United States Patent [19]

Takahashi

[11] **3,809,008**

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[54] **APPARATUS FOR PROCESSING TISSUE FOR ELECTRON MICROSCOPE EXAMINATION**

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[73] Assignee: **Sakura Seiki Kabushiki,** Kaisha, Tokyo, Japan

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[52] **U.S. Cl.** **118/6,** 118/50, 118/416, 118/425, 118/429

[51] **Int. Cl.** **B05c 11/12**

[58] **Field of Search** ..... 118/4, 6, 7, 421, 425, 118/429, 50; 117/113, 114 R, 114 A, 114 B, 114 C, 116

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Primary Examiner—Morris Kaplan

Attorney, Agent, or Firm—Waters, Roditi, Schwartz & Nissen

[57] **ABSTRACT**

Apparatus for processing tissue for electron microscope examination comprises a series of processing reagent containing receptacles arranged in an annular row, a rotary transfer disc for transferring a tissue-containing basket along the series of receptacles successively one by one to dip the basket and the tissue contained therein into each of the reagents in the receptacles, and means for maintaining the atmosphere surrounding some of the reagents at sub-atmospheric pressure when the basket and the tissue therein are being dipped therein for processing, the sub-atmospheric pressure improving and accelerating ingress and impregnation of the reagents into the tissue being processed.

