Nov. 23, 1965 J. B. McCORMICK 3,218,896

SPECIMEN FREEZING DEVICE FOR USE WITH A MICROTOME

Filed April 8, 1963 2 Sheets-Sheet 1

Fig. 1

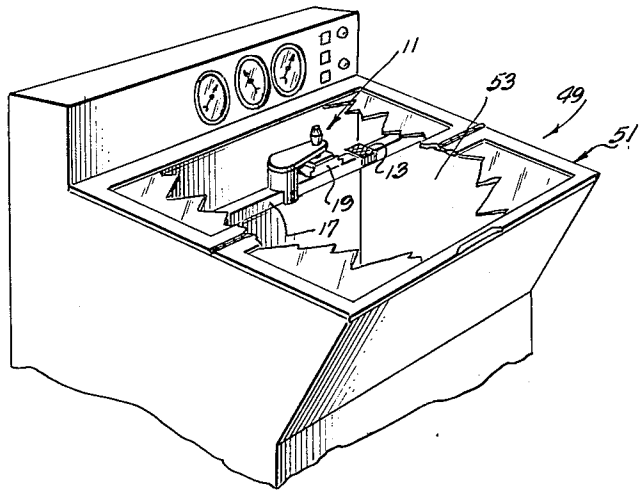


Fig. 2

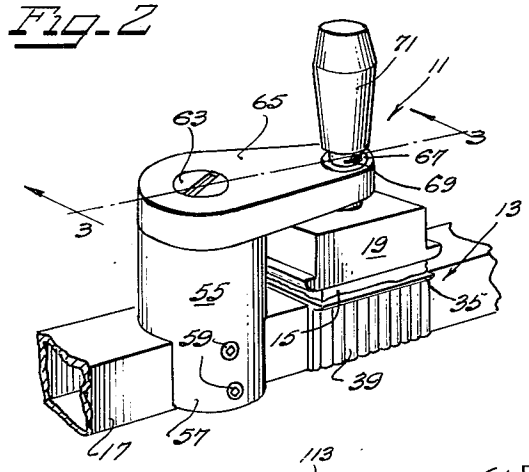


Fig. 3

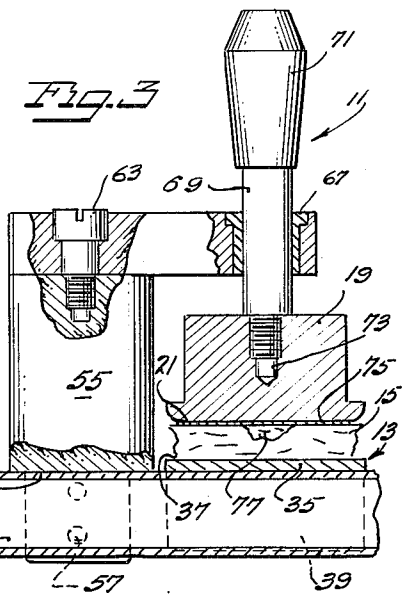
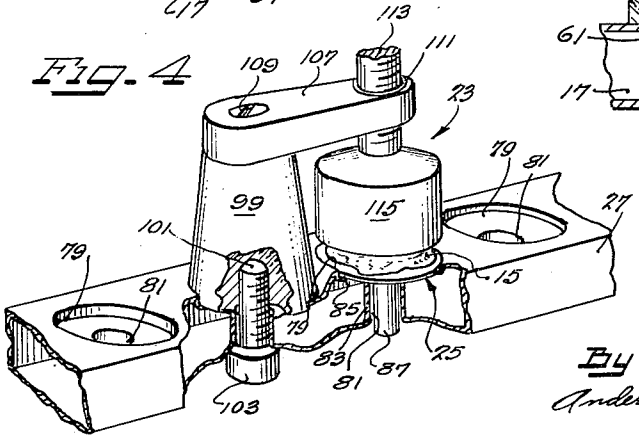


Fig. 4



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Filed April 8, 1963 2 Sheets-Sheet 2

