

Nov. 19, 1968

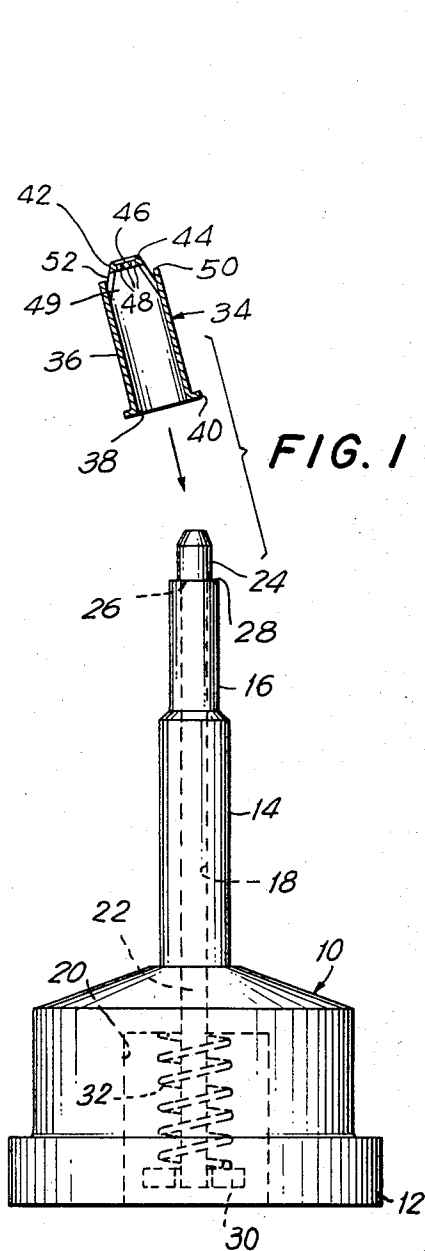
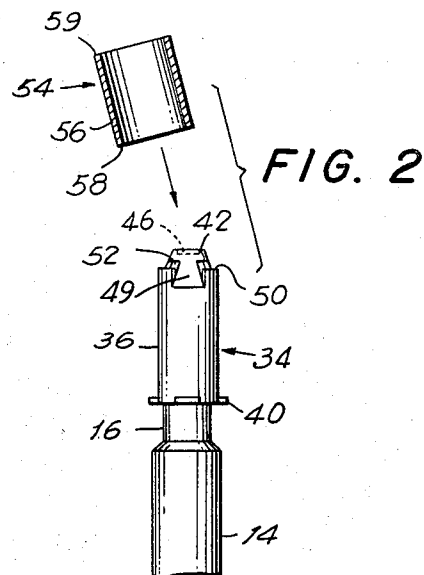
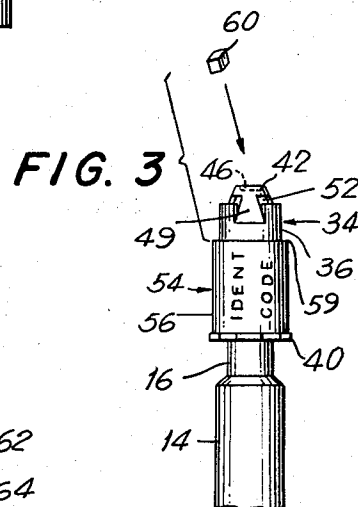
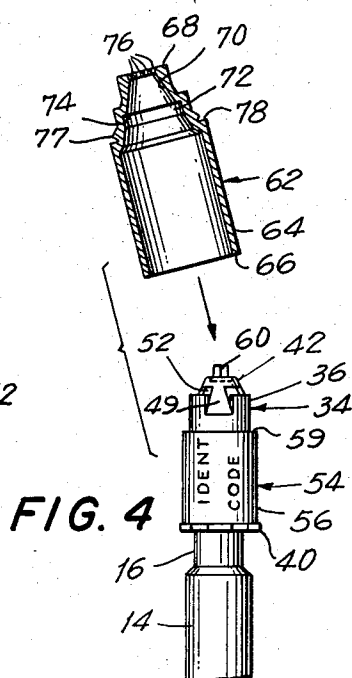
J. ISREELI ET AL

