MICROTOME CRYOSTAT

HM 505 E

INSTRUCTION MANUAL

MICROM International GmbH
Robert- Bosch-Str. 49

D- 69190 Walldorf

Microtome Cryostat HM 505 E

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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 ommissions 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

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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.

MICROM Ser. No. :.....

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

Issued on November 21, 2000

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Intended Use

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OPTION Vacutome

EC Certificate of Conformity

Name and address of the manufacturer:
MICROM International GmbH
Robert-Bosch-Straße 49
D-69190 Walldorf

Product designation: Microtome Cryostat
Type reference: HM 505 N-[V-P]; HM 505 E-[V-P]

The designated product complies with the laid down regulations:

73/23/EWG with amendments
89/336/EWG with amendments

The designated product complies with the EC regulations by strictly observing the following norms:

"Guideline of the Council for the alignment of the legal instructions of the member countries on electrical means of production for the use within certain voltage limits"

International Norm	European Norm	German Norm	VDE-classification
IEC 1010-1:1990-09 +A1:1992-09 + A2:1995-06	EN 61010-1:1993 +A2:1995	DIN EN 61010-1:1994-03 +A2:1996-05	VDE 0411 Teil1:1994-03 +A1:1996-05

"Guideline of the Council for the alignment of the legal instructions of the member countries on electro magnetic compatibility"

European Norm	German Norm	VDE-classification
EN 61326:1997	DIN EN 61326:1998-01	VDE 0843 Teil 20:1998-01
EN 61326:1997/A1:1998	DIN EN 61326/A1:1999-05	VDE 0843 Teil 20/A1:1999-05

The VDE Prüf- und Zertifizierungsinstitut (EU reference no. 0366), Merianstr. 28, D-63069 Offenbach has checked and certified the product. The marks licences entitle MICROM International GmbH to use the VDE labels opposite.

Marks licence no.: 97329 G

Marks licence no.: 95180 F

Hans Heid
Managing Director

Walldorf, 01 July 2002

SAFETY PRECAUTIONS

A T T E N T I O N !

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!

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°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.

HAZARD OF MALFUNCTION

To avoid the hazard of malfunction of an instrument, it must only be operated in a controlled electromagnetic environment. This means, that transmitters such as mobile phones must not be operated in their close vicinity.

HAZARD OF BIOLOGICAL DANGER

Specimens used during the intended operation of the instrument might potentially be infectious. For this reason, it is recommended to observe the general laboratory regulations concerning protection against danger of infection.
Information on decontamination media, their use, dilution and effective range of application can be read in the Laboratory Biosafety Manual : 1984 of the World Health Organization.

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.

1 - 2	**TECHNICAL SPECIFICATIONS HM 505 E**
Cooling of microtome	temperature range ..... +5°C - -35°C
chamber (at +20°C ambient temp.)	..... 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:	..... 100 V/12 A +/-10% 50 - 60 Hz
	..... 110 V/12 A +/-10% 50 - 60 Hz
	..... 115 V/12 A +/-10% 50 - 60 Hz
	..... 120 V/12 A +/-10% 50 - 60 Hz
	..... 127 V/12 A +/-10% 50 - 60 Hz
	..... 200V/6 A +/-10% 50 - 60 Hz
	..... 220 - 230V/6 A +/-10% 50 - 60 Hz
	..... 240V/6 A +/-10% 50 - 60 Hz
Pollution degree	 2
Overvoltage category:	 II
Dimensions:	..... 775x800x1200 mm (wide/deep/high)
Weight:	..... 135 kg

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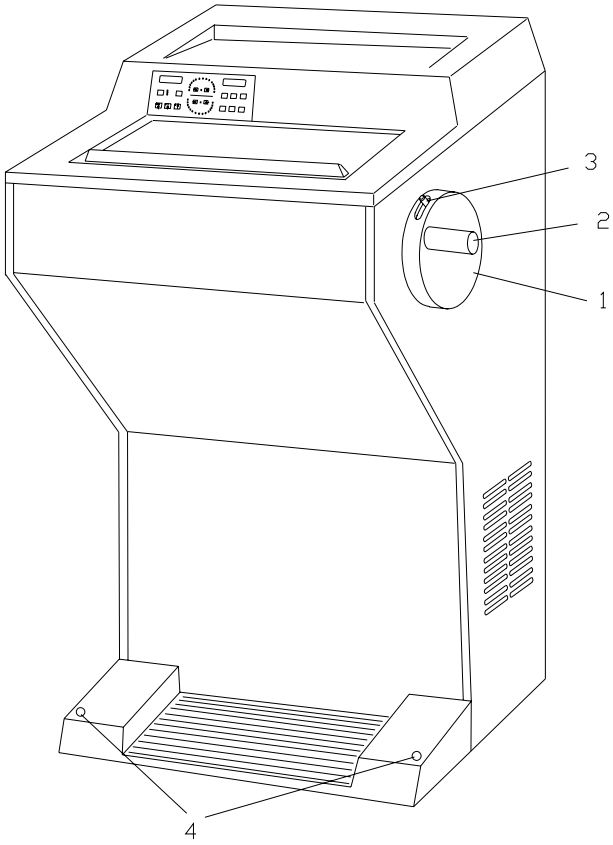

Fig. 1

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).

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

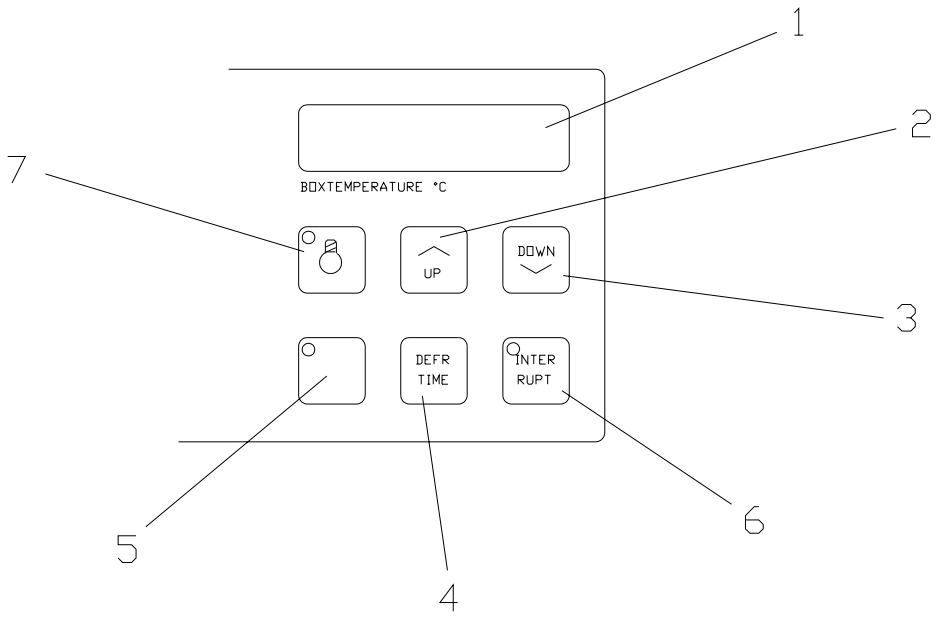

Fig. 2

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.

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).