Fig. 7

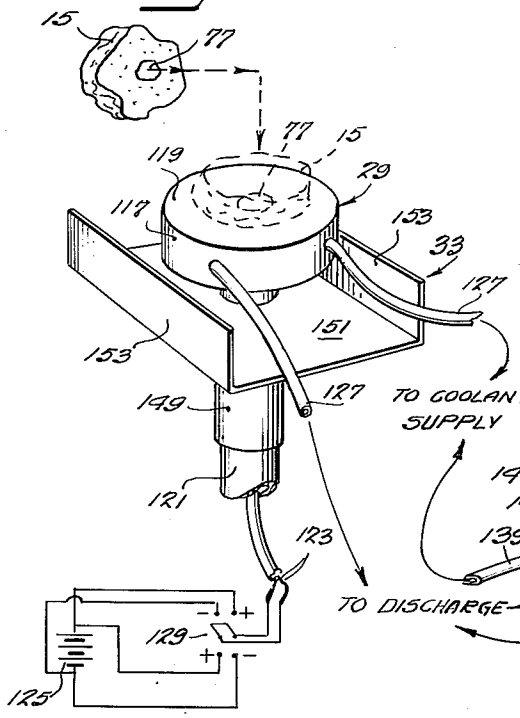


Fig. 8

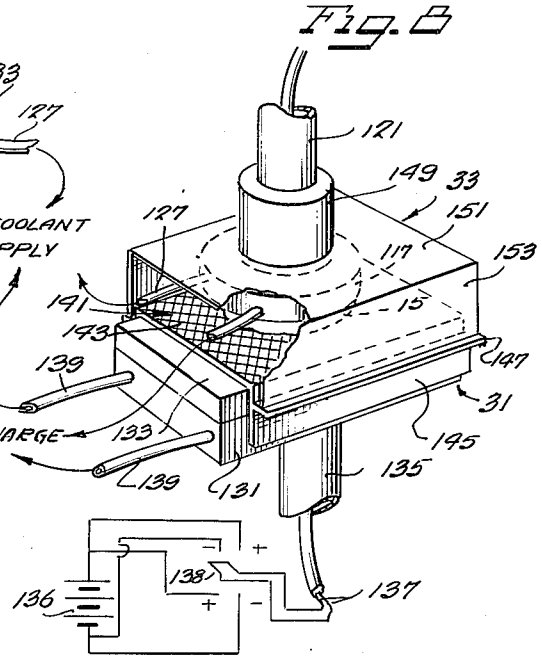


Fig. 5

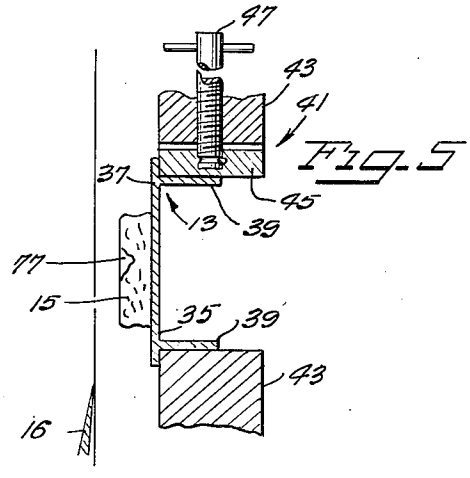
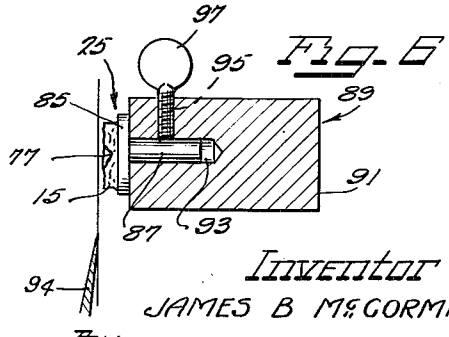


Fig. 6



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3,218,896

SPECIMEN FREEZING DEVICE FOR USE WITH A MICROTOME

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Filed Apr. 8, 1963, Ser. No. 271,137
11 Claims. (Cl. 83—15)

This invention relates to the freezing of specimens incident to the sectioning thereof in a microtome or similar instrument.

Specimens of material such as animal tissue are conventionally prepared for sectioning in a microtome by being embedded in a paraffin block or, alternatively, by being frozen to some form of carrier. The paraffin block or carrier is then mounted on the microtome either by being secured to the reciprocating chuck of the microtome, by means of which the specimen is moved into and out of engagement with a stationary knife, or by being supported on a stationary portion of the microtome in a position in which the specimen is engaged by a reciprocally movable knife. Regardless of the manner in which the specimen is mounted on the microtome, the relative movement between the carrier and the knife causes the knife to describe parallel cutting planes through the specimen.

In order to obtain sections satisfactory for microscopic examination through the use of a microtome, the face of the specimen presented to the knife ideally should be flat and lie in a plane parallel to the path of relative movement between the knife and specimen, i.e., parallel to the cutting planes which the knife describes in the specimen. This insures that the sections initially removed from the specimen will be of uniform thickness and, therefore, usable in the microscopic examination of the specimen.

When the freezing technique is used to prepare a specimen for sectioning, the face of the frozen specimen presented to the microtome knife has been found to be generally of irregular surface contour and configuration owing to the irregular shape of the unfrozen specimen. Accordingly, in order to render this irregular face suitable for the removal of satisfactory sections, it is customary to trim the face of the specimen by removing a sufficient amount of material to render the face flat and parallel to the aforementioned cutting planes. This preliminary removal of material, of course, causes a delay which is particularly undesirable in the examination of tissue removed by surgical biopsy, where the patient is maintained under anesthetic in surgery pending the outcome of the examination.

Also, and particularly in the sectioning of tissue specimens, a selected area of the specimen may be of particular interest to the pathologist and it may therefore be desirable to provide a section of the specimen for microscopic examination which includes a portion of this selected area. Ideally, therefore, the specimen should be so mounted on the microtome that a section including the selected area of interest may be most easily removed. However, when the specimen is frozen in such a manner that the selected area is located on the outwardly presented face of the specimen so as to be initially engaged by the cutting knife, much or all of the area might be removed by the preliminary trimming necessary to provide the face with a suitable surface. On the other hand, if the seletced area is disposed inwardly of the specimen, it may be necessary to remove and discard a large portion of the specimen before a section including the area of interest is provided, thus again necessitating undue delay.

Furthermore, in the frozen specimen technique, it is frequently difficult to freeze the specimen to the carrier

in such a manner that a section including the selected area of interest will be taken along the most desirable cutting plane.

Finally, it is desirable to freeze tissue specimens as rapidly as possible since a rapid freezing rate produces smaller ice crystals in the specimen. Specimens are thus occasionally frozen by means of liquified gas, but this method usually requires the presence and maintenance in the laboratory of cylinders of gas under pressure. An alternate method whereby a refrigerated environment is used generally accomplishes the freezing at an undesirably slow rate.

The principal object of the present invention is to provide an improved method and apparatus for freezing a specimen incident to the removal of sections therefrom.

Another object of the invention is to provide a method ad appartus for freezing a specimen to a carrier in such a manner that a desired section can be removed therefrom with a minimum amount of preliminary adjustment and trimming of the specimen.

A further object of the invention is to provide a method and apparatus for freezing a specimen to a carrier such that a particular area of interest of the specimen will be at least partially located in a predetermined cutting plane passing through the specimen.

A still further object of the invention is to provide an improved method and apparatus for rapidly withdrawing heat from a specimen.

Other objects and advantages of the invention will become apparent with reference to the following description and accompanying drawings.