3,411,481

APPARATUS FOR THE INFILTRATION OF TISSUE

Filed Dec. 28, 1964

4 Sheets-Sheet 1



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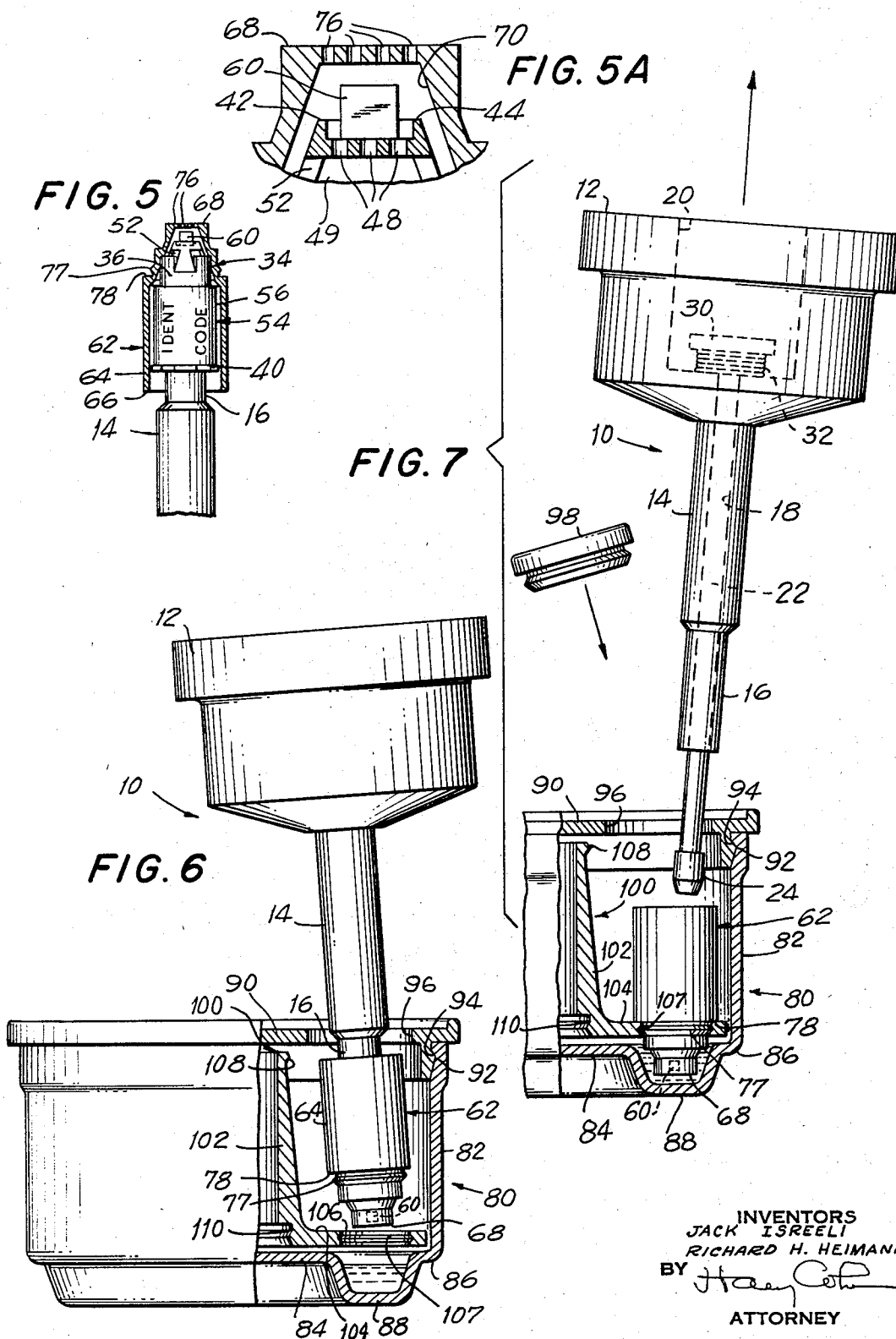
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4 Sheets-Sheet 2



