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Leica
MICROSYSTEMS

Leica EM FC6

Operating Manual

Order Number 16201032
Leica EM FC6-GA-E-05/06

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Important Note

Leica reserves the right to change technical specifications as well as manufacturing processes without prior notice. Only in this way is it possible to continuously improve the technology and manufacturing techniques used to provide our customers with excellent products.

Issued by:

Leica Mikrosysteme GmbH
Hernalser Hauptstrasse 219
A-1170 Vienna

Leica EM FC6

Operating Manual

Leica EM FC6 Serial Number:

Date of purchase:

For the instrument serial number, please refer to the name type label on the back of the instrument!

Please read this instruction manual carefully before operating the instrument.

Contents

Symbols and abbreviations used in this manual

Attention, take extra care.

Important information for the user.

Abbreviations

CU = control unit
n.i. = not illustrated
E-W = East - West
LN2 = Liquid Nitrogen
N-S = North - South
RC = Rapid Cooling
RT = room temperature

1 Safety Instructions

Liquid Nitrogen (LN_2)

When working with liquid nitrogen (LN_2) please bear in mind LN_2 is extremely cold. It boils at -196 °C. Nitrogen gas (GN_2) escapes at very low temperature from the boiling LN_2. Both LN_2 and GN_2 as well as cooled elements (e.g. pipes, valves, hoses, containers or stoppers) can cause severe frost bite and burns to the skin and eyes.

When LN_2 evaporates, it expands in a ratio of 1:700. 1 litre LN_2 produces almost 1 m^3 of GN_2. Care should therefore be taken to ensure that when large quantities of nitrogen evaporate (e.g. when transferring LN_2), the room should always be well ventilated.

Removing LN_2 waste: dump LN_2 into an outdoor pit or container filled with gravel, where it will evaporate rapidly and safely.

GN_2 is odourless and tasteless and will be inhaled like air. GN_2 is non-toxic, but a high GN_2 content in the air (> 78%) reduces the oxygen-content (< 21%) and produces immediate fainting and deep unconsciousness without any previous symptoms.

When there is doubt about the adequacy of ventilation, use an oxygen analyser (0 to 25% scale) to check for oxygen. The content of oxygen must not drop below 18%.

If an unconscious person stays in a low oxygen environment then death may occur.

If breathing stops, apply artificial respiration at once and notify doctor and ambulance immediately.

For the reasons given above, never put LN_2 Dewars in a closed storage room or chamber. The evaporation rate from Dewar vessels can rise to several litres a day if they are defective due to improper handling or to natural wear over many years of use.

Always keep the working area well ventilated.

Bring objects at room temperature carefully into contact with LN_2. Initially an insulating gas layer is formed preventing a large transfer of heat. During this initial period little LN_2 evaporates. However, once the object has cooled down there may occur unexpected strong boiling and spurting of LN_2.

In the case of burns from LN_2 splashes, rinse the affected skin immediately with plenty of water at hand temperature. For serious burns arrange for a skin specialist to see them at once.
In the case of LN_2 affecting the eyes, rinse immediately with water at hand temperature and arrange for an eye specialist to see it at once.

Never use glass Dewar vessels in the lab (especially glass Dewars larger than 2 litres capacity) without complete metal envelope: Glass Dewars often burst for no obvious reason or due to unintentional mishandling (e.g. contact with metal instruments etc.). Never work without open protective glasses when using LN_2 in a glass Dewar.

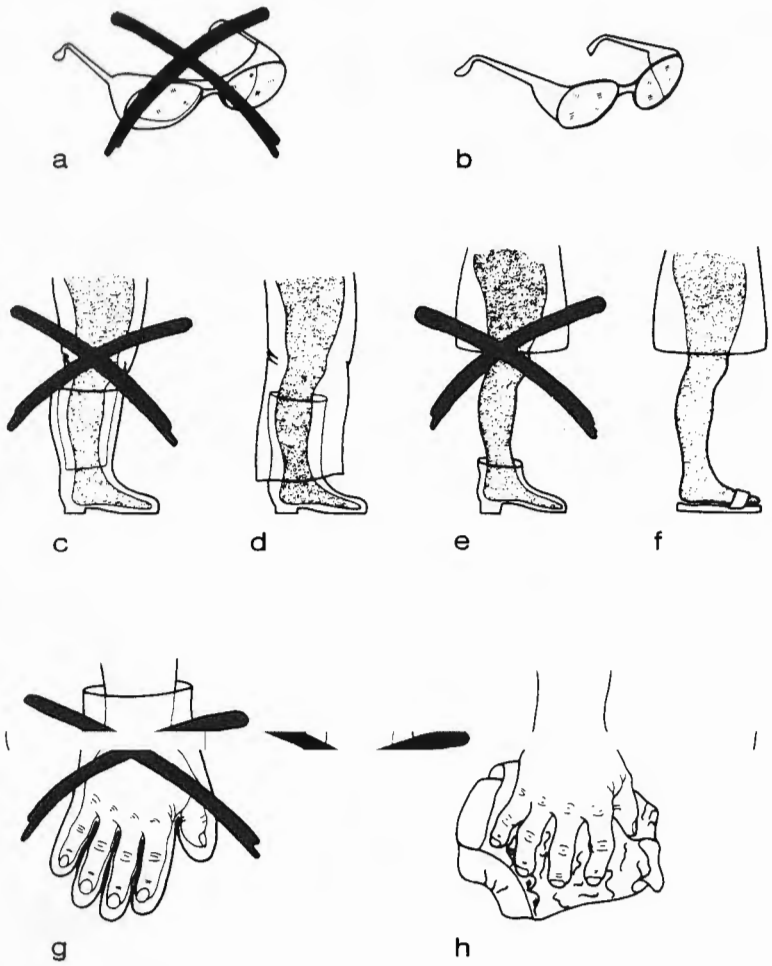


Fig.1.1: When working with LN_2 avoid protective glasses (a), boots (c), walking shoes (e) and protective gloves (g) out of which the LN_2 cannot easily escape if entered.LN_2 splashing into the closed protective glasses (a), open boots (c), shoes (e) or protective gloves (g) evaporates suddenly and can cause serious burns.
Always use protective glasses (b) with side protection which are open at the top and at the bottom. Only use boots if you have loose (not narrow) trousers coming outside the boots (d) and completely covering the gap. Wear only open slip-on sandals (f) in the lab, no walking shoes or court shoes. Always wear cufless trousers if you wear slip-on sandals.
Never wear protective gloves when pouring LN_2 or when putting the Dewar head on the Dewar vessel. Just use an open flannel cloth (h) to protect your hands from the cold. Gloves should be used only to grasp dry cold parts. They are unsuitable for LN_2 work.

Only use metal Dewars specifically designated for storage of LN_2, since only containers of this kind exclude risks during storage. For routine cryopreparation metal troughs (1 cm styrofoam insulation), styrofoam containers or plastic troughs are eminently suitable and ensure low risk cryopreparation.

Check the evaporation rate of your metal Dewar regularly every three months and compare these rates with the rate given by the manufacturer. The evaporation rate of an undamaged metal Dewar should be well below 1 litre of LN_2 per day. Defective Dewar vessels with higher evaporation rates are a safety risk, and should be taken out of work or repaired.