In the drawings:

FIGURE 1 is a fragmentary, partially broken-away perspective view of an instrument incorporating a device showing various of the features of the invention;

FIGURE 2 is an enlarged fragmentary perspective view of the device and a portion of the instrument of FIG. 1;

FIGURE 3 is an enlarged partially broken-away sectional view taken along line 3—3 of FIG. 2;

FIGURE 4 is a fragmentary partially broken-away perspective view of an alternate embodiment of the device shown in FIGS. 1 through 3;

FIGURES 5 and 6 are schematic views showing specimens mounted in microtomes in accordance with the method of the invention; and

FIGURES 7 and 8 are fragmentary partially broken-away perspective views of an alternate embodiment showing various of the features of the invention.

The present invention contemplates the use of a carrier adapted to be supported so as to form part of a microtome and, as such, to occupy a predetermined position in relation to the relative path of movement between the cutting instrument of the microtome and the carrier support. The specimen is preferably frozen to the carrier with one of its faces adhering to a surface of the carrier. Prior to the freezing of the specimen, however, the opposite face of the specimen is engaged by a flat surface so disposed relative to the carrier that, when the carrier is placed within the microtome with the specimen frozen thereto, the face of the specimen which was engaged by the flat surface lies in a plane parallel to the relative path of movement of the cutting instrument of the microtome and the carrier support. This flat face of the specimen is presented outwardly away from the carrier and is exposed so as to be initially engaged by the cutting instrument of the microtome as the sectioning is commenced.

Thus, since the outwardly presented face of the specimen is flat and lies in a plane parallel to the relative path of movement of the cutting instrument of the microtome and the carrier support, the sections initially

taken from the specimen are of uniform thickness. Also, by properly disposing the specimen on the carrier, as hereinafter described, a selected area of interest of the specimen can be accurately located on the outwardly presented flat face thereof so as to afford easy and convenient removal of a section containing a portion of the selected area without the liklihood of destroying, or rendering unusuable, all or a portion of the area.

Very generally, a device **11** of the principal embodiment of the invention as shown in FIGURES 1 through 3 of the drawings is utilized with a carrier **13** adapted to support a specimen **15** with one face of the specimen presented outwardly for engagement by a cutting instrument **16** of a microtome. The carrier **13** is adapted to be integrated into a microtome whereby it occupies a predetermined position with relation to the relative path of movement between the cutting instrument **16** of the microtome and that portion of the microtome on which the carrier is supported. The carrier is also adapted to be supported on a thermally conductive refrigerated bar **17** prior to the mounting thereof in the microtome, heat being thereby withdrawn from the specimen through the carrier by means of the bar.

The device **11** is supported on the bar **17** and includes means **19** defining a flat surface **21** placed in engagement with the outwardly presented face of the specimen prior to the freezing of the specimen, with the disposition of the flat surface **21** thereby determining the disposition of the plane in which the outwardly presented face of the specimen lies. The flat surface **21** is so disposed relative to the carrier **13** that, when the specimen is frozen and the carrier is mounted on the microtome, the outwardly presented flat face of the specimen lies in a plane parallel to the relative path of movement of the specimen and cutting instrument of the microtome.

A device **23** shown in FIGURE 4 and constituting an alternate embodiment of the invention is utilized with a modified form of carrier **25** and refrigerated bar **27**.

In the embodiment of the invention shown in FIGURES 7 and 8, a member **29** defining a flat surface and a carrier **31** are provided, each of which is cooled by means of a thermoelectric module. A positioning device **33** is provided for insuring that the outwardly presented surface of the specimen as engaged by the member **29** will be disposed in predetermined relation to the carrier **31**.

Referring now more specifically to the principal embodiment of the device as shown in FIGURES 1 through 3, the carrier **13** is preferably formed of a metal having a high thermal conductivity and includes a flat plate **35**, one face **37** of which is adapted to receive and support the specimen **15**. This face **37** is roughened as by shallow cuts to enable the specimen to adhere thereto when frozen and, while shown in the drawing to be flat and to lie in a single plane, may however be of various contours and configurations if desired.

Depending from the opposite surface of the plate **35** in normal disposition to the face **37** and parallel to one another are a pair of spaced-apart flanges forming skirts **39**. The skirts **39** facilitate the mounting of the carrier in a microtome, and also aid in the dissipation of heat from the specimen and plate **35** when the carrier is supported on the bar **17**, as will hereinafter be described.

The carrier **13** may either be mounted on the movable chuck of a microtome, or mounted on a stationary portion of a microtome in a position which will place it in proximity to a reciprocally movable knife. In the illustrated embodiment, the carrier is particularly adapted to be clamped within a movable chuck **41** such as that shown in FIGURE 5. The chuck **41** comprises a pair of wall members **43** disposed in a vertically spaced fixed position relative to one another so as to define parallel horizontally disposed opposing faces, and a clamping member **45** carried adjacent one of the wall members and adapted to be urged away from the one fixed wall

member by a screw **47**. The clamping member **45** is also provided with a generally horizontally disposed face, and is moved by the screw **47** in a vertical direction normal to the faces of the wall members and clamping member. Likewise, when the chuck is reciprocated, its movement defines a path normal to the aforementioned faces.

The carrier **13** is mounted in the microtome with the skirts **39** disposed horizontally intermediate the clamping member **45** and the opposite wall member **43**. As so disposed, the specimen-receiving face **37** of the carrier is vertically disposed and in parallel relation to the relative path of movement between the chuck **41** and knife **16**, i.e., when the knife is stationary, the path of movement of the chuck.

It will be noted, therefore, that the position of the carrier relative to the chuck of the microtome and, hence, to the relative path of movement between the carrier support or chuck and knife of the microtome, is fixed. Hence, in positioning the means **19** defining the flat surface **21** so as to render it engageable with the specimen in such a manner that the outwardly presented surface of the specimen will be parallel to the above-mentioned relative path of movement, each portion of the carrier is capable of serving as a point of reference. As hereinafter explained, however, the plate **35** is utilized as a reference.

