EMCorp

Electron Microscope Supplies Division

Micro Cut

H1200 Vibrating Microtome

Instruction Manual

H1200 VIBRATING MICROTOME

INSTRUCTION MANUAL

Contents :-

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RUBBER VIBRATION DAMPING PADS

Place between the existing rubber feet of the H1200 Vibrating Microtome and the table top to further reduce vibration and improve performance.

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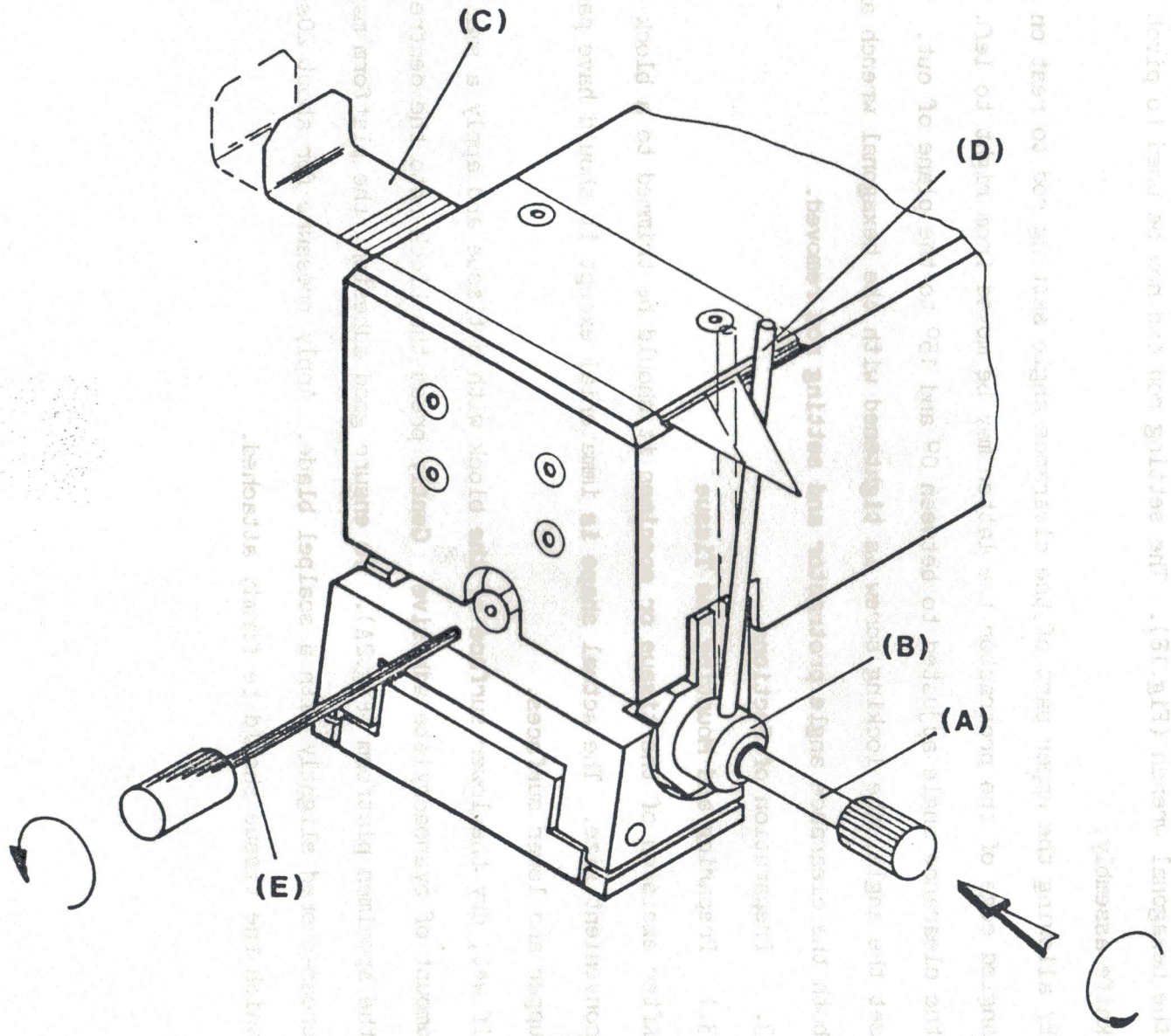