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FIG. 8

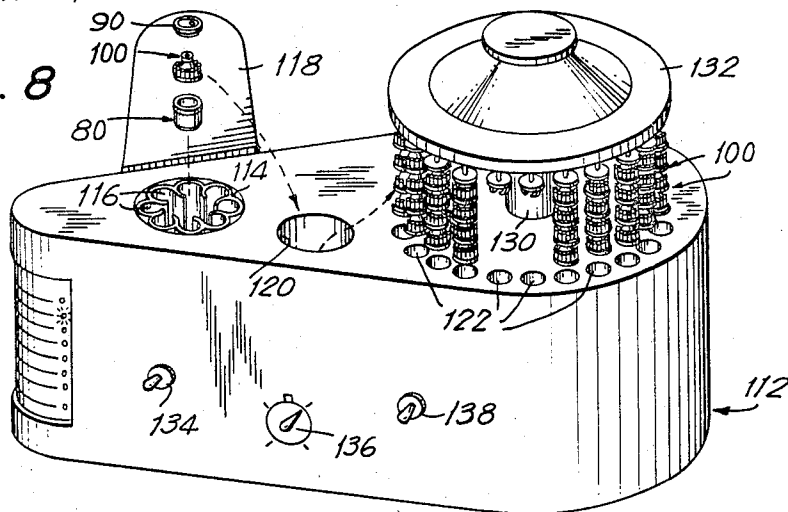


FIG. 9

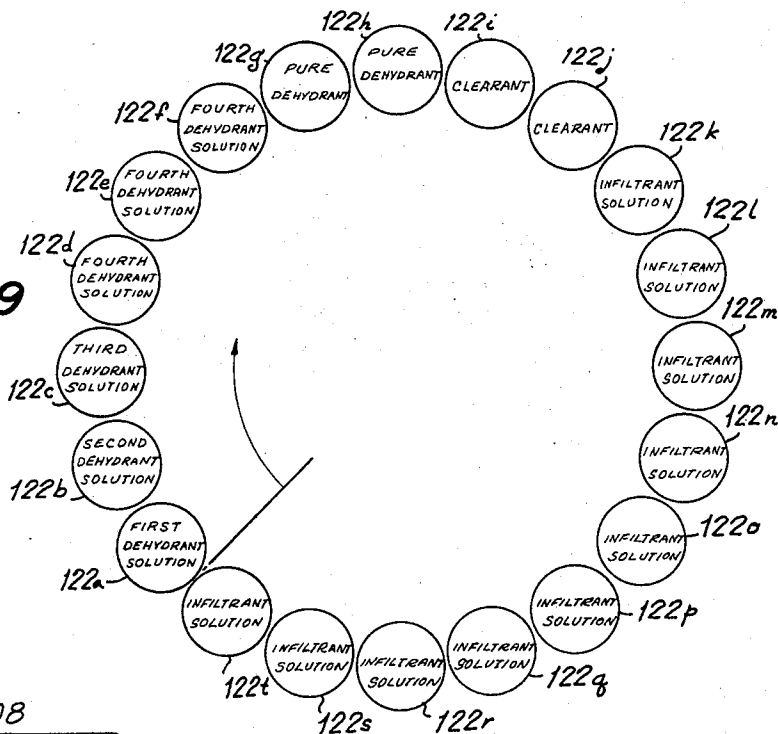
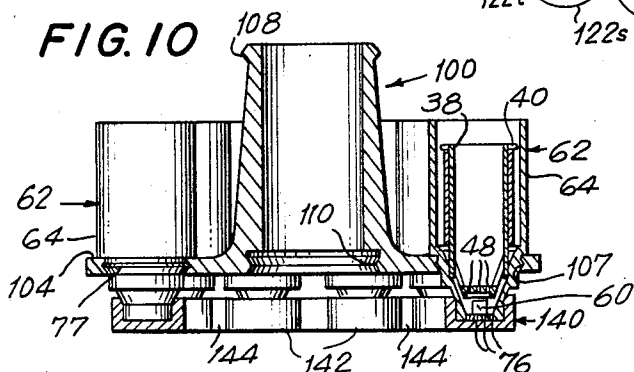


