



Instruction Manual

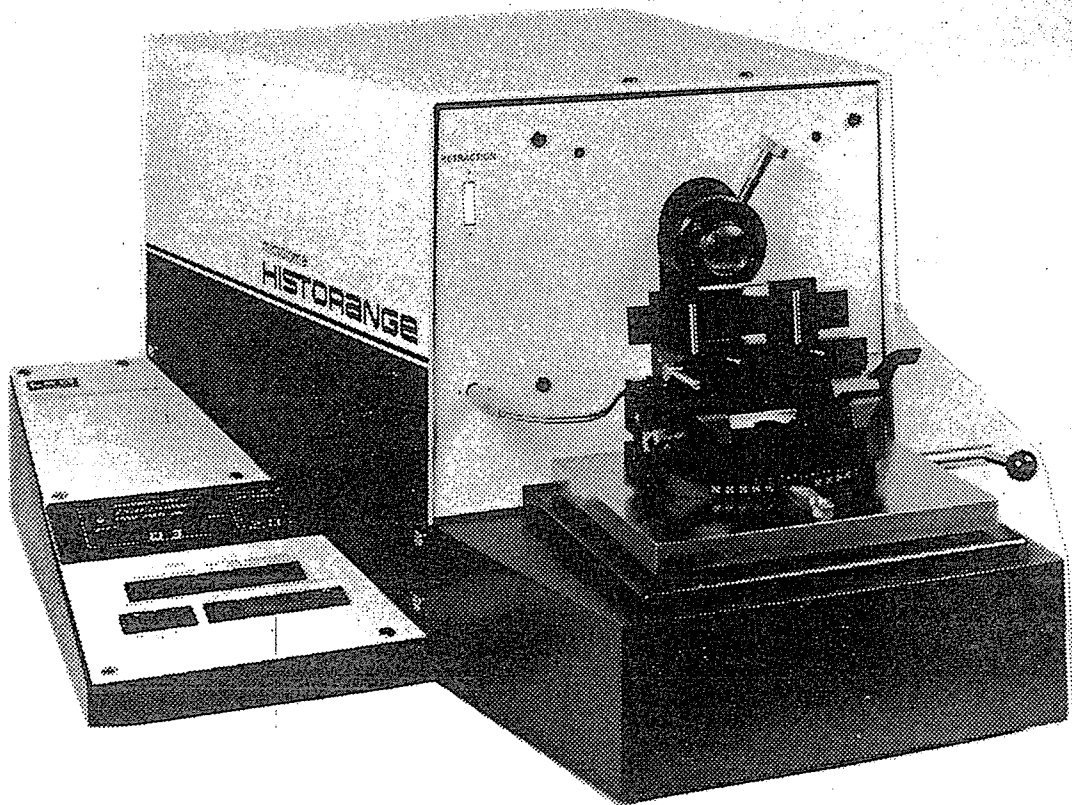
LKB 2218 HistoRange Microtome

90 01 3575

Instruction Manual

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PLEASE READ THIS MANUAL CAREFULLY BEFORE ATTEMPTING TO USE THE MICROTOME

This manual describes the LKB 2218 HistoRange microtome. Intended for the microtome user this manual deals with the appearance of the HistoRange and contains all necessary information regarding its operation and installation.

If you have any comments on this manual, we will be pleased to receive them at:

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The warranty included in the conditions of delivery is valid only if the product has been used according to the instructions supplied by LKB-Produkter AB.

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INTRODUCTION

The LKB 2218 HistoRange is a microprocessor-controlled microtome designed to cut sections for light microscopy with precision, accuracy and speed.

It is also designed with particular concern for operator comfort, convenience and ease of operation.

HistoRange has been quality engineered to ensure the high resolution and low distortion inherent in modern plastic embedding materials as well as to cope with many of the barriers to improved resolution associated with traditional paraffin embedding techniques. HistoRange enables, for example, convenient wet sectioning with a specially designed knife stage and a large retraction cycle.

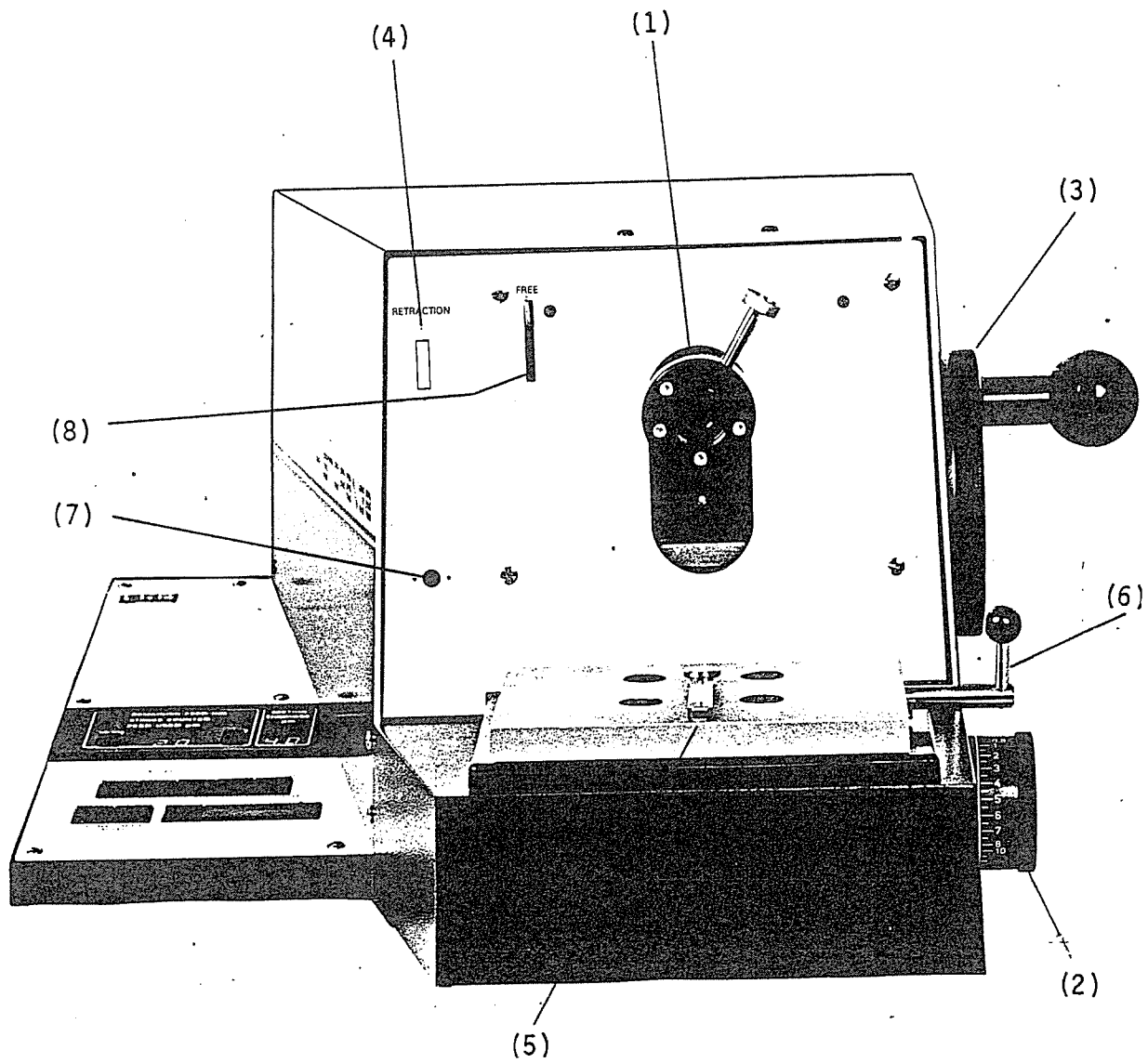
Designed to simplify sectioning with the help of a microprocessor, HistoRange includes a series of unique features: pushbutton control of cutting range and thickness, continuous display of stroke count, feed used and remaining feed, fingertip and/or pedal control of cutting cycle and start/stop and autotrimming possibilities. HistoRange also provides a unique backlighting system to facilitate your approach. Based upon a stable and reproducible knife advance system, HistoRange offers a wide range of cutting speeds and a rapid retraction-return cycle. It can be operated automatically or manually.

To accommodate a variety of demands for easy sectioning, HistoRange also offers a wide range of accessories, such as knife stages, specimen holders, orientation systems and wet section system etc.

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2218 HistoRange basic unit

GENERAL DESCRIPTION

A specimen block is mounted onto an appropriate holder. This holder is then inserted into the HistoRange slide (1) which moves vertically. The speed of the slide movement can be controlled either automatically by the speed control (2) or manually by a handwheel (3). The lever inside the handwheel releases the motor for less resistance when using the microtome in the manual mode.