Fig. 1

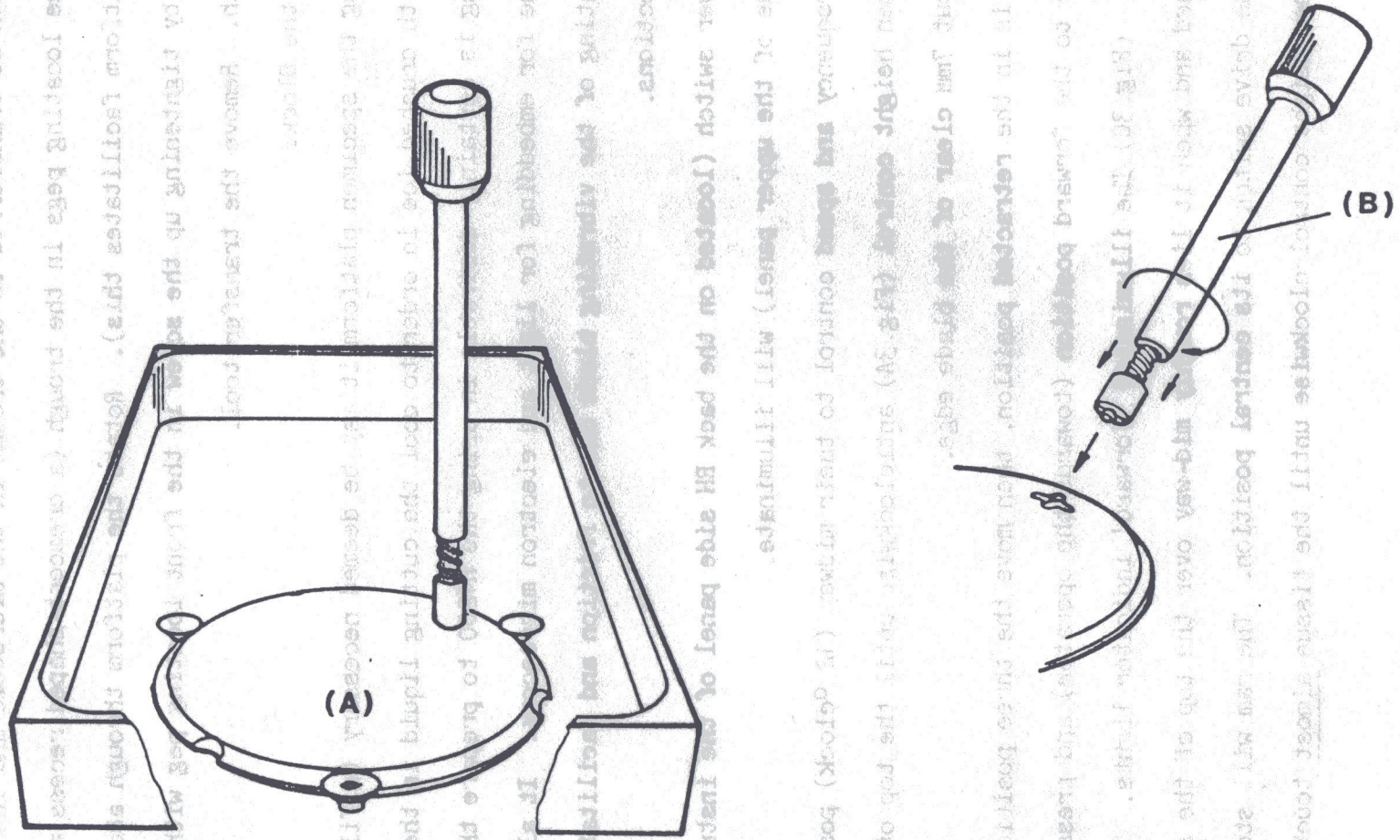


Fig. 2

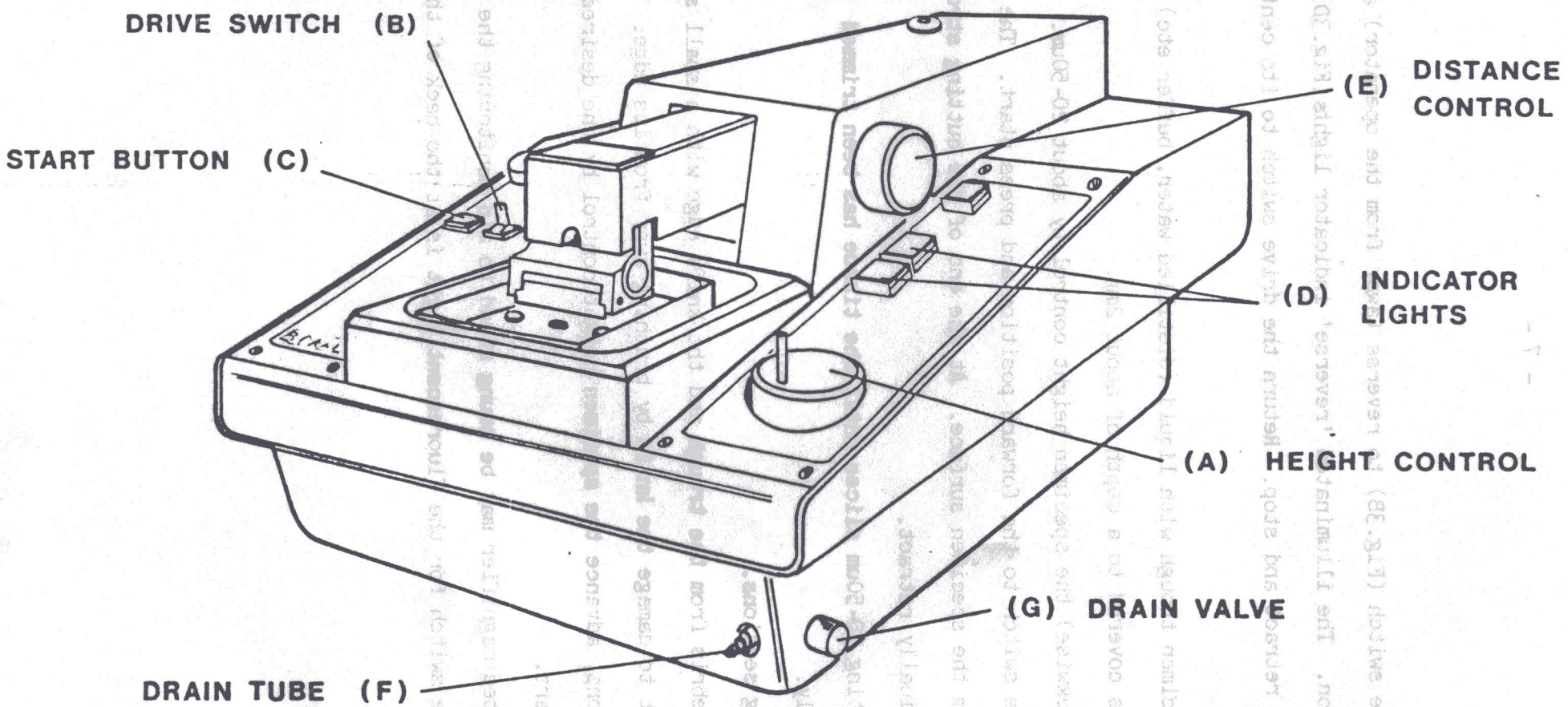

DRIVE SWITCH (B)
START BUTTON (C)
(E) DISTANCE CONTROL
(D) INDICATOR LIGHTS
(A) HEIGHT CONTROL
(G) DRAIN VALVE
DRAIN TUBE (F)

