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(54) CELL SORTING DEVICE AND METHOD OF MANUFACTURING THE SAME

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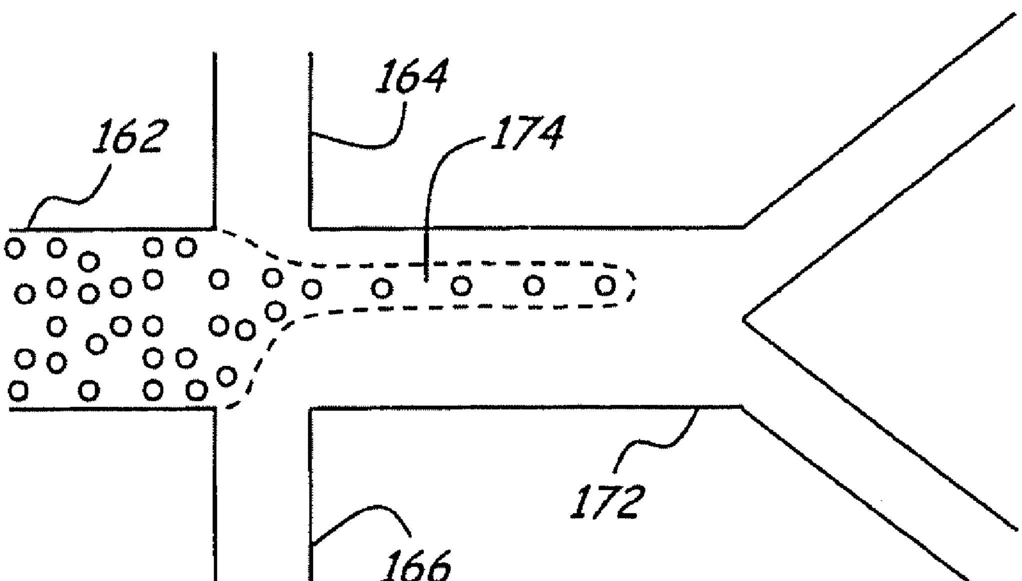
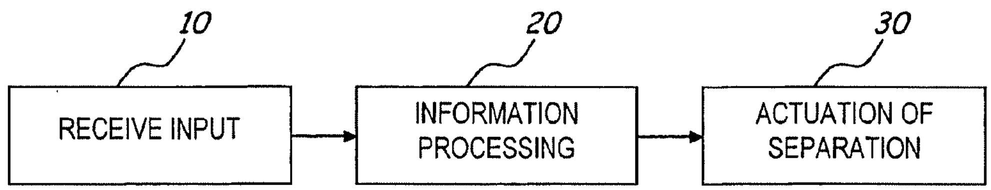
(57) **ABSTRACT**

A system, method and apparatus employing the laminar nature of fluid flows in microfluidic flow devices in separating, sorting or filtering colloidal and/or cellular particles from a suspension in a microfluidic flow device is disclosed. The microfluidic flow device provides for separating a particle within a suspension flow in a microfluidic flow chamber. The chamber includes a microfluidic channel comprising at least one inlet port for receiving a suspension flow under laminar conditions, a first outlet port and a second outlet port. The chamber further includes an interface for translating a particle within the channel. The first outlet port receives a first portion of the suspension exiting the said channel and the second outlet port receives the particle in a second portion of the suspension exiting the channel.

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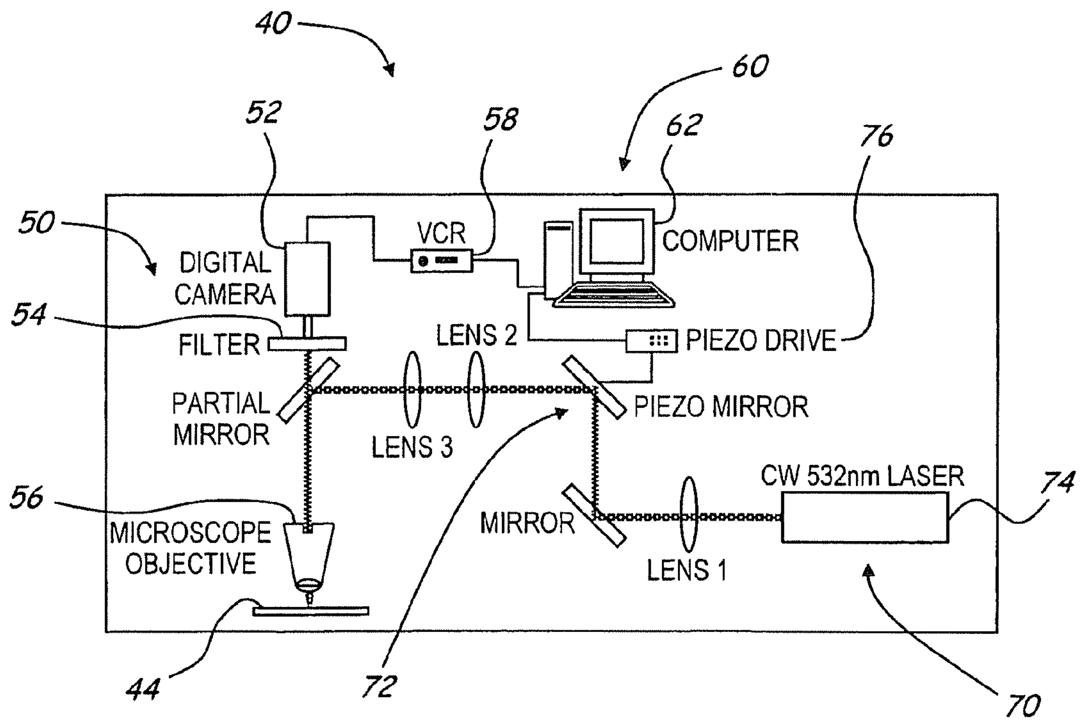
RECEIVE INPUT
INFORMATION PROCESSING
ACTUATION OF SEPARATION

FIG.1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DIGITAL CAMERA
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FILTER
LENS 2
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PARTIAL MIRROR
LENS 3
PIEZO MIRROR
CW 532nm LASER
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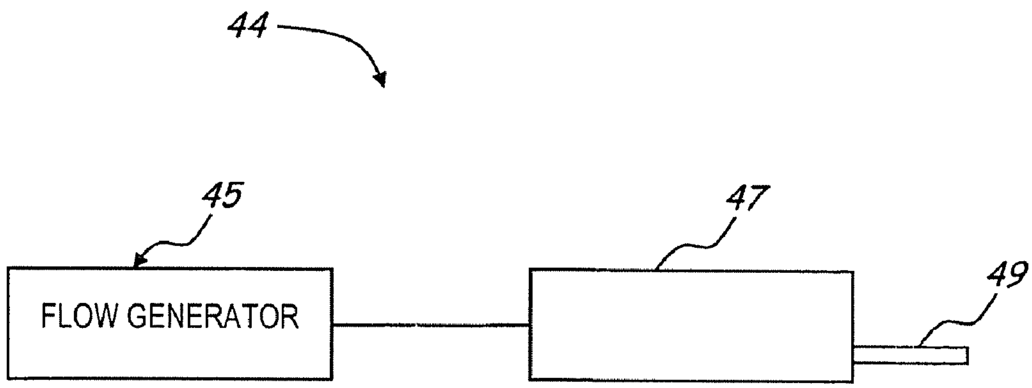
MICROSCOPE OBJECTIVE
MIRROR
LENS 1
44
72
70

FIG.2


44
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FLOW GENERATOR
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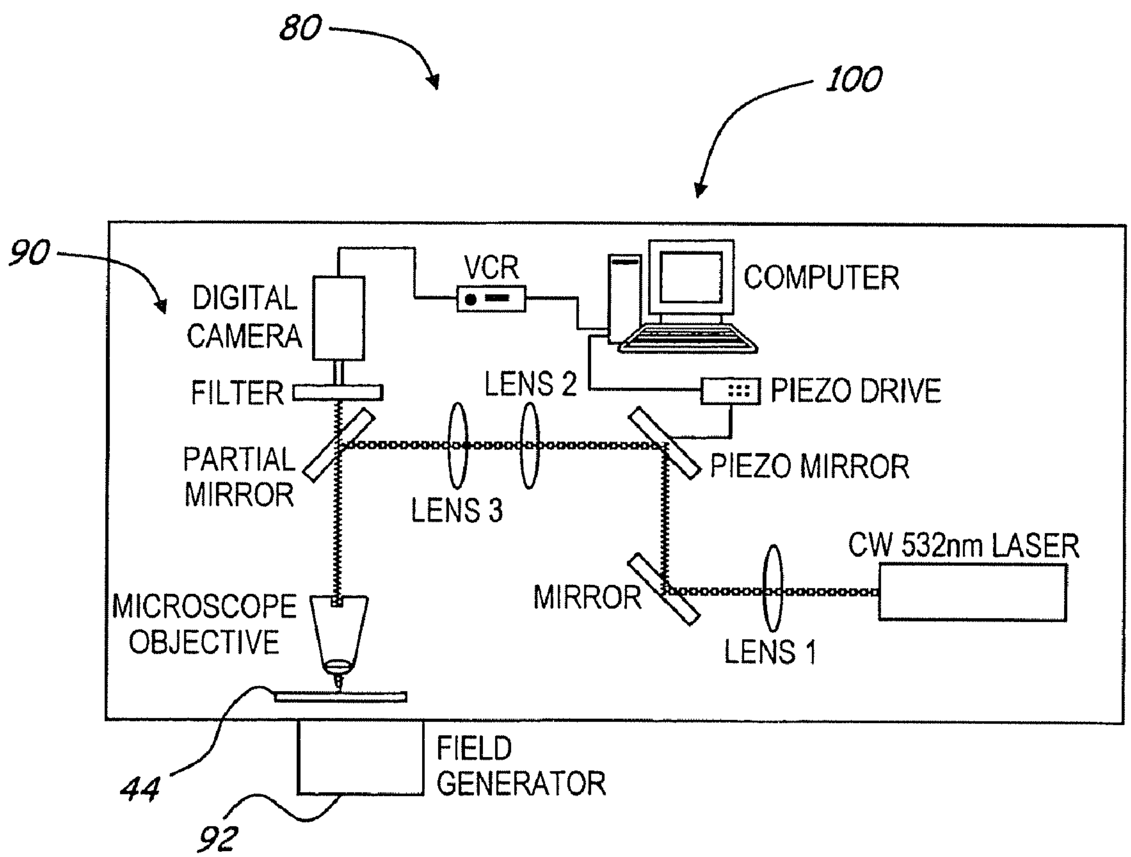
49

FIG.2A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CW 532nm LASER
MICROSCOPE
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MIRROR
LENS 1
FIELD
GENERATOR
44
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FIG.3

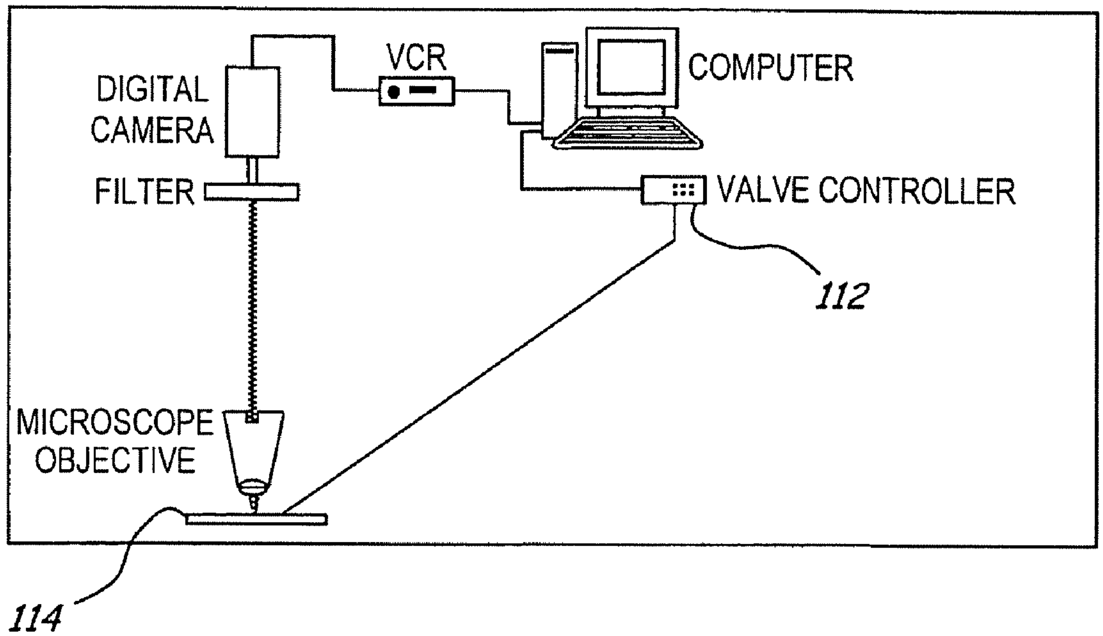
DIGITAL CAMERA
VCR
COMPUTER
FILTER
VALVE CONTROLLER
112
MICROSCOPE OBJECTIVE
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FIG.4

