Leica EM KMR2
Operating Manual

Order Number 177032
Leica EM KMR2 - GA-E-06/03

Leica
MICROSYSTEMS

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1. Safety instructions

Prior to the operation of the Leica EM KMR2 read the instruction manual carefully.
Always use safety goggles during operation to avoid eye injury.
Clean the instrument regularly with a brush to remove glass splinters.
Store broken knives safely in the knife boxes provided.
Do not leave knives laying around or on the Leica EM KMR 2.
Used knives should be put into a marked “sharps” box for safe disposal.

Symbols in this manual and their meaning

Warning:
take extra care.

Notes:
Important information for the user.

Fig.1

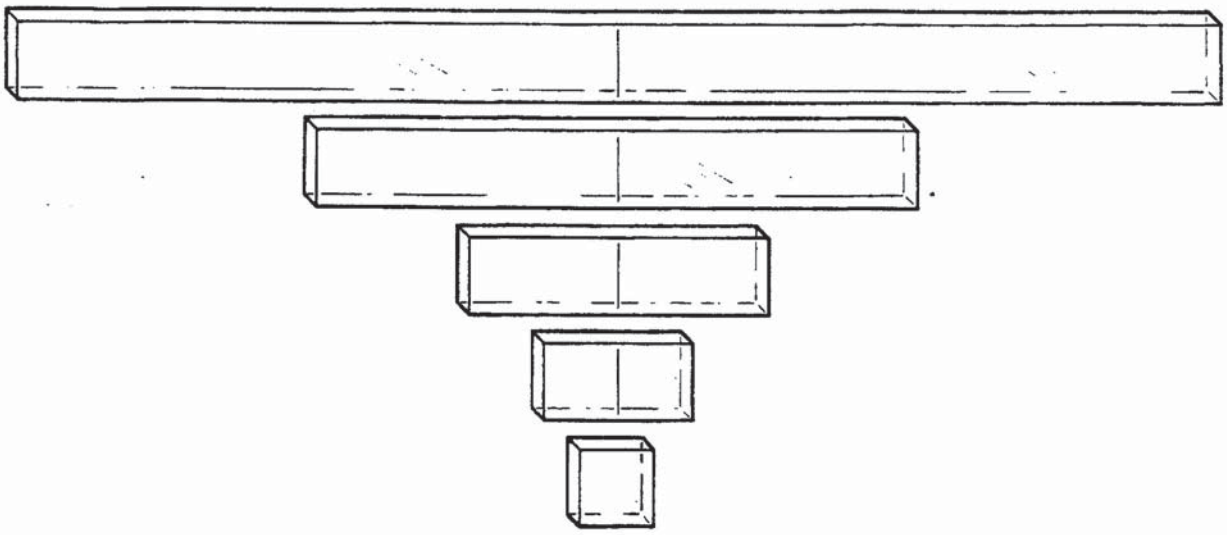


Fig.2

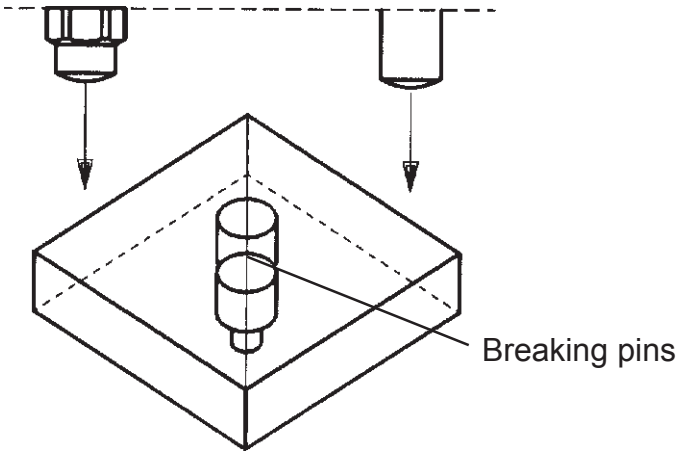


Fig.3

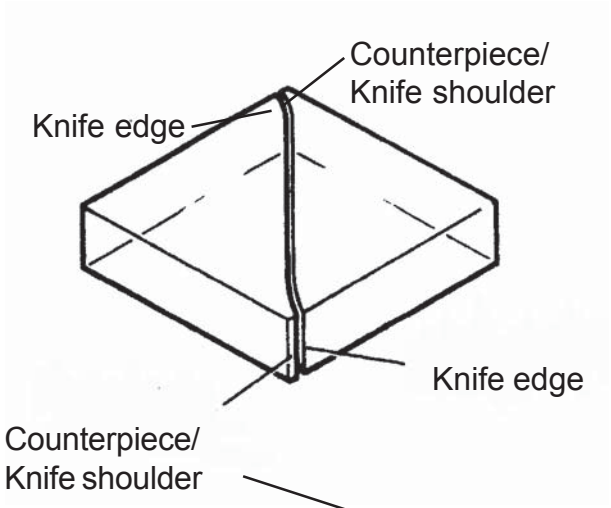
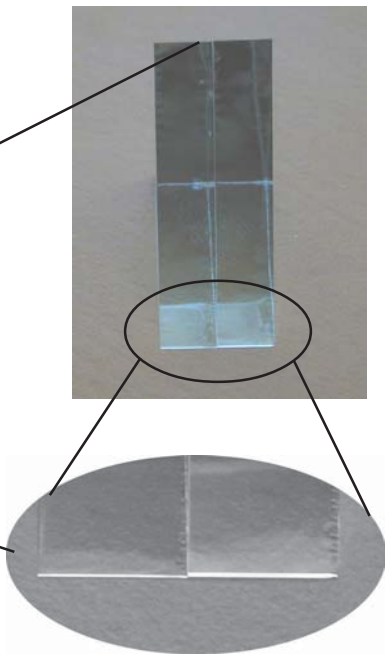


Fig.3a



2. Principles of Knifemaking

2.1 The Balanced Break method (Fig.1)

In the balanced break method, an original Leica glass strip 400mm in length (6.4, 8 or 10mm thick), is scored and broken into two equal halves, each 200mm long. With an equal mass of glass on each side of the score the break is balanced and the freshly fractured surfaces are plane.
By continuing to divide each piece produced into two equal halves, up to 16 squares can be made.
All squares produced have straight sides and precise right angled corners unlike squares produced from sequential breaking of a glass strip which have curved surfaces.

2.2 Scoring and breaking principles

Producing good glass knives routinely depends on a supply of reproducible squares, an accurately positioned score and controlled pressure precisely applied to make the break. The LEICA EM KMR2 gives the user a choice of two different scores, each one factory set to produce the optimum break.

The long score is used to break squares out of glass strips as well as to score squares to break knives. As a general rule, the knife edge is straighter and the counterpiece (knife shoulder) is small when the fracture occurs close to the corner.

The short score was suggested for cryo knives as the free break is longer resulting in the sharpest, longest useable knife edge (Griffiths et al 1983: Tokuyasu 1986).

Each score is preset and equidistant from the corners of the square.
During the break the glass sits on two breaking pins and is also held from above by two more pins **(Fig.2)**.
Both lower pins are fixed and one of the upper pins is fixed too, the other can be moved up and down by means of the breaking lever and actually initiates the breaking of the glass. The break follows the score line as far as it goes and then a free break occurs. The direction of this free break is determined by the mass of the glass on either side of the break and the breaking forces.
The free break curves to the edge of the square resulting in one knife and one flat-edge counterpiece (knife shoulder) opposite the knife edge **Fig.3**.
When the score runs centrally through a square a very small counterpice (< 0.2mm) is obtained and the knife angle is very close to 45° **(Fig.3a)**.

This is the optimal result for cryo knives. For resin sectioning we set the knife shoulder a little larger (approx. 1mm), to produce a larger knife angle which is more stable for resin sectioning.

