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(12) **United States Patent**
Fauver et al.

(10) **Patent No.: US 6,991,738 B1**
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(54) **FLOW-THROUGH DRUM CENTRIFUGE**

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(73) Assignee: **University of Washington**, Seattle, WA (US)

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B01D 21/26 (2006.01)
B01D 37/02 (2006.01)

(52) **U.S. Cl.** **210/771**; 210/778; 210/780; 210/787; 210/791; 210/360.1; 210/380.1; 210/393; 210/396; 210/402; 118/55; 118/58; 422/72; 494/37; 494/43; 494/58

(58) **Field of Classification Search** 210/770, 210/778, 780, 802, 783, 787, 803, 360.1, 210/393, 394, 396, 402, 771, 791, 380.1; 118/55, 58; 422/72, 101; 427/2.1, 2.13; 494/36, 37, 43, 58
See application file for complete search history.

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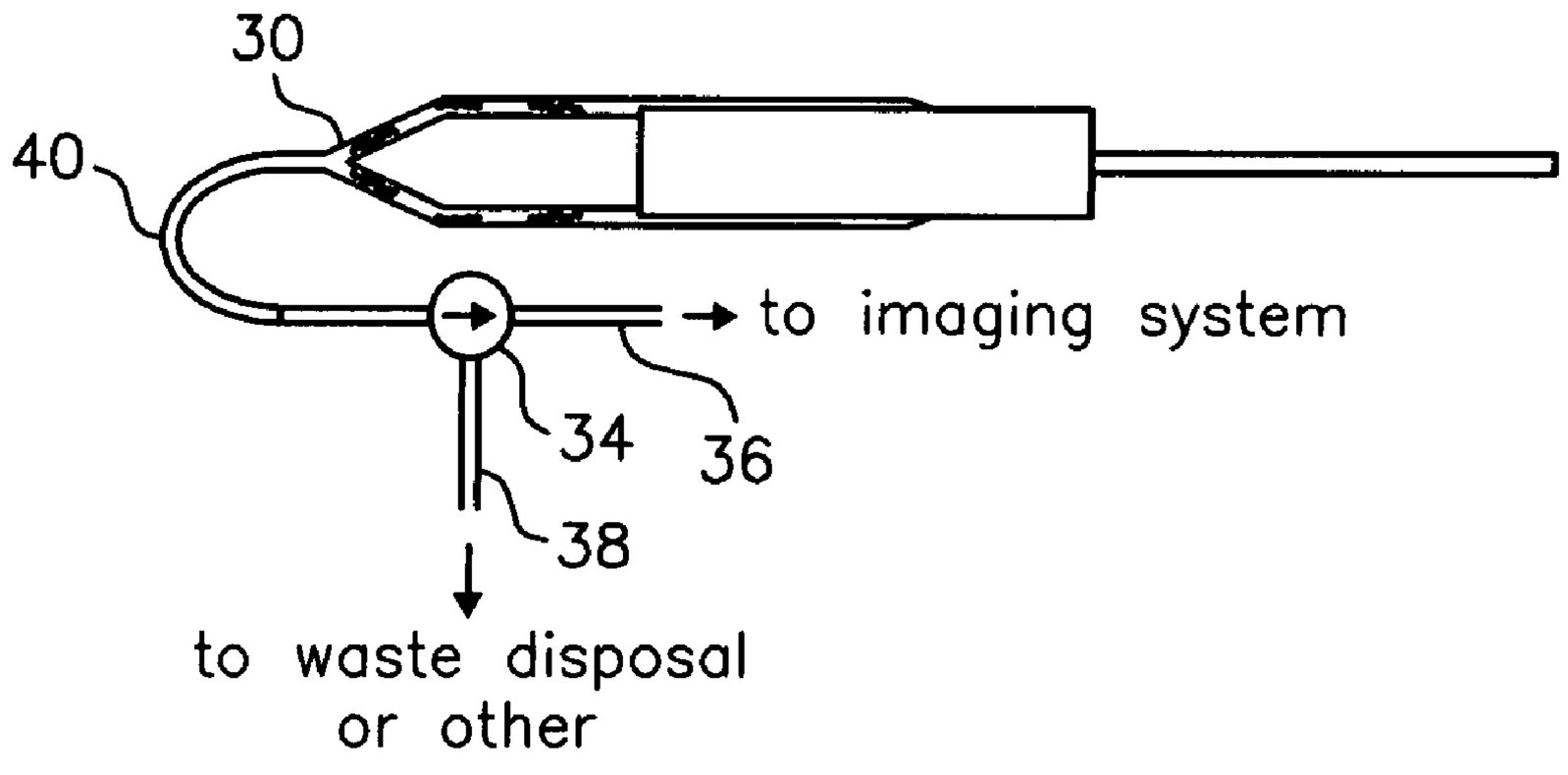
(57) **ABSTRACT**

A process includes coating a cylinder having an inner wall and a cylinder axis with a gel coating on the inner wall. Then a specimen mixture including solvent is made to flow through the cylinder while the cylinder is being continuously rotated. The specimen mixture is initially directed to flow along the cylinder axis and such that specimen particles from the specimen mixture are accelerated off the cylinder axis toward the inner wall, so as to form a film of specimen particles embedded into the gel coating.

17 Claims, 9 Drawing Sheets



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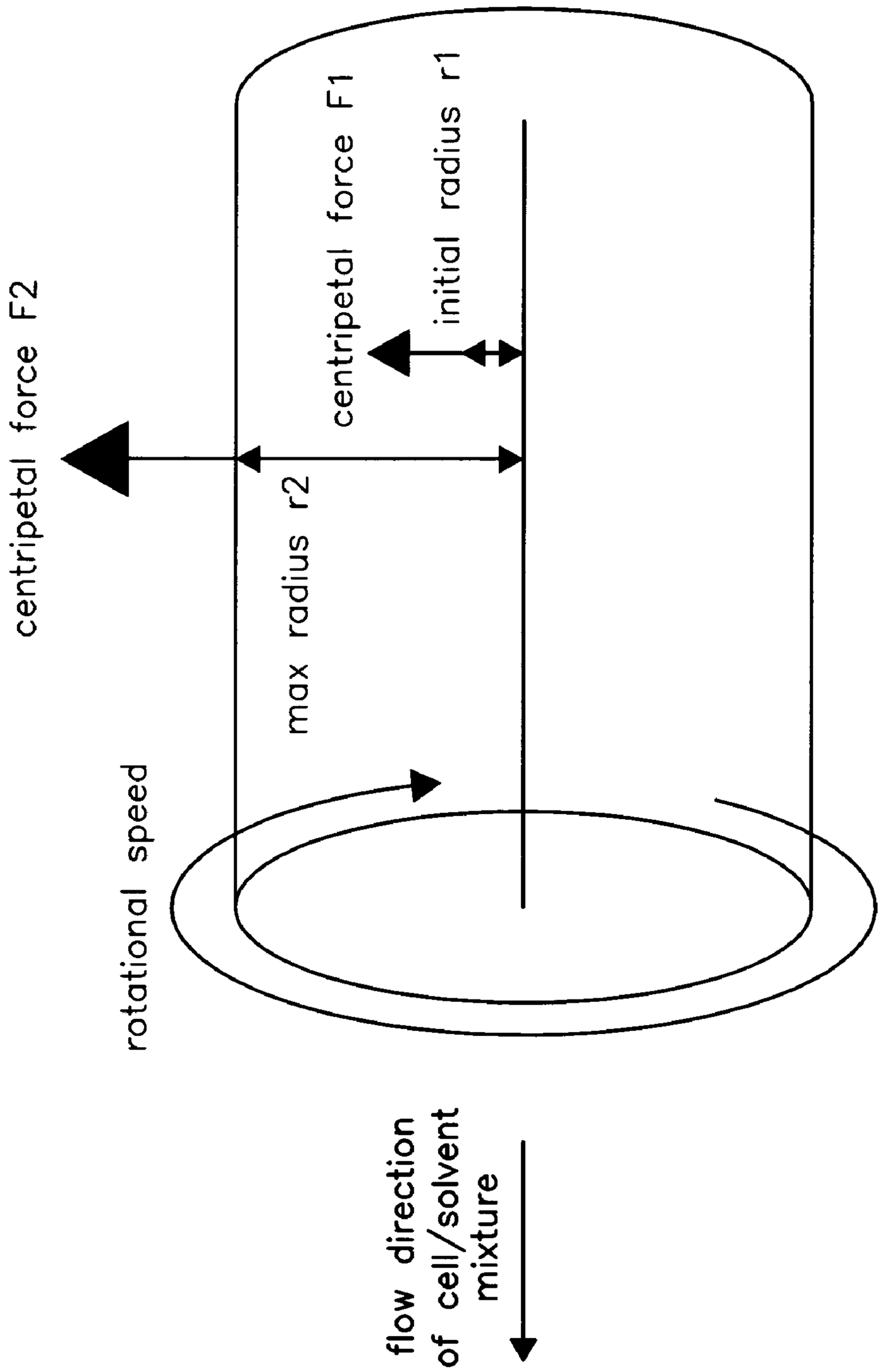


FIG. 1
(Prior Art)

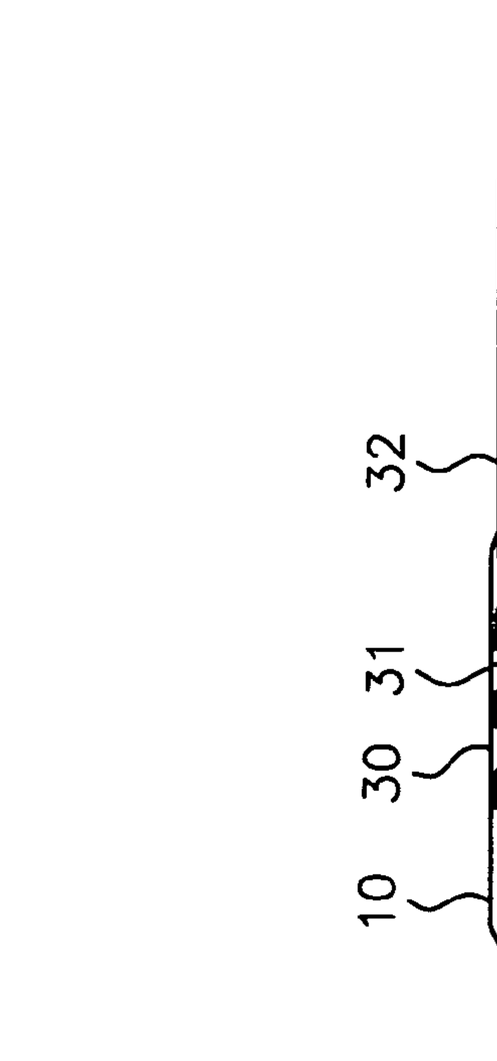
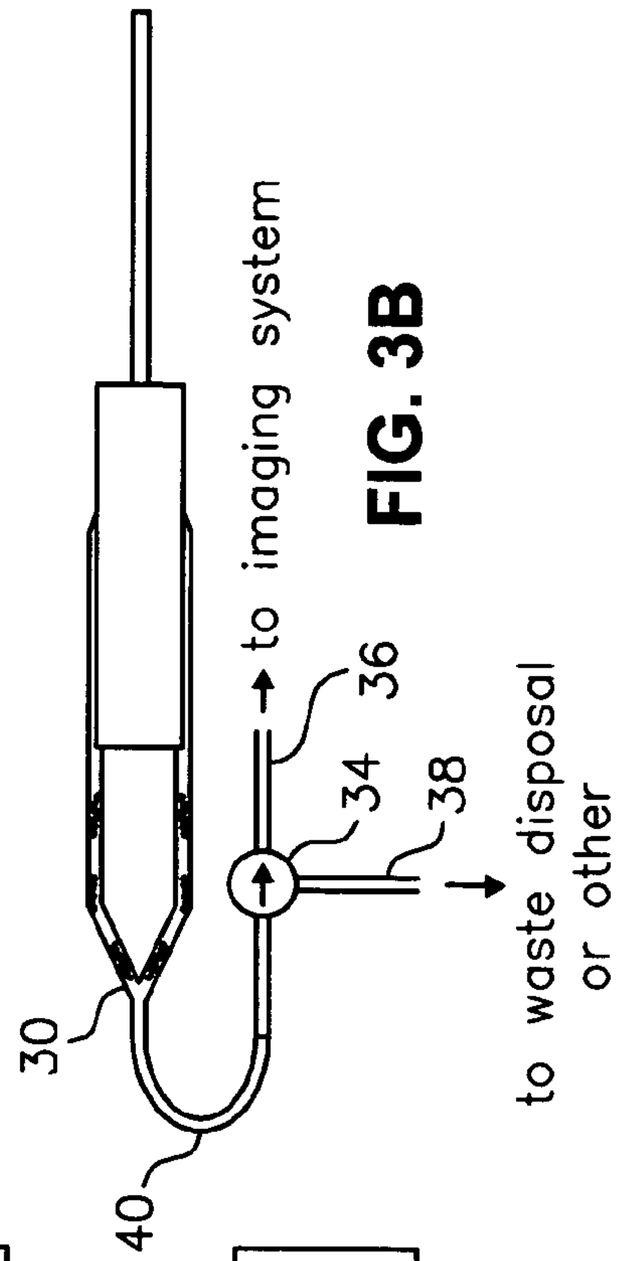