2-4 CUTTING MOVEMENT AND RETRACTION

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

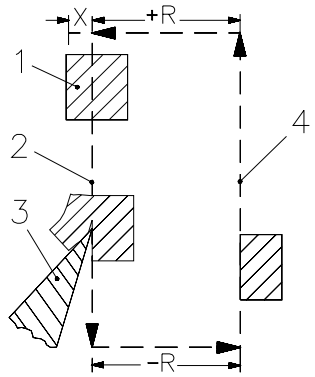

Fig. 3

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.

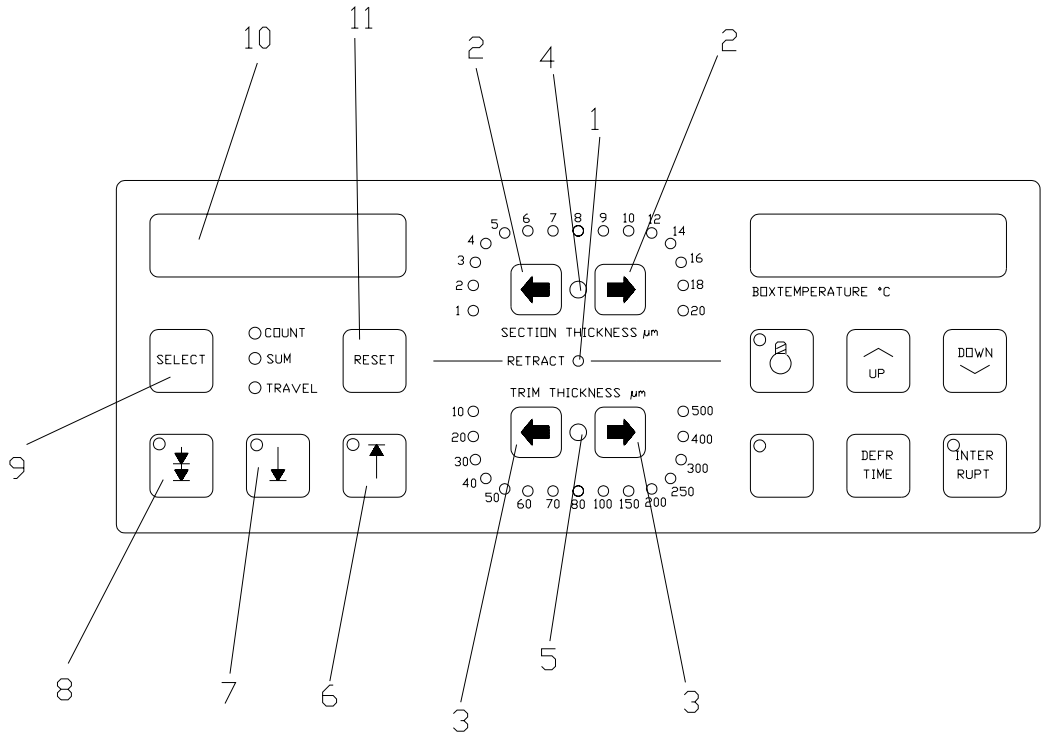

Fig. 4

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 section thicknesses is divided into 2 ranges:

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

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

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°C and -35°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, CO_2 and the like, which generate a freezing temperature of below -35°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 protuding 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 µm. 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 µm. 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 µm. 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°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.

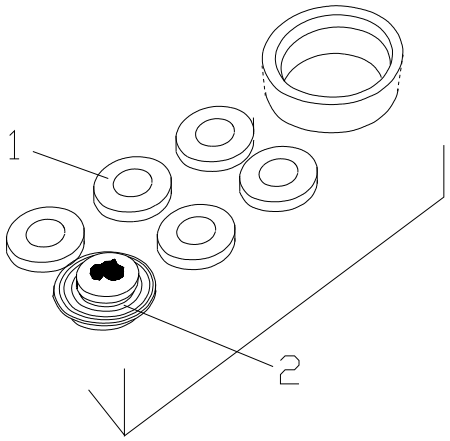

Fig. 5a

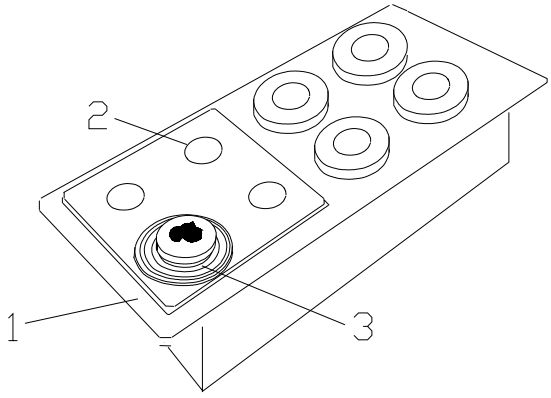

Bild 5b

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).

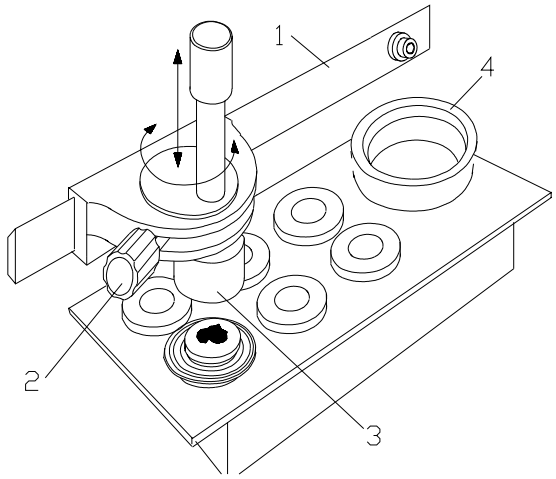

Fig. 6

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° 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.