Fig.4

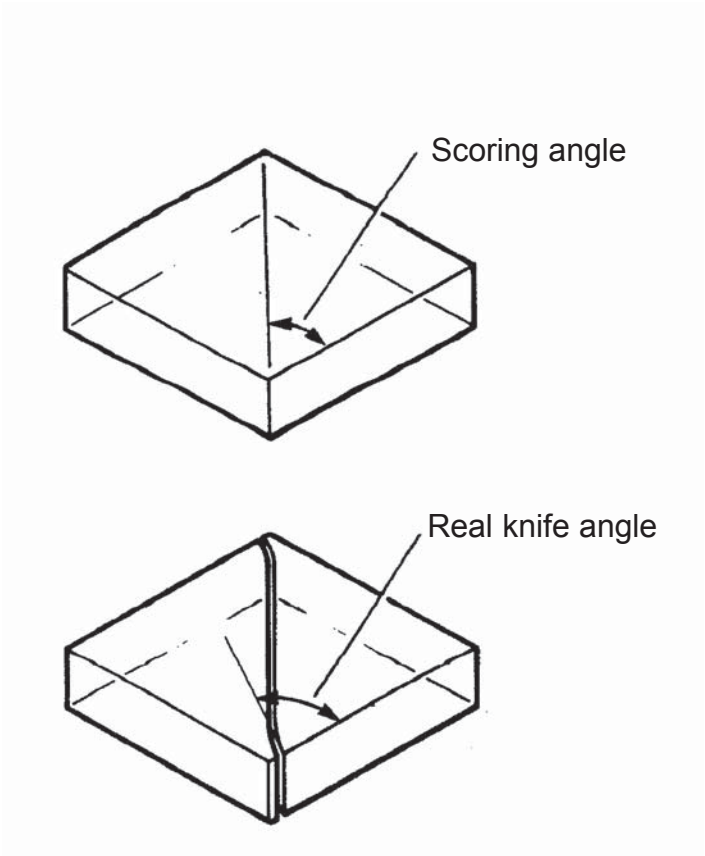
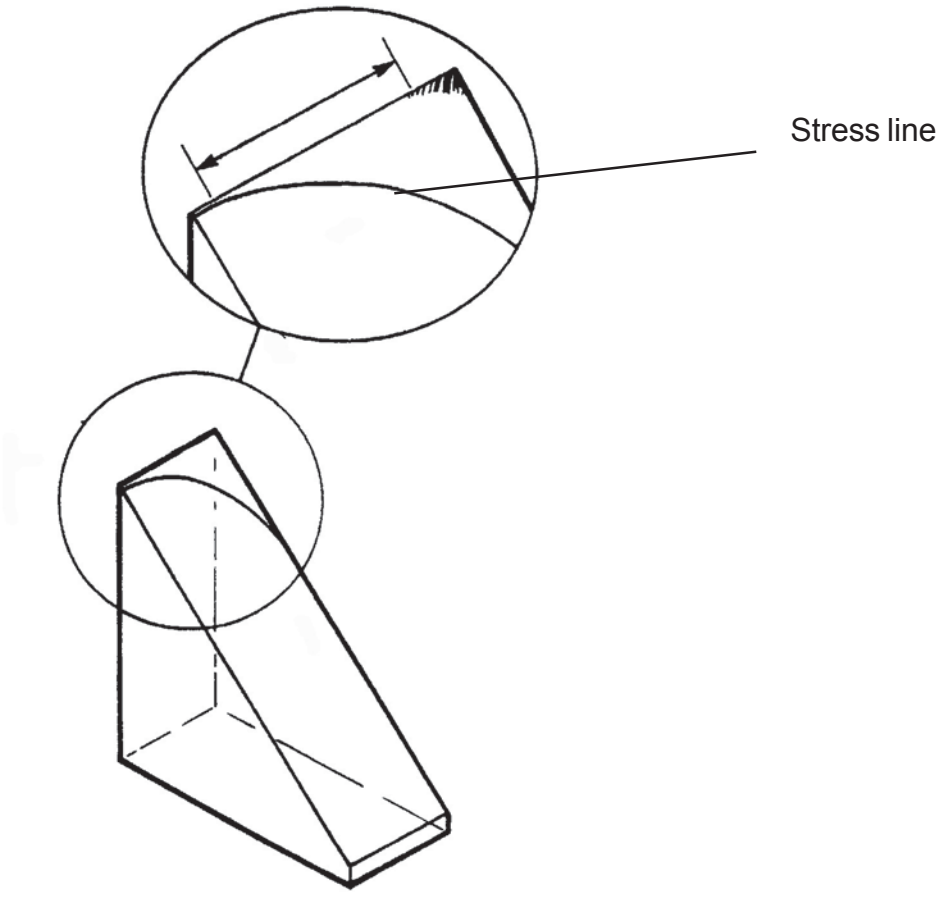


Fig.5



2.3 The real knife angle

When scoring the square all scores stop some distance from the corner.
When pressure is applied under the score, the fracture is initiated and is seen first as a deepening of the score. The fracture extends towards the corners of the square following the line of the score. Where the score ends and the break is ”free” the fracture deviates from the line of the score to curve away from the corner, towards one of the edges of the square.This results in the real included angle of the knife being somewhat greater than the angle of scoring.
The real angle of the knife increases as the score is moved further from the diagonal. This is when the knife shoulder becomes larger.
For example, when preparing knives from a square, the real angle of the knife is close to 45° when the knife shoulder is smaller then 0.2mm.
Increasing the height of the knife shoulder (> 0.2mm) results in an even larger knife angle which can be over 55° **(Fig. 4).**

2.4 Length of useful edge

When a glass knife edge is examined under darkfield illumination using a stereo microscope (or on LEICA Ultramicrotomes using the back light, **chapter 5.4**), it can be seen that the central part is most useful for ultrathin sectioning. The right side of the edge has visible marks (saw teeth) which reduce the quality of the knife, and the left corner is also unsuitable for sectioning because of the stress line **(Fig.5)**.
The useful knife edge starts where the stress line does not touch the edge until the part where the stress marks (saw teeth) can be seen.

Note:
When less force has been used to break the knife the stress line falls away rapidly from the knife edge and fewer saw teeth can be seen.

Note:
The useful knife edge is 30% longer on knives produced from 8mm thick glass compared to 6.4mm thick glass!

Fig.6

Fig.7

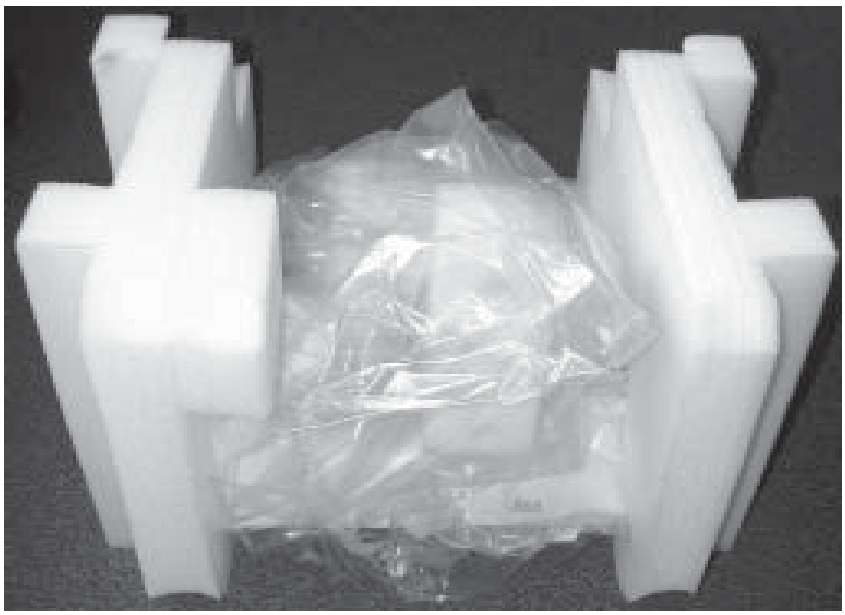


Fig.8

Side view Fig.8

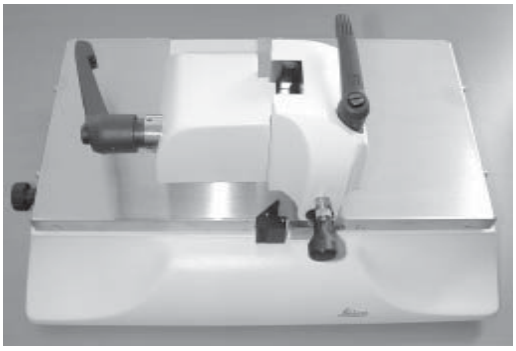
3. Unpacking and Installation

3.1 Unpacking

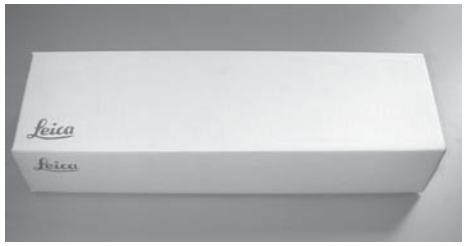
The Leica EM KMR2, complete with all accessories is packed in a single carton as shown in **Fig.6**.
Before unpacking ensure that the box is the correct way up. Having opened the carton, take the bag with the accessories and the dust cover out of the carton.
Carefully remove the Knifemaker together with the foam plastic **(Fig.7).**
Remove the foam transport lock of the scoring head **(Fig.8).**
Check all items against the packing list and examine each item for transport damage.

3.2 Packing list

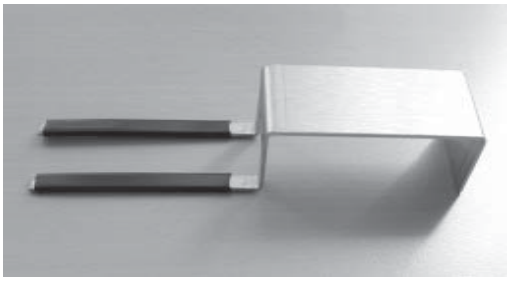
LEICA EM KMR2,
dust cover and Allen key (no picture)



Box glass strips (6.4 x 400 x 25mm)
(30 strips)

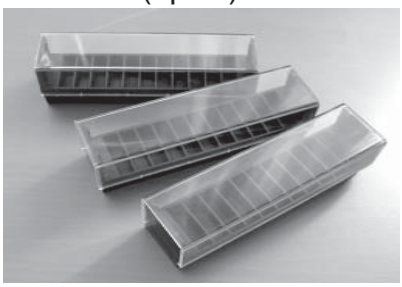


Knife handling fork (1pc.)

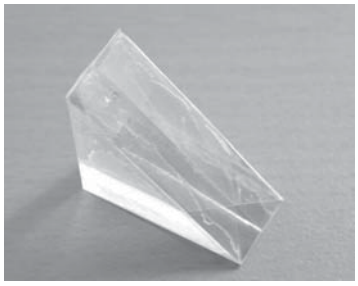


Spare scoring wheel cartridge (1pc.)

Knife box (3pcs.)



