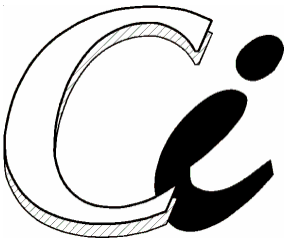

CAMPDEN INSTRUMENTS LIMITED

INSTRUCTION MANUAL

FOR

752 VIBROSLICE TISSUE CUTTER
(Manual tissue transport)

752/M VIBROSLICE TISSUE CUTTER
(Motorised tissue transport)

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The information provided in this document is intended to be a guide on how to use the Vibroslice instrument safely and effectively and is subject to change without notice. Campden Instruments does not accept liability for any damages arising from this document or the information contained in it. Campden Instruments will not accept liability for damages arising out of misuse of this instrument.

Read and understand this instruction book before using the instrument. Only competent and capable personnel should use the instrument.

This document should be retained for future reference as it contains the name and address of the manufacturer within the EC.

PACKAGING

Please retain original packaging for future use.

Instruments will not be accepted for service or repair unless the unit is returned adequately and properly packaged.

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INTRODUCTION.

The Campden Instruments Vibroslice was designed to cut 50-700µm thick slices of fresh brain tissue, but it has proved useful with a wide range of other tissues such as kidney or liver. It is also useful with fixed tissue where sections down to 20µm thick help with the penetration of reagents for histochemistry.

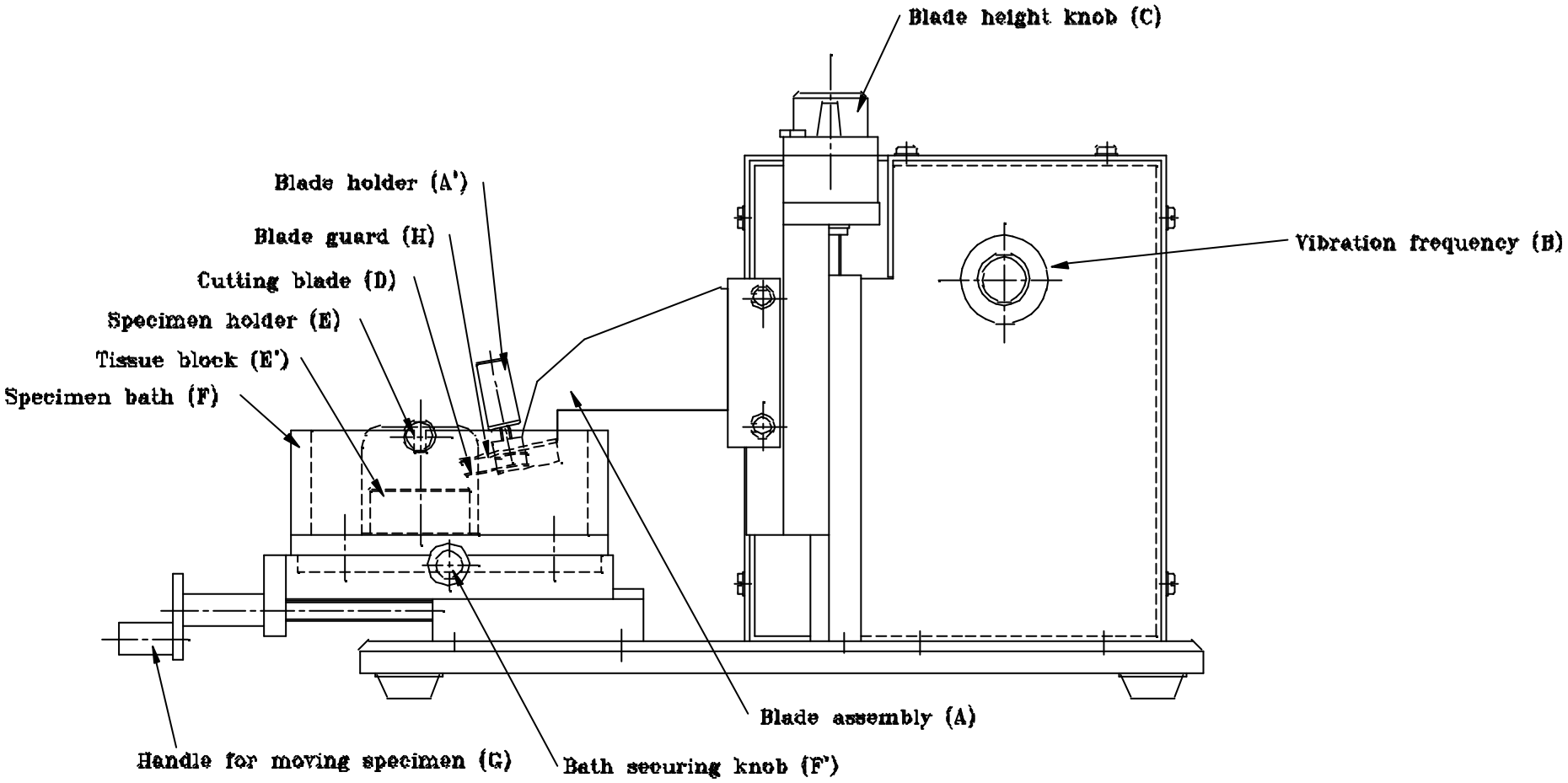
Vibrating blade slicers such as the Vibroslice appear to cause less damage than some alternative methods such as tissue choppers, this has proved valuable in preserving neurones in certain brain regions.

