. 7.8). The specimen holder can then be turned approx.  $8^\circ$  in each direction by means of the orienting lever (fig. 7.7). After having adjusted the specimen as desired, fix this position by means of the clamping lever (fig. 7.8).

The orienting lever (fig. 7.7) is also used to clamp the specimen stage into the orienting specimen holder.

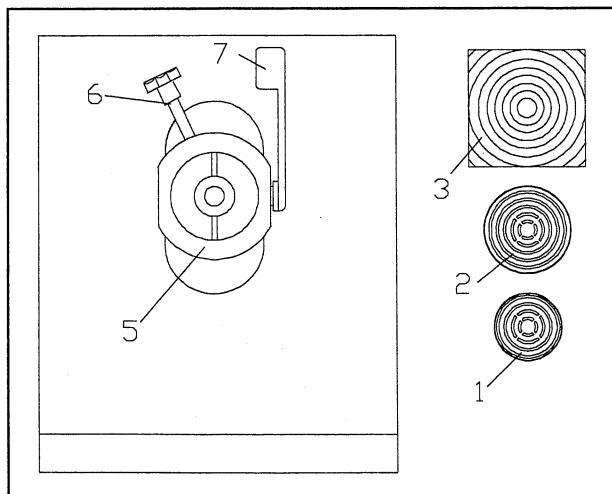


Fig. 7

2-10 KNIFE CARRIERS

2-10-1 STANDARD KNIFE CARRIER

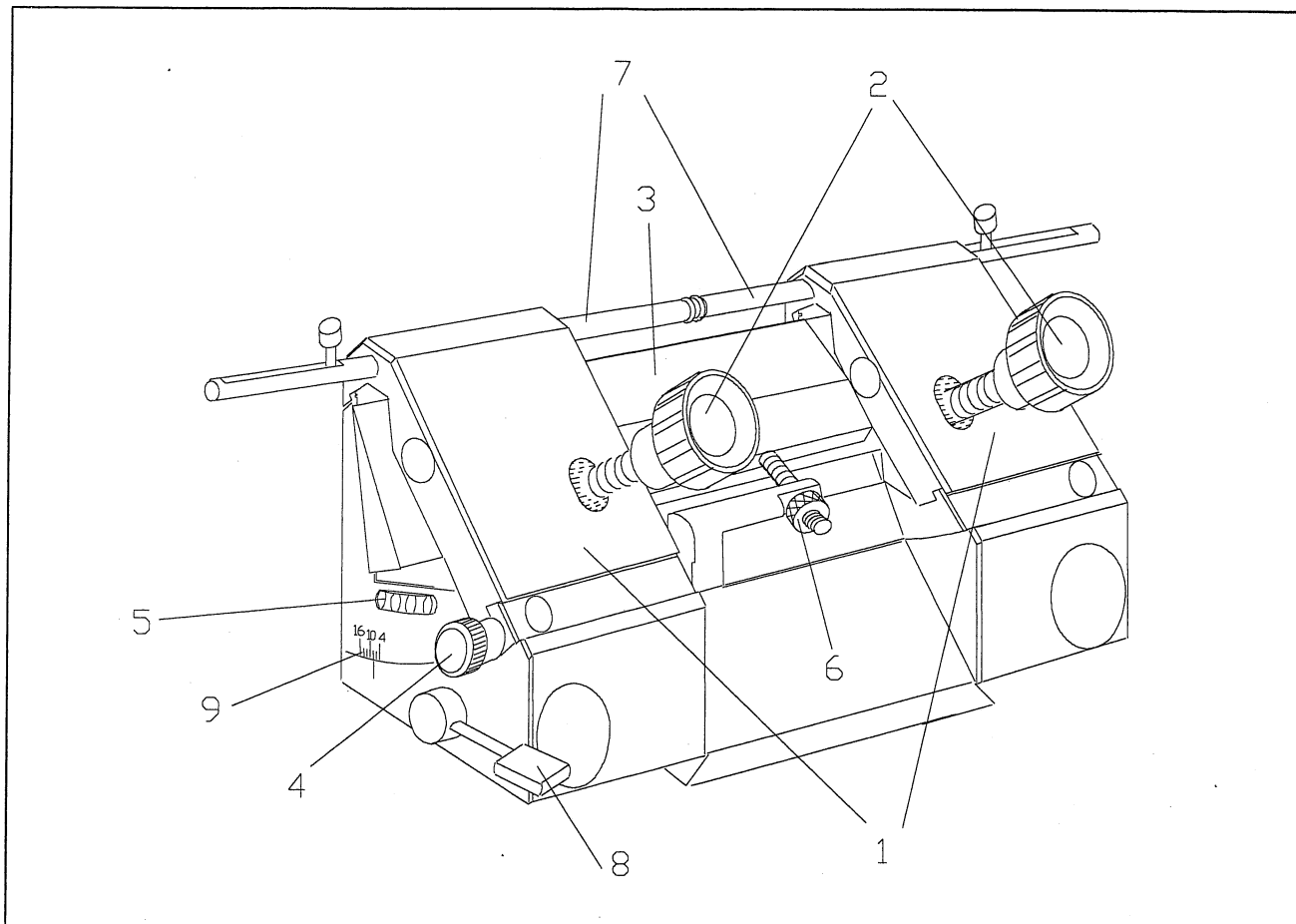


Fig. 8

The standard knife carrier of the Cryostat HM 505 E is easy to use, can be moved forwards or backwards and is equipped with knife guards for user safety while adjusting knife and specimen. This reduces the danger of injury !

The microtome knife can be inserted either from the side or from the front. It depends whether one or two clamping plates (fig. 8.1) are removed. For this, loosen the clamping screws (fig. 8.2) on the clamping plates (fig. 8.1). Turn the knob (fig. 8.4), which is situated on the left side of the microtome, in a clockwise direction to lift the anti-roll plate (fig. 8.3) forwards and insert the knife. To put the anti-roll plate (fig. 8.3) against the knife,

turn the knob (fig. 8.4) in a counter-clockwise direction. To adjust the knife edge in relation to the anti-roll guide, use the knurled screws (fig. 8.5) on the right and left side of the knife carrier. Especially note the parallel alignment of knife edge and anti-roll guide edge. Then, tighten the clamping screws (fig. 8.2) to fix the knife in its position.

The knurled screw (fig. 8.6) on the anti-roll guide allows the fine adjustment of the height of the anti-roll guide in relation to the knife edge.

If a cutting area of the knife carrier is no longer usable, the knife can be moved approx. 2-3 cm to the left or right side by loosening the clamping screws (fig. 8.2).

## OPERATING INSTRUCTIONS

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**CAUTION!** The knife carrier is equipped with two knife guards (fig. 8.7) that can be moved sideways. They should be pushed together in the middle when the knife and specimen are adjusted. In this way, the danger of injury from the knife can considerably be diminished.

To move or fix the knife carrier on the dovetail guide, loosen the clamping lever, which is situated on the right side on the base plate of the microtome. This also allows the rough adjustment of knife carrier to the specimen.

The clearance angle between cutting edge and specimen can be shifted and adjusted to the respective requirements of the tissue and the knife.

Loosen the clamping lever (fig. 8.8) on the left side of the knife carrier and move the upper part of the knife carrier on the knife carrier base. The adjusted clearance angle can be read on the scale (fig. 8.9) on the left side. Then turn the clamping lever (fig. 8.8) upwards to lock in the new clearance angle.

