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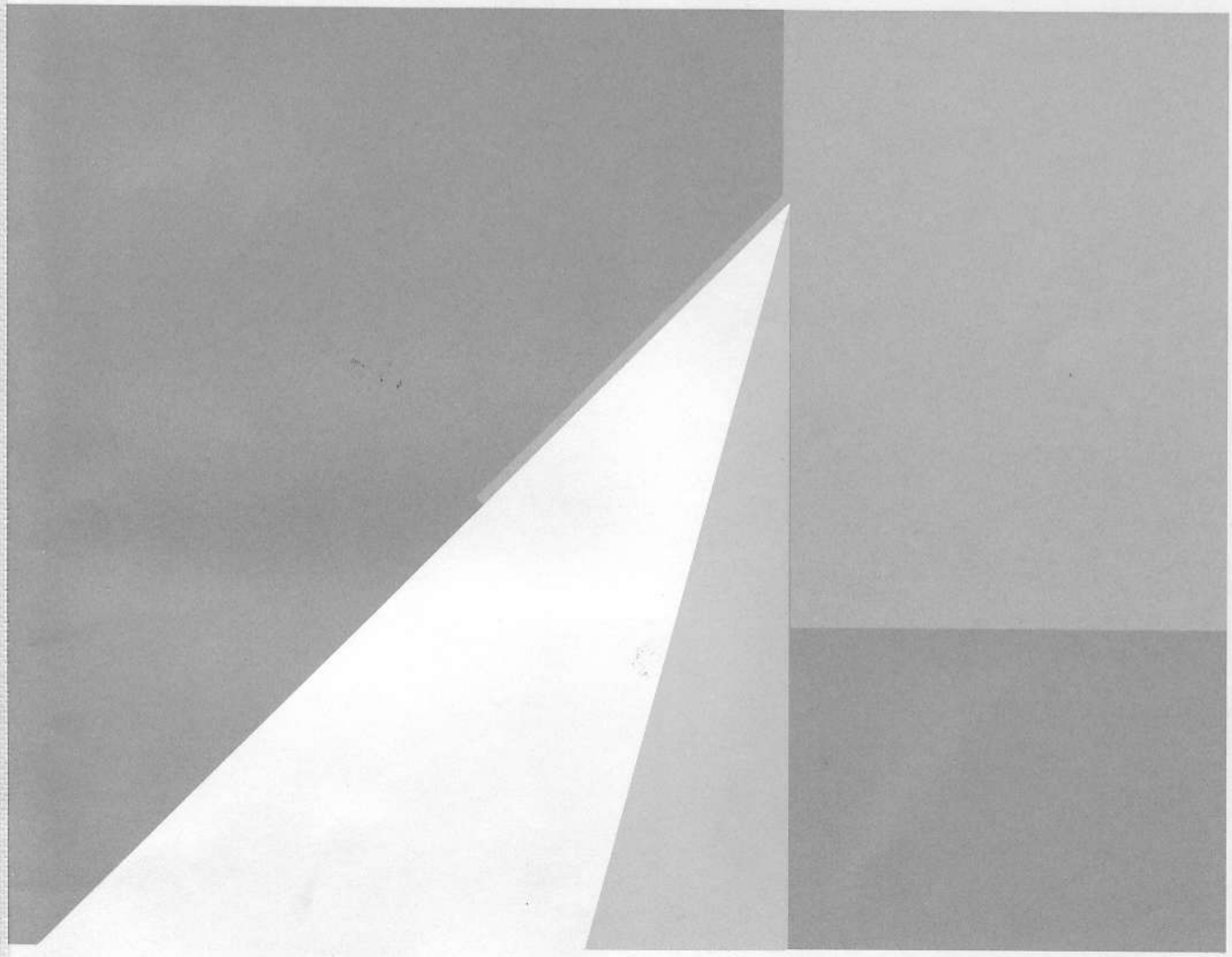
AO Microtome



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AO® American Optical
SCIENTIFIC INSTRUMENT DIVISION
BUFFALO, NY 14215

EFFECTIVE USE AND PROPER CARE OF THE MICROTOME

AO® American Optical
SCIENTIFIC INSTRUMENT DIVISION
BUFFALO, NY 14215

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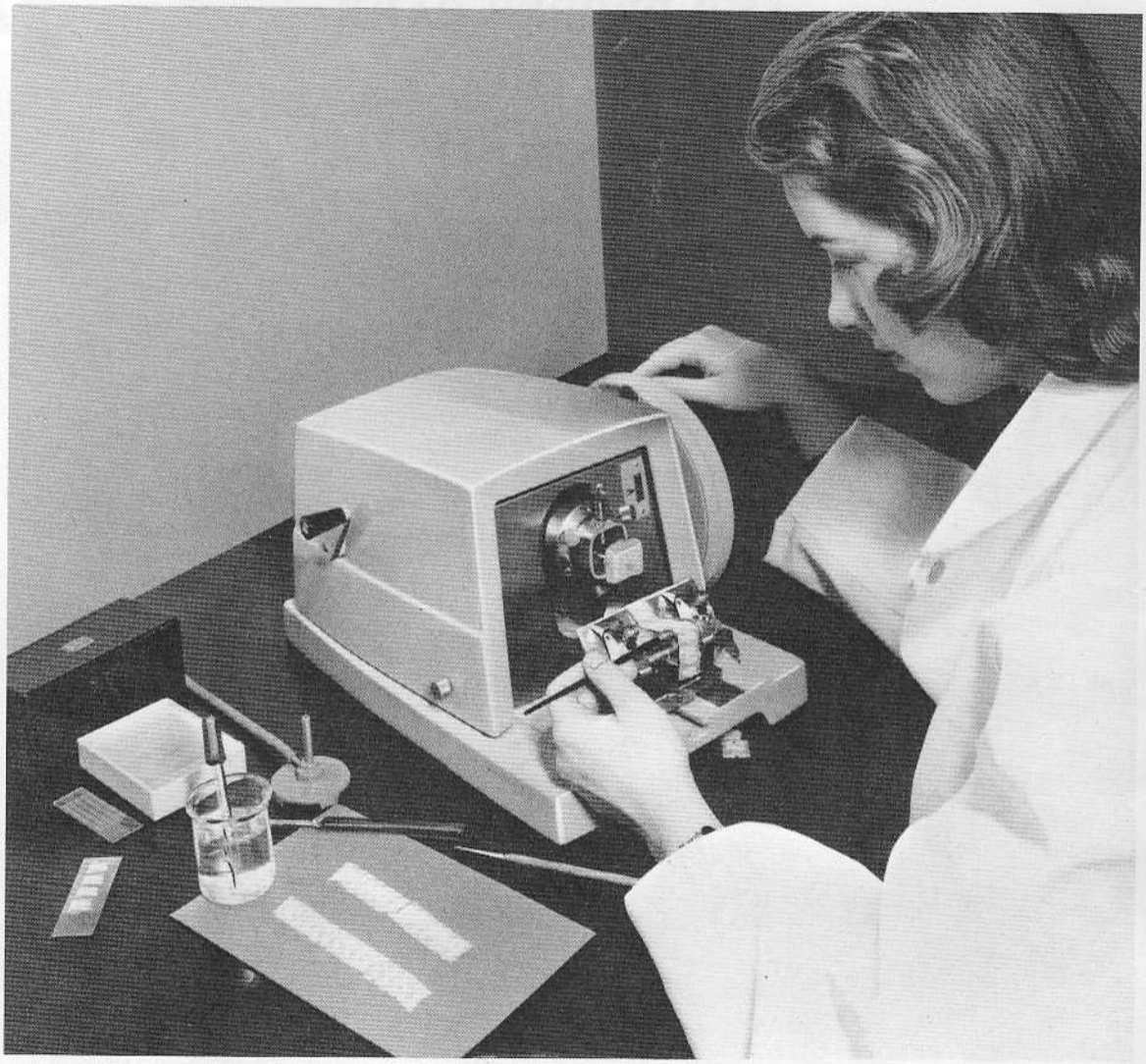
LB 455 149

"Given a substantial microtome, good results are largely a matter of personal skill in sharpening knives and manipulating the microtome." – Lucas (1927)



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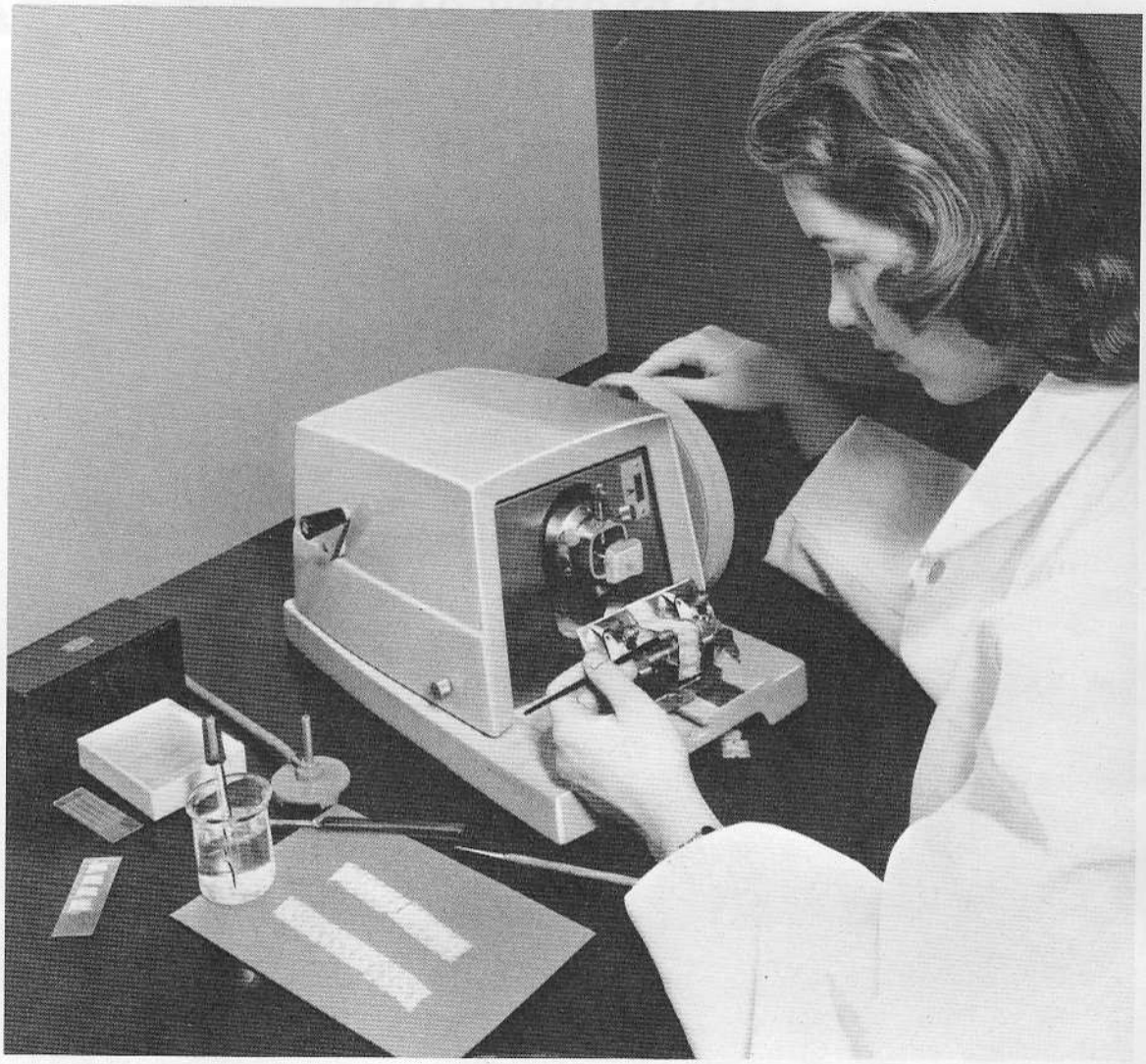

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I. INTRODUCTION

Microtomes are precision instruments designed for cutting materials into sections thin enough for examination with a microscope.

Successful sectioning requires:

1. Properly prepared material. Some specimens may be sectioned as they are found; many require extensive pretreatment and embedding in a supporting medium. The supporting medium must match the physical character of the specimen and have properties suitable for the cutting procedure to be used.
2. A sharp knife. Poorly prepared material can sometimes be sectioned with a good knife, but a poor knife may fail to cut, or ruin the best material.
3. The correct microtome. Different kinds of microtomes are available for different uses and the choice should depend on the application. With proper care AO Microtomes will give many years of service, but abuse will ruin precision instruments. Unless very old, damaged, or mistreated, the microtome is rarely the cause of poor sections.
4. A skilled operator. Most failures observed in microtomy could have been avoided by an experienced microtomist. With perfect material, a sharp knife, and properly adjusted microtome, automatic sectioning would be possible. Otherwise the operator must be able to recognize and correct difficulties as they arise. No technician should be expected to section improperly prepared material. For training courses see Steedman (1960).

The objectives of this manual are to provide directions for the use and care of AO Microtomes, call attention to some of the special problems of microtomy, to share our research and experience on the sectioning process, and to coordinate and outline the basic literature in this field. Detailed instructions may be found in the separate manuals for the microtomes.

II. CORRECTING DIFFICULTIES ENCOUNTERED IN SECTIONING

This check list should help the operator to overcome many of the common difficulties. When the suggested correction is not adequate, the corresponding part of the manual should be read and, as needed, other more complete sources (Chapter VIII).

Do not return the microtome to the factory unless it is damaged, or old and obviously worn and none of these suggestions work.

A. DIFFICULTIES COMMON TO ALL METHODS

Irregular sections, skipped sections, or thick and thin sections, are usually the result of insufficient tilt (fig. 1B) of the knife, that compresses the block on the return stroke, or of too much tilt which scrapes off the section instead of cutting it. Correct by turning the knife holder to give the proper clearance angle (fig. 1A), between the cutting facet of the knife and the specimen.

Scored, grooved, smeared and deformed sections are often caused by a dull knife. Regular, lengthwise scratches, and splits in sections are usually caused by a defect in the knife edge, although they may result from dirt or hard material in the specimen. Moving the knife to an unused area, or replacing with a sharper knife may restore good sectioning.

Sections that fall out of the matrix or show a different amount of compression than the embedding medium frame — indicate that the supporting, embedding medium is inadequate. Mushy appearing sections indicate insufficient dehydration or clearing. Re-embed the specimen in a more suitable material for better sections. (See Gray, 1958; Chapter VIII).

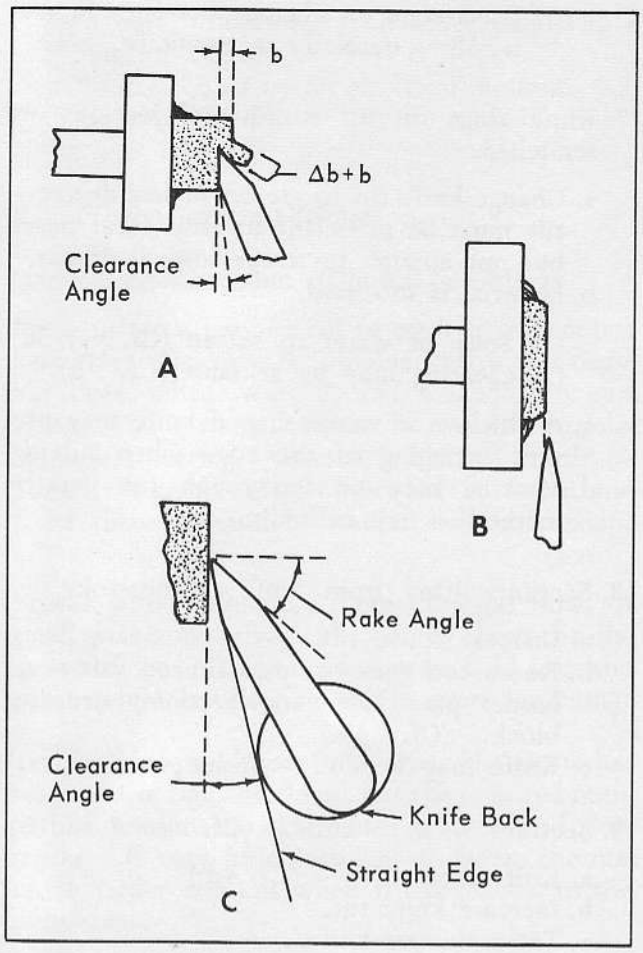


Fig. 1. A. Diagram showing clearance angle and increase in section thickness from compression. B. Wedging effect when there is no clearance angle. C. How to set the clearance angle.

B. DIFFICULTIES OF SPECIAL METHODS*

1. Paraffin Embedded Material
(See also Chapter IV F)

1. Ribbon fails to form.
   a. Room is too cold or paraffin too hard.
      (1) Use softer (lower melting point) paraffin.
      (2) Warm knife slightly by blowing the breath on it, or immerse in warm (not hot) water.
      (3) Place a desk lamp so that the light and heat fall on the knife and block.
   b. Tilt the knife less.
   c. Cut thinner sections.
   d. Knife may be too dull – resharpen.
   e. Dip block into a softer paraffin and trim so that a thin layer of softer paraffin remains on the upper and lower edge of the block.
   f. Unroll the section and hold it lightly against the knife with a camel's hair brush. If the first few sections can be held down, the ribbon will often form and follow.