FIG. 3A



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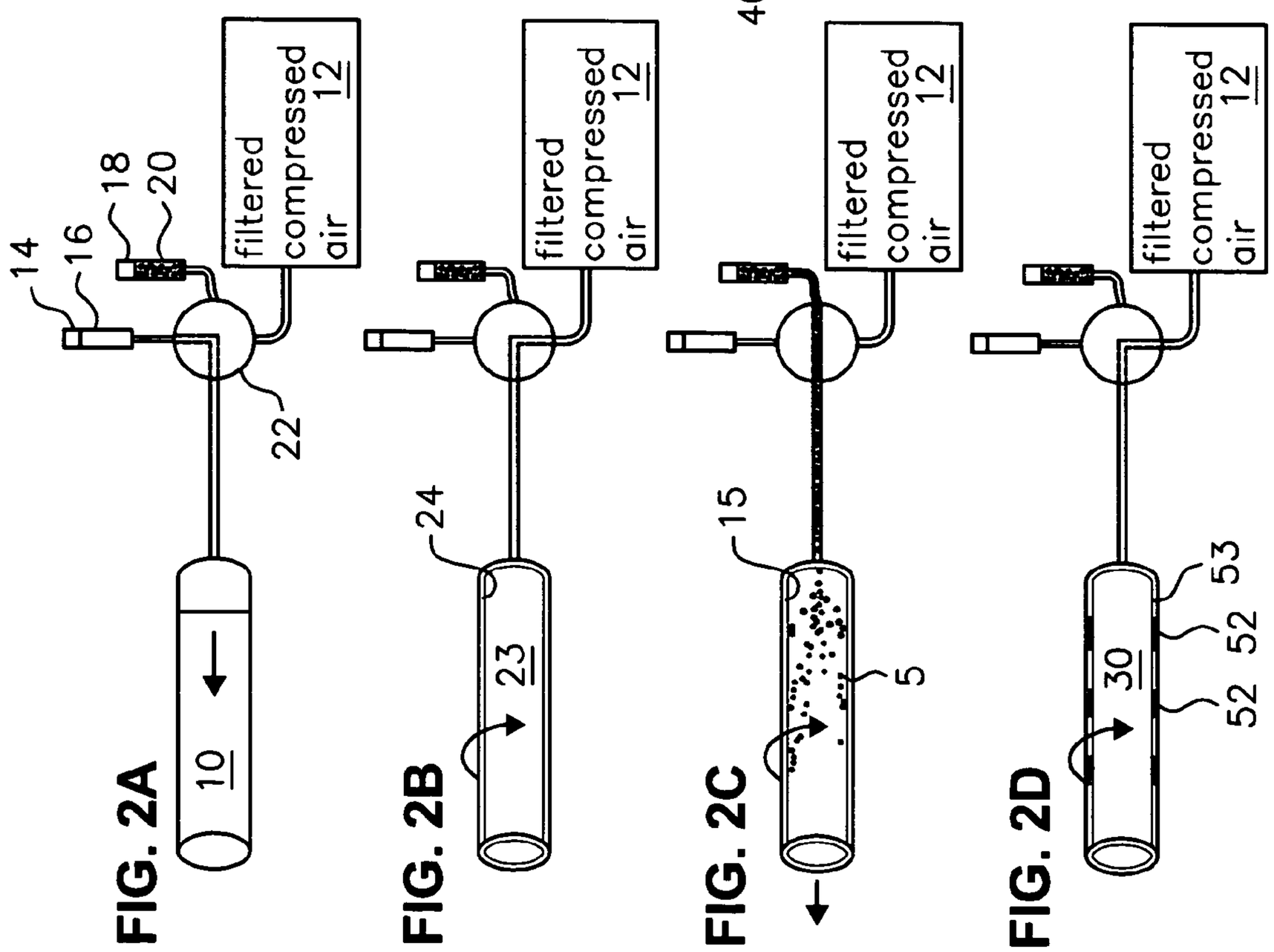


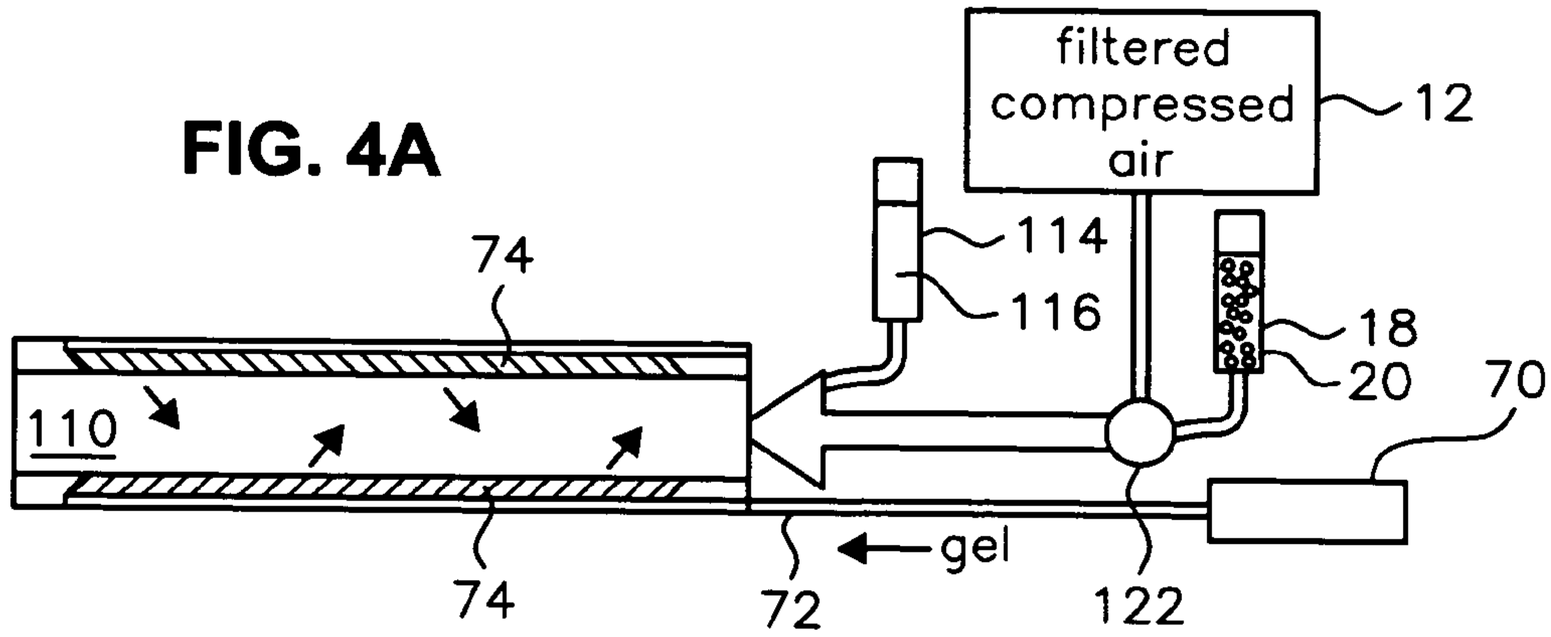
FIG. 2A

FIG. 2B

FIG. 2C

FIG. 2D

FIG. 4A



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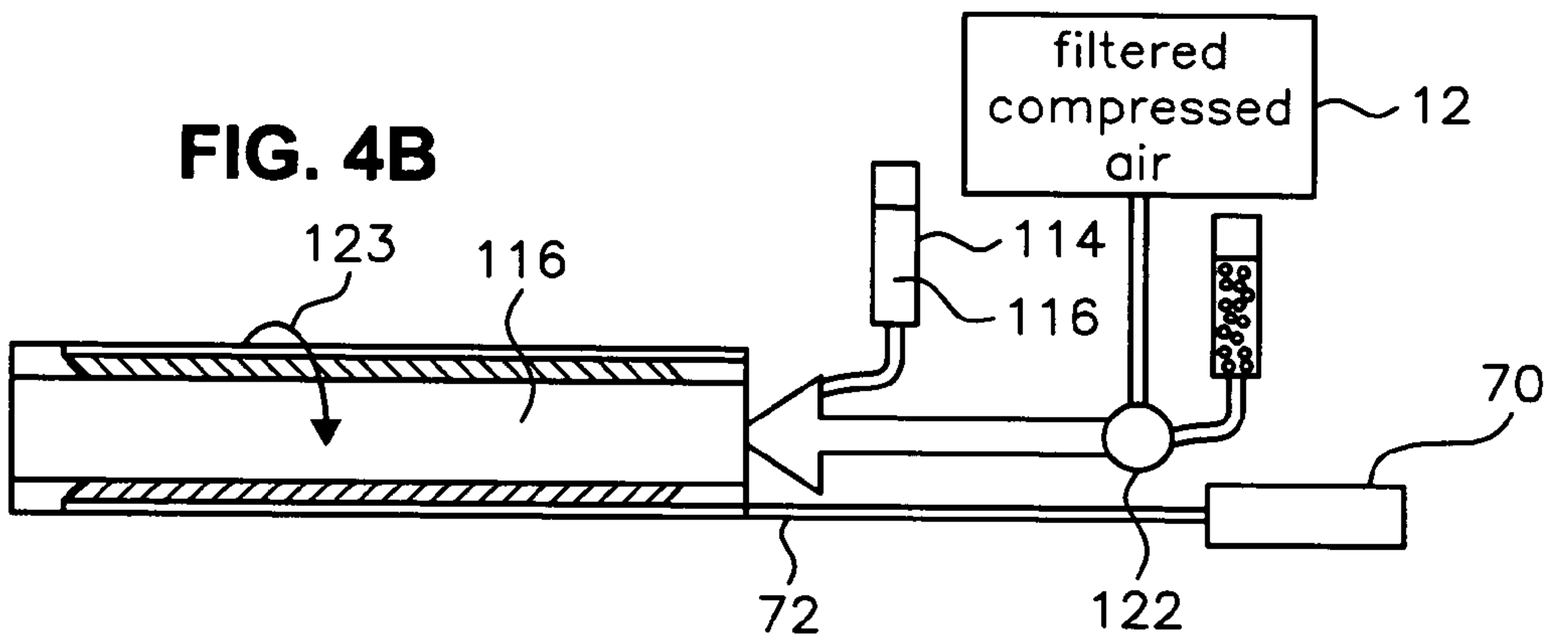