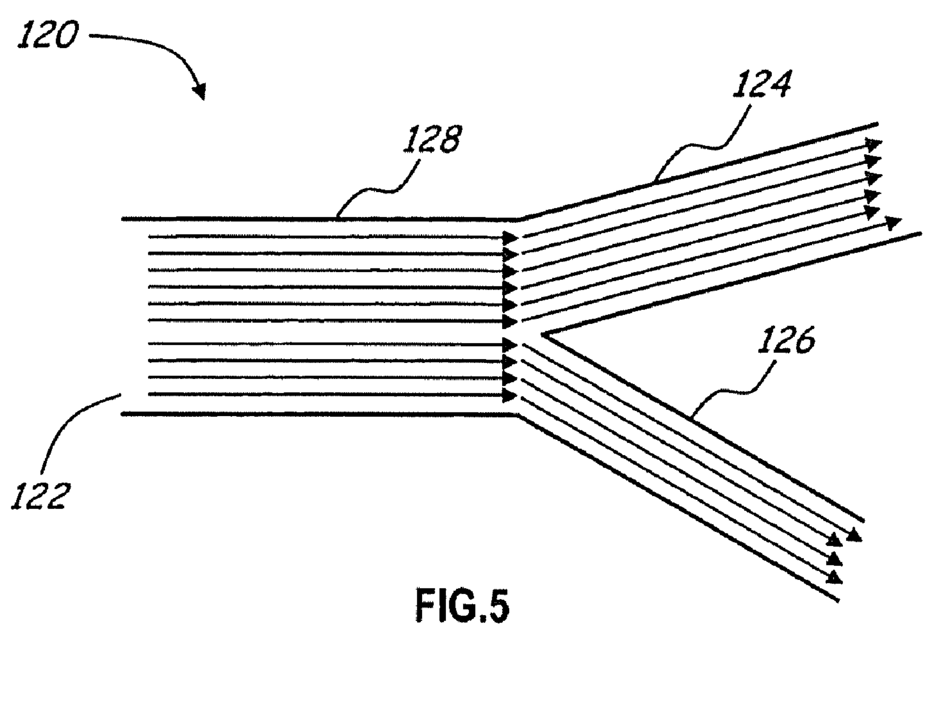
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FIG.5

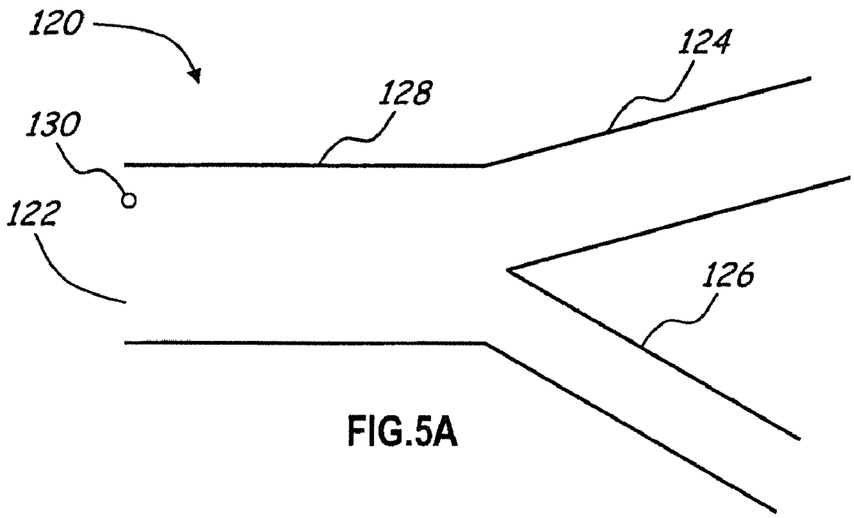


FIG.5A

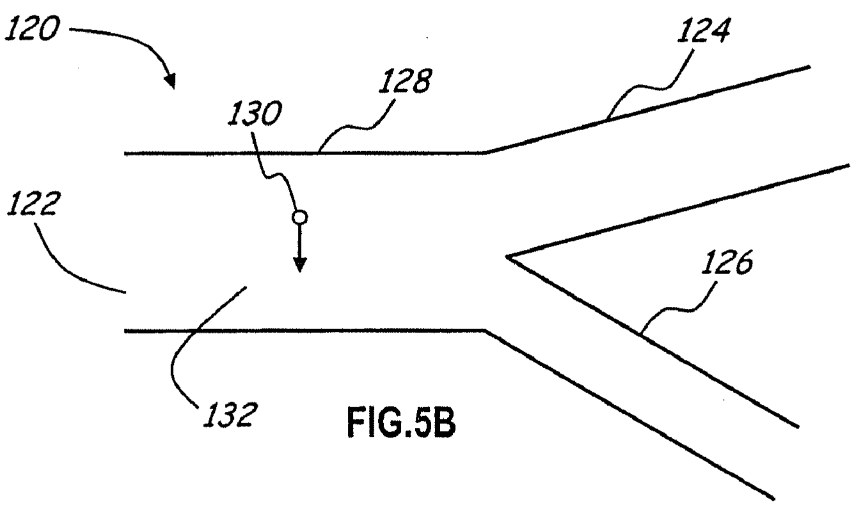


FIG.5B

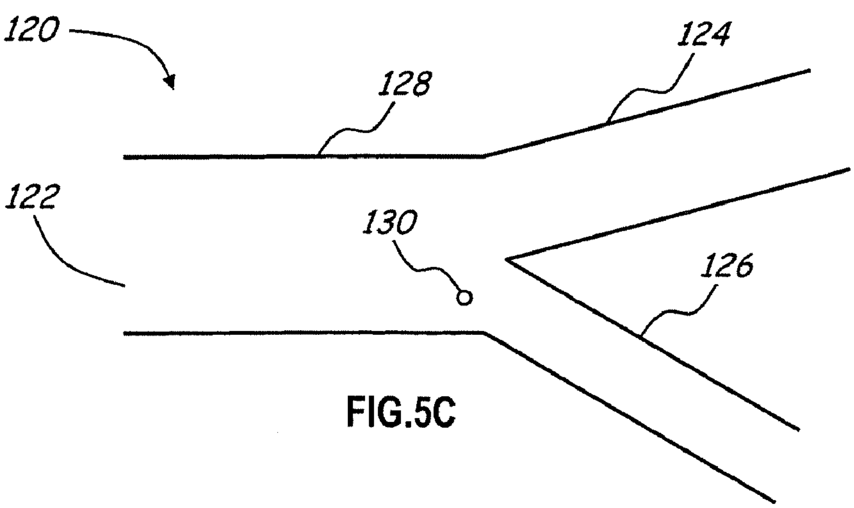


FIG.5C

FIG.6

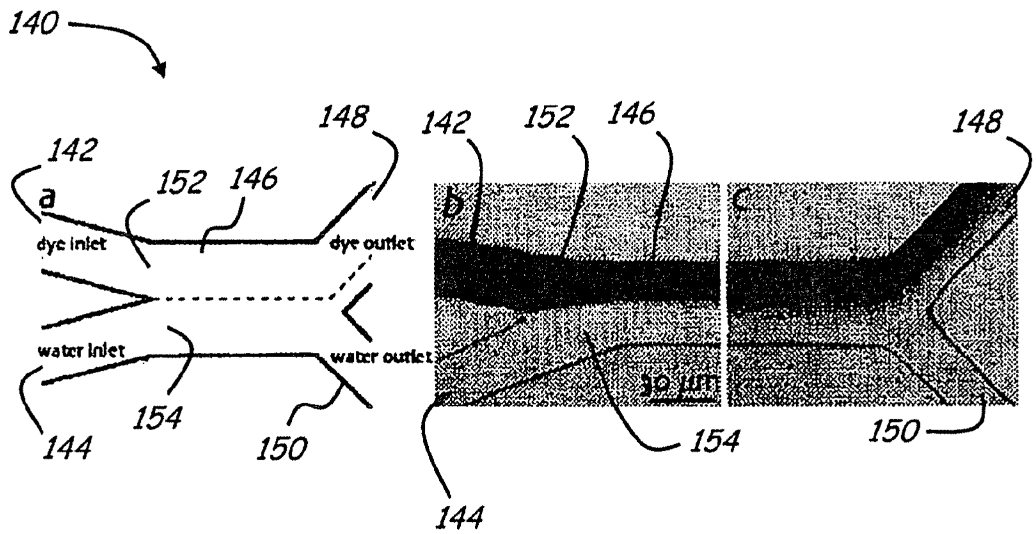


FIG.6A

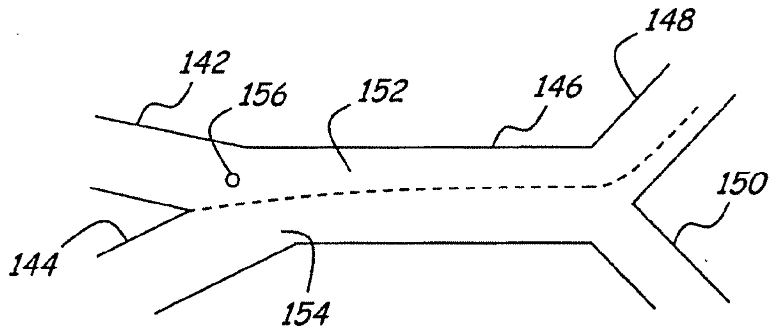


FIG.6B

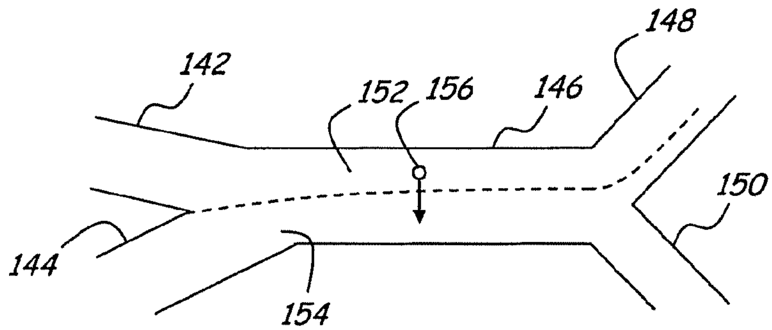
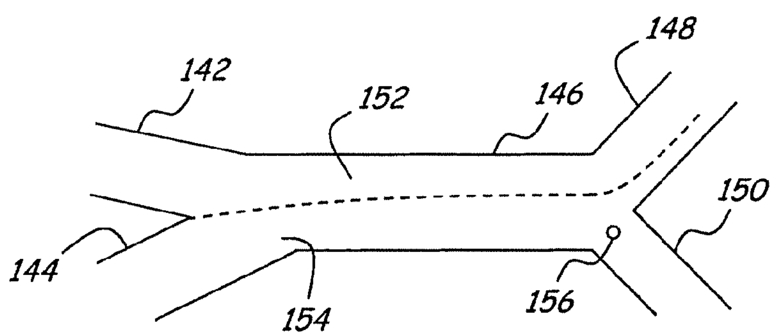