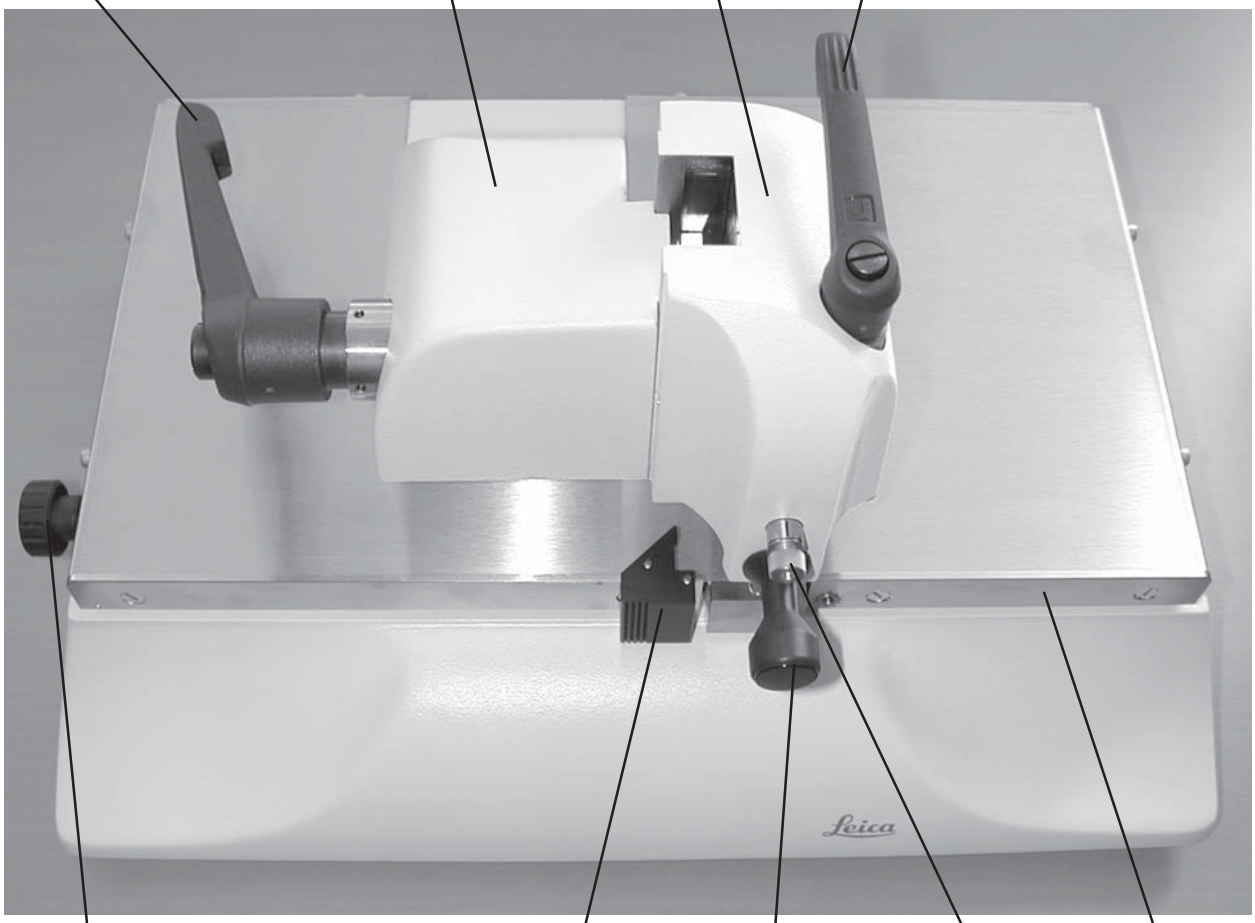
Pair of knives produced with this
LEICA KMR2

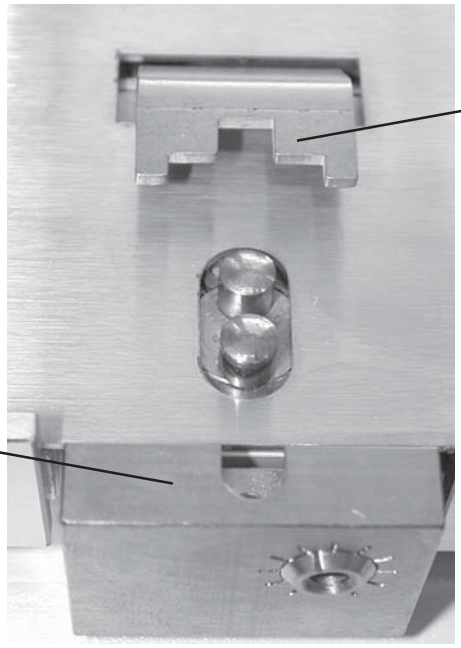


3.3 Installation

Place the Leica EM KMR2 on a sturdy laboratory bench or a table where it will not be affected by movement from other laboratory equipment, such as a centrifuge.
The Leica EM KMR2 is completely assembled in the factory, so the instrument is ready to use out of the box.

Fig.9





4. Description of the LEICA EM KMR2

The scoring and breaking mechanism for knife making are assembled on a heavy, vibration-absorbing base.

The parts are shown in Fig.9:

1. Clamping lever: is used to lower and raise the scoring head and also to clamp the scoring head to the clamping head (No.2). For user-preferred positioning the lever can be adjusted by pulling it horizontally to the left, rotating it and pushing it back.
The best position is horizontal when clamped with glass in place.This way the same scoring pressure can always be applied during the knife breaking process.

2. Clamping head: carries the scoring head and the clamping lever.

3. Scoring head: carries all necessary elements for scoring and breaking the glass.
The scoring head is lowered into position by means of the clamping lever (No.1).

4. Breaking lever: is used to break the glass after scoring. By turning the lever slowly clockwise a pin moves against the glass to break it. This lever can be positioned by the user by pulling it vertically upwards, rotating it and pushing it back.

5. Ruler: acts generally as a stop for the glass strip. The glass strip is pressed against the ruler before it is clamped. The left hand ruler acts also as a track for the moveable stop with its four click stop positions.

6. Scoring mark selector: can be set by rotating to two different scoring lengths.
The long score (22 mm) is for scoring the glass strips to break squares for making routine knives for resin sectioning.
The short score (12 mm) is used to score squares for making high quality knives for cryo sectioning.
When the long score is used for making knives the quality of the knife is high and also more reproduceable.

7. Scoring shaft: is pulled out with reasonable speed to the endstop to score the glass before breaking.

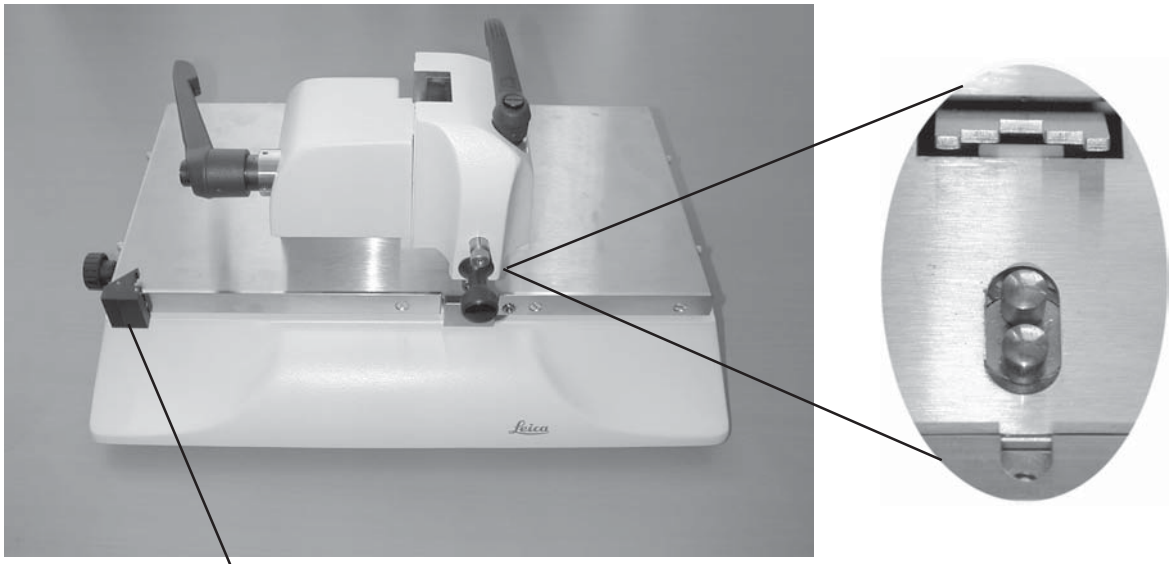
8. Moveable stop with its four cklick positions is mounted on the left hand ruler and is used to break the 400 mm long glass strips according the balanced break method.

9. Rotatable knob: is used to move the rear stop (No.11) forwards to locate the glass strips and squares against the ruler.

10. Front stop: is to hold and position squares during scoring and breaking of knives.

11. Rear stop: is used to hold and position the glass strips and the squares against the front stop (No.10) when breaking knives from the square.

Fig.10



Moveable stop at
position 1.