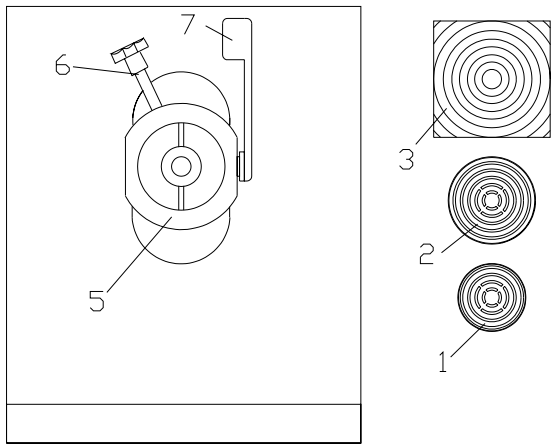

Fig. 7

2-10 KNIFE CARRIERS

2-10-1 STANDARD KNIFE CARRIER

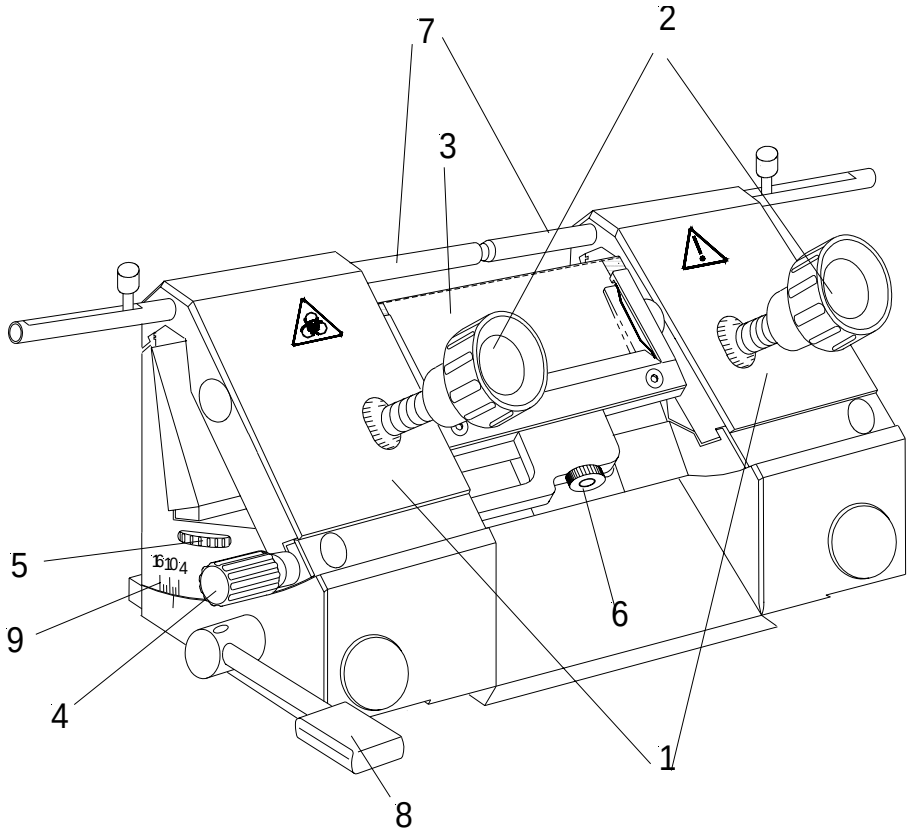

Fig. 8

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).

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° or more.

2-10-2 DISPOSABLE BLADE CARRIER EC

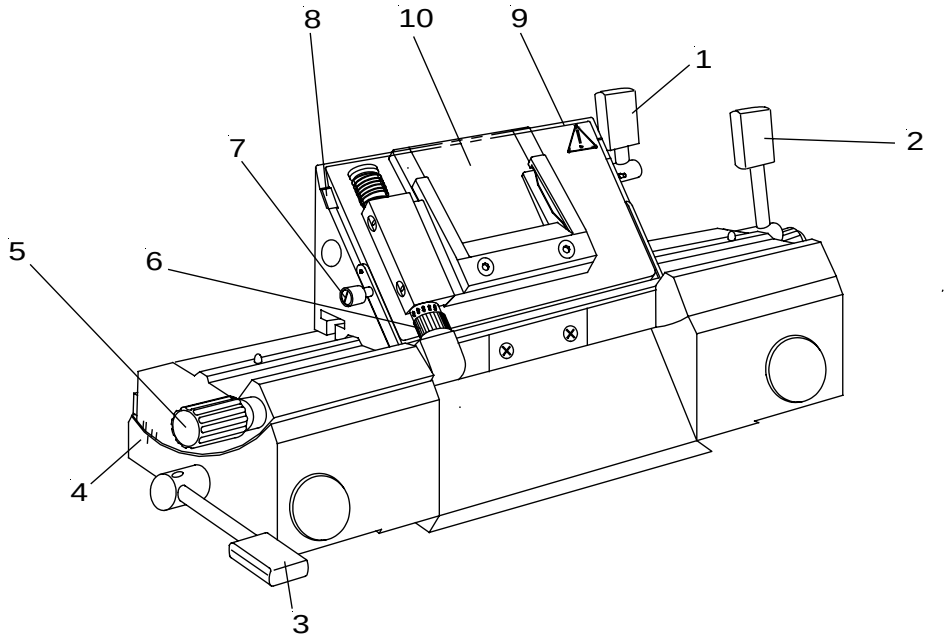

Fig. 9

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.

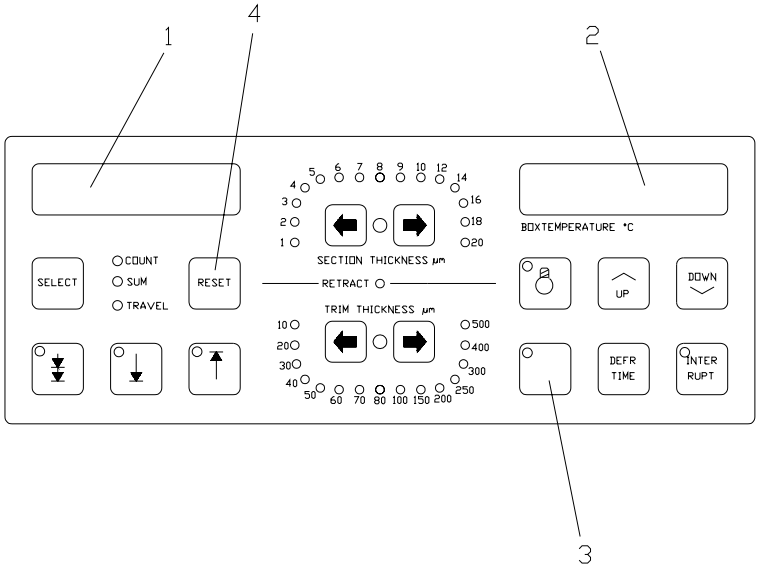

Fig. 10

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°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° 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 protuding 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°C. Above this temperature, the automatic approach system cannot be activated. Press button (fig. 10.3) to clear the error code E-06.

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	402490
1 Allan key 3 mm	362230
1 Allan key 5 mm	362260
1 Allan key 6 mm	362120
1 brush 12 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>	
∅ 30 mm	715110
∅ 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°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°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°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 groves 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 protuding 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 need 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.

- 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.

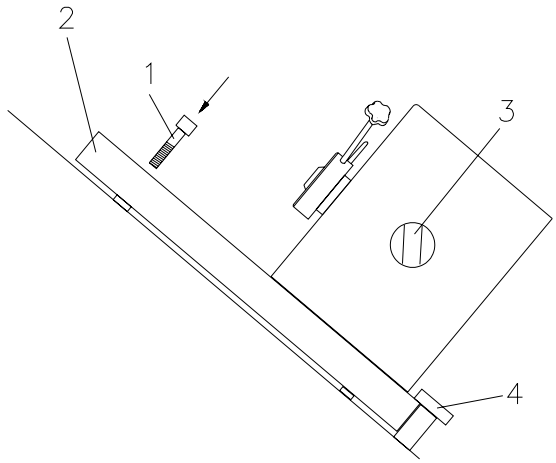

Fig. 11

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

5-2 CLEANING AND CARE OF THE MICROTOME

Carefully clean and dry the dismounted microtome. As there is condensated 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.

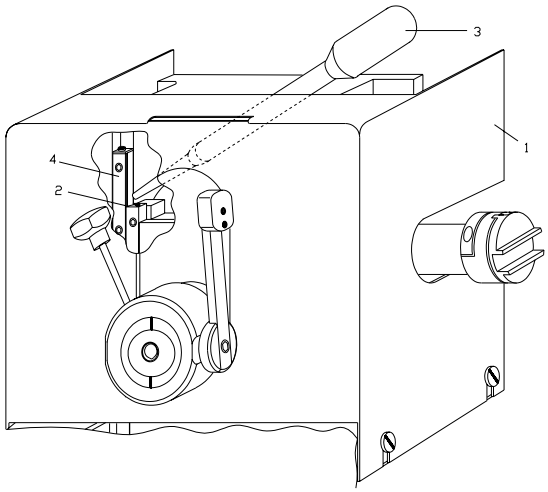

Fig. 12

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.