**NOTE!** By experience, usable cuts are only achieved at a clearance angle of  $10^{\circ}$  or more.

2-10-2 DISPOSABLE BLADE CARRIER EC

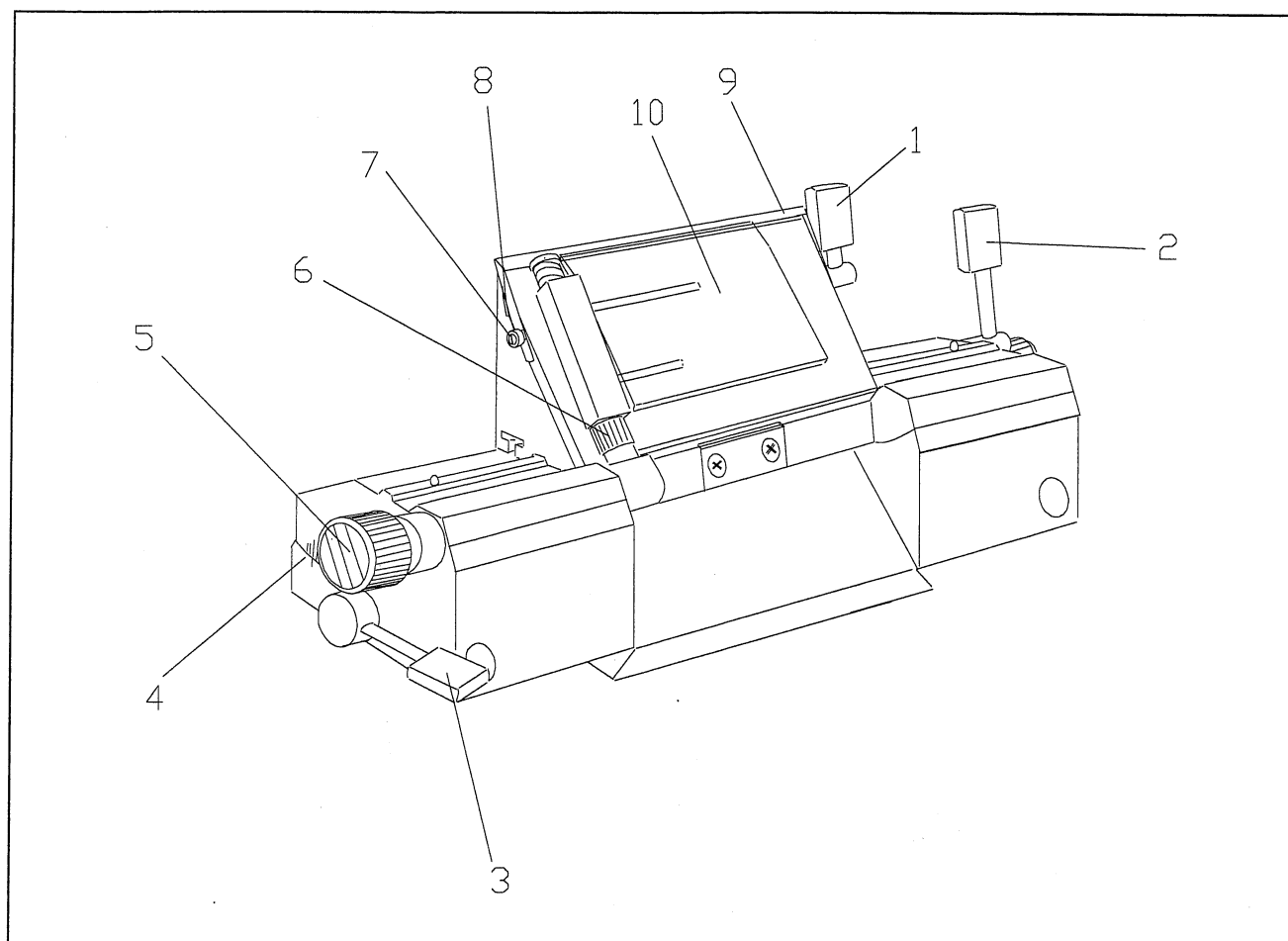


Fig. 9

With the disposable blade carrier EC, all commercially available low profile blades of 80 x 8 mm and a facet angle of approx. 35° as well as high profile blades can be clamped.

Insert the blade into the slot behind the clamping plate. When using high profile blades, first loosen the lever (fig. 9.1) and remove the spacer strip (fig. 9.8). The clamping plate can be opened and tightened by means of the lever (fig. 9.1). For the full use of the blade, the entire upper part of the carrier can be moved sideways by loosening the right lever (fig. 9.2).

The knife carrier is equipped with a knife guard (fig. 9.9). Turn the knob (fig. 9.8) to put the knife guard over the blade. This reduces the danger of injury considerably!

Turn the knob (fig. 9.5) to move the anti-roll plate (fig. 9.10) forwards and backwards. The knurled screw (fig. 9.6) allows the adjustment of the edge of the anti-roll plate in relation to the blade edge.

The lever (fig. 9.3) is used to fix or change the clearance angle adjustment.

To move or fix the knife carrier on the dovetail guide, loosen the lever, which is situated on the right side on the base plate of the microtome.