FIG.6C



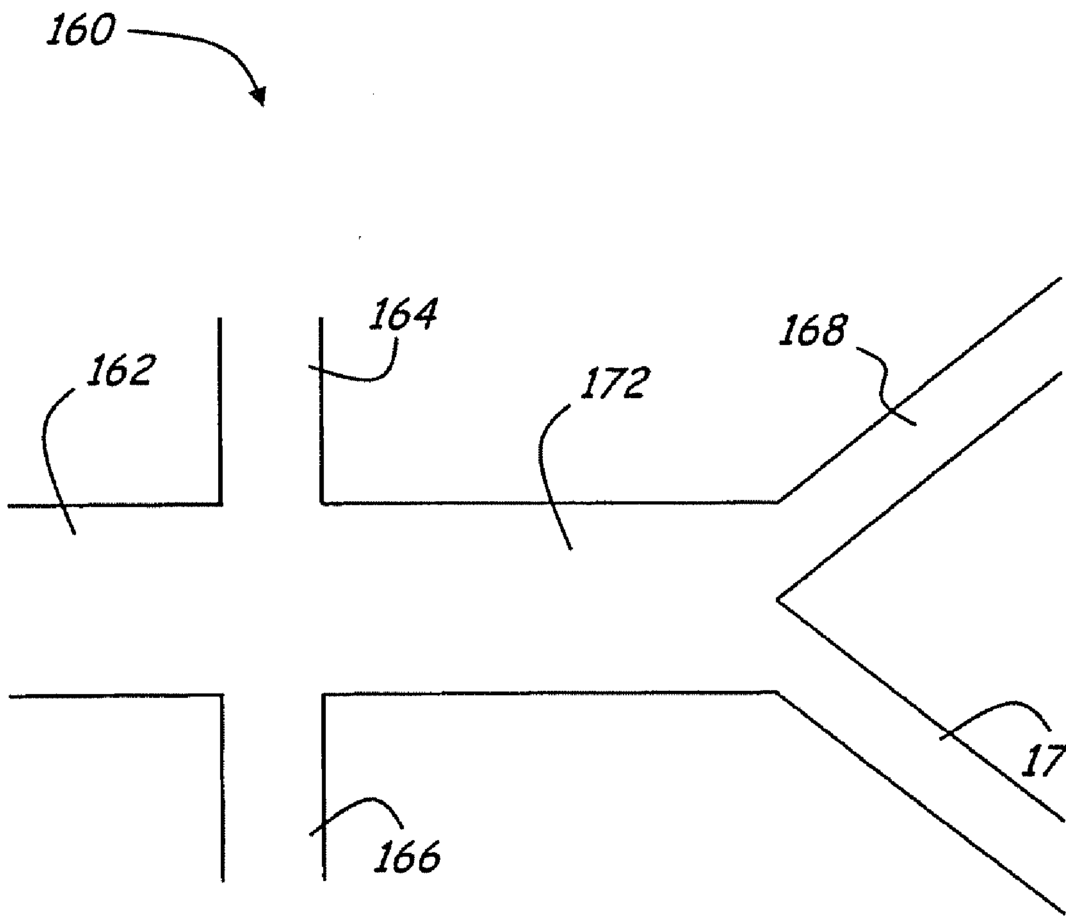


FIG.7

FIG.7A

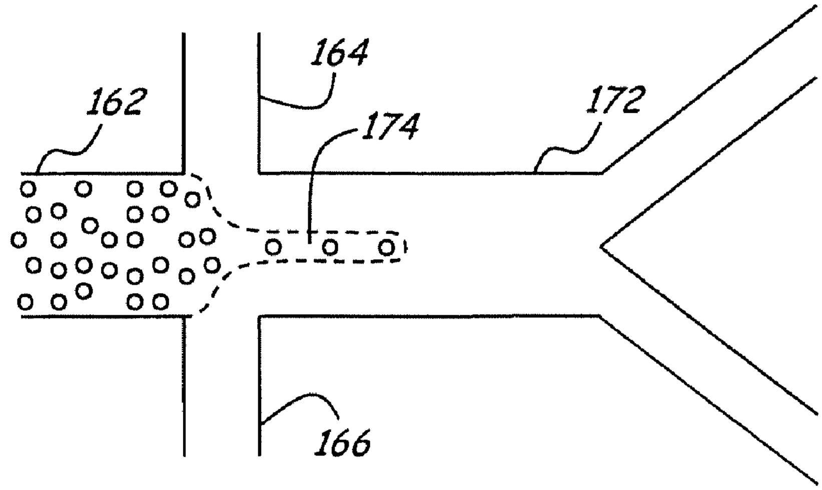


FIG.7B

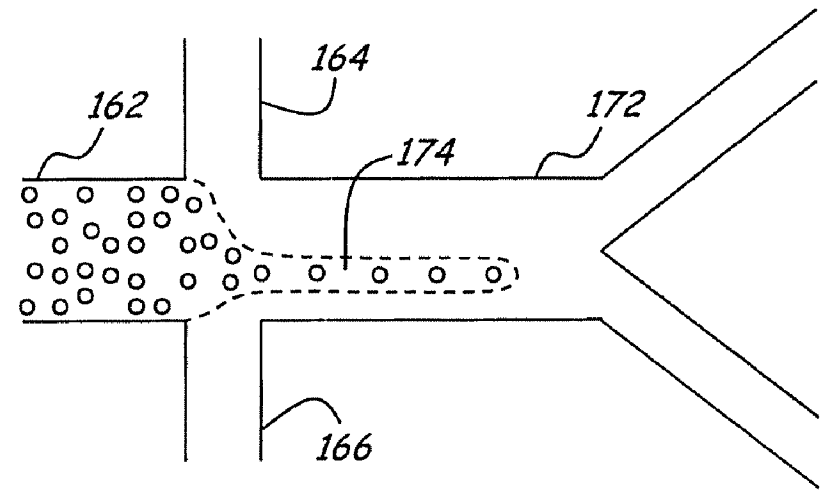
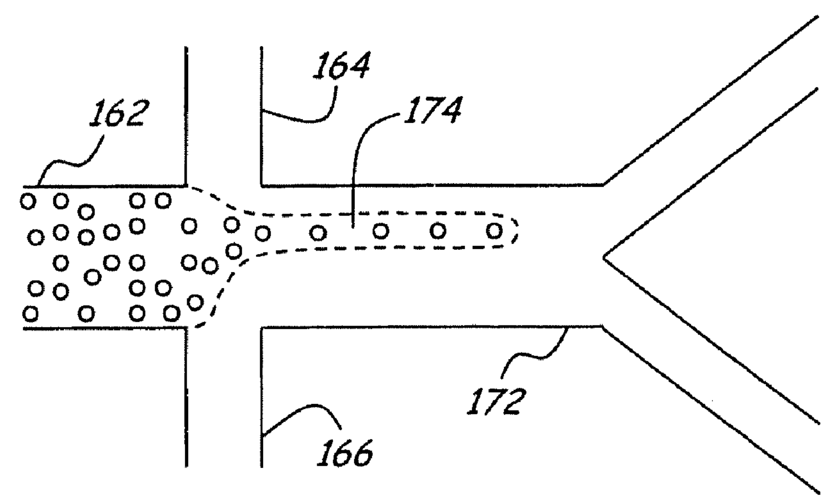


FIG.7C



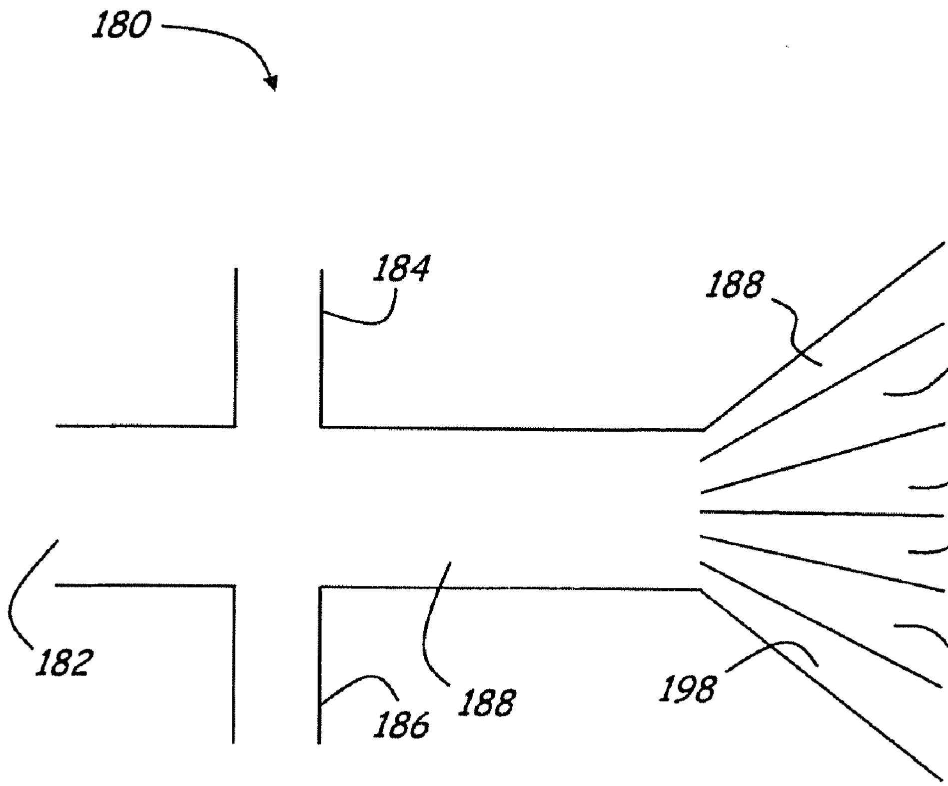

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FIG.8

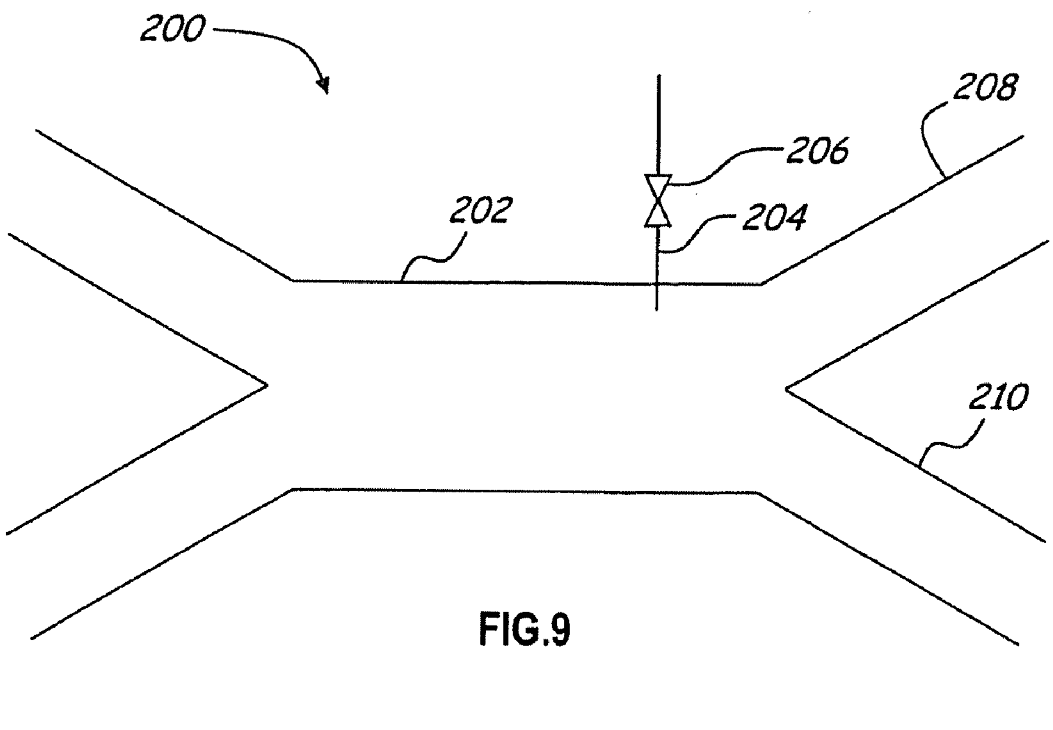
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FIG.9