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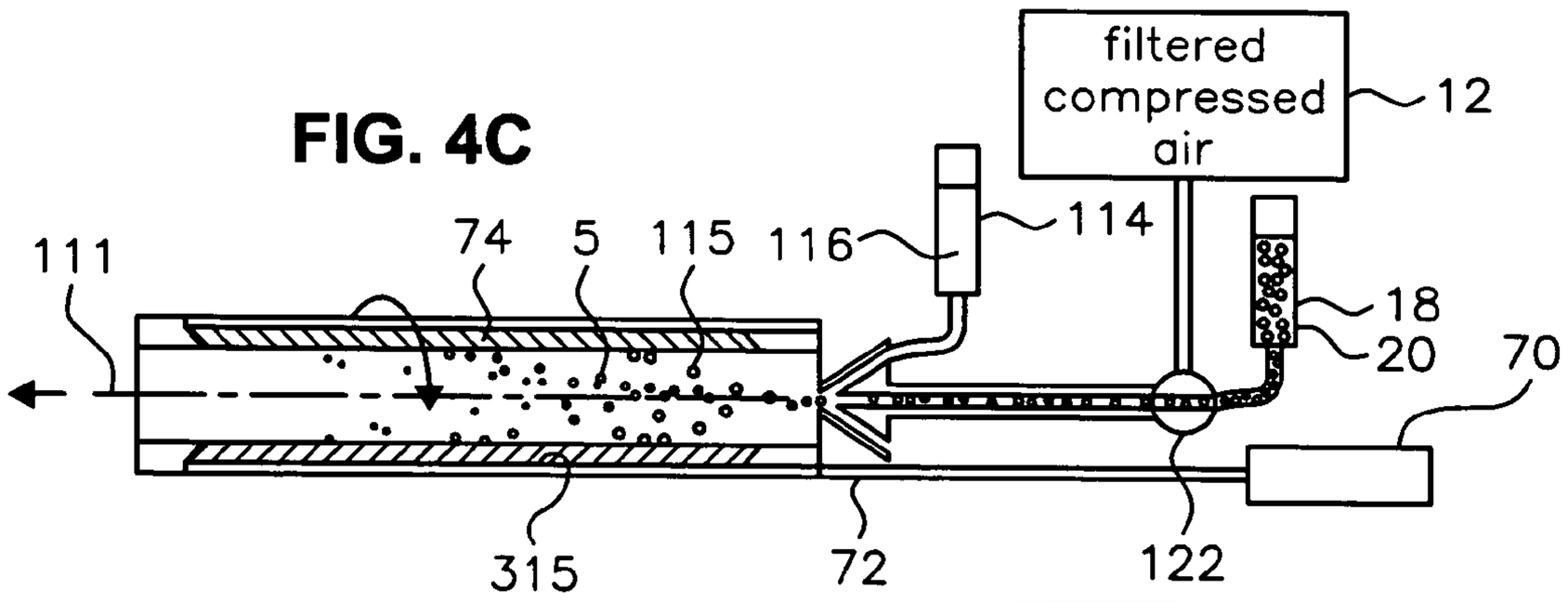

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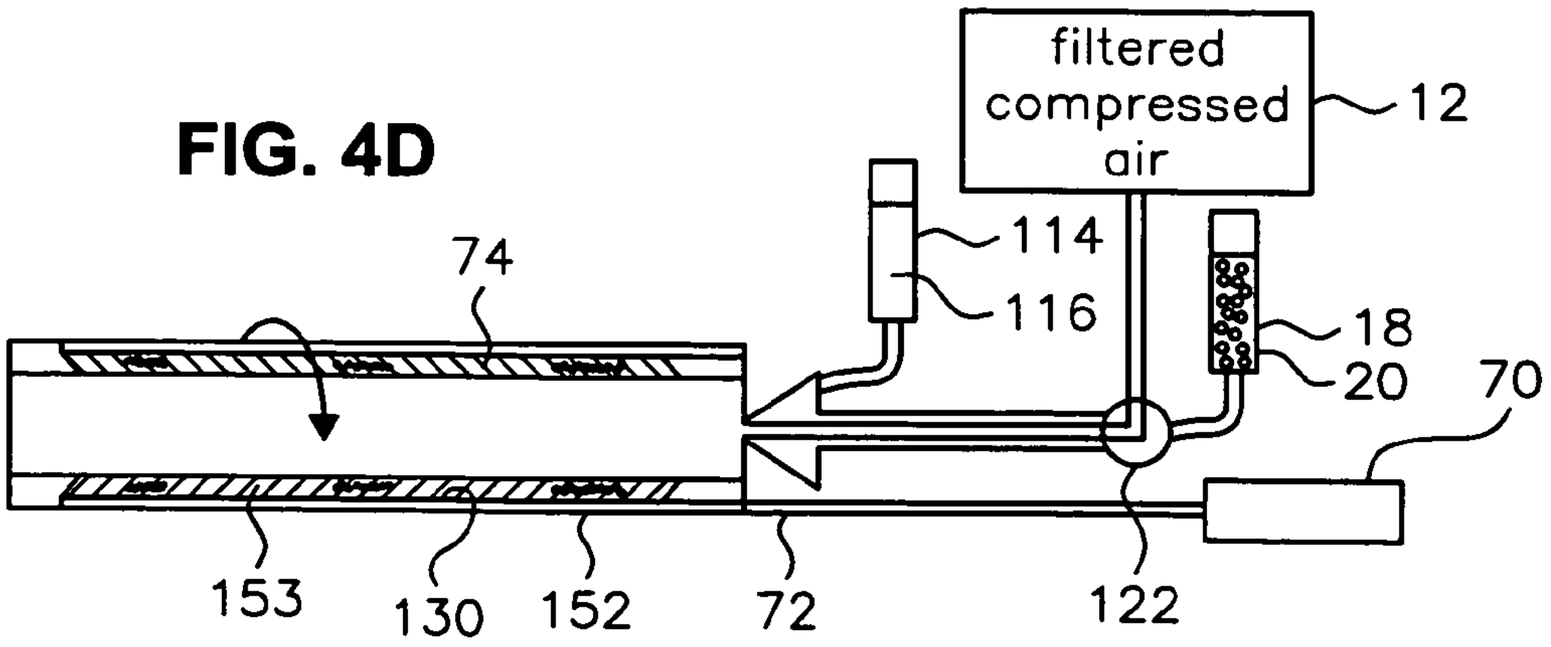


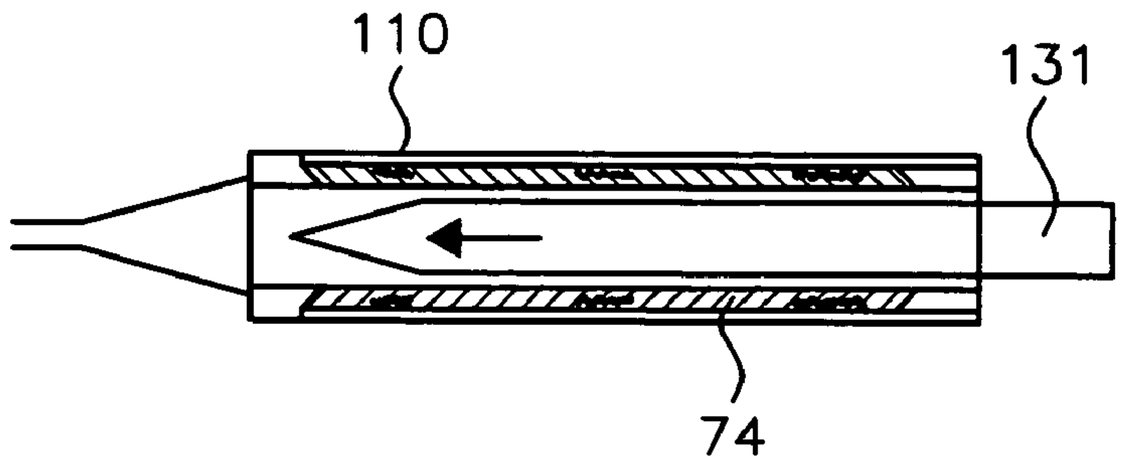
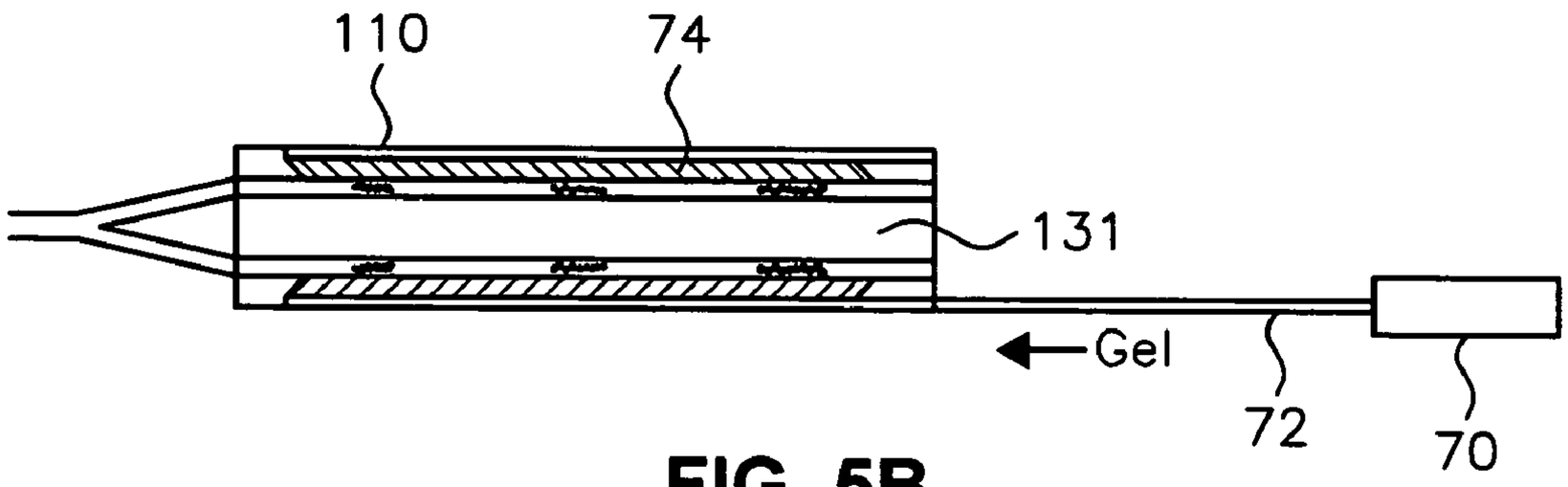


FIG. 5A



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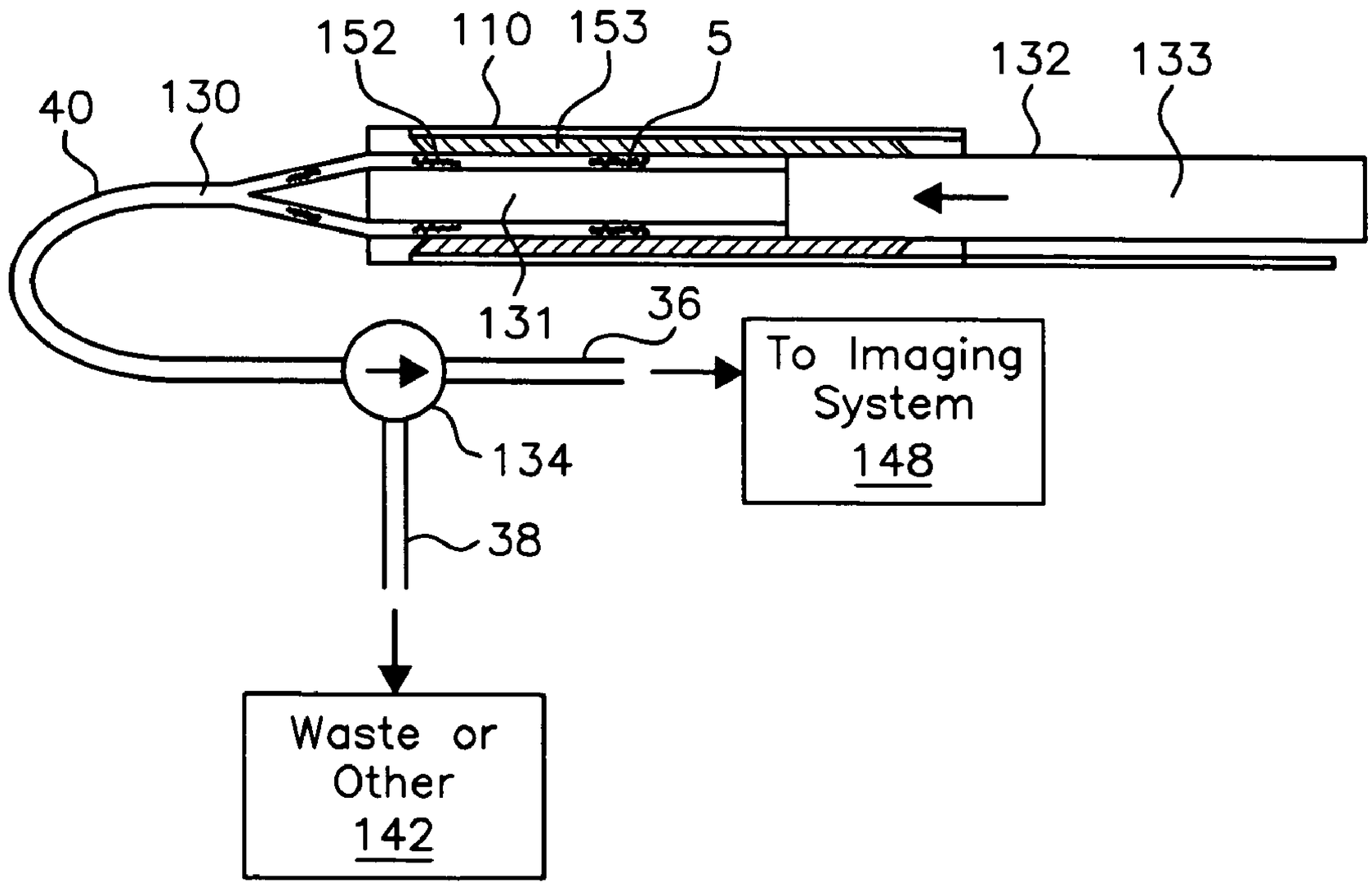