**NOTE!** By experience, usable cuts are only achieved at a clearance angle of 10° or more.

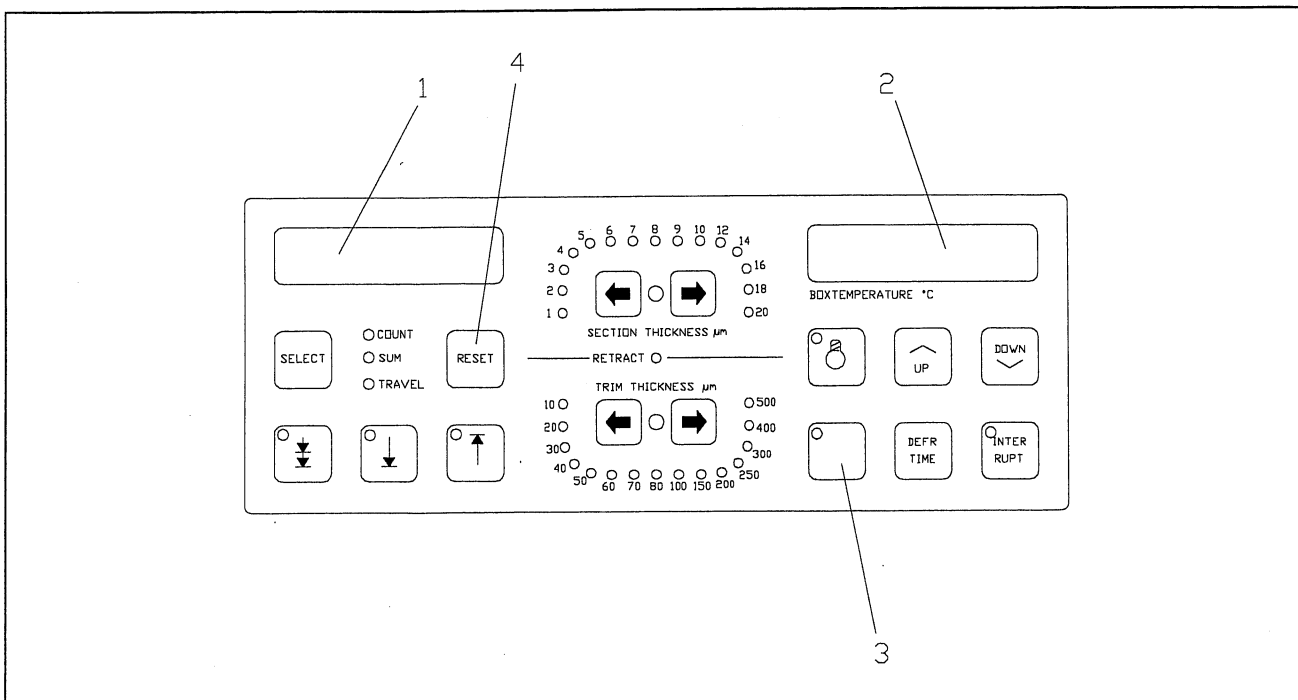


Fig. 10

## 2-11 ERROR CODE INDICATION

The instrument has an error code indication to define faster and better possible malfunctions.

The error codes E-01, E-02 and E-04 refer to the function of the integrated feed mechanism and to the condition of the battery buffer for storing data and is shown on the left display (fig. 10.1). The error code E-03 refers to the temperature of the cooling chamber and is shown on the right display (fig. 10.2). The error codes E-05 and E-06 refer to the automatic approach system and are shown on the right display (fig. 10.2).

### 2-11-1 DEFINITION OF THE ERROR CODES

The error code E-01 is shown on the left display (fig. 10.1) and indicates that there is a malfunction in the power amplifier of the instrument.

The user now knows that the integrated feed mechanism is not ready to operate.

In this case, a trained service technician must be called.

The error code E-02 is also shown on the left display (fig. 10.1) and indicates that the battery for the stored data is empty. This means that the indication of the real time clock as well as of the defrost timer is wrong. However, the instrument can be operated.

After a few seconds the error code E-02 disappears automatically. The variable values are then cleared. The set value of the temperature is adjusted to a default value of  $-35^{\circ}\text{C}$ . To select other values, press buttons (fig. 2.2 or 2.3)

The battery should then be replaced by a trained service technician as soon as possible.

The error code E-03 is shown on the right display (fig. 10.2), if the temperature of the cooling chamber is higher than  $0^{\circ}$  when turning on the instrument. This error code indicates that no cooling process was carried out for a longer time caused by power failure or turning off the instrument. In these cases, the instrument warms up and the cooling chamber as well as the microtome with knife carrier of the instrument gets wet or humid.



To avoid that the wet microtome is cooled down and consequently the guides freeze solid, the error code E-03 is shown on the display. Now the microtome together with the knife carrier must be dried (see part 5).

The user is to make sure that the microtome is in a perfectly dry condition. Then press button (fig. 10.3) to start the cooling process. The error code E-03 goes off and the cooling process starts.

The error code E-04 is shown on the left display (fig. 10.1) if the back end position of the integrated feed system is not reached within 2 min. after turning on the instrument. The instrument can be operated, however, the back end position is not defined. For this, press button (fig. 10.3). The error code E-04 goes off.

The error code E-05 is shown on the right display (fig. 10.2) in case the activating signal for the automatic approach system has already been recognized before the automatic approach system was started. This might be possible, in case a specimen has already been in contact with the knife edge, e.g. by protruding fibers. If this cause of trouble is eliminated, press button (fig. 10.3). The error code E-05 goes off and sectioning can be continued.

However, if there is no obvious explanation for the error code E-05, please call a service technician.

The error code E-06 is also shown on the right display (fig. 10.2) in case the actual temperature is warmer than  $-5^{\circ}\text{C}$ . Above this temperature, the automatic approach system cannot be activated. Press button (fig. 10.3) to clear the error code E-06.