As previously mentioned, the carrier **13** is also adapted to be supported on the refrigerated bar **17** which is effective to withdraw heat from the carrier and specimen prior to the mounting of the carrier in the microtome. The bar **17** preferably forms part of an instrument **49** such as that shown in U.S. patent application Serial No. 232,475, now Patent No. 3,204,424, of which James B. McCormick is the inventor. Briefly, the instrument **49** comprises a cabinet **51**, the walls of which define a well or chamber **53**. A plurality of refrigeration coils (not shown) are disposed behind the walls defining the well **53** and form a network for the circulation of a refrigerant. The bar **17** in the form of a hollow pipe of rectangular cross-section extends across the well **53** adjacent the upper end thereof and is interconnected to the coils so as to constitute a conduit for the refrigerant and form part of the aforementioned network. The bar **17** is thus maintained at a low temperature and serves as an effective means for withdrawing heat from the carrier and specimen.

The carrier is supported on the bar **17**, as shown in FIGURES 1 through 3, with the plate **35** resting on the upper wall of the bar and the skirts **39** straddling the side walls of the bar. Preferably, the skirts **39** are spaced a distance only slightly greater than the width of the bar so that there will be a removal of heat from the specimen and carrier via the skirts as well as by conduction through the plate **35**. The carrier **13** is slidable on the bar **17**, a feature which renders it particularly adapted for use with the device **11**, hereinafter described.

The device **11** is adapted to be supported on the bar **17** and includes a pedestal **55** which is generally cylindrical in shape and is formed of a rigid material, preferably a metal so as to enhance the thermal-conductivity of the device. The lower end of the pedestal is broached to provide a slot to receive the bar **17**, the slot having a width closely approximating the width of the bar so that the side wall portions **57** defining the slot will straddle the bar when the device is supported thereon. Each side wall portion is provided with a pair of threaded holes to receive set screws **59** engageable with the side walls of the bar to detachably affix or clamp the pedestal thereto and leave the pedestal **55** shiftable to various locations along the bar **17**.

The aforementioned slot is so provided in the pedestal **55** that a surface **61** defining the upper wall of the slot is disposed normal to the longitudinal axis of the pedestal **55**. Consequently, when the pedestal is clamped to the bar with the surface **61** of the slot resting upon the upper wall of the bar, the longitudinal axis of the pedestal is normal to the upper surface of the bar and, hence, to the speci-

men-receiving surface 37 of the carrier 13 when the carrier is supported on the bar.

The upper end of the cylindrical pedestal 55 preferably lies in a plane disposed normal to the longitudinal axis of the pedestal and is provided with a threaded hole coaxial with the longitudinal axis of the pedestal to receive a machine screw 63 which secures an arm 65 to the upper end of the pedestal. The arm 65 as shown in the illustrated embodiment comprises a flat plate, preferably formed of metal, having a slightly tapering configuration as it extends away from the pedestal. One end of the arm is provided with a countersunk hole to receive the machine screw 63 which passes through the arm and into the threaded hole of the pedestal, the screw being effective to maintain the arm firmly on the pedestal while permitting pivotal or swinging movement of the arm about the longitudinal axis of the pedestal in a plane normal to the axis.

The opposite end of the arm 65 is provided with a hole lined with a sleeve 67, the sleeve being adapted to receive a rod 69, hereinafter described, which carries the surface-defining means 19. The axis of the hole defined by the sleeve 67 is disposed normal to the longitudinal axis of the arm 65 and, hence, parallel to the longitudinal axis of the pedestal 55, but normal to the upper surface of the bar 17.

The rod 69 as shown in the drawings is of generally circular cross-sectional configuration, and is slidably disposed within the sleeve 67. The upper end of the rod is enlarged to provide a knob 71 by means of which the rod may be raised and lowered, and the lower end of the rod is threaded to facilitate attachment thereto of the surface-defining means 19 in the form of a block, referred to in greater detail shortly. As can be seen best in FIGURE 3, the length of the rod 69 intermediate its threaded end and the knob 71 is such as will permit the block 19 to be elevated a sufficient distance above the top wall of the refrigerated bar to allow a specimen-supporting carrier positioned on the bar to be inserted beneath the block, and also to permit the block to be lowered into engagement with the top wall of the bar 17 when a carrier is not disposed beneath the block. In the latter position, which is the position of the block when the device 11 is not in use, that block 19, which is formed of a material of relatively high thermal conductivity, is maintained at a low temperature approximating that of the bar 17 and thus is able to quickly extract heat from the specimen when placed in contact therewith.

The block 19 is formed of a rectangular piece of metal having a ledge projecting outwardly from the lower edge of each of a pair of opposing sides. The lower surface 21 of the block is flat, smooth and imperforate and adapted to engage the upper surface of the specimen 15, and a threaded hole 73 is provided in the upper surface of the block to receive the lower threaded end of the rod 69, the axis of the hole 73 being disposed normal to the specimen-engaging face 21 of the block.

It will be noted, therefore, that the specimen-engaging face 21 of the block is normal to the longitudinal axis of the pedestal and, accordingly, parallel to the upper wall of the bar 17. Hence, the specimen-engaging face of the block will be parallel to the specimen-receiving face 37 of the carrier 13 when the carrier is disposed on the bar in underlying relation to the block, and the surface of the specimen 15 engaged by the block will be caused to become parallel to the specimen-receiving face of the carrier. Since the specimen-receiving face of the carrier is disposed in parallel relation to the relative path of movement between the carrier support and cutting instrument of the microtome when the carrier is mounted thereon, the external or outwardly presented face of the specimen is also so disposed, as is desired.