Fig.11

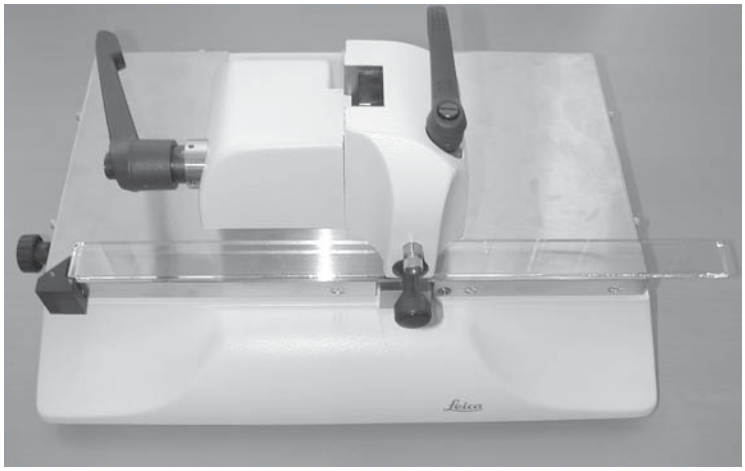
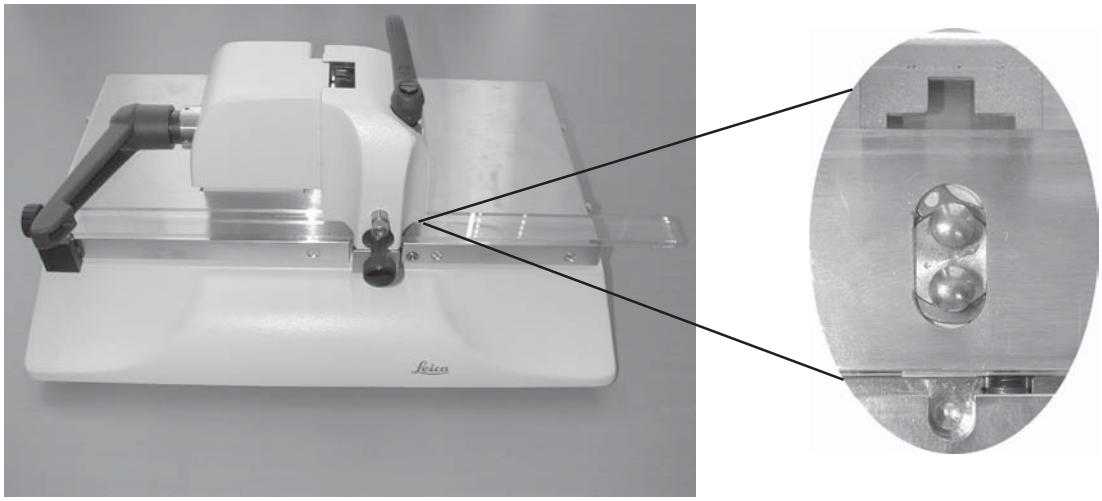


Fig.11a



5. Knifemaking

5.1 Hints

For the best results from your Leica EM KMR 2 it is recommended that glass strips supplied by Leica are always used. Leica glass strips for ultramicrotomy are produced from specially selected glass, the thickness and quality of which is precisely controlled. Only strict tolerances ensured by careful quality control allows breaking of two high quality knives from one square.
All strips supplied by Leica are 400mm long and 25mm wide.
They are available in thicknesses from 6.4mm, 8mm and 10mm.
Leica glass strips are washed individually wrapped in tissue paper, and packed in a strong carton. When handling the strips, avoid contacting the narrow edges as these may finally form the knife edge.
The strips and squares can be held by the wide upper and lower surfaces.
Never touch the corners of a partly broken strip or square as these will form the knife edge.
Cleaning the glass strips is in general not necessary because the strips are pre-cleaned wrapped in tissue paper and kept away from dust in the carton.
However, if there is a need to clean the glass:
wash each strip seperately in cool tap water which has a low concentration of a mild laboratory detergent added to it. Use a soft brush or cloth and take care not to touch the sharp edges of the strip.
Completely rinse off all detergent, then rinse thoroughly with distilled water. Dry gently with a clean, soft, lintfree cloth or place it in a drying oven

Warning:
glass knives and glass strips have sharp edges - handle with great care!

5.2 Making squares

Set the moveable stop by pressing it on the left hand side to release it and move it to **position 1 (Fig.10).** Move the rear stop **(Fig.9, No.11)** by means of the rotatable knob **(Fig.9, Nr.9)** backwards as shown in **detail Fig.10.**
Place the 400mm long glass strip onto the KMR 2 and push it against the moveable stop, which is in **position 1 (Fig.11).** At the same time press the glass strip against the rulers with the rear stop by means of the rotatable knob **(detail Fig.11a)**. Now lower the scoring head down onto the glass strip by rotating the clamping lever and tightening it. **(Fig. 11a)**. Move rear stop back again.

Note: for reproducible results always lock the clamping lever to the same position

Note: lowering the clamping head orientates the glass strip horizontal (parallel) to the surface of the instrument.

To be sure lower the clamping head 2 or 3 times onto the glass before clamping. Do not hold the glass strip anytime during this procedure, the glass must be free for self-orientation!

I

Fig.12

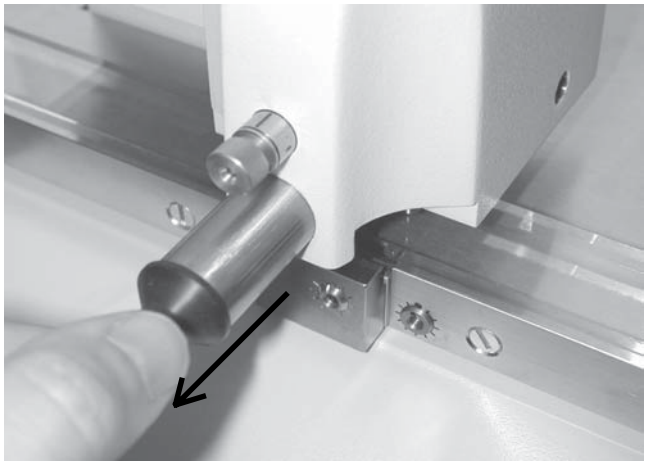


Fig.13

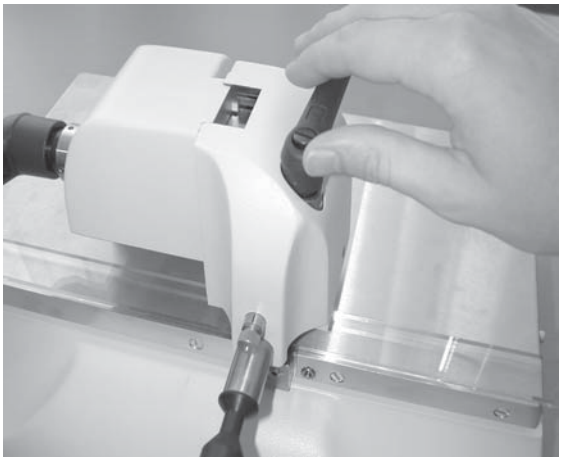


Fig.14

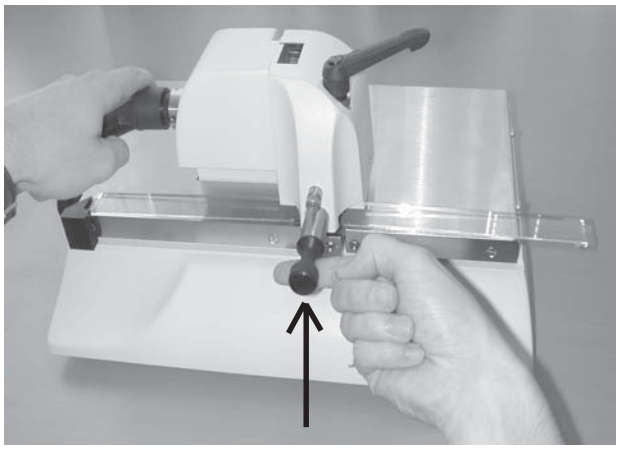


Fig.15

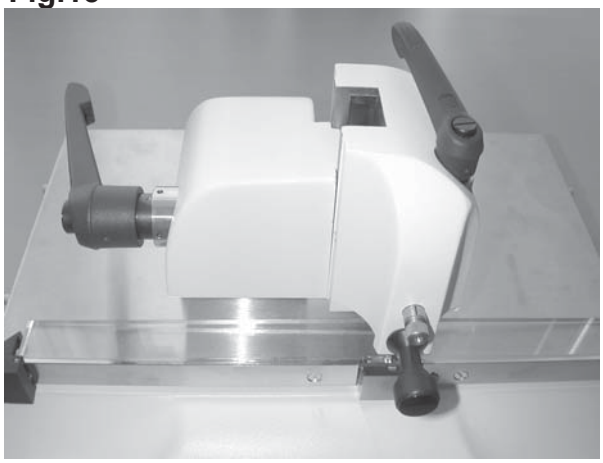
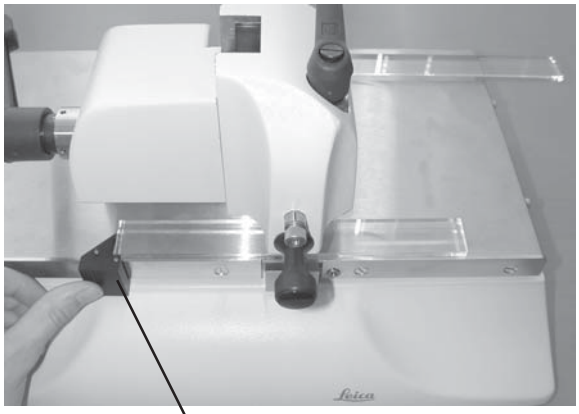
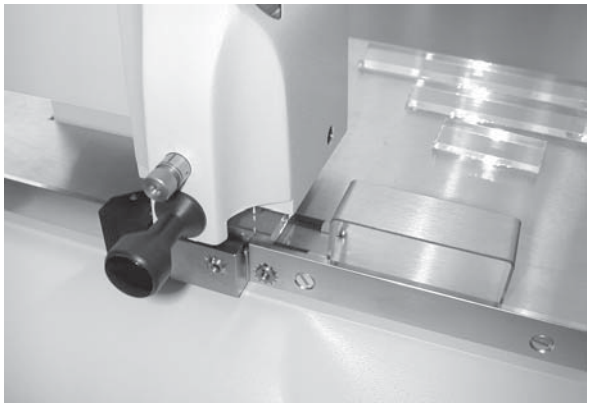


Fig.16



Moveable stop at position 2.

