

US Patent & Trademark Office

Patent Public Search | Text View

United States Patent Application Publication

20250256276

Kind Code

A1

Publication Date

August 14, 2025

Inventor(s)

Harley; John et al.

PARTICLE MANIPULATION SYSTEM WITH MULTISORT VALVE

Abstract

A particle manipulation system uses a MEMS-based, microfabricated particle manipulation device which has a sample inlet channel, output channels, and a movable member formed on a substrate. The device may be used to separate a target particle from non-target material in a sample stream. In order to improve the sorter speed, accuracy or yield, the particle manipulation system may also include a microfluidic structure which focuses the target particles in a particular portion of the sample inlet channel. The multiple outputs may be implemented using structures outside of, and in addition to, the microfabricated sorter. These may include pneumatic valves, which may transfer the contents of one fluid receptacle to another. This may enable simple and inexpensive serial sorting.

Inventors: Harley; John (Santa Barbara, CA), SHIELDS; Kevin (Goleta, CA), GRUMMITT; Daryl (Santa Barbara, CA), Li; Lily (Goleta, CA), Finnen; Ben (Goleta, CA), NAIVAR; Mark (Goleta, CA)

Applicant: Miltenyi Biotec, Inc. (Gaithersburg, MD)

Family ID: 96661449

Assignee: Miltenyi Biotec, Inc. (Gaithersburg, VA)

Appl. No.: 19/048874

Filed: February 08, 2025

Related U.S. Application Data

us-provisional-application US 63552798 20240213

Publication Classification

Int. Cl.: B01L3/00 (20060101)

U.S. Cl.:

CPC **B01L3/502707** (20130101); **B01L3/502738** (20130101); **B01L3/502761** (20130101); B01L2200/0652 (20130101); B01L2300/123 (20130101); B01L2400/0487 (20130101); B01L2400/0638 (20130101)

Background/Summary

CROSS REFERENCE TO RELATED APPLICATIONS [0001] This nonprovisional US patent application claims priority to U.S. Provisional Patent Application Ser. No. 63/552,798, filed Feb. 13, 2024. This prior application is incorporated by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0002] Not applicable.

STATEMENT REGARDING MICROFICHE APPENDIX

[0003] Not applicable.

BACKGROUND

[0004] This invention relates to a system and method for manipulating small particles in a microfabricated fluid channel.

[0005] Microelectromechanical systems (MEMS) are very small, often moveable structures made on a substrate using surface or bulk lithographic processing techniques, such as those used to manufacture semiconductor devices. MEMS devices may be moveable actuators, sensors, valves, pistons, or switches, for example, with characteristic dimensions of a few microns to hundreds of microns. A moveable MEMS switch, for example, may be used to connect one or more input terminals to one or more output terminals, all microfabricated on a substrate. The actuation means for the moveable switch may be thermal, piezoelectric, electrostatic, or magnetic, for example. MEMS devices may be fabricated on a semiconductor substrate which may manipulate particles passing by the MEMS device in a fluid stream.

[0006] Thus, a MEMS device may be a movable valve, used as a sorting mechanism for sorting various particles from a fluid stream, such as cells from blood. The particles may be transported to the sorting device within the fluid stream enclosed in a microchannel, which flows under pressure. Upon reaching the MEMS sorting device, the sorting device directs the particles of interest such as a blood stem cell, to a separate receptacle, and directs the remainder of the fluid stream to a waste receptacle.

[0007] MEMS-based cell sorter systems may have substantial advantages over existing fluorescence-activated cell sorting systems (FACS) known as flow cytometers. Flow cytometers are generally large and expensive systems which sort cells based on a fluorescence signal from a tag affixed to the cell of interest. The cells are diluted and suspended in a sheath fluid, and then separated into individual droplets via rapid decompression through a nozzle. After ejection from a nozzle, the droplets are separated into different bins electrostatically, based on the fluorescence signal from the tag. Among the issues with these systems are cell damage or loss of functionality due to the decompression, difficult and costly sterilization procedures between sample, inability to re-sort sub-populations along different parameters, and substantial training necessary to own, operate and maintain these large, expensive pieces of equipment. For at least these reasons, use of flow cytometers has been restricted to large hospitals and laboratories and the technology has not been accessible to smaller entities.

[0008] A number of patents have been granted which are directed to such MEMS-based particle sorting devices.

[0009] For example, U.S. Pat. No. 6,838,056 (the '056 patent) is directed to a MEMS-based cell

sorting device, U.S. Pat. No. 7,264,972 b1 (the '972 patent) is directed to a micromechanical actuator for a MEMS-based cell sorting device. U.S. Pat. No. 7,220,594 (the '594 patent) is directed to optical structures fabricated with a MEMS cell sorting apparatus, and U.S. Pat. No. 7,229,838 (the '838 patent) is directed to an actuation mechanism for operating a MEMS-based particle sorting system. Additionally, U.S. patent application Ser. No. 13/374,899 (the '899 application) and Ser. No. 13/374,898 (the '898 application) provide further details of other MEMS designs. Each of these patents ('056, '972, '594 and '838) and patent applications ('898 and '899) is hereby incorporated by reference.

SUMMARY

[0010] One feature of the MEMS-based microfabricated particle sorting system is that the fluid may be confined to small, microfabricated channels formed in a semiconductor substrate throughout the sorting process. The MEMS device may be a valve which separates one or more target particles from other components of a sample stream. The MEMS device may redirect the particle flow from one channel into another channel, when a signal indicates that a target particle is present. This signal may be photons from a fluorescent tag which is affixed to the target particles and excited by laser illumination in an interrogation region upstream of the MEMS device. Thus, the MEMS device may be a particle or cell sorter operating on a fluid sample confined to a microfabricated fluidic channel, but using detection means similar to a FACS flow cytometer.

[0011] A substantial improvement may be made over the prior art devices by having at least one of the microfabricated fluidic channels route the flow out of the plane of fabrication of the microfabricated valve. A valve with such an architecture has the advantage that the pressure resisting the valve movement is minimized when the valve opens or closes, because the movable member is not required to move a column of fluid out of the way. Instead, the fluid containing the non-target particles may move over and under the movable member to reach the waste channel. Furthermore, the force-generating apparatus may be disposed closer to the movable valve, resulting in higher forces and faster actuation speeds. As a result, the time required to open or close the valve may be much shorter than the prior art valve, improving sorting speed and accuracy. The systems and methods disclosed here may describe such a microfabricated particle sorting device with at least one out-of-plane channel. Furthermore, because of the small size of the features used in such a device, a fluidic focusing mechanism can dramatically improve the performance of the device by urging the particles into a portion of the fluidic channel. By locating the particles, the uncertainty is diminished, which may improve the sort speed and accuracy.