2. Crooked ribbons.
   a. When sections are wedge-shaped the sides of the block are not trimmed parallel.
   b. Edge of block not parallel to knife edge.
   c. Try another part of the knife – sometimes irregularities of the knife edge cause crooked ribbons.
   d. The paraffins at one side of the block may be softer than at the other side, especially if the material has been re-embedded in a paraffin of different hardness – re-embed the material and stir the melted paraffin.
   e. One side of the block may be warmer than the other, from a radiator, lamp or draft. Let the block cool and place the microtome where the temperature will be uniform.

3. Sections vary in thickness or are skipped.
   a. Knife not tilted enough to clear facet or bevel, or tilted too much, and tissue is compressed until the inevitable expansion gives a thick section.
   b. Some of the clamping set screws on the block or knife holder are not tight or knife holder block not clamped firmly.
   c. Microtome worn through lack of lubrication, or not in adjustment.
   d. Very large blocks or blocks with hard regions may spring knife edge while sectioning – soak block in water to soften, use other methods for softening the material or embed the celloidin. The block will soak more quickly if the paraffin is trimmed off one side to expose the tissue. Very little water is absorbed, but this process sometimes makes possible cutting hard or tough material.
   e. Paraffin is too soft – use higher melting point paraffin, or cool block and knife.

4. Sections compressed, wrinkled, and jammed together.
   a. Knife too dull.
   b. Room too warm – cool trimmed block and knife in very cold, or ice water immediately

*See also Steedman (1960)

before sectioning, or by rubbing surfaces with an ice cube, or re-embed in harder paraffin.

c. Knife tilt too slight, so facet bevel rubs over block – increase tilt.
d. Knife edge gummed with paraffin – wipe both sides with finger or cotton moistened with xylene.
e. Soak block, before cutting, from an hour or two to over night, in water, or 10% glycerin in 60% alcohol (Baker, 1941). Lendrum (1944) adds aniline.
f. Cutting too rapidly – very thin sections should be cut slowly. Increase section thickness.
g. When the specimen and not the paraffin is compressed, infiltration is incomplete or the specimen is softer than the paraffin.

5. Sections crumble and specimen may tear out.

a. Material incompletely dehydrated or not properly cleared.
b. When soft and mushy, material incompletely infiltered – reinfilter and embed. (Salvage rarely possible if material was incompletely dehydrated.)
c. Alcohol not completely removed by clearing fluid.
d. Object too long in paraffin bath or paraffin too hot.
e. Subject hard and brittle because of clearing fluid. Try toluene in place of xylene or a mixture of toluene and cedar oil.
f. When the specimen shatters and falls out of the wax, it is too hard for the paraffin. Use a harder wax or wax mixture.
g. Try celloidin embedding, or a rubber or asphalt mixture with paraffin for fragile material.
h. Try dioxan method for dehydrating.

6. Split ribbon or lengthwise scratches in ribbon.

a. Nicks in knife – use another part of knife or rehsarpen knife.
b. Use less tilt of knife so it will cut rather than scrape.
c. Knife edge dirty. (Cf. 4d.)
d. Object may be too large for paraffin method – use celloidin.
e. Hard particles in block may cause scratching.

(1) Dirt in paraffin – filter or decant melted paraffin.
(2) Crystals from killing fluid (mercuric chloride) when washing was insufficient.
(3) Calcareous or silicious particles in materials – decalcify or desilicify.

7. Knife rings on up stroke and sections are scratched.

a. Change knife tilt to greater or less degree – tilt must be sufficient to clear facet bevel, but not enough to scrape instead of cut.
b. Material is too hard.

(1) Soak in water to soften (Cf. 4e, 3d).
(2) Clearing may be at fault (Cf. 4b).

c. A thicker or wedge-shaped knife may prevent springing of the edge when cutting.
d. Material may be too tough for paraffin method – try celloidin.

8. Sections lifted from knife on upstroke.

a. Increase knife tilt.
b. Room too warm or paraffin too soft – try harder paraffin – cooler room; or cool block. (Cf. 4b.)
c. Knife may be dull – resharpen.

9. Sections stick to knife. (Cf. also 4 and 5).

a. Knife edge dirty – (Cf. 4d.)
b. Increase knife tilt.
c. Try a sharper knife.
d. Knife edge facets may be corroded – repolish.
e. Clean knife edge and rub a very little light machine oil on facets.

10. Undulations in the surface of the section.

a. Tighten all set screws on knife and block holders and see that knife holder is clamped fast to microtome base.
b. Lessen excessive knife tilt to prevent vibration. (Cf. 7c, 3.)

11. Scratching noise during cutting.

a. Material may be too hard, or small regions of material may be hard. (Cf. 5c, d, 6e.)

12. Sections fly and stick to parts of microtome or other nearby objects because of static electricity formed from the friction of cutting. This usually occurs only in winter when the air is very dry.

a. Increase humidity of room by boiling water in an open pan, or burn a Bunsen burner in the room.

b. Ground microtome to a water pipe with a wire or a chain.
c. Ionize the air by an electrical method. (Cf. Chapter IV, G).

2. Frozen Section Technic

Unenclosed Microtomes (Clinical or Sliding)

Fresh material may be cut as soon as frozen, but better sections may be obtained after the tissue has been killed, washed, and soaked in a gum syrup. Tissue fragments may be mounted in gelatin before cutting. Material in alcohol should be passed through a series of alcohols to water, and fixed tissues should be washed before freezing.

Freeze with moderately rapid CO_2 gas flow. A small glass tumbler with an opening about the diameter of the freezing head may be held over the tissue while freezing to aid in even hardening.

Test cutting conditions and, when the tissue has reached the right hardness, cut the required number of sections quickly with an even and slow stroke. It may be convenient to freeze the material harder and cut when it has thawed to the right stage.

The knife must be cooled to prevent the sections sticking to it. Sections may be removed with a camel's hair brush and placed in distilled water. When using a chisel-shaped blade, hold it against the chest or body to brace the arms and make the cut by swaying the body.

Very hard or dense tissue may not be cut at less than 18-20μm. The average thickness is often 15μm. Considerable skill is required for cutting thinner sections.

All set screws holding the freezing equipment and knife must be tight to avoid vibration when cutting frozen sections.

CRYOSTAT MICROTOMES

Sectioning is easier and thinner sections may be obtained when the microtome and tissue are maintained at the same temperature by refrigeration. For optimal sectioning the cryostat should be set to the proper temperature allowing sufficient time for temperature to stabilize before use. The microtome is used much the same as at room temperature. See section IV, IVB.

3. Celloidin Embedded Material

The knife should slice through the material with a slant angle of about 10° - 35° to the direction of the cut, and the knife should be tilted more than required for paraffin embedded material.

1. The chief difficulty comes from trying to cut improperly prepared material. Adequate impregnation of a large organ like a hemisphere of a brain may take a year. Improperly hardened blocks cannot be sectioned successfully. Pressure methods involving heat speed up the process and are available when warming will not injure the material.

2. Lengthwise scratches or splits in the section may be due to:
   a. Nicks in the knife – use a different part of knife or resharpen.
   b. Particles of hard material in the block.
      (1) Dust or dirt in the celloidin stock solution – let stand and use only upper portion after the particles have settled or filter the stock solution.
      (2) Calcareous or silicious deposits in the material – decalcify or desilicify.

3. Specimen falls out of section, is mushy and soft.
   a. Dehydration was incomplete.
   b. Infiltration incomplete – reinfilter, re-embed, and harden.
   c. Harden block if too soft, in chloroform, or a mixture of equal parts of 95% alcohol and glycerine.

4. Variation in thickness of sections.
   a. Loose screws on knife or block holders – tighten all set screws. Avoid strain on the knife from unequal tightness of the knife holding screws.
   b. Knife holder depressed or raised by the hand while sectioning – hold knife block so as not to move it vertically while cutting.
   c. Knife not tilted enough to clear facet of cutting bevel.
   d. Knife too dull.
   e. Microtome worn and out of adjustment.
   f. Material not hardened properly – cf. 3c.
   g. Slight drying of block between sections.

III. THE MICROTOME KNIFE

A. HISTORICAL

The cutting edge of an ideal microtome knife would be the straight line formed by the intersection of two planes, the cutting facets. The angle between the planes is called the bevel angle and is greater than the wedge angle between the sides of the knife. Such an ideal edge is not possible because the inhomogeneous structure of the steel results in a slightly rounded edge. The radius of curvature of this edge was measured from paraffin impressions of the knife by Kisser (1927). He considered a radius of curvature of 0.3 to 0.35μm a good approach to geometrical sharpness.[2] Schmeritz (1932) recommended that the radius of curvature be between 0.1 and 1.0μm. Ardenne (1939) would limit the radius of curvature to 0.1μm.

The cutting edge of a very sharp knife, when examined by reflected light under 100 magnifications, will appear as a very fine discontinuous line varying slightly in width. Higher magnifications of around 500X will give this edge a finely serrated appearance. von Mohl (1857) recommended sharpening the microtome knife until the two planes of the cutting facets come together to give such a minimum reflection; his criterion of sharpness is still used. Most microtomists recommend a magnification of 100 diameters for this purpose. Exact recommendations vary widely from 40X (Chamberlain, 1925) to 700 - 1000 diameters (Funk, 1910). Julian (1903) recommended the same criterion of sharpness, and pointed out further that the actual cutting edge must be thinner than the material to be cut. If the cutting edge is thicker than the cells, for instance, they will be destroyed rather than sectioned. Ssobolew (1909) emphasized the importance of proper hardness of the knife temper and that a fine edge could only be obtained by using fine honing material.

The proof of a sharp knife, according to Apathy (1912), was the ability to cut a paraffin ribbon at 2μm with no compression. This criterion depends on the paraffin as well as the knife (Cf. Chapter IV and V, section D). Bensley and Bensley (1938) also recommend polishing the edge until no reflection can be seen from the actual cutting edge. They test with paraffin at 3μm and advise the operator not to try the knife on hair or skin as these rather difficult tests may spoil the edge.

More critical tests utilize multiple beam interference microscopy (Giuntini and Edlinger, 1954; Hallen, 1954; Bull, 1958). While sharpening the knife, or at failure to section, examination of the condition of the edge with a microscope may save both time and material.

The size and general shape of the microtome knife have become established by use. The standard microtome knife has a wedge angle of 15° to 18° and the bevel angle between the cutting facets for knives of American manufacture varies between 27-32°. The width of the two facets which make the cutting edge of the knife has been recommended from 0.1 to about 0.6 millimeter (Malone, 1922; Nageotte, 1926; Franz, 1929). The early double concave knife is rarely used now, even for hand sectioning, and the modern microtome knife is either wedge-shaped with slightly hollow ground side or plano-concave. The plano-concave knife is used primarily for celloidin sectioning. Apathy (1912, 1897) recommends facets be of unequal length and his recommendation is supported by Kisser (1926) and Löw (1932). Such asymmetrical edges have not been required generally nor entered commercial practice because the increase in cost is not justified. The user also objects to the inconvenience of having to set the knife in accordance with which side is toward the block.

The nature of the edge is important; for cutting hard, dense material, such as wood, a very smooth edge is required with no fine serrations visible at 200 diameters (Bailey, 1937). The edge depends, of course, on the fineness of the abrasive material used for forming it. Julian (1903) called attention to this limitation of sharpening, and it was emphasized by Funk (1910). Since then it has been customary to choose fine grained hones or abrasives separated by decantation so as to retain only the finer sized particles for knife sharpening.

A glass plate seems to have had the longest and most successful use as a surface on which to sharpen the microtome knife (von Mohl, 1857). Bishop (1954) vibrates the glass plate with a motor as an aid to sharpening. Instead of using a large plate Lendvai (1909) recommended three pieces of plate glass about the size of an ordinary hone for use with different grades of abrasives.

[2]One micrometer (μm) = 0.001 millimeter (formerly called micron).

Apathy (1912) and Krause (1926) used glass with Vienna chalk as a polishing agent. Nageotte (1926) preferred a horizontal rotating glass annulus (1 revolution per second), and Franz (1929) developed a sharpening machine with a rotating glass disc and a clamp to hold, oscillate, and turn the knife. A vertical rotating glass wheel was recommended by Long (*n.d.*). The method was developed further by Garland (1935), Uber (1936) and Hillier (1951). Hallen (1954) prefers a cast iron lap and Bell (1958) a bronze lap impregnated with fine abrasive to glass plates.

A water motor and, later, an electric motor were used by Funk (1910) to drive a reciprocating motion for moving the stone to and from the operator about 3 strokes a minute to lighten the work of sharpening the knife. Malone (1922) advocated wide leather strops with specially prepared surfaces and used with increasingly finer graded abrasives. Weller (1924), Chamberlain (1925) and Evenden (1938) report having used Carborundum Hones followed by finer grained hones or stropping on canvas or leather.

There has been no agreement on the advisability of using a strop. Many of the experts protest against stropping while others recommend that it be done. When the edge is honed really sharp no gain will come from using a strop. On the other hand, if the edge consists of fine serrations, gentle stropping will bring the serrations into line and improve the cutting ability of the knife. Careless use of the strop will spoil the best edge. The surface of the strop must be free from dust or other abrasive material larger than a micrometer in diameter, because larger particles destroy the edge. It is very important that the strop itself be supported so that it will not give, nor that the knife is pushed down into the strop. Either procedure rounds the cutting edge instead of sharpening it and quickly spoils its cutting ability. The strop, even if it contains abrasive embedded in it, cannot replace the hone.

B. EXPERIMENTAL

The microtome knife must be made of a good grade of steel of proper hardness. A soft knife fails to hold an edge. Too hard a knife is brittle, so that tiny pieces of the edge are likely to be broken out during the sectioning of hard material and when sharpening the knife. The composition of the steel in the cutting edge is very important and, unless the right balance of the different phases of steel is obtained in tempering, the knife will neither take nor keep a good edge. AO Microtome Knives are finished to close limits of hardness and each knife is etched and examined with a microscope before final sharpening to make sure that the components of the steel are present in proper proportions.

While past experience has taught certain basic principles, it is clear from the above historical summary that there is no single generally accepted procedure for taking care of the microtome knife. von Mohl's (1857) early sharpness criterion, utilizing the light reflected from the cutting edge, is one of the best. A really smooth, well-polished edge shows only a slight reflection: a narrow, straight, and unbroken bright line. Any imperfections in the edge give an irregular, interrupted line, and unless the knife is properly sharpened to a true thin edge, a broad reflection appears.

A convenient method for routine use is to place the knife on a block like Fig. 2 and illuminate with a lamp, Fig. 3A. The polish of the facet can be examined with the knife on the B position, or the knife can be placed on the stage of the microscope, Fig. 3B. To see nicks, examine the edge in silhouette when illuminated from the microscope mirror (Richards 1950).

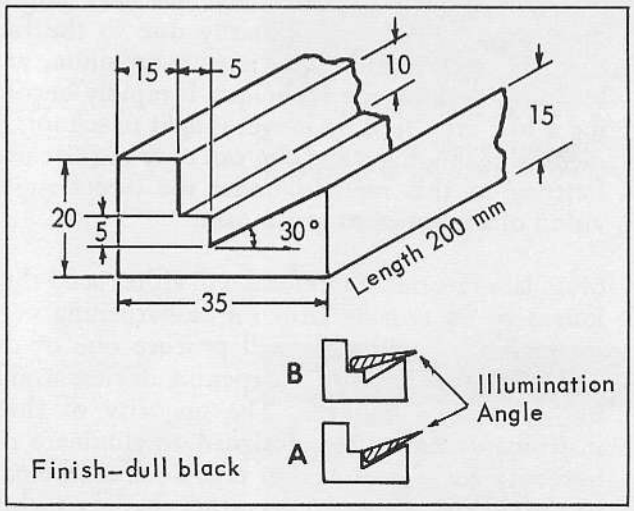


Fig. 2. Block for supporting microtome knife (dimensions in mm). A. Position for examining sharpness. B. Position for observation of polish.

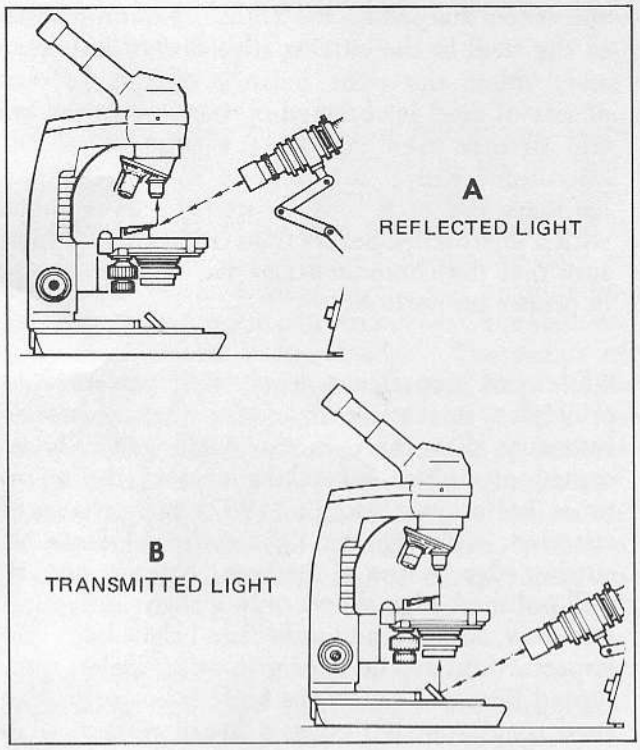


Fig. 3.