10 Claims, 9 Drawing Figures

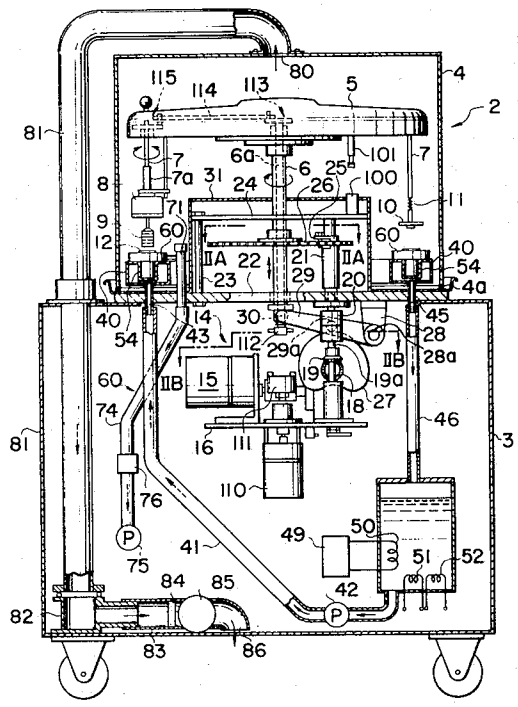


FIG. 1

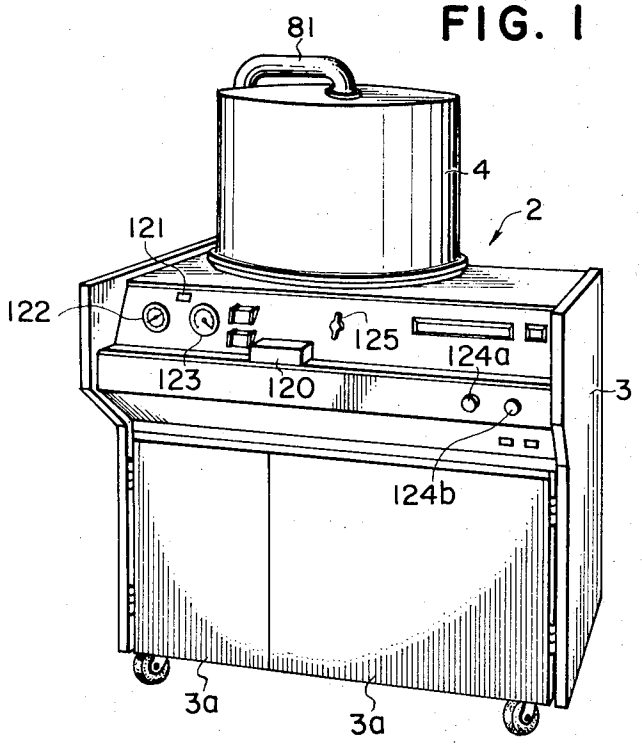

81
4
2
121
122
125
124a
123
120
3
124b
3a
3a

FIG. 3

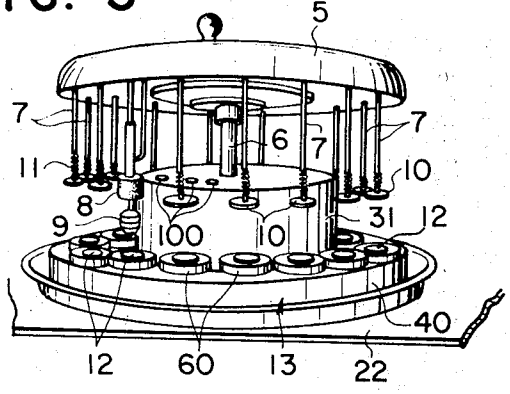

5
7
6
7
7
11
10
8
9
100
10
31
12
40
12
60
13
22

FIG. 2

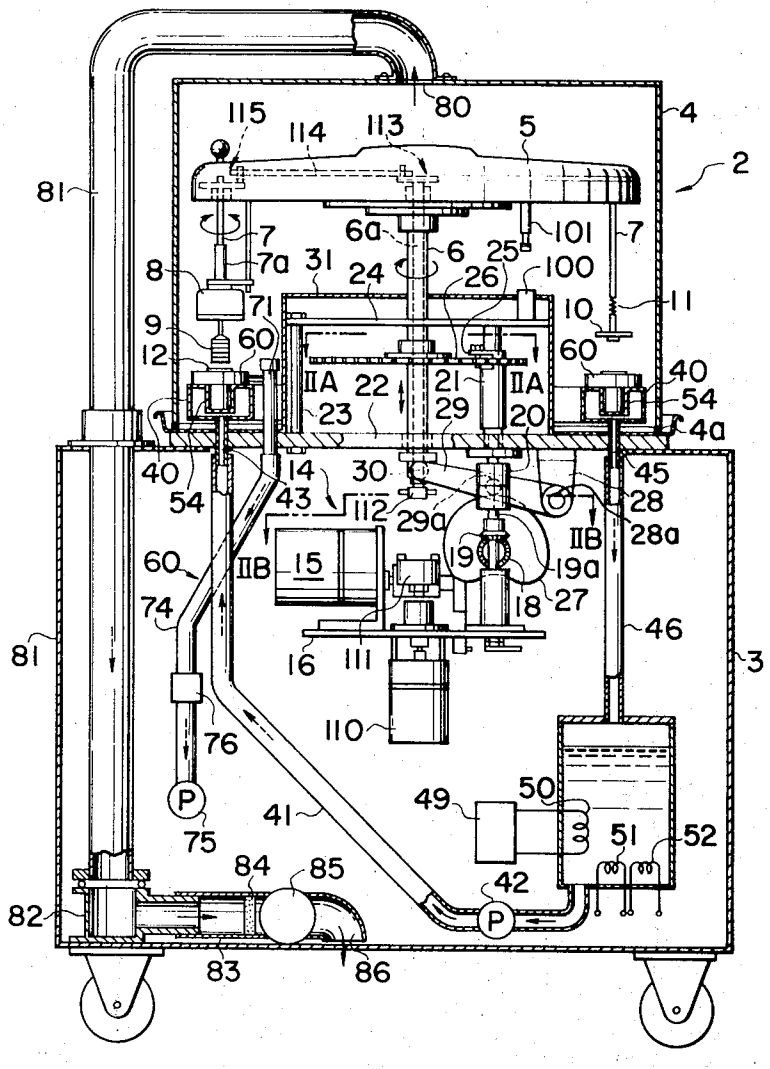
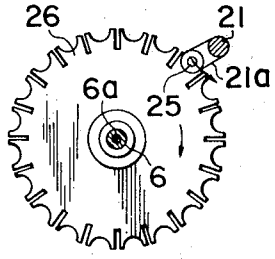

115
114
113
80
5
4
2
81
7
6a
101
7
8
7a
31
24
6
25
26
100
10
11
9
71
60
12
60
40
IIA
22
21
IIA
54
23
29
20
4a
40
14
30
45
54
43
112
29a
28
IIB
28a
60
IIB
15
19
19a
74
18
27
46
81
16
111
3
110
76
49
50
P
41
75
51
52
84
85
42
82
P
83
86