As the Vibroslice contains exposed moving parts and by its nature a blade capable of cutting tissue it is important adequate safety precautions are taken.
This instrument contains moving parts. Care must be taken not to interfere with their motion.
The blades used with this instrument are extremely sharp and should be handled with care. Dispose of used blades carefully.
Observe manufacturers guidelines when handling chemicals.
Spillage - If the cutting lubricant/preserving liquid , e.g. physiological saline, is spilt over the instrument it is important for electrical safety reasons to ensure that the instrument remains safe to use. To avoid the possibility of electrical shock if a spillage occurs, the unit should be switched off at the mains electrical outlet and disconnected before touching the instrument. The instrument should be inspected and tested if necessary by a suitably qualified technician before it is put into further use.

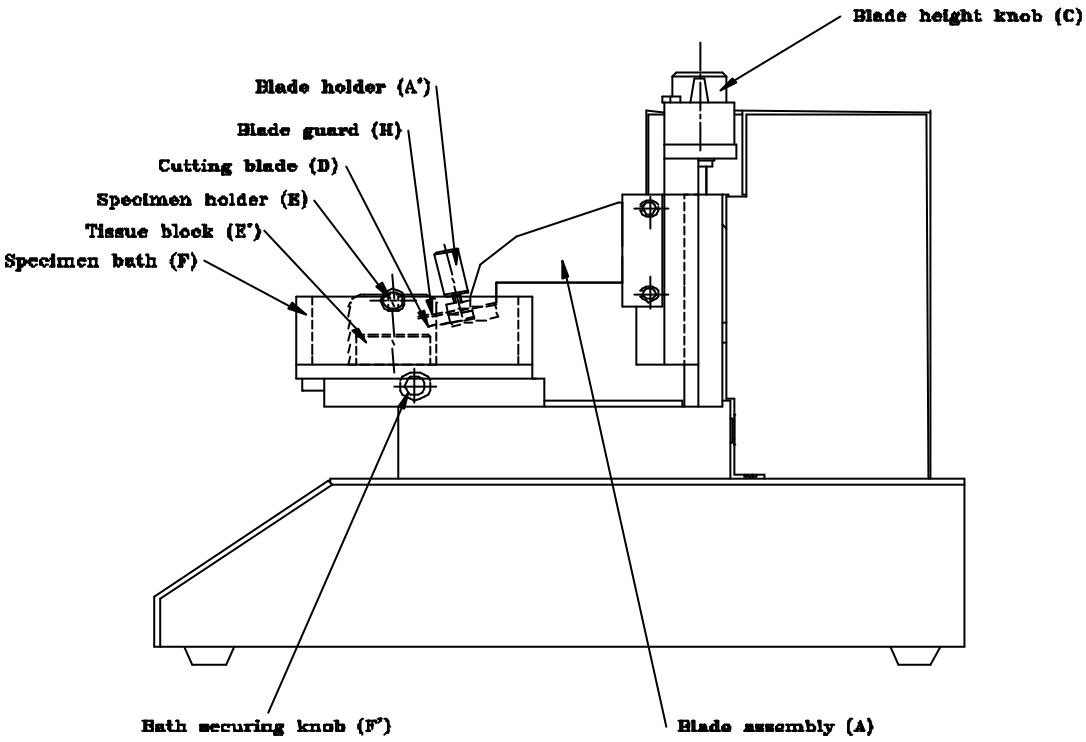
This instrument must not be operated unless it is adequately earthed (grounded).