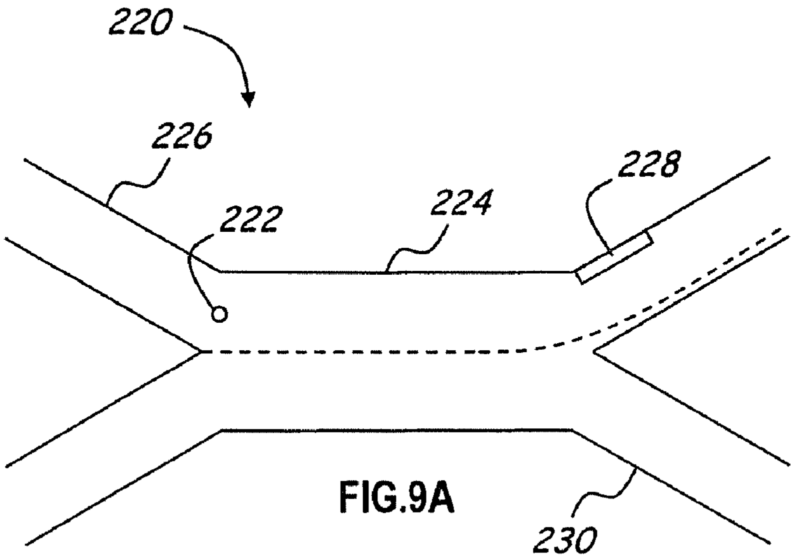

FIG.9A

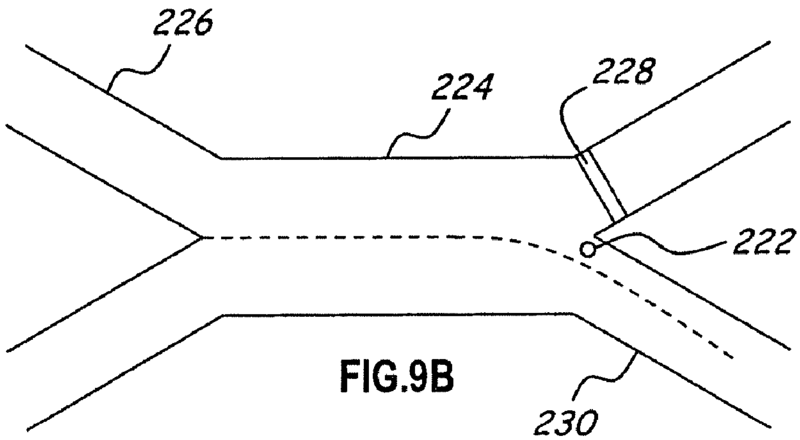

FIG.9B

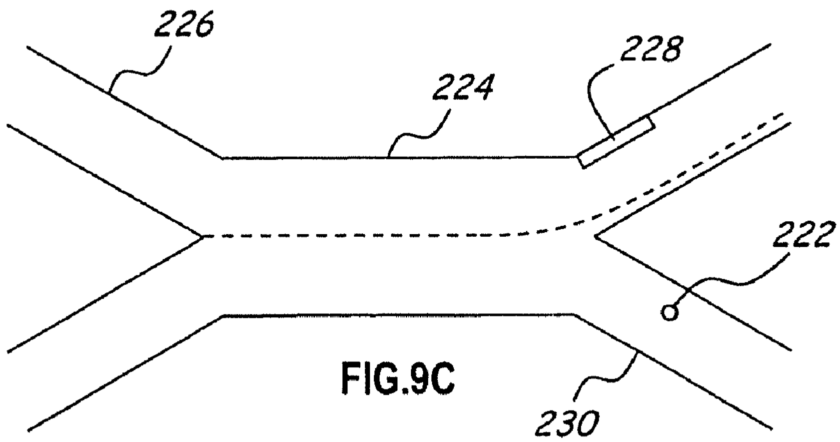

FIG.9C

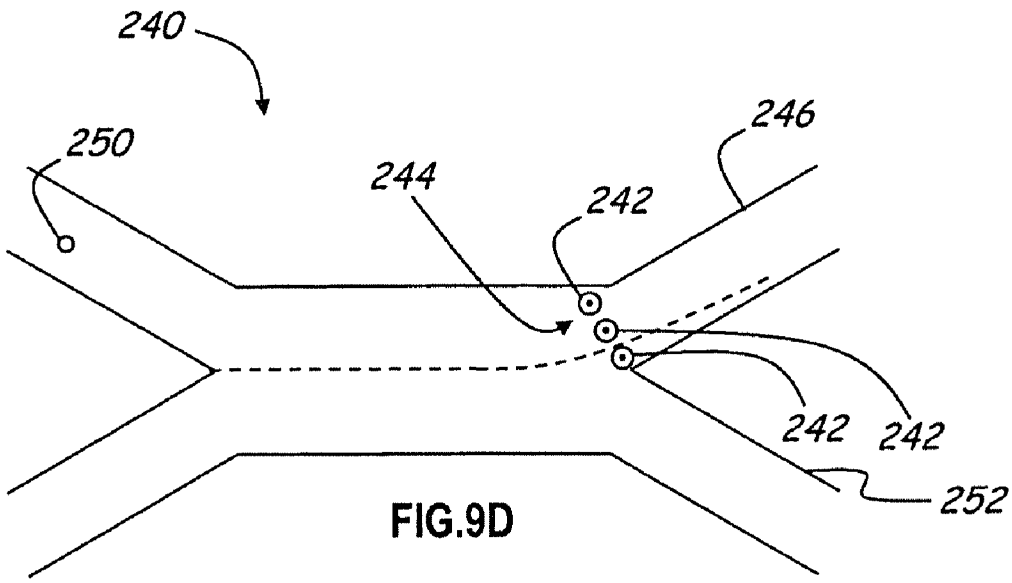

FIG.9D

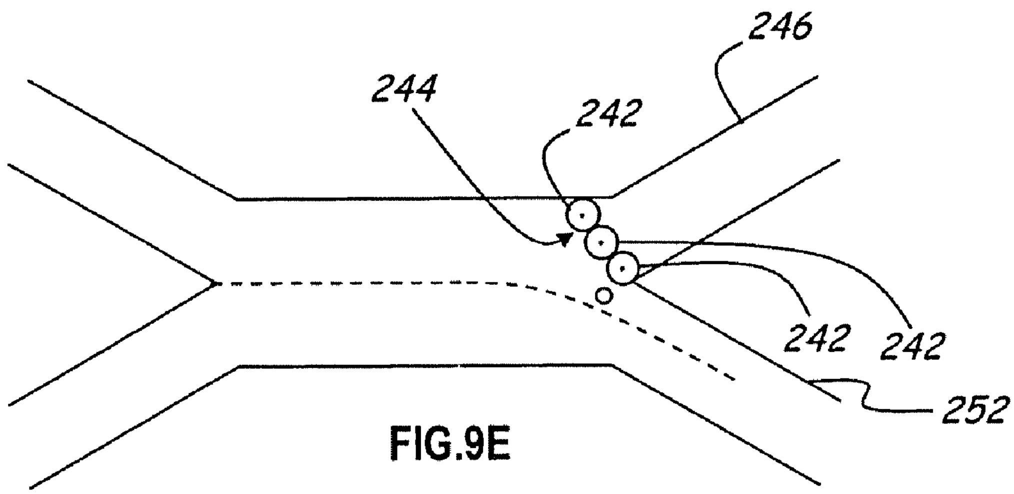

FIG.9E

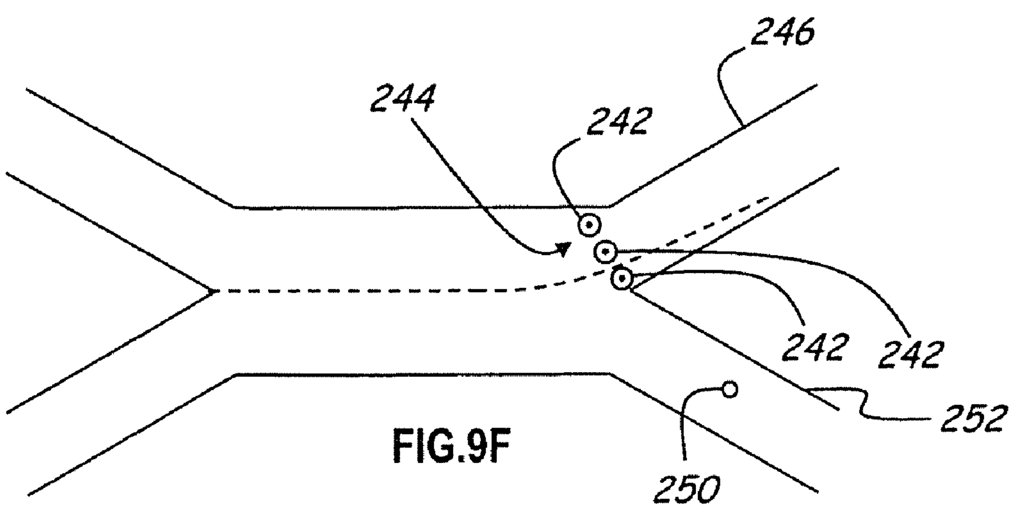

FIG.9F

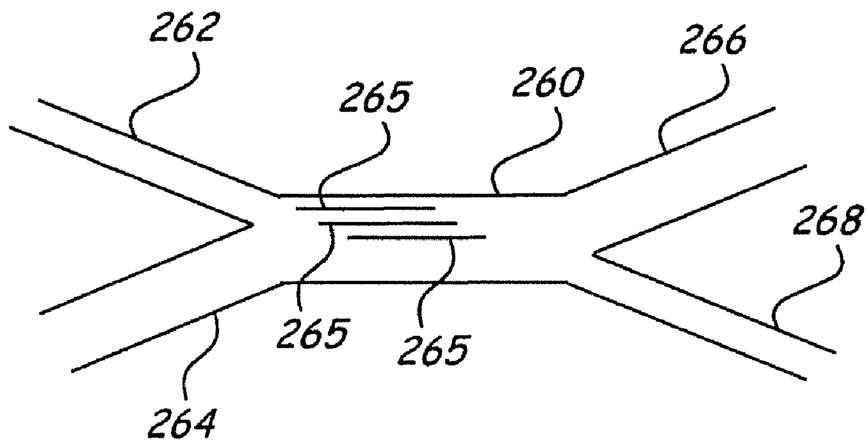

FIG.10

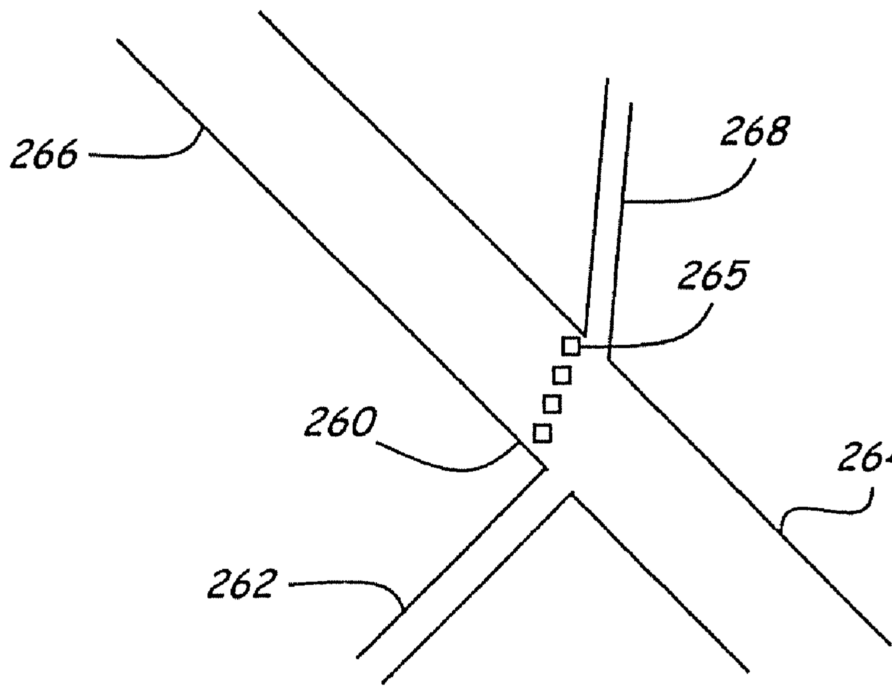

FIG.11

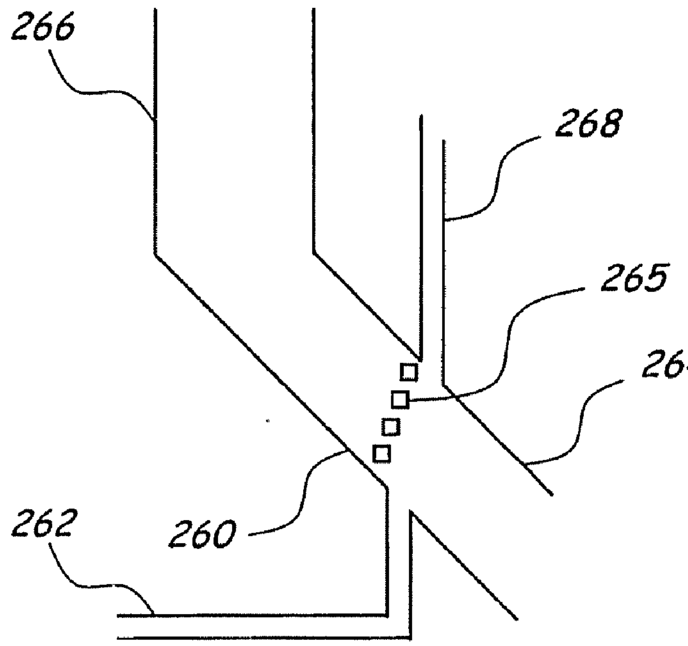

FIG.12

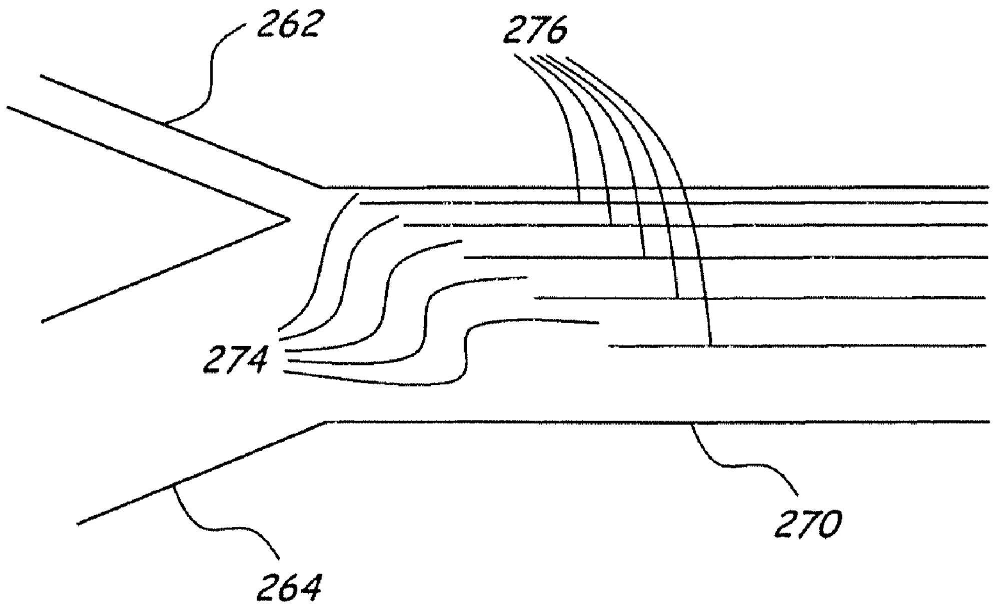

FIG.13

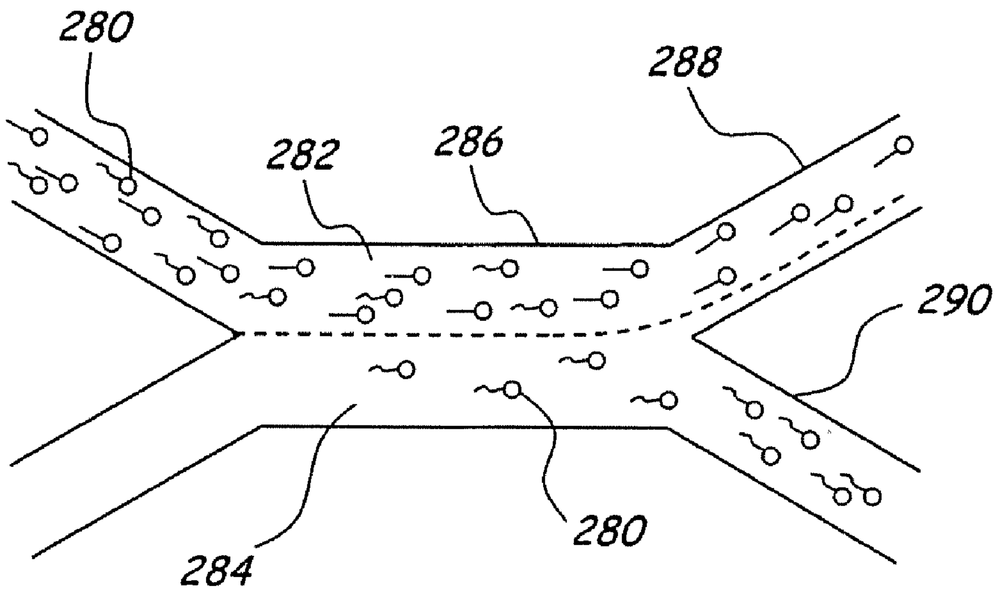

FIG.14

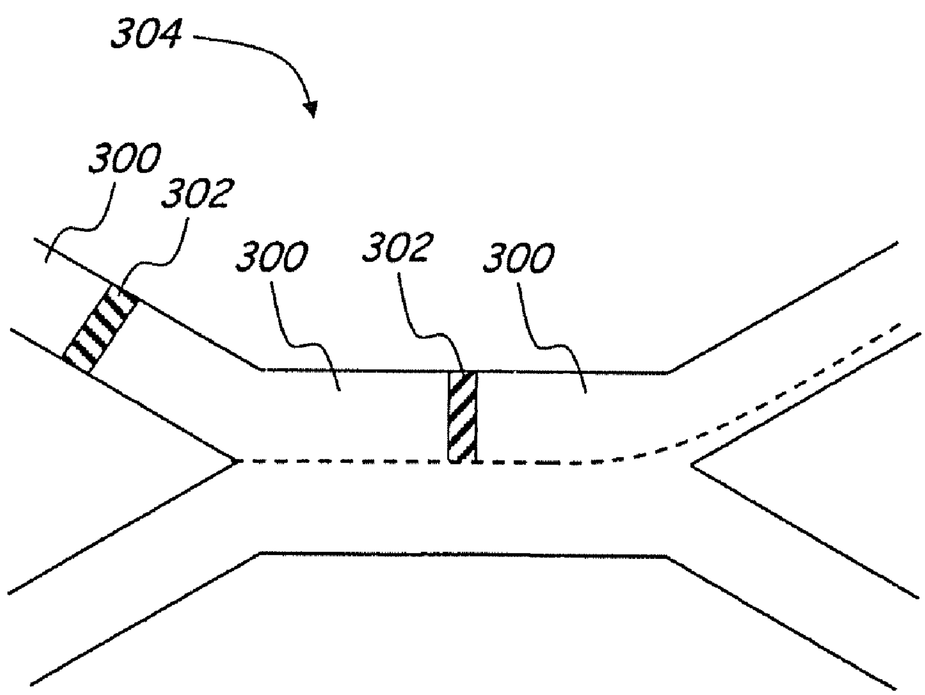

FIG.15

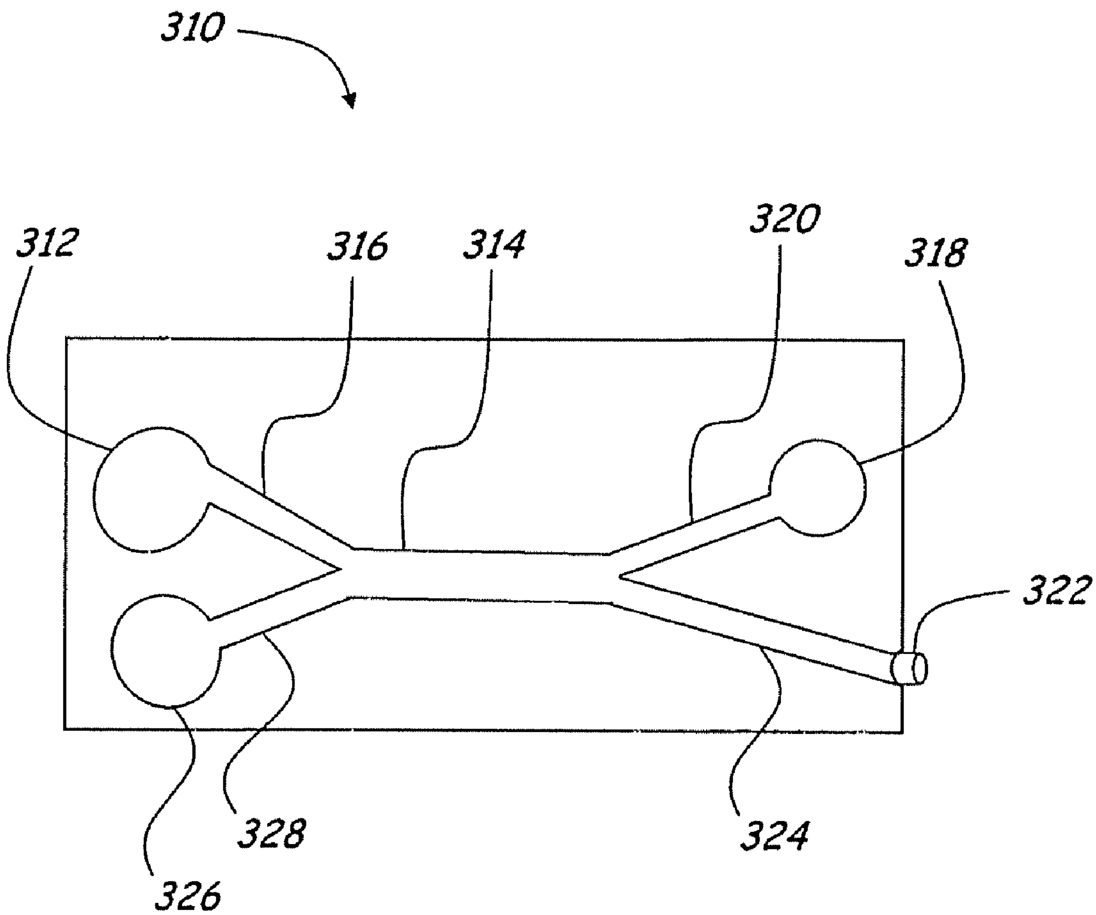

FIG.16

CELL SORTING DEVICE AND METHOD OF MANUFACTURING THE SAME

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the priority benefit of U.S. Provisional Patent Application Ser. No. 60/354,372 filed on 4 Feb. 2002 is herein incorporated in its entirety.

FIELD OF THE INVENTION

[0002] The present invention relates to a general class of devices that uniquely employ laminar flows in separating, filtering or sorting colloidal or cellular particles from a suspension within microfluidic devices.

BACKGROUND OF THE INVENTION

[0003] Microfluidic flows are particularly useful due to their ultra laminar nature that allows for highly precise spatial control over fluids, and provides both unique transport properties and the capability for parallelization and high throughput. These qualities have made microfluidic platforms a successful option for applications in printing, surface patterning, genetic analysis, molecular separations and sensors. Specifically, the effective separation and manipulation of colloidal and cellular suspensions on the microscale has been pursued with keen interest due to the tremendous multidisciplinary potential associated with the ability to study the behavior of individual particles and cells. Devices that employ electric fields to direct flow for the purpose of sorting and manipulating populations of cells have been realized and in some cases have demonstrated potential to achieve efficiencies comparable to their conventional analog, fluorescent activated cell sorters (FACS).

SUMMARY OF THE INVENTION

[0004] The present invention relates to a system, method and apparatus employing the laminar nature of fluid flows in microfluidic flow devices in separating, sorting or filtering colloidal and/or cellular particles from a suspension in a microfluidic flow device. In one embodiment, a microfluidic flow device is provided for separating a particle within a suspension flow in a microfluidic flow chamber. The chamber includes a microfluidic channel comprising an inlet port for receiving a suspension flow under laminar conditions, a first outlet port and a second outlet port. The chamber further includes an interface for translating a particle within the channel. The first outlet port receives a first portion of the suspension exiting the channel and the second outlet port receives the particle in a second portion of the suspension exiting the channel.

[0005] An alternative microfluidic flow device for separating a particle from a suspension flow into a second fluid flow is also provided. The microfluidic flow device includes a microfluidic channel comprising a first inlet port for receiving the suspension flow, a second inlet port for receiving the second fluid flow, a first outlet port and a second outlet port. The channel is adapted to receive the suspension flow and the second fluid flow under laminar conditions. The device further includes an interface for translating a particle from the suspension flow to the second fluid flow. The first outlet port is adapted to receive at least a portion of the suspension flow exiting the channel and the second outlet port is adapted to receive the particle in at least a portion of the second fluid flow exiting channel.

[0006] A method of separating a particle within a suspension is also provided in which a suspension flow is received in a microfluidic channel under laminar conditions. A particle in the suspension is translated within the suspension flow. A first portion of the suspension flow exits through a first outlet port, and the particle exits in a second portion of the suspension flow through a second outlet port.

[0007] Another method of separating a particle from a suspension flow is provided in which a suspension flow and a second fluid flow are received in a microfluidic channel. The suspension and the second fluid flow under laminar conditions in the channel. A particle is separated from the suspension flow into the second fluid flow. At least a portion of the suspension flow exits through a first outlet port, and the particle exits in at least a portion of the second fluid flow through a second outlet port.

[0008] A cartridge is also provided for use in system to separate a particle from a suspension flow. The cartridge comprises a microfluidic channel including an inlet port for receiving a suspension flow under laminar conditions, a first outlet port and a second outlet port. The cartridge further comprises an interconnect for connecting the cartridge to the system. The microfluidic channel is adapted to receive the suspension flow and provide an environment for translating the particle within the suspension flow. The first outlet port is adapted to receive a first portion of the suspension flow, and the second outlet port is adapted to receive the particle in a second portion of the suspension flow.

[0009] An alternative cartridge is further provided for use in system to separate a particle from a suspension flow into a second fluid flow. The cartridge comprises a microfluidic channel including a first inlet port for receiving the suspension flow, a second inlet port for receiving the second fluid flow, a first outlet port and a second outlet port. The channel is further adapted to receive the suspension flow and the second fluid flow in the channel under laminar conditions. The cartridge further comprises an interconnect for connecting the cartridge to the system. The microfluidic channel is adapted to provide an environment for translating the particle from the suspension flow to the second fluid flow. The first outlet port is adapted to receive at least a portion of the suspension flow, and the second outlet port is adapted to receive the particle in at least a portion of the second fluid flow.

[0010] A system for separating a particle from a solution in a microfluidic flow device is also provided. The system includes a detector, an information processor and an actuator. The detector monitors a microfluidic channel of the microfluidic flow device and provides an output to the information processor. The information processor processes the output to determine if the particle is present. If the particle is present, the information processor triggers the actuator to translate the particle within the channel.