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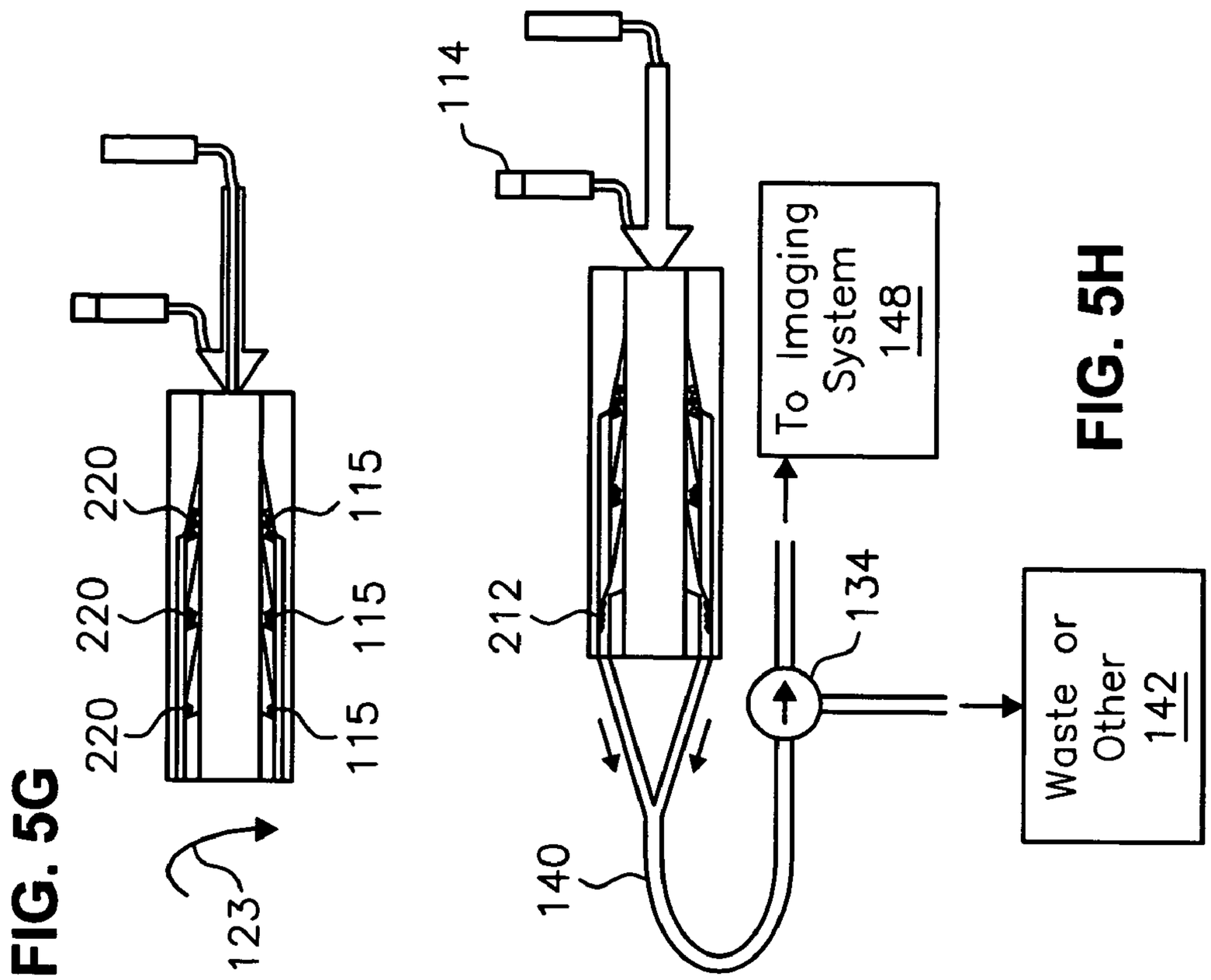

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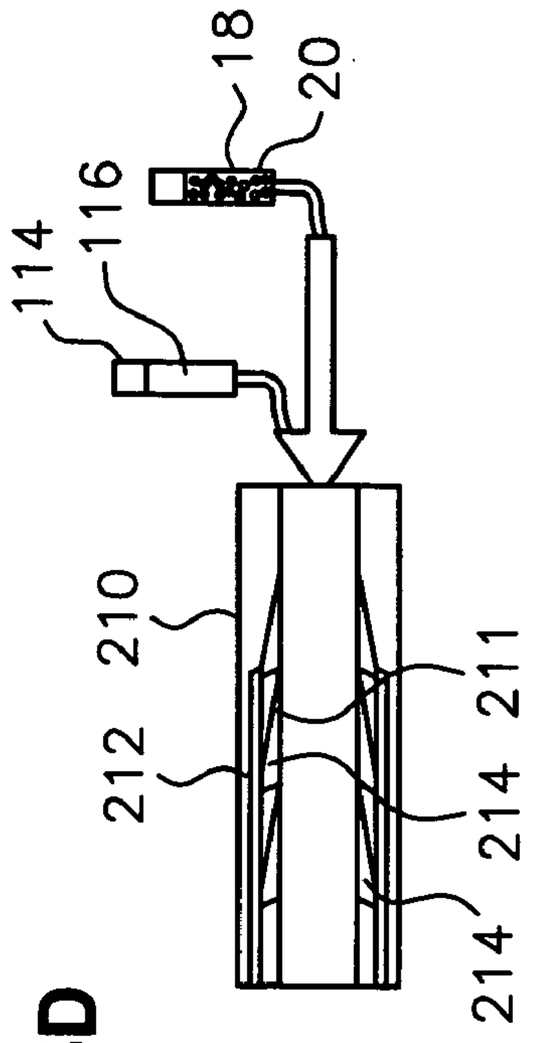


