United States Patent

Blum

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[54] **CRYOGENIC MICROTOME APPARATUS**

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[51] **Int. Cl.**.....**B26d 7/08,** B26d 4/22

[58] **Field of Search**.....83/170, 171, 915.5, 167

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Primary Examiner—Donald G. Kelly

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[57] **ABSTRACT**

Microtome apparatus with parts specially formed and arranged for cutting thin and ultra thin tissue sections under cryogenic conditions. The microtome is provided with an insulated enclosure or container for the cryogenic fluid and a stationary, but adjustable, cutting knife. The swinging fulcrum arm of the microtome has at its outer end a U-shaped specimen holder so designed to enable the specimen holder assembly to straddle one wall of the container. Thus, the stationary knife edge is horizontally aligned with the fulcrum arm pivot point without need for a sealed container-wall fulcrum arm passageway.

21 Claims, 3 Drawing Figures

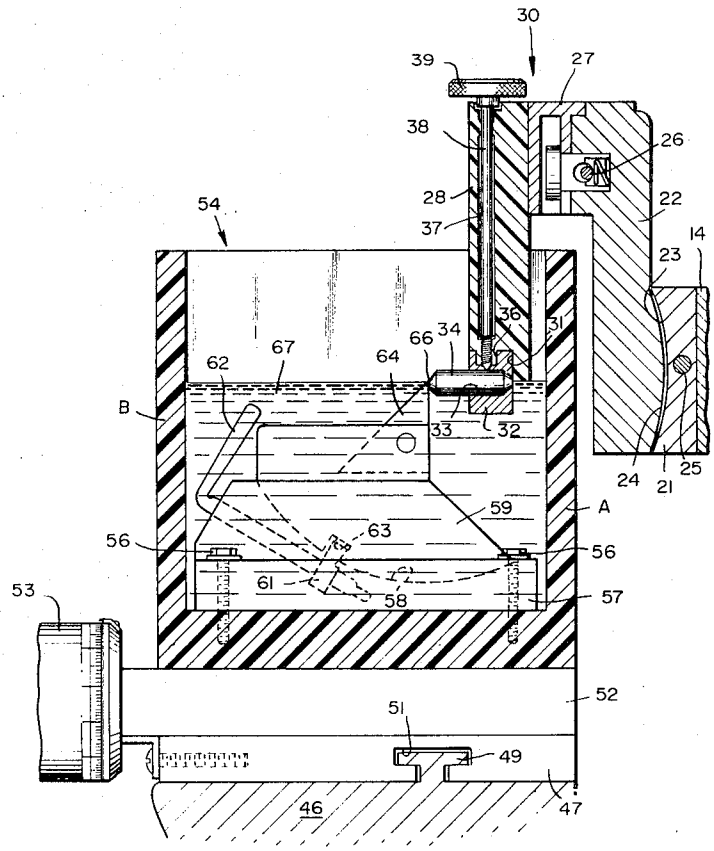

FIG. 1

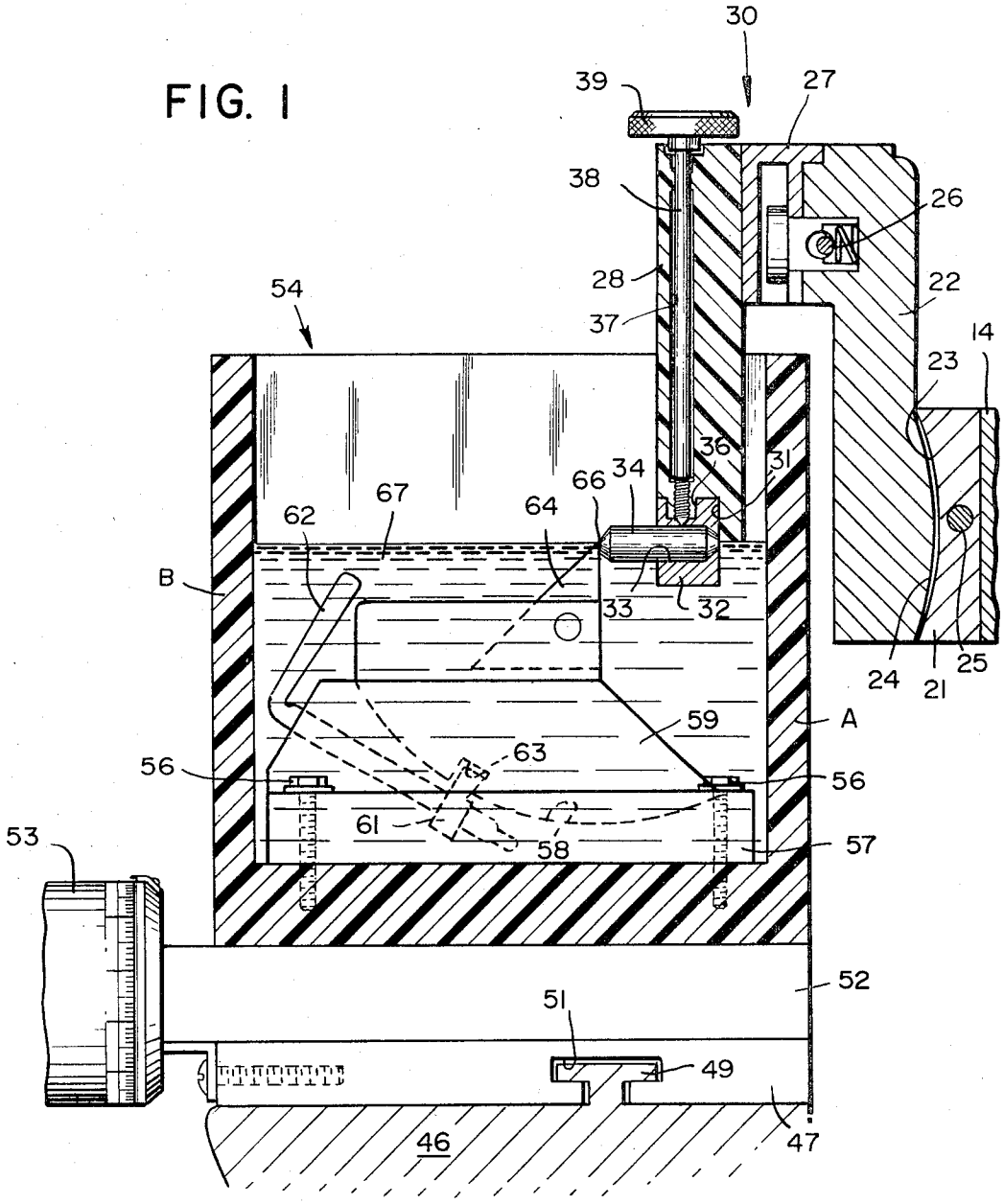

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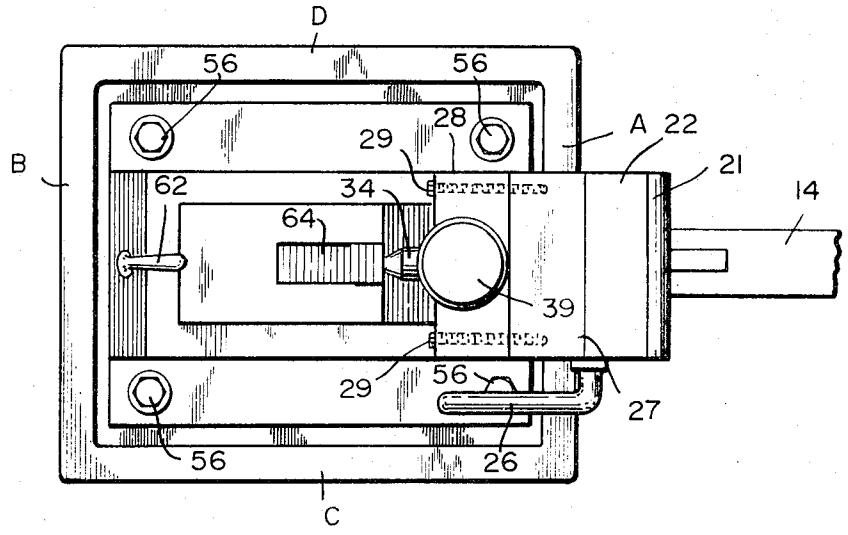
FIG. 2