FIG. 2A



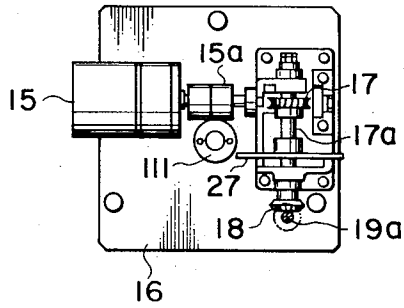


FIG. 2B

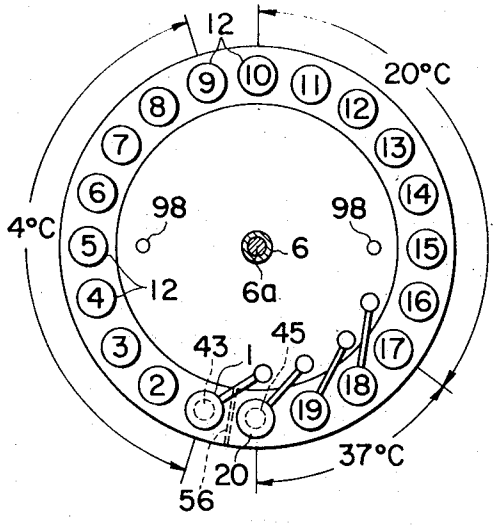


FIG. 4

FIG. 5

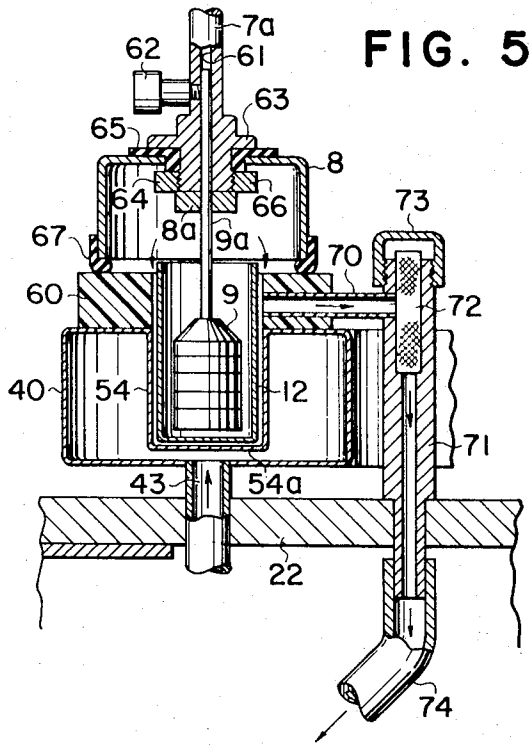

7a
62
61
63
65
8
64
66
73
67
8a
9a
70
60
9
72
40
54
12
71
43
54a
22
74

FIG. 7

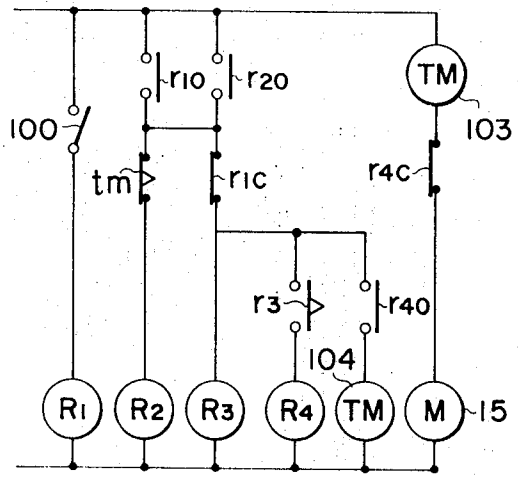
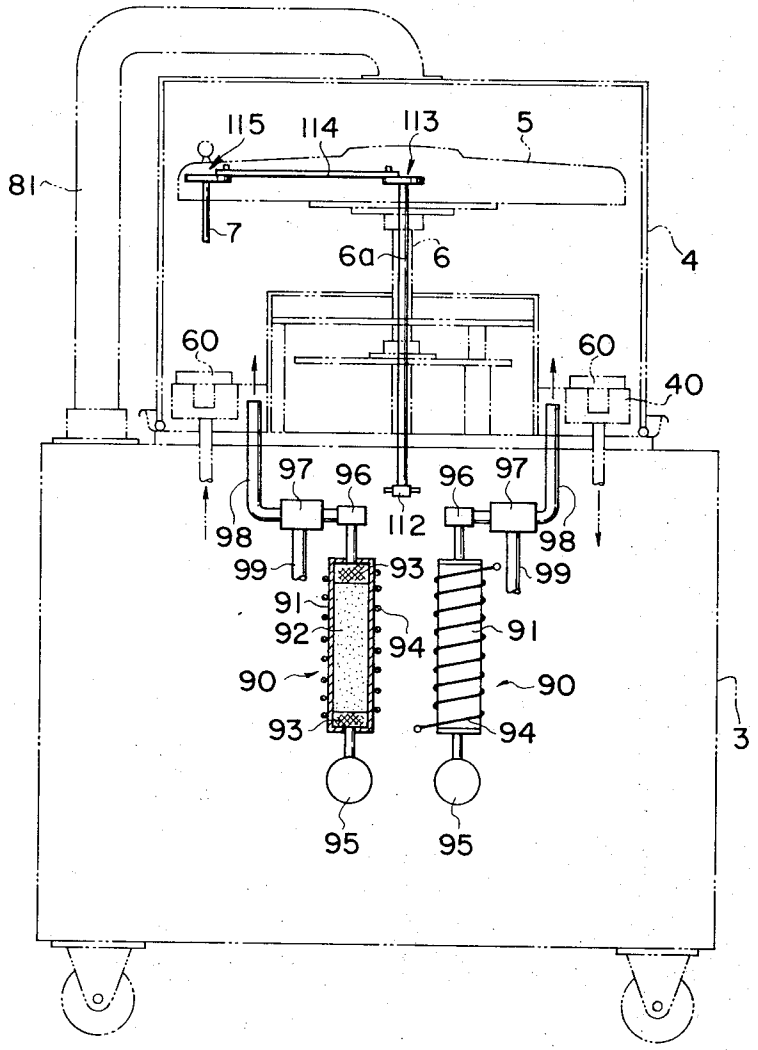

r10
r20
TM
100
103
tm
r1c
r4c
r3
r40
104
R1
R2
R3
R4
TM
M
15

FIG. 6


115
114
113
5
81
7
6a
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4
60
60
40
97
96
96
97
98
112
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91
92
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91
90
90
93
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3
95
95

APPARATUS FOR PROCESSING TISSUE FOR ELECTRON MICROSCOPE EXAMINATION

BACKGROUND OF THE INVENTION

This invention relates to apparatus for processing tissue for electron microscope examination and, more particularly, to an apparatus for preparing histologic tissue for subsequent examination by an electron microscope.

It is well known that, for carrying out histologic examination of part of a living body, a tissue specimen of the living body is cut from the body and sliced into a number of thin pieces, which are fixed, dehydrated and otherwise processed or treated in a tissue processing apparatus. After having been processed by the processing apparatus, the thin pieces or specimens are again sliced into extremely thin pieces, which are used for subsequent electron microscope examination.

Heretofore known tissue processing apparatus generally comprises a series of receptacles respectively containing different liquid reagents therein and arranged in an annular endless row, and a rotary indexing transfer disc disposed above the receptacles in concentric relation to the annular row and having on the peripheral portion thereof at least one basket suspended therefrom and adapted to receive therein tissue to be processed, the basket and, therefore, the tissue therein being dipped into the different liquid reagents contained in the successively arranged receptacles one by one when the transfer disc is rotated or indexed and moved up and down.