[0011] A microfluidic chemical dispenser for dispensing a fluid flow into a plurality of receptacles is further provided. The dispenser comprises a first inlet port, a second inlet port, a third inlet port, a central channel, a plurality of outlet ports, and a modulator. The channel is adapted to receive, under laminar conditions, a first fluid flow through the first input

port, a second fluid flow through the second input port and a third fluid flow through the third input port. The second input port is positioned at a first angle to the first input port, and the third input port is positioned at a second angle to the first input port. The modulator modulates the flow rates of the second and third fluid flows to dispense the first fluid flow into a plurality of outlet ports.

[0012] The foregoing and other features, utilities and advantages of the invention will be apparent from the following more particular description of a preferred embodiment of the invention as illustrated in the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] FIG. **1** depicts a flow diagram of an actuated process of separating a colloidal or cellular particle from a suspension in a microfluidic flow device;

[0014] FIG. **2** depicts a block diagram of an exemplary system for separating a colloidal or cellular particle from a suspension in a microfluidic flow device;

[0015] FIG. **2***a* depicts a block diagram of a microfluidic flow network that may be used in conjunction with the system depicted in FIGS. **2**, **3** and **4**;

[0016] FIG. **3** depicts a block diagram of an alternative system for separating a colloidal or cellular particle from a suspension in a microfluidic flow device;

[0017] FIG. **4** depicts a block diagram of another alternative system for separating a colloidal or cellular particle from a suspension in a microfluidic flow device, wherein the system controls a valve actuator to separate the particle from the suspension;

[0018] FIG. **5** depicts a fluid flow path in one example of a microfluidic flow chamber;

[0019] FIG. **5***a* depicts a particle entering the microfluidic flow chamber depicted in FIG. **5** via an inlet port;

[0020] FIG. **5***b* depicts the particle depicted in FIG. **5***a* being moved within a central channel of the microfluidic flow chamber depicted in FIG. **5**;

[0021] FIG. **5***c* depicts the particle depicted in FIG. **5***a* exiting the central channel of the microfluidic flow chamber depicted in FIG. **5** via an outlet port;

[0022] FIG. **6** depicts side-by-side laminar fluid flows in the central channel of the microfluidic flow chamber depicted in FIG. **5**;

[0023] FIG. **6***a* depicts a particle entering the central channel via an inlet port of the microfluidic flow chamber in the first fluid flow depicted in FIG. **6**;

[0024] FIG. **6***b* depicts the particle depicted in FIG. **6***a* being moved within the central channel of the microfluidic flow chamber from the first flow to the second flow;

[0025] FIG. **6***c* depicts the particle depicted in FIG. **6***a* exiting the central channel of the microfluidic flow chamber in the second flow via an outlet port;

[0026] FIG. **7** depicts an alternative example of a microfluidic flow chamber;

[0027] FIG. **7***a* depicts side flows pinching a central flow of a suspension at the entrance to a central channel of the microfluidic flow chamber depicted in FIG. **7** to orient the flow of suspension in the center portion of the channel;

[0028] FIG. **7***b* depicts side flows pinching a central flow of a suspension at the entrance to a central channel of the microfluidic flow chamber depicted in FIG. **7** to orient the flow of suspension in the bottom portion of the channel;

[0029] FIG. **7***c* depicts side flows pinching a central flow of a suspension at the entrance to a central channel of the microfluidic flow chamber depicted in FIG. **7** to orient the flow of suspension in the top portion of the channel;

[0030] FIG. **8** depicts another example of a microfluidic flow chamber including a plurality of outlet ports for sorting colloidal and/or cellular particles in a suspension;

[0031] FIG. **9** depicts a microfluidic flow chamber including a mechanical actuator for separating a colloidal and/or cellular particle in a suspension, wherein the mechanical actuator comprises a valve;

[0032] FIG. **9***a* depicts an alternative example of a microfluidic flow chamber including a mechanical actuator for separating a colloidal and/or cellular particle in a suspension, wherein the mechanical actuator comprises a valve;

[0033] FIG. **9***b* depicts the particle being separated from the suspension via the valve of the microfluidic chamber depicted in FIG. **9***a* being closed to divert the particle into an alternative outlet port;

[0034] FIG. **9***c* depicts the particle exiting the alternative outlet port of the microfluidic chamber depicted in FIG. **9***a* and the valve retracting to its open position;

[0035] FIG. **9***d* depicts another alternative example of a microfluidic flow chamber including a chemical actuator for separating a colloidal and/or cellular particle in a suspension, wherein the chemical actuator comprises a chemically actuated valve;

[0036] FIG. **9***e* depicts the particle being separated from the suspension via the valve of the microfluidic chamber depicted in FIG. **9***d* being swollen closed to divert the particle into an alternative outlet port;

[0037] FIG. **9***f* depicts the particle exiting the alternative outlet port of the microfluidic chamber depicted in FIG. **9***d* and the valve shrinking to its open position;

[0038] FIG. **10** depicts a series of suspensions being introduced into a microfluidic flow chamber in series separated by buffers;

[0039] FIG. **11** depicts an alternative non-actuated microfluidic flow device for separating colloidal and/or cellular particles from a suspension;

[0040] FIG. **12** depicts another alternative non-actuated microfluidic flow device for separating colloidal and/or cellular particles from a suspension;

[0041] FIG. **13** depicts an exemplary non-actuated microfluidic flow device for sorting colloidal and/or cellular particles from a suspension by size;

[0042] FIG. **14** depicts an alternative non-actuated microfluidic flow device for separating motile cellular particles from a suspension;

[0043] FIG. **15** depicts an exemplary non-actuated microfluidic flow device for separating colloidal and/or cellular particles from a suspension; and

[0044] FIG. **16** depicts a cartridge including a microfluidic flow chamber.