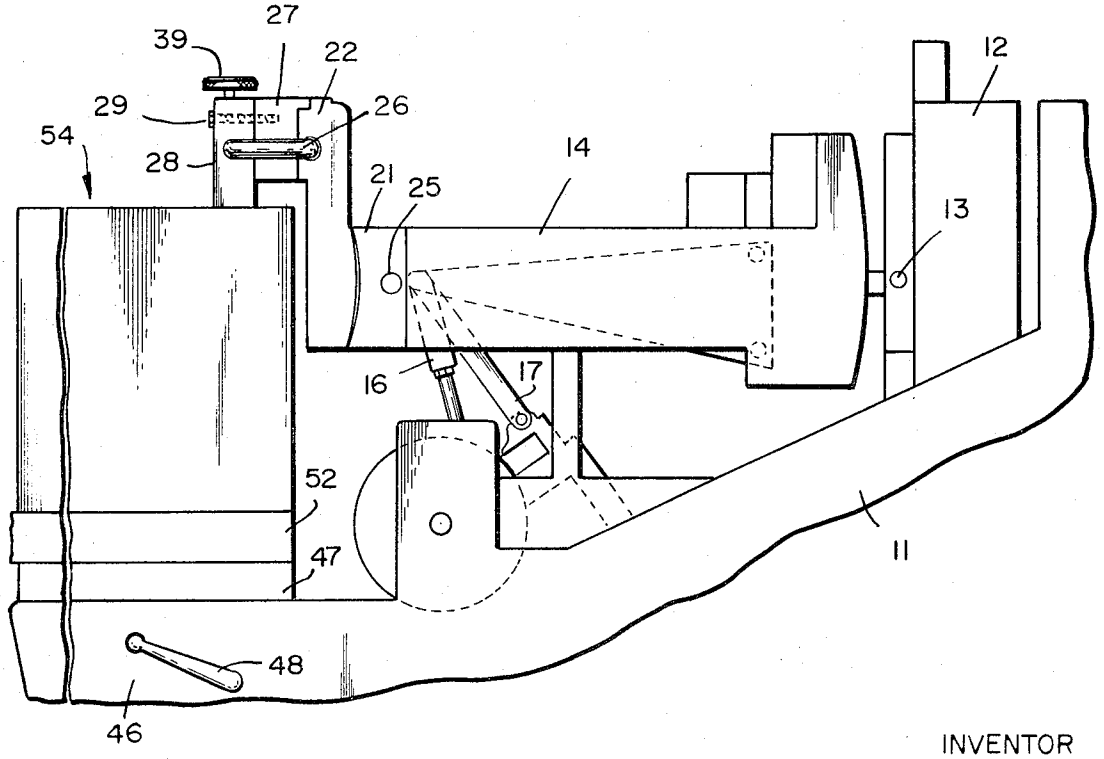


FIG. 3

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CRYOGENIC MICROTOME APPARATUS

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to microtomes and, more particularly, to apparatus for cutting frozen thin sections of biological cells and tissues, or the like, under cryogenic conditions to be examined by electron microscopy or light microscopy. An important feature of the invention is the provision of means for converting general purpose microtomes or ultramicrotomes that cut thin tissue sections under ambient conditions into instruments which will perform the tissue sectioning process under cryogenic conditions.