## EQUIPMENT LIST

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### 2-12 ACCESSORIES

#### 2-12-1 STANDARD EQUIPMENT

The microtome cryostat HM 505 E is equipped with the following accessories:

	Cat. No.
2 specimen stage, 30 mm round	715110
1 specimen stage, 40 mm round	715120
6 Cover caps for freezing stations	402500
1 hex head wrench 5 mm	362450
1 hex head wrench 6 mm	362430
1 brush 8-10 mm wide	334160
1 cryostat oil no. 407 100 ml	350040
1 freezing compound 100 ml for low and medium temperatures	350100
1 handwheel handle	424100
1 section waste tray and brush shelf	502090
1 centering grip to align the orienting specimen holding device	424210
1 screwdriver	362270
1 instruction manual	386240

**2-12-2 ADDITIONAL EQUIPMENT (OPTIONAL)**

	Cat. No.
Heat extractor	524500
 <u>Specimen stages:</u>	
Ø 30 mm	715110
Ø 40 mm	715120
50 x 50 mm	715170
55 x 55 mm	715180
60 x 55 mm	715190
70 x 55 mm	715200
 <u>Knife carriers:</u>	
Standard knife carrier	705460
Anti-roll guide, complete	565730
Anti-roll plate, 60 mm (standard)	532520
Disposable blade carrier EC	705470
Anti-roll guide, complete	565790
Anti-roll plate, 38 mm	532770
Specimen fine orientation	546140
Vactuome	542130
<u>Specimen clamps for sectioning paraffin:</u>	
Standard specimen clamp	546130
Universal cassette clamp	546060
Adapter for standard specimen clamp and universal cassette clamp	532040
Adapter top for TT-specimen stage system	449330
Clamping device for rectangular TT-specimen stages	715520

## PART 3 THEORY OF OPERATION OF THE CRYOSTAT

### 3-1 COOLING OF CHAMBER AND DEFROSTING

The chamber of the cryostat is cooled by a refrigeration machine. In the rear upper area of the microtome chamber is the evaporator which the cold coolant flows through. Due to the heat extraction from the microtome chamber a cooling down to  $-35^{\circ}\text{C}$  can be achieved.

On the left side of the microtome chamber of the basic version there are six freezing stations to quickly freeze-on specimens onto specimen stages.

If the instrument is equipped with an active deep freezing device, there are eight freezing stations in the microtome chamber. The four front stations can be cooled down to  $-55^{\circ}\text{C}$  by means of a Peltier cooling element. The temperature of the rear four stations adapt themselves to the prevailing chamber temperature.

Use the heat extractor to accelerate the freezing of the specimen.

The temperature of the microtome chamber can be adjusted continuously down to  $-35^{\circ}\text{C}$ . For this, pre-select the desired temperature as set value on the operating controls.

The actual value of the microtome is constantly measured and sent to the control unit which causes the refrigeration to switch on and off.

Inevitably, when working on the microtome the dry cold air of the microtome chamber mixes with the warm humid air outside the chamber. Frost forms on the finned evaporator. The increasing thickness of the frost reduces the efficiency of the evaporator. For this reason, the daily defrosting of the evaporator becomes necessary.

To carry out the defrosting process, let hot coolant gas flow through a bypass into the evaporator. Set the time of the daily defrosting on the operating controls. The period of the defrosting takes approx. 10 min. and varies according to the thickness of the frost.

Possible frost built-up on the microtome or on the knife carrier are not removed by defrosting the instrument. However, the frost recedes after defrosting as now the evaporator is fully efficient again.

To avoid condensation the sliding window is heated.

### 3-2 MICROTOME WITH RETRACTION

The microtome of this cryostat is a rustproof rotary microtome. The cutting movement is carried out either manually by turning the handwheel or by means of the motorized cutting drive. The rotary movement results in the vertical movement of the cylinder head which carries the specimen holder with the specimen. The specimen is moved up- and downwards close to the knife edge. The knife is clamped securely into the knife carrier.

The section thickness is delivered at the upper reversal point of the vertical movement. The specimen holder together with the specimen moves to the front by the size of the section thickness, which can be selected on the operating controls.

With the downward movement of the specimen, sectioning is carried out. During the return travel, the specimen is drawn back automatically to protect knife and specimen, which means that the knife can be used longer and that the specimen is protected against artefacts.

The number of sections made is indicated on the section counter. After each downward movement of the specimen holder, the number on the section counter increases by 1. The section counter can be re-set to zero at any time.

The sum of sections made can also be seen on the display. For this, trimming value and sectioning values are added up. Section counter and sum of section thicknesses can be reset to zero at any time by means of the RESET-button.

Moreover, the remaining travel to the front end position can also be shown on the display. The remaining travel, which is still available for sectioning, is shown in microns.

### 3-3 SPECIMEN COARSE FEED AND TRIMMING STAGES

After changing the specimen, moving the knife or knife carrier, it is necessary to adjust the specimen to the knife edge again. This can easily be done by means of the specimen coarse feed and the defined trimming values.