A knife for use in the average biological or medical laboratory should be sharpened until the edge appears free from serrations at 100 diameters and the reflection from the edge shows only a narrow and almost unbroken line.

C. SHARPENING

In the modern laboratory the practice of sharpening microtome knives by hand has been largely discontinued. This is primarily due to the fact that the technician's time is at a premium, and secondly because the technique is rapidly becoming a lost art. It is no longer taught in schools of medical technology, and one can only acquire proficiency in this method under the direct supervision of a competent technician.

Most laboratories, therefore, will either send their knives to an outside firm for resharpening or if the volume justifies it, will procure one of the several automatic knife sharpening devices available in today's market. The majority of these instruments have been designed to eliminate the necessity for a great deal of technique on the part of the technician, requiring rather that the technician only be able to recognize, by microscopic examination, the proper development of the sharpening process.

As previously mentioned in this publication, the glass plate technique has had the longest and most successful use as a surface on which to sharpen the microtome knife. The more popular instruments in use today all use the glass plate or a variation of that method. One of these is the American Optical Model 925, and while specific reference will be made to that in the following paragraphs, the techniques will generally apply to other similar sharpeners.

In preparing the knife for sharpening it should be examined under the microscope to determine the extent of damage to the edge. Severe nicks may require that the knife be returned to the manufacturer for reconditioning. The alternative to this is considerably extended sharpening times, but experience will enable the technician to determine what action must be taken. The initial sharpening of a new or reconditioned knife on any sharpener must follow the complete process as outlined in the reference manual for that sharpener. It is unlikely that the facet angles produced by factory methods can be exactly duplicated, and it is imperative that the angles which are characteristic of that sharpener be established on the edge of the knife. This will facilitate subsequent sharpenings which will be of shorter duration, and will make it possible under certain conditions to simply polish the cutting facet without going through the full process.

PROCEDURE

1. Follow setup instructions as described in the reference manual to install all necessary components of the sharpener.

2. Install hone plate in the position as prescribed for the coarse honing procedure.

3. Apply abrasive to the hone plate in a quantity sufficient to extend beyond the ends of the knife which is to be sharpened. If the abrasive is oil based rather than a paste, it should be first stirred or shaken until thoroughly mixed to provide the proper mixture of lubricant and abrasive.

4. Install the knife into the knife holder following the manufacturer's instructions in the reference manual. Most will require that the knife be centered as precisely as possible in the knife holder to maintain balance.

5. Set the timer for minimum of thirty minutes, close the cover and turn the instrument on. The sharpener should never be operated with the cover open, and in some instances will have an interlock on the cover to prevent this.

6. At the end of thirty minutes remove the knife from the instrument, clean with xylene or other suitable agent, and examine microscopically to determine the progress of the sharpening. Figures 5 and 6 show the typical appearances of the facet under the microscope when illuminated as illustrated in Fig. 3A. If necessary continue coarse honing after cleaning the hone plate and applying fresh abrasive. As the abrasive discolors, indicating the presence of metal it should be removed since the metal particles could damage the facet.

7. When it is determined by microscopic examination that the coarse sharpening procedure has been completed, the hone plate should be removed from the instrument, thoroughly cleaned of the coarse abrasive, and reinstalled as prescribed by the manufacturer for fine honing.

8. After installing the hone plate apply an amount of the fine abrasive in sufficient quantity to extend beyond the ends of the knife to be sharpened.

Fig. 4. AO 925 Automatic Knife Sharpener

9. Install the knife in the knife holder again following the manufacturer's instructions.

10. Set the timer for a minimum of fifteen minutes, close the cover and start the instrument.

11. After fifteen minutes remove the knife, carefully clean with xylene or other suitable agent using a soft cloth to prevent damage to the edge. Always wipe towards the edge rather than following the length of the knife.

12. The facet should show a high polish and be relatively even for the full length of the knife. It is possible that the facets may be of different widths on opposing sides of the knife, and this will be explained later in this text.

13. Figures 9 through 12 show the progress of the development of the polished facet on the knife.

14. Examine the knife microscopically to determine that the facet has been properly developed and polished, and any fine nicks remaining after coarse honing have been removed.

15. If further fine honing is necessary follow the steps as outlined above after first cleaning the hone plate and applying fresh abrasive. The presence of metal particles in the fine honing compound is more critical than during the coarse honing procedure.

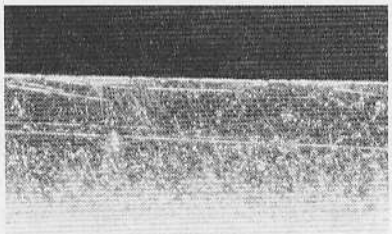


Fig. 5. Illustrated above is an "intermediate" stage in the coarse honing cycle.

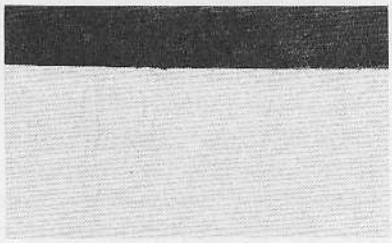


Fig. 6. Coarse honing complete.

Fig. 9. Appearance of an AO knife edge after proper coarse honing. Note that there is just a single facet, the surface of which is evenly ground and uniform in appearance.

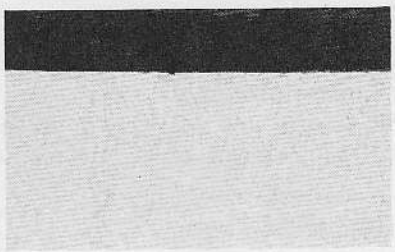


Fig. 9

Fig. 10. Illustrated here is an "initial" stage in the fine honing cycle. The second cutting facet is beginning to become evident near the edge of the knife.

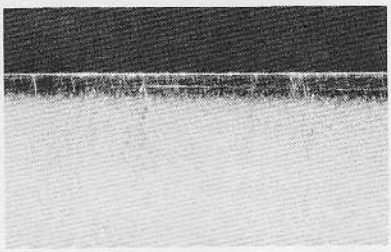


Fig. 10

Fig. 11. The fine facet is now clearly apparent in this "advance" stage photograph. In contrast to coarse honing, the fine honing action proceeds from the front edge of the knife to the back.

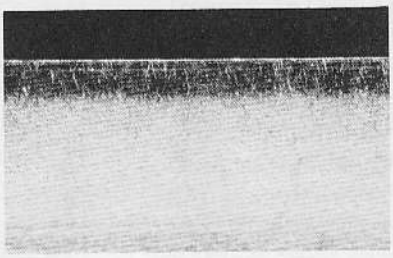


Fig. 11

Fig. 12. Fine honing complete. Note the even width of the fine facet (from end to end of knife). Small nicks have been satisfactorily removed. Microscopic "S" - shaped lines on fine facet are a normal result of polishing action and do not affect cutting qualities.

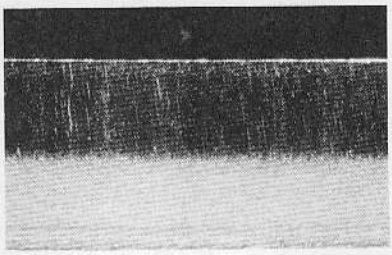


Fig. 12

The procedure for reconditioning microtome knives is generally identical to the original manufacturing process. Repeated sharpening of the knife will tend to extend the width of the facet, and also increase the facet angle appreciably. As stated elsewhere in this text, the facet angle on knives of American manufacture can vary between 27 and 32 degrees. To reestablish the relationship between the facet angle and wedge angle of the knife, the manufacturer will grind the wedge angle, thereby reducing the thickness of the knife at the heel, and also decreasing the dimension from the heel to the edge. The reduction in thickness of the knife at the heel on some sharpeners will have the effect of presenting the knife at a slightly different angle to the honing plate. When this occurs a wider facet will be produced on one side of the knife than on the other. This will in no way affect the cutting qualities of the knife, but simply means that some slight adjustments will have to be made with the knife in the microtome knife holder to establish the proper clearance angle for cutting. Some vendors will recondition knives by grinding only a quarter to one half inch back from the edge. This is not a generally accepted practice, and causes problems in resharpening knives. When sharpening methods are changed within a laboratory, it is generally recommended that the knives be reconditioned to manufacturer's specifications before attempting to sharpen on a different instrument. Repeated reconditionings will reduce the dimensions of the knives eventually to the point where they can no longer be reconditioned or resharpened on many instruments. The manufacturer will generally advise you of this fact when your knives are are returned for reconditioning.

After using the knife it should be cleaned and wiped dry before being put away. In many laboratories the knife will stay in good condition until it is needed again when it is put back in its box after thorough drying. In other laboratories where the atmosphere is more corrosive, it may be necessary to oil the knife in order to prevent corrosion. A good grade of light, *neutral* oil should be used. Under unusual conditions of humidity or corrosive atmosphere some of the regular anti-rusting greases may be required to protect the knife.

Glass and diamond knives are used for ultrathin sectioning and glass knives are used for fine cutting on rotary microtomes (Behnke & Rostgaard, 1963; Clevenger, 1964).

Marsh (1878) observed "Of not less importance than the microtome is the section knife, to be used in conjunction with it. How perfect soever the former, and whatever the dexterity of the operator, unless he be provided with a suitable, well made knife, he will never succeed in obtaining satisfactory results." It is poor economy to skimp on keeping a microtome knife in good condition. No amount of direction can take the place of experience in sharpening a knife. It is an art that can be learned and if knives are to be sharpened by the operator it *must* be learned. The alternative is sending the knives to the manufacturer for proper sharpening.

IV. METHODS

A. THEORY OF CUTTING

The sharp knife with a narrow edge probably wedges, rather than shears off the sections. Splitting is possible although it probably does not occur with hard gels. Tearing may be a reasonable explanation for cutting. Molecular splitting from the wedging of the secondary valences and crushing on a fine scale may occur with some materials, according to Bailey (1937), who concludes " . . . in colloidal material like wood, the mechanism of cutting is sub-microscopic tearing, crushing or a combination of both, rather than of the splitting of molecular separation types." Bailey found that wood sectioning required a very smooth knife edge, free from serrations and highly polished.

von Ardenne (1939) analyzed cutting and stated that the thinness of the section was limited by deformation and inner destruction. Resistance to cutting depends on 1) the adhesion which varies with temperature and the cutting angle; 2) resistance to deformation and 3) shearing pressure. The coefficient of friction depends upon 1) the quality of the object cut; 2) the original quality of the facet surface of the knife and 3) the pollution of the facet surface. By combining Euler's formula for breaking strength with a formula for inertia he was able to predict that about 1μm will be the approximate limit of thinness for ordinary sectioning.

The mechanism of separation of the section from the block and the effect of the embedding medium will be different for various materials and offer many opportunities for further investigation.

B. POSITIONING THE KNIFE IN THE MICROTOME

The actual cutting facets of a knife are very small in proportion to the knife as shown by a scale drawing, Fig. 13. The angle between the two cutting facets is established by the sharpening method. When the knife is set on the microtome it must be tilted so that there is clearance between the cutting facet next to the block of tissue and the surface of the block of tissue, Fig. 1A. If this tilt is not adequate, as shown in Fig. 1B, the surface of the block is forced down from the wedging effect of the cutting facet and no section results. The next time that the knife passes over the tissue this compression is increased and a partial section or no section may be obtained. However the tissue is soon so compressed that it suddenly expands and the next section is very thick. Skipping of a section, or the cutting of alternate thick and thin sections is usually due to insufficient or to excessive knife tilt. The tilt can be obtained by trial and error.

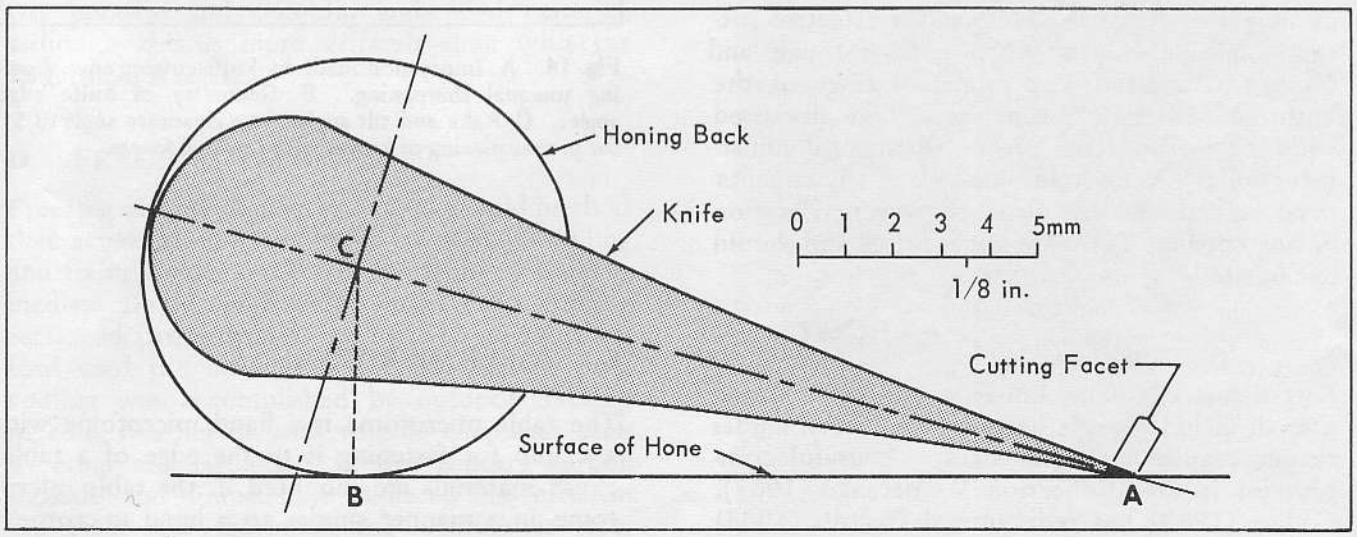


Fig. 13. Knife and honing back drawn to scale to show extent and formation of the cutting bevel and facets.

Not only is the clearance angle important but also the angle between the outer facet and the line perpendicular to the block at the point of cutting. This is called the rake angle, Fig. 14C.

The use of knives with unequal cutting bevels, with different and perhaps more advantageous rake angles, have been advocated by Apathy (1912), Walsam (1916), Kisser (1926) and Löw (1932). The gain from these special edges, despite the extra difficulty of using them, has not been enough to bring them into general use. The tilt angles are shown on Fig. 14C when unequal bevel angles are used.

Sometimes because of sharpening method or reconditioning, the facet on one side is longer than that on the other. If this is true, as in Fig. 14A, which shows the cross section of the edge after the knife has been pushed into a piece of thin lead and examined under the microscope, it is then necessary to tilt the knife for the proper clearance angle in accordance with which side of the knife is toward the specimen, Fig. 14C.

The standard holders on rotary microtomes maintain the edge of the knife at right angles to the direction of the cut. With sliding microtomes, it is possible to set the edge of the knife at an angle to the direction of cut. The slicing cut is advantageous for celloidin embedded and for hard materials. When the slice angle is small, then the wedging of the knife in the tissue is less, as shown in Fig. 15. This smaller effective cutting angle is helpful when cutting tough and brittle materials. The proper settings of the knife for different specimens will be discussed with the methods for cutting them. All adjustments on the knife holder and block adjustments must be tightened by hand to prevent vibration during cutting. Tools are not required and should not be used.

Soft tissues can be sectioned with a knife having a small included angle between the facets; harder tissues require stronger edges. A useful compromise is 28-30° (Section V, Marengo, 1967). Collins (1969) has rediscovered Heard's (1953) suggestion of etching the facets for smoother sectioning.

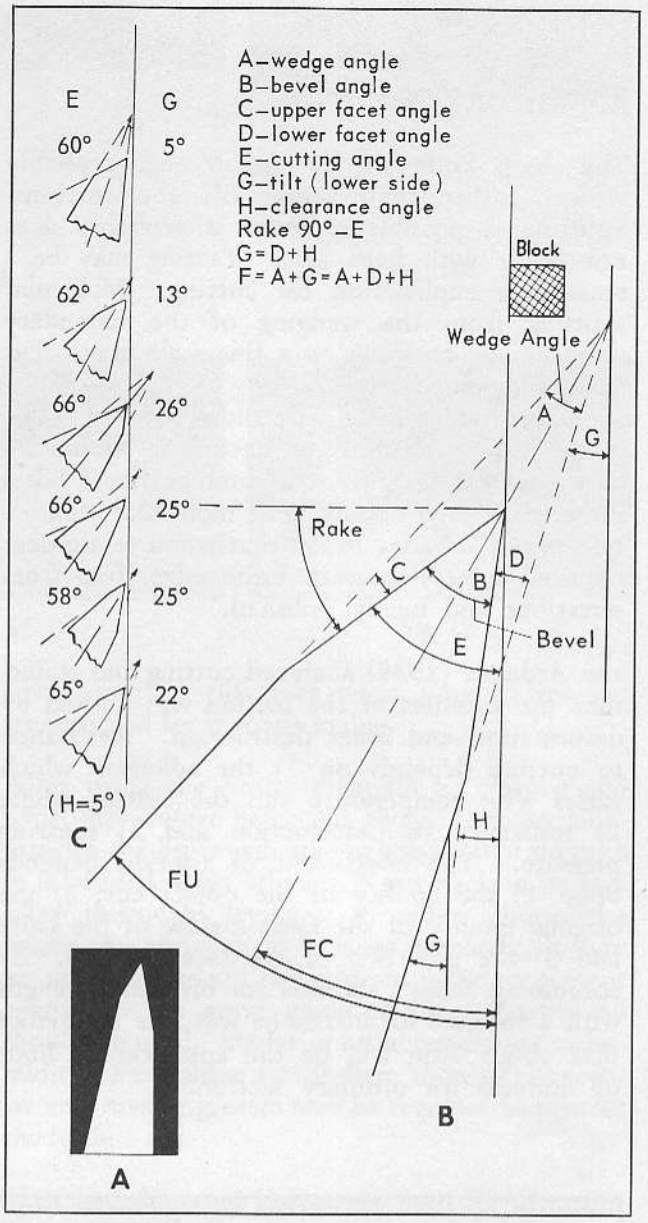


Fig. 14. A. Impression made by knife cutting edge showing unequal sharpening. B. Geometry of knife edge angles. C. Rake and tilt angles for a clearance angle of 5° for proper placing of knives with unequal facets.