2. Description of the Prior Art

Prior U.S. Pat. concerned with cryogenic microtomy and the like comprise: Nos. 2,292,973, 2,670,657, 3,218,896, 3,220,290, 3,296,821, 3,462,969, 3,491,638 and 3,495,490.

Most of the foregoing prior art patents disclose structures which are complex, expensive, and inconvenient to manipulate, thereby militating against their adoption for widespread use. Some of these devices require special auxiliary cooling equipments, sealing elements in the wall of the cryogenic vessel, complicated mounting means for either the specimen or the microtome knife and, in some cases, a complete microtome apparatus designed especially and exclusively for cryogenic microtomy.

SUMMARY OF THE INVENTION

In overcoming the disadvantages and drawbacks of the prior art apparatus, the apparatus described and claimed herein comprises a simple insulated enclosure or vessel for the cryogenic fluid within which the microtome cutting knife is located. The cryogenic container is removably mounted on the frame of a standard microtome or ultramicrotome. Connected to the free end of the fulcrum arm of the microtome, in place of the normally located specimen holder, is a new inverted U-shaped specimen holder assembly. The specimen holder assembly is shaped to straddle one wall of the cryogenic container and to support a frozen tissue specimen in the interior of said container, said specimen being movable relative to the cutting edge of the microtome knife in the conventional manner. By this means, there is avoided the problem of providing a seal in the wall of the cryogenic container for an element to move therethrough. Thus, a conventional microtome or ultramicrotome which performs its function in normally ambient temperature can be converted into a cryogenic microtome or ultramicrotome by the simple replacement of two parts of the apparatus; namely, the standard specimen chuck and the standard knife stage.

By providing for the inverted U-shaped attachment to the fulcrum of the regular microtome, the specimen is supported in a location in relation to the cutting edge of the microtome without any interference or even contact with the cryogenic container, in the same or comparable operating condition as the specimen is supported when the microtome apparatus performs its function under ambient conditions. When the cryogenic attachments are connected to the microtome, the specimen is or can be aligned with the pivot point of the fulcrum so that it travels the same

substantially straight line path for the short distance that it moves across the cutting edge of the microtome knife. By this means, the same conditions of specimen cutting are maintained, whether under ambient temperatures or under cryogenic temperatures, thereby assuring the production of uniform thin tissue sections as may be required under either of those temperature conditions.

Accordingly, the arrangement of the conventional microtome where the cutting edge of the microtome knife and the pivot point of the fulcrum are usually arrayed in approximately or substantially the same horizontal plane is preserved, notwithstanding the interposition of a box-like container for the cryogenic fluid between said cutting knife and said pivot point. This is accomplished by forming the end of the fulcrum in an inverted U-shaped structure which straddles and moves freely relative to the intervening wall of the container and the tissue specimen is brought into the proper location vis a vis the cutting edge of the knife as if there were no intervening wall. Thus, the standard microtome can either be built with the fulcrum having said inverted U-shaped specimen holding assembly as a permanent part thereof, or the fulcrum can be built with a conventional removable tissue specimen chuck in place of which is removably connected a replaceable inverted U-shaped specimen holding assembly.

Furthermore, the cryogenic container with a microtome knife located therein can be removably mounted on the standard microtome in place of the usual removable microtome knife stage assembly. In either form, the standard instrument is adaptable for use as a conventional microtome under ambient temperatures, and also for cryogenic sectioning, with the microtome knife and the tissue specimen in substantially the same operating locations with which the experimenter or technician is familiar.