To move the specimen holder electronically forwards or backwards, use the corresponding buttons on the operating controls.

After the specimen and knife are adjusted, further gradual feed for trimming can be carried out by using the function <trimming>. Whenever pressing the button <trimming>, the motorized feed is released. The same happens, when the function <trimming> is stored and the handwheel is turned.

### 3-4 AUTOMATIC APPROACH SYSTEM

The automatic approach system is used for the fast and exact approach of the specimen towards the knife edge.

The function of the automatic approach system is limited to the temperature range between -5°C and -35°C.

Up to now, this procedure has been carried out manually and has been quite time-consuming.

The automatic approach system uses the intrinsic capacity of the specimen to notice a first contact between specimen and knife edge. If specimen and knife edge get in contact with each other, a very low current is measured via a pre-amplifier, which is placed directly behind the specimen holder. The signal form of the detected current is transferred via the outlet of the pre-amplifier to the micro-processor. A corresponding software recognizes the threshold value causing an immediate interruption of the horizontal drive. The threshold value is also determined regarding the temperature.

The optimal function of the automatic approach system is based on the fact that the knife is conductively connected with the knife carrier base, whereas the specimen holder is separated by an isolating piece.

## PART 4 WORKING WITH THE CRYOSTAT

### 4-1 PREPARING THE MICROTOME CHAMBER

Before sectioning, the microtome chamber should be at a stable temperature around the desired cutting temperature. The temperature of the knife is determined by the cooling of the microtome chamber.

All tools which are necessary to take off sections or to manipulate the specimen must also be cooled, as the section will stick to them. For this reason, the required working accessories should always be stored on the shelves or brush tray in the microtome chamber.

The heated sliding window should be closed during breaks and while preparing work to avoid warm air getting into the chamber. Consequently, more favourable work conditions with a lower frost built-up on the microtome and knife are achieved.

### 4-2 FREEZING-ON THE SPECIMEN

The specimen is frozen-on to the specimen stage with clinging grooves with a freezing compound.

To freeze-on specimens, use the fast freezing device on the left side of the chamber.

When using the fast freezing device put some freezing compound onto the specimen stage, which should be outside the chamber at a low positive temperature. Then put the specimen onto the stage and surround it with some freezing compound.

Then insert the specimen stage into one of the fast freezing stations.

If the instrument is equipped with the active deep freezing device (optional equipment), eight freezing stations are installed in the cooling chamber. The front four stations can actively be cooled. Start the function <deep freezing> in due time before freezing-on the specimen (see part 2-9-2). It will take the deep freezing device approx. 2 - 3 min. to achieve a temperature of about -55°C.

If the instrument is equipped with a heat extractor (additional equipment) the specimen can be frozen faster and more evenly. Put the heat extractor onto the specimen from above. This way, the specimen is frozen at the same time from above and below, which reduces the formation of artefacts considerably (see 2-9-3, specimen holder using a heat extractor).

**4-3 TEMPERATURE LIST FOR CRYO-SECTIONING**

The optimal cutting temperature of a specimen depends on the respective characteristics of the tissue especially on the fat content. The following table won by experience recommends cutting temperatures for some typical applications:

Range A -10 to -20°C

Liver  
Kidney  
Spleen  
Thyroid  
Lymph Node  
Uterine Curettings  
Tongue  
Testicle

Range B -20 to -30°C

Muscle  
Breast without fat  
Brain  
Bone Marrow  
Lungs  
Intestine  
Prostata  
Cervix  
Uterus  
Pancreas  
Adrenal  
Skin without fat

Range C -30 to -60°C

additional cooling with  
liquid nitrogen or dry ice  
might be necessary

Adipose  
Omentum  
Breast with fat  
Skin with fat  
Cheese  
Butter  
Industrial Waxes  
Soft Rubber



### 4-4 ORIENTATION AND TRIMMING OF SPECIMEN

Clamp the specimen stage together with the frozen specimen into the specimen holder. Tighten the orienting lever (fig. 7.7) in a clockwise direction. Loosen the clamping lever (fig. 7.8) to have the possibility to adjust the specimen parallel in relation to the cutting edge by means of the orienting lever (fig. 7.7). Then press the clamping lever (fig. 7.8) backwards. With the motorized coarse feed (see 2-7-1 coarse feed) specimen and knife edge can roughly be adjusted.

The process of the first approach between specimen and knife edge can also be carried out via the automatic approach system (see 2-7-2, automatic approach system).

Then carry out a further gradual feed by using the function <trimming>. Turn the handwheel in a clockwise direction resulting in the first contact between knife and specimen. Continue this thus achieving the desired level. Any waste should be wiped away in an upward direction with a brush.