VACUTOME FOR CRYOSTAT HM 505 SERIES STRETCHING AND SUCTION DEVICE

INSTRUCTION MANUAL

MICROM International GmbH
Robert-Bosch-Str. 49

D- 69190 Walldorf

Microtome Cryostat HM 505 E

MICROM International GmbH
Robert-Bosch-Str. 49

D- 69190 Walldorf

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.

Errors and ommissions excepted. Subject to amendment.

WARRANTY

This MICROM product is warranted against defects in material or 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.

Validity

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 cryostat, the number of this instruction manual and the date of issue.

This instruction manual is available in the following languages:

	Cat. No.
German:	387 090
English:	387 100
French:	387 400

MICROM International GmbH
Robert-Bosch-Str. 49

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Microtome Cryostat HM 505 E

MICROM International GmbH
Robert-Bosch-Str. 49

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INTENDED USE

Dear customer,

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

Only skilled or specially trained personnel must work with this instrument. Please observe the listed and marked safety messages as well as the regulations of your respective lab.

MICROM Ser. No.:.....

Instruction Manual No. 387100

Issued on November 21, 2000

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SAFETY PRECAUTIONS

C A U T I O N !!

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 belonging to this Vacutome 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 BLADE

If possible, the specimen should be clamped in before the blade is inserted into the blade carrier. Before changing the blade carrier, always remove the blade! Never try to catch a dropping blade! Be careful when sectioning and/or removing sections.

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 -40°C.

HAZARD OF RADIOACTIVE RADIATION

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

HAZARD OF INFECTION

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

HAZARD OF MALFUNCTION

To avoid the hazard of malfunction of an instrument, it must only be operated in a controlled electromagnetic environment. This means, that transmitters such as mobile phones must not be operated in their close vicinity.

HAZARD OF BIOLOGICAL DANGER

Specimens used during the intended operation of the instrument might potentially be infectious. For this reason, it is recommended to observe the general laboratory regulations concerning protection against danger of infection.
Information on decontamination media, their use, dilution and effective range of application can be read in the Laboratory Biosafety Manual : 1984 of the World Health Organization.

PART 1 INTRODUCTION

1-1 DESCRIPTION OF THE VACUTOME

The unit consists either of a special magnetic disposable blade carrier MV or of a disposable blade carrier EV both of which are equipped with a vacuum anti-roll hood for the stretching and/or suction process.

Furthermore, the system is equipped with a filter including a reusable insert as well as five disposable filter bags.
In addition, the Vacutome comprises a vacuum generating aggregate with its control elements.

Stretching or disposing of is controlled by the suction window. The power of the required suction flow can be set via an operating knob, which is placed on the right side of the housing above the handwheel.

1-2 TECHNICAL SPECIFICATIONS

Max. specimen size:	28 mm
Max. section thickness to be disposed of:	80 µm
Max. section thickness to be stretched:	80 µm
Min. section thickness to be stretched:	1 µm
Cryostat blades for magnetic disposable blade carrier MV:	60 x 19 mm
Low profile blades for disposable blade carrier EV:	80 x 8 mm
High profile blades for disposable blade carrier EV:	76 x 14 mm
Clearance angle adjustment for magnetic disposable blade carrier MV:	27° - 32°
Clearance angle adjustment for disposable blade carrier EV:	10° - 12°
Operating temperature range of the cooling chamber:	from -5°C up to -35°C
Volume of disposable filter:	0,27 l
Volume of reusable filter:	0,27 l
Power requirements:	supply via cryostat according to the respective power supply version
Operating temperature range:	+5°C to +40°C (at max. 60% rel. humidity of the air) altitude up to 2000 m M.S.L.

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PART 2 OPERATING INSTRUCTIONS

2-1 INITIAL TURN-ON

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

Further information on the initial turn-on of a cryostat can be obtained from the enclosed instruction manual Microtome-Cryostat HM 505 N or HM 505 E.

To start working with the Vacutome, first mount the blade carrier into the dovetail guide and clamp it by means of the clamping lever, which is placed on the right side of the base plate of the microtome.

Cool the cryostat chamber down to the desired temperature.
Wait approx. 1,5 h, before starting cutting. During this time, the blade carrier adjusts itself to the cooling chamber temperature.

Meanwhile, place either the reusable filter (fig. 7.6) or a disposable filter bag (fig. 7.3) into the housing of the filter.
For this, unscrew the screw-type cap (fig. 7.4) from the filter housing (fig. 7.2) and mount the desired filter insert into the screw-type cap (fig. 7.4).

After having inserted one filter type, connect the connecting branch of the filter housing with the connecting bow (fig. 7.5) leading to the blade carrier.

2-2 SETTING THE VACUUM FOR STRETCHING SECTIONS AND DISPOSING OF SECTION WASTE

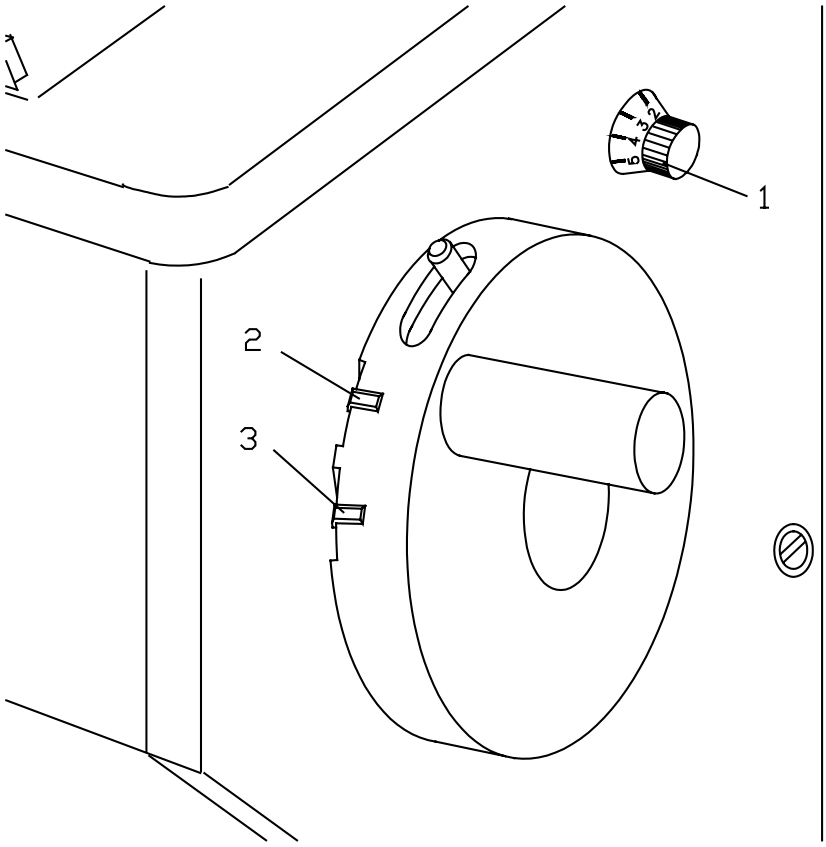

Fig. 1

The suction power of the required vacuum can be selected on the operating knob (fig. 1.1), which is placed above the handwheel. The user sets the desired value depending on the desired function (stretching or suction process), section thickness and size of the specimen.