During the upward-return stroke of the specimen slide (1) the specimen is retracted to prevent contact between the specimen and knife edge. When the specimen is retracted the retraction lamp is lit (4).

A knife stage is attached to the knife feed table (5) with a fast locking lever (6). The knife feed table is fed continuously toward the specimen head by a stepping motor which drives a micrometric screw.

Knife stages are supplied with a back lighting system for fast and easy approach and alignment. The light is connected at (7).

The mechanical locking mechanism (8) prevents damage and ensures safety when changing the specimen block and/or knife.

INSTALLATION

After the HistoRange has been unpacked, ensure that the transport lockings are removed.

- 1) Remove the wooden plank under the HistoRange. Three bolts hold this plank in place.
- 2) Remove the metal plate which locks the specimen slide to the knife feed table. To remove this plate, remove the two screws to the front panel, loosen the locking lever in the knife feed table and gently pull out the metal plate.

Before the mains cord is connected, ensure that the correct voltage is selected on the mains voltage selector located on the rear panel.

SPECIFICATIONS

Advance system

Microprocessor-controlled stepping motor with a total feed range of 20 mm (0.5-50 μ m advances). Multiple advances are used for rapid approach and single advances for fine approach. The knife stage is automatically returned to the start position at the end of the advance. A blinking light indicates reset in progress. The knife stage can also be returned to any position within the feed range.

Cutting

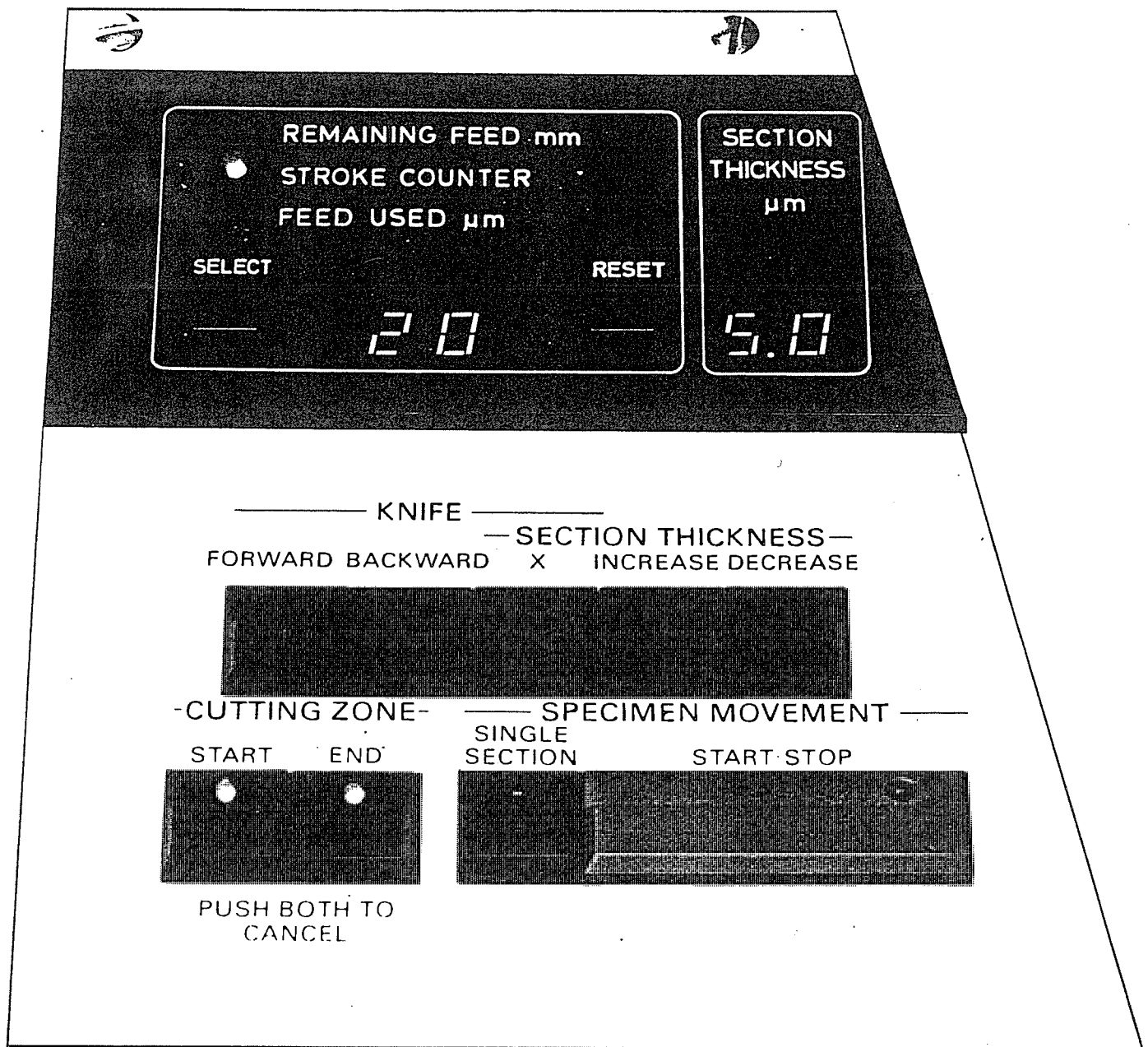
- Cutting speed - Continuously adjustable from 0.5 - 200 mm/s with a potentiometer wheel. Can be adjusted while sectioning.
- Cutting zone Pushbutton control for optimized cutting speed during sectioning and rapid return cycle (50mm/s greater than preset cutting speed).
- Cutting cycle - Automatic control of single or continuous cycles by pushbuttons or remote control foot pedal.
Manual control by balanced rotary wheel.
- Cutting stroke - 50 mm
- Retraction - 200 microns
- Cutting force - 10 kg
- Section thickness - 0.5 - 50 μ m, can be adjusted during cutting cycle. Feed intervals of 0.5 μ m (for section thickness 0.5 - 5.0 μ m), 1.0 μ m (for section thickness 5 - 10 μ m), 5.0 μ m (for section thickness 10 - 50 μ m).

Knife adjustments

- Standard knife stage - Accepts all conventional steel knives (base width up to 13 mm), disposable steel knives, tungsten carbide knives and LKB HistoKnife adapters.
Distance between clamps: 60 mm
Parallel Height Adjustment: 30 - 40 mm
Clearance Angle: 0-15°
- Glass knife stage - For Histoknives (25 - 38 mm) and triangular glass and diamond knives (up to 12 mm).
Lateral adjustments: 25 mm
Rotation: $\pm$ 30°
Clearance angle: 0-15°

Specimen adjustments

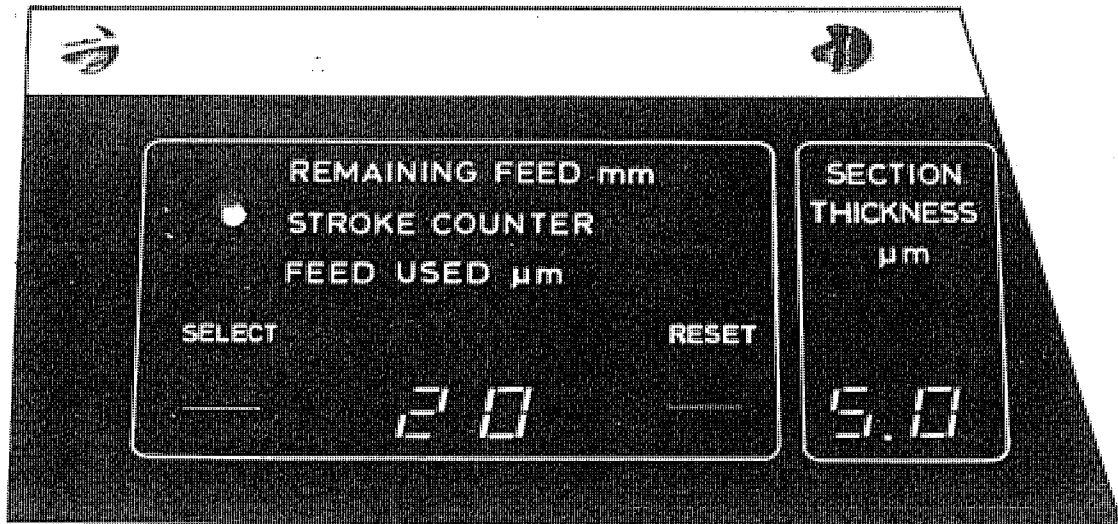
- Standard specimen clamp - Accommodates standard specimen holders (25x25mm) large block holder (40x40mm), LKB HistoMold adapters JB-4 chucks and similar holders, LKB UM specimen holders and specimen orientation arc.
- Ball-joint holder - For complete orientation of any size block. Accommodates standard Holders I and II and large block holders, (40x40 mm).
- Specimen orientation arc - +100 vertical orientation with 360° rotation possibilities. Mounted on Standard Specimen clamp. Accommodates same holders as Standard Specimen Clamp.
- Approach light - Standard backlighting on both knife stages to facilitate knife specimen approach.
- Digital display - Remaining feed, stroke counter or feed used. Section thickness.
- Electrical data - Voltage: 110, 115, 120, 220, 240 V $\pm$ 10% 50/60 Hz.
- Power consumption - 110 VA
- Dimensions (LXWXH) - 550 x 455 x 300 mm.
- Weight - Instrument: 50 kgs; shipping pack: 70 kgs.