### 4-5 SECTIONING AND TAKING OFF SECTIONS

For sectioning, pre-select the desired section thickness by means of the buttons on the operating controls (see 2-6 setting section thickness and trimming thickness). Put the anti-roll plate against the knife by means of the knob. Turn the handwheel in a clockwise direction to carry out sectioning. The section slides into the space between the knife and the anti-roll guide.

Then remove the anti-roll guide by means of the knob. The section sticks to the knife surface. Transfer the specimen now onto a slide by using a brush or tweezers.

### 4-6 HOW TO AVOID MALFUNCTIONS

To cut usable sections, the following points are of utmost importance:

- Condition of knife edge, probably move it horizontally to the left or right side.
- Check adjustment of anti-roll guide and correct it if necessary.
- Carefully remove frost from front- and backside of the knife and from the anti-roll plate (i.e. with ethanol, acetone or the like).
- Tighten all clamping screws and clamping levers on the knife carrier and specimen holder.
- Select the correct cutting temperature according to the specimen (see 4-3, temperature list for cryo-sectioning).
- Select the appropriate freezing compound.

**CAUTION!** If the specimen was frozen with liquid nitrogen or similar freezing techniques, the specimen must adapt itself to the cutting temperature.

- Carefully select the required knife material and profile.
- Adjustment of knife height.
- Adjustment of proper clearance angle. Select a clearance angle adjustment of 5-15° according to the facet angle of a steel knife.
- Select a proper cutting speed: The harder the material, the slower the cutting speed!
- Take care in bringing knife and specimen together.
- The most protruding point of the specimen must be recognized for optimal operation of the automatic approach system.
- The specimen must not be covered at all or too thick by freezing compound.

- Moreover, the specimen stage as well as the knife edge and the microtome base must have a certain conductivity. For this reason, the original equipment such as knife carrier and specimen stages should be used only.

- The cylinder head of the microtome is connected with a sensitive electronic detecting device. The temperature sensor on the left side of the microtome housing is also connected with such a device. Malfunctions might occur after electrostatic discharges on one of the above-mentioned parts.

**Recommendation:** Before touching the cylinder head or temperature sensor, touch another metallic part, e.g. knife carrier, inner wall of chamber or waste tray to guarantee a safe discharge.

- When working with the active deep freezing device, sufficient time must have been passed to allow this device reaching -55°C.

For optimal sectioning, the knife and anti-roll guide must be cleaned of paraffin waste. Only use a sharp knife and exactly adjust the anti-roll guide in relation to the knife edge.

**NOTE!** During defrosting, remove the specimens from the cooling chamber, as the temperature inside the chamber will increase.

Do not leave or store tissue inside the cryostat over a longer period. Due to a power failure or other unexpected malfunctions of the instrument the specimen might be damaged.

## PART 5 MAINTENANCE OF THE CRYOSTAT

### 5-1 SHUTTING-OFF FOR CLEANING

Cleaning of the cryostat depends on how frequently the instrument is used. The more it is used, the more it needs to be cleaned. However, it is recommended to shut the instrument off every 6 - 8 weeks.

This should be done as follows:

- Turn off the main switch; pull the mains plug off.
- Remove or suck off cold section waste.
- Remove the knife from the carrier and put it into a knife case, remove section waste tray, specimen stages and working tools. Loosen the clamping lever on the right side of the base plate of the microtome and remove the knife carrier towards the front. Lift the two side accessory shelves in the front, pull them out of the rear pegs and also remove them.
- Suck off again cold section waste.
- The warming up of the microtome chamber can actively be accelerated by using a hair dryer.

**CAUTION!** Before dismounting the microtome, first remove the temperature sensor from its holder, which is installed on the left upper side on the microtome.

- Disconnect the warm microtome as follows:

**CAUTION!** Turn the handwheel into the lower position, i.e. the specimen holder is also in the lower reversal point.

Turn out the socket head screw (fig. 11.1) in the middle of the front part of the microtome base plate with the hex head wrench 5 mm; then slightly lift the base plate (fig. 11.2) in the front and move it approx. 2 cm to the left side until the clutch on the right side of the microtome is free. Then pull the microtome to the front to loosen it from the rear clamp (fig. 11.4). Turn the microtome to the right side so that the frontside of the microtome faces the right side wall of the chamber; now the coded connector (16-lead) on the rear side of the microtome can be unplugged.

The connector (4-lead) for the automatic approach system must be disconnected as well.

Carefully take the microtome out and remove all loose waste immediately by using a brush

- Carefully clean and wash the interior. Open the stopper on the bottom of the microtome chamber. The waste detergent can be collected again at the end of the outlet. Insert the stopper after cleaning as otherwise cold air leaks out while in operation and the instrument will cover with frost.
- After having finished cleaning and maintaining (see 5-2, cleaning and care of the microtome) install the microtome in reverse order.