[0012] The particle manipulation device may further comprise a sheath fluid inlet in fluid communication with the sample inlet channel; and a focusing element coupled to the sheath fluid inlet, which is configured to urge the target particles into a particular portion of the sample inlet channel, wherein the focusing element comprises a microfabricated fluid channel with one substantially straight sidewall segment and an adjacent curved sidewall segment, wherein the straight and the curved sidewall segments define a fluid channel segment with a variable channel width. These variable channel width segments may define expansion/contraction cavities within the microfluidic channel, wherein the cavity is defined by the expanding portion followed by the contracting portion.

[0013] The particles suspended in the fluid stream may experience hydrodynamic forces as a result of these cavities. The first may be an inertial lift force, which is a combination of shear gradient lift resulting from the flow profile parabolic nature, and wall lift force. In addition, the particles may experience Dean flow drag: which is the drag force exerted on the particles as a result of the secondary dean flow induced by curved streamlines within the cavities. It is possible to balance these two forces by proper selection of the geometrical parameters of height, size, aspect ratio and placement with respect to the expansion/contraction cavities. Accordingly, these two forces may be balanced by introduction of the expansion-contraction cavities described below. This balance has not been achieved heretofore, but it may be achieved using the geometrical ranges set forth here.

[0014] The device may also be equipped with a particulate filter. The filter may further include filter barrier elements, wherein filter barrier elements are spaced so as to allow fluid to flow therethrough, but to trap debris and contamination flowing in the sample stream. The filter may also have a transparent layer above the filter elements, which may allow analysis, identification and removal of the contamination. Accordingly, the transparent layer may allow viewing of the material trapped in the filter.

[0015] Because of the effective focusing apparatus and filter element, the particles may arrive at the sorter free of debris or contaminants, and in a tightly confined streamline in a particular portion of the microchannel. In effect, because the particles are in a well-defined portion of the channel and with a well-defined velocity, some novel sort strategies may be brought to bear on the particles within the system. In particular, it is possible that a plurality of sort output paths may be provided, and each target particle may be directed into one of the plurality of sort output paths. The details of the current pulse delivered to the electromagnetic actuation means may determine which of a plurality of sort output paths the trajectory of the particle takes. In other words, in addition to a waste channel for non-target material, the target particle may be directed into one of a plurality of sort output channels. Such a multi-channel sorting valve (“multisort valve”), that is, a microfabricated particle sorting valve having a plurality of sort output channels, is described below.

[0016] Accordingly, a micromechanical particle manipulation device is described which is formed on a surface of a fabrication substrate. The device may include a microfabricated, movable member formed on the substrate, wherein the movable member moves from a first position to a second position in response to a sort waveform applied to an actuator, which generates a force to move the movable member, wherein the motion is substantially in a plane parallel to the surface of the substrate. The device may further include a sample inlet channel formed in the substrate and through which a fluid flows, the fluid including target particles and non-target material, wherein the flow in the sample inlet channel is substantially parallel to the surface. The device may also include at least three separate output channels including at least two separate sort output channels into which the microfabricated movable member diverts the target particles, and a waste output channel into which the non-target material flows, and wherein the flow in waste output channel is substantially orthogonal to the plane, and wherein the waste output channel is located directly below at least a portion of the microfabricated member over at least a portion of its motion, wherein the target particles are diverted into one of the at least two separate sort output channels by the movable member, depending on the characteristics of the sort waveform.

[0017] In other embodiments, the multiple outputs may be implemented using structures outside of, and in addition to, the microfabricated sorter. These may include pneumatic valves, which may transfer the contents of one fluid receptacle to another. This may enable simple and inexpensive serial sorting.

[0018] These and other features and advantages are described in, or are apparent from, the following detailed description.

Description

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] Various exemplary details are described with reference to the following figures, wherein:

[0020] FIG. 1 is a simplified plan view of a microfabricated particle sorting system in the quiescent (no sort) position;

[0021] FIG. 2 is a simplified plan view of a microfabricated particle sorting system in the actuated (sort) position;

[0022] FIG. 3a is a simplified plan view of a microfabricated particle sorting system showing the field of view of the detector, with the microfluidic valve in the quiescent (no sort) position; FIG. 3b

is a simplified illustration of a microfabricated particle sorting system showing the field of view of the detector, with the microfluidic valve in the actuated (sort) position;

[0023] FIG. **4a** is a simplified cross sectional view of a microfabricated particle sorting system in the actuated (sort) position, showing the flow of the sample stream into the sort channel which is in the same plane as the sample inlet channel; FIG. **4b** is a simplified cross sectional view of a microfabricated particle sorting system in the quiescent (no sort) position, showing the flow of the sample stream into the waste channel which is not in the same plane as the sample inlet channel; FIG. **4c** is a simplified cross sectional view of a microfabricated particle sorting system in the quiescent (no sort) position, showing the flow of the sample stream into the waste channel which is not in the same plane as the sample inlet channel, wherein the sample stream flows around the top and the bottom of the diverter;

[0024] FIG. **5** is a simplified plan view of a microfabricated particle sorting system in the quiescent (no sort) position, showing the stationary magnetically permeable feature;

[0025] FIG. **6** is a plan view of the actuation mechanism for the microfabricated particle sorting system, showing the functioning of the external magnetic field in combination with the stationary magnetically permeable feature;

[0026] FIG. **7** is a plan view of the actuation mechanism for the microfabricated particle sorting system, showing the functioning of the external magnetic field in combination with the stationary magnetically permeable feature, in the actuated (sort) position;

[0027] FIG. **8** is a plan view of the microfabricated particle sorting system in combination with a hydrodynamic focusing manifold;

[0028] FIG. **9** is simplified schematic diagram of the novel variable cross section focusing channel;

[0029] FIG. **10** is simplified schematic diagram of the forces operating in the novel variable cross section focusing channel; (a) shows the contours of the device and the streamlines therein; (b) shows the cross sectional dimensions and the flow direction; (c) shows the stable regions in the flow; and (d) shows the hydrodynamic forces acting on the particles;

[0030] FIG. **11a** is simplified schematic diagram of an embodiment of an acoustic focusing element acting on a microchannel of the particle manipulation device; FIG. **11b** shows the acoustic waves in the microchannel.