In a preferred method of utilizing the device 11, as hereinafter described, the surface 21 of the block 19 is placed in engagement with the outwardly presented surface of the specimen while the later is being frozen, i.e., while the latter is being rendered sufficiently solid to permit accurate sectioning thereof. To permit the specimen to be easily released from frozen engagement with the lower surface 21 of the block, it has been found to be helpful to coat this surface with a thin layer 75 of material, a chlorinated fluorocarbon such as Teflon being found to be highly satisfactory.

In the use of the device 11 shown in FIGURES 1 through 3, the pedestal 55 is secured to the refrigerated bar 17, and the device is normally maintained in an inoperative position during a period of non-use with the surface 21 of the block 19 resting on, and in heat-conductive engagement with, the upper surface of the bar so as to maintain the block at a low temperature. The carrier 13 may also be supported on the bar so as to maintain it at a low temperature as well.

When it is desired to remove a section from a specimen, particularly a section including an area of particular interest as designated at 77 in FIGURES 3 and 5, the specimen is placed on the specimen-receiving surface 37 of the carrier 13 with the area of interest 77 presented upwardly, i.e., with the area of interest located in the face of the specimen opposite to the face in engagement with the surface 37 of the carrier.

The carrier is then moved to a portion of a refrigerated bar 17 which underlies the specimen-engaging surface 21 of the block 19, the block being raised to accommodate the carrier and specimen and then lowered so that the surface 21 of the block rests upon the upper surface of the specimen, this being the surface of the specimen which includes the area of interest (FIG. 3). Preferably, the block 19 is urged downwardly onto the specimen with some pressure to insure that the face of the specimen will become flattened by the block and imparted with a complementary flat surface. The upper surface of the specimen thus lies in a plane parallel to the specimen-receiving surface of the carrier.

The specimen is preferably maintained with the block 19 resting upon its upper outwardly presented surface until the specimen becomes frozen, although it might be removed earlier if desired. Since the carrier and block were both maintained in contact with the bar prior to the introduciton of the specimen and, hence, were both maintained at a relatively low temperature, both are capable of withdrawing heat rapidly from the specimen. In addition, heat is removed through the carrier by conduction by virtue of its continuing contact with the bar 17.

When the specimen is frozen, the block is removed therefrom, such removal being facilitated by the coating 75 provided on the lower surface 21 thereof. As frozen, the outwardly presented face of the specimen is flat and lies in a plane parallel to the specimen-receiving surface 37 of the carrier and includes the area of interest 77. The carrier is then mounted in the microtome (FIG. 5) with the specimen-receiving surface 37 of the carrier, and hence the outwardly presented surface of the specimen, disposed in parallel relation to the path of relative movement between the carrier support (the chuck 41 in FIG. 5) and the cutting instrument 16. Hence, the initial section removed by the microtome will be of uniform thickness and, other conditions being satisfatcory, will normally be suitable for immediate mounting on a slide for microscopic examination.

It should be noted that while the device 11 and carrier 13 of the principal embodiment are shown as being formed with the surface defining means 21 of the block parallel to the specimen-receiving face 37 of the carrier, this particular relationship is not necessary and it would be possible for the specimen-receiving surface of the carrier to be, for example, convex. It is preferred, however, that some portion of the carrier be always maintained in the same position with relation to the relative path of movement of the carrier support and cutting instrument of the microtome when the carrier is mounted thereon, and that the specimen-engaging face of the block 19 be so disposed by the device 11 when the block is in

engagement with the face of the specimen as will render the outwardly presented face of the specimen parallel to the above-mentioned path of movement.

In the alternate embodiment of the invention shown in FIGURE 4, a device **23** is shown mounted on a refrigerated bar **27** which includes a plurality of circular depressions **79**, each of which is provided with a central hole **81** extending downwardly through the bar and defined by a cylindrical tube **83** sealed as by welding to the upper and lower wall of the bar to prevent the loss of refrigerant.

The circular depressions **79** are adapted to receive a carrier **25** comprising a circular plate or disk **85** having a diameter slightly smaller than that of the depression so as to permit the plate to rest conveniently within the depression. One face of the disk **85** is roughened to provide a specimen-receiving surface, and a stud **87** projects centrally from the opposite face of the disk **85**, the stud being adapted to occupy the hole **81** extending through the bar **27**. The longitudinal axis of the stud **87** is generally normal to the plate, and the stud is preferably of a length greater than the vertical thickness of the bar so as to permit the lower end of the stud to extend past the lower wall of the bar. The carrier can thus be easily removed from the bar by engaging the lower end of the stud and urging it upwardly, thereby elevating the disk **85** from the depression **79** to a position wherein it might be easily grasped.

The carrier **25** may be mounted on either a stationary or movable portion of a microtome but, in the preferred embodiment as shown in FIGURE 6, the carrier is mounted in a chuck **89** which is in the form of a cylinder **91** having a horizontally disposed hole **93** extending inwardly from one of its faces to receive the stud **87** of the carrier **25**. The axis of the hole **93** is normal to the relative path of movement between the chuck **89** and a cutting instrument **94** of the microtome. A set screw **95** having a flat finger-engaging portion **97** at its upper end extends inwardly into the block in a direction normal to the axis of the hole so as to engage the stud **87** and hold the carrier in place. With the carrier so mounted on the microtome, the specimen-receiving face of the carrier is parallel to the relative path of movement between the carrier support or chuck **89** and the cutting instrument **94**.