FIG. 10



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FIG. 11

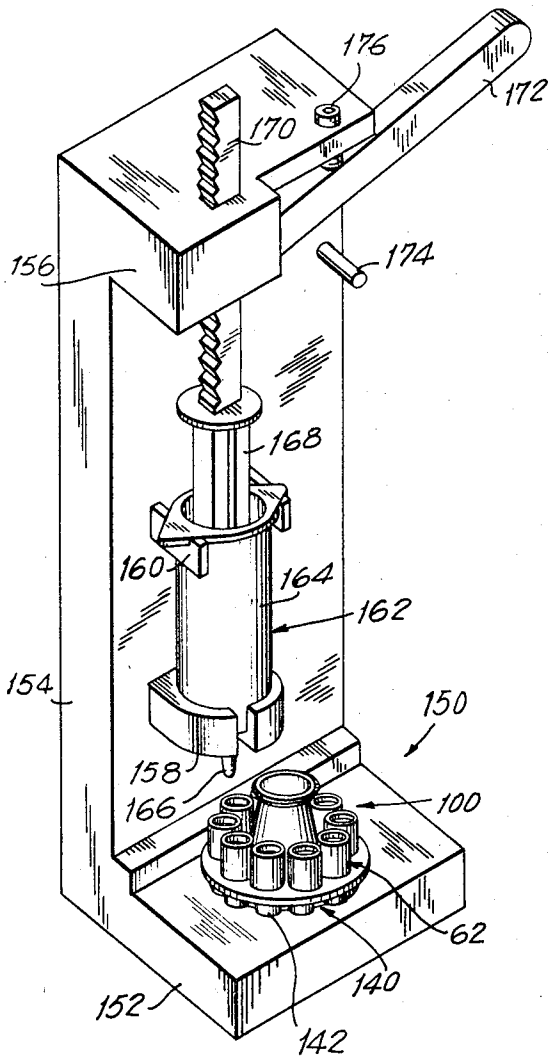


FIG. 12

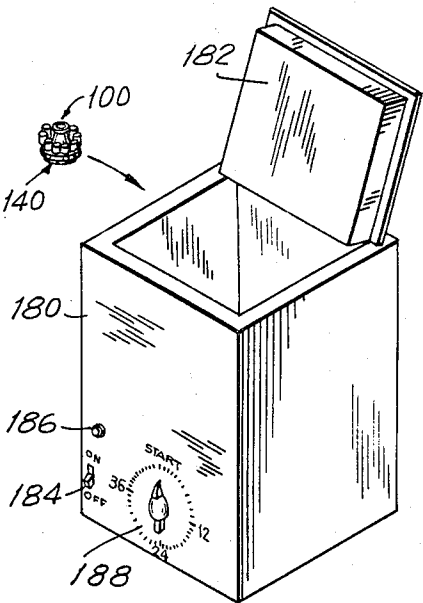


FIG. 13

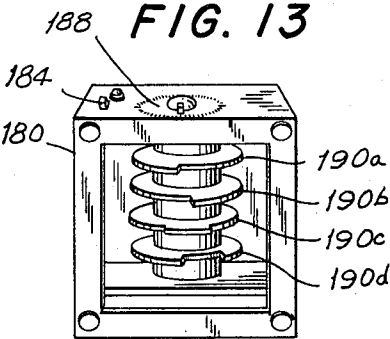


FIG. 14

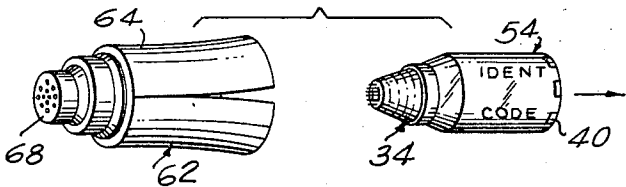


FIG. 15



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3,411,481

APPARATUS FOR THE INFILTRATION OF TISSUE
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ABSTRACT OF THE DISCLOSURE

The invention relates to apparatus used to prepare specimens of tissue for microtoming preparatory to examination and includes a two-piece interlocking specimen holder which cages the specimen for sequential fluid treatment. Mounting means for a plurality of holders and containers for the treating liquid are also provided.

This invention relates to an apparatus for the infiltration processing of tissue for histological examination, and is particularly suitable for processing tissue for examination by electron microscopy.

It is customary in preparing tissue for electron microscopy to commence the fixing of the tissue as soon as possible after the receipt thereof. The immediate fixation of the tissue not only preserves the cells and intracellular substances, but also prevents the post-mortem changes that take place after death due to putrefactive action of bacteria. Therefore, the gross tissue on receipt is customarily cut into cubes while coated with fixative, and is further processed by manual changes of fluid to infiltrate and encapsulate the tissue. A fixative customarily used is osmic acid, which has corrosive fumes.