[0031] FIG. **12** is a cross sectional view of a microfabricated particle filter that may be used with the microfabricated particle manipulation device described here;

[0032] FIG. **13** is a plan view of a microfabricated particle filter that may be used with the microfabricated particle manipulation device described here;

[0033] FIG. **14** is a simplified illustrative view of a plural sort valve in a first waste position;

[0034] FIG. **15** is a simplified illustrative view of a plural sort valve in a second sort position using long solenoid hold operation;

[0035] FIG. **16** shows the actuator in two different positions. FIG. **16A** is a simplified illustrative view of a plural sort valve in a second sort position using a normal (short) solenoid hold actuation FIG. **16B** is a simplified illustrative view of a plural sort valve in a second sort position using a normal (short) solenoid hold actuation;

[0036] FIG. **17** shows another embodiment of the multi-sort valve. FIG. **17A**, FIG. **17B** and FIG. **17C** show the actuator in three different positions;

[0037] FIG. **18** is an exemplary excitation profile for electromagnet for sorting into the first sort channel;

[0038] FIG. **19** is an exemplary excitation profile for electromagnet for sorting into the first sort channel;

[0039] FIG. **20** is a schematic of general sorting into multiple channels;

[0040] FIG. **21** is schematic of general sorting into multiple output channels;

[0041] FIGS. **22a**, **22b** and **22c** is schematic of general sorting into interposer based output channels;

[0042] FIGS. **23a**, **23b** and **23c** is schematic of another embodiment of sorting into interposer based output channels; and

[0043] FIG. **24** is a system level view of the multisort valve included in a particle manipulation system.

[0044] It should be understood that the drawings are not necessarily to scale, and that like numbers may refer to like features.

DETAILED DESCRIPTION

[0045] The system described herein is a particle sorting system which may make use of the microchannel architecture of a MEMS particle manipulation system. More generally, the systems and methods describe a particle manipulation system with a sample inlet channel and a plurality of output channels, wherein at least one of the plurality of output channels is disposed in a different plane than the sample inlet channel. In addition, these microfluidic devices are made with very tight tolerances and narrow separations, which can benefit significantly from focusing the suspended particles into a smaller portion of the flow channel. Coupled with a hydrodynamic focusing element, a stream of particles can be formed within the channels that has well-defined spatial and hydrodynamic properties. This well controlled situation may enable novel particle sorting protocols, such as the one described below. As will be made clear in the discussion below, this architecture has some significant advantages relative to the prior art.

[0046] In the figures discussed below, similar reference numbers are intended to refer to similar structures, and the structures are illustrated at various levels of detail to give a clear view of the important features of this novel device. It should be understood that these drawings do not necessarily depict the structures to scale, and that directional designations such as “top,” “bottom,” “upper,” “lower,” “left” and “right” are arbitrary, as the device may be constructed and operated in any particular orientation. In particular, it should be understood that the designations “sort” and “waste” are interchangeable, as they only refer to different populations of particles, and which population is called the “target” or “sort” population is arbitrary.

[0047] FIG. **1** is a plan view illustration of the novel microfabricated fluidic device **100** in the quiescent (un-actuated) position. The device **100** may include a microfabricated fluidic valve or movable member **110** and a number of microfabricated fluidic channels **120**, **122** and **140**. The fluidic valve **110** and microfabricated fluidic channels **120**, **122** and **140** may be formed in a suitable substrate, such as a silicon substrate, using MEMS lithographic fabrication techniques as described in greater detail below. The fabrication substrate may have a fabrication plane in which the device is formed and in which the movable member **110** moves.

[0048] A sample stream may be introduced to the microfabricated fluidic valve **110** by a sample inlet channel **120**. The sample stream may contain a mixture of particles, including at least one desired, target particle and a number of other undesired, nontarget materials. The particles may be suspended in a fluid. For example, the target particle may be a biological material such as a stem cell, a cancer cell, a zygote, a protein, a T-cell, a bacteria, a component of blood, a DNA fragment, for example, suspended in a buffer fluid such as saline. The sample inlet channel **120** may be formed in the same fabrication plane as the valve **110**, such that the flow of the fluid is substantially in that plane. The motion of the valve **110** is also within this fabrication plane. The decision to sort/save or dispose/waste a given particle may be based on any number of distinguishing signals. In one exemplary embodiment, the decision is based on a fluorescence signal emitted by the particle, based on a fluorescent tag affixed to the particle and excited by an illuminating laser. The distinction between the target particles and non-target material may be made in laser interrogation region **101**. There may be a plurality of laser interrogation regions **101**, although only one is shown in FIG. **1**. Details as to this detection mechanism are well known in the literature, and further discussed below with respect to FIG. **21**. However, other sorts of distinguishing signals may be anticipated, including scattered light or side scattered light which may be based on the morphology of a particle, or any number of mechanical, chemical, electric or magnetic effects that can identify a

particle as being either a target particle, and thus sorted or saved, or a nontarget particle and thus rejected or otherwise disposed of.

[0049] With the valve **110** in the position shown, the input stream passes unimpeded to an output orifice and channel **140** which is out of the plane of the sample inlet channel **120**, and thus out of the fabrication plane of the device **100**. That is, the flow is from the sample inlet channel **120** to the output orifice **140**, from which it flows substantially vertically, and thus orthogonally to the sample inlet channel **120**. This output orifice **140** leads to an out-of-plane channel that may be perpendicular to the plane of the paper showing FIG. **1**, and depicted in the cross sectional views of FIGS. **4a-4c**. More generally, the output channel **140** is not parallel to the plane of the sample inlet channel **120** or sort channel **122**, or the fabrication plane of the movable member **110**.

[0050] The output orifice **140** may be a hole formed in the fabrication substrate, or in a covering substrate that is bonded to the fabrication substrate. A relieved area above and below the sorting valve or movable member **110** allows fluid to flow above and below the movable member **110** to output orifice **140**, and shown in more detail in FIGS. **4a-4c**. Further, the valve **110** may have a curved diverting surface **112** which can redirect the flow of the input stream into a sort output stream, as described next with respect to FIG. **2**. The contour of the orifice **140** may be such that it overlaps some, but not all, of the sample inlet channel **120** and sort channel **122**. By having the contour **112** overlap the sample inlet channel, and with relieved areas described above, a route exists for the input stream to flow directly into the waste orifice **140** when the movable member or valve **110** is in the un-actuated waste position.

[0051] FIG. **2** is a plan view of the microfabricated device **100** in the actuated position. In this position, the movable member or valve **110** is deflected upward into the position shown in FIG. **2**. The diverting surface **112** is a sorting contour which redirects the flow of the sample inlet channel **120** into the sort output channel **122**. The output sort channel **122** may lie in substantially the same plane as the sample inlet channel **120**, such that the flow within the sort channel **122** is also in substantially the same plane as the flow within the sample inlet channel **120**. There may be an angle α between the sample inlet channel **120** and the sort channel **122**. This angle may be any value up to about 90 degrees. Actuation of movable member **110** may arise from a force from force-generating apparatus **400**, shown generically in FIG. **2**. In some embodiments, force-generating apparatus may be an electromagnet, however, it should be understood that force-generating apparatus may also be electrostatic, piezoelectric, or some other means to exert a force on movable member **110**, causing it to move from a first position (FIG. **1**) to a second position (FIG. **2**).