The device **23** itself comprises a pedestal **99** in the form of a truncated cone, the base end of which lies in a plane generally normal to the longitudinal axis of the pedestal and has a diameter slightly less than that of a depression **79** of the bar **27** so as to be accommodated therein. When so accommodated, the longitudinal axis of the pedestal is normal to the top wall of the bar **27**. A threaded hole **101**, disposed with its axis coaxial with the longitudinal axis of the pedestal **99**, extends upwardly into the base and is adapted to receive a machine screw **103** extending upwardly from the lower wall of the bar **27** through the hole **81** in the bar so as to secure the pedestal to the bar. The upper end of the pedestal lies in a plane parallel to the base end thereof and is provided with a threaded hole (not shown) similar to that of the pedestal of the principal embodiment so as to permit attachment thereto of an arm **107**, hereinafter described.

The arm **107** is similar to the arm **65** of the device **11** and is in the form of an elongated flat plate provided with a hole adjacent one end adapted to receive a machine screw **109** by means of which the arm is secured to the pedestal **99** for swinging movement thereon. The opposite end of the arm **107** is also provided with a hole within which is disposed a cylindrical sleeve **111** to receive a rod **113** which supports a block **115**, hereinafter described. The axis of the sleeve **111**, as was the case with the axis of the sleeve **67** of the principal embodiment, is disposed in parallel relation to the longitudinal axis of the pedestal **99**.

The rod **113** is slidably carried within the sleeve **111** and is preferably enlarged at its upper end to provide a knob (not shown). The lower end of the rod **113** is threaded to permit attachment to the block **115**. The rod **113** is of such a length as will permit the block to be lowered into engagement with the upper surface of the bar **27** when the device is not being used, such a position preferably placing the block in the base of one of the circular depressions **79**. The length of the rod is also such as will permit the block to be raised sufficiently to accommodate a specimen-supporting carrier **25** therebeneath.

The block **115** of the alternate embodiment is generally cylindrical in shape, but is provided with a somewhat convex upper surface. A threaded hole extends downwardly into the block and is adapted to receive the threaded end of the rod **113**. The lower end of the block **115** lies in a plane normal to the axis of the threaded hole and may be coated, as was the case with the block **19** of the principal embodiment, to permit the specimen to be easily released from the surface after it has been frozen thereto.

The device **23** is utilized in substantially the same manner as the device **11**, and hence, its method of use will not be further described.

In the embodiment shown in FIGURES 7 and 8, a member **29** defining a flat surface, and a carrier **31** are provided, each including a thermoelectric module. Accordingly, a mechanically refrigerated member such as a bar **17** or **27** is not utilized.

The member **29** and carrier **31** are used in a slightly different manner than the devices **11** and **23** and carriers **13** and **25**. In the use of the embodiment of FIGURES 7 and 8, the specimen is first placed upon the member **29** and, after the freezing of the specimen has been commenced, the specimen is transferred to the carrier **31** where the freezing is completed. Either or both of the units may be portable if desired.

More specifically, the flat surface-defining member **29** comprises a housing for a thermoelectric module (not shown), the housing being in the form of a circular plate **117**. The upper face **119** of the plate is adapted to receive the specimen **15** and serves as either a heat-absorbing or heat-dissipating surface depending upon the direction of current flow to the module. The face **119** may be provided with a coating in the manner of the block **19** of the device **11** if desired.

The plate **117** is mounted on the upper end of a hollow tube **121**, with the longitudinal axis of the tube being disposed generally normal to the plane of the upper face **119** of the plate. Suitable conductors **123** connected to a source of direct current such as storage batteries **125** extend from the plate through the tube **121**, the tube thereby serving as a conduit for the conductors and also as a handle by means of which the plate **117** can be easily supported.

In order to facilitate the dissipation of heat from the lower surface of the thermoelectric module when the upper surface **119** of the plate **117** is being maintained at a low temperature, so as to thereby provided a greater temperature differential on opposite sides of the module, conduits (not shown) adapted to carry a fluid coolant such as water are provided and extend adjacent the lower surface of the module. The conduits are connected to a suitable supply of fluid coolant by hoses **127**.

The circuit by means of which the thermoelectric module is connected to a source of direct current also includes, in addition to the module and batteries **125**, a switch means **129** having three principal positions, namely, a first position in which the direction of flow of current is such as will cause the module to maintain the upper specimen-receiving surface **119** of the plate **117** at a relatively low temperature; a second or off position in which no current flows from the battery to the module; and a third position in which the direction of flow of current to the module is such as will cause the upper surface of the plate to become heated. The purpose of heating the specimen-engaging face of the plate **117** is to facilitate the freeing of the frozen specimen

from engagement with the surface when it is desired to detach the member **29** from the specimen.

The carrier **31** includes a housing for a thermoelectric module (not shown), the housing being in the form of a rectangular block **131** having an upper face **133** which is also adapted to function as a heat-absorbing or a heat-dissipating surface depending upon the direction of current flow to the module. The block **131** is supported on one end of a hollow tube **135** with the longitudinal axis of the tube being disposed generally normal to the upper face **133** of the block. Electrical conductors **137** extend from the thermoelectric module downwardly through the tube **135** and are connected to a battery **136** through a three-way switch **138**. Hoses **139** connect fluid conduits (not shown) disposed adjacent the module to a suitable source of fluid coolant.

In the use of the carrier **31**, the specimen may be placed directly upon the face **133** of the block **131**, in which case the block might serve as a stationary specimen support for a microtome, relative to which support a cutting instrument might be caused to reciprocate. Alternatively, the specimen support may, as shown in FIGURE 8, be caused to receive an auxiliary carrier or saddle **141** similar to the carrier **13** shown in FIGURES 1 through 3, which saddle is chilled by the thermoelectric module of the support **31**.

The saddle **141** includes a flat plate **143** from which depend a pair of skirts **145**, as was the case with the carrier **13**. In contradistinction to the carrier **13**, however, the saddle **141** has a ledge **147** projecting outwardly from the outer side wall of each skirt **145** in parallel relation to the specimen-receiving surface of the plate **143** to receive an edge of the positioning device **33**, hereinafter described. The saddle **141** is adapted to be mounted in the movable chuck **41** of a microtome in substantially the same manner as the carrier **13**, as shown in FIGURE 5. When so mounted, the specimen-receiving surface of the plate **143** is parallel to the path of relative movement between the cutting instrument and specimen support of the microtome.