These and other novel features and advantages of the present invention will be described and defined in the following specification and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a vertical fragmentary section view, partly in elevation, of a portion of a microtome or ultramicrotome, showing schematically the cryogenic container and the inverted U-shaped specimen holder on the microtome fulcrum, some parts being omitted and other parts being shown in dotted outline;

FIG. 2 is a top view, in somewhat reduced size, of the portion of the apparatus shown in FIG. 1; and

FIG. 3 is a fragmentary side view, further reduced in size, of the apparatus herein, illustrating schematically the relative positions of the cryogenic container and the new specimen holder, and their relationship to the pivot point of the specimen fulcrum, some parts being omitted and some parts being shown in dotted outline.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Referring now to the drawings in detail, a fragmentary portion of a conventional microtome or ultramicrotome (both being hereinafter referred to generally as a "microtome"), is shown in FIG. 3 and comprises a base 11, upon which is mounted an upwardly extending frame 12 which contains a suitable mechanism, not

shown, but well known in various forms in the prior art, which serves as a pivot element **13** to which is connected the rear end of a fulcrum **14**. Fulcrum **14** is moved pivotally and reciprocably within a predetermined limited range by means of linkages **16**, **17** amongst others, and by various cams, not shown, for producing the desired reciprocating or oscillating vertical arcuate movements of said fulcrum **14** for its downward cutting pass and for its upward bypass motion. During the downward movement, the specimen is sliced by the cutting edge of the stationary knife and during the upward movement the fulcrum is slightly retracted so that the specimen will bypass the knife. During the next cycle, the fulcrum is advanced by means well known in the art to cause the knife to cut a succeeding slice off the specimen.

Instead of the conventional specimen holder which has been removably mounted on the forward end of fulcrum **14**, there is now substituted and removably mounted thereon an adapter block **21** which is firmly secured in position. Connected to block **21** is an upwardly extending fulcrum arm extension **22** which is made of suitable material such as cast aluminum or the like. The forward face of adapter block **21** has a concave recess **23** while the lower portion of extension arm **22** has a convex base **24** whereby said respective convex and concave surfaces provide means for adjustably locating extension arm **22** relative to fulcrum arm **14** after which arm **22** and block **21** may be secured in fixed position by means of a conventional lock **25**, operated by a suitable lever, not shown. Connected to the upper end portion of extension arm **22** is a spacer element **27** made of a suitable material such as cast aluminum or the like, said arm and spacer being secured together by a conventional lever lock **26**, or the like. Secured to the front vertical surface of spacer element **27** is a downwardly extending insulator-holder **28** by means of bolts **29** or the like. Insulator-holder **28** is made of a suitable thermally insulating material such as a phenolic resin or the like.

The combination of extension arm **22**, spacer **27**, and insulator-holder **28**, forms an inverted U-shaped specimen holder assembly, generally designated **30**. Mounted fast by suitable means in recess **31** in the lower end of insulator-holder **28** is a chuck holder **32** made of copper or other suitable highly thermally conductive material. Chuck holder **32** has a horizontal aperture **33** which accommodates the rear end portion of a tissue holder **34** made of solid copper or other suitable highly thermally conductive material. Chuck holder **32** has a vertical aperture **36**, the lower end of which communicates with aperture **33** in holder **34** and the upper end of which is aligned with vertical aperture **37** in insulator-holder **28**. Extending through apertures **37** and **36** is a rod **38**, the lower end portion of which threadably cooperates with the lower threaded end of aperture **37**. The upper end of rod **38**, which extends above insulator-holder **28**, terminates in a knob **39** which can be manually rotated in order to cause the lower end of rod **38** to secure tissue holder **34** firmly in position.

In some embodiments, specimen holder assembly **30** may be formed as a fixed or integral part of fulcrum **14** if such an arrangement is practicable or desired.

Insulator-holder **28** which is made of a thermally insulating material such as a phenolic resin or the like serves as a thermal barrier between the extremely low temperature contents of the cryogenic container and fulcrum **14**, thereby preventing thermal distortion of the latter which would otherwise prevent the production of dimensionally uniform tissue slices or sections. In some embodiments, additional parts of assembly **30** may be made of thermally insulating materials as may be practicable.