CONTROL PANEL

The control panel is logically arranged for simplicity of operation.

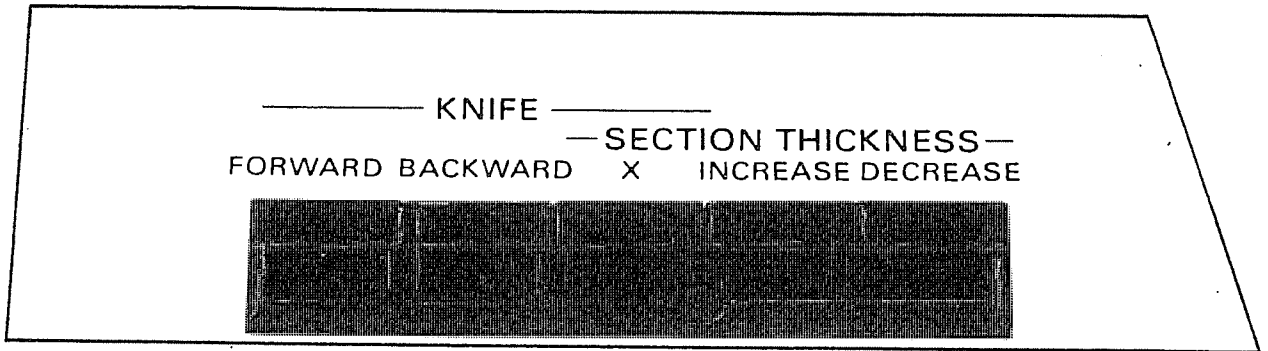
- (1) Display unit which indicates section thickness, remaining feed, number of strokes and feed used.
- (2) Selection and control of the horizontal knife movement.
- (3) Selection and control of the vertical slide movement.



Display

The display continuously shows one of the three parameters: remaining feed, stroke count or feed used. The LED of the activated parameter is illuminated. Switching from one parameter to another is done by pressing the SELECT button.

REMAINING FEED	Indicates the feed left (in mm) of a total horizontal feed range of 20 mm.
STROKE COUNTER	Indicates the number of sections cut. Useful for level sectioning.
FEED USED	Indicates the feed used in microns. Useful for monitoring the depth into the specimen block.
RESET BUTTON	The display function in use is reset when this button is activated. NOTE: If REMAINING FEED is activated when the reset button is pushed, both the display and the knife table will reset.
SECTION THICKNESS	Continuously shows the section thickness (in microns).



Knife feed function

The required section thickness (in microns) is programmed with the push-buttons INCREASE and DECREASE and displayed on the SECTION THICKNESS display.

The section thickness can be set from 0.5 to 50 microns in the following steps and ranges:

Step	Range
0.5 microns	0.5 - 5 microns
1.0 microns	5-10 microns
5.0 microns	10-50 microns

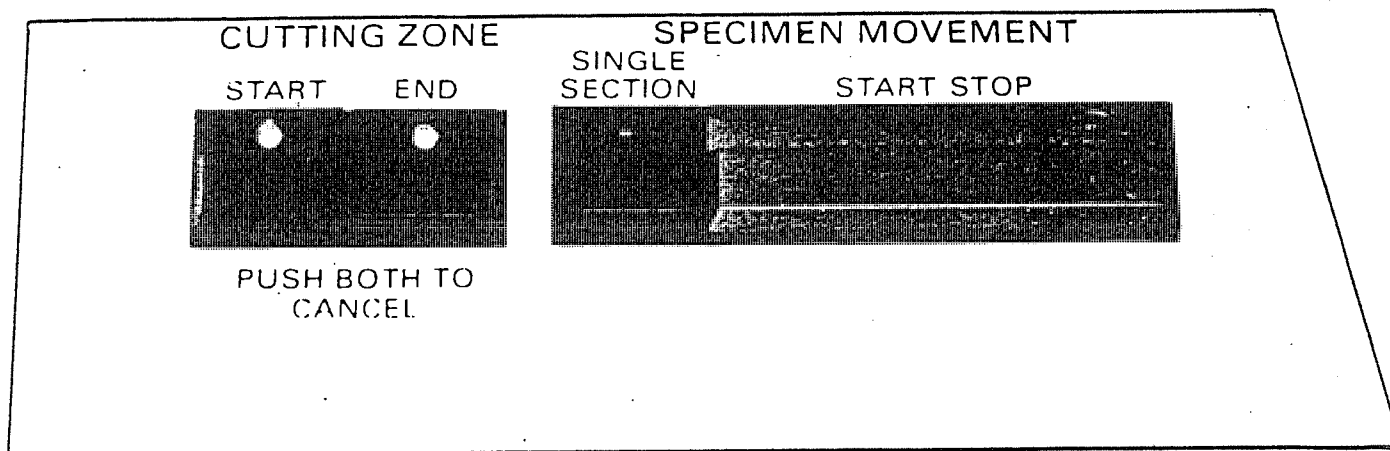
The thickness of the next section can be changed only if selected when in the downward stroke. Although a change in section thickness is displayed upon entry, the actual change is processed in the transition between the last section and the upward-return stroke.

The movement of the knife feed table is controlled by the pushbuttons FORWARD and BACKWARD. Each forward or backward step is equivalent to one preprogrammed section thickness.

The X-button is a "speed up" and a stop function.

By pressing the X-button together with the INCREASE, DECREASE, FORWARD, or BACKWARD buttons, respective functions are speeded up.

The X-button also cancels the reset function on the remaining feed display and stops the reset of the knife feed table. (Note. The X-button will not stop the automatic reset of the knife feed table when the mains is switched on.)



Slide function

The START/stop button activates the slide for continuous specimen movement.

The SINGLE SECTION button activates one complete cycle of specimen movement.

When these functions are activated they can also be controlled by foot pedal 2218-400.

A special cutting zone function has been incorporated to speed up the cutting cycle. This function is especially important as it saves much time when relatively slow cutting speeds are required - especially for serial sectioning of small blocks. When using the cutting zone, the slide moves with the preset cutting speed only within the selected cutting zone. The rest of the cutting cycle proceeds at a higher speed.

To set the cutting zone move the specimen slowly downwards. Push the START button when the specimen reaches a position where a selected cutting speed is required. Pass the knife edge to a point where the selected cutting speed is no longer required. Push the end button. The set cutting zone is cancelled by pushing the START and END buttons simultaneously.