The positioning device **33** facilitates an arrangement of the member **29** and saddle **141** such that the outwardly presented face of the specimen will be so maintained with relation to the saddle that it will be parallel to the relative path of movement between the specimen support and cutting instrument of a microtome when the saddle is mounted on the microtome.

More specifically, the positioning device **33** comprises a sleeve **149** slidably mounted on the hollow tube **121** of the flat surface-defining member **29** and having secured at its upper end a bracket **151** in the form of a flat plate disposed normal to the longitudinal axis of the sleeve **149** and having a pair of flanges **153** projecting from two of its opposing lateral side edges in the direction of the specimen-receiving surface of the member **29**. The outer edges of the flanges are disposed parallel to the plane of the specimen-receiving face **119** of the plate **117** and are adapted to engage the ledges **147** of the saddle **141** so as to render the longitudinal axis of the sleeve **149** normal to the plane of the specimen-receiving surface of the plate **143** of the saddle. The sleeve **149** is of a length sufficient to maintain the axis of the tube coaxial with the axis of the sleeve, thereby causing the specimen-receiving surface of the member **29** to be rendered parallel to the specimen-receiving surface of the auxiliary carrier or saddle **141**, thereby causing the specimen to be sandwiched between two parallel planes. Since the specimen-receiving surface of the saddle **141** is parallel to the path of relative movement between the carrier support and cutting instrument of the microtome when mounted thereon, the outwardly presented face of the specimen, as defined by the specimen-receiving face **119** of the member **29**, will also parallel to the above-mentioned path, as desired.

Of course, if the carrier **31** were used without the saddle **141**, the positioning device **33** would be effective to render the outwardly presented face of the specimen parallel to the path of relative movement between the specimen support and cutting instrument of the microtome when the carrier **31** was mounted thereon.

In utilizing the device of the embodiment shown in FIGURES 7 and 8, the specimen is placed on the specimen-receiving face **119** of the plate **117**, with an area of interest **77** being disposed in engagement with the face **119**. This represents a departure from the method used with the devices **11** and **23** wherein the specimen was first placed face up on a carrier **13** and **25**, and makes it possible to precisely locate the spot **77** on the outwardly presented surface of the specimen, with the spot occupying a particular disposition with respect to the plane of the outwardly presented face. The thermoelectric module of the member **29** is then energized to chill the face **119** and initiate freezing of the specimen, which freezing causes the specimen to adhere to the face **119** of the plate **117**.

When the specimen has become frozen to the face of the plate **117**, the member **29** is inverted into overlying relation to the carrier **31**, with the opposite face of the specimen engaging the surface of the plate **143** of the saddle **141**. The positioning member **33** is utilized to insure that the specimen-receiving face **119** of the plate **117** and the specimen-receiving surface of the saddle **141** are parallel. The thermoelectric module of the carrier support is then energized so as to chill the upper surface of the block **131** and initiate freezing of the lower face of the specimen.

When the specimen has become solid, it thus adheres to both the plate **117** of the member **29** and the plate **143** of the saddle **141**. To free the specimen from engagement with the plate **117**, the current to the thermoelectric module of the member **29** is reversed by means of the switch **129**, thus causing the specimen-receiving surface **119** of the plate **117** to be heated and melting the outwardly presented face of the specimen sufficiently to permit the face **119** to be separated therefrom.

The member **29** is then removed from engagement with the specimen, and the auxiliary carrier or saddle **141** is separated from the specimen support **31** and mounted in the microtome.

After the desired member of sections have been taken from the specimen, the saddle **141** may be replaced on the carrier **31** and the current to the thermoelectric module reversed by means of the switch **138**, thus heating the saddle slightly so as to free the specimen from engagement therewith and rendering the saddle suitable for use with another specimen.

A method and apparatus for the freezing of specimens for sectioning on a microtome have thus been described, which method and apparatus provide a considerable savings in time and effort while rendering the specimen particularly suitable for sectioning.

While various of the structural features of the invention have been shown and described, it should be apparent that various modifications may be made therein without departing from the scope of the invention.

What is claimed is:

1. A method of preparing a specimen for sectioning on a microtome, which microtome includes a carrier having a specimen-receiving surface, a support to which said carrier is adapted to be releasably secured in a predetermined disposition, a cutting instrument, and means for causing relative reciprocal movement between the carrier and cutting instrument along a given path, said method comprising placing the specimen between the specimen-receiving surface of the carrier and a flat surface supported adjacent thereto, said flat surface thereby defining a plane in which an outwardly presented face of the specimen lies, orienting the flat surface relative to the carrier so that when carrier is supported on the microtome in said

predetermined disposition, the plane of the outwardly presented face of the specimen is disposed parallel to the path of relative movement between the carrier and cutting instrument of the microtome, withdrawing heat from the specimen until it is frozen on and adheres to the specimen-receiving surface of the carrier, disengaging the flat surface from the frozen specimen, and securing the specimen-supporting carrier to the support of the microtome in said predetermined disposition.

2. A method of preparing a specimen having an area of particular interest for sectioning on a microtome, which microtome includes a carrier having a specimen receiving surface, a support to which said carrier is adapted to be releasably secured in a predetermined disposition, a cutting instrument, and means for causing relative reciprocal movement between the carrier and cutting instrument along a given path, said method comprising placing the specimen on the specimen-receiving surface of the carrier with the area of interest being located on the surface of the specimen opposite to that engaged by the carrier, engaging the opposite face of the specimen with means defining a flat surface, said flat surface thereby defining a plane in which a outwardly presented face of the specimen including the area of interest lies, orienting the flat surface relative to the carrier so that when the carrier is supported in the microtome in said predetermined disposition, the plane of the outwardly presented face of the specimen is disposed parallel to the path of relative movement between the carrier and cutting instrument of the microtome, withdrawing heat from the specimen until it is frozen on and adheres to the specimen-receiving surface of the carrier, disengaging the flat surface from the frozen specimen, and securing the specimen-supporting carrier to the support of the microtome in said predetermined position..