All electrical instruments and equipment should be periodically tested to ensure they remain safe to use. In some countries this may be a statutory requirement. Your local Health and Safety Executive (or equivalent) will be able to advise on this matter.

User-servicing is limited to changing the drive belts when necessary, and contains no other user-serviceable parts. Contact your dealer or Campden Instruments if you require assistance.


Blade height knob (C)
Blade holder (A')
Blade guard (H)
Cutting blade (D)
Specimen holder (E)
Tissue block (E')
Specimen bath (F)
Vibration frequency (B)
Blade assembly (A)
Handle for moving specimen (G)
Bath securing knob (F')

752 Vibroslice



752/M Vibroslice

Operation
Capital letters in parentheses refer to the illustrations.

1. Manual Vibroslice

Fix a new blade (D) in the slot in the blade assembly (A, A=) and fix the specimen holder (E) in the bath (F). It should be noted that the blade (D) is protected by a blade guard (H). Care should be taken when setting up the instrument and when replacing the blade. You may wish to pre-set the height of the first cut by lowering the blade until it just touches the stage (E=), and then raising it the required distance using the knob (C).

Remove the brain or other tissue and trim as necessary.

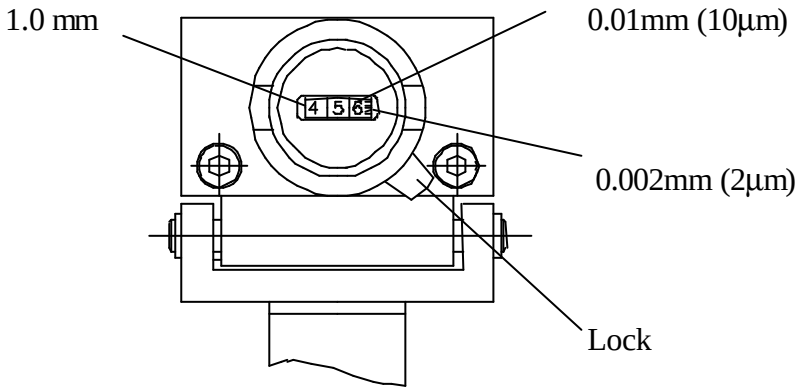
Glue the tissue block to the specimen holder (E=) using a thin film of Super glue adhesive.
Pour cold, freshly oxygenated physiological saline into the bath (F) until the tissue block is immersed.

Set the blade vibrating by depressing the non-latching foot switch and adjust the vibration frequency (B) as required.

Advance the tissue block onto the blade using the handle (G). When the blade begins to cut you may find it necessary to adjust the blade frequency to a more suitable rate.

When the cut is finished, the tissue block should be retracted from the blade by reversing the rotation of knob (G). You may wish to remove the tissue slice before retracting the tissue block.

Lower the blade by the required slice thickness. The numerals on the knob (C) represent 10µm, the small sub-divisions being 2µm as shown below.



Please note that the increments specified are nominal values only.

Repeat the above steps until you have sufficient slices, or you have run out of tissue.

2. Motorised Vibroslice 752/M

Operation of the 752M Vibroslice is generally the same as the 752 except that an electric motor removes the chore of repeatedly advancing and retracting the tissue bath by hand. The bath feed motor is controlled by a three-position switch which controls bath feed direction and a knob (graduated 1-11) which varies the speed of the bath advance.

Push the switch upwards for >bath advance= to cut the tissue. When the cut is finished set the switch to the centre >off= position. Reverse the bath by moving the switch down to >bath reverse=, and holding it down as this position is biased to >off=. Limit switches will stop the carriage automatically at the limits of travel.

The speed of the bath advance is continuously variable from approximately 0.28 to 4 mm/s.
The speed of the bath reverse is at the maximum 4 mm/s.

3.1 Blades.

Suitable blades include single segments of Valet strips, or other razor blades which fit in the blade holder. Spare blades (stainless steel or carbon steel) are available from Campden Instruments. Care should be taken when handling, inserting or removing blades.

3.2 Preparing Tissue

The interval between death and the immersion of the glued tissue should be kept as short as possible to minimise anoxic damage, however total failures are more often due to clumsy handling than to anoxia.