The applied vacuum for stretching sections and/or disposing of section waste is only active during the suction window. Outside the suction window, the selected vacuum is turned off by means of a valve.

The size of the suction window can be selected matching the size of the specimen.
Especially note that the lower limit of the suction window corresponds with the upper edge of the specimen, i.e. that the applied suction vacuum is turned off as soon as the section is completely cut.
Two sliders (fig. 1.2 and 1.3) situated on the rim of the handwheel, are used to set the size of the suction window. Both sliders (fig. 1.2 and 1.3) can be moved in either direction, to independently change the upper or lower suction window limit.
To reduce the size of the suction window, move the sliders (fig. 1.2 and 1.3) towards each others.
To increase the size of the suction window, move the sliders (fig. 1.2 and 1.3) in the opposite direction.
The selected limits of the suction window can be clearly noticed by the switching noise of the valve.

2-3 BLADE CARRIERS

2-3-1 MAGNETIC DISPOSABLE BLADE CARRIER MV

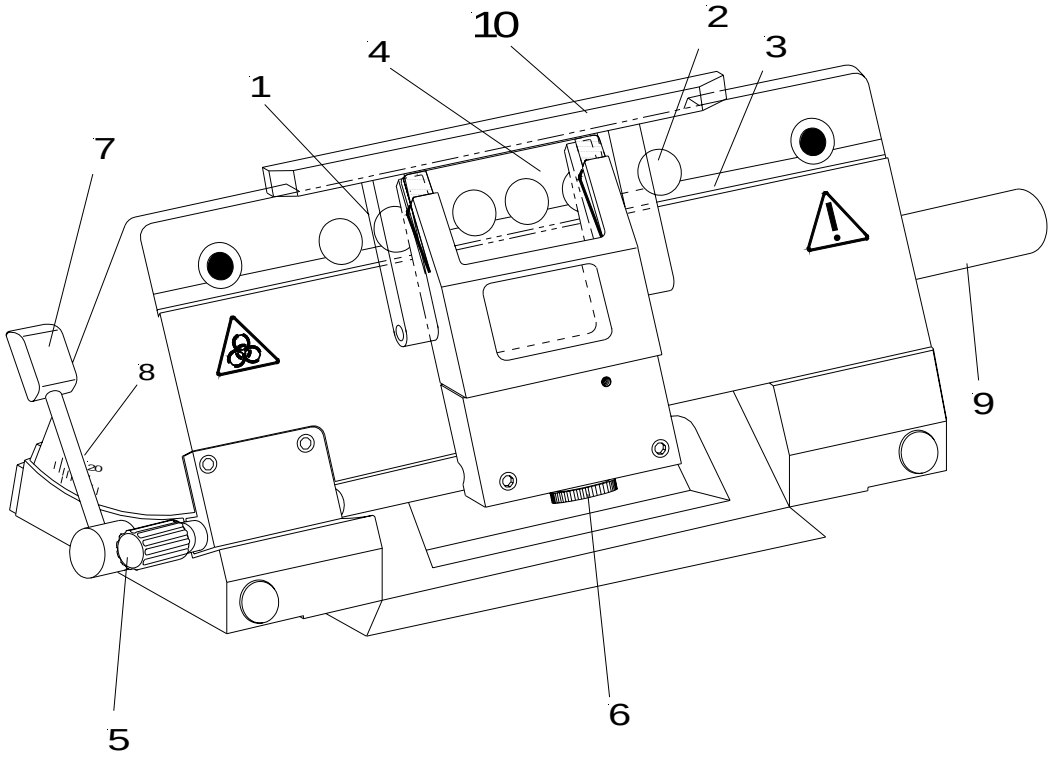

Fig. 2

With the magnetic disposable blade carrier MV, MICROM blades of 60 x 19 mm and a facet angle of approx. 33° can be clamped.
Insert the blade (fig. 2.1) onto the blade slider (fig. 3.2) and put it on the magnetic surface (fig. 2.2) of the blade carrier from the side. To move the blade sideways, press the blade slider downwards so that the blade is properly put against the ledge (fig. 2.3) of the blade carrier. Then take off the blade slider.

To move the blade to a new position, put on the blade slider (fig. 3.2) onto the blade and shift it. The blade slider must always be put on in a way that the inscription MICROM can be read, which guarantees that the slanted edge (fig. 3.5) of the blade slider shows to the backside of the blade.
Turning the grip (fig. 2.5) to move the vacuum anti-roll hood (fig. 2.4) forwards and backwards.

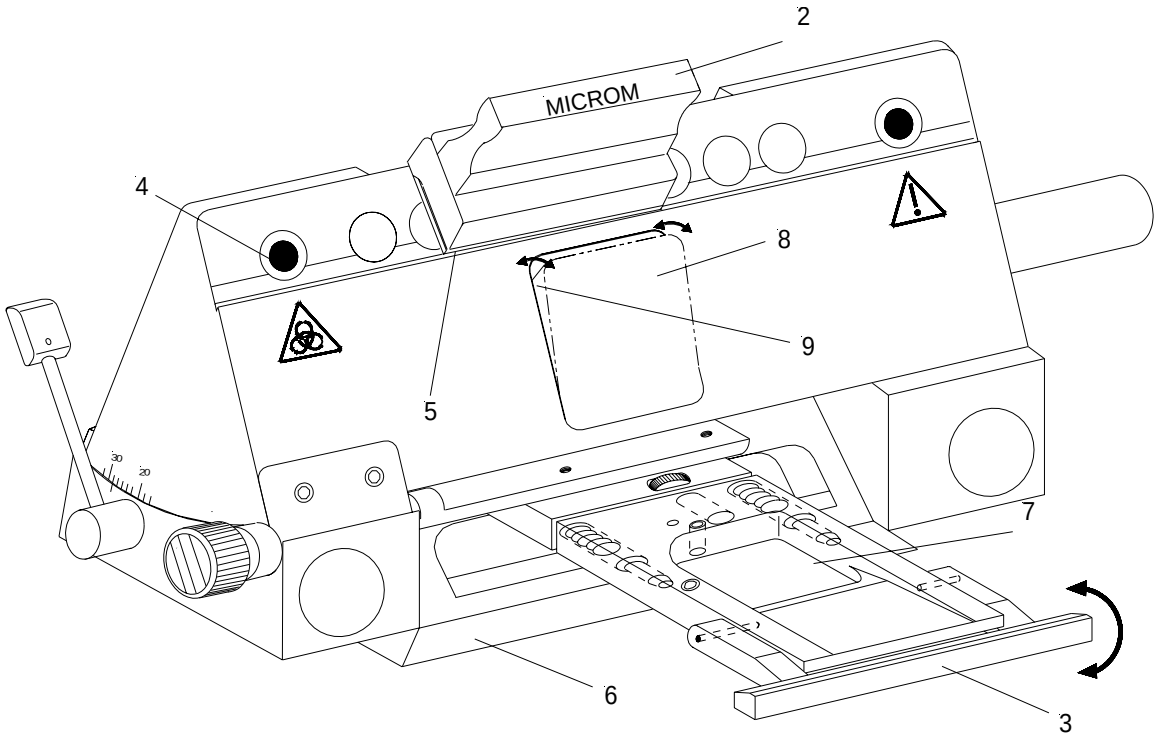

Fig. 3

The knurled screw (fig. 2.6) allows the adjustment of the edge of the vacuum anti-roll hood in relation to the blade edge.
A closed vacuum anti-roll hood (fig. 2.4) and a suitable vacuum (see part 2-2) generate stretching or suction effects. For this, the closed vacuum anti-roll hood (fig. 2.4) slightly tilts the underneath flap thus opening a suction slot (fig. 3.9).