[0052] More generally, the micromechanical particle manipulation device shown in FIGS. **1** and **2** may be formed on a surface of a fabrication substrate, wherein the micromechanical particle manipulation device may include a microfabricated, movable member **110** having a first diverting surface **112**, wherein the movable member **110** moves from a first position to a second position in response to a force applied to the movable member, wherein the motion is substantially in a plane parallel to the surface, a sample inlet channel **120** formed in the substrate and through which a fluid flows, the fluid including one or more target particles and non-target material, wherein the flow in the sample inlet channel is substantially parallel to the surface, and a plurality of output channels **122**, **140** into which the microfabricated member diverts the fluid, and wherein the flow in at least one of the output channels **140** is not parallel to the plane, and wherein at least one output channel **140** is located directly below at least a portion of the movable member **110** over at least a portion of its motion.

[0053] In one embodiment, the diverting surface **112** may be nearly tangent to the input flow direction as well as the sort output flow direction, and the slope may vary smoothly between these tangent lines. In this embodiment, the moving mass of the stream has a momentum which is smoothly shifted from the input direction to the output direction, and thus if the target particles are biological cells, a minimum of force is delivered to the particles. As shown in FIGS. **1** and **2**, the

micromechanical particle manipulation device **100** has a first diverting surface **112** with a smoothly curved shape, wherein the surface which is substantially tangent to the direction of flow in the sample inlet channel at one point on the shape and substantially tangent to the direction of flow of a first output channel at a second point on the shape, wherein the first diverting surface diverts flow from the sample inlet channel into the first output channel when the movable member **110** is in the first position, and allows the flow into a second output channel in the second position.

[0054] In other embodiments, the overall shape of the diverter **112** may be circular, triangular, trapezoidal, parabolic, or v-shaped for example, but the diverter serves in all cases to direct the flow from the sample inlet channel to another channel.

[0055] It should be understood that although channel **122** is referred to as the “sort channel” and orifice **140** is referred to as the “waste orifice”, these terms can be interchanged such that the sort stream is directed into the waste orifice **140** and the waste stream is directed into channel **122**, without any loss of generality. Similarly, the “sample inlet channel” **120** and “sort channel” **122** may be reversed. The terms used to designate the three channels are arbitrary, but the inlet stream may be diverted by the valve **110** into either of two separate directions, at least one of which does not lie in the same plane as the other two. The term “substantially” when used in reference to an angular direction, i.e. substantially tangent or substantially vertical, should be understood to mean within 15 degrees of the referenced direction. For example, “substantially orthogonal” to a line should be understood to mean from about 75 degrees to about 105 degrees from the line.

[0056] FIGS. **3a** and **3b** illustrate an embodiment wherein the angle α between the sample inlet channel **120** and the sort channel **122** is approximately zero degrees. Accordingly, the sort channel **122** is essentially antiparallel to the sample inlet channel **120**, such that the flow is from right to left in the sample inlet channel **120**. With valve **110** in the un-actuated, quiescent position shown in FIG. **3a**, the inlet stream flows straight to the waste orifice **140** and vertically out of the device **100**.

[0057] In FIG. **3b**, the valve **110** is in the actuated, sort position. In this position, the flow is turned around by the diverting surface **112** of the valve **110** and into the antiparallel sort channel **122**. This configuration may have an advantage in that the field of view of the detector **150** covers both the sample inlet channel **120** and the sort channel **122**. Thus a single set of detection optics may be used to detect the passage of a target particle through the respective channels. It may also be advantageous to minimize the distance between the detection region and the valve **110**, in order to minimize the timing uncertainty in the opening and closing of the valve.

[0058] The movable member or valve **110** may be attached to the substrate with a flexible spring **114**. The spring may be a narrow isthmus of substrate material. In the example set forth above, the substrate material may be single crystal silicon, which is known for its outstanding mechanical properties, such as its strength, low residual stress and resistance to creep. With proper doping, the material can also be made to be sufficiently conductive so as to avoid charge build up on any portion of the device, which might otherwise interfere with its movement. The spring may have a serpentine shape as shown, having a width of about 1 micron to about 10 microns and a spring constant of between about 10 N/m and 100 N/m, and preferably about 40 N/m

[0059] FIGS. **4a**, **4b**, **4c** are cross sectional views illustrating the operation of the out-of-plane waste channel **140**. FIG. **4c** is slightly enlarged relative to FIGS. **4a** and **4b**, in order to show detail of the flow around the movable member **110** and into the waste channel **142** through waste orifice **140**. In this embodiment, the waste channel **142** is vertical, substantially orthogonal to the inlet stream **120** and sort stream **122**. It should be understood that other embodiments are possible other than orthogonal, but in any event, the flow into waste channel **142** is out of the plane of the flow in the sample inlet channel **120** and/or sort channel **122**. As shown in FIG. **4a**, with the valve in the sort, actuated position, the inlet stream and target particle may flow into the sort stream, which in FIG. **4a** is out of the paper, and the waste orifice **140** is largely, though not completely, blocked by the movable member **110**. The area **144** (shown more clearly in FIG. **4c**) on top of the valve or movable member **110** may be relieved to provide clearance for this flow.

[0060] When the valve or movable member **110** is un-actuated as in FIG. **4b**, the flow of the sample inlet channel **120** may flow directly into the waste channel **142** by going over, around or by the movable member or valve **110**. The area **144** on top of the valve or movable member **110** may be relieved to provide clearance for this flow. The relieved area **144** is shown in greater detail in the enlarged FIG. **4c**. Thus, when the movable member **110** is un-actuated, the flow will be sent directly to the waste channel. When the movable member **110** is actuated, most of the fluid will be directed to the sort channel, although liquid may still flow over and under the movable member **110**.

[0061] Thus, the purpose of providing flow both under and over the movable member **110** is to reduce the fluid pressure produced by the actuator motion in the region behind the valve or movable member **110**. In other words, the purpose is to provide as short a path as possible between the high pressure region in front of the valve **110** and the low pressure region behind the valve. This allows the valve to operate with little pressure resisting its motion. As a result, the movable valve **110** shown in FIGS. **1-4c** may be substantially faster than valves which have all channels disposed in the same plane.

[0062] Another advantage of the vertical waste channel **142** is that by positioning it directly underneath a stationary permeable feature **130** and movable permeable feature **116**, the magnetic gap between the permeable features **116** and **130** can be narrower than if the fluidic channel went between them. The narrower gap enables higher forces and thus faster actuation compared to prior art designs. A description of the magnetic components and the magnetic actuation mechanism will be given next, and the advantages of the out-of-plane channel architecture will be apparent.