In treating or processing tissue with such a processing apparatus, difficulties have been encountered in rapidly and throughly impregnating the reagents into the tissue being processed because a fresh tissue generally has a characteristic of resisting rapid and uniform ingress of the reagents thereinto because it contains considerable quantities of air bubbles and moisture. It is also difficult to attain rapid and thorough impregnation of the reagent into the tissue especially when the reagent is a highly viscous liquid.

SUMMARY OF THE INVENTION

It is, therefore, an object of this invention to provide a new and improved apparatus for processing tissue for electron microscope examination which enables rapid impregnation of reagents into tissue being processed, thus realizing thorough and uniform processing of the tissue.

Another object of this invention is to provide an apparatus of the above stated nature wherein the local atmosphere surrounding at least one reagent is maintained at sub-atmospheric pressure while a tissue is being dipped in the reagent whereby air bubbles existing in the tissue are rapidly removed to leave void spaces which enables the reagent to rapidly enter the tissue for the processing thereof.

A further object of this invention is to provide an apparatus of the above stated nature wherein a basket containing tissue therein is held stationary for a while after it has been raised from a viscous processing reagent contained in a receptacle, whereby the viscous reagent remaining in the basket is allowed to drip into the receptacle, whereby carryover of the viscous reagent by the basket into the next reagent receptacle is prevented.

Still another object of this invention is to provide an apparatus of the above stated nature wherein reagent receptacles other than a receptacle in which a tissue basket is being dipped in reagent are covered by lids whereby evaporation of reagents in such receptacles is prevented.

A still further object of this invention is to provide an apparatus of the above stated nature wherein processing of tissue by reagents is carried out in a sealed hood from within which air is continually exhausted and which prevents dust infiltration.

It is also an object of this invention to provide an apparatus of the above stated nature wherein processing of tissue by reagents is carried out in a sealed hood into which dry air is always supplied.

According to this invention, briefly summarized, there is provided an apparatus for processing tissue for electron microscope examination comprising means for arranging a series of processing reagent containing receptacles in a row with equal distances therebetween, and transfer means for transferring a tissue-containing basket, the transfer means being located above the receptacles and mounted for indexing movement along the series of receptacles and for up and down movement to dip the basket and tissue therein into the processing agents successively one by one, said apparatus incorporating the improvement comprising sealing means associated with the transfer means and operative to seal temporarily from the outside the ambient atmosphere surrounding any receptacle in which the basket is being dipped in the reagent, and suction means associated with at least some of the receptacles to evacuate air from the atmosphere surrounding said some receptacles thus sealed.

The nature, principle, and utility of this invention will be apparent from the following detailed description of the invention with respect to a preferred embodiment thereof when read in conjunction with the accompanying drawings, in which like parts are designated by like reference numerals.

BRIEF DESCRIPTION OF THE DRAWINGS

In the drawings:

FIG. 1 is a perspective view showing principally the front exterior of an example of the processing apparatus according to this invention;

FIG. 2 is a front elevation, in vertical section, of the apparatus shown in FIG. 1;

FIG. 2A is a plan view taken along the line IIA — IIA of FIG. 2;

FIG. 2B is a plan view taken along the line IIB — IIB of FIG. 2;

FIG. 3 is a fragmentary perspective view showing a rotary transfer disc, a series of reagent receptacles, and other members associated therewith;

FIG. 4 is a diagrammatic plan view showing the receptacles disposed in an annular row;

FIG. 5 is a fragmentary front elevation, in vertical section, on an enlarged scale, showing one of the reagent receptacles, sealing cap, suction pipe and related parts;

FIG. 6 is a front elevation showing a dry air supply sytem and a basket oscillation system; and

FIG. 7 is a wiring diagram showing an electric circuit for controlling an indexing delay device.

DETAILED DESCRIPTION OF THE INVENTION

General Principle

Referring to FIG. 1, the apparatus for processing tissue for electron microscope examination according to this invention is generally designated by reference numeral 2 and comprises a lower housing 3 with front doors 3*a*, and a transparent upper cylindrical hood 4 mounted on the housing.

As shown in FIG. 2, there is provided a rotary transfer disc 5 within the hood 4. The disc 5 is rigidly supported on the top of a central rotary shaft 6 which is capable of vertical reciprocating motion and of intermittent rotary motion as is described hereinafter in detail. As is also shown in FIG. 3, the rotary disc 5 has on the lower surface thereof a series of vertical mounting rods 7 extending downward from the surface and arranged along the periphery of the disc 5 in angularly equally spaced-apart relationship. In one example, the number of such rods 7 is twenty. One of these rods 7 has on the lower end thereof a sealing cap 8 which will be described hereinafter in more detail. A basket 9 depends from within the cap 8, which basket is adapted to receive therewithin a tissue or specimen to be processed by the apparatus. Each of the remaining rods 7 has on the lower end thereof a lid 10 suspended from the rod by means of a flexible string 11.

Below the rotary disc 5, there are provided a series of beakers or receptacles 12 disposed in an annular row in vertically opposed relationship to the respective rods 7. The receptacles 12 are supported by a stationary structure 13 (FIG. 3) and are adapted to receive different kinds of processing liquids or reagents.

In the example shown, the number of the receptacles 12 is twenty which corresponds to the number of the rods 7. In the diagrammatical view of FIG. 4 showing the arrangement of the receptacles, the twenty receptacles are successively numbered 1 to 20.

At the beginning of operation of the apparatus, the rotary disc 5 is in a raised position as shown in FIGS. 2 and 3, so that the basket 9 and lids 10 are also in a raised position spaced apart upward from the receptacles 12. In this position, the rotary disc 5 is manually adjusted so that the rod 7 carrying the basket 9 is in vertical alignment with the receptacle 12 which is numbered "1" in FIG. 4.