It is, therefore, an object of this invention to provide an apparatus for the rapid and convenient processing of each cube of tissue as it becomes available for processing.

Another object is the provision of an improved capsule for the retention of each tissue cube.

Yet another object is the provision of a rack and a beaker for the handling of the capsules.

A feature of this invention is the provision of a capsule having a perforate well for the receipt of a tissue cube and a perforate cover which fits into the capsule to close the well and capture the tissue cube therein.

Another feature of this invention is the provision of a rack which will receive a plurality of capsules, either concurrently, or sequentially while disposed in a fixing beaker, and which can be joined to similar racks for further processing.

These and other objects, features and advantages will become apparent on consideration of the following disclosure taken in conjunction with the accompanying drawings in which:

FIG. 1 is an elevational view of bench mounting fixture, and an inner part of a multi-part capsule embodying this invention which is shown in longitudinal cross-section prior to disposition in the fixture;

FIG. 2 is a partial elevational view of the fixture, and an identification sleeve part of the multi-part capsule which is shown in longitudinal cross-section prior to disposition on said inner part on said fixture;

FIG. 3 is a partial elevational view of the fixture, and a sample cube prior to disposition on said inner part on said fixture;

FIG. 4 is a partial elevational view of the assembly of FIG. 3, and an outer part of the multi-part capsule shown in longitudinal cross-section prior to disposition on said assembly;

FIG. 5 is a partial elevational view of the assembly of FIG. 4; FIG. 5A is an enlarged detail of FIG. 5;

FIG. 6 is an elevational view showing the insertion of

the assembly of FIG. 5 into a rack in a receptacle containing a fixative;

FIG. 7 is an elevational view showing the removal of the mounting fixture from the multi-part capsule which is disposed in the rack in the fixative receptacle;

FIG. 8 is a perspective view showing an automatic processing mechanism for infiltrating the sample cubes with various processing fluids;

FIG. 9 is a diagrammatic plan view of the sequence of processing fluids after the fixative fluid;

FIG. 10 is an elevational view in partial section showing the rack into which a plurality of capsules have been disposed together a potting boot;

FIG. 11 is a perspective view of an assembly for inserting a potting compound into each capsule after the infiltration of the therein contained sample cube;

FIG. 12 is a perspective view of an oven for curing the potting compound;

FIG. 13 is a perspective view of the timing cams for controlling the sequential temperatures of the oven;

FIG. 14 is a perspective view showing the outer part removed from the remainder of the capsule after curing; and

FIG. 15 is an end view of the sample cube potted in the capsule after the slicing away of the tip thereof.

The sample cube of tissue is disposed within a multi-part capsule most conveniently with the aid of a mounting fixture 10. As shown in FIG. 1, the fixture comprises a base 12 having a central, upstanding post 14. The post has a distal portion 16 of reduced diameter. An axial bore 18 extends through the post 14 and merges into a cavity 20 in the base 12. A rod 22 is disposed within said bore 18 and cavity 20 for reciprocation therein. The upper end of the rod 22 extends beyond the top of the bore and has an enlargement or head 24 having a underside shoulder 26 which abuts the upperside shoulder 28 of the post 14. The lower end of the rod is threaded to a disc or button 30. A compression spring 32 is disposed on the rod above the button to bias the rod downwardly.

The inner part 34 is first slipped over the head 24 and onto the portion 16 of the fixture, as shown in FIG. 1. As shown also in FIG. 2, this inner part comprises a tubular body 36 which is open at one end 38 and has a segmented flange or plurality of projecting portions 40. The other end has a platform 42 with a peripheral upstanding ridge 44, which together form a dish-like receptacle or hollow 46. The platform 42 is perforated by a plurality of bores 48 parallel to the longitudinal axis of the inner part. The platform 42 is joined to the tubular body 36 by a plurality of webs 49 which merge with the tubular body below the end thereof to provide a plurality of shoulders 50. The webs are spaced apart by a plurality of lacunae 52. The lacunae 52 and the perforations 48 serve as passageways for fluid through the wall of the inner part 34.