[0063] FIG. **5** is a plan view of another exemplary embodiment of device **100** of the device **100**, showing the disposition of a stationary permeable feature **130** and further detail of the movable member **110**. In this embodiment, the movable member **110** may include the diverting surface **112**, the flexible hinge or spring **114**, and a separate area **116** circumscribed but inside the line corresponding to movable member **110**. This area **116** may be inlaid with a permeable magnetic material such as nickel-iron permalloy, and may function as described further below.

[0064] A magnetically permeable material should be understood to mean any material which is capable of supporting the formation of a magnetic field within itself. In other words, the permeability of a material is the degree of magnetization that the material obtains in response to an applied magnetic field.

[0065] The terms “permeable material” or “material with high magnetic permeability” as used herein should be understood to be a material with a permeability which is large compared to the permeability of air or vacuum. That is, a permeable material or material with high magnetic permeability is a material with a relative permeability (compared to air or vacuum) of at least about 100, that is, 100 times the permeability of air or vacuum which is about 1.26×10^{-6} H.Math.m.sup.-1. There are many examples of permeable materials, including chromium (Cr), cobalt (Co), nickel (Ni) and iron (Fe) alloys. One popular permeable material is known as Permalloy, which has a composition of between about 60% and about 90% Ni and 40% and 10% iron. The most common composition is 80% Ni and 20% Fe, which has a relative permeability of about 8,000.

[0066] It is well known from magnetostatics that permeable materials are drawn into areas wherein the lines of magnetic flux are concentrated, in order to lower the reluctance of the path provided by the permeable material to the flux. Accordingly, a gradient in the magnetic field urges the motion of the movable member **110** because of the presence of inlaid permeable material **116**, towards areas having a high concentration of magnetic flux. That is, the movable member **110** with inlaid permeable material **116** will be drawn in the direction of positive gradient in magnetic flux.

[0067] An external source of magnetic field lines of flux may be provided outside the device **100**, as shown in FIG. **6**. This source may be an electromagnet **500**. The electromagnet **500** may include a permeable core **512** around which a conductor **514** is wound. The wound conductor or coil **514**

and core **512** generate a magnetic field which exits the pole of the magnet, diverges, and returns to the opposite pole, as is well known from electromagnetism. Accordingly, the movable member **110** is generally drawn toward the pole of the electromagnet **500** as shown in FIG. 7.

[0068] However, the performance of the device **100** can be improved by the use of a stationary permeable feature **130**. The term “stationary feature” should be understood to mean a feature which is affixed to the substrate and does not move relative to the substrate, unlike movable member or valve **110**. A stationary permeable feature **130** may be shaped to collect these diverging lines of flux and refocus them in an area directly adjacent to the movable member **110** with inlaid permeable material. The stationary permeable feature **130** may have an expansive region **132** with a narrower throat **134**. The lines of flux are collected in the expansive region **132** and focused into and out of the narrow throat area **134**. Accordingly, the density of flux lines in the throat area **134** is substantially higher than it would be in the absence of the stationary permeable feature **130**. Thus, use of the stationary permeable feature **130** though optional, allows a higher force, faster actuation, and reduces the need for the electromagnet **500** to be in close proximity to the device **100**. From the narrow throat area **134**, the field lines exit the permeable material and return to the opposite magnetic pole of the external source **500**. But because of the high concentration of field lines in throat area **134**, the permeable material **116** inlaid into movable member **110** may be drawn toward the stationary permeable feature **130**, bringing the rest of movable member with it.

[0069] When the electromagnet is quiescent, and no current is being supplied to coil **514**, the restoring force of spring **114** causes the movable member **110** to be in the “closed” or “waste” position. In this position, the inlet stream passes unimpeded through the device **100** to the waste channel **140**. This position is shown in FIG. 5. When the electromagnet **500** is activated, and a current is applied through coil **514**, a magnetic field arises in the core **512** and exits the pole of the core **512**. These lines of flux are collected and focused by the stationary permeable feature **130** and focused in the region directly adjacent to the throat **134**. As mentioned previously, the permeable portion **116** of the movable member **110** is drawn toward the throat **134**, thus moving the movable member **110** and diverting surface **112** such that the inlet stream in sample inlet channel **120** is redirected to the output or sort channel **122**. This position is shown in FIG. 7.

[0070] Permalloy may be used to create the permeable features **116** and **130**, although it should be understood that other permeable materials may also be used. Permalloy is a well known material that lends itself to MEMS lithographic fabrication techniques. A method for making the permeable features **116** and **130** is described further below.

[0071] As mentioned previously, having the waste channel **140** and **142** directly beneath the movable member or valve **110** allows the movable permeable feature **116** to be disposed much closer to the stationary permeable feature **130**. If instead the waste channel were in the same plane, this gap would have to be at least large enough to accommodate the waste channel, along with associated tolerances. As a result, actuation forces are higher and valve opening and closing times are much shorter. This in turn corresponds to either faster sorting or better sorting accuracy, or both.

[0072] With the use of the electromagnetic actuation technique described above, actuation times on the order of 10 microseconds can be realized. Accordingly, the particle sorting device is capable of sorting particles at rates in excess of 50 kHz or higher, assuming 10 microseconds required to pull the actuator in, and 10 microseconds required to return it to the as-manufactured position.

[0073] Because of the microfabricated nature of particle manipulation device **100**, it lends itself to techniques that can make use of such an enclosed, well defined architecture. One such technique is illustrated in FIG. 8, wherein the microfabricated particle manipulation device may be coupled to a microfabricated fluidic focusing element **300**. The focusing element **300** may include at least one additional sheath fluid inlet channel **320** that provides a sheath fluid to the sample stream and also a z-focusing curve **330** coupled to the sheath fluid inlet channel **320**. The sheath fluid may be used to adjust the concentration or positioning of the target particles within the sample inlet channel. The focusing element **300** may be configured to urge the target particles into a particular portion of the

sample inlet channel **120**, such that the sorting process has fewer errors, as described further below. The focusing element **300** may be disposed in substantially the same plane as the movable member **110**, and may be formed in the same substrate surface as the movable member **110** and sample inlet channel **120**. The focusing element **300** may rely on inertial forces to focus the particles, as will be described further below. These forces may require relatively large flow rates to be effective. The microfabricated particle manipulation system with out-of-plane channel may be particularly suited to such an inertial focusing device, because of the narrow channels and high flow rates.