Do not leave LN_2 standing in open vessels where it can exchange with the room atmosphere. The boiling point of LN_2 (-196 °C) is lower than liquid oxygen's boiling point (-183 °C). When the exchange surfaces are extensive enough, oxygen from the air will be taken up in exchange for nitrogen. LN_2 with a high liquid oxygen content has a faintly bluish colour. **Concentrated liquid oxygen promotes vigorous burning!**

Make sure that your Dewar vessel is filled only with LN_2. Apply a note in the central distribution place stating clearly

ONLY LIQUID NITROGEN

or similar if different liquefied gases are delivered from there. Check the colour of cryogen: Bluish colour indicates the presence of a high percentage of liquid oxygen.
The concentration of liquid oxygen increases during long periods of storage as its boiling point (-183 °C) is higher than the boiling point (-196 °C) of LN_2.

HAZARD WARNING
LIQUID NITROGEN, LN_2

Suffocation

- Any vessel containing LN_2 is a potential hazard
- One litre LN_2 produces 700 litres N_2 gas
- N_2 gas is odourless and tasteless
- Oxygen levels can quickly drop in confined spaces due to displacement of oxygen by N_2 when using or dispensing large volumes of LN_2
- This can cause immediate fainting and unconsciousness
- **Always use LN_2 in well-ventilated areas**
- Treat it with respect!

Storage

- For reasons mentioned above do not store full LN_2 Dewars in confined spaces

Burns

- LN_2 boils at -196°C. It is extremely cold and can cause serious burns. Please read the safety instructions provided with all Leica products for the correct handling of liquid nitrogen!

2 Introduction to the LEICA EM FC6

2.1 Overview

The Leica EM FC6 is the low temperature sectioning system for the Leica Ultracut UC6 ultramicrotome. It is used for semi- and ultrathin cryosectioning of biological and industrial samples. This includes Frozen Hydrated Sectioning (FHS) of vitrified specimens, cryoprotected biological samples (Tokuyasu technique) and soft industrial specimens such as rubbers and polymers.

The Leica EM FC6 has been specifically designed for maximum ergonomy and ease of use. It has an integrated chamber illumination, allows to use an integrated antistatic device and provides a wide temperature range from –15 °C to – 185 °C for cryosectioning. All controls are via the control unit (CU) of the ultramicrotome to save workspace on the table and provide maximum comfort and ergonomy for the operator.

The EM FC6 system consists of

- Cryochamber with integrated LED illumination
- Dewar vessel on mobile trolley
- Liquid nitrogen pump
- Power supply
- Connecting cables, LN2 hose
- Antistatic electrode with holder
- Accessories box containing 2 rotating knife holders, 1 standard specimen holder, specimen carriers, special keys, tools and forceps

The „complete working outfit" contains in addition to the items mentioned above:

- Flat specimen holder
- Antistatic transformer, remote controlled

This allows to control the ioniser from either the „touch sensitive" or the „key pad" CU of the Ultracut UC6.

Recommended accessories are

- Set of cryotools
- Filling System for LN2 Dewar

Please read this instruction manual carefully before operating the instrument.

Fig. 2.1: Leica EM FC6 mounted on Ultracut UC6 with touch screen control unit.

2.2 Instrument Function

Operation of the EM FC6 is performed via the CU of the Ultracut UC6. Before quickly cooling down for trimming and sectioning, the cryochamber has to be unpacked, mounted on the EM UC6 and connected to the Ultracut UC6 CU. As the cryochamber is mounted on the chassis of the ultramicotome it is isolated from external vibrations. The contact free through-the-wall specimen arm provides optimum temperature and mechanical stability.

After connection of the LN2 hose to the LN2 filled Dewar via the automatic pumping system, the pump ensures a stable low temperature environment around the knife-specimen area by pumping LN2 into a tank in the EM FC6 wall. This LN2 is then heated and the evaporating gas is used to cool down chamber, specimen and knife. Temperature stability of specimen, knife and gas in the chamber are individually controlled by regulators close to knife and specimen. The desired temperature can be set, changed and stored by the operator via the control unit while the actual temperatures are displayed on the CU.

After finishing cryosectioning, an automatic bake-out function allows the cryochamber to heat up to +110 °C before returning to room temperature (RT).

2.3 Touch sensitive control unit

After connecting the EM FC6 to the touch sensitive control unit of the Ultracut UC6, the touch screen appears as shown in fig. 2.2.

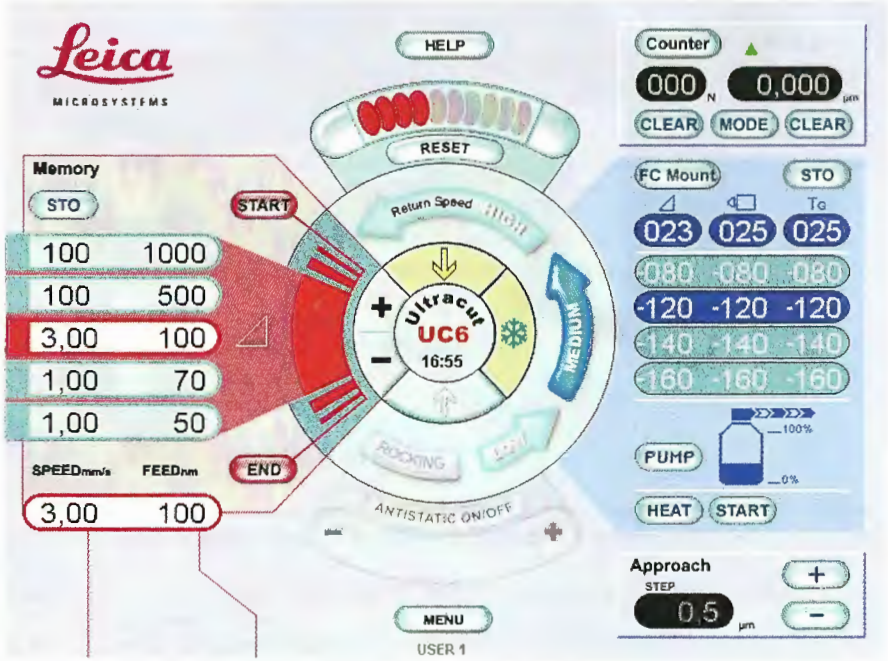


Fig. 2.2: Touch screen after connecting the EM FC6.

2.4 Key pad control unit

After connecting the EM FC6 to the the key pad control unit via the FC6 control device of the Ultracut UC6, the key pad appears as shown in fig. 2.3.