Fig.3

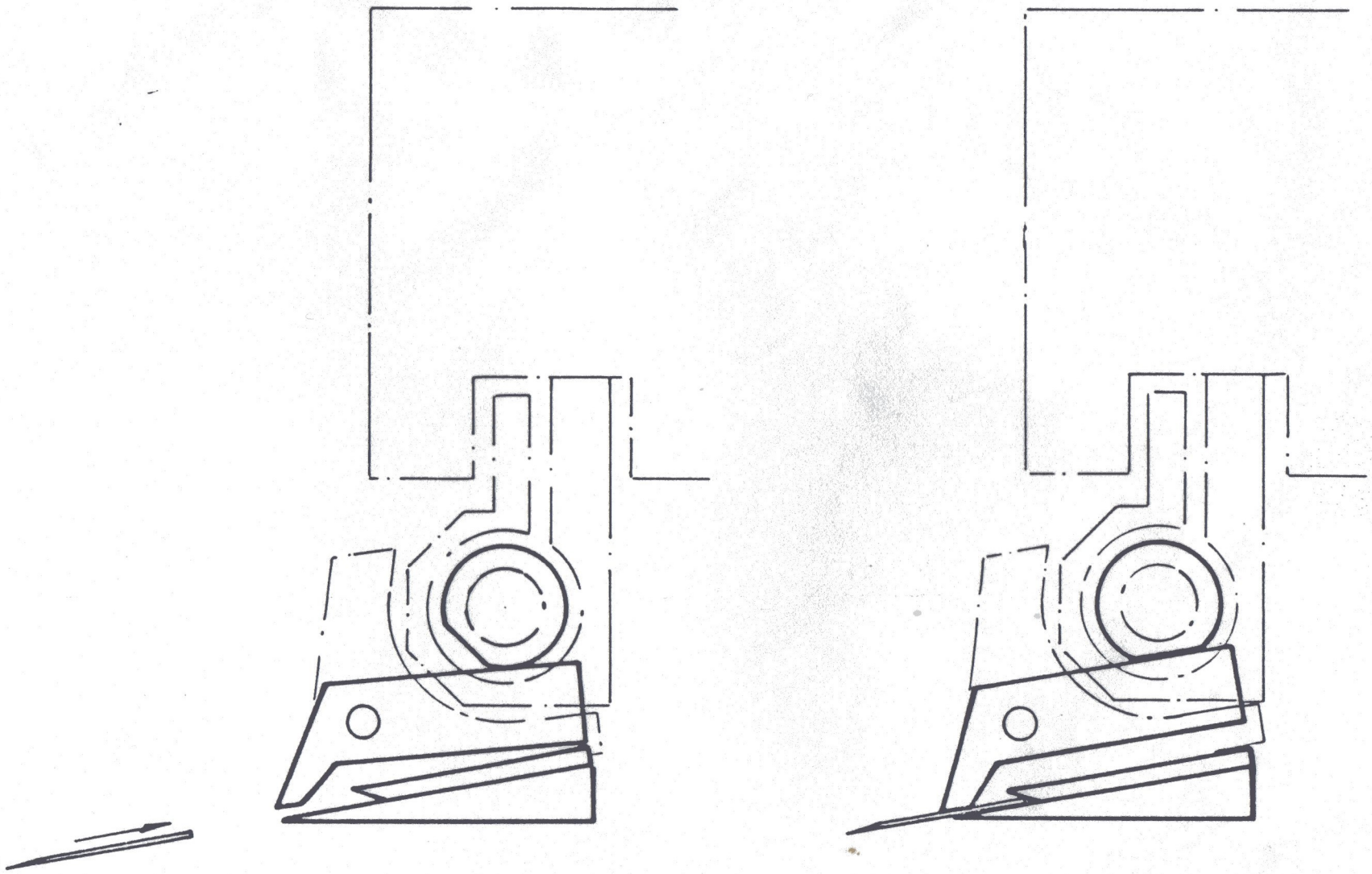


Fig.4

General Description of the H1200 Vibrating Microtome

The H1200 'Micro-Cut' is a vibrating blade microtome capable of preparing sections in the range 10 to 1000 micrometres from a wide variety of both biological and materials specimens.