The forward portion of base **11** of the microtome apparatus is formed as a platform **46** upon which is conventionally mounted a removable knife holder assembly with various positioning means for properly locating the cutting edge of the knife relative to the specimen on the fulcrum of the apparatus. Said conventional knife holder assembly is removed from stage **46** and in place thereof there is mounted a combination cryogenic vessel in the interior of which is located the knife holder assembly. Suitable means well known in the art are provided for adjustably securing the cryogenic vessel firmly in position on the stage of the apparatus.

The cryogenic vessel is supported on plate **47** slidably movable on platform **46**. Plate **47** can be secured firmly in the desired position relative to platform **46** by suitable means such as a lever lock **48** operating key **49** in slot **51**. Slidably movable on plate **47** is a stage **52** which is keyed by suitable means well known in the art to plate **47** for sliding movement relative thereto. The desired location of stage **52** relative to plate **47** is determined by micrometer adjustment element **53** or the like.

Mounted fast by suitable means upon stage **52** is a cryogenic vessel or container, generally designated **54**, made of a suitable thermally insulating material and having upwardly extending end walls A and B and side walls C and D. Mounted securely by means of bolts **56** to the floor of container **54** is a cutting knife assembly comprising a base **57** having a concave recess **58** which slidably accommodates the convex surface of knife holder **59**. Mounted in base **57** is a lock key **61** which is operated by lever lock element **62**, said key cooperating with recess **63** in knife holder **59** to secure the latter firmly in position. Mounted upon the upper portion of knife holder **59** is a microtome cutting knife **64**, the upper cutting edge of which is adjustably and firmly secured in the desired position by means well known in the art.

While cryogenic container **54** is illustrated in the drawings as being rectangular in shape, it is understood that it may take the form of other suitable shapes and contours, both externally and internally.

In operation, the cryogenic fluid **67**, such as liquid nitrogen, is poured from a suitable Dewar flask into container **54** to a level at or somewhat above the uppermost cutting edge of knife **64**. The tissue holder **34** which has previously been removed from the apparatus, is placed in the vertical position and the tissue specimen **66** is placed upon the end of the truncated conical tip thereof, at room temperature. Thereafter, the specimen is frozen onto holder **34** by connecting the latter by suitable means to a copper block freezing tool which has been cooled or is being kept cooled at liquid nitrogen temperature at about −196° C. Thus,

the tissue specimen **66** becomes firmly adhered to specimen holder **34**. Thereafter, specimen holder **34** is inserted into aperture **33** in chuck holder **32** and secured in position therein by means of rod **38**. The various components of the microtome apparatus are arranged whereby, when fulcrum **14** is arrayed substantially horizontally, specimen **66** is arrayed directly opposite the uppermost cutting edge of knife **64**. Requisite accommodations between knife **64** and specimen **66** can be made by the operator adjustably positioning knife holder **59** relative to base **57** and by adjustably positioning tissue holder **34** within aperture **33**.

Thereafter, the microtome is set in operation and fulcrum **14** moves pivotally and reciprocably to cause the frozen specimen **66** to pass against the cutting edge of knife **64** while both are submerged within the cryogenic liquid **67** within container **54**. During each downward pass of the specimen **66**, a slice of tissue is cut, the latter moving slowly down the sloping rear wall of knife **64**. During the upward movement of specimen **66**, fulcrum **14** has been slightly retracted so that the specimen does not make any contact with knife **64**. Just prior to the next subsequent downward pass of specimen **66**, fulcrum **14** has been advanced the predetermined microscopic amount to cause the specimen to be sliced again during the next downward pass. The successive frozen tissue sections that have moved down the sloping edge of knife **64** can then be lifted by a suitable tool, dried either at room temperature or freeze-dried in a suitable freeze-drying apparatus, after which the specimen is subjected to further electron or light microscopy or to other requisite analysis.