When the apparatus is put in operation, the disc 5 is lowered by the downward movement of the shaft 6 until the basket 9 containing a specimen therein is completely immersed in the reargent contained in the receptacle numbered "1". After a predetermined time interval, the shaft 6 and, therefore, the disc 5 are raised to the original position, and then the rotary transfer disc 5 is indexed to a position where the basket 9 is in vertical alignment with the adjoining or next receptacle indentified as "2" in FIG. 4. Then, the indexed disc 5 is again lowered by the downward displacement of the shaft 6 until the basket 9 is immersed in the liquid reagent contained in the receptacle numbered "2" for a predetermined time interval. The disc and, therefore, the basket 9 are again raised to the former position, and then similar indexing and up and down movement of the basket 9 is repeated until the basket has been immersed in the liquid reagent contained in the last receptacle numbered "20" in FIG. 4. During this operation, the liquid reagents in the receptacles are kept at predetermined different temperatures in a predetermined sequence. Thus, it will be seen that the tissue or specimen contained in the basket 9 successively undergoes chemical processing by the different kinds of liquid reagents.

A typical example of such a chemical processing is given in the following table.

Receptacle No.	Reagents	Time Interval in minute	Temp. in °C
1	2.5% Glutaraldehyde	120	4
2	0.2M Cacodylate Buffer (pH 7.4)	30	4
3	0.2M Cacodylate Buffer (pH 7.4)	30	4
4	0.2M Cacodylate Buffer (pH 7.4)	30	4
5	2% Osmic Acid	120	4
6	0.2M Cacodylate Buffer (pH 7.4)	30	4
7	50% Alcohol	10	4
8	50% Alcohol	10	4
9	70% Alcohol	15	4
10	95% Alcohol	15	20
11	100% Alcohol	30	20
12	100% Alcohol	30	20
13	100% Alcohol + Propylene Oxide (1 to 1)	15	20
14	Propylene Oxide	30	20
15	Propylene Oxide	30	20
16	Propylene Oxide + Epon 812 (2 to 1)	60	20
17	Propylene Oxide + Epon 812 (1 to 2)	60	20
18	Epon 812	60	37
19	Epon 812	60	37
20	Epon 812	60	37

ROTARY TRANSFER DISC ACTUATING MECHANISM

The intermittent rotary motion or indexing motion and the up and down movement of the rotary transfer disc 5 as described hereinbefore is effected by a rotary disc actuating mechanism generally designated by reference numeral 14 in FIG. 2.

The actuating mechanism 14 comprises an electric motor 15 stationarily mounted on a mounting base 16 rigidly secured to the inner wall of the housing 3. As also shown in FIG. 2B, the output shaft of the motor 15 is connected via a coupling 15*a* to a worm, not shown, which worm is in turn in mesh with a worm wheel 17. The worm wheel 17 has a shaft 17*a* on which is rigidly mounted a bevel gear 18. This bevel gear 18 is in mesh with another bevel gear 19 (FIG. 2) having a shaft 19*a*, which extends upward and connected via a coupling 20 to a rotary shaft 21. It will thus be seen that when the motor 15 is operated the shaft 21 is accordingly rotated.

On a base plate 22 rigidly mounted on the top of the housing 3, there is secured upright studs 23 one of which is shown in FIG. 2. These studs 23 have secured thereto a disc 24. The disc 24 and the base plate 22 cooperate to rotatably support the shaft 21.

As is clearly shown in FIG. 2A, the shaft 21 has a transverse arm 21*a* having a geneva pin 25, which engages inter-tooth spaces of a geneva wheel 26 successively. The geneva wheel 26 and pin 25 constitute a well known geneva mechanism for producing intermittent turning motion. The wheel 26 is mounted on the central shaft 6. It will thus be seen that when the motor 15 is operated, the shaft 6 and hence the transfer disc 5 undergo intermittent rotary motion or indexing motion.

As is shown in FIGS. 2 and 2B, the shaft 17*a* of the bevel gear 18 has rigidly mounted thereon a cam plate 27. On the other hand, a bracket 28 (FIG. 2) is secured under the base plate 22 and a lever 29 is pivotally mounted on the bracket at 28*a*. The lever 29 carries on an intermediate part thereof a cam follower 29*a* which rides on the peripheral surface of the cam plate 27. The free end of the lever 29 is pivotally connected at 30 to the lower end of the central shaft 6.

It will thus be seen that when the motor 15 is operated, the lever 29 is swung up and down by the cam plate 27 to cause the shaft 6 to move reciprocatively up and down. The geneva wheel 26 is mounted on the shaft 6 in a manner to transmit rotation to the shaft 6 but to be vertically slidable relative to the shaft. The disc 24, studs 23, shaft 21, geneva wheel 26 and other associated members are enclosed in a cover 31.

REAGENT TEMPERATURE CONTROL SYSTEM

The temperature control of the reagents is carried out by a heat transfer medium circulating system. As shown in FIG. 2, the circulating system comprises an annular hollow jacket member 40 through which a heat transfer medium, such as water, can flow. Part of the bottom of the jacket member 40 is communicatively connected to the delivery side of a pump 42 by a conduit 41 and an inlet pipe 43, and another part of the bottom of the jacket member 40 is communicatively connected to a reservoir 44 for storing the heat transfer medium through an outlet pipe 45 and a conduit 46. It will thus be apparent that the heat transfer medium in the reservoir 44 is circulated by the pump 42 through the conduit 41, the pipe 43, the interior of the jacket member 40, the pipe 45 and the conduit 46 back into the reservoir 44.