C. TABLE MICROTOME

The table microtome is a hand microtome with a clamp for fastening it to the edge of a table. Fresh materials are mounted in the table microtome in a manner similar to a hand microtome and are sectioned in the same manner. Harder materials are usually sectioned with a chisel

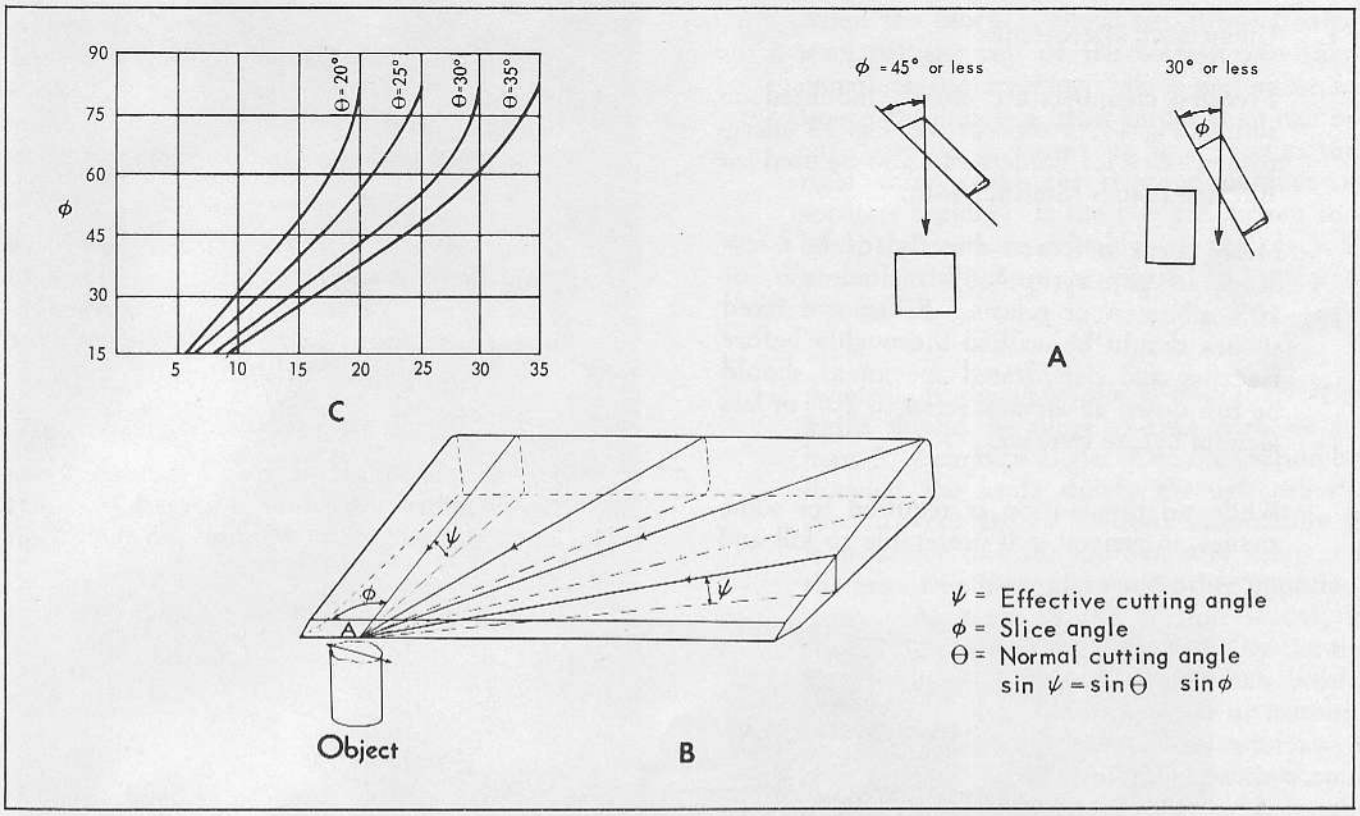


Fig. 15. A. Showing slice angle (ϕ) for square and rectangular blocks. B. Decreased wedging with small slice angles. C. The relations between them plotted with permission from data of Preston (1933).

shaped knife. A plane bit sharpened to a keen edge is satisfactory when very thin sections are not required. In cutting hard material the handle of the knife is held against the hip and the cut made by swaying the body. This gives a very firm hold and frozen sections may be cut. The table microtome, Fig. 16, is arranged to take the ordinary specimen holder and it is possible to cut paraffin and celloidin embedded material, although this is more difficult than with the larger microtomes.

D. FREEZING TECHNIC

Freezing and sectioning a tissue is a rapid method that avoids altering the tissue by solvents, killing and fixing fluids, and distortion from embedding media. Boyle cut frozen eyes in 1663, Stilling sectioned frozen spinal cord in 1842, and Rutherford used the method in the 1870's. At first, cooling was accomplished by outdoor freezing in cold weather or with volatile chemicals such as ether and rigolene. In this century carbon dioxide and Freon have replaced less efficient materials and thermoelectric cooling is now coming into use in microtomy. The chief advantages of freezing over embedding methods are rapidity (as needed when a tissue diagnosis determines an operation in progress), less distortion and freedom from the adverse affects of heat and solvents on the tissue.

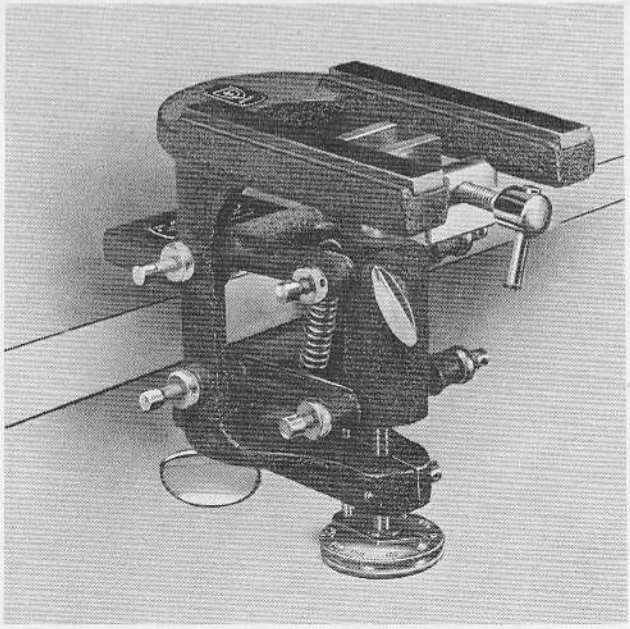


Fig. 16. AO Table Microtome.

1. Unenclosed Microtomes

Freezing chambers are usually mounted on sliding, Fig. 17, or shortstroke, Fig. 18 microtomes. Dry ice holders can also be used for freezing tissues (Smith, 1940).

Fresh tissue is frozen directly, or in a few drops of gum-syrup,[3] 5% melted agar, or 10% albumen or gelatin. Killed and fixed tissues should be washed thoroughly before freezing and dehydrated specimens should be run down an alcohol series to 20% or less alcohol before freezing.

While no preparation is required for some tissues, in general it is preferable to kill and harden the material and wash it well before freezing. Unless killing fluids are washed out or unless alcoholically dehydrated specimens are brought back to about 20% alcohol, it is difficult to freeze them properly.

Tissue damage from ice crystals formed in the cells may be reduced by embedding in gum sugar[3], 5% melted agar, or 10% albumen or gelatin. Spongy tissue should be embedded before cutting.

The sample to be frozen on a standard freezing chamber should be trimmed to about 2 x 2 centimeters or less and 3-5 mm thick.

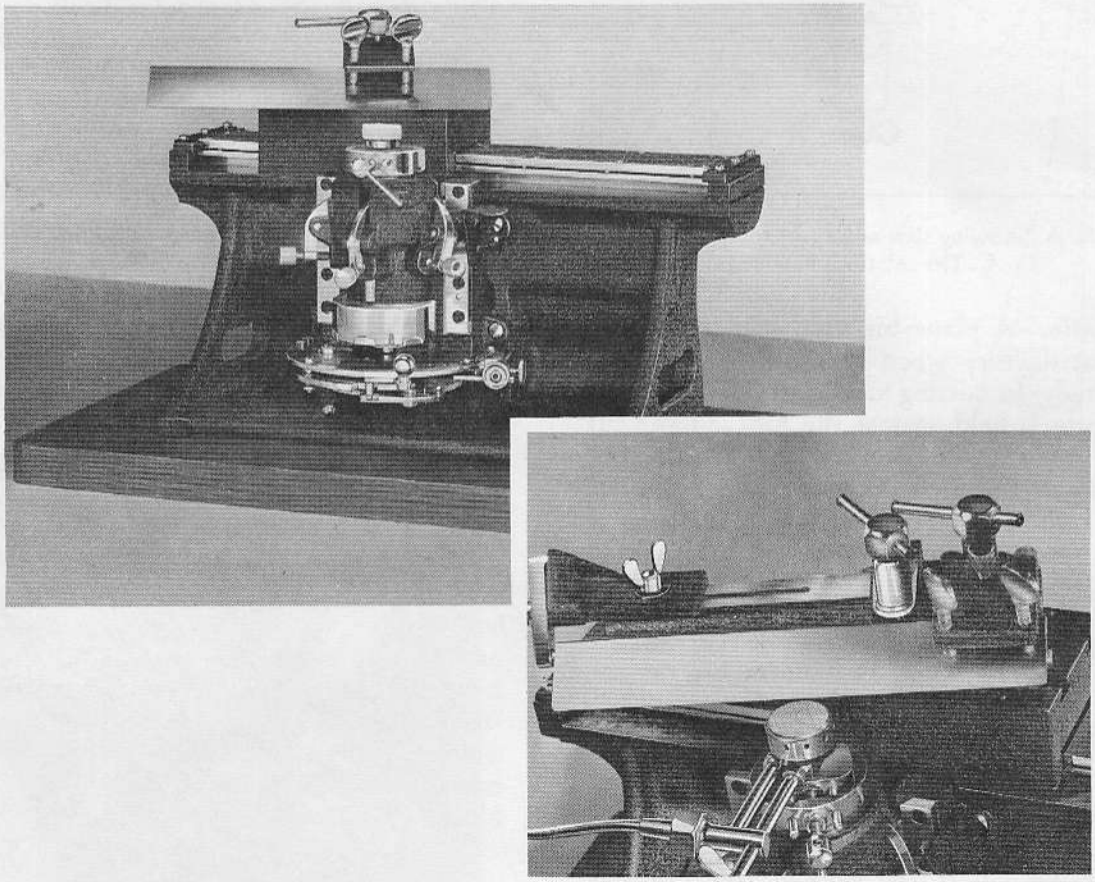


Fig. 17. AO 860 Sliding Microtome.

[3]Gum sugar is prepared by dissolving in 100 cc. of water, 100 grams cane sugar, 35 grams of gum acacia, and 0.1 gram thymol. Agar is recommended by Evenden and Schuster (1938).

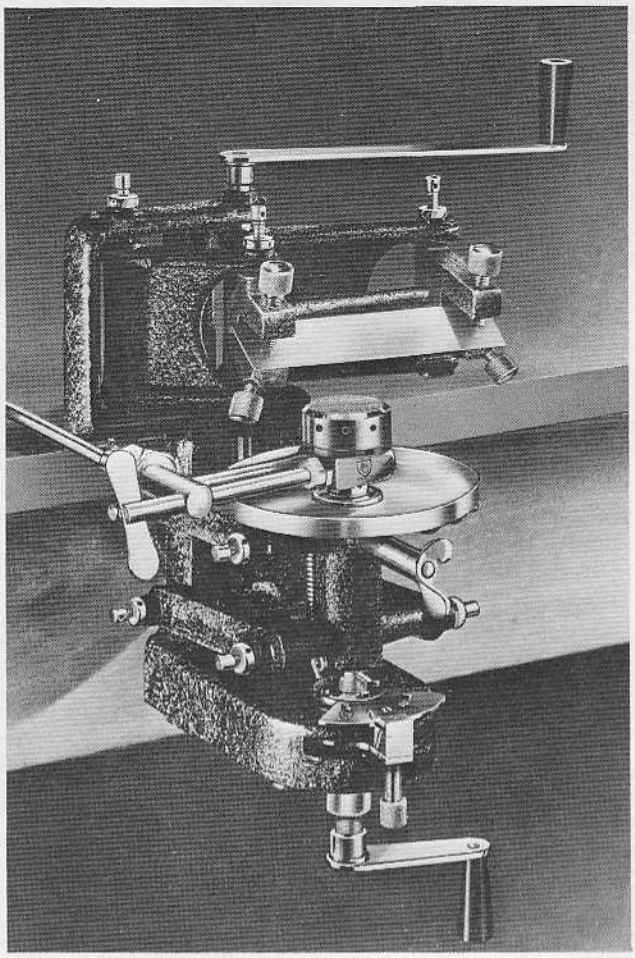


Fig. 18. AO 888 Automatic Clinical Microtomes and 930 Freezing Attachment.

Place a few drops of water, or better, of gum sugar, on the freezing chamber; place the tissue on this and add just enough fluid to surround it. Freeze the tissue slowly by turning on the CO_2 for a moment or two and turning it off. A series of successive jets freeze better and waste less gas than running it continuously. The tissue should be held flat against the chamber until freezing begins. If the freezing chamber is provided with a knife cooling attachment, the knife should be moved so that it is in the path of the cooling gas and deflects the gas onto the top of the block. This cools the knife and also assists in uniform cooling of the block. Some technicians facilitate freezing by holding an inverted medicine glass over the specimen when a deflector is not available. Stagg and Tappen (1963) insulated the holder from the microtome and used a perforated paper cup to facilitate freezing.

When the block is about two-thirds frozen, level off the top of the section and then complete the freezing. It is preferable to freeze the block a little harder than can be cut, then as it warms up and reaches the right stage, make the required number of sections rapidly. If the block is frozen too hard, the sections crumble. If the block is too soft, the tissues are injured and smeared together.

A medium slicing stroke is desirable. The knife should be tilted so that there is at least 5° clearance angle. Cut the section by drawing the knife slowly through the tissue. To cut frozen sections successfully it is necessary that the knife be very sharp and the edge free from nicks and other imperfections. As soon as the section is cut, it should be removed from the knife by the tip of the finger or with a camel's hair brush, and shaken into a dish of water or isotonic salt solution. The latter reduces cytolysis of the cells. On the other hand, with a cool knife and rapid cutting, three or four sections may be cut and transferred at one time from the knife to the storage dish. Alginate gel is used for serial sections by Lewis & Shute, 1963.

In attaching the tissue to the freezing chamber one should be careful not to get too much water around the tissue as shown at B in Fig. 19. Water forms hard ice which is liable to deflect the knife, causing uneven sections. Rather, keep the ice level below the surface of the tissues as shown at A in Fig. 19. If the tissue is spongy and is embedded only in water, hollow spots, as shown at C and D in Fig. 19, are liable to form hard ice crystals which tear the tissue

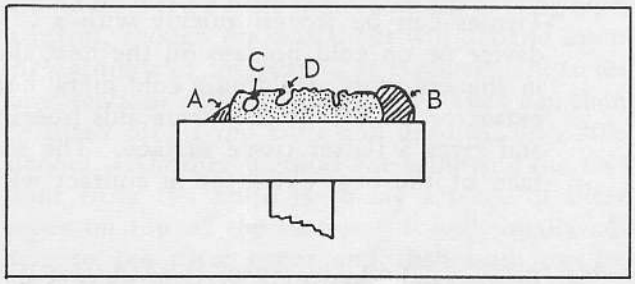


Fig. 19. Diagram Illustrating Difficulties With Freezing Technique. Cf. text.

as the knife goes through it. With such tissues it is much better to embed in one of the media advised, or at least to soak the tissue for a time in gum sugar. The gum sugar will not freeze hard and will lessen this type of tissue damage.

Rapid methods have been developed for hospital use.[4] Whole animals can be sectioned frozen with large microtomes (Patschke, 1968). Methods for staining, dehydrating and mounting frozen sections are also covered in the standard textbooks. Bush and Hewett (1954) collect the sections on a prestained film strip. Chapter II gives a check list of common difficulties with this method.

Skill and experience are required to make sections thinner than 15 μm with this technique.