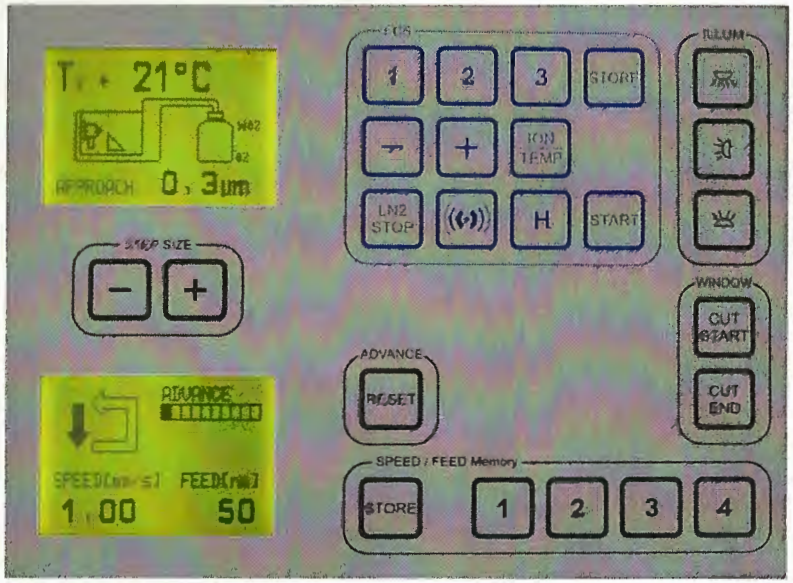


Fig. 2.3: Key pad control unit after connecting the EM FC6.

3 Installation

3.1 Packing list

LEICA EM FC6 Low Temperature Sectioning System contains:

EM FC6 cryochamber (Fig. 3.1)

Power supply, connecting cables

The **case for accessories** contains: **(Fig. 3.2)**
Rotating knife holder 2pcs.
Collet specimen clamp
Specimen carriers 50 pcs. with concentric rings
Specimen carriers 50 pcs. with slot
Preparation plate
Cryotool with M4 thread.
Allen key 3 mm,
Allen key 2mm
Special long forceps with insulation coating,
Lever, 2 pcs

Antistatic Electrode for FC6, incl. holder (Fig. 3.3)

Pressure-less automatic filling system (Fig. 3.4)

Insulated, threaded connecting hose (Fig. 3.4)

Dewar for 25l LN2 with special flange and mobile trolley and pump storage holder. (Fig. 3.4)

The **Complete Working Outfit** contains in addition:

Antistatic Transformer, connecting cables

Flat Specimen Holder.

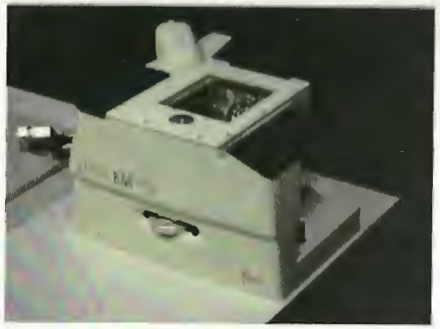


Fig. 3.1: EM FC6 cryochamber.

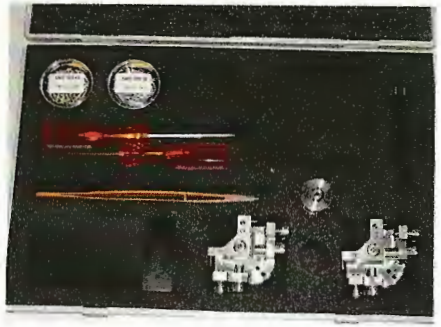


Fig. 3.2: Accessories supplied with the EM FC6.

Fig. 3.2: Antistatic Electrode in holder, both supplied with the EM FC6.

Fig. 3.4: Dewar, LN2 pump and LN2 hose.

Optionally available accessories:

Set of cryotools for frozen hydrated sectioning (Fig. 3.5)

Dewar Refilling System (Fig. 3.6).

Cryo Mesacut Mirror (Fig. 3.7)

Trimming Tool (Fig. 3.8).

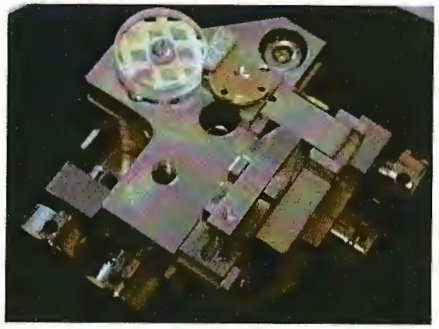


Fig. 3.5: Set of cryotools.

Fig. 3.6: Dewar Refilling System.

Fig. 3.7: Cryo Mesacut Mirror.

Fig. 3.8: Trimming Tool.

3.2 Preparation of the Ultracut UC6

Remove armrest, knife support and segment arc from the Ultracut UC6, as shown in fig. 3.9, 3.10 and 3.11. Switch on the instrument.

KNIFE STAGE
LOCK OPEN
TO MAX
HANDLE TOWARDS
OPERATOR

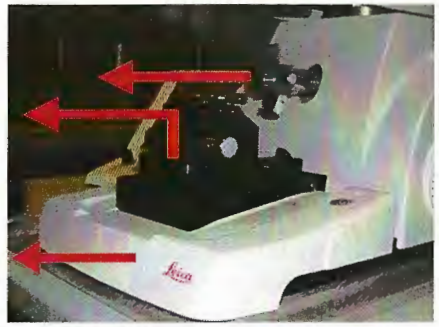


Fig. 3.9: Ultracut UC6 with armrest, knife support and segment arc.

Fig. 3.10: Ultracut UC6 after removal of armrest, knife support and segment arc.

Fig. 3.11: Armrest, knife support and segment arc after removal from Ultracut UC6.

Activate the FC Mount Function on the Ultracut UC6 by pushing the FC Mount button (fig. 3.12, red circle).

With the FC Mount Function of the Ultracut UC6, the knifestage will be centered both in N-S and E-W direction automatically. The backlight will switch off, the toplight will switch on. This will faciliate mounting the FC6.

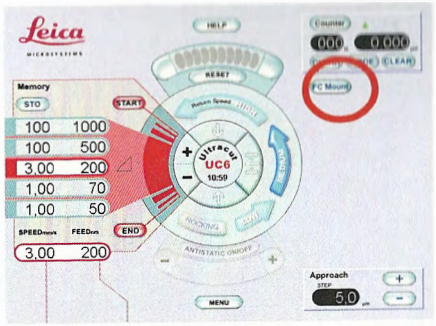


Fig. 3.12: UC6 touchscreen before mounting the FC6, showing FC Mount (red circle).

3.3 Unpacking

Open box and lift out FC6 cryochamber and LN2 hose as shown in fig. 3.11 to 3.13.

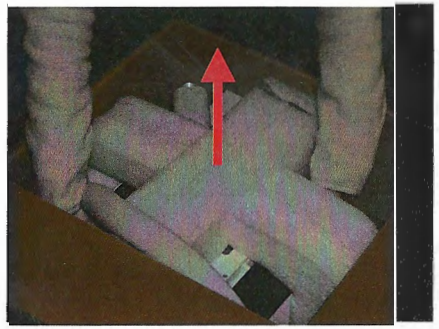


Fig. 3.13: Lifting out FC6 cryochamber with LN2 hose.

Fig. 3.14: Lifting out FC6 cryochamber with LN2 hose.

Fig. 3.15: FC6 cryochamber with LN2 hose in foam packaging.