The blade is clamped at a pre-set angle on a horizontally-disposed ram which runs on precision-engineered cross-roller linear bearings. This allows no vertical blade movement during cutting and enables the entire blade width to be used for wide, thin, distortion-free sections.

The blade holder is driven from side to side by a high power motor under pulse-proportional control. This provides a smooth positive action, even at low speeds, with hard tissues.

The specimen stage accepts tissues up to 40mm in diameter and the blade has a full 40mm max of forward travel. This can be varied for different sized pieces of tissue. Blade retraction is automatic after each cutting cycle.

A cooled liquid bath lubricates the blade, preventing the build-up of heat by friction and facilitating the retrieval of the sections.

Unpacking and Initial Installation

Carefully unpack the microtome and attach a 13A plug with 1 Amp fuse for the UK and 2 Amp fuse for USA.

Voltage selection 220/110V

1. Component Parts of the H1200

The microtome is composed of the following main parts :-

	Description	Parts No.
A)	The microtome body	
B)	The ram	
C)	The cooling bath	
D)	The trough	
E)	The specimen pallat	
F)	The rotating knife holder	
G)	The knife	
H)	The illuminated magnifier	

Additional tools and spares are :-

i) The specimen platform transfer tool (sprung loaded)
ii) Clearance angle protractor
iii) Clearance angle setting rod
iv) Hexagonal wrench
v) Blade clamping key
vi) Disposable blades
vii) Additional specimen pallets
viii) Cyanoacrylate adhesive

2. Setting up the Microtome

2.1 Inserting a Disposable Blade.

The H1200 uses disposable single edged blades which should be replaced regularly, particularly when cutting hard specimens or those containing crystalline materials.

If necessary the blades may be cleaned free from the protective film of oil by soaking them for 5min in chloriform in a Petri dish. Take care not to damage the edge of the blade.

To replace a blade in the microtome, first insert the blade clamping key (Fig 1A) into the pivot at the side of the knife assembly (Fig 1B) and twist the key through 90°. The jaws on the clamp will open and the old blade may be removed with a pair of curved forceps. Remove any tissue debris from the knife -clamping region and the inside surfaces of the jaws with 25% alcohol and a tissue.

With the knife clamp still open, slide a new blade from the dispenser and pick it up with curved forceps taking care not to touch the cutting edge. Insert the new blade in the clamping slot and ensure the back edge of the blade is flat against the back stop of the clamp (i.e. parallel to the jaws). While still holding the blade in position, turn the clamping key back through 90° to clamp the blade tightly. Remove the key.

2.2 Setting the Clearance Angle.

Insert the clearance angle protractor (Fig.1C) into the slot on top of the ram with the angled edge facing away from the operator . Insert the clearance angle setting rod (Fig.1D) in the hole of the pivot at the right hand side of the blade clamp. Undo the locking screw at the front of the blade clamp with the hexagonal wrench (Fig.1E). The setting rod can now be used to pivot the knife assembly.

By allowing the upper part of the clearance angle setting rod to rest on the angled edge of the protractor the latter may be moved from right to left and the clearance angle adjusted to between 0° and 15° to the plane of cut. Having set the angle, the locking screw is tightened with the hexagonal wrench and both the clearance angle protractor and setting rod removed.

3. Preparation of Sections

3.1 Preparing and Mounting the Tissue

After excision of the tissue or specimen it should be trimmed to a block of convenient size. The actual shape is immaterial except it should have parallel upper and lower surfaces.