A refrigerating unit 49 is associated with the reservoir 44 and has a refrigerant circulating coil 50 disposed in the reservoir 44 for cooling the heat transfer medium. The reservoir 44 has also associated therewith a main heating coil 51 and an auxiliary heating coil 52. These coils 51 and 52 may be in the form of an electric heating coil. When the temperature of the heat transfer medium is to be maintained at 4°C, for example, the refrigerating unit 49 is put in operation while the heating coils are not in operation. On the other hand, when the temperature of the heat transfer medium is to be maintained at 20°C, for example, the operation of the refrigerating unit 49 is stopped, and only the main heating coil 51 is energized, while, when the temperature of the heat transfer medium is to be maintained at 37°C, for example, the auxiliary heating coil 52 is additionary operated to increase the heating capacity.

As shown in FIGS. 2 and 5, the jacket member 40 is of a rectangular cross section and has in the upper surface thereof a series of pockets 54 disposed in an annular arrangement in circumferentially equally spaced-apart relationship. Each of the pockets 54 is in the form of a cylindrical recess with a bottom 54*a* and is configured to removably receive the beaker or receptacle 12 therein as is most clearly shown in FIG. 5. Thus, it will be apparent that the liquid reagents in the receptacles 12 received in the pockets 54 are cooled or heated by the heat transfer medium through the heat transfer across the walls of the receptacles 12 and pockets 54.

In order to maintain a constant-temperature condition of the liquid reagents in the receptacles, a temperature control device may be provided to detect the temperature of the heat transfer medium flowing through the jacket member 40 so as to control the refrigerating unit 49 and the heating coils 51 and 52.

In FIG. 2, the inlet and outlet pipes 43 and 45 are shown as provided at diametrically opposite portions of the annular jacket member 40 for the purpose of clarity, but these pipes are actually provided at positions corresponding to the positions of the receptacles numbered "1" and "20", respectively, as shown in FIG. 4. In this case, a partition wall 56 is provided to cause the heat transfer medium introduced through the inlet pipe 43 into the jacket member 40 to flow along the annular path within the member 40 to the outlet pipe 45.

REAGENT RECEPTACLE SEALING DEVICE

As is most clearly shown in FIG. 5, on the upper surface of the jacket member 40 there is secured an annular pad 60 made of a material such as synthetic resin, which is disposed around the opening periphery of each receptacle-receiving pocket 54. The height or thickness of the pad 60 is such that the sum of the thickness of the pad and the depth of the pocket 54 is slightly smaller than the height of the receptacle 12, whereby the top edge of the receptacle 12 projects upward beyond the upper surface of the pad 60 to facilitate insertion and removal of the receptacle into and out of the pocket 54.

The rod 7 carrying the sealing cap 8 and the basket 9 has on the lower end thereof a fitting 7*a* having a vertical through hole 61 therein. The basket 9 has a stem 9*a* extending upward therefrom and this stem 9*a* is slidably inserted into the hole 61 and secured in position by a set screw 62 transversely threaded in the fitting 7*a*. It will be seen that the vertical position of the basket 9 can be adjusted by loosening the set screw 62.

The fitting 7*a* has an enlarged part or flange 63 and male threads 64 below the flange. Under the lower surface of the flange 63, there is disposed a packing ring 65 and the cap 8 of an inverted cup-shaped form is placed on and around the ring 65. With the male threads 64 is engaged a nut 66 having female threads. When the nut 66 is tightened against the packing ring 65, the ring 65 is deformed to tightly hold the cap 8 on the fitting 7*a*. The lower periphery of the cap 8 is provided with an annular packing 67 which is adapted to tightly abut the upper surface of the pad 60 when the basket 9 is lowered to the position of FIG. 5 at which the basket 9 is completely dipped in the liquid reagent in the receptacle 12. It will be noted that, in the condition shown in FIG. 5, the interior space within the cap 8 and the pocket 54 is completely sealed from the outside. The cap 8 may have rigidly fixed thereto a bearing member 8*a* through which the stem 9*a* passes.

VACUUM SYSTEM

As shown in FIG. 5, there is provided a suction pipe 70 passing horizontally through the pad 60 and communicating at one end thereof with the interior space of the pocket 54. The other end of the pipe 70 is communicatively connected to a riser pipe 71 secured to the base plate 22 and having a strainer or filler 72 therein. The filter 72 can be inserted into the interior of the riser pipe 71 through the top opening thereof, and after the insertion, a top cover 73 is threadedly secured on the top of the pipe 71. As shown in FIG. 2, the

lower end of the pipe **71** is connected to a conduit **74** leading to a vacuum pump **75** through a solenoid valve **76.**

When processing the specimen in the first receptacle numbered "1", it is generally desirable to have the interior space of the receptacle evacuated because such an evacuation is effective to remove air bubbles and moisture existing in fresh tissue to be processed, and such removal greatly facilitates and improves ingress or impregnation of the reagent into the tissue. When the reagent is a viscous liquid such as "epon" as is used in the receptacles numbered "18", "19" and "20", the application of vacuum is also useful to improve impregnation of the viscous liquid into the tissue. From this point of view, the suction pipe **70** and the associated riser pipe **71** are provided for each of the pockets **54** receiving the receptacles numbered "1", "18", "19" and "20", as shown in FIG. **4.** The solenoid valve **76** is provided for each of the four suction pipes **70,** while the suction pump **75** is used commonly for the four suction pipes **70.** It will be apparent that, when the vacuum pump **75** is driven and one of the solenoid valve **76** is opened, the interior space of the cap **8** and, therefore, one of the receptacle **12** is evacuated and maintained at sub-atmospheric pressure to improve and accelerate the impregnation of the reagent.