FIG. 5D

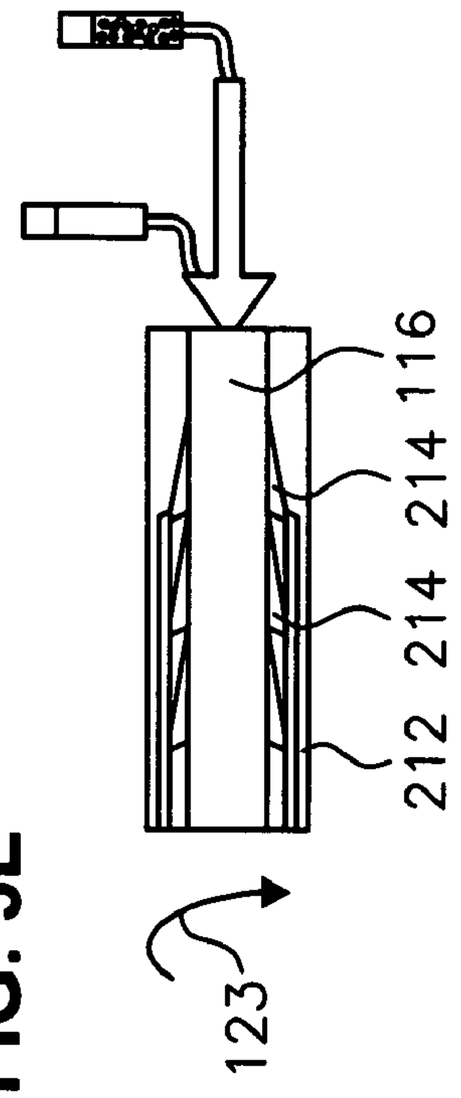


FIG. 5E

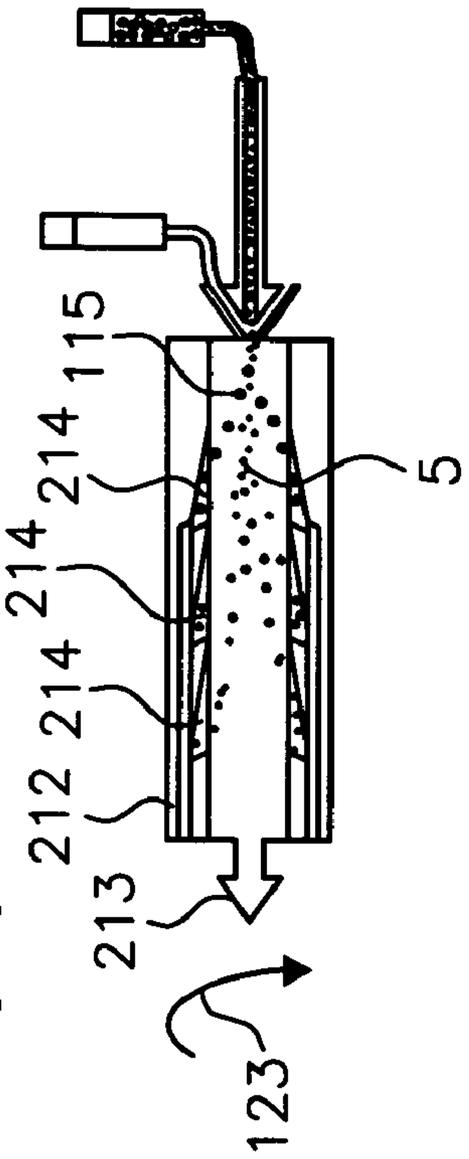


FIG. 5F

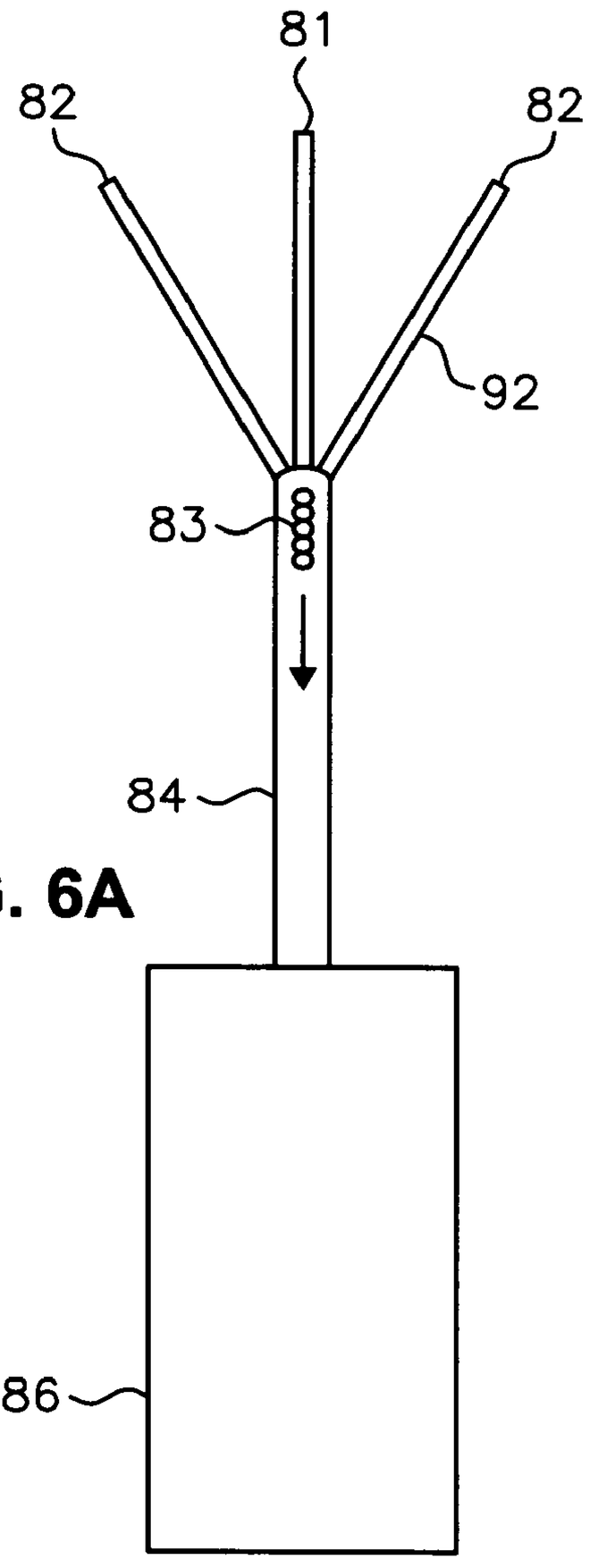
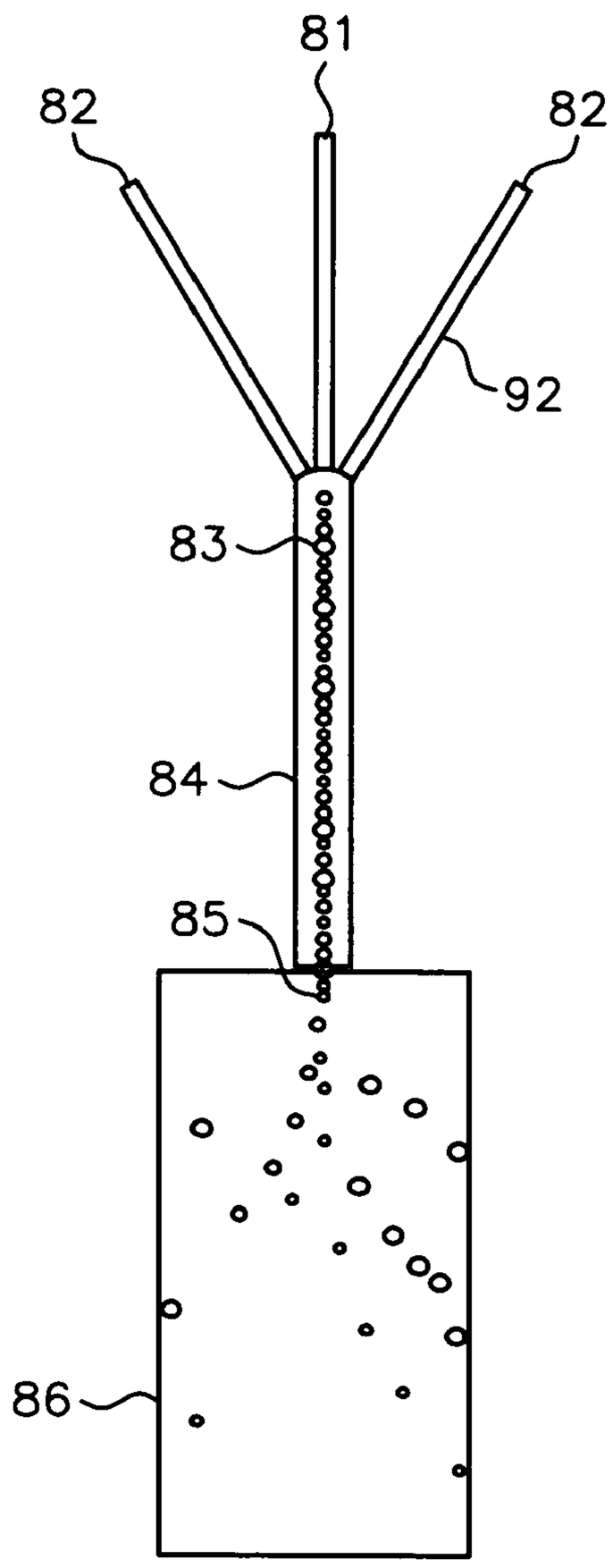