If wet, dry the lower surface of the block with a tissue and apply a small amount of cyanoacrylate adhesive. Gently press the block onto the centre of the specimen platform (Fig.2A). To ensure good adhesion, the platform may be cross-scored slightly with a scalpel blade. Apply pressure for about 20s after which the tissue should be firmly attached.

Pick up the platform with the transfer tool (Fig.2B) by pressing its sprung loaded end into the slot in the platform and turning the tool through 90°. The platform may then be transferred to the trough in the microtome and inserted inside the three locating pegs in the trough (a crescent-shaped recess at the edge of the platform facilitates this). Rotate the platform through about 5° then clamp it by tightening up the screw in the front locating peg with the hexagonal wrench. Remove the transfer tool.

3.2 Trimming the Block.

Before inserting the specimen platform it may be deemed necessary to fill the cooling bath with crushed ice in order to cool the cutting liquid in the trough. Cooling is certainly desirable in using the H1200 to prepare thin slices of tissue for embedding for light and electron microscopy. It also reduces any heating of the vibrating blade due to friction and facilitates retrieval of sections.

Turn on the power switch (located on the back RH side panel of the instrument. A light (RH side of the upper panel) will illuminate.

Set both the frequency and speed control to their midway (12 °clock) positions.

Turn the specimen height control (Fig.3A) anticlockwise until the top of the specimen is about 7mm clear of the blade edge.

Ensure the ram is in the retracted position, then move the three position drive switch (Fig.3B) to the forward position (towards the operator) and press the 'start' button. (Fig.3C) The illuminated 'forward' indicator lights. The ram will move forward and when it lies roughly mid-way over the top of the specimen block return the drive switch to its central position. The ram will stop.

Turn the specimen height control clockwise until the tissue <u>almost</u> touches the knife edge.

Set the drive switch (Fig.3B) to reverse (away from the operator) and press the 'start' button. The illuminated 'reverse' indicator lights (Fig.3D).

The ram will retract and stop. Return the drive switch to its central position.

Fill the specimen trough with liquid (distilled water, buffer etc) until the knife edge is covered to a depth of about 2mm.

Advance (clockwise) the specimen height control by about 20-50um.

Set the drive switch to the forward position and press start. The knife will begin to trim the specimen surface. At the end of the cutting stroke, the ram will automatically retract.

Continue cutting 20-50um slices until the tissue has been trimmed satisfactorily.

3.3 Cutting sections.

Remove any debris from the trough and the knife edge with a small sable brush. Take care not to damage the knife by brushing away from its edge.

To cut sections, advance the specimen height control by the desired thickness and press start.

The illuminated magnifier may be swung round to aid monitoring the sectioning process. The switch for the fluorescent light is at the back of the lens mounting.

The operator has full control over the frequency of vibration, the forward speed and the distance travelled by the ram. The distance control is mounted on the RH side of the ram housing (Fig.3E) and can be set at 25, 30, 35 or 40mm. Do not alter this control while the ram is moving and only when it is in the retracted position. The frequency and speed controls are set on the LH side of the top panel and are scaled 1 - 10. For calibrations see Tables 1&2. The optimum setting for frequency, speed and clearance angle must be determined by the operator through experience of his tissue. Generally the settings will be determined by the specimen hardness although to generalise, the following parameters would make a useful starting point :