EXHAUST SYSTEM

Referring to FIG. **2,** the upper cylindrical hood **4** has on the lower edge thereof an annular packing **4***a* which sealingly engages the bottom surface of an annular dish on the base plate **22.** The top wall of the hood **4** is formed with an exhaust opening **80** to which an exhaust conduit **81** is connected. The conduit **81** extends downward along the side surface of the hood **4** into the interior of the lower housing **3.** The lower end of the conduit **81** is connected by means of a connecting bend **82** to an exhaust pipe **83** having therein a filter element **84** and an exhaust blower **85.** The pipe **83** has an exhaust outlet **86.** It will be readily seen that, when the blower **85** is driven, irritating gases or vapours which are generated from the reagents into the interior space of the hood **4** will be exhausted through the conduit **81** and pipe **83** to the outside after having been filtered by the filter element **84.**

DRY AIR SUPPLY SYSTEM

As shown in FIG. **6,** the lower housing **3** of the apparatus encloses a pair of identical dry air supply devices **90.** Each device **90** comprises a cylindrical enclosure **91** containing drying agent **92** such as silica gel therein. The enclosure **91** has also therein a pair of air filters **93** at both ends thereof and has wound around the outer wall thereof an electric heating coil **94.** One end of the enclosure **91** is connected to the delivery side of an air blower **95,** and the other end thereof is connected through a strainer **96** to a three-way solenoid value **97.** From the valve **97,** a dry air supply conduit **98** extends upward into the interior space of the hood **4.** An exhaust pipe **99** extends from the valve **97.**

The dry air supply devices **90** are alternately operated for supplying dry air into the hood **4.** When one of the devices **90** is to be operated, the solenoid valve **97** thereof is so operated as to cause the supply conduit **98** to communicate with the strainer **96** and the blower **96** is put in operation. Thus, air is forced through the filter **93,** drying agent **92,** filter **93,** strainer **96** and valve **97,** and dry air is supplied into the hood **4** through the supply conduit **98.** When one device **90** is thus operating, the other device **90** undergoes a recovering cycle. That is, the solenoid valve **97** is changed over to communicate the strainer **96** with the exhaust pipe **99** and the heating coil **94** is energized. It will be seen that the drying agent **92** is heated by the coil **94,** and moisture accumulated in the agent **92** druing the dry air supply cycle is dissipated from the agent by operating the blower **95** and is carried away through the exhaust pipe **99** to the outside. By such alternative use of the dry air supply devices, dry air is continuously fed into the hood **4,** and the atmosphere within the hood is constantly maintained dry.

INDEXING DELAY DEVICE

When a processing reagent is a viscous liquid such as epon, carryover of the reagent into the next reagent tends to occur as the basket is raised from the former reagent and indexed into the next reagent because the viscous liquid has a tendency to adhere to the basket and cannot completely drip into the reagent receptacle immediately after the basket has been raised. Since the processing reagents in the receptacles are of different kind from reagent to reagent, the carryover of a reagent into the next reagent is not desirable.

In order to avoid such a disadvantage, an indexing delay device is provided according to this invention. As shown in FIG. **2,** the stationary disc **24** forming part of a framework for supporting the shafts **6** and **21** supports thereon a row of microswitches **100** only one of which is shown for clarity in FIG. **2.** In the example shown, three such microswitches **100** are provided at successively adjoining positions as shown in FIG. **3.**

Referring again to FIG. **2,** the transfer disc **5** has rigidly secured thereto a downwardly extending pin **101** for actuating the microswitches **100** one after the other. It is to be noted that when the basket **9** is at a position directly above a receptacle containing a first epon, i.e., at a position directly above the receptacle numbered "18" (see FIG. **4**), the actuating pin **101** is directly above the first microswitch **100.**

As the transfer disc **5** is lowered to immerse the basket **9** into the first epon (No. 18), the pin **101** is also lowered to engage the actuating member of the first microswitch **100** to close the same.

Referring to the diagram of FIG. **7,** when the switch **100** is closed, a relay R_1 is energized, and its normally open contact *r***10** is closed, while its normally closed contact *r***10** is opened. Then, a relay R_2 is energized through a normally closed timer switch tm and the closed contact *r***10** to close a normally open contact *r***20,** and the circuit passing through the contact *r***20,** timer switch tm, and relay R_2 makes a self-holding circuit.

The main motor **15** is controlled by a main timer **103,** and when the transfer disc **5** is lowered to a position where the basket **5** is completely dipped in the first epon (No. 18), the main timer **103** operates to open the circuit to stop the main motor **15.** After a preset time interval has elapsed, the main timer **103** again operates to activate the main motor **15,** whereby the transfer disc **5** begins to rise and the switch **100** is opened.

When this occurs, the relay R_1 is deenergized to close the contact *r***1C** so that a relay R3 is energized. This relay R_3 is a delay relay, and, after a predetermined time, for example, ten seconds, has elapsed, a normally

open relay contact *r*3 is closed so that a relay R_4 is energized. Then, a normally closed relay contact *r*4C is opened to stop the main motor **15**. It will be noted that a predetermined time has elapsed before the main motor **15** is stopped after it was restarted. This time interval enables the motor **15** to rotate, after it has been restarted, until the cam plate **27** assumes an angular position at which the transfer disc **5** is at its highest position.