**CAUTION!** The handwheel must be in the lower position when the microtome is introduced into the clutch.

## MAINTENANCE OF THE CRYOSTAT

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- Insert accessory shelves, accessories and knife carrier into the instrument.
- Connect the mains plug to the power outlet.  
Turn on the mains switch.
- Close the sliding window.

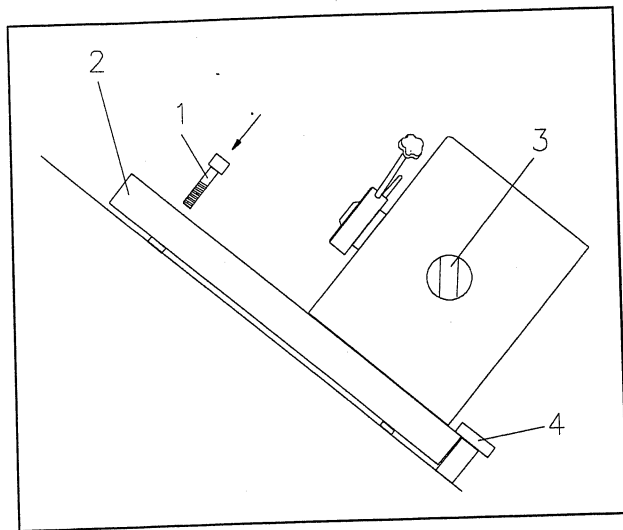


Fig. 11

**NOTE!** To maintain the cooling power of the instrument, clean the fins of the condenser at least once a year, if necessary more often.

## MAINTENANCE OF THE CRYOSTAT

### 5-2 CLEANING AND CARE OF THE MICROTOME

Carefully clean and dry the dismantled microtome. As there is condensed humidity inside the microtome dry it very carefully. A drying closet at temperatures up to +60°C can be used. Another possibility is to dry it by means of a hair dryer.

After each shutting-off or cleaning of the cryostat the cross roller bearings should be lubricated.

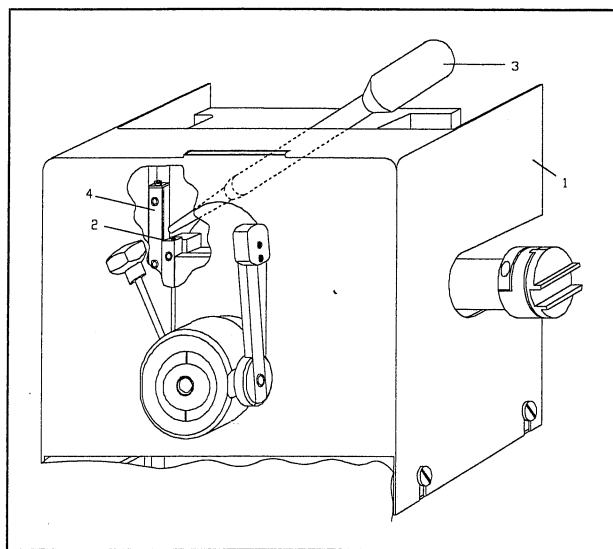


Fig. 12

By means of a pipette (fig. 12.3) the cross roller bearings (fig. 12.4) can be reached from an angle from behind below the housing (fig. 12.1).

Fill the pipette with a small amount of cryostat oil (cat. no. 350040). Put one or two drops into the space (fig. 12.2) of the cross roller bearings.

The monobloc should be in the lower position.

Also slightly lubricate the horizontal cylinder guide behind the specimen holder.

**NOTE!** For the examination and re-adjustment of the microtome a routine maintenance should be performed by a trained service technician once a year.

### 5-3 CHANGING THE FLUORESCENT LAMP

The fluorescent lamp of the cryostat is situated above the sliding window.

Using a mirror to change the fluorescent lamp would be of great help. To exchange the lamp, remove the sliding window.

First turn off the instrument and pull out the mains plug. Close the sliding window and slightly lift it. Then pull it to the front and fold it backwards. Put a soft pad underneath it.

**CAUTION!** Make sure that the sliding window does not slip down!!

On the left side, the lamp is inserted into a bracket and on the right side it is clamped onto the machine by a flexible clip.

Pull the lamp downwards out of this clip. Then pull it to the left side out of the bracket.

Change the lamp against a new one.

**NOTE!** Only use the lamp type, which was installed in the factory.

First insert the lamp into the bracket. Then carefully press the lamp back into the clip.

Carefully fold the window forwards and push it into the cryostat again. Take note that the cable for the heated sliding window is not squeezed.