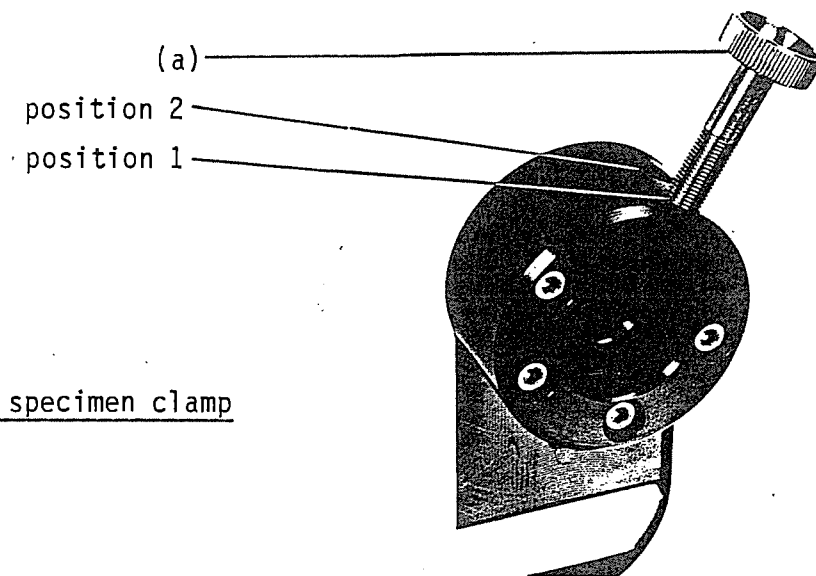


Fig. 1
Standard specimen clamp

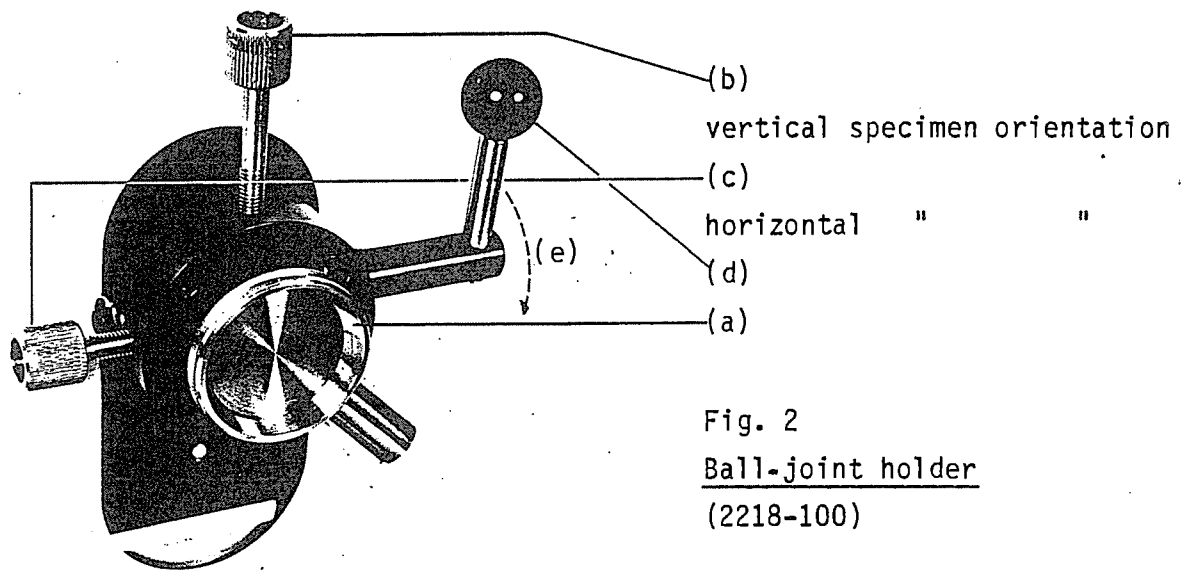


Fig. 2
Ball-joint holder
(2218-100)

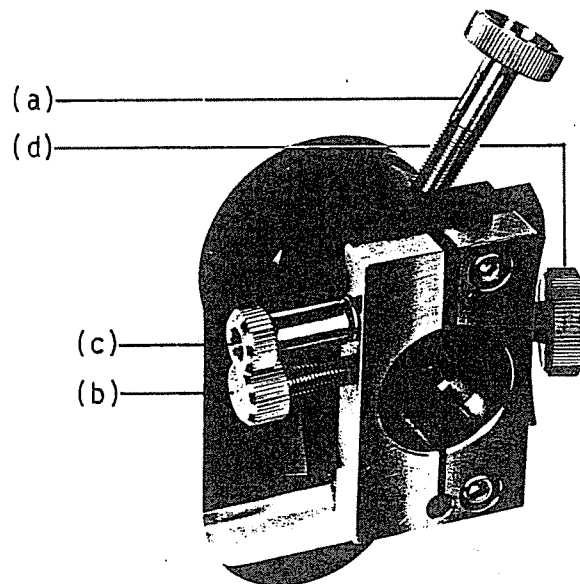


Fig. 3
Specimen
orientation arc

SPECIMEN HEADS

Standard specimen clamp (Fig. 1)

The standard specimen clamp is mounted on the slide of the microtome body. The following specimen holders can be mounted directly on the standard specimen clamp using screw (a) in position (1): orientation arc 2218-250, specimen holders 2218-150, 2218-151, 2218-152, LKB HistoMold adapter (2218-310) and JB-4 chucks. LKB Ultramicrotome specimen holders can also be mounted directly on the standard specimen clamp using screw (a) in position 2.

To remove the standard specimen clamp, loosen the allen screws and pull off.

Ball-joint holder (Fig. 2)

The ball-joint holder is a flexible orientation head for specimen holders 2218-110, 2218-111 and 2218-112.

To mount the ball-joint holder, remove the standard specimen head. The ball-joint holder is affixed with 4 allen screws. (If necessary, adjust the position of the center plate (a) for easy access to each of these screws). Tighten the allen screws.

Fit the specimen holder firmly onto the ball-joint center plate. Tighten each screw. For vertical specimen orientation use screw (b) and for horizontal orientation use screw (c).

The ball-joint orientation plate is released and locked with lever (d). Orientation adjustments can be made only when this lever is released. During operation the lever must be locked (e).

Specimen orientation arc (Fig. 3)

The specimen orientation arc is intended primarily for vertical orientation of plastic embedded specimens. It takes the same specimen holders and chucks as the standard specimen clamp (see above) and is mounted on the standard specimen clamp with screw (a) in position 1.

UM specimen holders are affixed with screw (b) and GMA chucks and HistoRange specimen holders 2218-150, 2218-151 and 2218-152 are affixed using screw (c). Screw (d) locks and releases the position of the arc. An adjustment scale is located on the lower left-hand side of the arc.