DETAILED DESCRIPTION

[0045] The processes and devices described herein relate to actuated or non-actuated separation of various colloidal and/or cellular particles from a suspension flowing under laminar conditions in a microfluidic flow device. The colloidal and cellular particles may include, for example, polymeric, inorganic or other abiotic colloidal particles, individual polymers, proteins, fragments of DNA or RNA, entire sections or genomes of DNA, cells including single-celled organisms, formed bodies such as they would appear in blood, viruses and the like. A microfluidic flow device, as used for the purposes of the present invention, refers to a microscale device that handles volumes of liquid on the order of nanoliters or picoliters.

[0046] Under "laminar" flow conditions, a fluid flows through a channel without turbulence. The quantification of laminar or nonturbulent behavior is typically done through calculation of the Reynolds number, $Re=\rho vD/\eta$, where ρ is the fluid density, η is the fluid viscosity, v is the fluid velocity, and D is some characteristic channel dimension (typically the channel width). If the Reynolds number is small (<1000) for typical channel geometries, then flow is laminar, reversible, and non-turbulent. For this reason, the diameter of the channel can be designed to account for the intended fluid properties and fluid velocity, or, equivalently, the fluid velocity can be determined by the fluid properties and the channel diameter.

[0047] FIG. **1** shows a flow diagram of a process for an actuated separation of colloidal and/or cellular particles from a suspension flowing through a microfluidic flow device under laminar conditions. In the receive input block **10**, an input is received from a sensor monitoring a target region for a particle of interest. The target region may be monitored to detect any known attribute (or absence thereof) that can be used to distinguish a particle from the remaining suspension. An imaging device such as a charge-coupled device (CCD) camera, for example, may be utilized to capture a stream of images that may be used to identify a particle by its particular morphological attributes or motility. Alternatively, signatures, fingerprints or indices such as a fluorescent signature, light scattering signature, optical fingerprint, X-ray diffraction signature or index of refraction, and the like, or any combination of these, may be used to distinguish the particle from the remaining suspension. Surface charges of particles may also be used to distinguish the particle by observing the reaction of the particle to an applied electric or magnetic field.

[0048] Further, the suspension or the individual particles may be pretreated, as known in the art, to enhance the recognition of the particles. The suspension may further be pretreated with an antibody that will bind specifically to a particular type of particle may be used to enable or enhance the recognition of the particle. A suspension of cells, for example, may be pretreated with antibody-decorated magnetic particles and passed through a magnetic field to distinguish the particles from the remaining suspension. Similarly, other recognition methodologies known in the art may be used to distinguish the particle of interest from the remaining suspension.

[0049] Information processing block **20** performs any processing steps necessary to distinguish the particle from the remaining suspension such as comparing received images or signals from the receive input block **10** to threshold values, e.g., size and shape. The information processing block **20** may include any required processing steps as known in the art to distinguish the particle of interest from the remaining suspension. The processing steps may vary depending upon the type of input received. The processing step, for example, may include simple recognition of a digital input value or may include complicated processing steps to detect whether a given input corresponds to the presence of a particle of interest.

[0050] After a particle is identified, the particle may be separated from the suspension by the actuation of separation block **30**. The actuation may include, for example, steering an optical trap such as via a piezoelectric mirror, an acoustic optic deflector, a diffraction grating, a holographically-generated trap, a static line trap, a dynamic line trap, an optical gradient, a microlens array, a waveguiding structure or other known optical steering mechanism. The actuation may alternatively include generating an electric field or a magnetic field. The actuation may also include a mechanical or chemical actuator. A mechanical actuator, for example, may include a pump, valve, gate, applied pressure and the like. A chemical actuator, for example, may include a hydrogel or similarly behaving material that reacts to a property sensed in the suspension that may indicate the presence or absence of a particle of interest.

[0051] Each of the functions shown in blocks **10**, **20** and **30** of FIG. **1**, however, need not be performed by distinct hardware components. A sensor, for example, may receive an input and perform the information processing on that input to determine if a particle of interest has been detected. An actuator may even perform each of the functions by directly reacting to a property being monitored (e.g., a pH responsive hydrogel may swell in response to a sensed pH level).

[0052] FIG. **2** shows one exemplary system **40** for separating a particle of interest from a suspension in a microfluidic flow device **44** utilizing an actuated separation technique. The system includes an detector system **50**, an information processing system **60** and an actuator system **70**. The detector system **50** includes an imaging system, such as a camera **52**, that may be used to image a field of view through a filter **54** and a microscope **56**. The detector system **50**, for example, may utilize a CCD camera to capture a stream of images of the microfluidic flow device through a microscope lens. In one particular embodiment, the camera **52** captures images at a rate of 30 images per second through a 100× objective. The images are recorded by a recording device, such as VCR **58**, and/or passed directly to an information processor, such as a computer **62**. Optionally, the identification of the particles may be aided by utilizing the laser **74** or another light source, such as a secondary laser, multiple other lasers, a broad spectrum lamp and the like, to irradiate the suspension to illuminate the particles of interest.

[0053] The information processor may include the computer **62**, a controller or other processor known in the art.

The information processor receives and processes the image data and distinguishes the particle of interest from the remaining suspension as described above. Once the particle is recognized, the information processor may trigger the actuator system **70** to separate the particle from the suspension.

[0054] The actuator system **70** may include a targeting device **72** to target a laser beam from a laser **74** on the microfluidic flow device **44**. The targeting device, for example, may include a piezo drive **76** to control a piezo mirror **78** to direct the beam of a laser **74**. The laser **74**, when focused on the particle, traps the particle. The optical trap may then be used to translate the particle between streams in the channel of the microfluidic flow device **44**.

[0055] Utilizing an optical trap as the means of actuation provides the capability for highly precise and immediately customizable individual separations Other applied fields, however, may also be utilized to translate particles from the primary stream to the secondary stream. Both electric and magnetic fields may be employed with appropriate suspensions to isolate individual or multiple particles. All colloidal particles and living cells carry with them a surface charge, which, in the presence of an electrical field results in electrophoresis. The electrophoretic force, or the migration of surface ions with an electric field, is sufficient to translate cells or particles from one stream to another. Similarly, if a particle or cell possesses a magnetic moment, it may be selectively translated in a magnetic field. Each of these fields could be applied continuously to fractionate particles or cells based on electrical or magnetic properties, or could be pulsed or applied discriminatively for custom separations.

[0056] As described above, the suspension or the individual particles may be pretreated, as known in the art. The pretreatment, for example, may enhance the response of the particle to an optical trap or electric or magnetic field. The suspension may further be pretreated with items, such as antibodies that will bind specifically to a particular type of particle may be used to enable or enhance the movement of the particle via an optical trap or electric or magnetic field. A suspension of cells, for example, may be pretreated with antibody-decorated magnetic particles and, thus, be easily moved by means of a magnetic field.

[0057] FIG. **2***a* shows further detail of a microfluidic flow device **44** that may be used in connection with a system **40**, **80** and **110** such as shown in FIGS. **2**, **3** and **4**, respectively. The microfluidic flow device **44** includes a flow generator **45**, which provides a pressure differential to induce fluid flows through the microfluidic flow device **44**. The pressure differential, for example, may be induced by any method known in the art such as, but not limited to, capillary forces; gravity feed; electro-osmosis systems; syringes; pumps such as syringe pumps (e.g., a kdScientific, model 200 syringe pump), peristaltic pumps and micropumps; valves such as three-way valves, two-way valves, ball valves and microvalves; suction; vacuums and the like. Further, although FIG. **2***a* shows the flow generator located upstream of a microfluidic flow chamber **47**, the flow generator may also be placed midstream in the microfluidic flow chamber **47** or downstream of the microfluidic flow chamber **47**. Further, the microfluidic flow chamber **47** preferably provides at least one output **49** with the collected particles separated from a suspension within the chamber. This output **49** may provide the collected particles as an end process or may provide the particles to a downstream network for further processing.

[0058] FIG. **3** shows an alternative system for separating a particle of interest from a suspension in a microfluidic flow device. The imaging system **90** and its operation is the same as shown in FIG. **2** except that the imaging system **90** further includes a field generator **92**. The field generator **92** induces an electric or magnetic field in the microfluidic flow device **44**. As the suspension flows through the device **44**, the movement of the particles of interest, whether induced by electric or magnetic properties of the particles themselves or by properties associated with a pretreatment of the particles, is captured by the imaging system **90** and identified by the information processor **100**.

[0059] FIG. **4** shows another system **10** for separating a particle of interest from a suspension in a microfluidic flow device **114**. In this system, the actuator system includes a valve controller **112** that controls the operation of a valve within the microfluidic flow device **114**. The valve, for example, may be opened to divert the flow of the suspension within the microfluidic flow device for a predetermined time after the recognition of the particle of interest. In this manner, the system separates the particle in a small portion of the suspension by diverting the suspension carrying the particle into an alternative outlet port. An example of such a valve is described below with respect to FIGS. **9***a*-**9***c*.

[0060] A particular microfluidic flow channel can be modeled to determine the flow path of a fluid flowing in a laminar manner through the channel. This is well known in the art and involves solving the Langevin equations, the Navier-Stokes equations or other equations of motion, which can be done manually or electronically. Commercial software tools are also available for modeling the laminar flow path of a fluid through any microfluidic flow channel. For example, CFDASE, a finite element modeling for computational fluid dynamics module available from Open Channel Foundation Publishing Software from Academic & Research Institutions of Chicago, Ill., and FIDAP, a flow-modeling tool available from Fluent, Inc. of Lebanon, N.H., can be used to model the laminar flow of a fluid through a particular microfluidic channel.

[0061] FIG. **5** shows an embodiment of a microfluidic flow chamber **120** in which a particle of interest may be separated from a suspension. The microfluidic flow chamber includes a single inlet port **122**, two outlet ports **124** and **126** and a central channel **128**. FIG. **5** further shows arrows depicting a modeled laminar flow of a particular fluid through the microfluidic flow chamber **120**. FIGS. **5***a*-**5***c* show a process for separating a particle **130** from a suspension flow in the microfluidic flow chamber **120** of FIG. **5**. FIG. **5***a* shows the particle entering the microfluidic flow chamber **120** via the inlet port **122** at which point it is identified as described above. The information processor initiates an actuator to direct the particle **130** into a desired portion of the flow stream **132** of the suspension in FIG. **5***b*. Thus, the particle **130** is directed to a portion of the flow in which it will exit the central chamber **128** through the second outlet port **126**, as shown in FIG. **5***c*.

[0062] FIGS. **6** and **6***a*-**6***c* show an alternative embodiment of a microfluidic flow chamber **140**, which includes two inlet ports **142** and **144**, a central channel **146** and two outlet ports

148 and **150**. As FIG. **6** shows, a first fluid **152**, indicated by dye, enters the central channel **146** via the first inlet port **142** and a second fluid **154** enters the central channel **146** via the second inlet port **144**. As described above, when the first fluid **152** and the second fluid **154** flow through the microfluidic flow chamber in a laminar manner, the fluids maintain separate streams and undergo minimal convective mixing. Rather, the mixing present is primarily due to molecular-scale diffusion, which for colloidal-sized particles is referred to as Brownian movement, as shown near the outlet port of the central channel. The system can be designed to minimize the diffusion that occurs within the central channel **146** by controlling the central channel **146** dimensions and the velocity of the fluid flowing through the channel **146**. In general, the diffusion distance x, can be expressed as $x \approx \sqrt{D \cdot t}$, wherein D is the diffusivity and t is the time. To a first order, the diffusivity is inversely proportional to the size of the particle. Therefore, to a first order, the channel residence time required to achieve complete mixing, $t \approx x^2 D^{-1}$, scales linearly with the particle diameter. Thus, by designing the microfluidic flow chamber dimensions for a particular flow rate of a fluid, a laminar two-phase flow may be used as an effective barrier against particle cross-transport. In the example shown in FIG. **6**, each of the inlet streams has a width of about 30 μm and the central channel has a length from the inlet ports to the outlet ports of about 3000 μm, the reduction of which will correspondingly reduce the diffusion within the channel **146** for a constant flow rate. Both of the fluids streams **152** and **154** shown are water. The first stream **152** includes a molecular dye (Methylene Blue), which has a diffusion coefficient on the order of about 1×10^{-5} cm^2/sec in water.