Lift the vacuum anti-roll hood (fig. 3.7), the flap beneath (fig. 3.8) falls back in place and the slot (fig. 3.9) gets closed.

The lever (fig. 2.7) is used to fix or change the clearance angle adjustment.

By experience, usable cuts are only achieved at a clearance angle between 24° and 30°. The adjusted clearance angle is shown on the scale (fig. 2.8).

During breaks or while working on the specimen or the specimen holder, the knife guard (fig. 3.3) should be used.

2-3-2 DISPOSABLE BLADE CARRIER EV

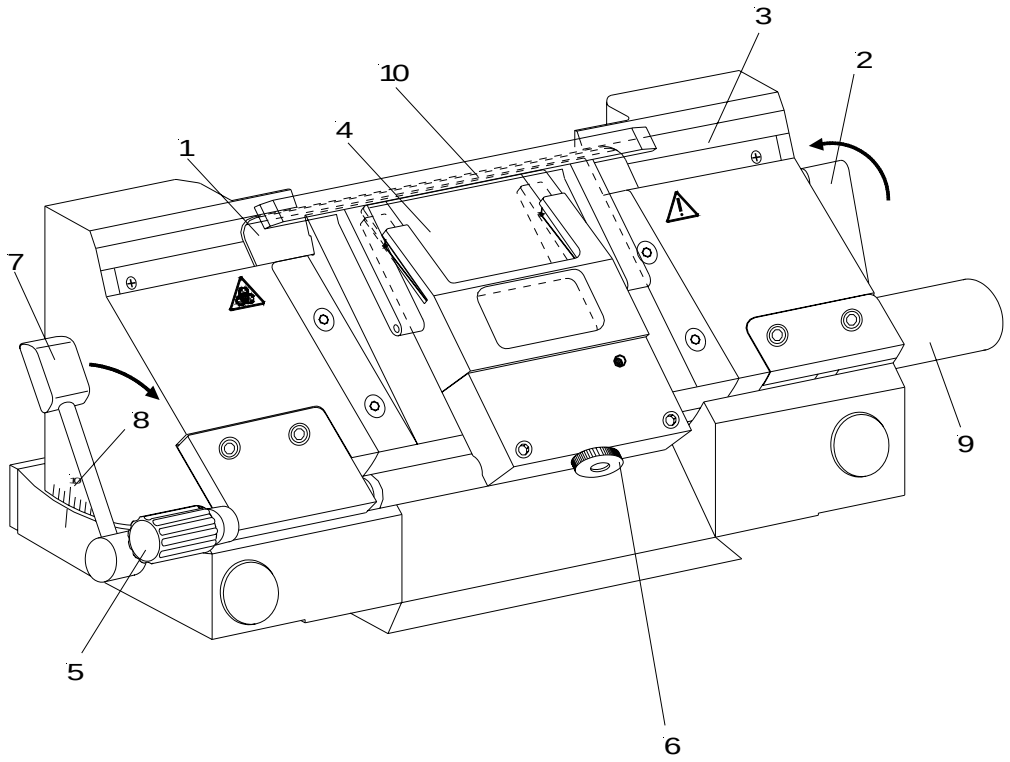

Fig. 4

With the disposable blade carrier EV, all commercially available low profile blades of 80 x 8 mm and a facet angle of approx. 35° as well as high profile blades of 76 x 14 mm can be clamped.

Insert the blade into the slot behind the clamping plate (fig. 4.1). When using high profile blades, first loosen the lever (fig. 4.2) and remove the spacer strip. The blade is clamped by means of a clamping plate, which is placed on the rear part of the blade carrier. This plate can be opened and tightened by means of the small lever (fig. 4.2).

Turning the grip (fig. 4.5), the vacuum anti-roll hood (fig. 4.4) can be turned forwards and backwards. The knurled screw (fig. 4.6) allows the adjustment of the edge of the vacuum anti-roll hood in relation to the blade edge.
A closed vacuum anti-roll hood (fig. 4.4) and a suitable suction vacuum generate stretching or suction effects.

For this, the closed vacuum anti-roll hood (fig. 4.4) slightly tilts the underneath flap (fig. 5.6) thus opening a suction slot (fig. 5.7).

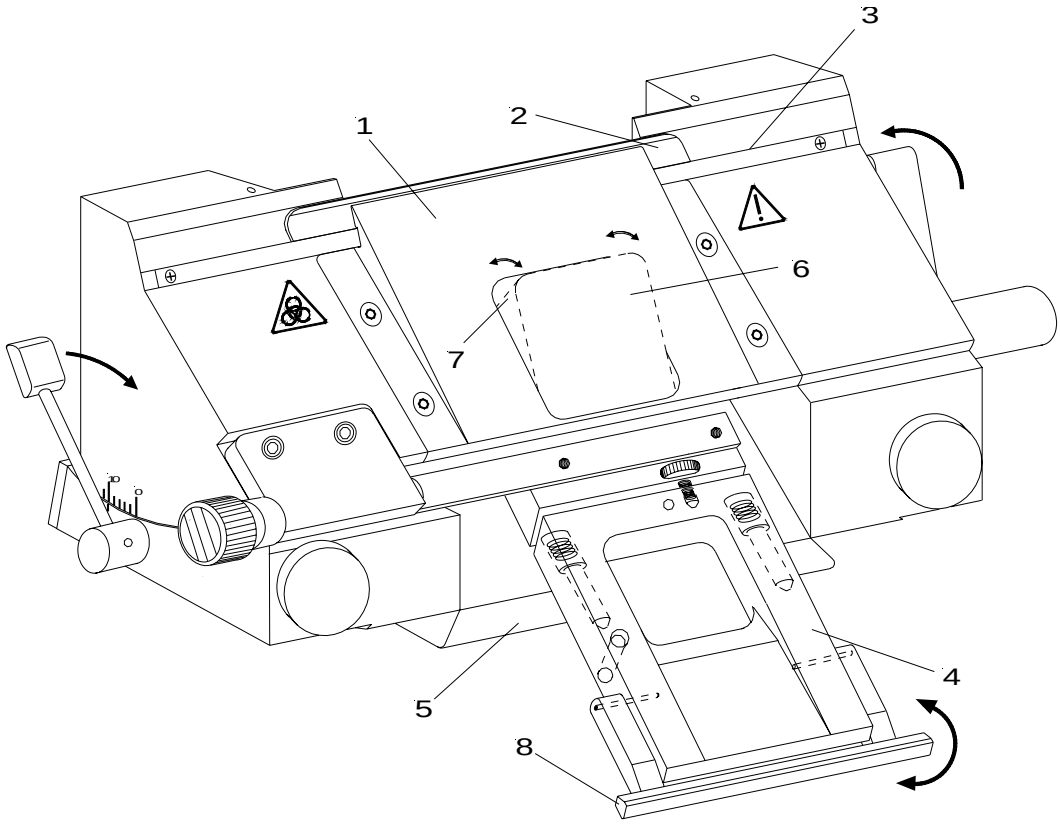

Fig. 5

Lift the vacuum anti-roll hood (fig. 5.4), the flap (fig. 5.6) beneath falls back in place and the slot (fig. 5.7) gets closed.
The lever (fig. 4.7) on the left side is used to fix or change the clearance angle adjustment.