Trimming the block is important because the orientation of the slices is determined by that of the surface glued to the stage. Parasagital cuts have been found useful for transverse slices of rat dorsal hippocampus (glue down the cut midline) and for preserving the planar dendritic trees of Purkinje cells in folia of the cerebellum close to the midline (a Parasagital initial cut should be made which removes the lateral lobes). In some cases it is easier to dissect the target structure free before mounting it on the stage. In others leaving the adjacent tissues on can give useful mechanical support (e.g. with transverse hippocampal slices); Tough connective tissue can cause trouble and should be cut off, or the block can be oriented to keep it out of the way of the cutting of the target structure. In a few special cases, additional support may be needed from external agents, such as embedding in Agar, or in formalised albumin (fixed tissues only).

3.3 Fixing Tissue

The block of tissue must be fixed firmly to the stage. Apply a thin film of cyanoacrylate (Super Glue) adhesive to the stage over an area large enough to accommodate the whole of the cut surface of the tissue block. The C2 grade supplied by Farnell Instruments, Leeds (UK) works well. It has a setting time of the order of tens of seconds, and it is strong enough to hold the tissue firmly, but weak enough to be scraped off the stage without too much difficulty when cleaning up.
Two common problems associated with fixing tissue are:

(i) The tissue block floats away during cutting. This means that the glue has not bonded properly. This is usually due to the specimen being too wet. Use filter paper to draw off the excess liquid from the block before placing it on the stage.
(ii)The glue forms a rigid film up the side of the block, interfering with the cutting process. This probably means too much glue has been used.

3.4 Preparing the Tissue Bath

Slices are cut under liquid to lubricate the blade as it cuts, and in the case of fresh tissue, to avoid anoxia. The tissue block should be covered to a depth of approximately one millimetre; much more than this reduces visibility due to rippling of the liquid surface.
Cutting fresh tissue under cold (<8 degrees centigrade) physiological saline improves the quality of recordings, presumably by reducing the metabolic rate and avoiding anoxic damage. Keeping the saline on ice while oxygenating it may be enough. However, the saline warms up remarkably during the cutting process (to 10 degrees centigrade in 5-15 min depending on conditions). An ice-cold stainless steel block in the bottom of the chamber can help, as can freezing the whole cutting chamber with a few mm of physiological saline in the bottom (some spare chambers are useful in this case). Chilling can cause condensation on the stage interfering with gluing the tissue block; either wipe it dry at the last moment, or keep it out of the freezer and insert it in the chamber just before slicing. In the latter case a silicone rubber insert can be used to leave a gap in the frozen saline. A simpler solution is to buy the 764 Peltier cooling unit which has been designed to fit onto the Vibroslice.

3.5 Speed adjustment

Three factors govern the cutting action: The frequency of the blade vibration (oscillation), the speed of advance of the tissue block onto the blade and the amplitude of the blade movement.
The vibration frequency rarely needs changing. The fastest setting is generally suitable for slices of fresh brain over 200µm thick. Thinner slices may benefit from slower vibration.
The rate of cutting, which is determined by the speed of the tissue advance, is one of the most important factors in successful slice cutting. It should be relatively slow (1 turn every few seconds). If it is too fast the blade tends to deform the tissue, often pushing it over rather than cutting it cleanly. What is meant by >too fast= depends on the local consistency of the tissue; it tends to be slower in tougher tissues. Sometimes local areas of tougher tissues may require withdrawing the blade a little before resuming the cut. It always pays to monitor the tissue block as the cut progresses to check that all is well; a magnifier or dissecting microscope may help, as will good illumination.
The blade movement amplitude is fixed by an eccentric cam to 1mm, this has proved suitable for most applications.
If the blade consistently deforms the tissue block rather than cuts it, several possibilities should be considered. The simplest is to use a shorter block of tissue so that the cuts are made closer to the rigid support of the stage. In extreme cases extra support may be needed, most simply by leaving adjacent tissues attached. In a few cases the tissue may need to be embedded in Agar, or perhaps fix a suitable support on the stage next to the tissue, such as cork or pith (not expanded polystyrene as this disintegrates in contact with cyanoacrylate adhesive). If tissue block deformation is a regular problem, it may be worth modifying the stage or blade holder, in which case contact Campden Instruments for advice.