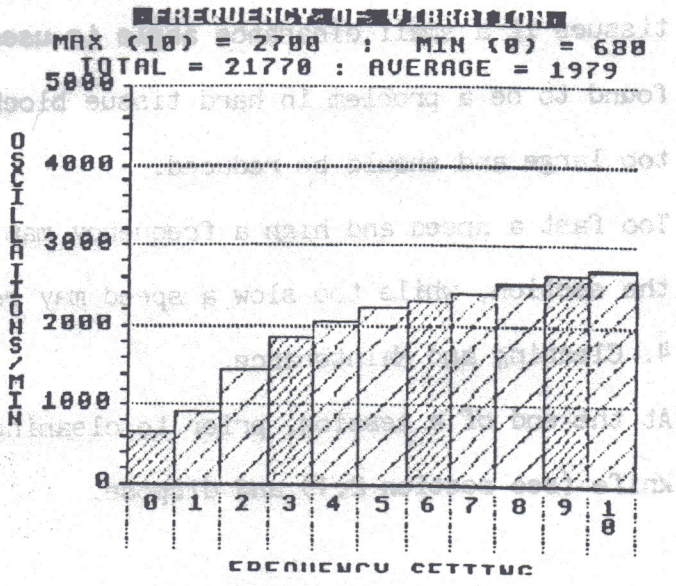
	H1200 Setting	Actual Value
Clearance angle	7°	7°
Frequency	5	2240 osc. min $^{-1}$
Speed	5	214mm. min $^{-1}$

Sections can be collected from the trough with a sable brush and transferred to fixative, alcohol distilled water etc.

Table 1.

Frequency of Vibration

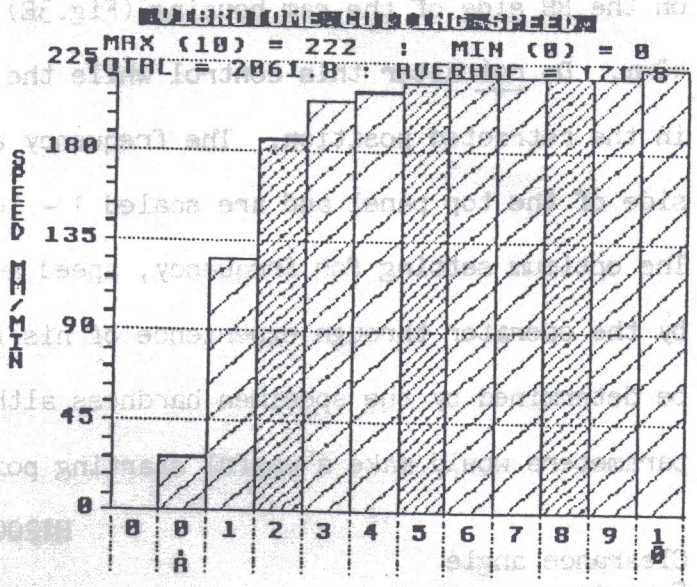
Frequency Setting	Oscillations min $^{-1}$
1	680
2	930
3	1440
4	1840
5	2060
6	2240
7	2320
8	2400
9	2520
10	2640
11	2700



6/15 - Spinal Nerve in gelatin S=.9 (30μ) f=3.4

Table 2.

Ram Speed Setting	Speed ($mm.min^{-1}$)
(0.8)	26.8
2	126
3	186
4	205
5	210
6	214
7	218
8	218
9	218
10	218
(11)	222



3.4 Sectioning Problems

Problems often encountered in vibrating micotomy include, 'chatter' (alternate thick and thin areas on the section), disintegration of the tissue, and failure to cut sections.

A combination of fast speed and frequency normally increases the amount of chatter in softer tissues. This may also be found to be a problem in softer tissues if a small clearance angle is used. Alternatively, if chatter is found to be a problem in hard tissue blocks the knife angle will be found to be too large and should be reduced.

Too fast a speed and high a frequency may cause, mechanical disintegration of the section, while too slow a speed may result in no sections being cut.

4. Cleaning and Maintenance.

At the end of a session, prior to cleaning of the instrument, always remove the knife (see section 2.1) and dispose.

Drain the ice-water from the cooling bath by fitting a length of tubing to the nipple on the front of the instrument (Fig.3F) and opening the tap on the RH side (Fig.3G). Drain into a flask.

Pipette liquid from the trough and remove the specimen platform using the hexagonal wrench. For routine operation it is sufficient to remove any large debris from the trough and wipe clean with a tissue. Occasionally, however, it may be considered necessary to remove the trough for thorough cleaning by ultrasonication. to facilitate this, remove the central screw using the hexagonal wrench.

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