FIG. 6A



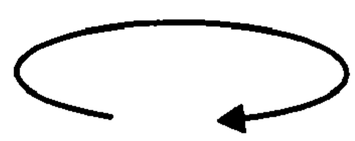


FIG. 6C

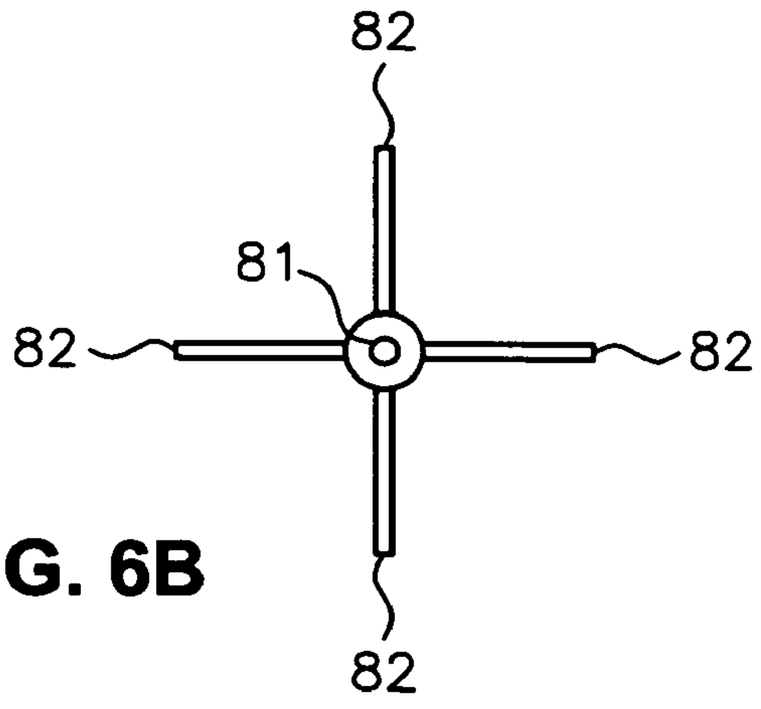


FIG. 6B

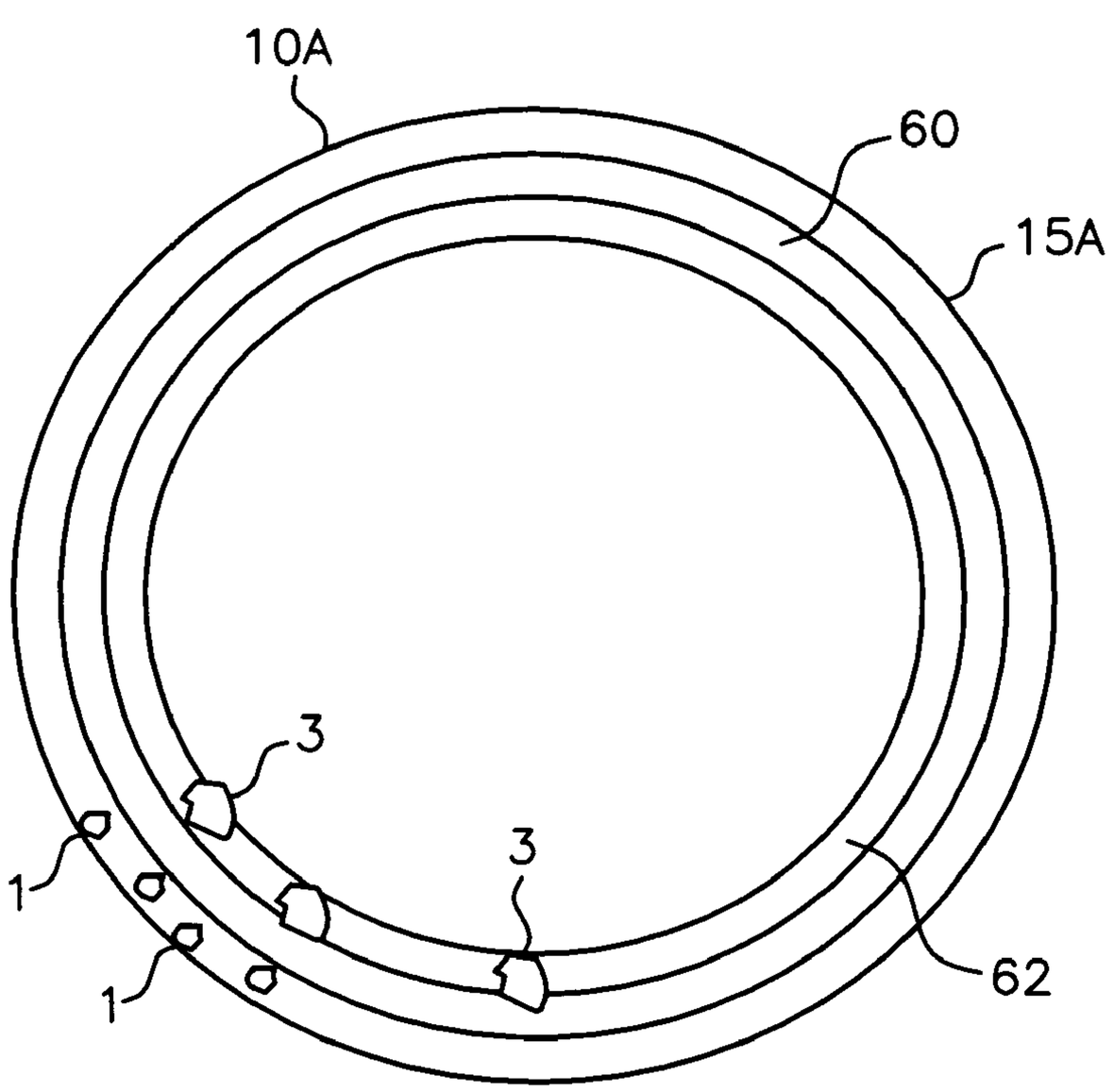


FIG. 7

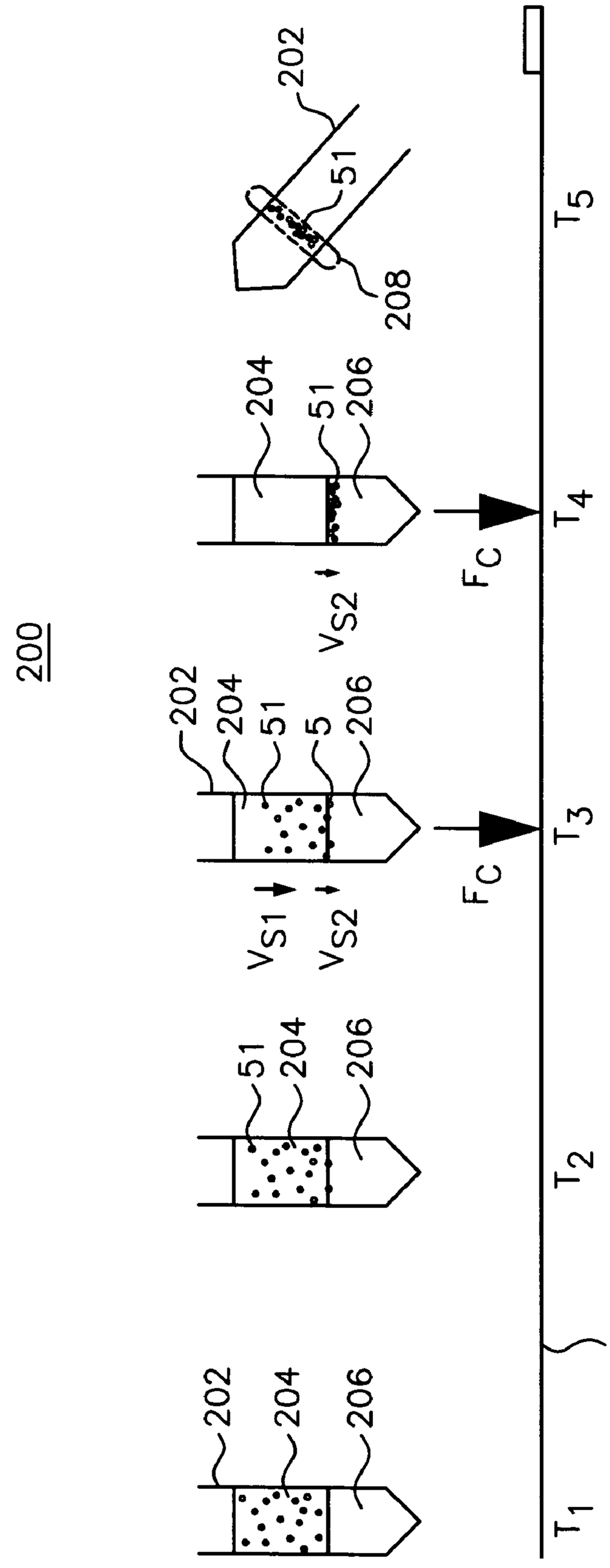


FIG. 8

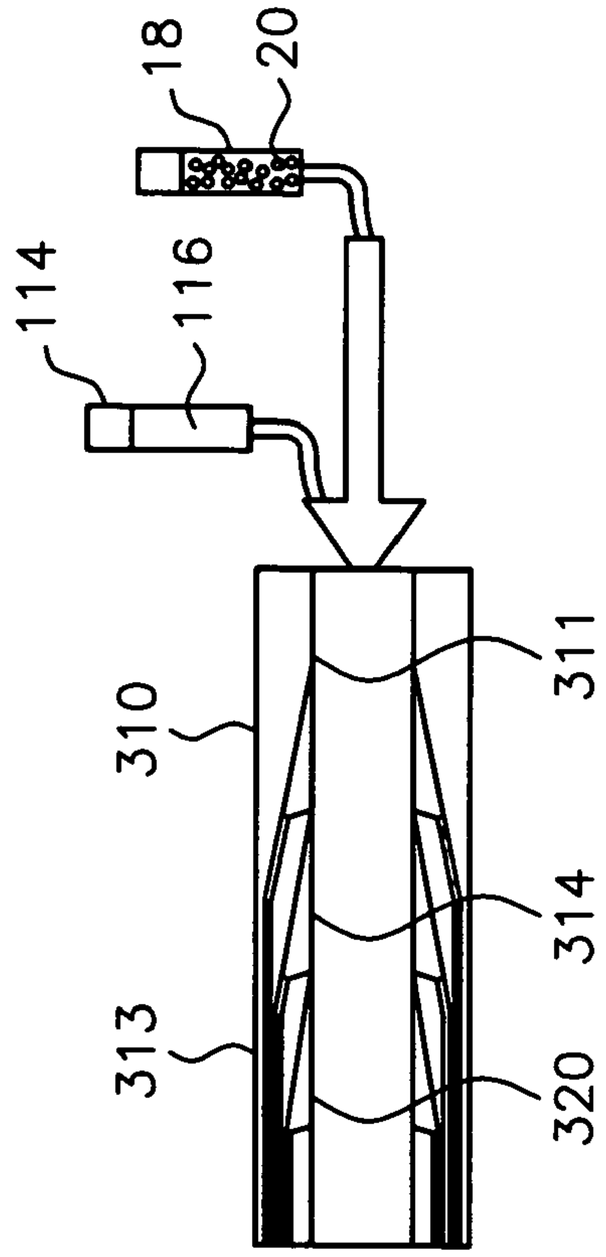


FIG. 9

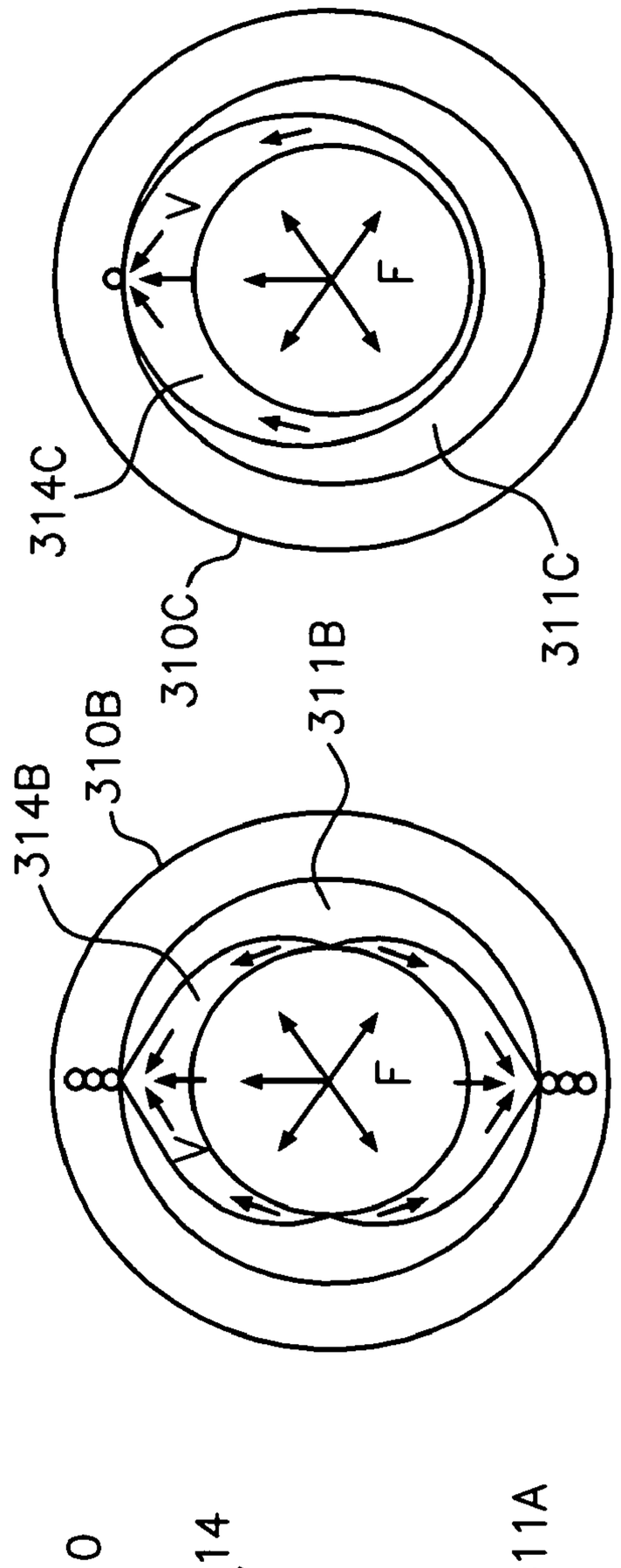


FIG. 9C

FIG. 9B

FIG. 9A

FLOW-THROUGH DRUM CENTRIFUGE

FIELD OF THE INVENTION

The present invention relates generally to specimen preparation for analysis, and more particularly, to biological specimen preparation for optical tomography.

BACKGROUND OF THE INVENTION

Specimen preparation for pathological analysis for detection of diseases such as cancer often affect specimen characteristics. Therefore, reliable procedures are needed in order to produce quality specimens, especially for analysis by automated devices. During preparation, unwanted contaminants must be removed in the specimen, while maintaining specimen characteristics, such as, for example, morphological details of biological cells.

SUMMARY OF THE INVENTION

The present invention provides a process and apparatus for separation of specimen particles. The process includes coating a cylinder having an inner wall and a cylinder axis with a gel coating on the inner wall. Then a specimen mixture including solvent is made to flow through the cylinder while the cylinder is being continuously rotated, wherein the specimen mixture is initially directed to flow along the cylinder axis and such that specimen particles from the specimen mixture are accelerated off the cylinder axis toward the inner wall, so as to form a film of specimen particles embedded into the gel coating.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** illustrates centripetal forces acting on a specimen in a microcentrifuge.

FIG. **2**A–FIG. **2**D schematically illustrate a drum centrifuge process for separation of specimen particles in accordance with the principles of the present invention.

FIG. **3**A and FIG. **3**B schematically illustrate a plunger mechanism used to eject a gel containing specimen particles into a microcapillary tube for later selection and imaging as used in one embodiment of the invention.

FIG. **4**A–FIG. **4**D schematically illustrate a process for separation of specimen particles using recessed wells in accordance with the principles of the present invention.

FIG. **5**A–FIG. **5**C schematically illustrate a plunger mechanism used to eject the gel containing specimen particles into a microcapillary tube for later selection and imaging as used in one embodiment of the invention.

FIG. **5**D–FIG. **5**H, schematically illustrate an alternate embodiment for a drum centrifuge having recessed wells for separation of specimen particles without a trapping material in accordance with the teachings of the present invention.

FIG. **6**A–FIG. **6**C schematically illustrate a system for using hydrodynamic focusing for centering specimen particles in a cylinder.

FIG. **7** schematically illustrates a cross-sectional view of an alternative embodiment of a drum centrifuge constructed in accordance with the present invention including a drum centrifuge with filters.

FIG. **8** schematically illustrates a process flow diagram of an alternative embodiment of a method for concentration of specimen particles in accordance with the present invention.

FIG. **9** and FIG. **9**A–FIG. **9**C schematically illustrate further ultimate embodiments for a drum centrifuge having recessed wells for separation of specimen particles without a trapping material in accordance with the teachings of the present invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The invention is described herein with respect to specific examples relating to biological cells. However, it will be understood that the examples are for the purpose of illustrating the principals of the invention, and that the invention is not so limited.

Referring now to FIG. **1**, centripetal forces acting on a specimen in a microcentrifuge are shown. Centripetal forces **F1**, **F2** are shown for an initial radius **r1** and a maximum radius **r2** respectively. Generally, centripetal acceleration a is defined by the equation:

$$a = \frac{v^2}{r} = \frac{(2\pi r\omega)^2}{r} = 4\pi^2\omega^2 r,$$

where r is the radius, and Ω is the rotational speed. The centripetal force is defined by the well-known relationship F=ma. Thus, if the product $\Omega^2 r$ is maintained, the centripetal forces acting on any specimen are the same for a standard centrifuge and a drum centrifuge.

In general, the centrifuging process of the invention allows specimen particles, such as biological cells, to be separated from debris according to size and density. Following the process of the invention as detailed below, it has been found experimentally that cells may advantageously be adequately centrifuged into a thixotropic optical gel, having a nominal viscosity of 10,600 poises, at 1000 rpm with a spin radius of 200 mm, yielding a product $\Omega^2 r = 200\times10^6$ [rpm^2][mm]. As an illustrative example, an equivalent centripetal acceleration can be achieved for a 2 mm radius if the rotational speed is 10,000 rpm. Rotational speeds up to 14,000 rpm are easily achieved with electric motor-based centrifuges. Higher rotational speeds can be generated with turbine-style motors, in some instances air pressure has been used to actuate these turbine-based rotation mechanisms.

Referring now to FIG. **2**A–FIG. **2**D, a procedure of how to use a drum centrifuge for separation of specimen particles, such as biological cells, in accordance with the teachings of the present invention is schematically illustrated. Referring now specifically to FIG. **2**A, there shown is a drum centrifuge **10**, a source of filtered compressed air **12**, a mixture container **18** holding a specimen mixture **20**, and a first selector valve **22**. A gel container **14** holding optical gel **16** is included for embedding particles in gel. The first selector valve **22** is coupled to the source of filtered compressed air **12**, the mixture container **18**, and the gel container **14**. The first selector valve **22** operates to selectively allow flows of optical gel, specimen mixture and filtered compressed air into the drum centrifuge **10** as the case may be.

The specimen mixture **20** may be any mixture including specimen particles to be prepared for analysis, especially, for example, a biological sample including a solvent and cell mixture where the cells are to be analyzed by automated or manual optical systems. One such system is described in pending U.S. patent application Ser. No. 10/716,744 to Fauver entitled "METHOD AND APPARATUS OF SHADOWGRAM FORMATION FOR OPTICAL TOMOGRAPHY," and published on Apr. 22, 2004 as publication num-

ber US-2004-0076319-A1. The disclosure of the patent application in publication number US-2004-0076319-A1 is incorporated herein by reference.

Referring now to FIG. **2**B, after injecting a small quantity of optical gel **16** into the drum centrifuge **10**, the optical gel **16** is centrifugated using a (not shown) motor as indicated by rotational arrow **23** to produce a uniform thin coating **24** on the inner wall of the drum centrifuge **10**. The coating thickness may range, for example, from 100 microns to 1 mm, depending on inner diameter of drum centrifuge **10**.