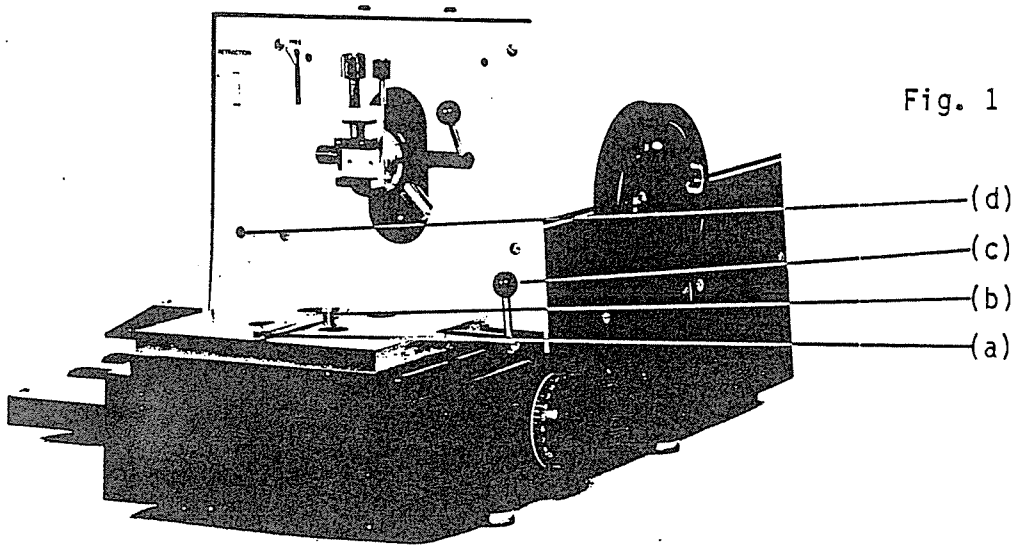


Fig.2
Standard knife stage

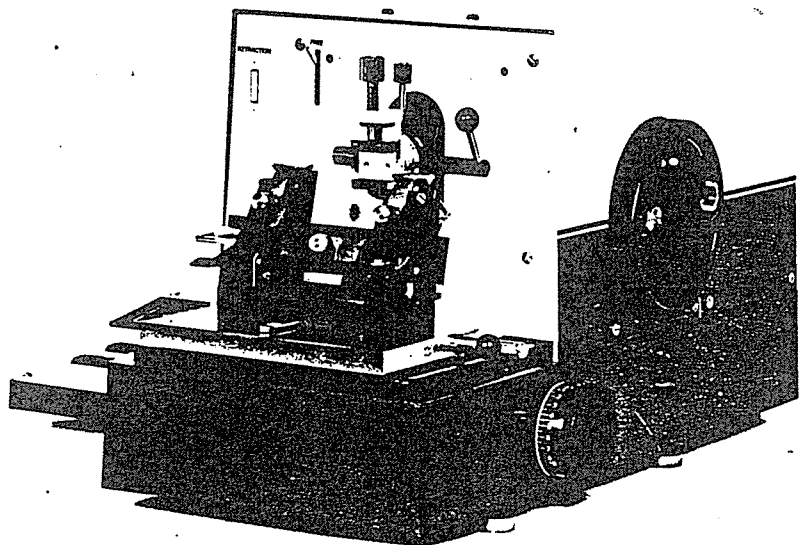
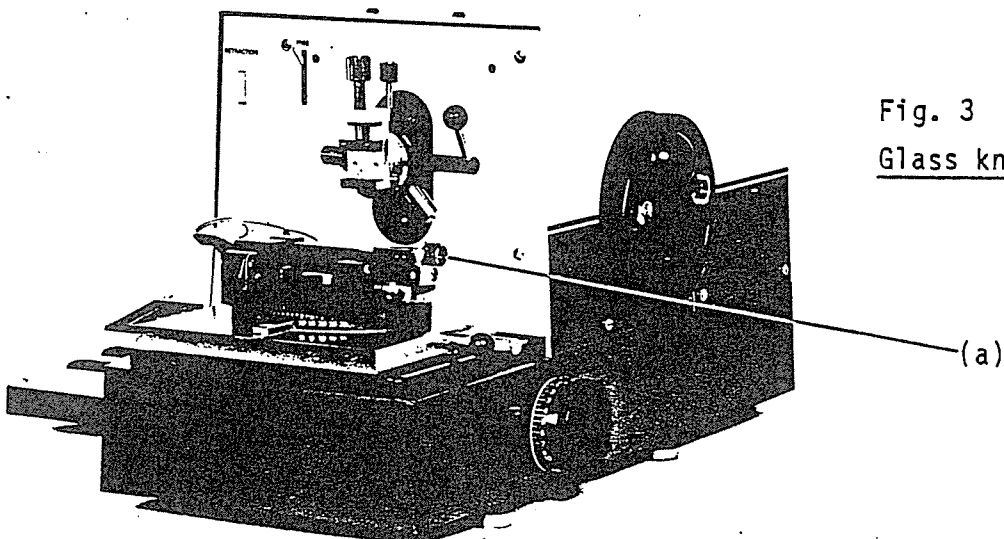


Fig. 3
Glass knife stage



KNIFE STAGES

Standard knife stage (Fig. 2)

The standard knife stage is intended for use with all steel knives (reusable and disposable) as well as with the LKB 2078 HistoKnife-adapters.

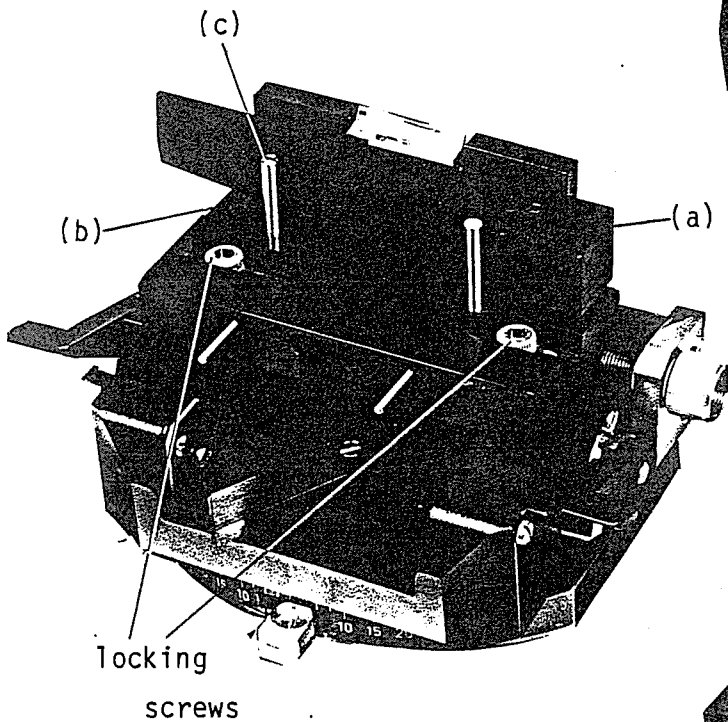
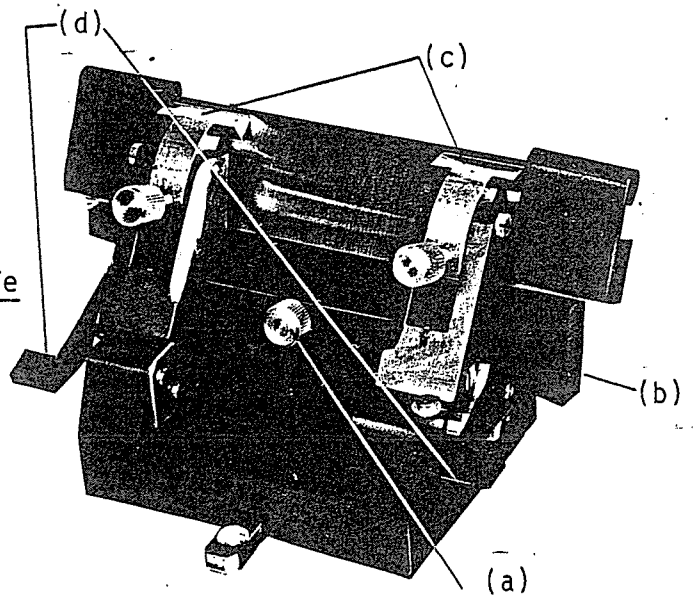
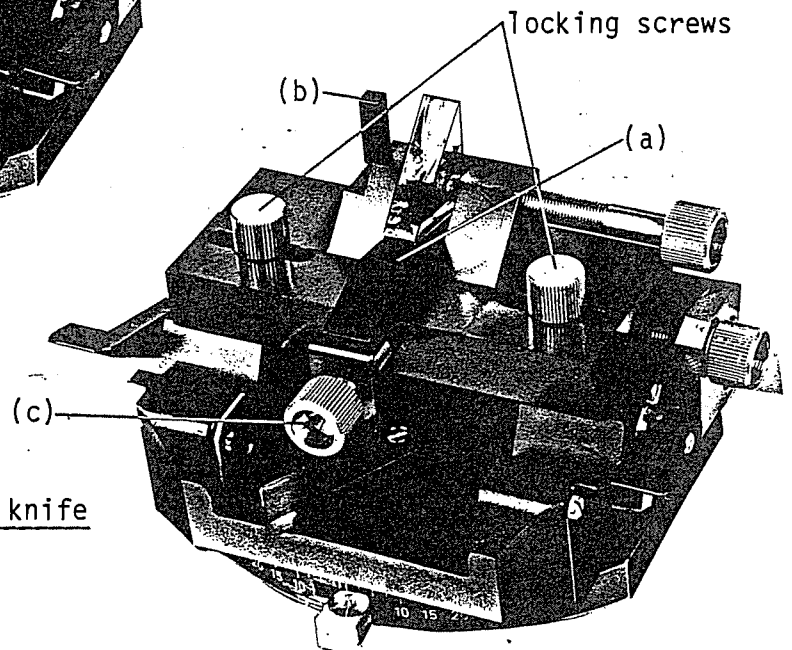
To mount this knife stage, slide the knife stage groove over the guide rail (Fig. 1,a) and locking knob (b) of the knife feed table. (If necessary, adjust the height of the locking knob to allow free passage). Lock the knife stage in the desired position with lever (Fig. 1,c) by pressing counterclockwise. Insert the backlight cable (Fig.1,d). Note: If rotation of the knife stage is desired, remove guide rail (a) and allow the knife stage to rotate around the locking knob.

Glass knife stage (Fig. 3)

The glass knife stage is supplied with a holder for triangle glass knives and the holder for Histoglass knives.

To mount the glass knife stage, slide the knife stage groove over the guide rail (Fig. 1,a) and locking knob (Fig. 1,b) of the knife feed table. (If necessary, adjust the locking knob to allow free passage). Lock the knife stage in the desired position with lever (Fig. 1,c) by pressing counterclockwise.

To replace a glass knife holder remove locking screws (see Fig. 2 & 3, page 16). Move the holder to the left by turning screw (Fig. 3,a) until it releases. Pull off. The desired knife holder is pushed into its track from the left, attached to screw (a) and tightened.

Fig. 1 Steel knifeFig. 2 HistoKnifeFig. 3 Triangular glass knife

KNIFE INSTALLATION

Steel knives (Fig. 1)

Steel knives are mounted directly on the standard knife stage.

To install a steel knife, slide in from the side or insert from above.

Adjust the knife height by opening screw (a) and adjusting the wedge (b) so that the knife edge is just above the clamps (c). Tighten screw (a) and the clamp screws.