Fig.17



Set the scoring mark selector **(Fig.9 No.6)** to its long mark.
Score the glass by fast and evenly pulling the scoring shaft as shown in **Fig.12.**
Rotate the breaking lever gently and slowly clockwise to initiate the
break as shown in **Fig.13.**
After the break, raise the scoring head completely by means of the clamping lever.
Support the head with a finger below the scoring shaft to prevent the head dropping onto the glass **(Fig.14).**
Push back the scoring shaft and bring the breaking lever to its starting point by rotating anticlockwise **(Fig.15).**
Place the right hand 200 mm long glass strip onto the working surface of the instrument as shown in **Fig.16.**
Move the stop with the 200 mm long glass strip to **position 2** as shown in **Fig.16** and push the glass strip against it.
Press the glass strip by means of the rear stop against the rulers, lower the scoring head by means of the clamping lever down onto the glass strip (2 or 3 times) and pull the clamping lever tight. Move rear stop back again.
Score, break and raise the scoring head as before.
The result is now two 100 mm long strips.
Place the right hand 100 mm long glass strip to the rear of the working suface of the instrument.
Move the stop with the 100 mm long strip to **position 3.**
Press the strip by means of the rear stop against the rulers, lower the scoring head and clamp it. Move rear stop back again.
Score, break and raise the scoring head as before.
The result is now two 50 mm long strips.
Place the right hand 50 mm long glass strip to the rear of the working surface of the instrument.
Move the stop with the 50 mm long strip to **position 4.**
Support the 50 mm glass strip by placing the handling fork under the glass strip for easier handling of the resulting squares as shown in **Fig.17.**
Press the strip by means of the rear stop against the rulers, lower the scoring head and clamp it. Move rear stop back again.
Score, break and raise the scoring head as before.
The result is now two 25 mm squares as shown in **Fig.18.**

Fig.18

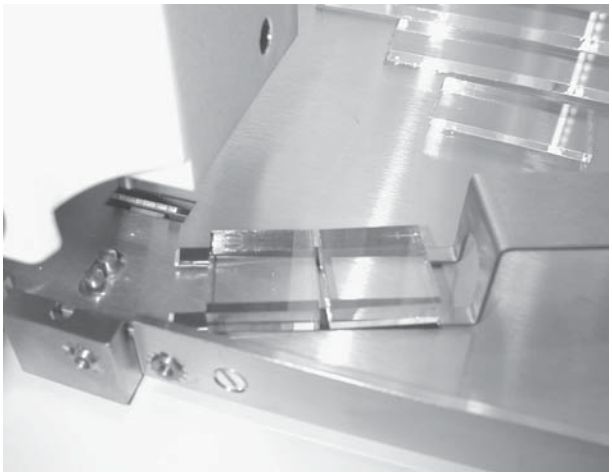


Fig.21

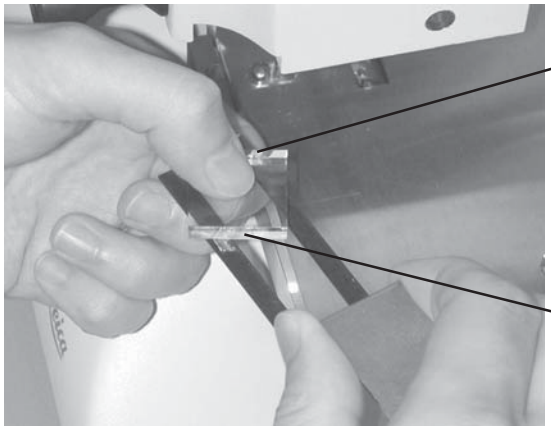


Fig.22

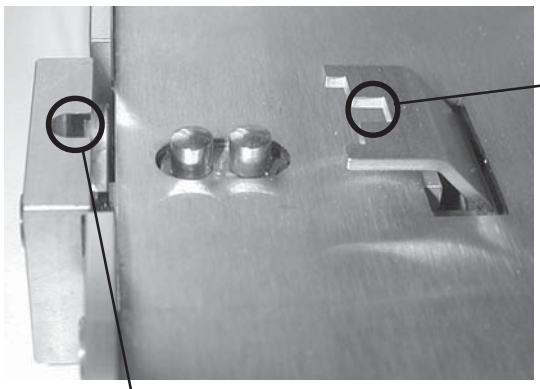
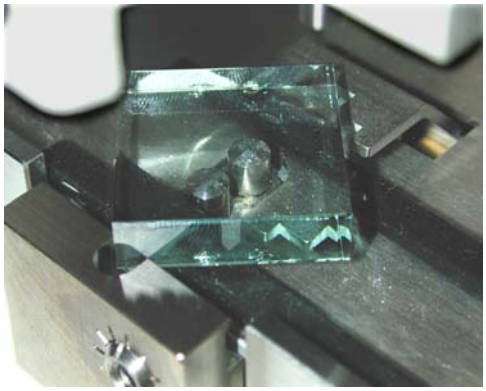


Fig.23



Note: As the left end of the left hand 200 mm glass strip is machine broken by the glass manufacturers and therefore geometrically not perfect do not use this square for breaking knives (Fig.19). Also, the outer square of the right hand 200 mm glass strip is not used to make knives (Fig.20) for the same reason.

Fig.19

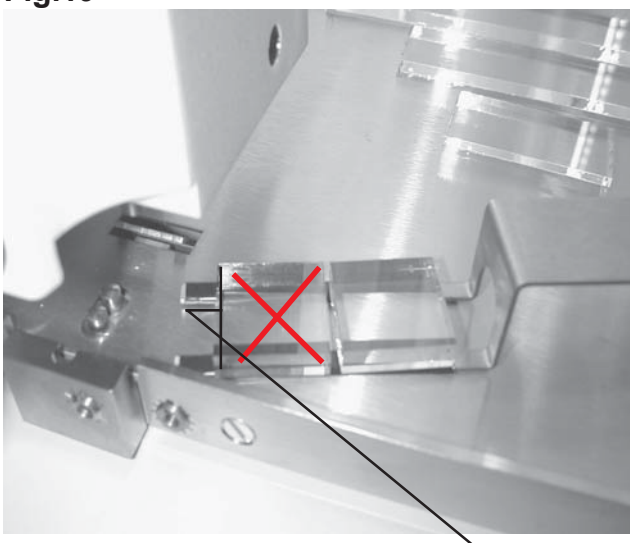
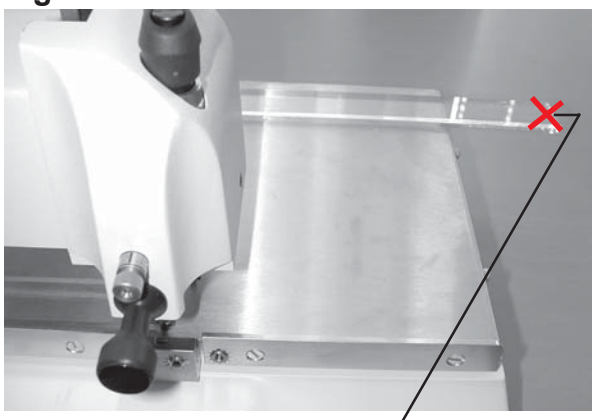


Fig.20



End broken by
manufacturers - not perfect!

5.3 Making knives from square

Set the moveable stop to position 1 as it is not used for breaking knives.
Scoring head is raised completely, breaking lever turned anticklockwise to its start position, scoring shaft is pushed back and the rear stop is moved back.

Place a square onto the handling fork and rotate it a **quarter turn clockwise (Fig.21)** to produce the **knife edges fom the freshly broken sides of the glass.**
Place the square using the handling fork onto the two pins and make sure that the corners of the square are positioned into the notch of the front and rear stops **(Fig.22).**
Move the rear stop by means of the rotatable knob carefully against the square making sure you do not touch the corners.
The square is now held firmly between the two stops. Open and close the rear stop 2 or 3 times to ensure the glass is correctly positioned.
The handling fork lies loose underneath the square **(Fig. 23).**
Lower the scoring head by means of the clamping lever down onto the square 2 or 3 times then pull clamping lever tight.
Set the scoring mark selector to its short or long mark.
Score the glass by quickly and evenly pulling the scoring shaft to the end stop.
Rotate the breaking lever gently and **very slowly** clockwise to initiate the break.
The slower the better.

Note: The best knives are made using the lowest breaking pressure.
They may take a minute or more to break!

Fig.24

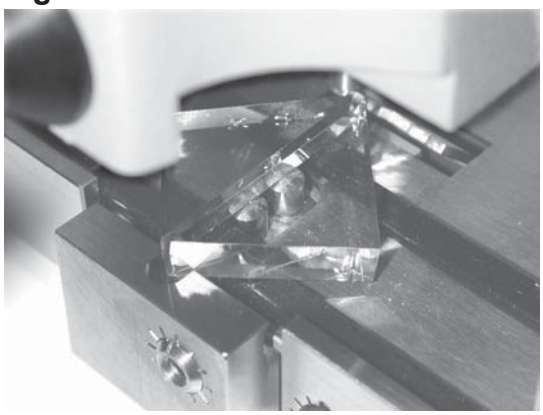


Fig.25

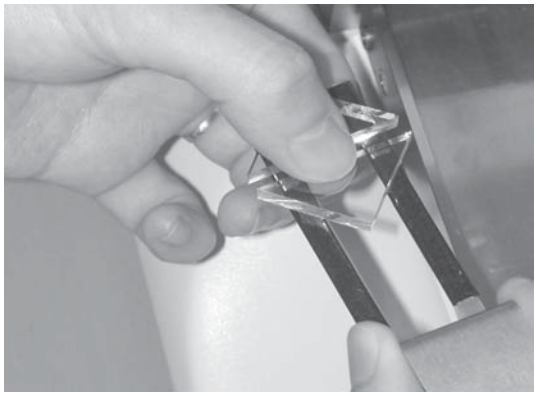


Fig.26

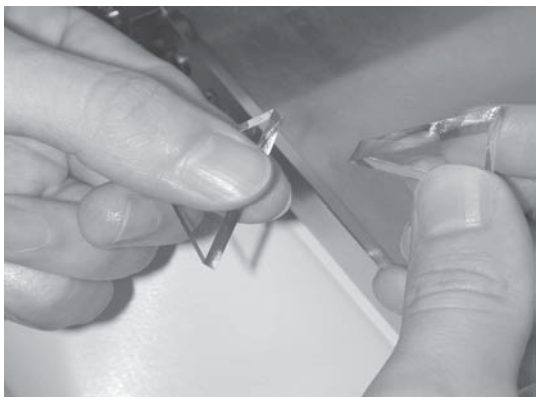
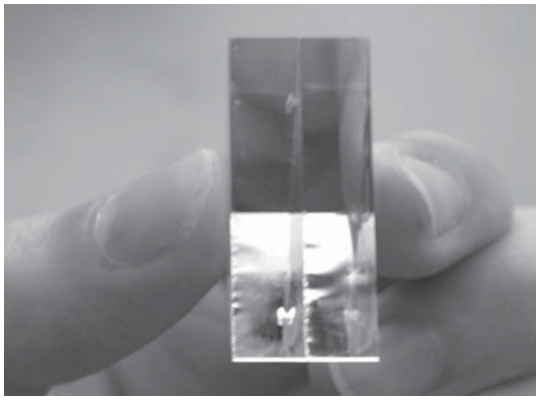