After the inner part is disposed on the fixture 10, a tubular sleeve 54 is slipped over the inner part 34, as shown in FIG. 2. The inner diameter of the wall 56 of the sleeve is substantially equal to the outer diameter of the tubular body 36 of the inner part. The outer diameter of the wall 56 is smaller than the outer diameter of the segmented flange 40. One end 58 of the sleeve rests on the segmented flange 40, and the other end 59 is spaced from the shoulders 50 of the inner part 34. The outer surface of the sleeve may have a suitable identification code or number printed thereon.

After the sleeve is disposed on the inner part, a sample cube of tissue 60 is disposed into the hollow 46, as shown in FIG. 3. The cube 60 was previously cut from a gross portion of tissue while under a fixative fluid, such as osmic acid. The cube may be conveniently positioned with a pair of tweezers held at arm's length, if desired, by the technician. That is to say, it is not necessary for the

technician to have his eyes in close proximity to the cube 60 and the hollow 46.

After the sleeve is disposed on the hollow part, a sample 62 is fitted onto the inner part 34, as shown in FIG. 4. This outer part is advantageously made of a substantially transparent material, so that the identification number printed on the sleeve 54 may be read therethrough. The outer part 62 has a tubular body 64, which is open at one end 66. The other end is closed by a transverse portion or platform 68 which is connected to the body by several annular transitions, to form a substantially cone shaped inner cavity therein. As also shown in FIG. 5, the apex portion 70 of the cavity accommodates the platform 42, ridge 44 and cube 60. An inner cylindrical portion 72 accommodates the end of the tubular body 36 and has a transverse shoulder 74 against which the shoulder 50 abuts. The end 59 of the sleeve is disposed below the base of the conical cavity. The peripheral surfaces of the segmented flange 40 engage the inner surface of the tubular body 64, to frictionally hold the capsule assembly together. The platform 68 is perforated by a plurality of bores 76. The cube of tissue is thus captured between the two perforate platforms 42 and 68. The ridge 44 precludes the cube from being caught and pinched between the adjacent conical surfaces of the inner and outer parts as they are interfitted. The outer surface of the outer part between the platform 68 and the body 64 includes a ridge or bead 77 and a transverse shoulder 78.

The cube is processed by a further immersion in a fixative liquid in a container assembly 80. The assembly comprises a beaker 82 having a bottom with a raised central portion 84, a raised outermost annular portion 86, and a depressed medial annular portion or trough 88. A cover 90 is snap-fitted into and onto the beaker by an interlocking bead and groove arrangement 92 and 94 respectively. The cover 90 has a hole 96 which may be closed by a plug 98 snap-fitted thereto. Disposed within the beaker is a rack 100 having a tubular spindle portion 102 and an annular platform portion 104. The platform has a plurality of tubular holes 106 thereon, each to accommodate a capsule, and here shown as ten in number. Each hole has a groove 107 in the wall thereof to interlock with the bead 77 on the respective outer part 62. The upper end of the spindle has an annular bead 108, while the lower end of the spindle has an annular groove 110 to snap-lock with the bead 108 on a companion rack as will be described later.

The trough 88 is fitted with a fixative liquid, and a fixture with a cube containing capsule is inverted and is inserted through the hole 96 in the cover into the beaker 82. The rack may be rotated within the beaker, as by poking it with the capsule, until an empty hole 106 therein is below the cover hole 96. Alternatively, the cover may be rotatably mounted to the beaker, so that the hole 96 may be displaced over a stationary rack 100. The capsule is inserted into this hole, the bead 77 snap-locking into the groove 107. The disc 30 on the rod is pushed, extending the head 24 from the post 16, as shown in FIG. 7, thereby releasing the capsule. The two platforms 42 and 68, and the cube which is caged therebetween, are completely submerged in the fixative liquid, which passes through the perforations 48 and 76. The cover serves to minimize the exposure of the technician to the fumes from the fixative liquid. The multi-part capsule and the container assembly may be made of polyethylene. The outer part 82 may advantageously be made transparent so that the code identification on the sleeve may be read therethrough.

The automatic processor 112 is shown in FIG. 8. This processor includes a well 114 in which is mounted a rack having a plurality of tubular guides 116, each of which will accommodate a plurality of stacked beakers. The rack is mounted in suitable bearings and is rotated by a suitable oscillatory drive, all not shown. The well is closable by

a cover 118. The rack shown here has eight guides, each of which will hold three beakers. Eight timer-indicators are mounted on the housing to monitor the use of the oscillating rack. Each timer may be manually set, as the corresponding guide has one or more beakers inserted therein, for the interval that it is desired to oscillate those beakers, and will in due course indicate the completion of such interval. A two hour immersion of a 2 mm. cube is typical. Beakers may be inserted and removed from the guides ad liberatum.