A gel, such as Nye OC431A optical gel or equivalent optical gels, may advantageously be used for embedding cells. The gel lines the wall of the drum centrifuge so that the cells adhere to the gel where they strike the gel-coated inner surface. Such optical gels may advantageously comprise commercially available index matching materials. The preferred gel index of refraction is the index of the mating materials during subsequent viewing or imaging. If the gel is used between two dissimilar materials, the preferred gel index is usually equal to the geometric mean of the indices of the two materials. Thixotropic gels are very useful for the purpose of embedding the particles in a film as a result of centrifugation. This allows the separation based on size and density to be maintained during flow of the specimen mixture **20**. Commercial sources of optical gel include Nye Optical Gels, Dymax Corp, and Cargille Labs.

Referring now to FIG. **2**C, the specimen mixture **20** is made to flow through the drum centrifuge **10** along its axis **11** while being rotated. Specimen particles **5**, such as cells, from the specimen mixture **20** are accelerated toward the inner wall **15** of the drum centrifuge **10**, with the smallest, lowest mass particles taking the longest time to reach the inner wall **15**. The time taken to reach the inner wall **15** determines where along the axis **11** of the centrifuge that a particular particle will be embedded into the thin layer of gel lining the wall.

The initial radial spread determines the starting centripetal acceleration, though even cells exactly on axis of the centrifuge are unlikely to stay there since the only restoring forces acting on these cells are hydrodynamic focusing forces. Hydrodynamic focusing forces may advantageously be altered as desired by changing flow rate. Once a cell starts to move off axis, it will be increasingly accelerated toward the wall of the drum centrifuge due to an inherent instability in the system. Flow rate has an upper limit due to the finite length of the tube used for centrifugation. The radial spread of the cells as they flow through the drum centrifuge determines the resolution of separation of specimens along the drum centrifuge wall, with the smaller radial spread leading to better separation resolution. Vortexing of the fluid in addition to the hydrodynamic focusing keeps cells or other particulates in the center of the drum centrifuge. The centripetal force acting on the particles forces them out toward the wall of the drum centrifuge and into the optical gel, where they are trapped. Laminar flow of the specimen mixture **20** must be maintained prior to injection into the drum centrifuge.

Referring now to FIG. **2**D, after flow through of the specimen mixture **20** is complete, the drum centrifuge is flushed with filtered compressed air to accelerate removing solvent by evaporation. After the excess solvent is removed, a concentrated film **30** of particles, cells, for example, adhere to the inner wall **15**. If optical gel has been added the concentrated film **30** will also include optical gel. If necessary, the concentrated film **30** may be centrifugated after the drum centrifuge is flushed to remove excess solvent and trapped bubbles. Specimen particles embedded in the concentrated film **30** register onto the inner wall **15** so as to form sections containing specimen particles **52** and sections with sparse or negligible specimen particles **53**.

In one useful embodiment, the cells may advantageously be injected slightly off the drum centrifuge axis to ensure that a minimum centripetal acceleration is experience by all cells from the start of injection. If necessary, the specimen mixture **20** may advantageously be recycled through the drum centrifuge to increase the chances of particles being centrifuged into the gel. Alternatively, a fresh specimen mixture may be injected before removing the specimen deposit. If the solvent isn't immediately evaporated, it is air blown after the flow-through cycle is completed. The subsequent airflow helps to remove solvent from the gel by evaporation.

Referring now jointly to FIG. **3**A and FIG. **3**B, a schematic of a plunger mechanism **32** used to eject the concentrated film **30** of optical gel and specimen particles into a microcapillary tube **40** for later selection and imaging as used in one embodiment of the invention is shown. A second selector valve **34** may advantageously be used to select a desired section of the film **30** while rejecting others. The plunger mechanism **32** has two sections including a plunger tip **31** and a base **33**. The plunger tip **31** advantageously has a diameter matched to that of the inner diameter of the film **30**, so that the film **30** is not actuated by the plunger tip **31**, but so that there is very little gap left between the film **30** and the plunger tip **31**. The plunger base **33** has its diameter matched to that of the inner diameter of the drum centrifuge **10**, so that the film **30** is forced forward. The plunger tip **31** maintains the registration of the specimen particles **5** during plunging. In this way the separation order is maintained, and specimens of interest may advantageously be conveniently injected into a microcapillary tube for imaging by a second selector valve **34** used to select which components are sent to an imaging system **48** and which are sent to waste disposal or other processing **42**. Preferably, in operation, the sections containing specimen particles **52** will be sent to the imaging system, while the sections with sparse or negligible specimen particles **53** will be routed for disposal or other processing.

Referring now to FIG. **4**A–FIG. **4**D, there illustrated is a schematic for a drum centrifuge having recessed wells for separation of cells in accordance with the teachings of the present invention. Note that materials used are consistent with those described hereinabove with reference to FIG. **2**A–FIG. **2**D. Referring now specifically to FIG. **4**A, there shown is a drum centrifuge **110** with recessed wells **74**, a source of filtered compressed air **12**, a mixture container **18** holding a specimen mixture **20**, a selector valve **122** and a solvent container **114** holding solvent **116**. The selector valve **122** is coupled to the source of filtered compressed air **12** and the mixture container **18**. The selector valve **122** operates to selectively allow flows of specimen mixture and filtered compressed air into the drum centrifuge **110** as the case may be. A syringe pump **70** is connected to the recessed wells **74** by connecter **72**. The syringe pump **70** operates to inject gel through connecter **72** into the recessed wells **74**.

Referring now to FIG. **4**B, after injecting a solvent **116** into the drum centrifuge **110**, the solvent **116** is centrifugated using a (not shown) motor as indicated by rotational arrow **123**. The centrifugation continues through the next step in the process.

Referring now to FIG. **4**C, the specimen mixture **20** is made to flow through the drum centrifuge **110** along its axis **111** while being rotated. Specimen particles **5**, such as cells **115**, from the specimen mixture **20** are accelerated toward

the inner wall **315** of the drum centrifuge **10**, with the smallest, lowest mass particles taking the longest time to reach the inner wall **315**. The time taken to reach the inner wall **315** determines where along the axis **11** of the centrifuge that a particular particle will adhere to the wall or be embedded into the thin layer of gel in the recessed wells **74** lining the wall **315**, as the case may be.

Referring now to FIG. **4**D, after flow through of the specimen mixture **20** is complete, the drum centrifuge is flushed with filtered compressed air to help remove solvent by evaporation. When the excess solvent is removed a film **130** of particles, as for example, cells, and optical gel remains in the recessed wells **74**. If necessary, the film **130** may be centrifugated after the drum centrifuge is flushed to remove excess solvent and trapped bubbles from the film **130**. Note that, due to the operation of the centrifuge drum **110** specimen particles are separated into the film **130**, wherein the specimen particles **5** register onto the inner wall **315** so as to form sections containing specimen particles **152** and sections with sparse or negligible specimen particles **153**.

Referring now jointly to FIG. **5**A, FIG. **5**B and FIG. **5**C a schematic of a plunger mechanism **132** used to eject the film **130** of optical gel and specimen particles into a microcapillary tube **40** for later selection and imaging as used in one embodiment of the invention is shown. A second selector valve **134** may advantageously be used to select a desired section of the film **130** while rejecting others. The plunger mechanism **132** has two sections including a plunger tip **131** and a base **133**.

Actuation of the plunger tip **131** prevents rolling or mixing of the film **130** during ejection. The plunger tip **131** advantageously has a diameter matched to that of the inner diameter of the film **130**, so that, when inserted, the plunger tip **131** does not actuate the film **130**, but so that there is very little gap left between the film **130** and the plunger tip **131**.

Referring particularly to FIG. **5**C, there shown is a step in the process wherein a small volume of the film **130** including the sections containing specimen particles **152**, is ejected from the recessed wells **74** onto the surface of the plunger tip **131**. The plunger base **133** has its diameter matched to that of the inner diameter of the drum centrifuge **110**, but smaller than the inner diameter of the recessed well portion of the drum centrifuge so that the film **130** is forced forward. The plunger tip **131** maintains the registration of the specimen particles **5** during plunging. In this way the separation order is maintained, and specimens of interest may advantageously be conveniently injected into a microcapillary tube for imaging by a second selector valve **134** used to select which components are sent to an imaging system **148** and which are sent to waste disposal or other processing **142**. Preferably, in operation, the sections containing specimen particles **152** will be sent to the imaging system, while the sections with sparse or negligible specimen particles **153** will be routed for disposal or other processing.

Referring now to FIG. **5**D–FIG. **5**H, alternate embodiments for a drum centrifuge having recessed wells for separation of specimen particles without a trapping material in accordance with the teachings of the present invention are schematically illustrated. Note that materials used are consistent with those described hereinabove with reference to FIG. **2**A–FIG. **2**D. Referring now specifically to FIG. **5**D, there shown is a drum centrifuge **210** with recessed particle trapping wells **214** having associated well valves **212**, a mixture container **18** holding a specimen mixture **20**, and a solvent container **114** holding solvent **116**.

Referring now to FIG. **5**E, a solvent **116** is injected into the drum centrifuge **210**. The solvent **116** is centrifugated using a (not shown) motor as indicated by rotational arrow **123**. The centrifugation continues through the next step in the process.

Referring now to FIG. **5**F, the specimen mixture **20** is made to flow through the drum centrifuge **210** along its axis **213** while being rotated. Specimen particles **5**, such as cells **115**, from the specimen mixture **20** are accelerated toward an inner drum **211**, with the smallest, lowest mass particles taking the longest time to reach the inner drum **211**. In operation, the recessed particle trapping wells **214** in the surface of the inner drum **211** are used to catch specimen particles **5** as they sediment out of the flow stream. As in the configuration described with respect to FIG. **5**A, specimen particles **5** are trapped in recessed particle trapping wells **214** at different positions along the drum axis depending on their size and density. The centrifugal force acting on the specimen particles **5** serves to concentrate them in each well, with each well itself acting like a small centrifuge tube.

Referring now to FIG. **5**G, each of the particle trapping wells **214** is angled so that force vectors due to flow and centrifugal force cause the particles to localize near its associated well valve **212**. The associated well valve **212** at the apex **220** of the well that is the point of localization of particles, is closed for all steps until the specimen mixture **20** is depleted, or when the wells are full of particles.

Referring now to FIG. **5**H, at this point the associated well valve **212** is opened and, either additional flow of solvent, or continued centrifugal force, or both operate to redirect the particles out of the well once their associated well valves are opened. Separate pathways for each well may be provided, or all wells may lead to a common tube **140** for the particles to exit the drum centrifuge. Actuation of the associated well valves **212** in sequence allows particle flow with separation based on the grouping of said particles according to size and density. The stream of particles exiting the drum centrifuge may advantageously be subsequently disposed of **142** or directed for analysis **248**.

Referring now to FIG. **9** and FIG. **9**A–FIG. **9**C, there illustrated are further alternate embodiments for a drum centrifuge having recessed wells for separation of specimen particles without a tapping material in accordance with the teachings of the present invention. Note that materials used and the operational processes of the drum centrifuge and recessed wells are consistent with those described hereinabove with reference to FIG. **5**D–FIG. **5**H, except as specifically noted below. Refining now specifically to FIG. **9**, there shown is a drum centrifuge **310** with an inner drum **311** including at least one recessed particle trapping well **314** having at least one associated well valve **313**, a mixture container **18** holding a specimen mixture **20**, and a solvent container **114** holding solvent **116**.

As in the configuration described with respect to FIG. **5**A, specimen particles are trapped in recessed particle trapping wells **314** at different positions along the drum axis depending on their size and density. The centrifugal forces, F (as shown in FIG. **9**A–FIG. **9**C) acting on the specimen particles serve to concentrate them in each well, with each well itself acting like a small centrifuge tube.

Referring now to FIG. **9**A, an enlarged crossectional end view of an embodiment of the drum centrifuge **310** of FIG. **9** is shown. Each of the particle trapping wells **314** is angled so that force vectors, V, due to flow and centrifugal force cause the particles to localize near its associated well valve **313**. The associated well valve **313** at the apex of the well that is the point of localization of particles, is closed for all

steps until the specimen mixture **20** is depleted, or when the wells are full of particles. The associated well valve **313** is opened and, either additional flow of solvent, or continued centrifugal force, or both operate to redirect the particles out of the well once. Separate pathways **312** for each well may be provided, or all wells may lead to a common tube for the particles to exit the drum centrifuge (as best shown in FIG. **5**D). Actuation of the associated well valves **313** in sequence allows particle flow with separation based on the grouping of said particles according to size and density. While the number of particle trapping wells **314** may vary both along the length and around the circumference of the drum centrifuge **310**. The plurality of wells may advantageously comprise four wells evenly spaced around the circumference of the drum centrifuge **310** at a location **320** along the length of the drum centrifuge **310**. In another embodiment as shown in FIG. **9**B, the at least one well may advantageously comprise two wells **314**B included in inner drum **311**B, wherein the wells are evenly spaced around the circumference of the drum centrifuge **310**B. Yet another embodiment comprises a single well **314**C in inner drum **311**C as shown in FIG. **9**C at a location **320** along the length of the drum centrifuge **310**C.