To adjust the clearance angle, unlock the levers (d) and tilt the knife to the appropriate angle. A scale is located on the left side of the knife stage. 10-12° are suitable angles for sectioning wax with standard steel knives (C type). As much as 15° can be appropriate for work with disposable steel knives. Knife protectors (2218-205) are available to guard against sharp protruding knife edges.

Histoknives (fig. 2)

The Histoknife holder supports 25 and 38 mm wide glass knives. All thicknesses of glass (up to 10 mm) can be used with this holder. The HistoKnife holder is also designed to accommodate the HistoTruf (2218-202,203).

To adjust the opening of the knife holder, use the four allen screws on the back of the knife holder. Note that there are two positions (with separate screw holes) for these screws. The outer position is for 38 mm knives and the inner position for 25 mm knives.

To install a HistoKnife, place the knife in the opening of the holder with the curved facet of the knife facing forward. Insert wedge (a) between the knife and the support pins. For sectioning stability it is important that knives are of an appropriate height and that the wedge supports the knife evenly.

If the wedge does not rest firmly on both pins, it is possible to alter the wedge space by adjusting the position of the left hand pin.

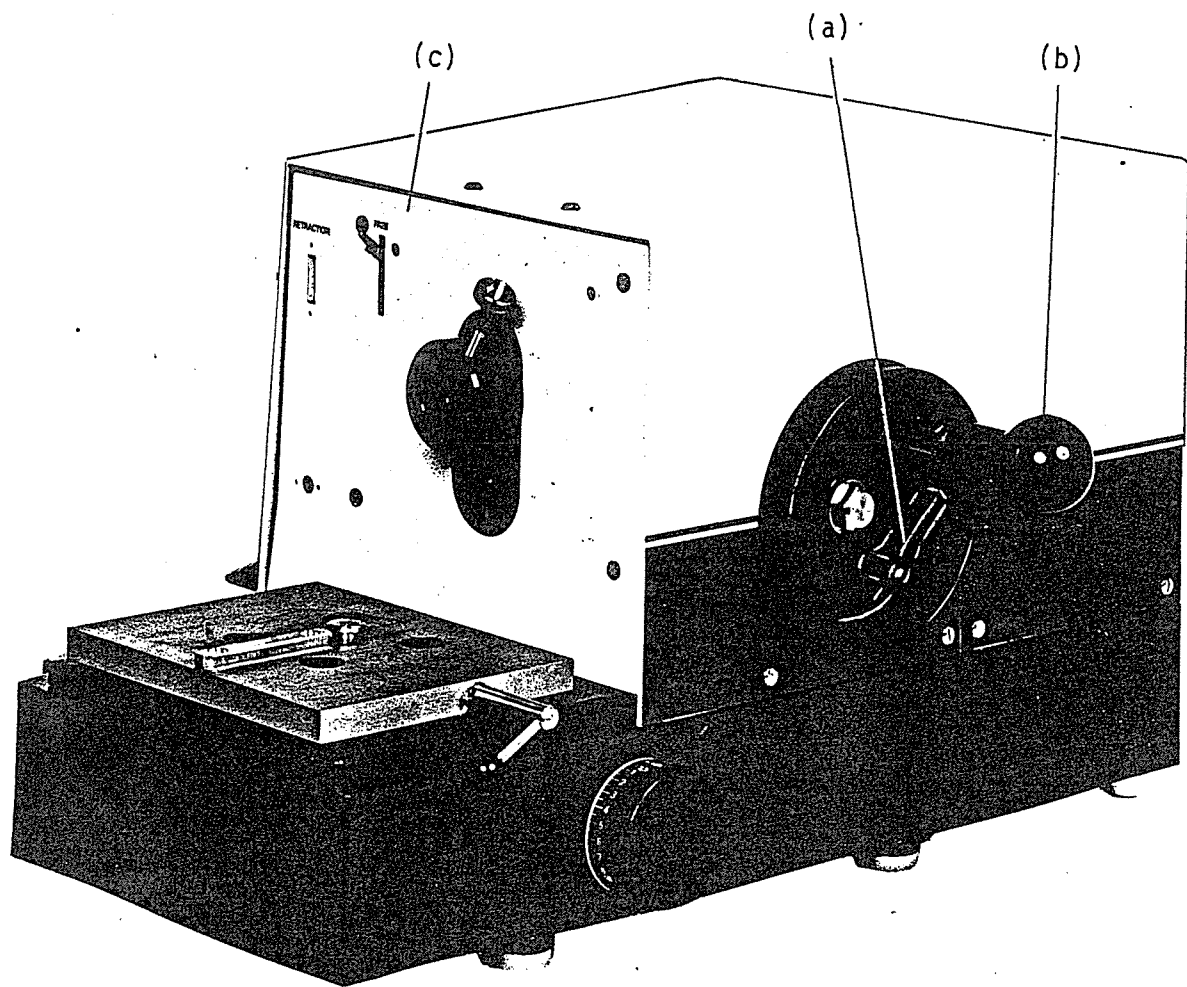
To adjust the position of this pin, release the pin lock with an allen key (b) and reposition with a screwdriver (c). Use the allen key to lock the pin in its new position. (b).

For mounting HistoTrufs, see page 22.

Triangular glass knives (Fig. 3)

Triangular glass knives are mounted on the glass knife stage with a triangle knife holder. This holder is constructed to support 45°, 50° and 55° glass knives. 45° are supported directly on the base of the knife holder. 50° and 55° knives are supported with wedge (a).

To ensure that the knife edge is always at the rotation center, regardless of the clearance angle, use the knife height guide (b) on the left-hand side of the holder. To adjust the height of 50 and 55° knives, it is possible to slide the wedge forward or backward slightly with screw (c).



SPECIMEN-SLIDE MOVEMENT

Handwheel

The HistoRange can be operated either automatically or manually. Rotation of the handwheel for sectioning is clockwise. Lever (a) connects the handwheel to the motor. For automatic operation, this lever must be locked. To lock lever (a) press clockwise, or counterclockwise. (The locking position of the lever can be changed by pulling out and releasing at the desired locking point). When operating manually this lever is usually locked. If, however, a lighter rotation movement is desired, the lever can be released. Handle (b) can be folded into the wheel when not in use.

Specimen retraction

During the upward-return stroke, the specimen moves backward from its sectioning position and a RETRACTION light is lit. During the downward sectioning stroke, the specimen is in its sectioning position and the RETRACTION light is off. NOTE: Do not approach the specimen block unless the light is off and the specimen is in its sectioning position. If an approach is made during the specimen retraction cycle, 250 μ m extra will be cut off the face of the block during the first stroke.

Specimen slide lock

The specimen slide can be locked in both its upper and lower positions. To lock in the upper position, turn the handwheel clockwise until the RETRACTION light goes off. Move bar (c) down to its lock position. To lock in the lower position slide the bar to its lock position just before the retraction light goes on.