The energization of the relay R_4 also causes its normally open contact *r*40 to close, whereby an indexing delay timer **104** is set in operation. After its preset time, which allows the epon adhering to the raised basket to drip completely into the receptacle, has elapsed, the timer contact tm is opened, whereby the contact *r*20 is opened with the result that the relay R_4 is deenergized to close the contact *r*4C, and the main motor **15** is operated again to index the basket to the position of the second epon. At the positions of the second and third epons (Nos. 19 and 20), the same operation as described above is repeated.

BASKET OSCILLATION SYSTEM

For the purpose of promoting the processing, the basket **9** is oscillated or turned reciprocatively when it is being dipped in the reagents. The system for this purpose is driven by an auxiliary motor **110** secured to the mounting base **16** as shown in FIG. **2**. The motor **110** has a vertical output shaft on the top of which is secured a drive coupling **111**. As is also shown in FIG. **6**, the central shaft **6** is a hollow shaft and a core shaft **6***a* is slidably passed through the shaft **6**. The lower end of the core shaft **6***a* has a driven coupling **112** on the lower end thereof. On the top of the core shaft **6***a* there is provided a crank mechanism **113** and a connecting rod **114** is pivoted at one end. The other end of the rod **114** is connected to a rocking mechanism **115** which is in turn connected to the upper end of the rod **7** associated with the basket **9**.

It will be seen that when the transfer disc **5** is lowered, the driver coupling **112** engages the drive coupling **111**, and the rotary motion is transmitted from the motor **110** to the core shaft **6***a* to oscillate the rod **7** and, therefore, the basket **9** which is now in the reagent. Thus, an agitating action is created to promote processing.

GENERAL CONTROL

As is apparent from the foregoing description, the indexing or intermittent motion and up-and-down movement of the transfer disc **5** is carried out by the operation of the main motor **15**, and this main motor is controlled automatically by the main timer **103**. This main timer **103** is, for example, of the type comprising a punched tape and a tape reader useing a photoelectric sensing device.

As shown in FIG. **1**, the apparatus **2** has on the front side thereof an enclosure **120** for enclosing such a timer device. The apparatus may also include a vacuum indicating pilot lamp **121**, a temperature gauge **122** for the heat transfer medium, and a vacuum gauge **123**. The apparatus may also include operation keys **124***a* and **124***b* for raising and lowering the hood **4** relative to the housing **3** for the charge and discharge of the tissue into and out of the apparatus as well as a knob **125** for change-over between "automatic" and "manual" control.

I claim:

1. In an apparatus for processing tissue for electron microscope examination, comprising means for arranging a series of processing reagent containing receptacles in a row with equal distances therebetween, transfer means for transferring a tissue-containing basket, said transfer means being located above said receptacles and mounted for indexing movement along the series of receptacles and for up and down movement to dip the basket and, therefore, the tissues therein into the processing reagents in the receptacles successively one by one, the improvement comprising sealing means associated with said transfer means and operative to seal temporarily from the outside the ambient atmosphere surrounding any one of the receptacles in which the basket is being dipped in the reagent, suction means associated with at least some of the receptacles to evacuate air from the atmosphere surrounding said some receptacles thus sealed, series of processing reagent containing receptacles being arranged in an annular endless row, said transfer means being in the form of a rotary disc capable of being rotated around a center coaxial with the center of said annular endless row, said rotary disc having along the periphery thereof a series of mounting means of annular arrangement with equal distances therebetween corresponding to those between said receptacles, wherein one of said mounting means carries said basket and each of the remaining mounting means carries a lid which is adapted to cover and close an associated receptacle when the rotary disc is lowered to dip the basket in the reagent, thereby to prevent evaporation of the reagents contained in the receptacles other than that cooperating with the basket, a hood enclosing said rotary disc, receptacles, basket and lids and means for exhausting gases and vapors within said hood, and means for supplying dry air into the interior space of said hood.

2. The apparatus as claimed in claim **1** wherein said sealing means comprises a sealing cap coaxially mounted above said basket and adapted to be lowered to sealingly confine therein a receptacle in which the basket is lowered for dipping in reagent.

3. The apparatus as claimed in claim **2** wherein each of the receptacles has therearound an annular pad on which said sealing cap engages sealingly.

4. The apparatus as claimed in claim **2** wherein said sealing cap and said basket are in rigid connection with each other.

5. The apparatus as claimed in claim **1** wherein said suction means has associated therewith means for actuating the same in response to a predetermined down movement of said transfer means.

6. The apparatus as claimed in claim **1**, further including means for delaying the indexing movement of the transfer means from one receptacle to the next receptacle, whereby excess reagent remaining in the basket raised from said one receptacle is allowed to drip into the latter.

7. The apparatus as claimed in claim **1** wherein each of the lids is suspended from the associated mounting means through a flexible suspension means.

8. The apparatus as claimed in claim **1**, further including means for maintaining the reagents in the series of receptacles at respectively predetermined temperatures.

9. The apparatus as claimed in claim **8** wherein said maintaining means comprises means for circulating a heat transfer medium in heat transfer relationship to be receptacles.

10. The apparatus as claimed in claim **1**, further including means for reciprocatively moving the basket when it has been dipped in the reagent.

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