Remove LN2 hose and take away foam packaging as shown in fig. 3.16 to 3.18.

Fig. 3.16: Removing foam packaging from FC6.

Fig. 3.17: Removing foam packaging from FC6.

Fig. 3.18: Removing foam packaging from FC6.

Before mounting the EM FC6 on the Ultracut UC6, remove the transport locking screws on rear side and bottom knife plate of the cryochamber.

Fig. 3.19: Transport locking screws on FC6 rear side and bottom.

Remove transport locking screws as shown in fig. 3.19 and 3.20, using the 3 mm Allen key.

Keep the transport locking screws in the case for accessories for potential future shipping of the cryochamber.

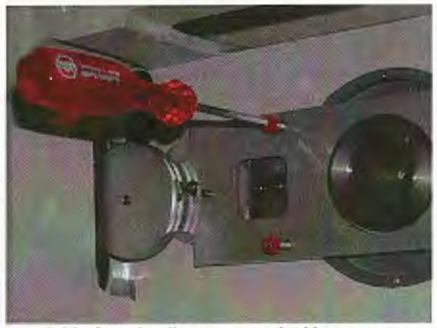


Fig. 3.20: Opening the transport locking screws on FC6 rear side and bottom with 3 mm Allen key.

Before mounting the EM FC6 on the Ultracut UC6, remove the transport locking screws on rear side and bottom knife plate of the cryochamber.

3.4 Mounting the EM FC6 onto the Ultracut UC6

Move the knifestage of the UC6 into the E-W center position (using the FC Mount button of the touch screen controller, or with the E-W control wheel).

Take FC6 chamber with both hands and slide it on the chassis of the Ultracut UC6 as shown in fig. 3.21 to 3.23.

KNIFE STAGE LOCK OPEN
HANDLE TOWARDS OPERATOR

Fig. 3.21: Mounting the FC6 cryochamber on the Ultracut UC6.

Turn the handwheel of the Ultracut UC6 to move the specimen arm to the lowest position.

Guide the cryo specimen arm (a in fig. 3.22) into the specimen arm of the Ultracut UC6.

Slide the FC6 cryochamber into the chassis until you reach the end stop (fig. 3.23).

Lock the cryo specimen arm of the FC6 cryochmaber to the specimen arm of the Ultracut UC6 by turning the locking screw either manually or using the 3 mm Allen key as shown in fig. 3.22.

Observe the gap between cryo specimen arm and cryochamber (2 red arrows in fig. 3.22). The distance of the gap should be 0.5 to 1 mm.

Fig. 3.22: The cryo specimen arm (a) has been guided onto the specimen arm of the UC6.

Fig. 3.23: Slide the FC6 cryochamber onto the UC6 until you reach the end stop.

Adjusting the distance between specimen arm and cryochamber should be done as follows:
Press the cryochamber to the right side and north against the UC6 base plate (red arrows). The distance will be adjusted by turning the adjusting screw on the front side of the FC6 cryochamber using the 2 mm Allen key. For accurate setting the 0.5mm spacer (transparent plastic foil) can be used.

The distance adjustment only has to be done only during the initial installation.

Fig. 3.24: Adjusting the distance between cryo specimen arm and cryochamber.

Lock the FC6 cryochamber to the chassis of the Ultracut UC6 by turning the lever „a" clockwise until its end stop position (a in fig. 3.25).

Make sure the FC6 is firmly connected by gently pulling in the south direction.

To increase the clamping remove lever „a" by unscrewing slot srew. Place lever an eighth counterclockwise on the clamping shaft and lock FC6 again. Repeat procedure until FC6 cannot be removed by gently pulling in south direction.
Fix lever by tightening the slot screw.

Lock the knife plate of the FC6 cryochamber to the knife stage of the Ultracut UC6 by turning the lever b clockwise (b in fig. 3.25).

Move the cryo specimen arm up and down by turning the handwheel to make sure that there is no contact to the cryo chamber.

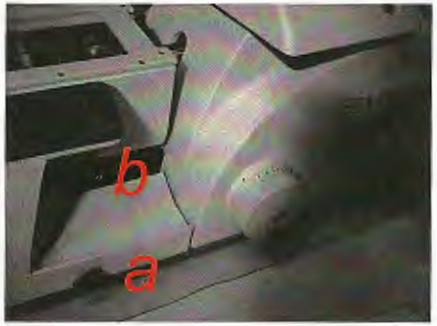
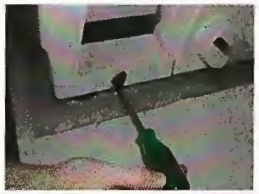


Fig. 3.25: Levers for locking the cryochamber to the chassis (a) and the knife plate to the knife stage (b) in open position.



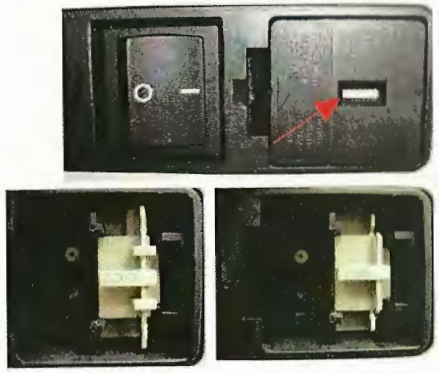
Removing lever „a" by unscrewing slot screw.

Clamping shaft

Adjust tightness of the connection by repositioning lever „a" an eighth counterclockwise.

3.5 Electrical Connections

Before connecting the power supply to the mains check the voltage selector on the rear panel of the power supply.
To change the voltage selector switch off the main switch, open cover with a screwdriver, remove white insert with a screwdriver, turn insert until desired voltage is indicated, change fuses, two pieces with the same value, mount insert and cover again.
Fuses: 230V with 3.15AT
115V with 6.3AT



Connect the power supply (fig.3.27) to the mains.

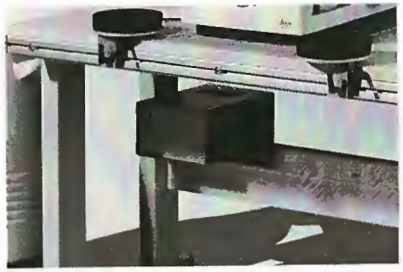


Fig. 3.27: EM FC6 power supply on out-of-the-way-shelf.

Connect hot plug of the power supply to the multiway connector on the rear side of the ultramicrotme (b in fig. 3.28).

Connect hot plugs of cryochamber (d in fig. 3.28) and pump (a in fig. 3.28) as shown in fig. 3.28.

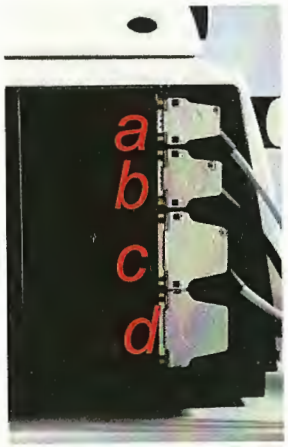


Fig. 3.28: Multiway connector with hot plugs for pump (a), power supply (b), control unit (c) and cryochamber (d).