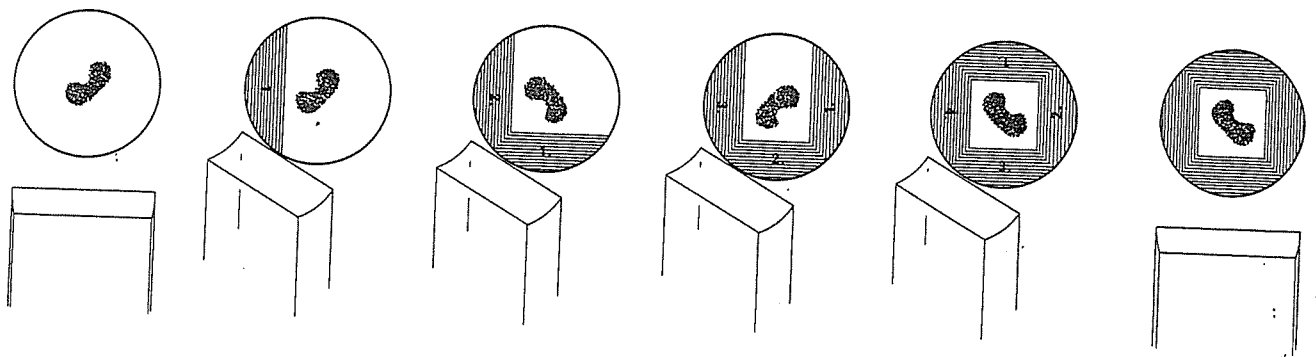


Fig. 1 Pyramid trimming

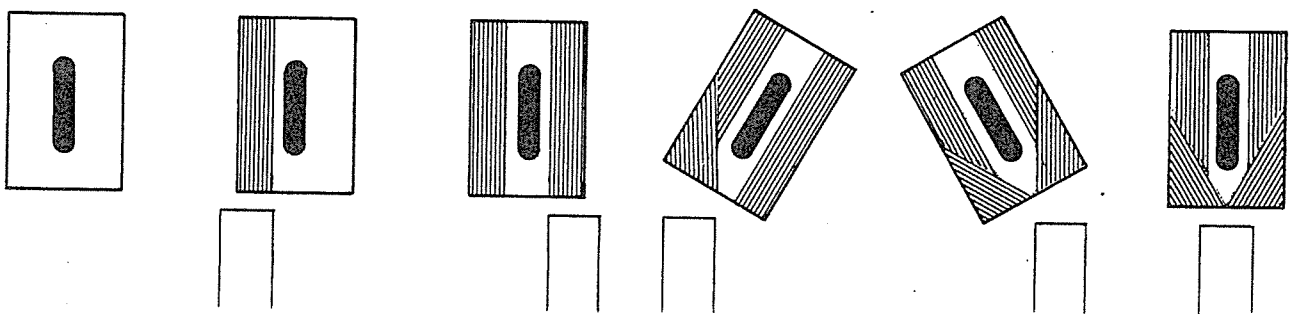


Fig. 2 Mesa trimming

OPERATING HINTS

Knife approach and alignment

For a precision knife-to-specimen approach, it is possible to observe - with the help of the HistoRange back lighting system - a thin line of red light between the knife edge and the specimen block face. The light, observed directly from above, gives a clear indication of both the parallel orientation of the knife and the specimen as well as the distance between the knife and the specimen. During the approach, rock the handwheel (without entering the retraction cycle) and advance the knife by pressing the FORWARD button. For continuous advances press the X- and FORWARD buttons simultaneously. If the blockface reflects, it is also possible to monitor the approach by observing the reflection of the knife edge on the block face.

Trimming

Manual rough trimming can be performed on the HistoRange by turning the handwheel clockwise until the retraction light goes out. Without entering the retraction cycle, rock the handwheel while pressing the FORWARD button. To speed up the advance, press the X-button simultaneously. Note. Do not press the FORWARD + X buttons unless the specimen is above the knife edge.

Autotrimming

For efficient trimming of hard specimen blocks, it is possible to use a rapid automatic cutting speed together with a thin section thickness. The HistoRange cutting speed and section thickness can be altered at any time during autotrimming.

Pyramid and mesa trimming (Fig. 1 and 2)

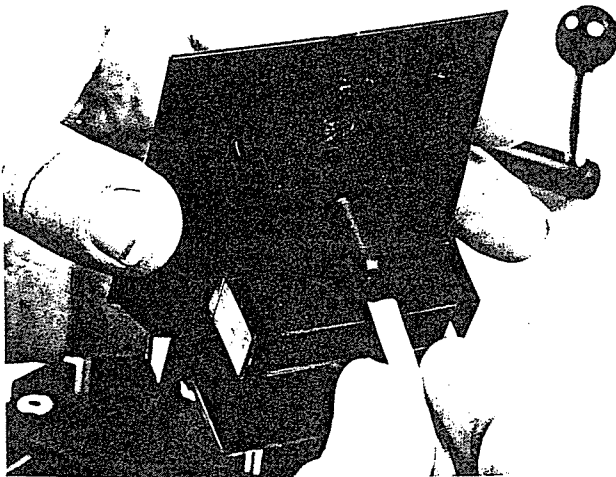
HistoRange will easily cut a pyramid block (minimal cutting surface) using glass knives. The oblique sides of the pyramid are cut by turning the knife stage to an appropriate angle and rotating the specimen block. Mesa trimming is especially useful for sectioning hard plastic embedding materials where it is not only important to minimize the overall cutting surface but also to reduce the surface of initial knife impact. For mesa trimming, adjust the clearance angle to 10-15°. Align the specimen block parallel to the knife edge. Cut off excess plastic manually or by autotrimming (as described above).

Wet sectioning with Histoknives

Wet sectioning has proven to be an excellent method for the serial sectioning of small wax-embedded specimens, where thin ($1-3\mu\text{m}$), high quality sections are required. This method can also be used to facilitate the production of sections from polymer-embedded specimens. The design of the HistoRange glass knife stage and the glass knife holder (to easily accommodate HistoTrufs), together with a large retraction cycle and an appropriate cutting zone, make HistoRange particularly suitable for this technique.

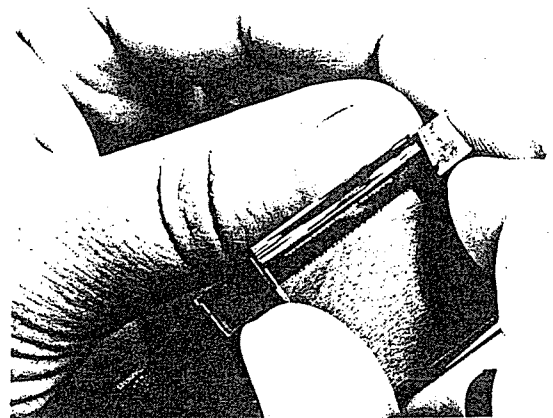
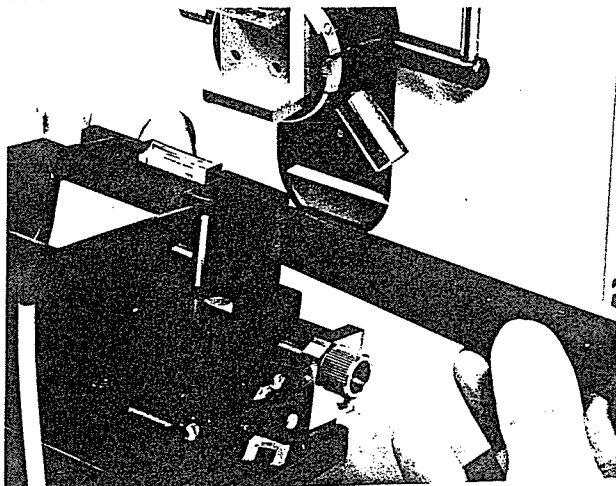
Wet sectioning is performed using the glass knife stage with a HistoKnife holder and Truf. Additional accessories include: Syringe and tubing, dental wax, spatula, spirit lamp and a camel hair brush.

Note: Before sectioning wax embedded specimens, cut off excess embedding medium from the sides of the specimen. Cut the leading and trailing edges parallel to one another, leaving a 2mm margin between the specimen and cutting edge.

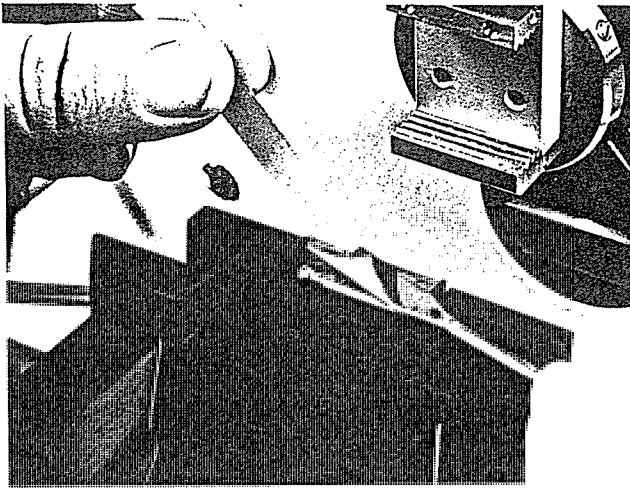


1. Before mounting the HistoTruf, connect the silicon tubing.

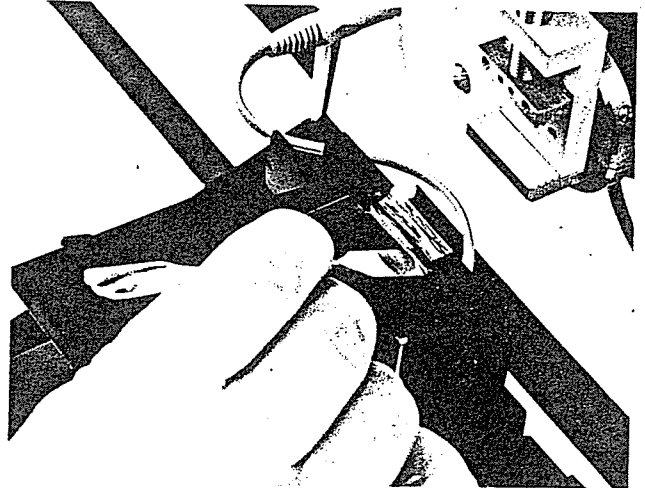
2. Press the HistoKnife firmly onto the HistoTruf with the curved knife facet facing towards the Truf.