2. Cryostat Microtomy

Enclosing the microtome in a constant temperature chamber holds the tissue and knife at the same temperature, which avoids distortion from uneven freezing and makes for easier recovery of the section. Linderstrom-Lang and Morganson (1938) are usually credited for this advance in frozen sectioning technique.

The cryostat, Fig. 20, has a built-in automatic refrigeration unit that can be set to the temperature desired. The microtome is made of stainless steel or corrosion resistant materials and is lubricated with an oil made for cold use. Inside the cold chamber is a heat sink bar for the tissue holders which keeps them cold and aids freezing the tissue to be cut. The cryostat should be turned on long enough before it is to be used to reach the proper working temperature.

Tissues can be frozen quickly with a CO_2 device or on cold holders on the heat sink in the cryostat. Holding a cold metal heat extractor on top of the tissue aids freezing and gives a flatter tissue surface. The surface of the heat extractor in contact with

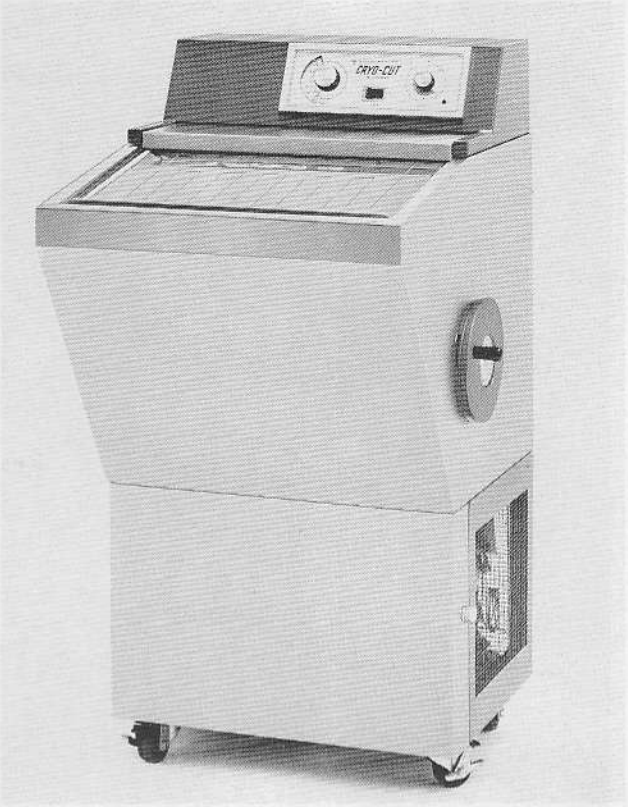


Fig. 20. AO Cryocut

the tissue should be sprayed, as needed, with a releasing compound to prevent sticking*. The tissue should be trimmed before mounting on the microtome. The knife is positioned and tilted to provide adequate clearance angle (III and IV,2) A sharp knife is required for good sections and the general manipulation of the microtome is the same as outside the cryostat. A tissue flattening device (anti-roll guide) is set parallel to the knife edge and it is usually possible for a skilled operator to make nearly undistorted sections as thin as 2 μm. The sections remain flat on the cold knife.

For histochemistry, the section can be moved into the desired container, or onto a cold slide, with a camel's hair brush. When a warm slide or cover glass (from room temperature) is brought close to the section on the knife, the section will usually jump onto the glass, thaw and stick well enough

[4]Cf. Evenden and Schuster (1938), Geschickter et al (1931). Gradwohl and Krajian (1940), Hjort and Moulton (1931), or Marshal (1940).

*E.g. Aerosol Zinc Stearate Plastic Mold Spray, Chicago, or similar releasing agent.

for direct staining. When permanent mounts are required a small amount of an adhesive material should be placed on the slide before putting the section onto the slide or cover glass. Sections may be stored frozen.

Many tissues section easily at -20°C, although some sectioning is done at -95°C (Agnew & Bullara, 1970). Soft tissues such as brain, and fatty tissues cut better at colder temperatures from -15°to -35°(Pearse & Bancroft, 1966). Fragments of tissues (curettings, small glands, etc.) can be held together with blood serum and often section better at about -5°C. Hardened, killed and fixed tissues cut less easily than fresh tissues and should be thoroughly washed before freezing. Blocks of 5 mm cross section and 4-6 mm thick are good sizes for frozen material for cutting. Skill is needed for sectioning large pieces of tissue.

Various fixing and killing agents may be used: acetone, acid-alcohol, Carnoy, 4% neutral formalin, etc., to precede staining, or the sections may be stained with toluidin blue or other appropriate stains for immediate study. For permanent preparations the staining should be followed by dehydration and mounting. Temporary mounts can be made with corn syrup, glycerine, glycerine jelly, etc. Mounting media which accept undehydrated sections are also available. Some laboratories use one section immediately and save another for the usual slower histological techniques.

Examination and diagnosis can be done in very few minutes when the Cryostat, dish of stain and microscope are located close to the operating suites. Normal tissues are rarely kept, thus saving time, materials and storage space. Malignant, or doubtful tissues can be preserved in damp or wet storage, or processed into embedded blocks. Cryostat sections can be good enough for routine use with considerable economic advantage. (Russell *et al* 1961; Hanske 1963; Funkhauser *et al* 1966).

Physiological investigations are possible within the Cryostat, or adjacent cold chamber. Clyde (1962) uses a carrier system to take the sections outside the Cryostat.

E. CELLOIDIN METHOD

The celloidin method is preferable for large tissues and organs and for hard or delicate materials. The celloidin is often not removed from the tissues and holds the delicate structures permanently together. The material to be cut is embedded in one of the commercial celloidins (cellulose nitrate); the slow burning kinds are preferable. The disadvantages are the longer embedding time, unless one of the rapid methods is used; the fact that serial sections cannot be cut, so that each section has to be handled individually; and the fact that stains which do not stain celloidin may be required, unless the celloidin is removed from the mounted section.

For materials not injured by moderate amounts of heat, the rapid process (Walls, 1932) facilitates impregnation and preparation of the tissue. The slower, cold process takes more time and does not damage the tissue. It may not be possible to embed a hemisphere of a brain or a whole lung and harden it sufficiently for cutting in less than six months to one year.

The chief difficulty in celloidin sectioning arises from trying to cut improperly prepared material. Inadequately hardened blocks cannot be sectioned successfully. The blocks should be dense enough to cut at the required thickness. Unless the block is sufficiently hardened, the sections are uneven and distorted. If the block is too hard, irregularities are apt to occur. The surface of the block and the surface of the knife should be kept wet with 70% alcohol, and as soon as the sections are removed, they should be placed into alcohol or, the block may be lubricated with cedar oil.

The sliding microtome is the instrument of choice for cutting celloidin material, Fig. 17. A slice angle of 10° to 40° is ordinarily used. The knife should be tilted a little more than for cutting with paraffin, though the actual tilt will depend upon the hardness of the tissue. It is convenient to let large sections roll up on the knife. They can then be lifted off of the knife and unrolled into 70% alcohol. Another method for removing the sections from the knife is to lay a piece of filter paper on top of the section. It will usually adhere to the filter paper and then both can be placed in a Stender dish. If the filter papers are numbered they may be kept in order. Another simple procedure is to stamp a number on the

margin of the section with a commercial numbering machine as each one is cut (Rasmussen, 1940).

The knife for celloidin sectioning must be very sharp because any irregularities in the edge will leave marks on the section. Likewise the section and the materials for embedding must be kept free from dust, because dust particles catching on the edge of the knife spoil the section, particularly when very thin sections are required. Siliceous or calcareous material must be removed before sectioning. The most common cause of irregularities in cutting is partial drying of the surface. If it is necessary to stop sectioning for even a short time the block should be covered with absorbent cotton and saturated with alcohol or else removed and placed in alcohol or other storage liquid.

The annoyance of keeping the block and knife wet with alcohol has led to the development of the so-called dry process (Walls, 1936). After the tissue is embedded and hardened, the block is soaked in an oil; e.g., cedar oil, which lubricates the block during cutting. The sections as they are removed from the knife are placed in the same oil. The oil soaked block may be cut on a rotary microtome as no slicing cut is required. The method has been found exceptionally good for making sections of difficult organs, such as of the entire mammalian eye.

Special knife holders are available, so that the knife can be turned to give a slicing stroke, and may be used for cutting small celloidin blocks on a rotary microtome. The position of the block and knife is such, however, that it is difficult to keep the surfaces properly lubricated with alcohol so that the rotary microtome is not very satisfactory for this type of sectioning. If much sectioning in celloidin must be done on a rotary microtome, it is advisable to use the dry method, which does not require a slicing stroke.

Celloidin blocks are sometimes embedded in paraffin to hold them for sectioning. At some medical laboratories they are placed on the freezing chamber and frozen before sectioning. The size of the section which may be cut depends on the size of the microtome and knife and the skill of the operator.[5] A brief check list of the common difficulties found in the use of the celloidin method will be found in Chapter IIB. Means for counting and numbering the sections are available (Duddy & Curran 1962; Brody & Tanski 1964).

F. PARAFFIN METHOD

Blocks of material embedded in paraffin may be cut rapidly on a rotary microtome, and successive sections adhere to each other to form a ribbon which facilitates handling and mounting the sections. The objections to the paraffin method involve the limitations due to the nature of paraffin itself and possible injury to delicate tissue from the elevated temperature during infiltration. Paraffin is a mixture of hydrocarbons which solidifies into characteristic types of crystals, varying to some degree with the proportion of harder and softer hydrocarbons present. The peripheral crystals are oriented with respect to the cooling surface, while the center of the mass forms a meshwork (Dempster, 1941, 1942ac). Different samples of paraffin have different plastic points, the plastic point being the lowest temperature at which permanent deformation may be made without fracture. A paraffin with low plastic point appears more translucent, is less brittle, but compresses more in sectioning. The hardness of paraffin depends on its plastic point, which lies a few, but variable number of degrees, below the melting point. Consequently, the plastic point, or more roughly, the melting point of the paraffin has to be adapted to the temperature of the room in which the sectioning is being done.[6] In warm rooms higher melting point paraffins must be used than in colder laboratories. The alternative is to condition the paraffin by the addition of various materials. Bayberry wax may be added to paraffin to improve its plasticity and cutting qualities. Rubber is often added to paraffin to improve it (Hance, 1963). Certain waxes may be added to paraffin to harden it without increasing its melting point (Waterman, 1939). Thorough infiltration is important and should be done in a vacuum to remove air from the specimen for critical work with some tissues. Paraffin blocks harden with time according to Steedman (1960) and when thin sections are to be cut, the blocks should be allowed to harden at least overnight.

[5]For methods cf. the chapter by Wasbottom in Bensley and Bensley (1938). For distortion cf. Dempster (1942b).

[6]Gallagher (1934) gives a temperature table on p. 228. For a 45°C paraffin, room temperatures should be 70° to 80°F for sections from 13 to 24 μm; for 52°C paraffin, 62° to 75°F for 3 to 13 μm sections.

In addition to matching the hardness of the paraffin to the temperature at which it is to be sectioned, it is necessary to match the hardness of the paraffin to the hardness of the tissue. The hardness of the tissue will depend upon the preparatory treatment and its structure. A Penetrometer was made, Fig. 21, to study these variations. The material to be tested is placed under the standard (A.S.T.M. asphalt) needle and the height of the index read on the millimeter scale. The distance which this standard needle point penetrates is proportional to the hardness.[7]

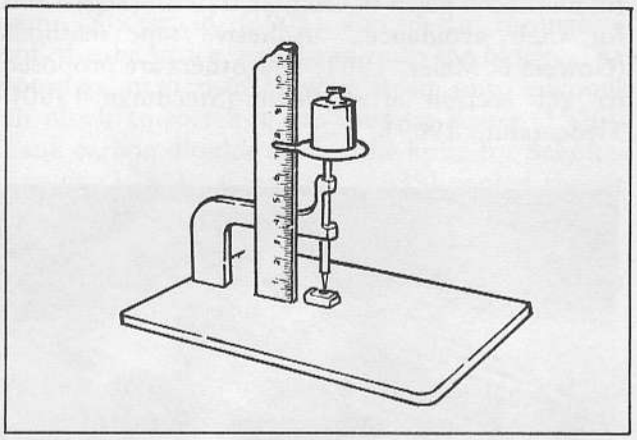


Fig. 21. Penetrometer for measuring hardness of specimens.

A paraffin used for some experimental work (Tissuemat 56) gave a penetration of 1.7mm with a 100 gram weight. A hardened paraffin[8] gave a reading of 2.3mm. Kidney tissue from a guinea pig was used to find out the effect of the usual procedures on the tissue. The fresh tissue failed to support the weight of the needle and pan (2 grams). After hardening the tissue in Bouins Fluid the penetration was 5.4mm. After dehydrating in absolute alcohol the 100 gram weight only drove the needle in 2.2mm; after clearing in xylene, 1.7mm; and after embedding in wax 1.0 mm. This shows great hardening of the fresh tissue due to the procedures of preparing it for sectioning, although care was used to keep to a moderately rapid schedule. During infiltration the temperature was maintained at the melting point of the wax.

The actual process of sectioning leads to distortion so that the sections will be a little bit thicker and a little shorter than the block itself. This distortion results from the nature of the paraffin, the tissue, and the action of the knife. Large crystals are sectioned or pushed apart by the wedging of the knife, especially when the paraffin is brittle and has a high plastic point. This upsetting of crystals gives the velvety appearance to the section.[9]

When the crystals are not of proper size to fit closely to the tissue, they cannot support the tissue adequately and local deformation will occur, giving a section which cannot be completely flattened out. Small folds show when the thin parts were smaller than the size of the paraffin crystals and compress less than the paraffin.

The crystals show clearly in polarized light. Photomicrographs in black and white do not record all the color differences and lose clearness. All microtomists should examine a section from each batch of paraffin with a polarizing microscope.* A finer crystalline paraffin gives adequate support. When the crystal structure is too large and inhomogeneous, that batch should be rejected.

Warm paraffin shrinks as it cools and compresses the tissue in the block. Tissue that is harder than the paraffin withstands this pressure, but soft or spongy tissue may be under considerable strain. When sectioned the tissue tends to expand to the shape and size it had before compression, and if confined by the paraffin around it, pleating or wrinkling results. The wax mixture[8] decreased 13 per cent in volume and 2.4 per cent in a linear direction on cooling to room temperature. Despite the fact that attention was called to shrinkage by Krause (1926) and Kisser (1927), little ac-

[7]Hardness and testing methods have not been extensively developed for paraffin cf. Williams (1940). Steedman (1960) used another method.

[8]From Waterman (1939) Paraffin (Parowax) 80%, stearic acid 16%, spermacetti 3%, bayberry 1%. Melting point 46°C.

[9]For further details the reader is referred to Dempster (1941, 1942ac). I am greatly indebted to Professor Dempster for a preview of his excellent work and for his friendly criticism during the progress of my experiments.

*A binocular biobjective (Greenough) Microscope with POLAROID above and below the section is suitable for this use.

count is given to it in current publications.[10] Stowell (1941) reported that Parowax shrinks in volume 14.3 per cent on cooling from 59° to 20°C. Shrinking can also vary with the attachment to the slide and the drying temperature (Smith 1962).

Unless the hardness of the paraffin is adapted to the temperature at which the cutting is done and the nature of the tissue, good sections may not be expected, regardless of the excellence of the microtome and sectioning knife. When the material has been properly dehydrated, it is possible to re-embed it in another paraffin should the first not prove satisfactory. Poorly prepared tissue can rarely be salvaged. Several changes of the melted paraffin should be used to wash out the former paraffin.

The microtome knife should be set at 90° to the direction of the cut (no slice angle) and tilted to as little clearance as will give good sections (2° to 6° cf. V, 4). The knife edge should not show any pronounced serrations when examined at 100 diameters, although the edge need not be so smooth for paraffin as for some other methods. The possibility of cutting with only a fair edge prevents many technicians from getting excellent sections, because they do not keep the microtome knife in the best condition.

The block must be trimmed so that the edges parallel to the knife are straight and parallel to each other; otherwise, the ribbon will not be straight and the distortion will be increased. A camel's hair brush (pencil type) is used to handle the ribbon. It may be necessary to hold the first few sections onto the knife with gentle pressure of the brush until the ribbon forms. Then the end of the ribbon is raised with a dissecting needle and placed over the brush. This is withdrawn from the knife as the ribbon lengthens until it becomes too long to handle. The ribbon lengths are placed in order on smooth paper or in a shallow box until they are mounted on slides. Some experience is necessary to cut serial sections without the loss of parts of the ribbon. Should the brush be carelessly allowed in contact with the knife, it will be spoiled rather than the knife. The use of a dissecting needle in place of a brush would damage the knife edge with this type of accident. Serial sections may be cut on a sliding microtome but less conveniently, as the hand supporting the end of the ribbon must move back and forth with the knife. (See page 2.)

Since the paraffin method is used more than any other at the present time, other problems of sectioning will be considered in the next chapter. Temperature control methods will be discussed in a following section. A check list of the more common difficulties encountered with the paraffin method is given in Chapter II, with suggestions for their avoidance. Adhesive tape methods (Gowers & Miller, 1961) and others are proposed to aid section arrangement (Steedman 1960), Tiedemann, 1969).