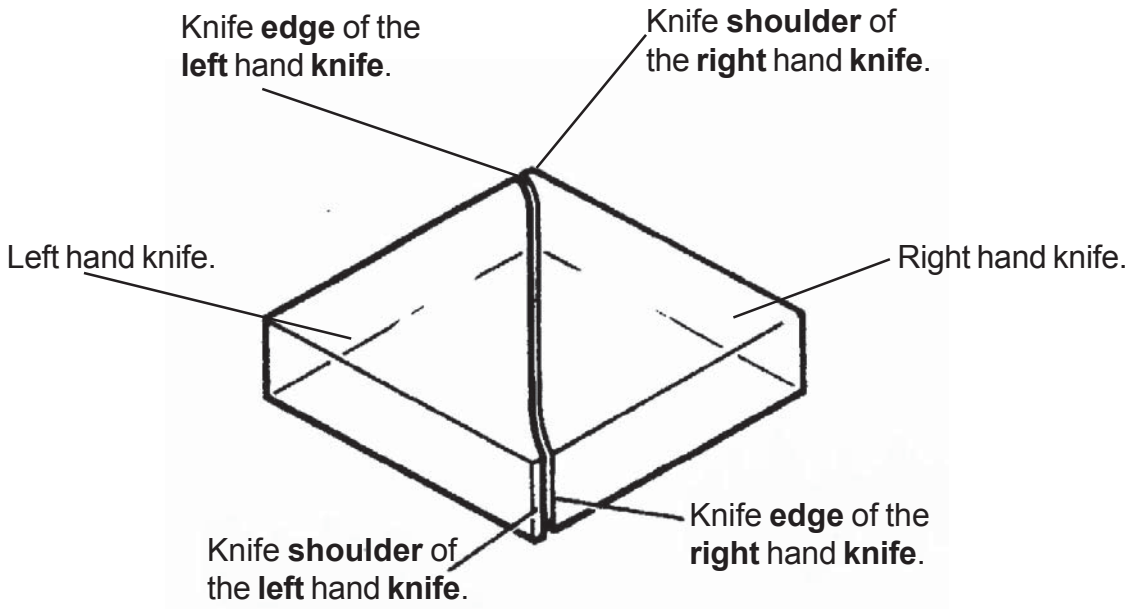
Fig.27



After breaking raise the scoring head completely by means of the clamping lever.
Support head with finger below the scoring shaft to prevent it droping onto the glass.
Push back the scoring shaft and bring the breaking lever to its starting point by rotating anticlockwise.
Two knives are now broken from the square **(Fig.24)**.
Remove the knives with the hand fork.
We have now produced 2 knives, one on the left and one to the right.

Each of the knives have a knife edge and a knife shoulder **(Fig.24a)**.

Fig.24a



Keep the knives together as a 'pair'.
Take them from the handling fork as shown in **Fig. 25.** Do not allow the knives to touch.
Turn the right hand knife **(Fig.26)** to bring the knife edge side by side with the knife edge of the left hand knife.
Both knives are know placed together as a 'pair' and visual evaluation can be carried out **(Fig.27)**.

Fig.28

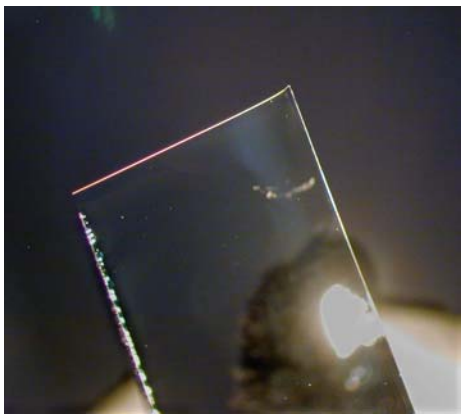


Fig.29

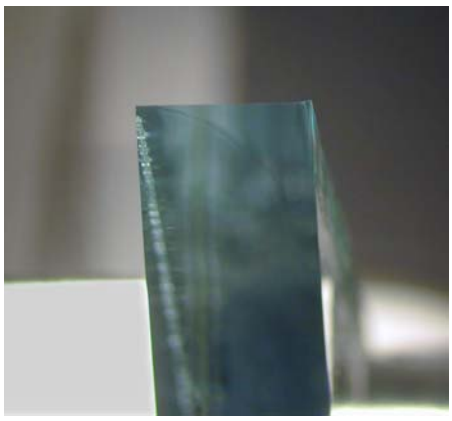


Fig.30

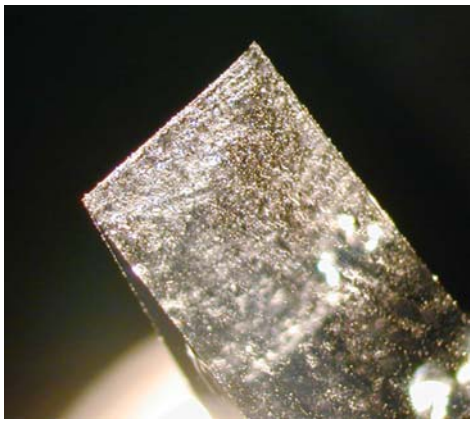


Fig.31

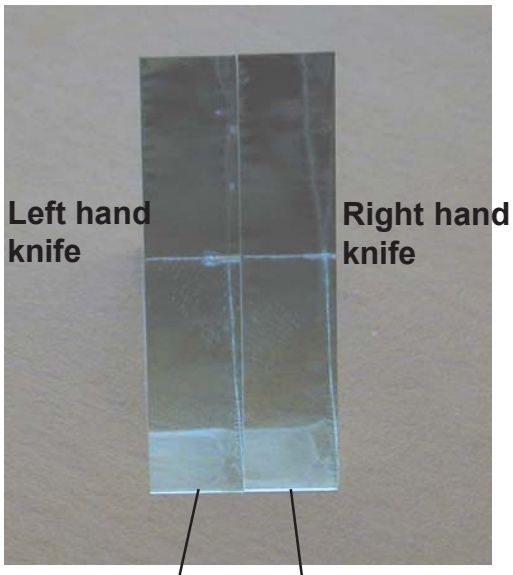
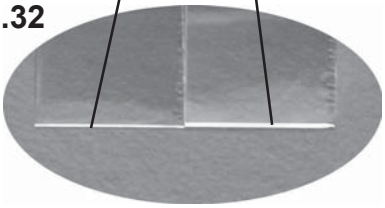


Fig.32



5.4 Evaluation of the knife edge

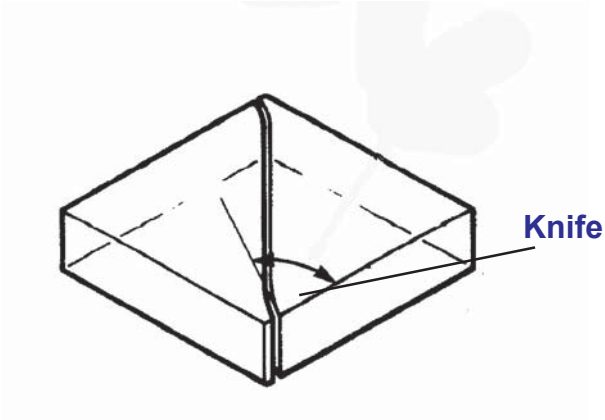
After making a pair of knives, evaluation of the quality can be carried out with the Ultramicrotome (Leica Ultracut UCT / R / S).
Using the backlight illumination and setting the clearance angle to maximum a fine white line can be seen **(Fig.28)**.
The image of the line indicates the quality of the knife edge, which must be straight, free of any dirt such dust, grease and finger prints and free of glass splinters.
The top light of the ultramicrotome can also be used for checking knife quality **(Fig.29).**
An example of a knife which should not be used is shown in **Fig.30**.
This has been picked up incorrectly leaving a finger print over the knife edge.

In **Fig.31** a pair of knives is shown, broken and placed side by side according to chapter **5.3**.
The detail **(Fig. 32)** shows the knife shoulder of both knives
The right hand knife edge was opposite the left hand knife shoulder during breaking and the left hand knife edge was opposite the right hand knife shoulder.

Note: the sharpest knife edge is the one opposite the smaller knife shoulder!

The knife edge opposite a small knife shoulder (< 0.2mm) has a knife angle very close to 45°, as recommended for cryo-ultramicrotomy **(Fig.32a)**.
(*Griffiths et al. 1983: Tokuyasu 1986*).