[0063] Further, as shown by the dashed line in FIG. **6***a*, a portion of the second fluid stream **154** can exit the central channel **146** via the first outlet port **148** while the remainder of the second fluid **154** exits via the second outlet port **150**. If the first fluid **152** is a suspension including suspended particles and the second fluid **154** is a clean solvent, for example, the portion of the solvent that exits the first outlet port **148** along with the suspension **152** acts as an additional barrier to cross-contamination of the streams through diffusion. Thus, particles that diffuse into this portion of the solvent stream may still exit the central chamber **146** via the first outlet port **148**, as shown in FIG. **6**.

[0064] The steady state flow-based particle barrier can be penetrated, however, by providing an actuator to move a particle **156** across the barrier. A selective activation of an electric, magnetic or optical field, or any combination of these fields, for example, may be used to move the particle **156** from one stream to another stream. Alternatively, a mechanical actuator, such as a valve, pump, gate or applied pressure may be employed to move the particle from one stream to another stream. Although described here for parallel flows, the flows traveling in arbitrary orientations, including opposite directions, are possible.

[0065] FIGS. **6***a*-**6***c* show a particle **156** being separated from the first inlet stream **152** into the second inlet stream **154** in the embodiment shown in FIG. **6**. In FIG. **6***a*, a suspension enters the central channel **146** from the first inlet port **142**, and a second fluid **154**, such as a solvent, enters the central channel **146** from the second inlet channel **144**. The suspension **152** and the second fluid **154** flow in a laminar manner through the central channel **146**. The suspension stream **152** and a portion of the second fluid stream **154** exit the central channel **146** via the first outlet port **148**. The remaining portion of the second fluid stream **154** functions as a collection stream and exits the central channel **146** via the second outlet port **150**. A particle **156** suspended in the suspension stream **152** is shown entering the central channel **146** from the first inlet port **142**, where it is identified as described above. In FIG. **6***b*, the particle **156** is shown being separated from the suspension stream **152** into the second fluid stream **154**. The particle **156** may be separated from the suspension **152** via an electrical, magnetic, mechanical or chemical actuator such as described above. In FIG. **6***c*, the particle **156** is shown exiting the central channel **146** via the second outlet port **150** in the second fluid stream **154** for collection.

[0066] FIG. **7** shows another embodiment of a microfluidic flow chamber **160** in which a particle of interest may be separated from a suspension. The microfluidic flow chamber **160** includes three inlet ports **162**, **164** and **166**, two outlet ports **168** and **170** and a central channel **172**. In this example, a suspension including suspended particles enters from the first inlet port **162**. Other fluid streams, such as a pair of solvent or buffer fluid streams enter the central channel **172** from either side of the first inlet port **162**. As shown in FIGS. **7***a*-**7***c*, the relative flow rates of each inlet port may be modulated to vary the resulting incoming stream **174** into the central channel **172**. In FIG. **7***a*, for example, the relative flow rates of the streams in the second inlet port **164** and the third inlet port **166** are relatively equal and pinch the flow from the first inlet port **162** at a neck and form a narrow stream of the first fluid approximately down the center of the central channel **172**. By varying the flow rates of the second and third inlet streams **164** and **166**, the width of the first fluid stream **174**, i.e., the suspension, can be narrowed down to the width of a single particle. Thus, the inlet sample suspension **174** may be "prefocused" into a narrow, or even single file, particle stream surrounded on either side by a potential collection stream. This allows for a decrease in the lateral distance, i.e., distance perpendicular to the flow direction, a particle must be moved away from the suspension stream to be captured in the collection stream and, thus, an increase in sorting efficiency.

[0067] FIG. **7***b* shows the embodiment of FIG. **7**, wherein the flow rate of the third inlet port **166** is less than the flow rate of the second inlet port **164** and prefocuses the inlet particle stream in the lower half of the central chamber **172**. Conversely, FIG. **7***b* shows the embodiment of FIG. **7**, wherein the flow rate of the third inlet port **166** is greater than the flow rate of the second inlet port **164** and prefocuses the inlet particle stream in the upper half of the central chamber **172**. The relative flow rates of the three inlets can thus be modulated to control the particle stream within the central channel.

[0068] FIG. **8** shows yet another embodiment of a microfluidic flow chamber **180** in which a particle of interest may be separated from a suspension. As in FIG. **7**, the microfluidic flow chamber **180** includes three inlet ports **182**, **184** and **186** and a central channel **188**. The chamber **180** of FIG. **8**, however, includes six outlet ports **188**, **190**, **192**, **194**, **196** and **198**. The number of outlet ports shown in FIG. **8** is merely exemplary and may include any number of outlet ports greater than or equal to two. In this example, the plurality of outlet ports may be used to sort a plurality of particles into various outlet ports. Different types of particles, for example, may be sorted into different outlet ports. Alternatively, the plurality of outlet ports may be used to individually sort the same type of particles into different outlet ports. In yet another embodiment, the side flows may be modulated as described above to dispense particles, chemicals and/or fluids (e.g., reagents) into multiple outlet ports for use in various downstream applications or networks.

[0069] Alternatively, the incoming streams may be prefocused prior to entry into the microfluidic flow chamber, or the side inlet ports may be arranged to enter the central channel downstream of the first inlet port.

[0070] FIG. **9** shows an embodiment of a microfluidic flow chamber **200** in which a particle of interest may be separated from a suspension via a mechanical actuator. As shown in FIG. **9**, the central channel **202** includes a side channel **204** through which incoming fluid flow is controlled by a valve **206**. After a particle is detected, the valve may be opened to vary the fluid flow within the central channel **202** and divert the suspension along with the particle away from the first outlet port **208** into the second outlet port **210**. Alternatively, the valve **206** may be closed or the flow through the valve may be merely adjusted to divert the particle into the desired outlet port. Similarly, the valve **206** may be positioned on the opposite side of the central chamber **202** and may obtain a similar result by providing or modulating the flow in the opposite direction.

[0071] FIGS. **9***a*-**9***c* show yet another embodiment of a microfluidic flow chamber **220** in which a particle of interest may be separated from a suspension via a mechanical actuator. As shown in FIG. **9***a*, the particle **222** enters the central channel **224** in the suspension via the first inlet port **226**. In FIG. **9***b*, the valve **228** activates after the particle is identified as described above and redirects the particle **222** into the second outlet port **230**. Then, in FIG. **9***c*, after the particle **222** has exited the central channel **224**, the valve **228** retracts and the fluid stream flows return to their steady state condition.

[0072] FIGS. **9***d*-**9***f* show an exemplary microfluidic flow chamber **240** in which a particle of interest may be separated from a suspension via a chemical actuator. As shown in FIGS. **9***d* **9***f*, the microfluidic flow chamber **240** includes a chemical actuator material **242**, such as a hydrogel, that swells or shrinks in reaction to an attribute associated with a particular particle of interest (e.g., pH). Hydrogels, such as these are known in the art. Beebe, David J. et al, "Functional Hydrogel Structures for Autonomous Flow Control Inside Microfluidic Channels, Nature, vol. 404, pp. 588-90, (Apr. 6, 2000), for example, discloses hydrogel actuators that may be used in the present embodiment.

[0073] FIGS. **9***d*-**9***f* show a chemically actuated valve **244** including the chemical actuator material **242**. In FIG. **9***d*, for example, the chemical actuator is in its normal condition in which the valve **244** is open and the suspension flows through the first outlet port **246**. FIG. **9***e* shows the chemical actuator in its active state in which the chemical actuator material **242** is swollen in response to a detected attribute, effectively shutting off the first outlet port **246** and the suspension flows through the second outlet port **252** and allowing the particle **250** of interest to be collected. Although FIG. **9***e* shows the chemically actuated valve **244** completely closing off the first outlet port, the swelling of the chemically actuated material **242** may also merely create a barrier to particular-sized particles while allowing the remainder of the suspension to pass into the first outlet port **246**. Where the individual valve members are angled toward the second outlet port **252**, the blocked particles **250** may be conveyed to the second outlet port **252** for collection. FIG. **9***f* further shows the chemically actuated valve **244** returned to its open condition after the detected particle **250** has passed into the second outlet port **252**.

[0074] Alternatively microfluidic flow devices may employ laminar flows and specific microgeometries for non-actuated separation of colloidal and/or cellular particles in fluid suspensions. The geometry of these devices has been designed to act similarly to a filter without the use of membranes or sieves which are highly susceptible to clogging and fouling. Such devices will also be capable of replacing the centrifugation step common to many biological processes upon a chip surface. With a microscale alternative to centrifugation available, a host of multi-step biological processes such as bead-based assays and cell counting using dying techniques will be able to be performed within microfluidic devices.

[0075] As demonstrated in FIGS. **1012**, specific channel geometries may be created to take advantage of the laminar nature of fluids flowing in microchannels. In each of these designs, the particle suspension enters the central channel **260** through a first inlet port **262**. A second fluid stream, such as a solvent stream, enters the channel **260** through a second inlet port **264**, which meets the first inlet port **262** at any angle. Because of the laminar nature of microfluidic flows, these streams will generally not mix convectively. The central channel **260** further includes microscale obstacles **265**. Molecular debris small enough to fit through the openings formed by the microscale obstacles **265** will be carried down the first outlet port **266**. Due to the presence of microscale obstacles, however, any particles larger than the separation of the obstacles will be shuffled toward the second outlet port **268** and exit the central channel **260** with a portion of the second fluid stream. The designs shown here do not depend upon relative channel size, instead the presence of the microscale obstacles at or near the confluence of the two (or more) inlet streams alter the direction of flow for any particulate matter in the suspension inlet stream(s).

[0076] FIG. **13** further shows a configuration for sorting particles in the suspension by size and produces a size fractionation effect by designing the size of the gaps **274** between the guides **276** to increase away from the first inlet port **262**, by which the suspension is introduced into the central channel **270**. By gradually increasing the widths of the gaps **274** moving away from the first inlet port **262**, particles of increasing size flow into the guides **276** and may be collected individually.

[0077] FIG. **14** shows yet another embodiment of a non-actuated separation of motile particles within a suspension between laminar flows. In this embodiment, motile particles **280** entering in the suspension flow **282** move within the suspension flow and can pass from the suspension flow **282** into the second fluid stream **284** without the need of an actuator to separate the particles **280** from the suspension flow **282**. In this manner, the motile particles **280** may enter the second fluid stream **284** and exit the central channel **286** through the second outlet port **290** instead of the first inlet port **288**. For example, in a suspension **282** containing sperm, the active sperm may move on their own into the second fluid stream **284** for collection, while inactive sperm are carried out of the central channel **286** with the suspension **282** via the first outlet port **288**.

[**0078**] Non-actuated separation of colloidal and/or cellular particles from a suspension in a microfluidic flow device presents a very simple approach to microfluidic separations or enrichments of colloidal and/or cellular particles because it relies upon the condition native to fluids flowing on the microscale, regardless of flow rate or channel morphology: laminar flows. Furthermore, the selection of materials for the construction of these devices is irrelevant, thus they may be incorporated into microfluidic devices constructed on any substrate.

[**0079**] FIG. **15** shows another example of a microfluidic flow chamber in which a series of discrete sample suspensions **300** are combined into a single laminar flow. In this example, a plurality of discrete samples **300** form the single sample flow. The sample flow further preferably includes buffers **302** between each discrete sample **300** to prevent cross-contamination between samples **300**. In this manner, a single microfluidic flow chamber **304** can separate particles from a series of samples to increase throughput. The series of discrete sample suspensions may, for example, be created using a microfluidic dispenser as shown and described above with reference to FIG. **8** in which individual samples are directed into a plurality of outlet ports and combined downstream into a series of discrete sample streams.

[**0080**] FIG. **16** shows a cartridge **310** that may be plugged into, or otherwise connected to, a system for separating one or more colloidal or cellular particles from a suspension. The cartridge **310** may be reusable or disposable. The cartridge may include a sample reservoir **312**, or other inlet mechanism, for receiving a fluid suspension. The sample reservoir **312** is connected to a central channel **314** via a first inlet port **316**. The cartridge further includes a waste receptacle **318**, or other outlet mechanism, connected to the central channel **314** via a first outlet port **320** for receiving the suspension after it has passed through the central channel **314** for the removal of one or more particles of interest. A collection receptacle **322** is also connected to the central channel **314** via a second outlet port **324** for receiving the particles collected from the suspension. The collection receptacle **322** may include a reservoir or other means for holding the collected particles or may include a channel or other means for providing the collected particles to downstream networks for further processing.

[**0081**] The cartridge **310** may also include a second inlet reservoir **326** for receiving a second fluid, may receive the second fluid from an external source in the system, or may not utilize a second fluid at all, such as described with reference to FIG. **5**. If used, the second fluid may include a fluid such as a buffer or a solvent (e.g., water, a saline suspension and the like) or a reagent (e.g., antibody tagged particles, fluorescent tags, lysing agents, anticoagulants and the like), or any combination thereof. Indeed, the fluid requirements may be system-specific and may be matched to the intended application and mode of use. The second inlet reservoir **326** or receptacle for receiving a second fluid, if used, may be connected to the central channel **314** via a second inlet port **328**.