[0074] FIG. **8** depicts the microfabricated focusing element **300** which may be used to focus the particles in a certain area within the fluid stream. As the name suggests, the sheath fluid inlet channel **320** adds a sheath fluid to the sample stream, which is a buffering fluid which tends to dilute the flow of particles in the stream and locate them in a particular portion of the stream. The combined fluid then flows around a focusing element **300** coupled to the sample inlet channel **120**. The focusing element **300** may include here a z-focusing curve **330**, which tends to herd the particles into a particular plane within the flow. This plane is substantially in the plane of the paper of FIG. **8**. The combined fluid in the focusing element **300** then passes another intersection point, a “y-intersection point” **350**, which introduces additional sheath fluid above and below the plane of particles. At the y-intersection point **350**, two flows may join the z-focus channel **330** from substantially antiparallel directions, and orthogonal to the z-focus channel **330**. This intersection may compress the plane of particles into a single point, substantially in the center of the stream. Accordingly, at the y-intersection point **350** the target particles may be compressed from a plane to a stream line near the center of the z-focus channel **330** and sample inlet channel **120**. Focusing the particles into a certain volume tends to decrease the uncertainty in their location, and thus the uncertainty in the timing of the opening and closing of the movable member or valve **110**. Such hydrodynamic focusing may therefore improve the speed and/or accuracy of the sorting operation.

[0075] In one exemplary embodiment of the microfabricated particle manipulation device **100** with hydrodynamic focusing illustrated in FIG. **8**, the angular sweep of z-bend **330** is a curved arc of about 180 degrees. That is, the approximate angular sweep between the junction of the sheath fluid inlet channel **320** and the y-intersection point **350**, may be about 180 degrees. Generally, the radius of curvature of the z-bend **330** may be at least about 100 microns and less than about 500 microns, and the characteristic dimension, that is the width, of the channels is typically about 50 microns to provide the focusing effect. In one embodiment, the radius of curvature of the channel may be about 250 microns, and the channel widths, or characteristic dimensions, for the sample inlet channel **120** and z-bend channel **330** are on the order of about 50 microns. These characteristic dimensions may provide a curvature sufficient to focus the particles, such that they tend to be confined to the plane of the paper upon exit from the z-focus channel **330** at y-intersection point **350**. This plane is then compressed to a point in the channel at the y-intersection point **350**. Accordingly, the y-intersection **350** flows along with the z-focusing element **330** may urge the particles into a single stream line near the center of the microfabricated sample inlet channel **120**.

[0076] FIG. **9** shows another embodiment of a focusing element, **600**. In this embodiment, the focusing element **600** includes a plurality of segments having a variable lateral dimension or cross section. The variable cross section portion of the channel serves to urge or focus the particles into a particular portion of the stream flowing in the channel. The discussion now turns to the design and performance details of this variable cross section focusing channel as applied to the above described microfabricated particle sorter **100**.

[0077] The novel flow channel may possess portions of variable cross section, wherein the variable cross section arises from the shapes of the sidewalls of the flow channel. These variable portions may have one sidewall which is substantially straight with respect to the flow direction, and an adjacent side wall which is not straight, or at least not parallel to the substantially straight portion. In particular, this adjacent sidewall may be triangular or parabolic in shape, deviating away from the straight sidewall in an expanding region, to a point of maximum channel width, before coming

back to the nominal distance between the sidewalls in a contracting region. The expanding portion, maximum point, and contracting portion may constitute what is hereafter referred to as a fluid “cavity” **620** in the microfabricated channel. Accordingly, the variable channel width segments may define expansion/contraction cavities **620**, **620'** within the microfluidic channel, wherein the cavity is defined by the expanding portion followed by the contracting portion.

[0078] The cavity **620** should be understood to be in fluid communication with the microfabricated fluid channel, such as sample inlet channel **120**, such that fluid flows into and out of the cavity **620**. It should be understood that this cavity **620** may be a two-dimensional widening of the channel in the expanding region, and narrowing of the channel in the contracting region. This shape of geometry is shown schematically in FIG. 9.

[0079] The variable cross section focusing channel **600** may be used instead of the curved focusing channel **300** shown in FIG. 8. That is, the variable cross section focusing channel **600** may be used in place of the z-focusing curve **330**, or in place of the entire focusing element **300**. The variable cross section focusing element **600** may be disposed upstream of the moveable member sorting device **110**.

[0080] The cavity **620** may have a length of L , which may be the distance between the expanding and contracting portions. More particularly, the variable cross section portion, cavity **620**, may have an expanding region **625** and a contracting region **627** disposed over a distance L with a high point **623** between them. The high point **623** may be the point of maximum lateral extent of the channel **600**, that is, the portion of widest channel width. As shown in FIG. 9, the variable cross section focusing channel **600** may include a plurality of expanding and contracting regions, such as **620** and **620'** shown in FIG. 9. The expanding and contracting regions may be arranged in different ways with respect to a turn that is made by the channel as it directs the sample fluid from the sample input **310** to the valve mechanism **100** or **110**.

[0081] Because of this shape, and expanding region **625** followed by a contracting region **627**, the variable cross section focusing channel **600** may encourage various eddies, motions and hydrodynamic forces within the focusing element.

[0082] FIG. 9 illustrates quantities that will be used to discuss the various design parameters and their resulting hydrodynamic behaviors in further detail below. H is the height of the variable cross section portion cavity, and L is the length of the cavity portion. W is the nominal width of the sample inlet channel **120** (channel without the expanding and contracting cavities). H/W is the aspect ratio of the variable cross section cavity portion with respect to the nominal channel width. The pitch P is the distance between one cavity **620** and a subsequent cavity **620'**.

[0083] As mentioned previously, various hydrodynamic effects may result from this variable cross section geometry, and these are illustrated in FIG. 10. These effects may result in a geometry induced secondary flow focusing. Particles experience two forces in the flow. The first may be an inertial lift force, which is a combination of shear gradient lift resulting from the flow profile parabolic nature, and wall lift force. In addition, the particles may experience Dean flow drag: which is the drag force exerted on the particle as a result of the secondary dean flow induced by curved streamlines. It is possible to balance these two forces by proper selection of the geometrical parameters of height, size, aspect ratio and placement. Accordingly, these two forces may be balanced by introduction of the expansion-contraction cavities **620** of a particular size, shape and distribution, in the variable cross section element **600**. The combination of geometrical parameters determines whether there is a balance between these forces or not and where in the channel are the equilibrium nodes or points where the net force on the particles is zero.

[0084] As a result of these balanced forces, particles may be focused in one position within the channel using the cavities **620**, **620'** shown in FIG. 9, as the particles are brought to a two dimensional focused state.