Referring now jointly to FIG. **6**A and FIG. **6**B, there schematically illustrated is a front view and end view respectively of a system for using hydrodynamic focusing for centering specimen particles in a cylinder. After concentration of specimen particles in the desired medium, such as a solvent, using the centrifugation methods described hereinabove, a high concentration (e.g. approximately 50% specimen particles by volume) of a cell-medium mixture **81** is injected into a flow tube **84**. A second medium **82** is injected to form a focusing flow stream as, for example, by injecting into four or more ports **92**. The second medium **82** advantageously comprises a medium, such as a solvent, without specimen particles. At least two pairs of opposing flow streams of the second medium **82** serve to focus and center the cell-medium mixture **81** along two orthogonal axes, resulting in specimen particles **83** centered within the microcapillary flow tube **84**. Alternatively, the focusing flow stream may be a focused annular ring of solvent flow around the cell/solvent mixture.

Ideally, laminar flow without rippling is achieved for hydrodynamic focusing (Reynolds number Re<4 to 25 [See Transport Phenomena by Bird, Stewart, Lightfoot. John Wiley & Sons 1960]) in accordance with the relationship,

$$Re = \frac{\rho\langle v\rangle D}{\mu},$$

where ρ is density, <v> is average (characteristic) flow velocity, D is characteristic length and μ is (absolute) viscosity. In the case of a circular cross-section tube, the characteristic length D is the inner diameter of the microcapillary flow tube **84**.

Referring now to FIG. **6**C, a side view of the system for using hydrodynamic focusing for centering specimen particles in cylindrically-shaped medium as shown in FIG. **6**A is schematically illustrated. Once the cell concentration has been increased, the cell-medium mixture **81** is injected substantially simultaneously with the four or more flow streams of medium **82** at a constant rate. Specimen particles **81** in solvent, typically xylene, are injected simultaneously with pure solvent **82** to produce a focusing of specimen particles prior to injection in the drum centrifuge. The focused cell-solvent stream **83** flows through tube **84** until it enters the drum centrifuge **86** where the cell stream is no longer confined as shown at point **85** by the smaller diameter of tube **84**. Producing a tightly focused cell-solvent stream at point **85** reduces errors in separation of specimen particles based on size and density.

Referring now to FIG. **7**, a cross-sectional view of an alternative embodiment of a drum centrifuge constructed in accordance with the present invention including a drum centrifuge with filters is schematically illustrated. A drum centrifuge **10**A includes an inner wall **15**A, a first filter **60** and a second filter **62**. The first filter **60** may advantageously comprise a membrane of sufficiently sized filter pores to pass both particles of no interest **3** and particles of interest **1**. The second filter **62** may advantageously comprise a membrane of smaller sized filter pores to trap particles of no interest **3** and pass particles of interest **1**. Thus, when the tube is rotated particles of interest are forced to the inner wall **15**A. The particles of interest can then be recovered by removing the filters and subsequently flushing the drum centrifuge **10**A or mechanically recovering the particles of interest **1**.

Now referring to FIG. **8**, a process flow diagram of an alternative embodiment of a method for concentration of cells in accordance with the present invention is schematically illustrated. For illustrative purposes, the process is described as proceeding along a relative time line **210**. At step T_1 a tube **202** has a bottom section filled with a high viscosity substance **206**. The tube **202** also has an upper section with cells **51** in suspension in low viscosity supernatant **204**, which has a density less than the cells. The high viscosity substance **206** may advantageously have a density slightly less than or equal to the density of the cells **51**. For example, the high viscosity substance may comprise a thixotropic gel and further, the gel may have a viscosity greater than about 100000 cps. The low viscosity supernatant **204** may advantageously comprise a solvent, such as, for example, xylene or an equivalent substance.

At step T_2 cells **51** begin to sediment out of the low viscosity supernatant **204** into the high viscosity substance **206** due to gravitational forces. At step T_3, a centrifugal force F_c is applied to produce acceleration which produces a drag-limited velocity of the cells in the direction of the high viscosity substance **206**. The sedimentation rate in the low viscosity solvent is referenced as V_{s1} and the sedimentation rate in the high viscosity substance is referenced as V_{s2}. The difference in sedimentation rates is determined by difference in viscosities of the two substances, and the density of the substances relative to that of the individual cells. The centrifugal force F_c is applied until, at step T**4**, substantially all of the cells **51** are embedded in the high viscosity substance **206**. At step T_5, the low viscosity supernatant **204** is removed, leaving a layer of high cell concentration **208** embedded in the high viscosity substance **206**.

The concentration of the cells, or other specimen particles, where the high viscosity substance **206** comprises a medium that is suitable for optical imaging provides advantages in addition to aiding optical imaging. For example, the high viscosity substance helps to prevent biofouling because the cells are encapsulated and have little exposure to the inner surface of a tube, such as a glass microcapillary tube, to which they could adhere. The use of a high viscosity substance **206** also facilitates extraction of cells from a container with very low loss of cells. This is an advantage over other practices where cells are concentrated in a pellet by centrifugation, causing retention of some solvent with the

pellet, or where the cells stick to the inside of a centrifuge tube and are difficult, if not impossible, to recover.

The invention has been described herein in considerable detail in order to comply with the Patent Statutes and to provide those skilled in the art with the information needed to apply the novel principles of the present invention, and to construct and use such exemplary and specialized components as are required. However, it is to be understood that the invention may be carried out by specifically different equipment, devices and algorithms, and that various modifications, both as to the equipment details and operating procedures, may be accomplished without departing from the true spirit and scope of the present invention.

What is claimed is:

1. A process for separation of specimen particles, the process comprising coating a cylinder having an inner wall and a cylinder axis with an optical gel coating on the inner wall by injecting an optical gel into the cylinder and centrifuqating the optical gel to produce a uniform coating of the optical gel on the inner wall, then flowing a specimen mixture including solvent through the cylinder while the cylinder is being continuously rotated, wherein the specimen mixture is initially directed to flow along the cylinder axis and such that specimen particles from the specimen mixture are accelerated off the cylinder axis toward the inner wall, so as to form a film of specimen particles embedded into the uniform coating of the optical gel, and ejecting the film of specimen particles embedded into the uniform coating of the optical gel using a plunger mechanism having a tip and a base where the tip has a diameter matched to that of the inner diameter of the film of biological cell specimen particles embedded into the gel, so that the film of specimen particles embedded into the uniform coating of the optical gel is not actuated by the plunger tip, but so that there is a gap left between the film of specimen particles embedded into the uniform coating of the optical gel and the plunger tip, and wherein the plunger base has its diameter matched to that of the inner diameter of the cylinder, so that the film of specimen particles embedded into the uniform coating of the optical gel is forced forward when the plunger mechanism base engages the inner wall of the cylinder.

2. The process of claim **1** further comprising the step of removing the solvent from the film of specimen particles embedded into the uniform coating of the optical gel.

3. The process of claim **1** wherein the film of specimen particles embedded into the uniform coating of the optical gel include specimens of interest in a separation order and wherein the plunger tip maintains the separation order during plunging.

4. The process of claim **1** further comprising the steps of injecting the film of specimen particles into a microcapillary tube and operating a selector valve to discard portions of the film of specimen particles having negligible specimen particles.

5. The process of claim **1** wherein the uniform coating of the optical gel has a thickness that ranges from 100 microns to 1 mm.

6. The process of claim **1** including the step of blowing compressed air through the cylinder to remove solvent before ejecting the film of specimen particles embedded into the uniform coating of the optical gel.

7. The process of claim **1** wherein the specimen mixture comprises a biological sample including a solvent and cell mixture.

8. A process for separation of specimen particles, the process comprising the steps of:

injecting optical gel into a centrifuge having an inner wall;

centrifuging the optical gel to produce a coating of optical gel on the inner wall;

flowing a specimen mixture including solvent through the centrifuge while the centrifuge is rotated such that specimen particles from the specimen mixture are accelerated toward the inner wall and embedded into the coating of optical gel;

removing solvent from the coating of optical gel and specimen particles to form a film of specimen particles in optical gel; and

ejecting the film of specimen particles in optical gel into a microcapillary tube by using a plunger mechanism having a tip and a base where the tip has a diameter matched to that of the inner diameter of the film of specimen particles in optical gel, so that the film of specimen particles in optical gel is not actuated by the plunger tip, but so that there is a gap left between the coating and the plunger tip, and wherein the plunger base is matched to engage the inner wall, so that the film of specimen particles in optical gel is forced forward when the base engages the inner wall of the centrifuge.

9. The process of claim **8** wherein the optical gel is selected from the group consisting of optical gels, oils, fluids, polymer and epoxy.

10. The process of claim **8** further comprising the step of operating a selector valve to discard portions of the film of specimen particles in optical gel having negligible specimen particles.

11. The process of claim **8** wherein the film of specimen particles in optical gel includes specimens of interest in a separation order and wherein the plunger tip maintains the separation order during plunging.

12. The process of claim **8** wherein the coating has a thickness that ranges from 100 microns to 1 mm.

13. The process of claim **8** wherein the specimen mixture comprises a biological sample including a solvent and cell mixture.

14. A process for separation of specimen particles, the process comprising the steps of:

coating a cylinder having an inner wall and a cylinder axis by injecting an optical gel into the cylinder and centrifugating the optical gel to produce a uniform coating of the optical gel on the inner wall, wherein the optical gel has a selected index of refraction matched for subsequent imaging of the specimen particles;

flowing a specimen mixture including solvent through the cylinder while the cylinder is being continuously rotated, wherein the specimen mixture is initially directed to flow along the cylinder axis and such that specimen particles from the specimen mixture are accelerated off the cylinder axis toward the inner wall, so as to form a film of specimen particles embedded into the uniform coating of the optical gel; and

ejecting the film of specimen particles embedded into the uniform coating of the optical gel from the cylinder into a microcapillary tube for imaging by an imaging system.

15. A flow-through centrifuge system comprising:

a centrifuge having a gel-coated inner surface;

a mixture container for holding a specimen mixture coupled to the centrifuge, wherein the specimen mixture is injected into the centrifuge and rotated to embed specimen particles into the gel-coated inner surface of the centrifuge to form a film of gel and specimen particles; and

a plunger mechanism having a tip and a base where the tip is sized so that there is a gap left between the film of gel and specimen particles and the tip when the tip is inserted into the centrifuge, and wherein the base is sized so that the film of gel and specimen particles is forced forward when the base engages the inner surface of the centrifuge.

16. The flow through centrifuge system of claim 15 further comprising:

a microcapillary tube coupled to receive the film of gel and specimen particles; and

a selector valve for selecting a section of the film of gel and specimen particles.

17. The system of claim 15 wherein the specimen mixture comprises a biological sample including a solvent and cell mixture.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

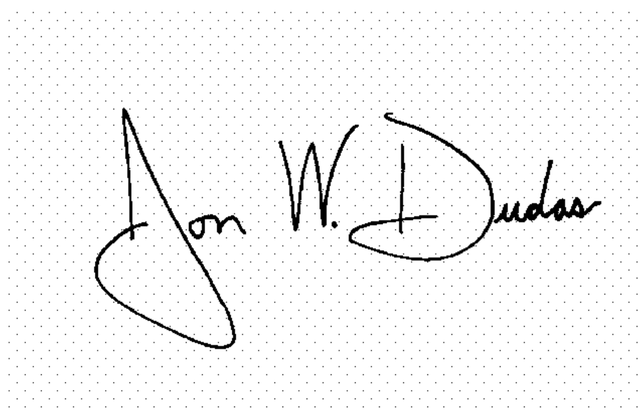
PATENT NO. : 6,991,738 B1
APPLICATION NO. : 10/964513
DATED : January 31, 2006
INVENTOR(S) : Fauver et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 9, lines 20 and 21 Replace “centrifuqating” with --centrifugating--.

Signed and Sealed this

Ninth Day of January, 2007



JON W. DUDAS
Director of the United States Patent and Trademark Office