3.6 Connecting the LN2 pump

Connect the LN2 hose with its threaded connection to the FC6 cryochamber (fig. 3.29) and to the pump (fig. 3.30).

The clean Dewar vessel has to be filled with LN2 according to the safety precautions.

Remove the yellow protective cup from the lower end of the pump. Slowly lower the pump into the Dewar. Hold the pump for a while until the strong boiling decreases and carefully lower the pump until it is completely immersed.

Fig. 3.29: Threaded connection of the LN2 hose to cryochamber.

Fig. 3.30: Threaded connection of the LN2 hose to pump.

The clean Dewar vessel has to be filled with LN2 according to the safety precautions.

3.7 Connecting the antistatic device

Connect the remote control cable and the antistatic electrode to the antistatic transformer as shown in fig 3.31 a and b.

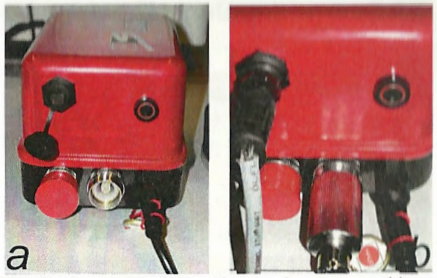


Fig. 3.31 a and b. Antistatic transformer, rear side without (a) and with cables connected (b).

Place the antistatic transformer on the out-of-the-way-shelf as shown in fig. 3.32.

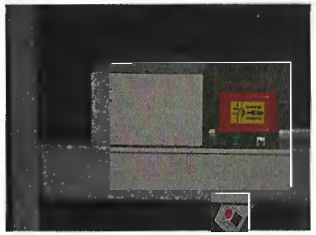


Fig. 3.32: Antistatic transfomer on out-of-the-way-shelf.

Connect the remote control cable to the control unit as shown in fig. 3.33.

Fig. 3.33: Touch screen control unit, rear side with remote control cable of antistatic device connected.

Place the antistatic electrode with its holder into its position on the cryochamber (fig. 3.34).

Fig. 3.34: Antistatic electrode with holder on cryochamber.

The touch screen of the control unit now appears as shown in fig. 3.35.

The intensity of the ionizer is controlled by pushing +/- buttons on the ANTISTATIC ON/OFF field.

3.8 Using the antistatic device

For trimming:

For perfect ribbons of sections a perfect trimmed sample is mandatory.

It is recommended to use the antistatic device for trimming, because it eliminates the sticking of the chips on the specimen and on the blade.
Set the intensity for trimming at the full voltage = all 10 indication marks are illuminated.

For sectioning:

Set the intensity to 5 indication marks. If the sections tend to stick on the knife edge, increase the intensity step by step until the sections begin to float in a nice ribbon over the knife edge. If the sections begin to lift up, decrease the intensity step by step.

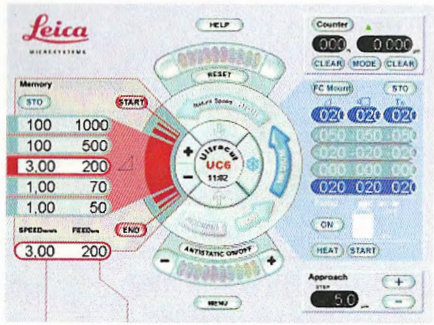


Fig. 3.35: Touch screen with EM FC6 and antistatic device connected, pump inserted in full Dewar .

The electrode will lose its power if its metal tip becomes covered with ice or debris. If this occurs, just clean the ionizer tip with a fine brush with the antistatic device switched off.

Attention: The electrode tip carries high voltage and must not be touched or allowed to touch any parts of the cryoultramicrotome while operation.
Switch off the antistatic device prior to any manipulation in the chamber.

Never heat the chamber with the electrode inside.

4 Operation

4.1 Inserting the cryo specimen holder

Insert the cryo specimen holder into the specimen arm as shown in fig. 4.1.

Lock the cryo specimen holder with the torque limited screw (a in fig. 4.1) using the 3 mm Allen key.

Fig. 4.1: Inserting the cryo specimen holder before locking it with the torque limited screw (a).

If the cryo holder is locked in the 0° position initially, subsequent reading of the next position (turned 90° for trimming) will be easy.

4.2 Inserting knives into the knife holder.

Set the desired clearance angle (typically 6°) as shown in fig. 4.2.

Insert the desired knives (e.g. a Diatome diamond trimming tool and a Diatome 35° cryo immuno knife for Tokuyasu technique specimens, as shown in fig. 4.3).

Fig. 4.2: Setting the clearance angle to 6°.

Lock the knife by turning the locking screw clockwise as shown in fig. 4.3

Fig. 4.3: Locking the knives, here showing a Diatome diamond trimming tool and a Diatome 35° cryo immuno knife.

Clearance angle setting and locking/unlocking of knives can also be done with the knifeholder in the cooled cryochamber.

4.3 Inserting the knife holder

The knife plate in the cryochamber carries a pin (red arrow in fig. 4.4) that corresponds to the hole in the knifeholder (red arrow in fig. 4.5).

Fig. 4.4: Pin in the knife plate (arrow).

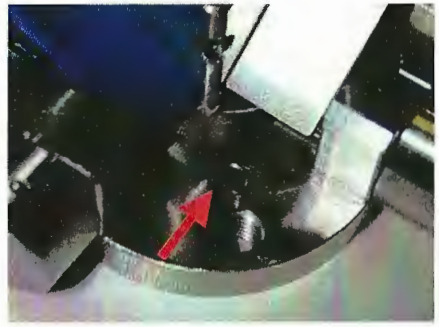


Fig. 4.5: Hole in the knife holder (arrow).

Insert the rotating knife holder containing knives into the chamber as shown in figs. 4.6 and 4.7.

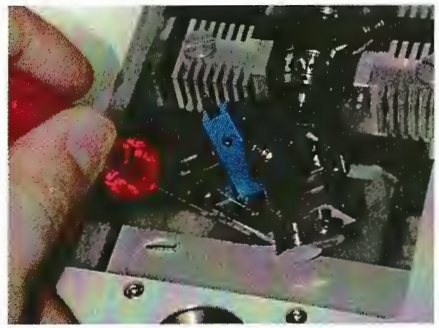


Fig. 4.6: Inserting the knife holder into the cryo chamber.

The rotating knife holder is now in the " loading position" (fig. 4.8). It can only be inserted in this position.

Fig. 4.7: The knife holder is inserted into the cryochamber.

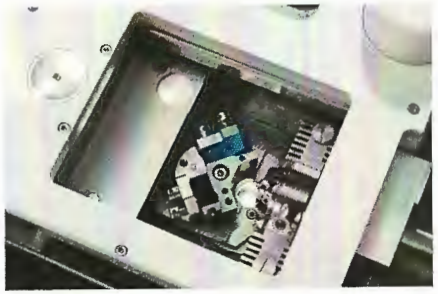


Fig. 4.8: Knife holder in loading position.

In the loading position the knife holder cannot be locked but is held in position by a magnet.