[0085] FIG. 10 is simplified schematic diagram of the forces operating in the novel variable cross section focusing channel; (a) shows the contours of the device and the streamlines therein; (b)

shows the cross sectional dimensions and the flow direction; (c) shows the stable regions (equilibrium nodes) in the flow channel without cavities; and (d) shows the hydrodynamic forces acting on the particles as a result of curved streamlines in the cavities;

[0086] As shown in FIG. **10** (a), the cavities **620** in focusing element **600** are generally triangular cavities with a height of H and a base of $2H$. In other words, the cavities may be two adjacently placed equilateral triangles. The width, W , of the nominal channel before and after the cavities **620** and **620'**, is used as a scale factor, to parametrize the quantities as discussed below. The apex of the triangle may be smoothed to discourage bubbles becoming trapped at the apex.

[0087] The cross section of the channel is shown in (b) along with the flow direction in the channel. The inertial focusing effects are shown in FIG. **10**(c). An equilibrium position exists for particles in a straight channel with the same non-varying cross section. The expansion-contraction cavities create an out of plane secondary flow (dean flow) which balances the inertial drag force and changes the equilibrium nodes, as shown in (d). Accordingly, an equilibrium position for the particles will exist as shown in FIG. **10**, as shown in (c).

[0088] Alternatively, the focusing element may be an acoustic focusing structure. Such a structure is shown in FIG. **11a, b**. FIG. **11a** shows an acoustic focusing structure which is achieved by actuating the PZT acoustic transducer element **700** seated under, for example, the sample inlet channel **120** on the microfabricated particle manipulation device **100**. The PZT element **700** may be operating at its resonant frequency. The resonating PZT may launch a bulk acoustic pressure wave **710** into the microfluidic channel **120**, as is shown in FIG. **11b**. This acoustic pressure wave **710** may drive the particles **5** suspended in the flow to the low pressure node in the center of the channel **120**.

[0089] But in any case, because the focusing element tends to herd the particles into a well-defined portion of the sample stream, the uncertainty in gate timing and particle trajectory may be reduced. Accordingly, a multisort system such as described above may be an ideal application for the particle focusing structures described above, because it can make use of the predictable fluid trajectory of the target particles.

[0090] A filter element may be added for the purpose of retaining undesired particles, and placed upstream of the hydrodynamic focusing elements and the movable member **110** of the valve. FIG. **12** shows one such device, with parallel filter elements to allow more filter area and also robustness to filter clogging.

[0091] FIG. **12** is a cross sectional illustration of a microfabricated filter. The filter may be used in, for example, a cell sorting system as described below. In FIG. **12**, a sample stream may include at least one debris particle **5**, suspended therein. The sample stream may be admitted to the filter structure **1** through an inlet channel **12**, from which it may flow laterally across the face of the substrate **10** as shown by the arrows in FIG. **13**. The flow may traverse a series of filter barriers **22**, **24** which are arranged so as not to seal the channel to the flow of the sample stream, but to trap particles of a particular size which may be suspended in the sample stream. In FIGS. **18** and **19**, these filter barriers may be disposed in a staggered arrangement across the width of the channel. However, no barriers extend entirely across the channel so as to seal it against the flow. Instead, the sample stream may flow between the staggered barriers **22** and **24** which may be separated by a distance d . Accordingly, particulate debris with a dimension greater than d may be trapped in the filter **1**.

[0092] As shown in FIG. **12**, the microfabricated channel with filter barriers **22**, **24** may be sealed on top by another layer or substrate **30**. This layer or substrate **30** may be optically transparent, allowing radiation to pass through and impinge upon the trapped particle **5**. The transparent layer **30** may comprise at least one of quartz, sapphire, zirconium, ceramic, and glass. The transparent layer **30** may allow analysis and characterization of the particulate debris found in the sample stream. Such information may be important in identifying and correcting the source of the contamination. FIG. **12** shows evaluation of trapped particle **5** by an analysis unit **40**, such as a

microscope or spectrometer. The analysis technique may include investigation of specular, diffractive, refractive behaviors of the particle **5**, for example. Accordingly, the filter system may include an optical microscope which is disposed adjacent to the filter and is configured to image the particulates intercepted by the plurality of barriers, through the transparent layer **30**. Alternatively, the analysis tool may be a spectrometer which is disposed adjacent to the filter and is configured to analyze the particulates intercepted by the plurality of barriers, through the transparent layer. In other embodiments, x-ray diffraction, crystallography, or other methods may be used to analyze the trapped debris through the transparently layer **30**.

[0093] FIG. **13** is a plan view of the microfabricated filter **2**. FIG. **13** shows effectively the staggered arrangement of the filter barriers **22** and **24**. In one embodiment, each filter barrier **22** extends less than the full diameter, but more than one-half of the diameter of the channel. Accordingly, by staggering pairs of like filter barriers **22**, **24** one behind the other, the channel remains open to the passing of the sample stream but will trap particles of debris with a dimension larger than the distance between the barriers. In other embodiments, the filter barriers **22**, **24** may extend less than $\frac{1}{2}$ the distance across the channel, such that the fluid may flow between the barriers but particulate debris may not. Accordingly, in some embodiments, at least one of the plurality of barriers has a rectangular shape, and there is a varying distance between opposing barriers.

[0094] The plan view of FIG. **13** shows a plurality of parallel paths **32**, **34**, **36** and **38** each with filter barriers **24**, **26**. It should be understood that although the paths **32**, **34**, **36** and **38** may have the same shape of filter barriers **24**, **26** as shown, or they may be different. In some embodiments, the filter barriers may be the same in the parallel paths **32**, **34**, **36** and **38**. In other embodiments, the filter barriers may be different. The paths are shown as being in parallel, but this is also exemplary only, and some filter barrier shapes **32**, **34**, **36** and **38** may be placed serially before or after other filter barrier shapes. It should be appreciated that since the filter barriers are fabricated lithographically, the shapes may be made arbitrarily complex.

[0095] The sample stream may again be input to the filter **2** through an input channel **12**, from which it may flow laterally across the face of the substrate **10** as shown by the arrows in. **32-38**. The flow may traverse a series of filter barriers **22**, **24** in each of the channels **3-38**, which are arranged so as not to seal the channel to the flow of the sample stream, but to trap particles of a particular size which may be suspended in the sample stream. In channels **32-38**, these filter barriers may be disposed in a staggered arrangement across the width of the channel. However, no barriers extend entirely across the channel so as to seal it against the flow. Instead, the sample stream may flow between the staggered barriers **22** and **24** which may be separated by a distance d . Accordingly, particulate debris with a dimension greater than d may be trapped in the filter barriers **22**, **24**.

[0096] In channels **32-38**, the filter barriers may be simple rectangles, similar to filter barriers **22**, **24** in FIGS. **12** and **13**. In other embodiments, the barriers may have different shapes, such as a tapered shape, narrowing from base to tip, triangular or sawtooth. The filter barriers **34** may lean into or away from the flow. The different shapes and orientations may have different behaviors in terms of effectiveness in trapping particles. Each type of filter shape creates a specific flow circulation around it which traps particles based on their characteristics such as the relative rigidity or stiffness of the particle, or how round or rod-shaped a particle is.