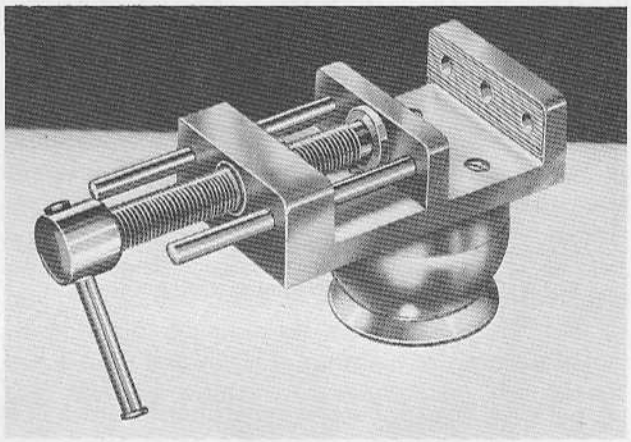


Fig. 22. AO Object Clamp.

G. STATIC ELECTRICITY ELIMINATION

One great inconvenience of paraffin sectioning, particularly in winter or at other times when the atmosphere is dry, is the flying of sections and their sticking to parts of the microtome and other nearby objects, due to the static electrical charge generated by the friction of cutting. Raising the humidity by boiling water in the room or burning a Bunsen burner near the microtome may alleviate the difficulty. Sometimes grounding the microtome to a nearby water pipe with a wire or chain will carry the electricity away. A wet belt is used by Church and Kroeger (1970) to reduce static charge on ribbons or sections.

[10]Kisser (1927) gives formulae for mixing paraffins and gives the following figures for volume shrinkage to 18°C room temperature: 36°, 8.8%; 40°, 10.3%; 45°, 10.6%; 50°, 11.1%; 55°, 12.6%; 60°, 13.8%. See also Smith 1962; Steedman (1960).

H. TEMPERATURE CONTROL

An air conditioned room maintained at the optimum temperature would be ideal for sectioning. Paraffin sections can be cut in a cryostat (IV D2). The traditional method is to immerse the block and the knife in ice water, dry them, and cut before they warm unduly. Krause (1908) used liquid carbon dioxide in a Dewar flask for cooling the blocks until they were sectioned. (Undue shrinkage from over cooling should be avoided.)

Vapor jets have been used for cooling the knife. von Lendenfeld (1901) forced air through a coiled tube in ice water and onto the block. The converse method of blowing steam onto a wooden block to soften it was used by Kisser (1926). Tank carbon dioxide cooled the knife for Schultz-Brauns (1931). Crossmon (1935) cooled the air by forcing it through pipes surrounded by a freezing mixture before it was blown onto the knife. Schechtman (1941) used tank CO_2 arranged so that it also flowed over and cooled the block.

Jackets have been used to surround the knife, and either hot or cold material was forced through the jackets. It is not practical to drill holes in the knife because the weakened knife is apt to crack during the process of tempering. Heating the knife in this way by steam was proposed by van Walsen (1894). Ice water was circulated through a knife jacket by Stoss (1891), who also used a cold air jet on the block. Held (1897) circulated water through a jacket surrounding the block holder. Separate lines to the knife and to the block holder were used similarly by Land (1914). Wagener (1967) used CO_2 cooling. A cooling channel was built into a safety razor blade holder by Craig and Wilson (1935). Duffield (1941) has proposed a box to fit the knife closely, which may be filled with warm or cold water as required. A box surrounding the knife was used to cool the knife with dry ice by Hueper (1933).

Since it is not always feasible to cool the knife or the entire room, a cabinet can be used to cool the microtome. Foot and Strobel (1905) built a box completely around their microtome with hand holes for manipulation and cooled by ice. Grave and Glaser (1910) placed an ice tray over a hollow truncated pyramid so that cold air flowed over the microtome. A large cardboard carton is placed over the microtome by Hance (1937) with a container of dry ice over a hole in the top of the carton. By adjusting the apertures between the dry ice container and the carton, to control the amount of carbon dioxide passing into the box, the temperature is regulated. The front end of the box was left open for access to the instrument.

Dry ice was used by Palmer and McDonald (1963) and Perkins (1963) used rubber balloons filled with cold water for cooling the knife and block. Freon, instead of CO_2, was used by Batsakis (1963) and thermoelectric methods are proposed by Okamoto & Mizuno (1963),Rutherford (1964) and others.

I. RAZOR BLADE HOLDERS

The razor blade holder, Fig. 23, provides a means for using inexpensive safety razor and similar blades in place of a standard microtome knife. Like substitute methods generally, it is not so satisfactory as a regular knife, but with care it is possible to make good sections, even at 1μm. These are used in large beginning classes in technique to avoid damage to microtome knives, to save the students the cost of knives, and to cut materials where the edge is rapidly destroyed.

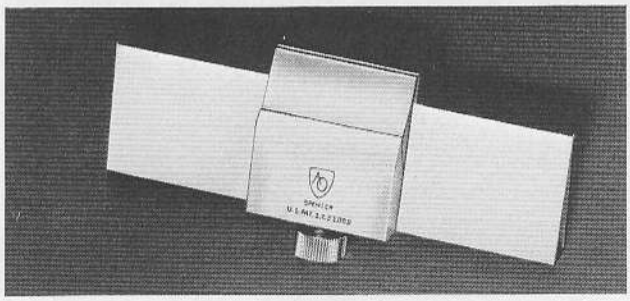


Fig. 23. AO Razor Blade for Rotary Microtomes.

The thicker types of flat safety razor blades are more satisfactory than the wafer-thin type. The blade should be inserted so that the cutting facet is just beyond the edge of the holder.

As soon as the edge is worn, a new blade should be used. It is rarely worthwhile to resharpen safety razor blades.

Disposable Microtome Blades are available which use a specially designed holder replacing the standard holder on the microtome (Fig. 24). The holder clamps the blade in a manner which insures rigidity and eliminates vibration. Since the blade is always clamped in the same position, and at the proper clearance angle to the specimen, knife tilt adjustments are not necessary. The design also makes it possible to change knives during sectioning without the loss of a section.

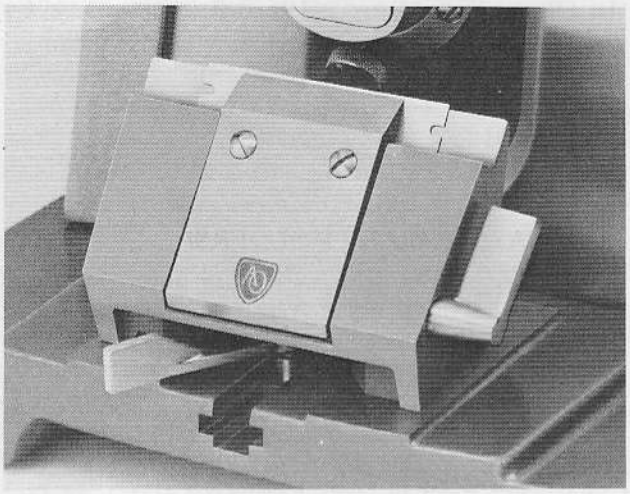


Fig. 24. Disposable Blade Holder

A glass knife holder (Fig. 25) allows the use of glass knives in the rotary microtome. The holder is clamped in the standard microtome knife holder. It is recommended for use with the ultra thin section adapter (829) on the AO Model 820 Microtome.

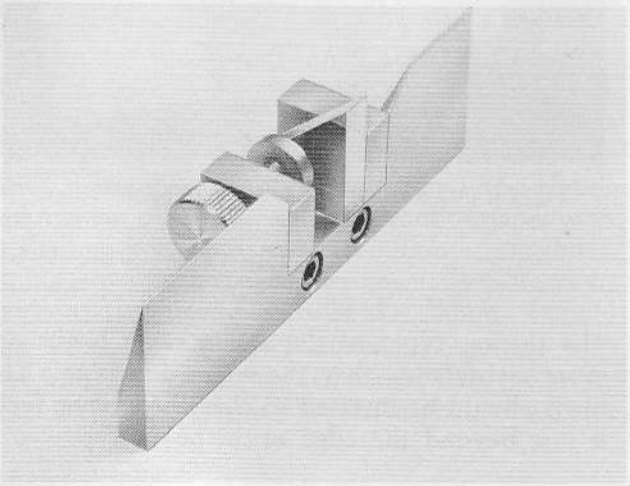


Fig. 25. Glass Knife Holder

[12] Cf. Lucas (1927)

J. SECTIONING OR SURFACING HARD MATERIALS (METALS, PLASTICS, ETC.)

Soft metals, plastics and other materials not harder than about 18 Brinnell may be sectioned or surfaced with a microtome. The upper limit depends on the brittleness or plasticity of the material. The thickness of the section is limited by the nature of the material and the size of the specimen. Sometimes thinner 5μm sections can be cut and with others thicker 15μm sections may cut better. The specimen should be no larger than the area to be examined.

Some materials can be clamped in the holder and sectioned. Other specimens require some support. One useful method is to place the material between two pieces of pith. Paper and film are cut when only a millimeter or so extends beyond a smooth, jaw clamp. A sliding microtome is preferable for sectioning plastics and a slicing cut may succeed when a square cut fails, Fig. 17.

Plastics with glass or mineral fibers or other hard materials usually cannot be sectioned with a microtome.

Rubber is a difficult material. Some rubbers can be sectioned after freezing hard and allowing to thaw enough for cutting. Some spongy and hard rubbers cannot be sectioned and only the surface can be examined with a vertical illuminator – a fresh surface can sometimes be obtained by breaking after freezing the specimen.

Lead alloys and other soft metals may be surfaced smooth enough for etching and microscopic study with a microtome more rapidly than by the usual grinding and polishing methods.[12] Other metals may require only polishing after surfacing. Great care is necessary with magnesium alloys and other metals which surface harden by cold working, to stop before the surface becomes so hard that it suddenly destroys the knife edge.

Metals may be embedded in methacrylate, other soft plastics or Bakelite. If the latter, a pure resin should be used rather than one mixed with asbestos or other fibers that will unduly wear the knife edge. Small pieces may be clamped directly in the jaws of the holder Fig. 22. The cutting should be moderately slow and only one or two microns removed at a time. For surfacing, the large Rotary Microtome is preferable to a Sliding Microtome because of the very solid construction of the knife holder and the feed mechanism.

Hard rubber, wall board, pressed wood, soldered joints, horn, bone, and similar substances have been sectioned with microtomes. The materials embedded in hard paraffin, soft plastics, or even melted sulphur are held in a clamp. When the section is desired rather than smoothing the surface, a large Sliding Microtome, Fig. 17 is preferred. A slicing stroke with a considerable slice angle is often advantageous, Fig. 15. The resourceful operator will soon find from experience the best means of handling a given material. Presoaking in water or oil facilitates cutting when such treatment will not interfere with the subsequent use to be made with the material (Lendrum, 1944). Wood has been steamed during cutting by Kisser (1926). It is usually easier to cut thin sections of a hard material; e.g. Harlow (1940) recommends that wood be cut at 3-5μm. Other special methods are described in the technical books listed in the bibliography.

When a long slicing type of stroke is not required, the razor blade holder and thicker type of blades are used for sectioning various hard and difficult materials, because a few cuts can dull the knife and it may be more economical to replace blades than to resharpen a standard knife. The AO Holder, Fig. 23, is strong enough to hold a flat blade during the hardest cutting that the edge of the blade will withstand.

Phillips (1961) uses a diamond knife when sectioning metals.

K. THE RATE OF CUTTING

The rate at which the section is cut influences its quality. Frozen sections cut better slowly. A slow even cut is desirable when cutting celloidin embedded material. Any hesistance during the cut may leave knife marks on the section. Some paraffin blocks of tissue must be cut slowly to obtain the best section, while others give a better ribbon if the microtome is run at a slightly greater speed. Undue speed invariably results in sections of poor quality.

More time is saved by cutting slowly and carefully than rushing through several yards of sections in the hope that a good one can be found in the lot.

The motor drive for AO Rotary Microtomes should not be run faster than sixty cuts per minute.

Each material sectioned on the microtome has an optimum cutting speed which should be determined experimentally; as it depends on the nature of the material, the cutting edge, the angle of the knife and the thickness of the section. With further research and control it may be possible ultimately to predict these rates rather than depend on trial as at present.

Oscillating or vibrating modifications of microtomes have been used for sectioning bone, teeth and mineralized tissues (Southern & Thomas, 1962; Burgstedt 1964; Eschenbrenner & Eggleston, 1964). Baud & Morgenthaler used a modified dental technique and methacrylate embedding proved useful for Siglitz (1964). See also Brain, 1966.

V. MINIMIZING DISTORTION

WITH SPECIAL REFERENCE TO THE PARAFFIN METHOD

An ideal section would be of the thickness indicated by the microtome feed setting and of the same size and shape as the tissue in the block. This rarely occurs. The killing, fixing and embedding process results in an average linear shrinkage of one-third; sometimes two-thirds (Ambrosias, 1955; Bahr, 1955; Bahr et al, 1957; Dempster, 1941; Miles and Linder, 1952; and Ross, 1953). The actual cutting causes distortion when the supporting medium is inadequate (paraffin: section IV, and Dempster, 1941). Aumonier (1938) concluded from comparison of 5-15μm with 25μm-sections that knife sharpness was the limiting factor in the distortion. Distortion can be a considerable source of error in three dimensional reconstructions.

A further difficulty is the lack of good measuring means for the thickness of the sections. The problem is discussed by Clemmens (1950), Gettner and Ornstein (1956) and Lange and Engström (1954). Interference microscopy (including channel spectra) offer the highest precision, Richards (1959).

Some methods and instruments were developed in 1939 for the evaluation of distortion in paraffin embedded tissue and this work will now be described.

A. PRECISION OF THE MICROTOME

A section of thickness different from the setting of the advance of the microtome would indicate error in the microtome or distortion from the cutting of the specimen.

Before making any experiments on actual cutting with the microtome, the feed mechanism was tested by fastening a dial gauge firmly and inflexibly to the frame of the microtome and reading the movement of the specimen holder by the dial gauge. The gauge was graduated in single microns and had been calibrated with an interferometer. The average error of the microtome feed, found with the microtomes used, was within ± 0.1μm.

The thickness of successive 10μm sections of heart tissue cut on a No. 820 Precision Rotary Microtome at 27°C using a standard knife set to a clearance angle of 6° gave a standard deviation (σ) of 0.3μm and the sections were within a range of 1.1μm. Similarly, using a No. 852 Sliding Microtome, the range was 0.6μm and the standard deviation was 0.2 m. Consequently, the variation in successive sections is due to the cutting process and the inhomogeneity of the tissue, rather than the microtomes used in the experiments. As these were in no way specially chosen, similar results may be expected from other AO instruments of the same type.

B. MEASURING SECTION THICKNESS AND CUTTING FORCE

The traditional method for measurement of section thickness cuts a measured length (e.g., a milimeter) of the block and divides this length by the number of sections obtained (Pusey, 1939). Unless a considerable amount is cut the precision of this method is low, and it does not differentiate errors in the specimen advancing mechanism of the microtome from the distortion of cutting.

John (1929) used an Optometer (200g point pressure) and found that section thickness averaged one-sixth greater than the setting of the microtome, depending on the material sectioned and the microtomes used. The available commercial testing machines were unsatisfactory for measuring the thickness of paraffin sections, because the pressure on the measuring point deformed the section.

Consequently, we turned to optical methods and designed an interferometer. A piece of glass was ground flat optically and the section mounted without fixative on one end of it. One end of a special cover glass also having an optically flat surface was placed on the edge of the tissue and the other end rested on the glass base. When illuminated with monochromatic light, Fig. 26, definite dark and light bands are seen, Fig. 27. The number of these bands, or fringes, is counted with the aid of a small telescope and when sodium light is used multiplying this number by 0.29 gives the true thickness in micrometers. The method is precise to one fringe, or about 0.3 of a micrometer.[13]

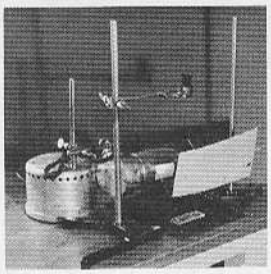


Fig. 26. Interferometer for measuring the thickness of sections.

Fig. 27. Interference fringes seen with the Interferometer of Figure 26.

For studying variations on the surface, a cover glass was used with two small hyperhemispherical lenses of about 1mm diameter attached to one end, and the third one attached to the center of the other end. As this is moved across the tissue, the single contact follows the surface, the number of fringes between fiducial marks changes accordingly, and the interferometer serves as a profilometer. The cover glass weighs only a fraction of a gram; consequently it is unlikely that this force causes any deformation of the tissue. The only disadvantage is that the method is slow, because only two sections (one at each end) can be measured at a time, and it requires time to mount the section on the glass and care to avoid distortion.

[13]The base piece was of thick glass, 26 x 75mm, with the under side ground to avoid reflections from the non-flat surface. The 25 x 38mm cover glass was ground wedge-shaped to avoid interfering reflections. A piece of black photographic wrapping paper was placed under the instrument. Williams (1930) describes the use of interferometers.