By experience, usable cuts are only achieved at a clearance angle of 10° or more. The adjusted clearance angle is shown on the scale.

During breaks or while working on the specimen or the specimen holder, the knife guard (fig. 3.3) should be used.

2-4 ACCESSORIES

2-4-1 STANDARD EQUIPMENT

The Vacutome for the microtome cryostat HM 505 series is supplied with the following accessories:

	Cat. No.
1 Blade carrier (type as ordered)	
1 Reusable filter insert	468050
5 Disposable paper bags	281030 /1 piece
1 Cleaning hose	570230

2-4-2 ADDITIONAL EQUIPMENT (OPTIONAL)

Magnetic disposable blade carrier MV	705590
Disposable blade carrier EV	705580

PART 3 THEORY OF OPERATION

The Vacutome includes a special blade carrier with which a vacuum can be applied just below the blade edge for stretching sections or the disposal of section waste. For this, the blade carrier features a slot that can be opened and closed by means of the vacuum anti-roll hood through an inserted flap.

A vacuum pump, installed in the cryostat, generates the vacuum needed for the suction. A filter system is placed between vacuum pump and blade carrier. This system can either be equipped with a reusable filter or a disposable paper bag.
The suction force of the vacuum can be changed by means of an electronic control. Moreover, the set vacuum is only effective during the suction window due to an in-line valve, which is controlled by the set suction window limits.

3-1 STRETCHING SECTIONS

The compression of sections when gliding onto the blade is a well known phenomena during sectioning in general and especially during cryo-sectioning. However, this can be enormously reduced by using a Vacutome with a special blade carrier.
For this, a vacuum can be set below the vacuum anti-roll hood via an operating knob, which is situated on the left side of the instrument above the handwheel. This vacuum anti-roll hood acts as a traditional anti-roll guide. By means of the suction vacuum prevailing in the slot between vacuum anti-roll hood and blade edge, the cut is drawn on the air cushion over the blade thus immensly reducing the compression. The section remains on the blade in stretched form.
The vacuum for stretching sections is turned off immediately after reaching the end of the specimen via an in-line valve, however, depending on a correct setting of the suction window.
Turn the vacuum anti-roll hood backwards to transfer the cut onto a slide.

3-2 DISPOSING OF SECTION WASTE

The Vacutome with special blade carrier is also highly suitable for the disposal of debris.
For this, the vacuum anti-roll hood is used as described in part 3-1. Close the vacuum anti-roll hood to open the slot on the blade carrier by means of the flap.
Continue cutting and the penultimate section is pushed forwards and reaches the area of the suction slot. From here the section gets through the blade carrier and the tubings into the filter system.
Collected section waste can now easily be removed.

PART 4 WORKING WITH THE VACUTOME

4-1 PREPARING THE VACUTOME

First insert the blade carrier onto the dovetail guide as already described in part 2-1 and clamp it with the clamping lever. Select a clearance angle of approx. 28 - 30°.

Insert the blade onto the blade carrier. Put it on the magnetic surface of the magnetic disposable blade carrier MV from the side.

NOTE! To move the blade sideways, press the blade slider downwards so that the blade is properly put against the ledge of the blade carrier.

When using the disposable blade carrier EV, insert the blade between front and rear clamping plate and tighten it via the clamping lever on the right side of the instrument.

Clamp the specimen stage together with the frozen specimen into the specimen holder and tighten it via the orienting lever.

After having adjusted the specimen, set the suction window limits.
Please note that the lower limit of the cutting window corresponds with the upper edge of the specimen.

4-2 ORIENTATION AND TRIMMING OF SPECIMENS

With the clamping lever loose, you have the possibility to adjust the specimen parallel in relation to the cutting edge via the orienting lever.
(See part 2-9-2 and part 4-4, Instruction Manual of Microtome Cryostat HM 505 N or HM 505 E).
While trimming, determine the correct force of the vacuum by continued cutting and varying it via the operating knob, which is placed above the handwheel.

The setting is optimal, when the last section remains stretched on the blade, while the penultimate section gets into the suction slot when starting a new cut.

4-3 SECTIONING AND TAKING OFF SECTIONS

For sectioning with a cryostat HM 505 E, pre-select the desired section thickness on the corresponding buttons of the operating panel (see part 2-6, Instruction Manual Microtome Cryostat HM 505 E).
For sectioning with a cryostat HM 505 N, select the desired section thickness on the knob on the right side of the microtome (see part 2-6, Instruction Manual Microtome Cryostat HM 505 N).

In addition, set the desired vacuum on the operating knob above the handwheel.
Put the vacuum anti-roll hood (fig. 2.4) against the blade by means of the lever (fig. 2.5) Turn the handwheel or use the motorized cutting drive of the HM 505 E (see part 2-7, Instruction Manual Microtome Cryostat HM HM 505 E) to carry out sectioning.
At the same time, the vacuum prevailing in the slot between vacuum anti-roll hood and blade draws the section over the blade. The section remains stretched on the blade. Open the vacuum anti-roll hood to transfer the section onto a slide.

4-4 HOW TO AVOID MALFUNCTIONS

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

- Condition of blade edge, probably move it horizontally to the left or right side.
- Check adjustment of vacuum anti-roll hood and correct it, if necessary.
- Carefully remove frost from front- and backside of the blade and from the anti-roll hood (i.e. with ethanol, acetone or the like).
- Tighten all clamping screws and clamping levers on the blade carrier and specimen holder.
- Select the correct cutting temperature according to the specimen (see part 4-2, Instruction Manual Microtome Cryostat HM 505 N or HM 505 E).
- Select the appropriate freezing compound.

CAUTION! If the specimen was frozen with liquid nitrogen, the specimen must adapt itself to the cutting temperature.

- Select a suitable clearance angle. When using a magnetic disposable blade carrier MV select a clearance angle between 24° and 30°. For high and low profile blades the clearance angle must be between 5° and 15°.
- Select a proper cutting speed:
  The harder the material, the slower the cutting speed.
- Take care in bringing knife and specimen together.
- Make sure that the filter is changed or cleaned regularly allowing a perfect airflow.
- Exactly adjust the suction window to the specimen size, to avoid inadvertent disposal of good sections.
- Keep the area around the flap tidy for optimal functionality.
- Set the suction vacuum according to the specimen and the selected section thickness.

For optimal sectioning, the blade and vacuum anti-roll hood as well as the flap must be cleaned of waste. Only use a sharp blade and exactly adjust the vacuum anti-roll hood in relation to the blade edge.

PART 5 MAINTENANCE OF THE VACUTOME

5-1 SHUTTING-OFF FOR CLEANING

Cleaning of the Vacutome depends on how frequently the instrument is used. However, it is recommended to shut the instrument off every 6 - 8 weeks.