4.4 Inserting the specimen

Once the cryochamber has cooled down to the desired temperature (e.g. -80 °C for trimming of Tokuyasu technique specimens), insert the specimen pin with the specimen (fig. 4.9).

Fig. 4.9: Inserting the specimen pin with the insulated forceps.

Make sure the knife holder is in loading position to avoid touching the delicate knife edge.

Lock the specimen pin in the cryo specimen holder as shown in fig. 4.10: Turn the small black lever in front clockwise while holding the cryo specimen holder in position with the small black lever in the back.

Fig. 4.10: Locking the specimen pin in the cryo specimen holder. Arrows indicate the direction of the force applied for locking.

Rotate the knife holder into „trimming position" as shown in fig. 4.11.

Lock the locking screw of the knife holder with the 3 mm Allen key.

Start trimming and sectioning.

To change from sectioning position 1 (e.g. the trimming knife) to sectioning position 2 (e.g. the sectioning knife), unlock the screw, rotate knife holder and lock again.

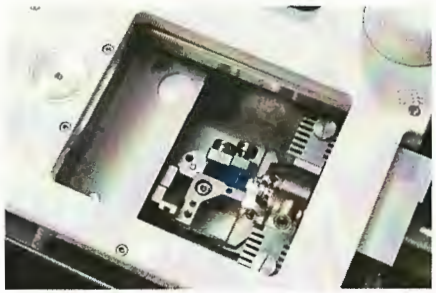


Fig. 4.11: Knife holder in sectioning position 1 (here the trimming position).

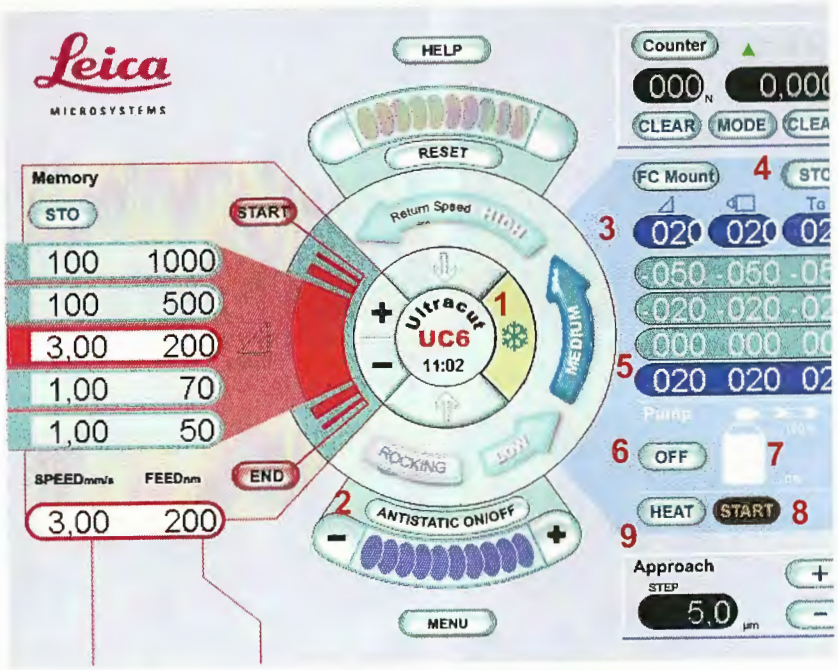


Fig. 4.12: Overview Touch screen. For description corrsponding to numbers see next page.

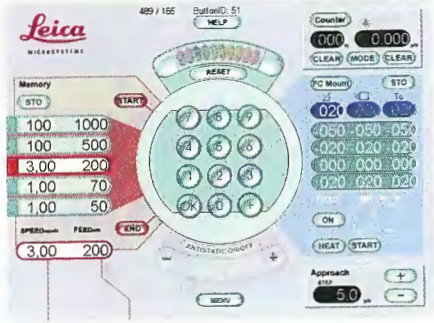


Fig. 4.13: Number pad for setting the desired temperature. After keying in the values are confirmed with „OK".

4.5 Functions of the touch screen control unit

1: LED chamber illumination. Touch to switch on or off. When activated, brightness can be controlled with + - buttons.

2: Integrated ioniser control.
Touch to switch on or off. When activated, intensity can be controlled with + - buttons.

3: Three individual readings for the actual temperature of specimen, knife and gas. Only the actual and not the set temperatures are shown.
Touch one, two or all three buttons to activate setting mode. When activated, the temperatures can be set.
A number pad appears on the screen. The desired temperature can be keyed in and must be confirmed with „OK".
If only one button was activated (e.g. knife), only the temperature of this button (in this case the knife) will be set. If all three buttons were activated, all three will be set together.

4: Store function. Touch to activate storing mode, followed by one of the four memory fields (5). The set temperatures of specimen, knife and gas are now stored in the desired memory field.

5: Memory fields. Touch to activate the prestored values.

6: Pump. Touch to switch on or off pump *(e.g. for refilling the Dewar it may be necessary to warm up the LN2 hose until it is flexible enough to me moved).*

7: LN2 level indicator. Indication of the filling status of the Dewar. An acoustic warning signal will sound if the Dewar is almost empty. Approx. 1 h working time is left.
The acoustic signal can be switched off by touching the Dewar symbol.

8: Start: Touch to activate cooling of the chamber to the set temperature. Touching a 2nd time will activate the „RC" mode (rapid cool mode). i.e. cooling down to lower temperatures is achieved faster.

9: Heat: Touch to activate automatic bake out of the chamber. The automatic safe guard will ask you to touch the button for more then 1 second to prevent activation by accident.

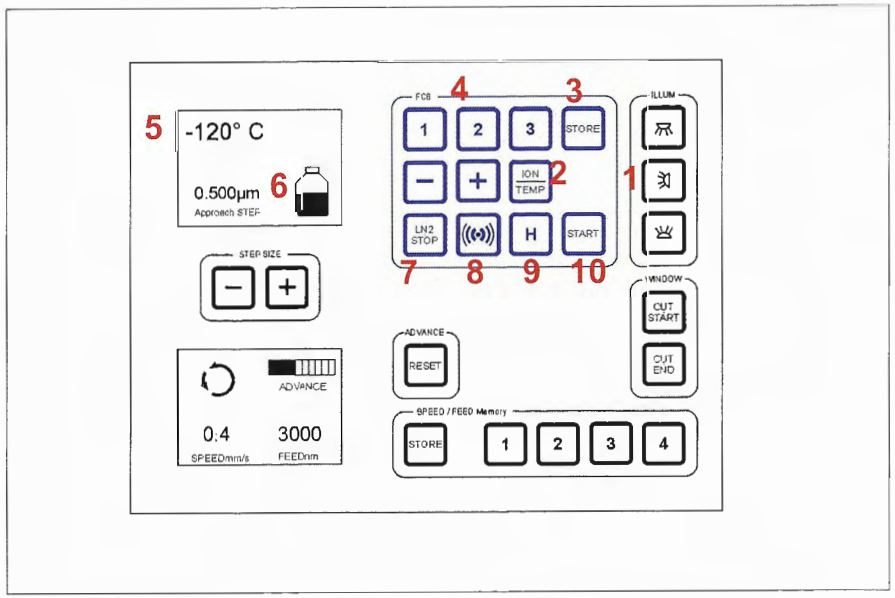


Fig. 4.14: Key pad control unit.