By providing an open top cryogenic container **54**, the tissue sectioning procedure is greatly simplified by virtue of the fact that important parts of the apparatus such as tissue holder **34** and knife **64** are readily accessible as are the adjustment elements for those parts. Furthermore, by providing an inverted U-shaped specimen holder assembly **30** which straddles wall A of cryogenic container **54** with one leg, extension **22**, outside said container, and the other leg, insulator-holder **28**, extending into the interior of said container, the tissue specimen **66** is arrayed in relation to the cutting edge of knife **64** in the same location as if said specimen were mounted in a chuck on the free end of fulcrum **14** in the conventional microtome. Said legs, elements **22** and **28**, are sufficiently spaced apart and are sufficiently long so that when they move reciprocably and vertically in unison with fulcrum **14** freely in respect of wall A of container **54**, there is no impingement against the top of wall A by spacer **27** or any other untoward interference between the components of assembly **30** and container **54**.

In the standard microtome or ultramicrotome, the various component parts are generally arranged so that a tissue specimen mounted on the free end of the fulcrum is usually arrayed directly opposite the cutting edge of the stationary knife when said fulcrum is in a substantially horizontal position. In order to preserve this optimum relationship, notwithstanding the replacement of the conventional knife holding stage by a cryogenic container having a wall that is now interposed between the fulcrum and the cutting knife, the provision herein of the inverted U-shaped specimen holder assembly mounted on the free end of the fulcrum brings about the requisite relationship between the tissue specimen and the cutting knife on the standard or conventional microtome without the necessity of constructing a special microtome to be used only for cutting frozen tissue specimens under cryogenic conditions. Thus, the present invention makes possible the use of a single all-purpose microtome with replaceable parts for adapting the apparatus to operating under both ambient temperature conditions and cryogenic conditions.

Although the present invention has been described with reference to particular embodiments and examples, it will be apparent to those skilled in the art that variations and modifications can be substituted therefor without departing from the principles and true spirit of the invention.

I claim:

1. Cryogenic equipment for a microtome having a pivoting fulcrum comprising a frame, a container made of insulating material, a microtome knife positioned in said container, an inverted U-shaped specimen holder assembly mounted on the free end of said fulcrum, the spaced legs of said specimen holder assembly straddling a wall of said container and movable reciprocably and freely relative thereto, the outer leg of said specimen holder assembly extending into said container, a tissue holder removably mounted on said outer leg in a position where a tissue specimen mounted on said tissue holder is sectioned by the cutting edge of said knife when said fulcrum moves through one of its reciprocating passes.

2. Cryogenic equipment according to claim 1 wherein at least a portion of said U-shaped specimen holder assembly comprises a thermal barrier for thermally insulating said fulcrum from the materials in said container.

3. Cryogenic equipment according to claim 1 wherein said outer leg is made of thermally insulating material.

4. Cryogenic equipment according to claim 1 wherein said U-shaped specimen holder assembly is removably mounted on said fulcrum.

5. Cryogenic equipment according to claim 1 wherein said container is removably mounted on said frame.

6. Cryogenic equipment according to claim 1 and further comprising a lock element for adjustably securing said U-shaped specimen holder assembly in a desired position relative to said fulcrum.

7. Cryogenic equipment according to claim 1 wherein said tissue holder is removably connected to said outer leg.

8. Cryogenic equipment according to claim 1 wherein said tissue holder is made of a highly thermally conductive material.

9. Cryogenic equipment according to claim 8 and further comprising a highly thermally conductive chuck on said outer leg in which said tissue holder is adjustably mounted.

10. Cryogenic equipment according to claim 1 and further comprising a locking element on said outer leg for securing said tissue holder in position, the position of said tissue holder being adjustable for locating the

tissue specimen on said holder in a determined position relative to the knife.

11. Cryogenic equipment according to claim **1** wherein the pivot point of said fulcrum is in approximately the same plane as the cutting edge of said knife.