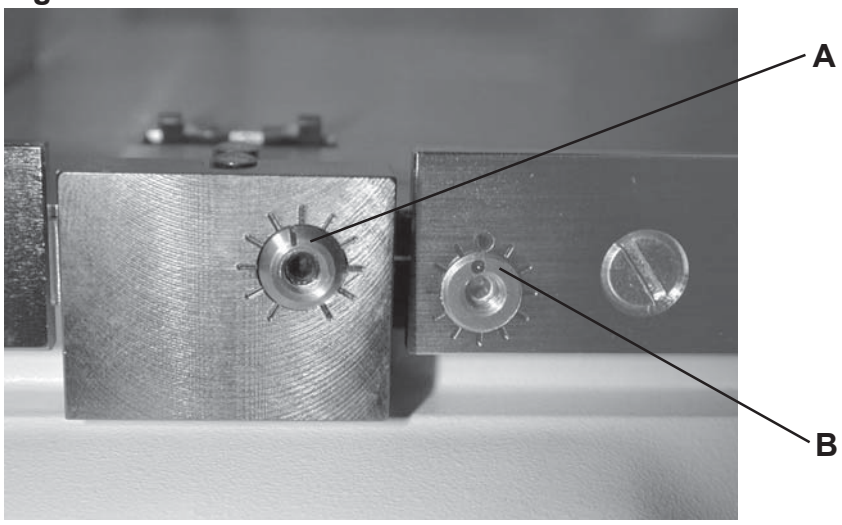
For resin sectioning a larger knife shoulder (approx. 1mm) is recommended.



6.1 Knife shoulder fine adjustment

The adjustment **'A'** and **'B'** as shown in **Fig.33** is used to adjust the size of the knife shoulder.
As mentioned earlier in this manual the sharpest knife edge is found opposite a very small knife shoulder.
Fine adjustments on the Leica EM KMR2 allow you to adjust the size of the knife shoulder for either resin or cryo sectioning.

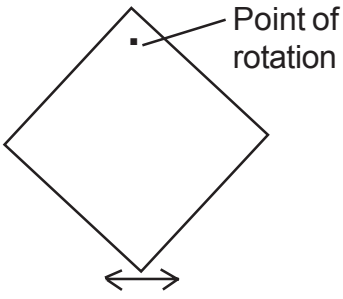
Fig.33



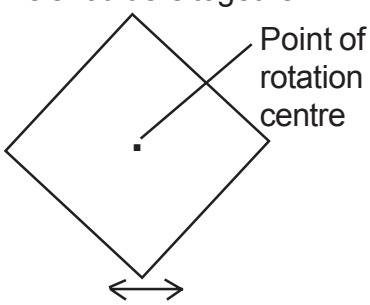
Fine adjustment A: rotatable one complete turn. This movement influences both knife shoulders contrary.
Turn it to the left: left knife shoulder becomes bigger, right knife shoulder becomes smaller.
Turn it to the right: right knife shoulder becomes bigger, left knife shoulder becomes smaller.

Fine adjustment B: rotatable a half turn to the right and a half turn to the left. This movement influences both knife shoulders simultaneously.
Turn it to the left: both knife shoulders become **bigger**.
Turn it to the right: both knife shoulders become **smaller**.

Step 1
A
Adjusts both knife shoulders to same size.



Step 2
B
Adjusts height of both knife shoulders together.



Start fine adjustment

If the Leica EM KMR2 is completely out of adjustment:
set the notch on the adjustment wheel **'A' and**
the point on adjustment wheel **'B'** to 12 o´clock using the 2 mm Allen Key provided.
This is then your starting point .

If the Leica EM KMR2 is adjusted for resin sectioning and you wish to have smaller knife shoulders for cryosectioning then your starting point is the factory setting,

To start with, follow the scoring and breaking procedure as described in chapter **5.2 and 5.3**.
Having a closer look at the knife shoulders (as described in chapter **5.4**), then proceed turning fine adjustment **'A'** or **'B'**.

Note:
Before you adjust 'A' or 'B'
make a note of its position so you can easily adjust back to the factory setting if necessary.

Note:
Always begin with fine adjustment 'B' to set both knife shoulders as small as possible.
Then use adjustment wheel 'A' to equalize either the left hand or the right hand knife shoulder.

Note:
Fine adjustments 'A' and 'B' are very sensitive - only a small turn within the scale is necessary.

Fig.34

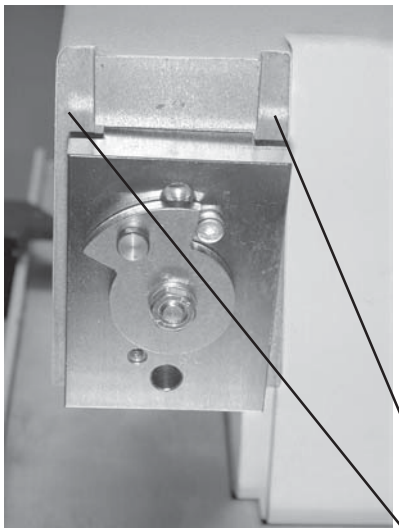
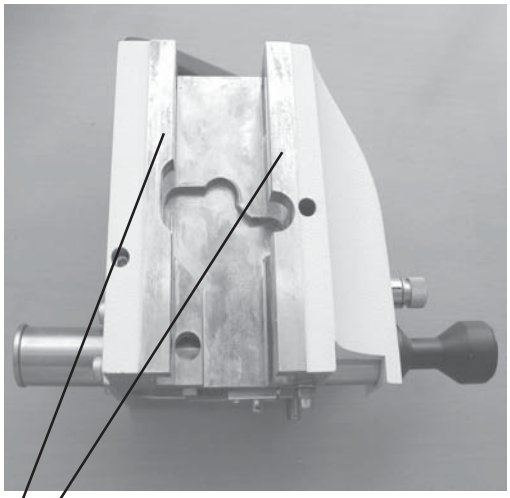
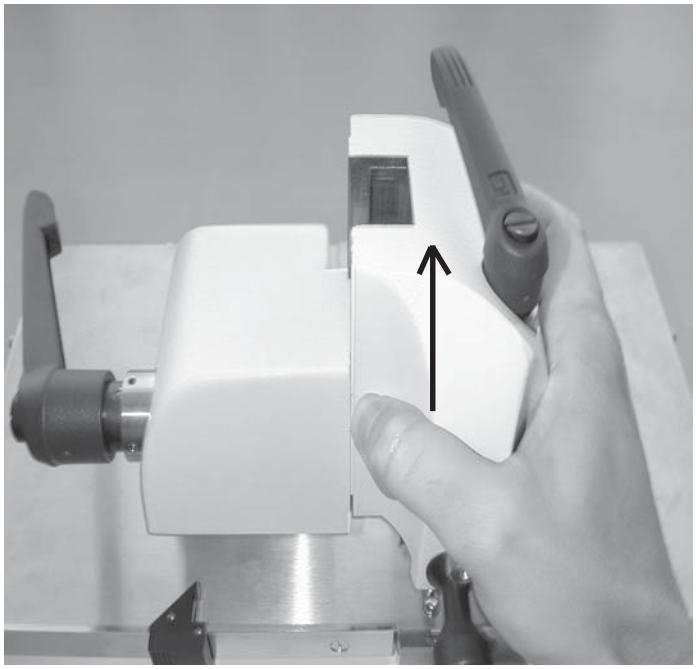


Fig.35



Sliding surfaces

Fig.36



7. Care and maintenance

The Leica EM KMR2 should always be kept clean.The breaking pins must be free of any glass particles. Use a brush or 'Dust off' for cleaning.
The sliding surface of the scoring and clamping head **(Fig. 34 and Fig.35)** must also be kept clean **and dry** . Use alcohol to keep the surfaces grease-free.
No grease or oil should be applied!
To clean the sliding surfaces lift up the scoring head as shown in **Fig.36** (Clamping lever in 'raised' position).
All surfaces are now accessible for cleaning.
After cleaning replace the scoring head.

7.1 Replacement of the scoring wheel cartridge

With frequent use the scoring wheel cartridge should be replaced around once a year .
To replace, raise the scoring head as shown in **Fig.36** and place it on the table upside down.
Using the 2 mm Allen Key (provided) to open the clamping screw for the scoring wheel cartridge as shown in **Fig.37** and pull out complete cartridge as shown in **Fig. 38**.

Fig.37

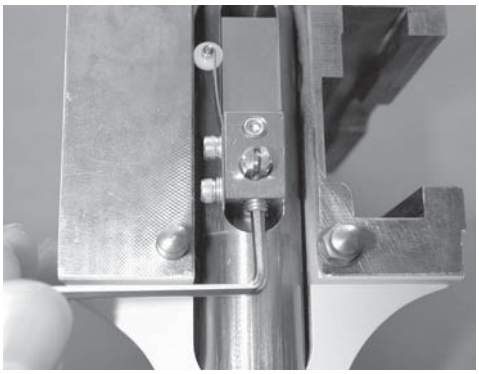
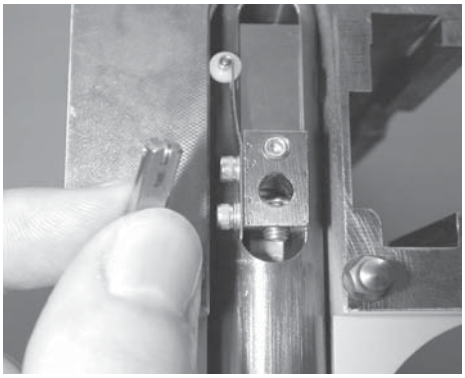


Fig.38



Insert a new scoring wheel cartridge with the **flat side facing the clamping screw** and clamp it. Replace the scoring head and make a 'test' score on a piece of glass strip.
The scoring pressure with the new scoring wheel cartridge will probably be too strong or maybe not enough. For adjustment see **chapter 7.2**.