[**0082**] The reservoirs or receptacles may include any interface for transferring a fluid known in the art. For example, the reservoir may be adapted to receive fluids from a syringe, either with or without a needle, from a tube, from a pump, directly from a human or animal, such as through a finger stick, or from any specially designed or standard fluid transfer coupling.

[**0083**] The microfluidic flow chambers described herein may be manufactured by a variety of common microelectronics processing techniques. A pattern of a shadow mask may be transferred to a positive or negative photoresist film spun upon a silicon wafer, a glass slide, or some other substrate, for example. This pattern may be sealed and used directly as the microfluidic network, replicated in another material, or further processed. The substrate may be further processed through subsequent wet etching, dry etching, molecular epitaxy, physical deposition of materials, chemical deposition of materials, and the like, or any combination of these or similar techniques. The final network may be used directly or reproduced through the use of a replication technique designed to produce a replica upon the master, such as by the pouring and curing, imprinting in or deposition of elastomers, polymers and the like. A pump or other means for introducing and controlling fluid flow within the fluidic network as well as a means for connecting the pump or pressure differential means may also be provided. The network can further be sealed, such as with a cover slip, glass slide, silicon wafer, polymer films or a similar substrate.

[**0084**] In one specific, nonlimiting example, a pattern on a shadow mask was exposed to ultraviolet light and transferred to a negative photoresist film spun upon a silicon wafer to a depth of approximately 5 μm. A two-part mixture of poly(dimethyl siloxane) (PDMS), which is commercially available from Dow Corning under the trade name of Sylgard 184, was poured and cured upon the silicon master to produce a flexible, biocompatible optically transparent replica. In addition to the PDMS channel network a flow apparatus comprising a syringe pump such as a kdScientific, model 200 syringe pump and a polymethyl methalacrylate (PMMA) flow introduction base. The PDMS channel network was placed upon the PMMA base, and holes were punched through the PDMS to provide access for the microchannels to the ports in the base. The network was further sealed with a cover slip. Because the PDMS forms a tight seal with both PMMA and glass, no additional bonding or clamping was required. The syringe pump was further fitted with 3 cm^3 plastic syringes (such as available from Becton-Dickson) joined to the base.

[**0085**] One embodiment of an optical trap and digital microscopy that may be used with the microfluidic flow devices described herein may incorporate a piezoelectric mirror (such as available from Physik Instrumente, model S-315) to simultaneously trap several particles by rapidly scanning a single laser beam (such as available from Spectra Physics, 532 nm, typically operated at 200 mW) among a number of positions to create a time-averaged extended trapping pattern. A Neofluar, 100×, oil immersion high numerical aperture objective (N.A.=1.30) can be used to focus the beam and create the optical trap. CCD images can be captured by a data acquisition board and processed by LabView (National Instruments) routines that may be customized to distinguish various visual particle or cell features for specific applications. Optical traps and digital microscopy are described in further detail, for example, in Mio, C.; Gong, T.; Terry, A.; Marr, D. W. M., Design of a Scanning Laser Optical Trap for Multiparticle Manipulation, Rev. Sci. Instrum. 2000, 71, 2196-2200.

[0086] While the invention has been particularly shown and described with reference to particular embodiment(s) thereof, it will be understood by those skilled in the art that various other changes in the form and details may be made without departing from the spirit and scope of the invention. One skilled in the art of microfluidic flows, for example, would recognize that downstream or upstream analogues of mechanisms described herein may be substituted for the particular exemplary structures disclosed herein.

1.-**71**. (canceled)

72. A microfluidic device for separating particles according to size comprising a microfluidic channel, and an array comprising a network of gaps within the microfluidic channel, wherein the device employs a field that propels the particles being separated through the microfluidic channel; and wherein a flux of the field from the gaps is divided unequally into a major flux component and a minor flux component into subsequent gaps in the network such that the average direction of the major flux components is not parallel to the average direction of the field, and, when particles are introduced into the array, particles having a size less than a predetermined critical size are transported generally in the average direction of the field, and particles having a size at least that of the critical size are transported generally in the average direction of the major flux component, thereby separating the particles according to size.

73. The microfluidic device of claim 72, wherein the array is an ordered array of obstacles.

74. The microfluidic device of claim 73, wherein the ordered array of obstacles comprises obstacles arranged in rows, wherein each subsequent row of obstacles is shifted laterally with respect to the previous row.

75. The microfluidic device of claim 73, wherein the ordered array of obstacles is tilted at an offset angle θ with respect to the direction of the field.

76. The microfluidic device of claim 72, wherein the field is fluid flow, electrical, electro-osmotic, gravitational, hydrodynamic, pressure gradient, or capillary action.

77. The microfluidic device of claim 76, wherein the field is a fluid flow.

78. The microfluidic device of claim 76, wherein the field is an electrical field.

79. The microfluidic device of claim 72, wherein the particles are single celled organisms, formed bodies as in blood, cells, viruses, nucleic acids, proteins, protein complexes, polymers, emulsions, or colloids.

80. The microfluidic device of claim 72, wherein the particles are DNA molecules.

81. The microfluidic device of claim 72, further comprising a first output, configured to accept particles having at least the predetermined critical size, and a second output, configured to accept particles smaller than the predetermined critical size.

82. The microfluidic device of claim 72, further comprising a boundary, such that, when particles having a size at least as large as the predetermined critical size are introduced into the array, the particles having a size at least as large as the predetermined critical size are transported generally to the boundary, thereby concentrating the particles at the boundary.

83. A microfluidic device for separating particles according to size comprising: a microfluidic channel, and an ordered array of obstacles within the microfluidic channel, wherein the device employs a field that propels the particles being separated through the microfluidic channel; and the ordered array of obstacles is asymmetric with respect to the average direction of the field, such that, when particles are introduced into the array, particles having a size less than a predetermined critical size are transported in a first direction, and particles having a size at least that of the critical size are transported in a second direction, wherein the first and second directions are different, thereby separating the particles according to size.

84. The microfluidic device of claim 83, wherein the ordered array of obstacles comprises obstacles arranged in rows, wherein each subsequent row of obstacles is shifted laterally with respect to the previous row.

85. The microfluidic device of claim 83, wherein the ordered array of obstacles is tilted at an offset angle θ with respect to the direction of the field.

86. The microfluidic device of claim 83, wherein the field is fluid flow, electrical, electrophoretic, electro-osmotic, gravitational, hydrodynamic, pressure gradient, or capillary action.

87. The microfluidic device of claim 86, wherein the field is a fluid flow.

88. The microfluidic device of claim 86, wherein the field is an electrical field.

89. The microfluidic device of claim 83, wherein the particles are single celled organisms, formed bodies as in blood, cells, viruses, nucleic acids, proteins, protein complexes, polymers, emulsions, or colloids.

90. The microfluidic device of claim 89, wherein the particles are DNA molecules.

91. The microfluidic device of claim 83, further comprising a first output configured to accept particles transported in the first direction and a second output configured to accept particles transported in the second direction.

92. The microfluidic device of claim 83, further comprising a boundary, such that, when particles having a size at least as large as the predetermined critical size are introduced into the array, the particles having a size at least as large as the predetermined critical size are transported generally to the boundary, thereby concentrating the particles at the boundary.

93. A method for separating particles according to size comprising:

introducing the particles to be separated into a microfluidic channel comprising a network of gaps within the microfluidic channel; and applying a field to the particles to propel the particles through the microfluidic channel, wherein a flux of the field from the gaps is divided unequally into a major flux component and a minor flux component into subsequent gaps in the network such that the average direction of the major flux components is not parallel to the average direction of the field, and particles having a size less than a predetermined critical size are transported generally in the average direction of the field, and particles having a size at least that of the critical size are transported generally in the average direction of the major flux component, thereby separating the particles according to size.

94. The method of claim 93, wherein the network of gaps is constructed from an array of obstacles.

95. The method of claim 94, wherein the array of obstacles is an ordered array of obstacles.

96. The method of claim 95, wherein the ordered array of obstacles comprises obstacles arranged in rows, wherein each subsequent row of obstacles is shifted laterally with respect to the previous row.

97. The method of claim 95, wherein the ordered array of obstacles is tilted at an offset angle θ with respect to the direction of the field.

98. The method of claim 93, wherein the field is fluid flow, electrical, electro-osmotic, gravitational, hydrodynamic, pressure gradient, or capillary action.

99. The method of claim 98, wherein the field is a fluid flow.

100. The method of claim 98, wherein the field is an electrical field.

101. The microfluidic device of claim 93, wherein the particles are single celled organisms, formed bodies as in blood, cells, viruses, nucleic acids, proteins, protein complexes, polymers, emulsions, or colloids.

102. The method of claim 101, wherein the particles are DNA molecules.

103. The method of claim 93, further comprising introducing the particles having at least the predetermined critical size into a first output and the particles smaller than the predetermined critical size into a second output.

104. The method of claim 93, further comprising transporting the particles having a size at least as large as the predetermined critical size to a boundary of the microfluidic channel, thereby concentrating the particles at the boundary.

105. A method for separating particles according to size comprising:

introducing the particles to be separated into a microfluidic channel comprising an ordered array of obstacles; and

applying a field to the particles to propel the particles through the microfluidic channel, wherein the ordered array of obstacles is asymmetric with respect to the average direction of the field, such that particles having a size less than a predetermined critical size are transported in a first direction, and particles having a size at least that of the critical size are transported in a second direction, wherein the first and second directions are different, thereby separating the particles according to size.

106. The method of claim 105, wherein the ordered array of obstacles comprises obstacles arranged in rows, wherein each subsequent row of obstacles is shifted laterally with respect to the previous row.

107. The method of claim 105, wherein the ordered array of obstacles is tilted at an offset angle θ with respect to the direction of the field.

108. The method of claim 105, wherein the field is fluid flow, electrical, electro-osmotic, gravitational, hydrodynamic, pressure gradient, or capillary action.

109. The method of claim 108, wherein the field is a fluid flow.

110. The method of claim 108, wherein the field is an electrical field.

111. The microfluidic device of claim 105, wherein the particles are single celled organisms, formed bodies as in blood, cells, viruses, nucleic acids, proteins, protein complexes, polymers, emulsions, or colloids.

112. The microfluidic device of claim 111, wherein the particles are DNA molecules.

113. The method of claim 105, further comprising introducing the particles transported in the first direction into a first output and the particles transported in the second direction into a second output.

114. The method of claim 105, further comprising transporting the particles having a size at least as large as the predetermined critical size to a boundary of the microfluidic channel, thereby concentrating the particles at the boundary.

115. A microfluidic device for concentrating particles, comprising a microfluidic channel, an array comprising a network of gaps within the microfluidic channel, and a boundary, wherein the device employs a field that propels the particles being concentrated through the microfluidic channel; and wherein a flux of the field from the gaps is divided unequally into a major flux component and a minor flux component into subsequent gaps in the network, such that the average direction of the major flux components is not parallel to the average direction of the field, and, when particles having a size at least as large as a predetermined critical size are introduced into the array, the particles are transported generally towards the average direction of the major flux component to the boundary, thereby concentrating the particles at the boundary.

116. The microfluidic device of claim 115, wherein the array is an ordered array of obstacles.

117. The microfluidic device of claim 116, wherein the ordered array of obstacles comprises obstacles arranged in rows, wherein each subsequent row of obstacles is shifted laterally with respect to the previous row; or the ordered array of obstacles is tilted at an offset angle θ with respect to the direction of the field; or a combination thereof.

118. The microfluidic device of claim 115, wherein the field is fluid flow, electrical, electro-osmotic, gravitational, hydrodynamic, pressure gradient, or capillary action.

119. The microfluidic device of claim 118, wherein the field is a fluid flow.

120. The microfluidic device of claim 118, wherein the field is an electrical field.

121. The microfluidic device of claim 115, wherein the particles are single celled organisms, formed bodies as in blood, cells, viruses, nucleic acids, proteins, protein complexes, polymers, emulsions, or colloids.

122. The microfluidic device of claim 115, wherein the particles are DNA molecules.

123. The microfluidic device of claim 115, wherein the microfluidic channel contains more than one array.

124. The microfluidic device of claim 115, further comprising an output, configured to accept particles from the boundary of the array.

125. A method for separating particles according to size comprising:

introducing the particles to be separated into a microfluidic channel comprising a plurality of obstacles within the microfluidic channel, wherein the obstacles are an ordered array of obstacles comprising obstacles forming rows, wherein each subsequent row of obstacles is shifted laterally with respect to the previous row; and

applying a field to the particles to propel the particles through the microfluidic channel, such that the average direction of the particles having a size at least that of a predetermined critical size is not parallel to the average direction of the field, and particles having a size less than the predetermined critical size are transported generally in the average direction of the field, thereby separating the particles according to size.

126. The method of claim 125, wherein the ordered array of obstacles is tilted at an offset angle θ with respect to the direction of the field.

127. The method of claim 125, wherein the field is fluid flow, electrical, electro-osmotic, gravitational, hydrodynamic, pressure gradient, or capillary action.

128. The method of claim 127, wherein the field is a fluid flow.

129. The method of claim 127, wherein the field is an electrical field.

130. The microfluidic device of claim 125, wherein the particles are single celled organisms, formed bodies as in blood, cells, viruses, nucleic acids, proteins, protein complexes, polymers, emulsions, or colloids.

131. The method of claim 130, wherein the particles are DNA molecules.

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