A second well 120 provides a supply of wash liquid for washing racks 100 after they have been removed from their beakers 80.

Twenty additional wells 122 are provided in a circular array. A mast 130 is journaled in the center of the array and is coupled to suitable drive means for rectilinear up and down movement, and rotational movement when in the up position. Attached to the top of the mast is a plate 132, which has a plurality of sockets which are not shown, each to receive and snap-lock with the top 108 of a spindle 102 of a rack 100. The mechanism is similar to that shown in the patent to Jack Isreeli, U.S. 2,872,894 issued Feb. 10, 1959. When a rack 100 is snapped to the plate 132 over the well 122 which is first in the processing sequence, it will subsequently be immersed in that well, oscillated up and down while immersed, removed from that well, swung over and down into the next adjacent well and in a similar manner, sequentially into each well.

A typical arrangement of processing liquids is shown in FIG. 9. With fifteen minutes of immersion in each well, the following processing schedule will result:

Beaker 122a, first dehydrant solution, 15 minutes, 1 beaker;
Beaker 122b, second dehydrant solution, 15 minutes, 1 beaker;
Beaker 122c, third dehydrant solution, 15 minutes, 1 beaker;
Beakers 122d-f, fourth dehydrant solution, 45 minutes, 3 beakers;
Beakers 122g-h, pure dehydrant, 30 minutes, 2 beakers;
Beakers 122i-j, clearant, 30 minutes, 2 beakers;
Beaker 122k-t, infiltrant solution, until removed, 10 beakers.

By this arrangement ten snapped-together strings of racks may be contemporaneously processed by the rotating plate assembly. The number of racks which may be snapped together to form a string is limited by the depth of the wells 122, which wells may be provided with removable beakers as liners. Four racks to a string are illustrated in FIG. 8. Thus, at the commencement of the processing operation, up to four racks may be affixed to the plate 132 over the beaker 122a for insertion therein. At the end of the first immersion interval, which has been illustrated at 15 minutes, but may advantageously be in the 10 to 20 minute range, these racks will be removed from the beaker 122a and shifted over to the beaker 122b for immersion therein. At this time, or at any subsequent pause between beakers, another string of racks may be snapped to the plate 132 over the beaker 122a for immersion therein; since there are ten infiltrant solution beakers 122k-t, ten immediately successive strings of racks may be contemporaneously processed; since when the initial string is halted in the beaker 122t the terminal string will be halted in the beaker 122k. The racks are left in the infiltrant solution beakers for at least the time required to fully impregnate them with the infiltrant medium.

The oscillatory well 114 and the first three beakers may be disposed within a refrigeration assembly, not shown, within the processor 112. If desired the first eight beakers may be refrigerated. A temperature of 6° C. is customarily maintained.

Suitable controls are provided to determine the operation of the processor 112; a switch 134 to turn the processor on, a timer 136 to indicate shifting time progress,

and a switch 138 to arbitrarily raise and stop the plate 132.

After the contained tissue cubes 60 have been fully impregnated with the infiltrant medium the rack 100 is removed from the infiltrant beaker and snapped-off the plate 132. The capsules are now prepared for potting. A rubber boot 140 is fitted onto the underside of the rack 100, as shown in FIG. 10. The boot comprises a plurality of cups 142 here shown as ten, equal in number to the number of holes 106 in the rack, and joined together in a ring by interconnecting webs 144. Each cup 142 is fitted over the cone shaped end of a respective capsule to close the perforations 76 in the platform 68 of the outer part 62.