The resistance to cutting was determined by attaching a tuning fork beside a sliding microtome, Fig. 28, and recording its vibrations on a moving record surface as a constant weight pulled the knife through the block. The difference in time required to pull the knife the length of the block, when the instrument ran free, and when a section was cut, is a direct measure of the resistance to cutting, or of the force required, since the force equals the mass times the acceleration. The force (gravity driving weight) and the mass remain constant so the delay in time is a measure of the acceleration. Permanent records were obtained by fastening a platen to the knife block of the microtome and recording the tuning fork trace on *Waxon Recording Paper.* Records were made with the microtome feed set to 10μm. The positions of the beginning and end of the block and of the tissue embedded in the block were marked on the paper before it was removed from the carriage. The tuning fork was adjusted so that the record started each time from the same position. The resistance of the paraffin and of the tissue could be determined separately from the records, Fig. 29A. The time intervals (0.01 sec.) were counted with the aid of a low power stereoscopic microscope, and the difference between the free run and a cutting run was expressed by $\triangle$. For each experiment three to five separate cuts were averaged, depending on the amount of variation shown.[14] With good cutting conditions $\triangle$ was small. When the knife was tilted too little or too much, the force used was insufficient to pull the knife through the block. Between these extremes the method gave interesting and useful information.

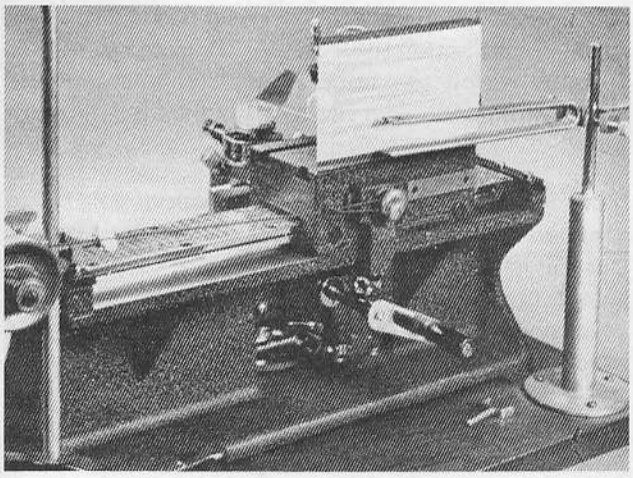


Fig. 28. Sliding Microtome arranged for measuring the resistance of cutting.

C. MATERIAL

Different specimens vary in their homogeneity, consequently in all of their cutting properties. It is difficult to find material sufficiently constant for a reference standard. To obtain a reasonably homogeneous reference material, one of the hardened paraffins made according to Waterman's method[8] was used. This was fairly homogeneous and satisfactory, but less dense than paraffin blocks. Cutting was done with blocks trimmed to 15mm square. While results from this material at the same temperature were comparable from time to time, it nowise represented actual conditions of cutting.

Three tissues were chosen for the experimental work, and large blocks prepared so that a number of tests could be made on the block. A typical block of human heart muscle with some arteriosclerosis was obtained from a local hospital. This was trimmed to 15 x 18mm with the actual tissue area of 11.5 x 13mm. The Penetrometer (100 gram weight) Fig. 21, showed that the tissue was much harder than the paraffin because the penetration into the tissue was only 0.4mm, while the paraffin was 1.5mm. It was difficult to get flat sections, because the paraffin frames prevented expansion of the wrinkled sections. The second specimen was a piece of cat testis, killed by Bouin's fluid, dehydrated in dioxan, and embedded in 55°C paraffin. A block was trimmed to 12 x 12mm and the tissue was an oval 9mm in the direction of the cut and 10mm at right angles to the direction of the cut. The hardness of the tissue was 0.7mm and of the block 1.2 mm penetration. The third section was of human uterus, killed in formalin, dehydrated in alcohol, and embedded in 60° paraffin. The block was trimmed to 14 x 21mm and the tissue was 6 x 16.5mm. The penetration of the tissue was 0.6 and of the paraffin 0.9mm. These specimens were used for the comparative work and others were used from time to time for general study.

[14]The decrease in rate of movement is due to 1) the resistance to rupture of the material being sectioned, 2) the friction between the knife and the paraffin and tissue and 3) the friction between the parts of the microtome. The latter are relatively constant so that standardizing against a free run was as satisfactory as a cut of block containing no tissue.

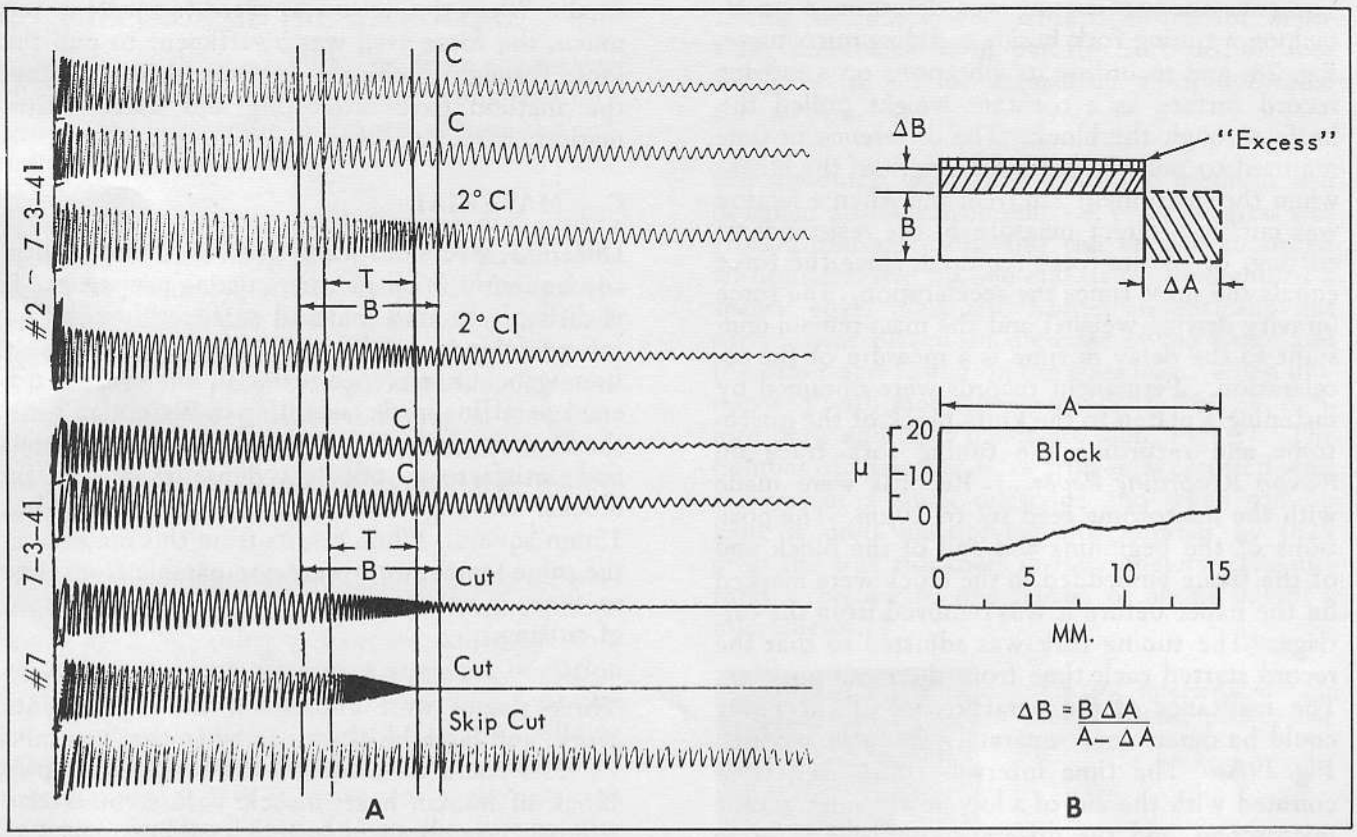


Fig. 29. A. Resistance to cutting record. B. Diagram illustrating distortion.

D. DISTORTIONS

Paraffin changes in size with temperature as described in section F, Chapter IV. Consequently, the thickness of the sections cut depends both on the temperature of the block at which they were cut and the thickness measured. The interferometer showed that the average section decreases approximately 0.9mm in thickness per °C, (3 fringes). Unless a different temperature is stated, the following discussion is based on records at or corrected to an average room temperature of 25° C. Sections were flattened for mounting with water at 45° C, and measurements made after drying.

The section is usually compressed in the direction of cutting, so that it becomes shorter and proportionately thicker while retaining the same width as the block. Taking into account the change in proportion, Fig. 29B, the predicted thickness can be computed from the formula given. This is true only for two dimensional change with the knife at right angles to the direction of cut. When a slice cut is made, the section will be distorted in all three dimensions (Dempster, 1941, 1942ac). Measurement of the thickness with the interferometer usually demonstrated greater thickness than this computed value, which will be called *excess thickness* in this report. With poor cutting conditions this excess thickness may bring the total thickness of the section 2½ times the thickness to which the microtome feed was set. The excess thickness was little influenced by temperature.

The increased thickness of the section, computed from the distortion of the section, depends on the nature of the knife edge, the tilt of the knife (clearance and rake angles), the temperature, and the material sectioned. The compression and thickness distortion decrease as the knife is sharpened with finer abrasives. Lightly stropping the knife improved its appearance under the microscope, but compression and distortion, particularly the *excess thickness,* was increased.

Sectioning tissue is quite different from sectioning paraffin blocks with no tissue. The relation of excess thickness, A in Fig. 30, and the computed increased thickness, B in Fig. 30, from distortion are shown with respect to the tilt of the knife. The tilt is expressed as the clearance angle and these results were obtained with standard factory-

sharpened knives. All sections were cut at 10μm on the sliding microtome with no slice angle (knife at 90°). With knife tilt up to 10° clearance angle, the heart tissue thickness was very close to the actual setting of the microtome, B in Fig. 30. The other tissues were thicker from the greater compression in length; A in Fig. 30, shows the excess thickness, or the difference between the total thickness measured with the interferometer and the thickness computed from compression. For all three tissues, this decreased rapidly to clearance angles of 4° - 8° and showed a definite minimum for each tissue.

The forces required for cutting, or conversely, the resistance to cutting as measured by the difference in time described in the previous section, is illustrated by C on Fig. 30. Less resistance is encountered as the clearance angle is increased to about 4°. Little further change occurs up to considerable tilt, when the resistance again increases, The heart tissue resistance to cutting increased rapidly at 10° clearance angle, and with 13° clearance angle skipping occurred, producing alternately thin and thick sections. All of the material so far investigated suggests that the optimum tilt angle ranges between 3° and 8° knife clearance. Within this limited range the resistance to cutting is minimal, and the measured thickness of the sections approaches a value very close to that calculated from measurement of the degree of section shortening; this is considered as a necessary condition for optimal sectioning.

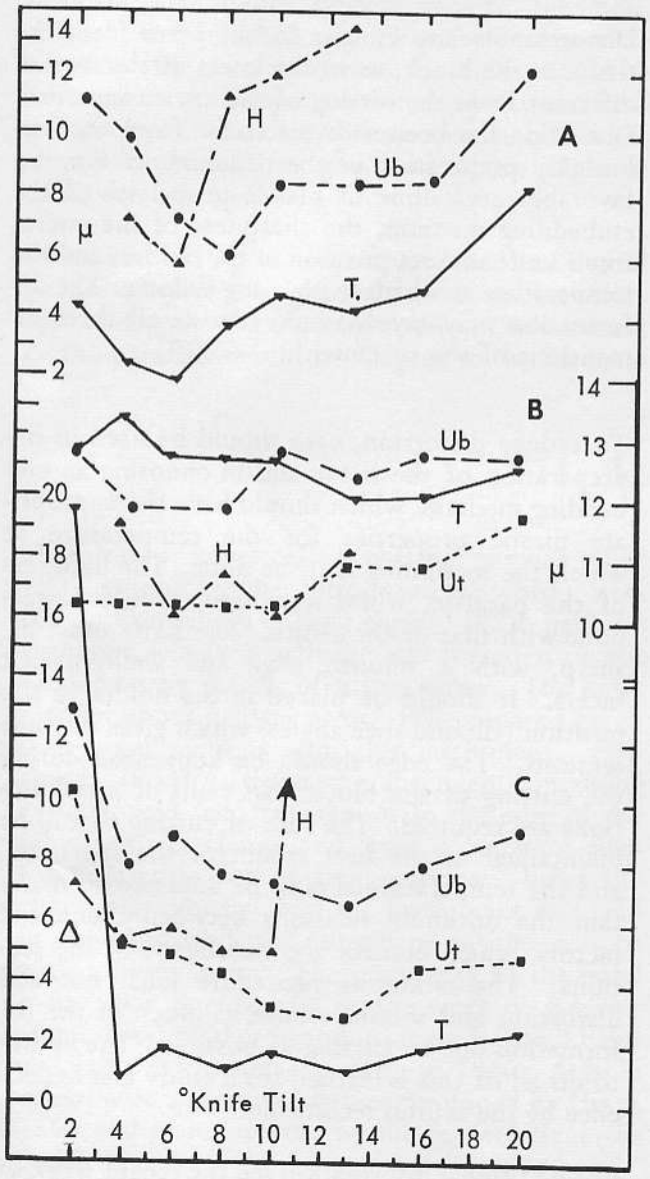


Fig. 30. Cutting resistance and compression with respect to knife tilt. A. Excess thickness. B. Thickness corrected for compression. C. Resistance to cutting. Cf. text.

With the knife set at the optimum clearance angle for a given tissue, it is possible to study the effect of different kinds of edges. A smooth, sharp, unstropped edge gave the least resistance to cutting ($\triangle$=0.3 to 1).

The resistance to cutting decreases with temperature, and the rate of the decrease is about the same as the change in thickness of individual sections with temperature, Fig. 31. The force re-

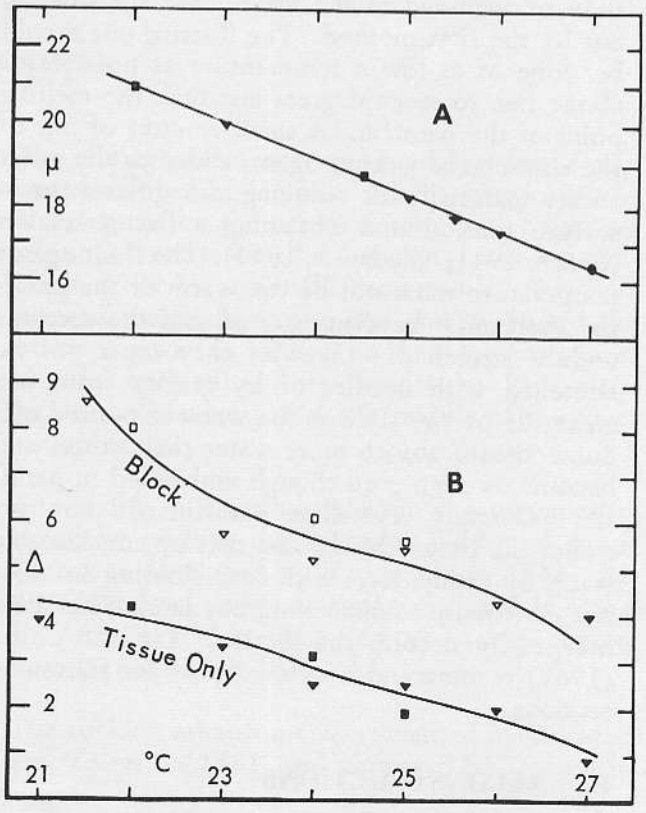


Fig. 31. Effect of temperature on (A) thickness of section and on (B) resistance to cutting.

quired, or conversely, the resistance to cutting of the tissue itself, is about the same as of the entire block and tissue, but the curve is slightly concave. These data were obtained with standard factory-sharpened knives and set to a clearance angle of 6° using the uterus tissue. A few scattered experiments with temperature gave essentially the same slope of curve for knives of different bevel. However, when the bevel was reduced to 17° the curve was much steeper. With such a knife it is difficult to cut in cool laboratories.

E. FLOATING OUT

Except under the most perfect conditions for cutting, the ribbon is formed with some irregularity, which must be flattened before the sections may be attached firmly and evenly to the slide. Two general methods are in use. The ribbon may be cut into convenient lengths, and placed on the slide, a few drops of water added to the slide, and the whole gently warmed until the sections expand to their full size. The other method is to place the pieces of the ribbon onto warm water and, after expansion, to recover them by placing a slide underneath the sections. A very thin layer of albumen fixatives may be rubbed onto the slide or included in the water used for floating out by the first method. The floating out should be done at as low a temperature as possible or about five to seven degrees less than the melting point of the paraffin. A small amount of one of the commercial wetting agents added to the water assists materially in reducing the differences in surface tension and obtaining a flatter section (Groat, 1941; Medawar, 1941). The floating out temperature must not be too warm or the paraffin itself may be disintegrated and the sections unduly stretched. Likewise they must not be stretched with needles or by gravity from too great tilt of the slide as the water is poured off. Some tissues absorb more water than others and become swollen even though embedded in paraffin. Coarsely crystalline paraffins do not surround the tissue closely and take up considerable water by capillarity. With care, floating out does not contribute to distortion, but careless handling may easily deform the section. Lin and Collet (1969) recommend a xylene spray for flattening sections.

F. RECONSTRUCTIONS

Earlier three dimensional reconstruction models were made from serial sections with little concern for errors from distortion, or actual knowledge of the true thickness of the sections. Recent work gives due consideration to correction or minimization of such errors; *e.g.* Dean and Magnum, 1945; Elias *et al*, 1951-71; Hennig, 1954, 1956; Merriam, 1957; Shields and Dean, 1949; Wüstenfeld, 1957. Heard (1951) describes a photographic method for the rectification of compression.