4.6 Functions of the key-pad control unit.

1: LED chamber illumination. Press to switch on or off.

2: Combined ioniser control / temperature control. Press to switch from temperature setting to ioniser setting and back.
When ioniser is activated, the ioniser intensity can be controlled with + - buttons next to the ioniser control / temperature button.
When temperature is activated, the gas temperature in the chamber can be set with +- buttons next to the ioniser control / temperature button.

3: Store function. Press to activate storing mode, followed by one of the three memory buttons (4). The set gas temperature is now stored in the desired memory button.

4: Three memory buttons. Press to activate the prestored value.

5: Reading for the actual temperature of the LN2 gas in the chamber.

6: LN2 level indicator. Indication of the filling status of the Dewar. An acoustic warning signal will sound if the Dewar is almost empty. Approx. 1 h working time is left.
The acoustic signal can be switched off by pressing the „acoustic alarm" button (8).

7: LN2 stop. Touch to switch on or off pump (e.g. for refilling the Dewar it may be necessary to warm up the LN2 hose until it is flexible enough to me moved).

8: Acoustic alarm button. Press to switch off acoustic alarm when Dewar is empty (6).

9: H-button: Heat. Press longer than two seconds to activate automatic bake out of the chamber.

10: Start: Press to activate cooling of the chamber to the set gas temperature. Touching a 2nd time will activate the „RC" mode. i.e. cooling down to lower temperatures is achieved faster.

4.7 Refilling the Dewar

The 25 l Dewar holds enough LN2 for a 1 day operation. It is recommended to keep the pump in the Dewar until the LN2 has been consumed. The LN2 consumption of the pump when not in use in less than 1 l per day.

Refilling can be done in 2 different ways:

1. Lift the pump slightly and refill the Dewar, using the Dewar Refilling System.

2. Take out the pump from the Dewar. The cold pump should always be kept in an upright position to avoid entrance of water and humid air into the valves. Use the yellow protective cap immediatly to close the lower end of the pump.
Before inserting the pump again, dry pump and take off yellow protective cap.

For ease of operation, one can use a second Dewar and place the pump immediately into the new, full Dewar.

Taking out the pump from the Dewar if the pump is not in use for a longer time:
The cold pump should always be kept in an upright position to avoid entrance of water and humid air into the valves. Use the yellow protective cap and the stroring bracket that is provided with the pump.

Before removing the pump from the Dewar, switch off pump and wait for a few minutes. The LN2 hose must be flexible enough to be moved.

4.8 Finishing work with the EM FC6

Close down and bake out

Pressing the „H" button (key pad CU) or touching the Heat button (touch sensitive CU) will activate the automatic bake out of the chamber.
The FC6 cryochamber will now heat up to +110 °C. The heating cycle will be switched off automatically and the instruments will go back to RT.
During the heating time (approximately 90 minutes), both the Ultracut UC6 and the FC6 must be connected to the mains.

The heating cycle can be stopped and started again any time by pressing the „H" button (key pad CU) or touching the Heat button (touch sensitive CU).

Before heating the chamber, to avoid any damage to components and knives:

- **take out all specimens.**
- **take out antistatic electrode.**
- **take out diamond knives.**
- **unlock torque limited screw holding the cryo specimen holder.** This will prevent mechanical damage of specimen holder and locking screw due to heat expansion.
- **unlock knife holder.** This will prevent mechanical damage of the locking screw due to heat expansion.
- **move stereomicroscope with stereocarrier to the side.** This will prevent steam / condensing water droplets on optical components.

Do not touch the parts inside the chamber during bake out. Risk of burns!

4.9 Maintenance of pump valves

Humidity in the valves can cause freezing and sticking of the valves during operation. To prevent this both valves in the block can be easily removed and cleaned with ethanol.

Remove both steel valves with a screw driver. The 2nd steel valve is located underneath the 1st valve.
Before inserting the valves again, check that the ball inside the valves is free.

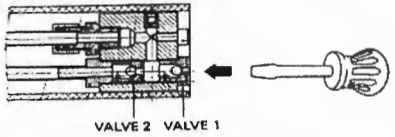


Fig. 4.15: Disassembly of pump valves.

5 Leica EM FC6 Set of Cryotools

5.1 Overview

The Leica EM FC6 Set of cryotools is used for handling frozen hydrated sections. The set consists of 4 parts: the base (with a flip-over bridge for preloading / releasing of grids), the spring loaded grid holder, the section press and the transfer container.

5.2 Instrument Function

The base of the cryotools is mounted on the rotating knife holder with a locking screw. A precooled grid is clamped in the spring loaded grid holder (2). The grid holder is shifted forward until the grid is close to the knife edge. Cryosectioning is carried out. Once the ribbon of frozen hydrated sections is on the grid, the holder with the grid is pulled back in south direction. The grid is then placed onto the flip -over bridge (3) and sections are pressed with the section press (4). After flattening the sections on the grid, the grid is transferred to a transfer container (5).

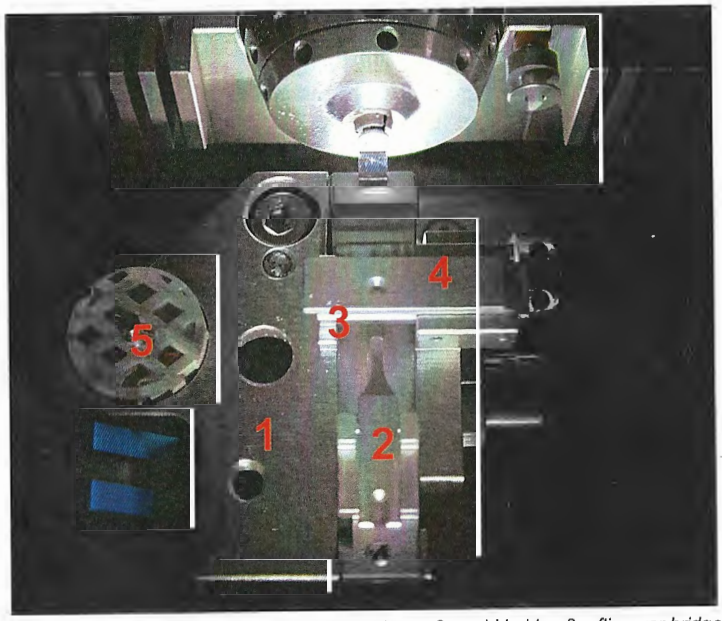


Fig. 5.1: Leica EM FC6 Set of cryotools. 1 = base, 2 = grid holder, 3 = flip-over bridge, 4 = section press, 5 = transfer container.

Insert cryo knife into knife holder as shown in fig. 5.2.