3.6 Slice Thickness

Slice thickness depends on why they are being cut. For most physiological studies on brain slices, the thickness is a compromise between anatomical integrity (better in thicker slices), and oxygenation of the tissue (better in thinner slices). There are several experimental and theoretical accounts of the >limiting thickness= of tissue slices, which is the thickest slice with a non-anoxic centre (eg; Warburg, 1930; Elliott, 1969; Harvey, Schofield & Brown, 1974). Most researchers usually use 400µm brain slices equilibrated with 95% O2, 5% CO2 at 35 degrees centigrade. If thicker slices are essential, then consider reducing the temperature to reduce the metabolic rate, or use a smaller animal. Slices of up to 750µm have been described (Halliwell, 1975). Thinner slices of around 100µm may be needed for applications involving direct visualisation of cells (Yamamoto & Chuko, 1978; Keenen et al, 1988; Konnerth, 1990), or where the problem is to improve the penetration of reagents for horseradish peroxidase labelling.

3.7 Removing Slices

Slices can be transferred as they are cut, or accumulated on the stage until the end of the run. A small stainless steel or plastic spatula ground to a fine edge resembling a chisel is useful to help slide the slice on and off. Alternatives are fine paint brushes to wrap the slices on or wide-mouthed Pasteur pipettes to suck the slice up in a column of saline.

References

1. Elliot, K. (1969) pp103-114 in Handbook of Neurochemistry vol 12 (Ed. Lajtha).
2. Halliwell,J.V. (1975) J.Physiol. 246, 91-93P.
3. Harvey, J.A., Schofield, C.N. & Brown, D.A. (1974) Brain Res. 76, 235-245.
4. Konnerth, A. (1990) Trends Neurosci. 13:321-323 (1990).
5. Keenan, C.L., Chapman, P.F., Chang, V.C. & Brown, T.H. (1988) Brain Res. Bull. 21,373-383.
6. Warburg, O. (1930) pp75-93 in The Metabolism of Tumours. New York.F
7. Yamamoto, C.A. & Chujo, T. (1978) Exp. Brain Res. 31, 299-301.

Maintenance

The robust construction of the Vibroslice means that little maintenance is necessary apart from keeping it clean. The stage should be scraped clean after each use, removing all traces of glue. All the wetted parts, especially the blade holder, should be rinsed in distilled water and dried.
Do not use any solvents such as acetone or chloroform.

After a long period of use, the drive belts may deteriorate and break. To check or change the belts, disconnect the instrument from the electrical supply and remove the ten screws securing the top cover. Remove the top cover and inspect the condition of the exposed belts - change as required. Note that the 752 manual version has two belts to drive the blade head and the 752/M motorised version has two additional belts to drive the bath slide. Replace the top cover and securing screws.

Specifications

Section Thickness:		Minimum 20µm (fixed tissue), 50µm (fresh tissue) Maximum 700µm
Step size: 10µm		
Chamber dimensions:		55mm x 82mm x 31mm
Chamber volume:		140ml.
Bath advance speed(752M):		0.28 to 4mm/sec continuously variable
Bath reverse speed:		4mm/sec
Vibration speed:		60-3000RPM
Vibration displacement:		1mm
Power requirements:		220V/240V, 50Hz, 0.5A
(factory set)		110V/130V, 60Hz, 0.5A
Permissible Voltage Tolerance:		+10%, -6%
Current Consumption:	752:	0.07A (Average)
	752/M:	0.09A (Average)
Power Ratings:	752:	15W
	752/M:	20W
Fuse Ratings:	752:	Mains 220V/240V ac, 250mA, type 'T' 20mm Mains 110V/130V ac, 500mA, type 'T' 20mm
	752/M:	Mains 220V/240V ac, 250mA, type 'T' 20mm Mains 110V/130V ac, 500mA, type 'T' 20mm

Each Vibroslice is supplied with a Tissue Holder and Bath, 10 individual stainless steel Blades, Foot Switch, Manual and Mains Lead.

Order Codes

Description	Order Code
Manual Vibroslice	752
Motorised Vibroslice	752/M
Peltier Cooling Bath for Vibroslice, (including tissue holder and control unit)	764
Blades for Vibroslice, stainless steel (pk of 50 individual blades)	752/1*
Tissue Holder	752/2A*
Tissue Bath	752/2B*
Tissue Holder and Bath assembly	752/2AB*
Blades for Vibroslice, carbon steel (pk of 50 individual blades)	752/3*
Drive belts for 752/M (pk of 8, two sets of 4)	752/4
Drive belts for 752 (pk of 4)	752/5
Blade guard, plastic (pk of 5)	752/6*

Items indicated * are common to both 752 & 752/M.

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