3. In an apparatus for freezing a specimen to a specimen carrier adapted to be secured within the chuck of a microtome, said apparatus including means defining a conduit through which a refrigerant is circulated, said conduit-defining means being adapted to support the specimen carrier with a face thereof presented outwardly for engagement with one surface of a specimen supported thereon, said conduit being thereby adapted to chill said specimen carrier and effect the withdrawal of heat from a specimen supported thereon through said one surface thereof, the improvement which comprises means supported adjacent said conduit-defining means and said specimen carrier including a member formed of a material having a relatively high thermal conductivity and movable between a first position in which it is in engagement with said conduit-defining means so as to be chilled thereby and a second position in which it engages a surface of the specimen supported on said specimen carrier other than the said one surface, said chilled member being adapted to effect the withdrawal of heat from the specimen through said other surface when in said second position.

4. In apparatus for freezing a specimen to a thermally conductive specimen carrier, which apparatus includes a refrigerated thermally conductive member adapted for heat-conductive engagement by a specimen carrier, the improvement which comprises a specimen-engaging member having a smooth imperforate flat surface adapted to engage a specimen disposed in contact with a specimen carrier in heat-conductive engagement with said refrigerated member and to impart to the specimen a complementary flat external surface during freezing thereof, and means mounting said specimen-engaging member on said refrigerated member so as to render said specimen-engaging member movable between an inoperative position and an operative position wherein it engages the specimen.

5. A combination according to claim 4 wherein said specimen-engaging member is formed of material of relatively high thermal conductivity.

6. A combination according to claim 4 wherein said mounting means is secured to said refrigerated member in such a manner as to render it shiftable to various locations thereon.

7. A combination according to claim 4 wherein the flat surface of the specimen-engaging member is adapted to be placed in heat-conductive engagement with the refrigerated member when in the inoperative position.

8. In apparatus for freezing a specimen to a thermally conductive specimen carrier, said apparatus including a refrigerated thermally conductive member having a generally horizontally disposed surface adapted to receive a specimen-carrier thereon in a predetermined position in heat-conductive engagement therewith, the improvement which comprises a support member fixedly mounted in a stationary position adjacent the carrier-receiving surface of the refrigerated thermally conductive member, means including a specimen-engaging member pivotally mounted on said support member for pivotal movement between a first operative position wherein it is in vertical alignment with the carrier-received surface of the refrigerated thermally conductive member and a second position permitting unobstructed vertical access to the surface, said specimen-engaging member having a flat smooth imperforate surface adapted to engage a specimen disposed in contact with the specimen-receiving surface of a specimen carrier disposed on said carrier-receiving surface of said refrigerated member, said flat surface of said specimen-engaging member being adapted to impart to the specimen engaged thereby a complementary flat external surface.

9. A combination according to claim 8 wherein the specimen carrier has a generally flat specimen-receiving surface adapted to be disposed in a predetermined plane when said specimen carrier is received on said thermally conductive member, and wherein said specimen-engaging member is mounted on said support member so as to locate said flat surface of said specimen-engaging member in a plane generally parallel to the plane of the flat specimen-receiving surface of the carrier when said specimen-engaging member is in said first operative position.

10. In apparatus for freezing a specimen to a thermally conductive specimen carrier having a generally flat specimen-receiving surface, said apparatus including a refrigerated thermally conductive member adapted to receive a specimen carrier thereon in a predetermined position in heat-conductive engagement therewith, the improvement which comprises a support member adapted to be detachably affixed on the refrigerated thermally conductive member, and means including a second thermally conductive member movably mounted on said support member, said thermally conductive member having a smooth imperforate flat specimen-engaging surface and being movable to a first position in which it is disposed in heat-conductive engagement with said refrigerated member, said movably mounted member also being movable to a second position in which the specimen-engaging surface thereof is adapted to engage a specimen disposed in contact with the specimen-receiving surface of a specimen carrier disposed in said predetermined position on said refrigerated member.

11. An apparatus for freezing a specimen for sectioning on a microtome, said apparatus comprising a refrigerated thermally conductive member, a specimen carrier adapted to be located in a predetermined position on said refrigerated thermally conductive member in heat conductive engagement therewith and to be removed from the refrigerated thermally conductive member and secured in operative position within the chuck of a microtome, said specimen carrier including means defining a flat surface adapted to lie in a predetermined plane when said specimen carrier is in said predetermined position on said refrigerated thermally conductive member, said specimen carrier also including an element depending from said flat surface-defining means adapted to engage said

refrigerated thermally conductive member and to prevent lateral movement of said specimen carrier relative thereto, a support member mounted on said refrigerated thermally conductive member adjacent said specimen-carrier, and a specimen engaging member movably mounted on said support member, said specimen engaging member including a smooth flat surface adapted to be placed in engagement with a specimen supported on said specimen carrier, said specimen engaging member being so mounted on said support member as to locate the smooth flat surface in a plane parallel to the plane of said flat surface of said specimen carrier when said specimen engaging member is in engagement with the specimen, whereby said smooth flat surface of said specimen-engaging member is adapted to impart to a specimen engaged thereby a complementary flat external surface so positioned with respect to said carrier that when said carrier with the specimen frozen thereto is subsequently secured in said operative position within the chuck of a microtome, said

complementary flat external specimen surface is disposed in substantial parallelism with the cutting plane of said microtome.

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