12. A microtome for sectioning biological tissues or the like under cryogenic conditions comprising a frame, a fulcrum mounted pivotally on said frame, a container of insulating material for accommodating a quantity of cryogenic fluid mounted on said frame, a microtome knife positioned in said container, first means on said fulcrum freely bypassing a wall of said container and extending into the interior of said container, and second means mounted on that portion of the first means extending into the interior of said container for supporting a tissue specimen to be sectioned by said knife.

13. Microtome according to claim **12** wherein the cutting edge of said microtome knife is in substantially the same plane with the pivot point of said fulcrum.

14. A microtome according to claim **12** wherein said first means moves freely relative to said container wall during the complete oscillating movement of said fulcrum.

15. A microtome according to claim **12** wherein at least a portion of said first means is made of a thermally insulating material for thermally isolating said fulcrum from said second means.

16. A microtome according to claim **12** wherein said first means has an inverted U-shaped form.

17. A microtome according to claim **12** wherein said first means is removably mounted on said fulcrum.

18. A microtome according to claim **12** wherein said second means comprises a highly thermally conductive metal.

19. A microtome according to claim **18** wherein said second means is removably mounted on said first means.

20. A microtome according to claim **12** wherein said container with said microtome therein is removably mounted on said frame.

21. A microtome for sectioning biological tissues or the like under cryogenic conditions comprising a frame, a fulcrum mounted pivotally on said frame, a container of insulating material for accommodating a quantity of cryogenic liquid mounted on said frame, a microtome knife positioned in said container, the cutting edge of said knife being located in substantially the same horizontal plane as the pivot point of said fulcrum, an inverted U-shaped specimen holder assembly mounted on the free end of said fulcrum and extending freely over and around one wall of said container, the outer leg of said assembly extending into the interior of said container, and a tissue holder mounted on the lower portion of said leg for moving a tissue specimen on said holder relative to the cutting edge of said knife.

* * * * *

Disclaimer

3,680,420.—*Josef Blum,* Norwalk, Conn. CRYOGENIC MICROTOME APPARATUS. Patent dated Aug. 1, 1972. Disclaimer filed Apr. 3, 1989, by the assignee RMC, Inc.

Hereby enters this disclaimer to claims 12, 13 and 14 of said patent.

[*Official Gazette January 30, 1990*]

REEXAMINATION CERTIFICATE (1094th)

United States Patent [19]

Blum

[11] B1 3,680,420

[45] Certificate Issued Jul. 11, 1989

[54] CRYOGENIC ULTRAMICROTOME APPARATUS

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[51] Int. Cl.[4] G01N 1/06
[52] U.S. Cl. 83/167; 83/171; 83/915.5
[58] Field of Search 83/915.5, 167, 170, 83/171

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[57] **ABSTRACT**

Microtome apparatus with parts specially formed and arranged for cutting thin and ultra thin tissue sections under cryogenic conditions. The microtome is provided with an insulated enclosure or container for the cryogenic fluid and a stationary, but adjustable, cutting knife. The swinging fulcrum arm of the microtome has at its outer end a U-shaped specimen holder so designed to enable the specimen holder assembly to straddle one wall of the container. Thus, the stationary knife edge is horizontally aligned with the fulcrum arm pivot point without need for a sealed container-wall fulcrum arm passageway.

REEXAMINATION CERTIFICATE ISSUED UNDER 35 U.S.C. 307

THE PATENT IS HEREBY AMENDED AS INDICATED BELOW.

Matter encosed in heavy brackets [ ] appeared in the patent, but has been deleted and is no longer a part of the patent; matter printed in italics indicates additions made to the patent.

AS A RESULT OF REEXAMINATION, IT HAS BEEN DETERMINED THAT:

The patentability of claims 1, 6, 10, 11, 16 & 21 is confirmed.

Claims 12-14 are now disclaimed.

Claims 2-5, 7-9, 15 and 17-20 are cancelled.

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