Fig.39

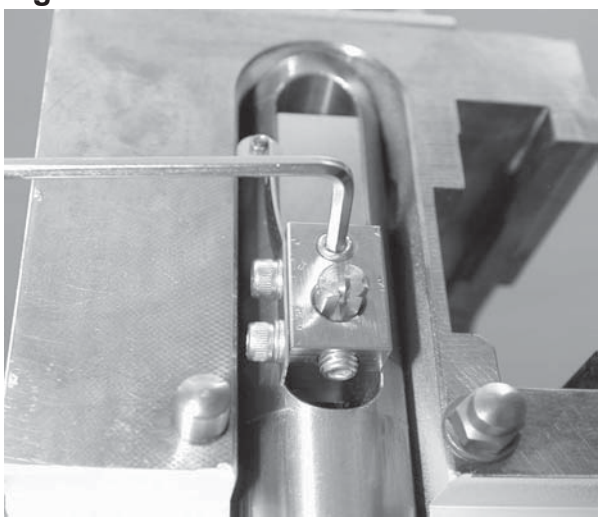


Fig.40

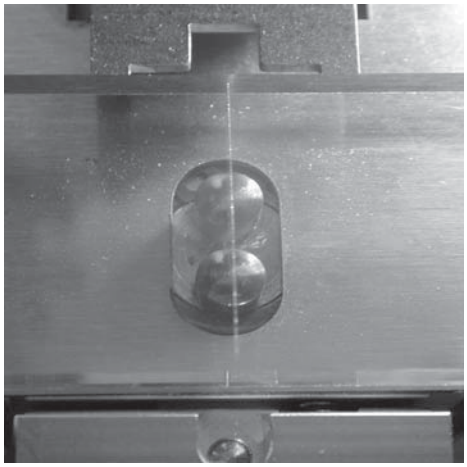
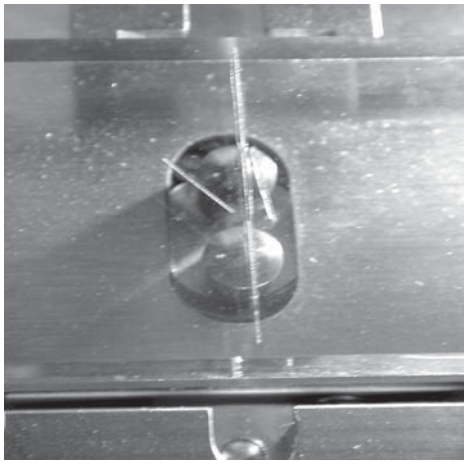


Fig.41



7.2 Adjusting the scoring pressure

Raise the scoring head clompletely as shown in **Fig.36** and place it on the table upside down.
By turning the screw **(Fig.39)** with the 2 mm Allen Key (provided) **clockwise** the pressure can be **increased**, **counter clockwise** the pressure can be **decreased**.
After turning the screw in the required direction (a quater to a half turn) replace the scoring head and make a test score on a piece of glass.
Repeat until the pressure is correct.

The pressure is correct **(Fig.40)** - one faint smooth line without glass splinters.
The pressure is too strong **(Fig.41)** - a groove and glass splinters can be seen.
The pressure is not enough when the scoring line is faint and interrupted.

Fig.42

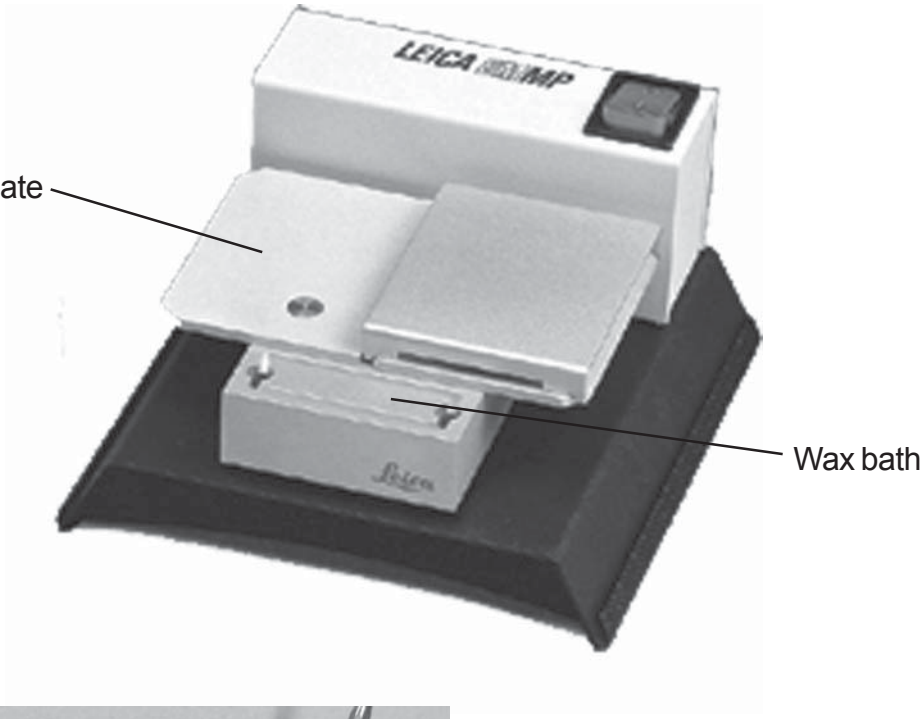


Fig.43

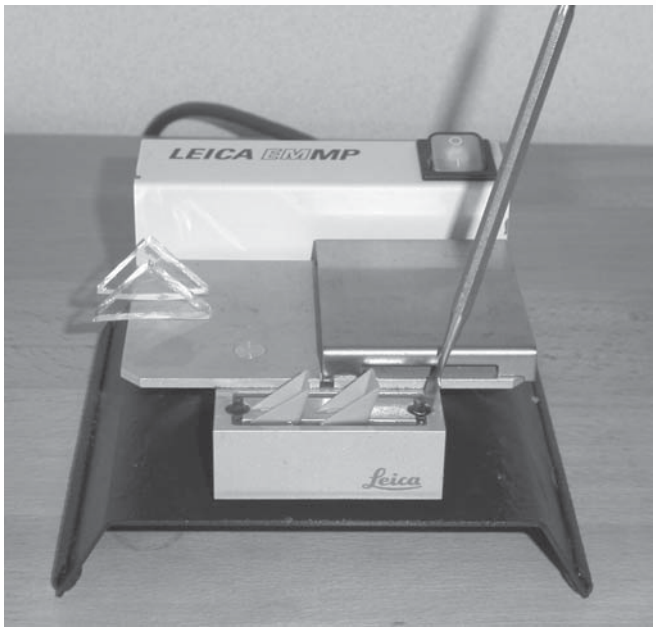
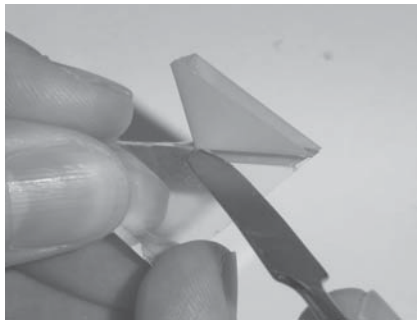


Fig.44

Fig.45



8. Mounting troughs (‘Trufs’) onto the knife

For room temperature ultrathin sectioning disposable plastic troughs - called ‘Trufs’ - are used to carry distilled water where the sections float after sectioning.
Fitting a Truf is simple if the Leica EM MP is used **(Fig.42)**.
Wax is melted in the bath of the Leica EM MP, glass knives are lightly warmed on the hotplate with the knife edge overhanging the edge of the hotplate as shown in **Fig.43**.
The attachment surfaces of the Truf are coated with molten wax by putting it onto the wax bath as shown in **Fig 43**.
The Truf is then brought into contact with the pre-warmed knife and slid up until it is levelled with the knife edge as shown in **Fig.44**. The Truf is held in this position for a second or two until the wax has set.
Additional wax is applied on the right side of the Truf using the spatula supplied with the Leica EM MP for further protection against leaks **(Fig.45)**.

9. Accessories and consumables

84 00 31 Glass strips 6.4 x 400 x 25mm (box of 30 strips)
84 00 32 Glass strips 8 x 400 x 25mm (box of 24 strips)
84 00 79 Glass strips 10 x 400 x 25mm (box of 18 strips)
84 00 42 Trufs 6.4 mm (500 pcs.)
84 00 45 Trufs 8mm (500 pcs.)
84 00 46 Trufs 10mm (500 pcs.)
89 50 32 Dust cover
70 52 25 Knife boxes (3 pcs.)
70 52 27 Scoring wheels for Knifemaker1 (3 pcs.)
70 66 99 Scoring wheel cartridge for Leica EM KMR2, KMR and Knifemaker II

Recommended accessories

70 54 01 LEICA EM MP 115/230VAC
complete with metal spatula and 500g dental wax.
Hotplate with 3 different temperatures for mounting TRUFS on glass knives and for staining and drying of semithin sections on glass slides.

Leica - best solution for EM sample preparation.
www.em-preparation.com

Leica EM TP Tissue Processor

Leica UCT Ultramicrotome

10. Tecnical specifications

Method of Operation:	Balanced break method
Score Marks:	Two, pre-set
Scoring Angle:	45°
Glass Strips:	Special glass for Ultramicrotomy, Cryo-ultramicrotomy and Histology Length 400 mm Width 25 mm Thickness 6.4 /8 /10 mm
Scoring Wheel:	Tungsten carbide in cartridge

Leica EM KMR 2

Dimensions and Weight:	Length:	400 mm
	Depth:	280 mm
	Height:	280 mm
	Weight:	11kg

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