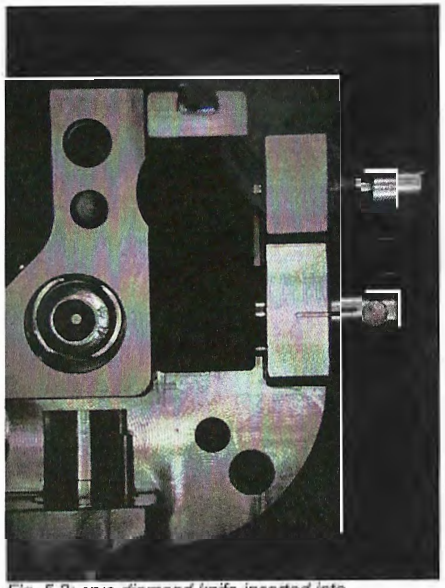


Fig. 5.2: cryo diamond knife inserted into knifeholder.

Mount base of cryotools on rotating knifeholder and lock (fig. 5.3).

Fig. 5.3: Locking the cryotools.

Insert grid holder and transfer container into base of cryotools and precool (fig. 5.4)

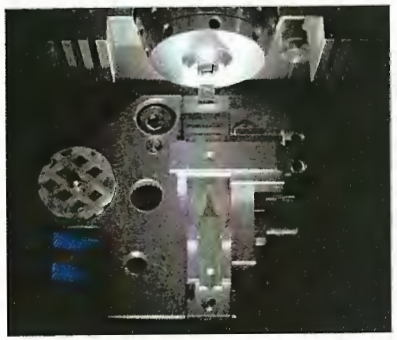


Fig. 5.4: gridholder and transfer container inserted into base of cryotools.

Flip section press over to the right (fig. 5.5)

Fig. 5.5: open section press.

Place grid on flip-over bridge (fig. 5.6)

Fig. 5.6: grid on flip-over bridge.

Open grid holder by pushing on the small indentation at the rear end with a small black lever or a pair of forceps. Move girdholder towards the grid and clamp it by releasing the clamping jaw (fig. 5.7).

Fig. 5.7: Clamping grid in grid holder.

Pull back grid holder towards you and flip bridge to the right using a pair of forceps (fig. 5.8).

Fig. 5.8: Flipping the bridge out of the way.

Push grid holder forwards until the grid has reached the desired distance from the knife edge (fig. 5.9).

Take extra care not to touch the delicate diamond knife edge.

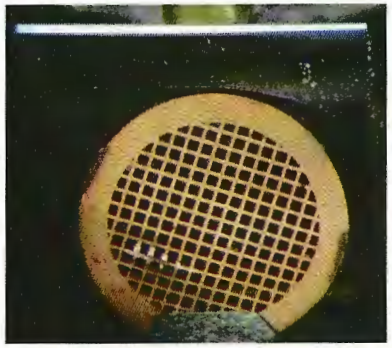


Fig. 5.9: Moving grid towards the knife edge.

Start cryo sectioning and collect the ribbon on the grid (fig. 5.10).

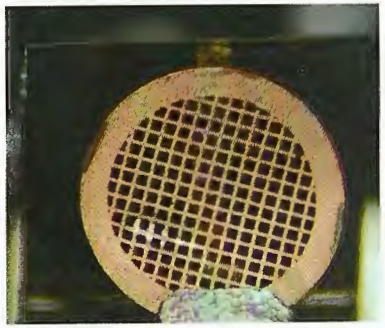


Fig. 5.10: Grid in section collection position.

Pull back grid holder.

Flip over bridge to the left.

Push forward grid holder until the grid is placed over the bridge (fig. 5.11).

Fig. 5.11: Grid ready to be pressed.

Close section press by using a pair of forceps (fig. 5.12).

Fig. 5.12: Close section press.

Flatten sections under the press by pushing on the small indentation in the center with a small black lever or a pair of forceps (fig. 5.13).

Apply pressure perpendicular to the surface to avoid sideways movement of the press.

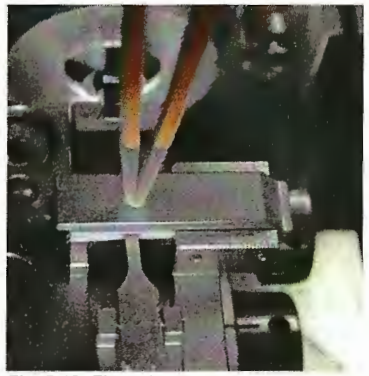


Fig. 5.13: Flattening sections.

Flip over section press to the right. (fig. 5.14)

Fig. 5.14: Flip over section press.

Release grid from the holder by pushing on the small indentation at the rear end with a small black lever or a pair of forceps and retract grid holder (fig. 5.15)

Fig. 5.15: Release grid from the holder.

Transfer flattened grid into the transfer container (fig. 5.16)

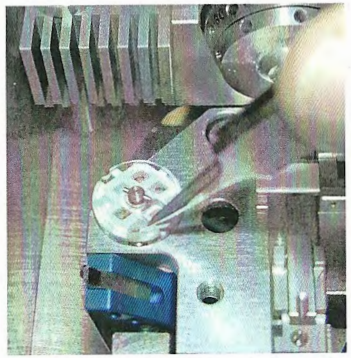


Fig. 5.16: Transfer grid into the transfer container.

The transfer container can be removed using a pair of forceps (fig. 5.17)

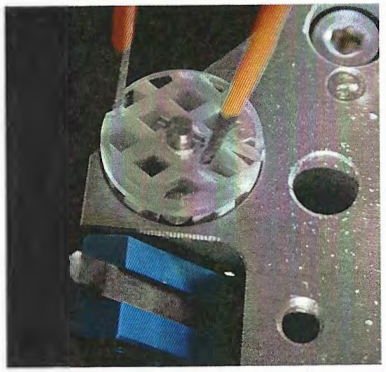


Fig. 5.17: Removing transfer container.

The ceramic surfaces of the section press should be cleaned with 100% ethyl alcohol before use.

EC Declaration of Conformity
EG Konformitäts-Erklärung
Déclaration CE de Conformité

We / Wir / Nous

Leica Mikrosysteme GmbH
Hernalser Hauptstrasse 219
A-1170 Wien Austria

declare in exclusive responsibility that the product
erklären in alleiniger Verantwortung, daß das Produkt
déclarons sous notre seule responsabilité que le produit

Model	**Leica EM FC6**
Modell	**Leica EM FC6**
modèle	**Leica EM FC6**
Type / Typenbezeichnung / type	**706001/656001/706030**

to which this declaration relates is in conformity with the following standards:
auf das sich diese Erklärung bezieht, mit den folgenden Normen übereinstimmt:
auquel se réfère cette déclaration est conforme aux normes:

EN 61010-1
EN 50081-1
EN 50082-1
EN 61000-4

following the provisions of directive
gemäß den Bestimmungen der Richtlinie
conformément aux dispositions de directive

89 / 336 / EEC	(Electromagnetic compatibility) (Elektromagnetische Verträglichkeit)
73 / 23 / EEC	(Low Voltage Equipment) (Niederspannungsrichtlinie)
89 /392 EEC	(Maschinery) (Maschinen)

Wien, 13. Dezember 2003

Dr. Reinhard Lihl
Entwicklungsleiter
R&D Manager
Chef du service développement