Lison (1937) and Pusey (1939) may be consulted for general methods.

G. CONCLUSIONS

Distortion is any change in the form from the tissue in the block, or in thickness of the section different from the setting of the microtome feed. Distortion has been shown to arise from the preliminary preparation of the tissue, shrinkage, unfavorable crystalline or plastic properties of the embedding medium, the sharpness of the microtome knife and its position in the holder, and the temperature at which the cutting is done. The deformation may involve one, two or all three dimensions of the specimen.

To reduce distortion, care should be used in the preparation of the tissue and in choosing an embedding medium, which should have the appropriate plastic properties for the temperature at which the sectioning is to be done. The hardness of the paraffin, when it is used, should correspond with that of the tissue. The knife must be sharp, with a smooth edge and well-polished facets. It should be placed in the holder to the position (tilt and slice angles) which gives the best sections. The edge should be kept clean during the cutting of the block, especially if serial sections are required. The rate of cutting should be maintained at the best speed for the specimen, and the temperature should be adjusted to maintain the optimum relations between the above factors, which control the excellence of the sections. The mounting procedure must not add distortion and should relieve as much of the deformation due to cutting as possible. The ability to do all of this is learned from study and experience by the skillful technician.

For additional information see the recent work of Steedman (1960) and for other aspects of distortions see: Gough, (1968); Lehmann, (1969); Tomasch, (1969).

VI. CONCLUSIONS

Microtomes are precision instruments and require care to keep them in proper condition. To insure optimal performance and long life of the instrument, the slideways should be cleaned with a soft cloth when cutting is finished or at the end of the day. All bearings should be properly oiled. The instrument must be kept clean, free from bits of embedding material and, when not in use, it should be covered or placed in a cabinet to prevent damage.

Unless the microtome knife is kept in excellent condition, it is not possible to cut good sections. Delicate and dense objects require the best, smooth, well-polished edge. For the best possible sections of a given specimen, the knife should be adjusted to the proper tilt for the particular kind of specimen. For an average specimen this tilt should be enough to give a clearance angle of 3°-8°.

When embedding is necessary, a medium should be chosen with the right grain or crystalline structure to support the object properly. The hardness of the paraffin should be adapted to the hardness of the specimen to prevent undue shrinkage, and to the temperature of the room at which the sectioning is to be done.

The types of distortion commonly found in section cutting have been discussed and recommendations from experimental work given, so that these distortions may be minimized.

This manual has been written to cover the use of the microtome and to give you the benefit of the experience of our own research laboratory. Section cutting depends on no single item. The formula for obtaining excellent sections is a precision microtome, a sharp knife, properly prepared specimens at the right temperature, and a skillful operator.

Microtomy is an art which is learned mainly from experience. This manual summarizes the experience of other for your use, but cannot replace the actual use of the materials. The beginner who understands and profits from mistakes is bound to become an expert technician.

VII. HISTORY[1]

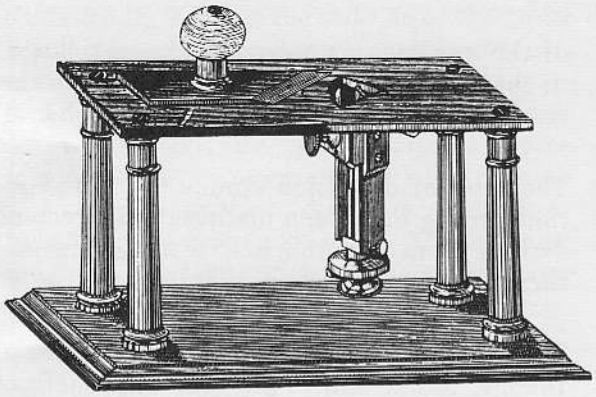
Microtomes were called cutting engines until 1839, when Chevalier introduced the word microtome. Cumming's cutting engine, 1770, held the specimen within a cylinder and forced it up for sectioning by means of a fine screw. The knife pivoted about a fixed pivot. Hill prepared excellent wood sections with this instrument. Pritchard, 1835, fastened the specimen containing cylinder and feed mechanism to the edge of the table with a clamp and used a separate two-handled knife for cutting sections. Later the clamp was omitted, and the instrument was held in one hand while the other hand cut the sections with a heavy razor. The popularity of the hand model was due largely to the work of Ranvier about 1880. Baker (London) manufactured hand and table microtomes as early as 1840. A table model with a slanting top was developed by His in 1866 and manufactured by the Societé Genevois in 1870.

The sliding microtome with a vertical feed screw was developed in 1798 by Adams, who obtained a slicing cut by the use of a knife with a slanting edge. Custace fastened the straight razor at an angle in 1799 to attain the same result. Queckett pivoted the knife at one end and supported the other on a way in 1848. The knife holder, adjustable for both tilt and slice angles, has been attributed to Schanze about the middle of the nineteenth century.

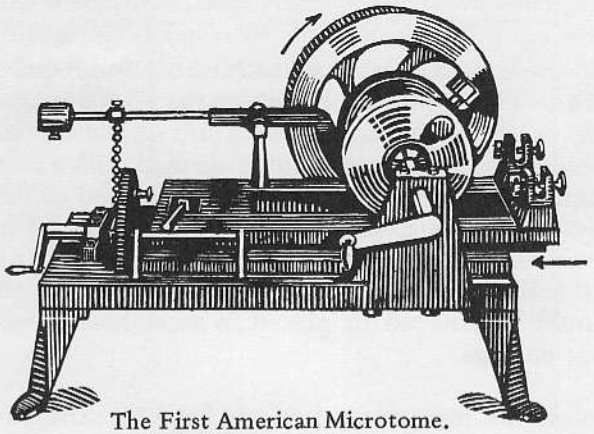
The sliding microtome wherein the specimen is raised by pushing the specimen holder up an incline was developed by Capanema in 1848. Rivet's improved design was manufactured in wood by Verick (Paris) in 1863, and in metal by Leyser-Brandt (Leipsig) in 1870.

The rocking microtome was invented by Caldwell and Trefall in 1881, and improved by Darwin in

[1]Cf. Behrens et al (1889). Gatenby and Painter (1937), Minot (1903), Romeis (1932), Smith (1915), Cowles & Richards (1947).



Adam's Cutting Engine, 1798



The First American Microtome.
Pfeifer's Rotary Microtome.

1885. The disadvantage of this design was the cutting of a curved cylindrical block surface, and led to the development of the flat-cutting type of rocking microtome in 1888.

Rotary microtomes were invented independently by Pfeifer at the John Hopkins University in 1883 and by Minot in 1886. Minot's model was manufactured by Baltzer (Leipsig) in 1887, and by the Franklin Educational Co. (Boston) in 1895.

In America, the sliding microtome with a triangular knife block was manufactured by Bausch and Lomb Optical Company in 1882, and an improved model three years later. Their first rotary microtome was manufactured in 1901, and the horizontal Minot Automatic Precision Microtome with stationary knife and sliding specimen holder and feed in 1909. Spencer Lens Company produced the Clinical Microtome in 1901, using the unequal parallelogram knife movement to give a slicing stroke. In 1910 the large Spencer Rotary Microtome first appeared, with increased precision of cutting obtained by separating the feeding mechanism from the driving mechanism (Ott, 1911). The Spencer Sliding Microtomes were developed during the middle of the nineteen twenties. Other kinds of Spencer microtomes are also manufactured. Instead of cataloging very large instruments it has been the practice of manufacturers in the United States to make specially designed instruments as required.

Many kinds of microtomes have been designed and tried. The more important types are summarized in Table I. All the principles except those in the circular and the peeling microtome are in common use at the present time. The peeling mi-

TABLE I

I. With movable knife

A. Object feed vertical

1. Knife is held in hand – table and hand models.
2. Knife moves around a vertical axis at one end, with the other end free or supported by a slideway.
3. Knife holder pivots on a parallelogram. Two unequal supports provide a slicing stroke.
4. Knife holder moves on a horizontal track.

B. Object feeds up an inclined plane (Capanema-Rivet model).

II. With stationary knife

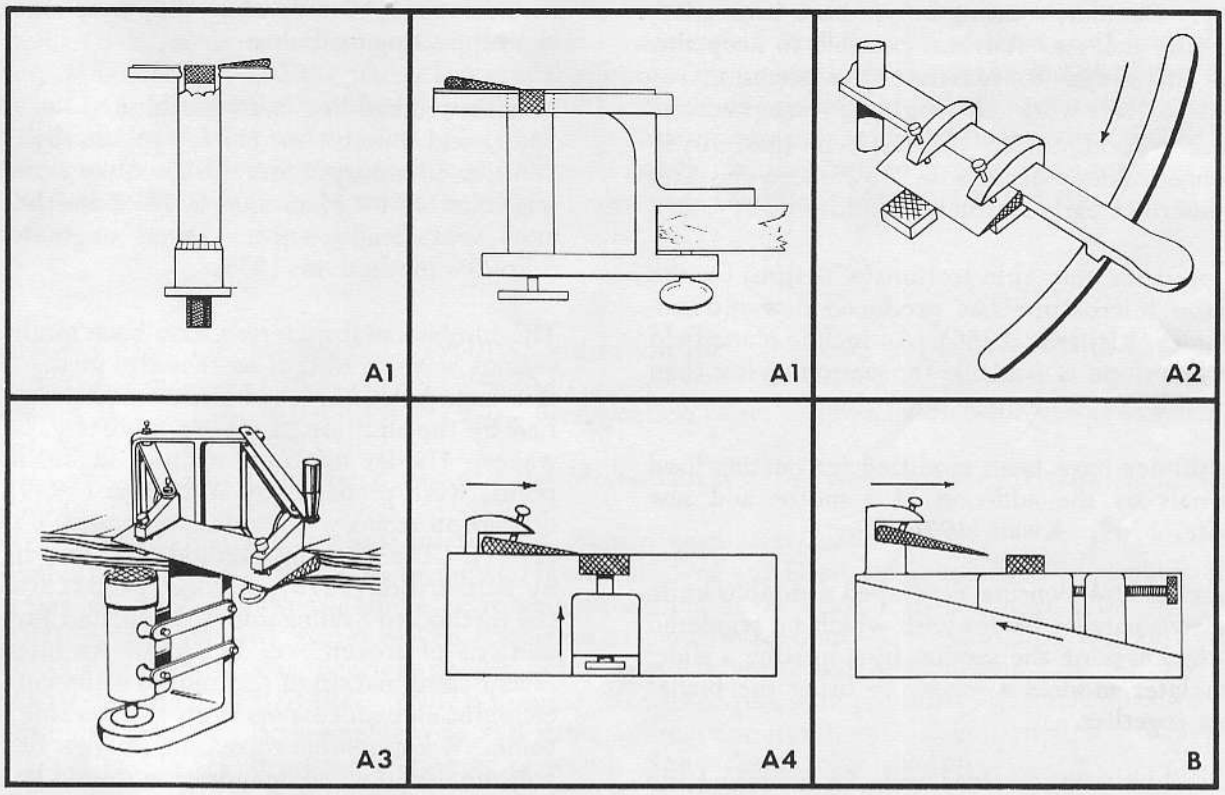
C. Knife vertical

5. Object moves along a vertical guide (Minot Rotary model).
6. Object holder moves around a horizontal axis (Rocking model). Sections are cut from cylindrically surfaced block.
7. Similar to 6 with a parallelogram object support to give flat sections (Flat-cutting Rocking Model).
8. Object rotates around a horizontal axis parallel to knife (Peeling method).

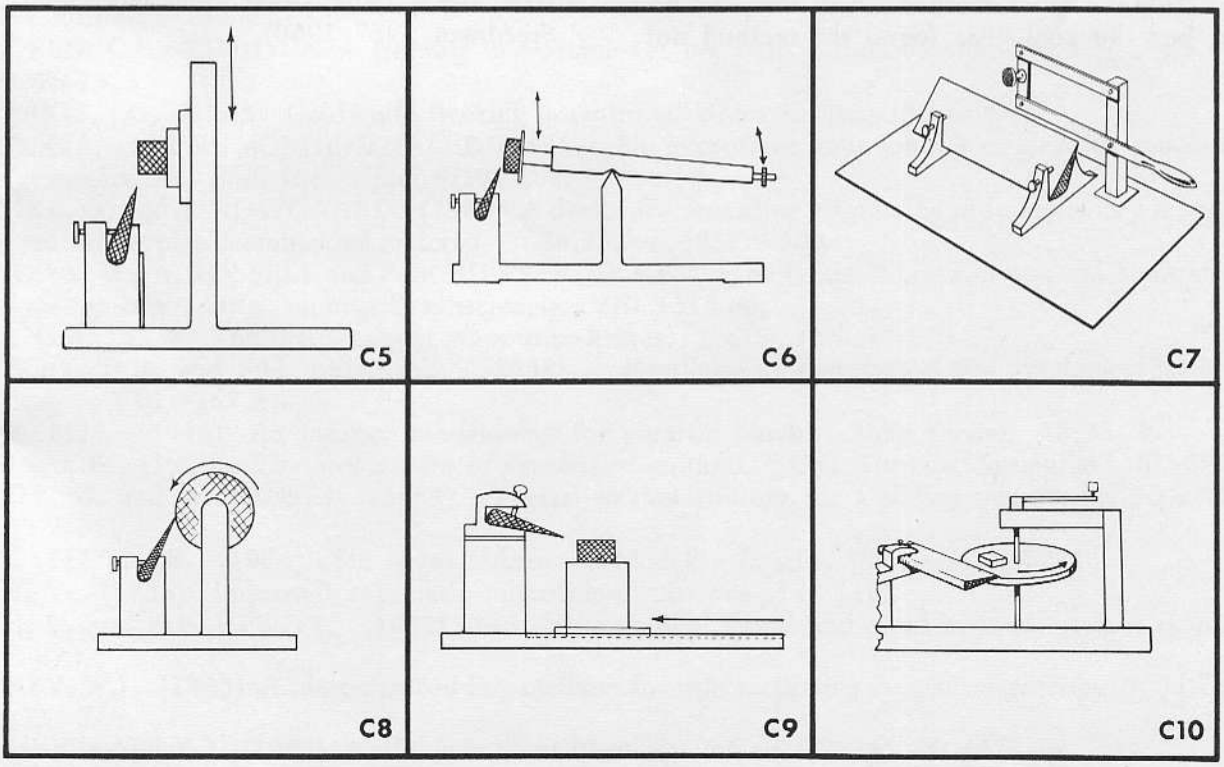
D. Knife horizontal

9. Object moves on horizontal ways.
10. Object rotates about a vertical axis.

I. WITH MOVABLE KNIFE



II. STATIONARY KNIFE



Types of microtome mechanisms.

crotome has been adopted by the wood veneer industry. Circular, rotating knives have been tried with little success. It is not possible to keep the blade sharp and to maintain the precision required for this work, although they are practical for use in food stores. The last type (No. 10) is no longer marketed. Krause (1926) also classifies and describes early European microtomes.

The need for very thin sections($<0.1\mu m$) for the electron microscope has produced new ultramicrotomes (Richards, 1956). An incline plane field with less slope is available for sectioning less than $1\mu m$ with the AO Rotary Microtome.

Microtomes have been modified for cutting hard materials by the addition of a motor and saw (Roofe, 1949; Kwan, 1970).

About 1839 Valentine developed a double knife with two parallel blades with which he regulated the thickness of the section by adjusting a slide, or in later models a screw, to bring the blades closer together.

The early cutting engines and the modern hand microtomes wedge the material to be sectioned into the feed cylinder of the microtome. Pith may be used to hold leaves and other softer tissues. This is a quick method useful for plant organs, but the zoologists found the method not satisfactory for animal tissues. The latter could be sectioned well only after they were embedded in a supporting medium.

Paraffin embedding was established by Klebs, 1869, and Bütchli in 1881; gelatin by Klebs, 1869, and later by Kaiser, 1880. Soap embedding was initiated by Flemming in 1873 and has been used sporadically since. Duval originated the celloidin method in 1879.

The fundamental materials have been modified in various ways to obtain more useful media. Paraffins have been hardened with ceresin and modified by the addition of rubber, bayberry, or other waxes. Harder mixtures without higher melting points were prepared by Waterman (1939). Hydrocarbon resins were added to paraffin by Groat (1941). The freezing method was used in 1871 by Rutherford (1873); Bardeen (1901) attributes the method to Stilling during 1846, and Boyle cut sections of frozen eyes in 1663. An interesting recent combination of techniques is the cutting of celloidin embedded tissues on the freezing microtome. Water soluble waxes and polyehtylene glycols are useful when dehydration should be avoided, and epoxys, methyl/methacrylate and other plastics are used in very thin sectioning. (Fall and Rawson, 1955; Glauert *et al*, 1956; Gray, 1954; Hale, 1952; Lillie, 1954; Miles and Linder, 1952; Steedman, 1957; 1960).

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The items given are intended to suggest current methods and to lead to detailed sources of information. Popular, non-technical, and the older, less available books are omitted. It is not the intent of this bibliography to give information on the preparation of materials or microscope slides. Certain general books marked by an asterisk (*) and Gray and Gray (1956) contain references to the bibliography in this field and may be consulted. If the references do not lead to an answer, you are invited to send your question or problem to American Optical Corporation, Scientific Instrument Division, Buffalo, N.Y. 14215.

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