A flowable, preferably transparent, potting compound is then dispensed into the open end of each capsule. A suitable dispensing fixture is shown in FIG. 11. The fixture 150 comprises a stand having a base 152, a back 154, and a top 156. The rack 100, with its rubber boot 140 on, is disposed on the base 152. A pair of lower brackets 158 and upper brackets 160 are fixed to the back 154 to support a dispensing syringe 162. The syringe comprises a cylinder 164 having an outlet 166, and a piston 168. A rack 170, and a ratchet therefor which is not shown, are mounted within the top 156, together with a crank arm 172 which is coupled to the ratchet to advance the rack a fixed increment for each stroke of the arm. A pin 174 and a set screw 176 limit the swing of the arm. The flowable potting compound is initially loaded into the cylinder 164, and predetermined quantities are then dispensed into each capsule by operating the arm 172. The compound passes through the perforation 48 in the platform 42 to fill up the cage in which the cube of tissue is captured, and at least a portion of the remaining interior of the capsule. The cup 142 precludes the loss of compound beyond the perforations 76.

After the capsules have been potted, the potting compound must be cured. A suitable curing oven is shown in FIGS. 12 and 13. The oven comprises an insulated enclosure 180, having a lid 182, and suitable heating means within and not shown. Also provided are a main power switch 184, a power-on indicator 186, and a resettable running time indicator 188. The indicator has shafted thereto four cams 190a-d, for controlling, in conjunction with suitable follower operated switches which are not shown, the temperature sequence of the oven. Customarily, the oven is run for a total of forty-eight hours, initially at 35° C., subsequently at 45° C., and finally at 60° C.

After curing, the capsules are removed from the rack 100 and the boot 140. As shown in FIG. 14, the outer part 62 is removed from the cube of tissue, the inner part 34 and the sleeve 54 which have all been joined together by the potting compound to form a convenient assembly for examination. The tip of the nose of the assembly is cut away, providing a cross-section of the infiltrated cube of tissue for microtomy, as shown in FIG. 15.

While we have shown and described the preferred embodiment of the invention, it will be understood that the invention may be embodied otherwise than as herein herein specifically illustrated or described, and that certain changes in the form and arrangement of parts and in the specific manner of practicing the invention may be made without departing from the underlying idea or principles of this invention within the scope of the appended claims.

What is claimed is:

1. A multipart capsule for supporting a specimen for preparation for examination, comprising an inner part having a tubular body and a first closed upper end; said first closed upper end having on its exterior upper surface means for receiving the specimen and limiting lateral movement thereof and a passageway for fluid through said first end; an outer part having a tubular body and a second closed upper end; said second closed upper end having a passageway for fluid therethrough; said outer

part being adapted to be fitted onto said inner part in telescoping relationship over said exterior upper surface; said outer and inner parts having mutual interlocking means for holding said parts together with said first and second upper ends in spaced apart relationship, for caging the specimen therebetween.

2. A capsule according to claim 1, wherein said respective passageways in said first and second closed upper ends each comprises a plurality of bores.

3. A capsule according to claim 1, further including an additional part having a tubular body bearing identification indicia thereon, and adapted to be disposed over said tubular body of said inner part and within said tubular body of said outer part.

4. A capsule according to claim 1, wherein said means on the upper surface of said first closed upper end for receiving the specimen and limiting lateral movement thereof comprises a downwardly directed recess.

5. An assembly comprising a multipart capsule according to claim 1, wherein said first and second closed upper ends form a cage, and further including a rack for supporting said capsule in an inverted position and including a horizontal element having a vertical hole therethrough for receiving a portion of said capsule including said cage.

6. An assembly according to claim 5 wherein said rack includes a vertical element having mutual interlocking means in the upper and lower ends thereof, whereby said upper end of said vertical element is adapted to be interlocked to the lower end of the vertical element of a companion rack and said lower end of said vertical element is adapted to be interlocked to the upper end of the vertical element of an additional companion rack.

7. An assembly according to claim 5, wherein said cage projects below the undersurface of said horizontal element.

8. An assembly to claim 5, wherein said hole in said horizontal element is one of a plurality of similar holes, each for receiving a respective multipart capsule.

9. An assembly according to claim 8, further including a container for receiving said rack and said capsule therein, having a first bottom portion for supporting the undersurface of said horizontal element and a second recessed bottom portion for receiving a liquid therein and for receiving said cage of said capsule in the liquid therein.

10. An assembly according to claim 9, wherein said holes are in an annular array, and said second recessed bottom portion is annular.

11. An assembly according to claim 10, further including a cover for said container having an aperture therein for the sequential passage therethrough of each of said capsules; said rack being movable within said container for sequentially presenting each of said holes under said aperture for the receipt of a capsule; and an auxiliary cover for closing said aperture.

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