This should be done as follows:

- Turn off the mains switch. Pull the mains plug off.
- Remove or dispose of cold section waste.
- Remove filter housing from connecting bow and clean it. Remove the filter. Clean it with appropriate cleaners or dispose of the paper bag.
- Remove the blade from the blade carrier and store it in safe place. Remove section waste tray, specimen stages and working tools. Loosen and pull out the two clamping levers (fig. 2.7) on the blade carrier and remove the blade carrier upwards. Lift the two side accessory shelves in the front, pull them out of the rear pegs and also remove them.
- Again dispose of 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 handle into the lower position, i.e. the specimen holder is also in the lower reversal point.

Turn out the socket head screw (fig. 6.1) in the middle of the front part of the microtome base plate with the hex head wrench no. 5. Then slightly lift the base plate (fig. 6.2) 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. 6.4). Turn the microtome to the right side so that the front side of the microtome faces the right lateral wall of the chamber. Now the coded connector on the rear side of the microtome can be unplugged. 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 afterwards at the end of the outlet tube (left side of the instrument). Insert the stopper after cleaning as otherwise cold air leaks out and the instrument will cover with frost.

- After having finished cleaning and maintaining (see 5-2), install the microtome in reverse order.

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

- Insert accessory shelves, accessories and blade carrier into the instrument.
- Connect the filter housing first with the tube on the suction side and then via the connecting bow with the blade carrier.
- Connect the mains plug to the power outlet. Turn on the mains switch.
- Close the sliding window.

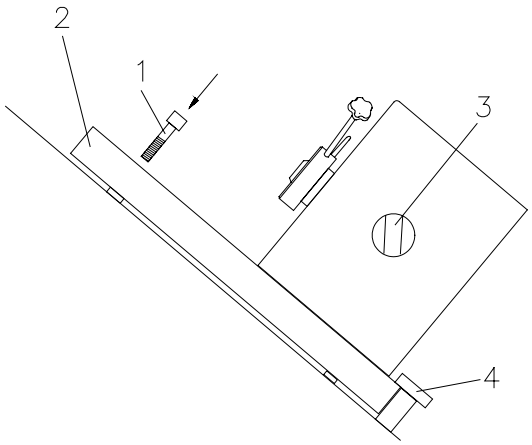

Fig. 6

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5-2 CLEANING AND CARE OF THE CRYOSTAT WITH VACUTOME

Carefully clean and dry the disconnected microtome. As there is condensated 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.
Before installing the microtome into the chamber again, use a syringe to lubricate the vertical cross roller bearings on either side of the specimen holder with the cryostat oil no. 407. Also lubricate slightly the horizontal cylinder guide.

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

5 - 3 CHANGING AND CLEANING FILTERS

To change or clean the filters, unscrew the screw-type cap (fig. 7.4) from the housing (fig. 7.2) and remove either the disposable paper bag (fig. 7.3) or the reusable filter (fig. 7.6) from the screw-type cap (fig. 7.2).

Dispose of the disposable paper bag (fig. 7.3) in a propriate way.
Empty the reusable filter (fig. 7.6) and clean it with appropriate cleaners.

Then insert either a new paper bag or the cleaned reusable filter into the screw-type cap (fig. 7.4).
Note that the disposable paper bag is **not folded up anymore.**

Again screw the screw-type cap onto the housing (fig. 7.2).

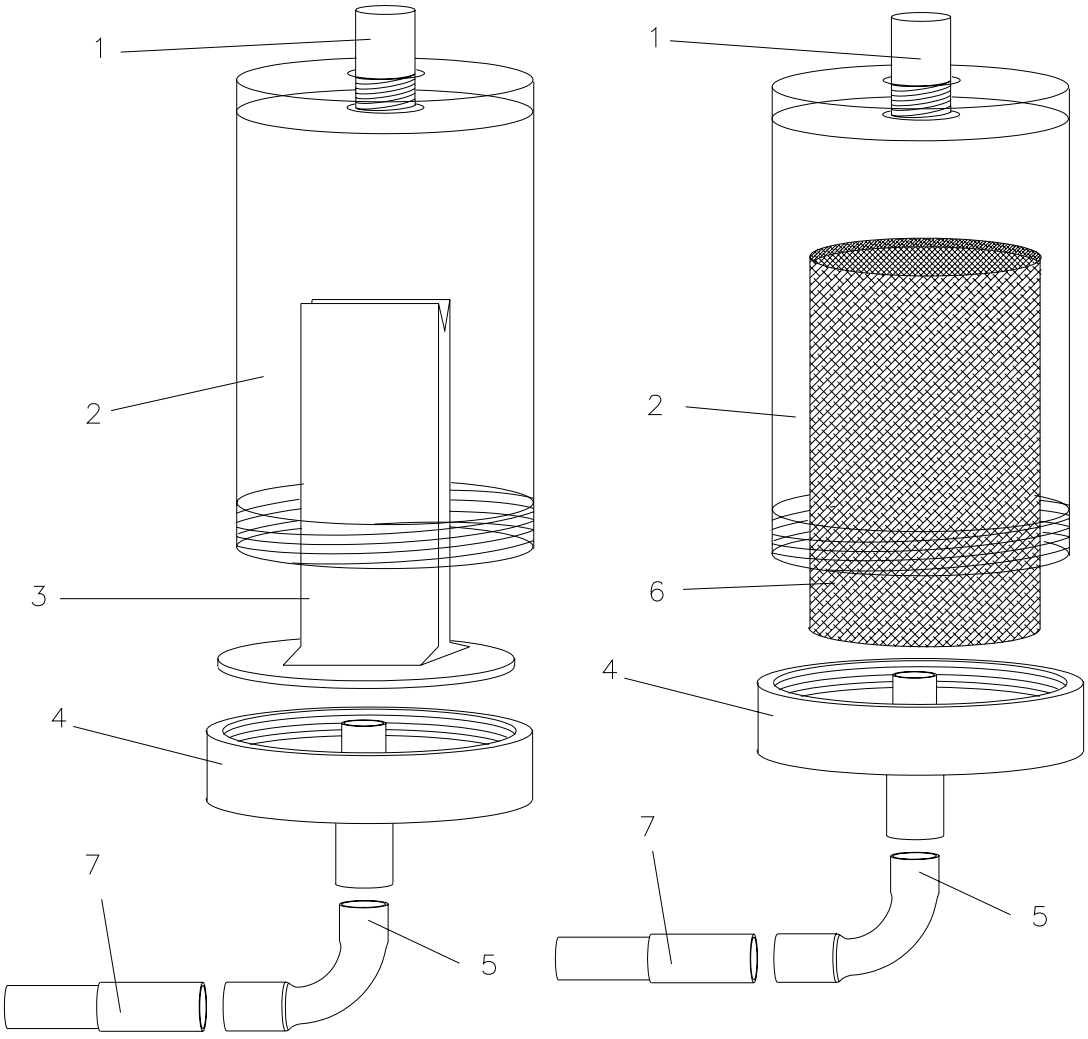

Fig. 7

1 = Connecting piece
2 = Housing
3 = Disposable filter bag
4 = Screw-type cap
5 = Connecting bow
6 = Re-usable filter
7 = Connecting piece

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