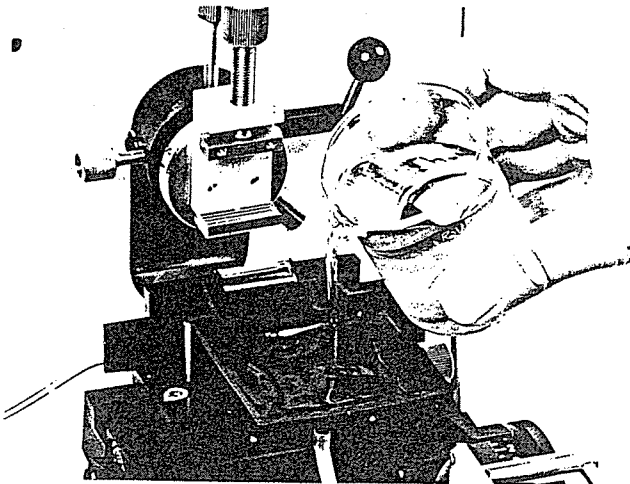
3. Slide the knife (with the Truf) into the opening of the glass knife holder. Ensure that the Truf rests firmly on the guide pins of the knife holder. Insert the wedge.



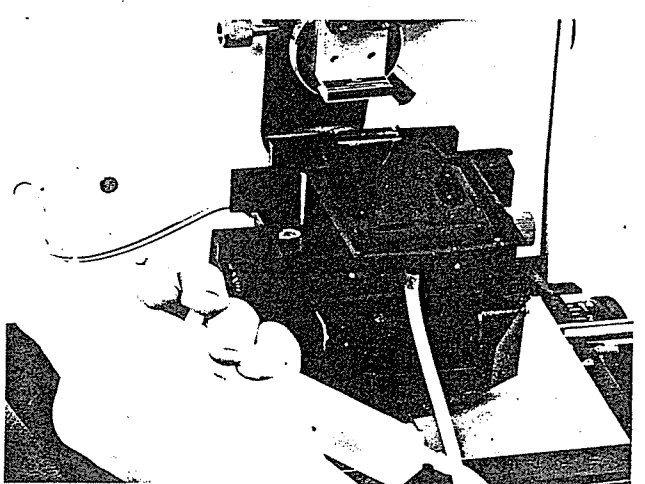
4.
To seal the knife and Truf warm a spatula and melt dental wax on the tip of the warm spatula.



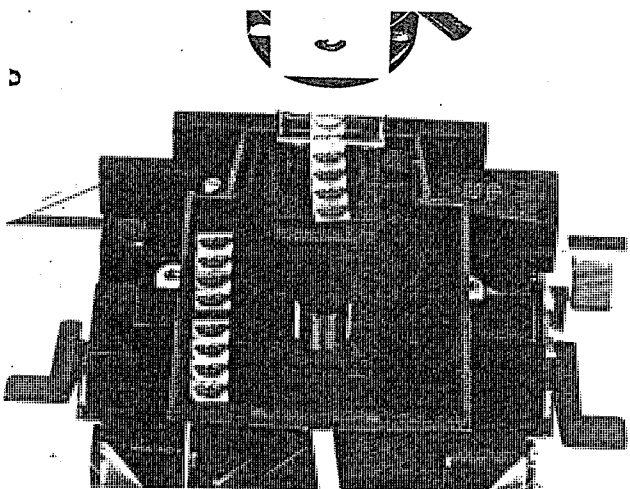
5.
Apply wax along the HistoKnife/Truf seam. Adjust the clearance angle to 4-6°.



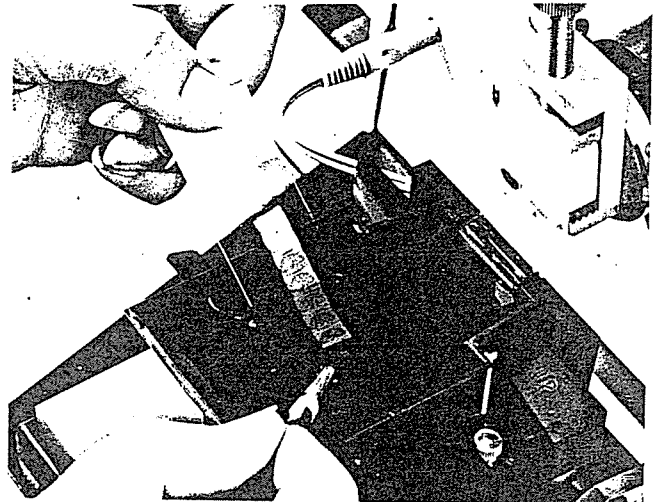
6.
Fill the Truf from above with the section float liquid.



7.
Use the syringe to adjust the liquid level to the knife edge. Note that too low a liquid level can cause compression in wax-embedded specimens.



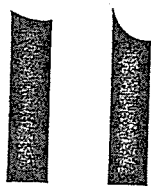
8.
To avoid compression and knife damage the cutting speed should be fairly low. (2-4 mm/s).



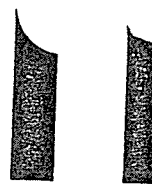
9.
To collect sections, submerge a glass slide into the liquid and carefully maneuver the ribbon of sections onto the slide.

Choosing a Histoknife

An optimally-shaped knife edge is the most critical requirement for successful sectioning with HistoKnives. A low knife edge is stronger and lasts longer - especially when sectioning hard materials. A higher knife edge, on the other hand, causes less compression especially when sectioning soft materials. The knife profile is not only dependent upon the fulchrum and the breaking positions of the knife, but also upon the thickness of the glass strip from which knives are produced. The quality of the knife edge can be easily examined in a microscope. A good edge will be smooth without nicks or other defects.



Effect of fulchrum
and breaking position



Effect of glass thickness
(same fulchrum and breaking
positions).

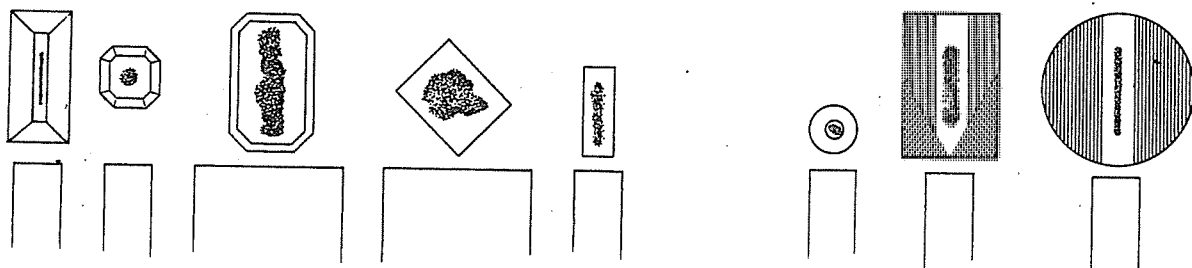
Sectioning plastics with glass knives

Plastic-embedded specimens can be sectioned with both triangular glass knives as well as with HistoKnives. Where the dimensions of the block permit, it is preferable to use triangular glass knives. The recommended angle of a triangular glass knife is 50°. For appropriate HistoKnife profiles see above. A glass knife prepared 1-2 days before sectioning may produce smoother sections than a freshly prepared knife.

Before sectioning it is advisable to trim off excess plastic (excess plastic increases the cutting surface and may cause sections to loosen from the microscope slide during the staining process.)

For trimming see page 21.

When mounting a plastic embedded specimen, it is important to reduce the surface of initial knife impact as shown below.



Water-soluble plastics

Non-water-soluble plastics

Water soluble plastics - The recommended cutting speed for water-soluble plastics is 20 mm/s. Should it be difficult to collect sections (i.e. due to curling), reduce the cutting speed to 4mm/s and use fine, dry forceps to hold and slightly stretch each section until it releases from the knife edge. It is also possible to eliminate curling by momentarily increasing the cutting speed.

Non-water soluble plastics - The recommended cutting speed for non-water-soluble plastics is 2mm/s. To minimize friction between the specimen block and the knife, apply a suitable wetting solution (i.e. ethanol, detergent solution) to the block face, knife edge and knife facet with a camel hair brush. To prevent folding, lead each section down the knife face with the wet brush. Collect sections with forceps.

Note: When detergents are used as wetting solution, sections must be rinsed with distilled water before mounting on a slide.