[0097] Because of the effective focusing apparatus of FIGS. **8-11** and filter element of FIGS. **12-13**, the particles may arrive at the sorter **100** free of debris or contaminants, and in a tightly confined streamline in a particular portion of the microchannel **120**. In effect, because the particles are in a well-defined portion of the channel and with a well-defined velocity, some unusual sort strategies may be brought to bear on the particles within the system. In particular, it is possible that a plurality of sort output paths may be provided, and each target particle may be directed into one of the plurality of sort output paths. The details of the current pulse delivered to the

electromagnetic actuation means may determine which of a plurality of sort output paths the trajectory of the particle takes. One embodiment of such a multi-channel sorting valve (“multisort valve”), that is, a microfabricated particle sorting valve having a plurality of sort output channels, is described below. In other words, in addition to a waste channel for non-target material and a sort channel for target particles, the target particle may be directed into one of a plurality of sort output channels.

[0098] FIG. **14** is a schematic illustration of a particle manipulation device **100'** which is adapted for the multisort embodiment. Particle manipulation device **100'** may be similar to particle manipulation device **100** in that it has a sample input channel **120**, leading to a movable member **110** which is the sorting device, and a waste channel **140** which flows generally orthogonally to the sample input channel **120**, and orthogonal to the plane of motion of the movable member **110**. More particularly, the waste channel **140** may be into the plane of the paper, and generally orthogonal to that plane.

[0099] However, in contrast to particle manipulation device **100**, particle manipulation device **100'** may have a plurality of sort output channels, all may be generally in the plane of the substrate. Shown in FIG. **14** is sort channel **123'** which is henceforth referred to as sort channel 1, and sort output channel **122'** which is henceforth referred to as sort channel 2. Comparison of FIG. **14** with FIG. **2** reveals that sort channel **122'** is largely similar in size and location to sort channel **122**. The new sort channel **123'** may be located below sort channel 2 **122'**, and may form a larger angle (generally around 60 degrees) to sample input channel **120**. Sort channel 2 **122'** may, as before, form an angle of about 45 degrees to the sample input channel **120**.

[0100] Accordingly, upon entering the sort device **100** and movable member **110'** a target particle **5** may flow into one of a plurality of sort output channels, depending on the results of the laser interrogation and the current pulse applied to the movable member **110'** via the electromagnetic actuator **500**.

[0101] It should be understood that the embodiment shown in FIG. **14** is exemplary only, and that the concepts here can be extended to any number of sort channels in addition to a first sort output channel, which may be disposed at other angles with respect to the first sort output channel.

[0102] As before, the particles may be identified based on a fluorescent signal detected in the laser interrogation region **101**. Depending on the identity of the particle, the decision can be made whether to direct it into sort channel 1 (**123'**), or sort channel 2 (**122'**), or to let it flow into the waste channel **140**. Depending on the outcome of the interrogation, the particle can be directed into the proper path by the choice of the details of the sort pulse applied to the electromagnet **500**, as will be described further below.

[0103] An important parameter in making the multisort device **100'** work properly may be the ratio of fluidic resistance in sort channel 1 compared to fluidic resistance of sort channel 2. In particular, sort channel 1 may be low-resistance path compared to sort channel 2. In other words, sort channel 2 (the nominal “ordinary”) sort channel may have high fluidic resistance compared to sort channel 1.

[0104] In the waste position depicted in FIG. **14**, the valve is not actuated and the sample stream flows directly into the waste channel **140**. Then, for sorting into sort channel 2, the electromagnet **500** may use a standard sort signal which may be relatively long, on the order 200 microseconds. In this period, sort channel 2 may be the only path available during the long sort pulse. Accordingly, the target particle **5** may flow into sort channel 2 if the gate is held in the position shown in FIG. **15** for a sufficiently long time. If there is no actuation at all, of course the particle will flow into the waste path and waste orifice **140**.

[0105] In other words, if the solenoid, and thus the gate or valve is held down for a relatively long time, the target particle may be forced down the only open path, into sort channel 2, despite its relatively high fluid resistance. With the valve in the position of FIG. **14**, the actuator cuts off flow to sort channel 1, and particles can only go to sort channel 2. Particles flowing in sort channel 2

will continue out of chip into a sort reservoir.

[0106] In contrast, in FIG. **16**, the scenario is shown schematically of sorting the target particle **5** into sort channel 1, using a relatively short gate sort signal. During this short gate, sorted particle enters the area between sort channel 1 and sort channel 2 (see FIG. **16A**). But before the particle is forced into sort channel 2, actuator is released and the particle flows into the lower fluidic resistance path, sort channel 1 (see now FIG. **16B**). The movement of the movable member **110'** may assist in moving the particle **5** along this lower path, as when the actuator relaxes, it is pulled downward by the restoring spring discussed above. Particles flowing in sort channel 1 will continue out of chip to another sort reservoir.

[0107] FIG. **17** shows another embodiment of the multisort actuator having 3 different positions. FIG. **17A** shows the multisort actuator in a position wherein the sort channel **120** flows directly into the waste channel **150**. FIG. **17B** shows the actuator directing the sort stream into the sort channel **120**. FIG. **17C** shows the actuation directing the sort channel into a second sort stream **120'** which is different from sort stream **120**. In this way, the multisort actuator may be used to sort a plurality of target species or moieties into separate, different storage receptacles. The actuator or movable member may move to a first, second and third position, to direct the target particles as desired between the plurality of output channels. It should be understood that these concepts may be extended to any number of channels and target species, and is not limited to those described here and illustrate in FIG. **17**. It should also be understood that the identity of the “sort” versus “waste” channels is arbitrary, such that the “sort” may also be understood as the “waste” channel, and the designations serve only to demonstrate that the populations being separated are different. Further, at least one of the first, second and third positions may be metastable. To assist in functioning, the restoring spring **115** may be non-linear in its spring constant over the range of its travel. Details as to its shape and behavior may depend upon the application. “Metastable” should be understood to mean that the movable member can be driven into the state, but will not linger in that position indefinitely without controlling the position of the movable member.

[0108] FIG. **18** is a qualitative illustration of the control signal/waveform which may be delivered to the electromagnetic device **500** to accomplish the sort into sort channel 2. As described above, the sort gate waveform may have a relatively long duration, between about 80 to about 200 microseconds. During this period, the only path available is from the sample inlet channel **120** into the sort channel 2. This duration is sufficient to cause the particle to overcome the fluidic resistance of sort channel 2, because there are no other paths open to it.

[0109] In contrast, FIG. **19** is a qualitative illustration of the control signal/waveform which may be delivered to the electromagnetic device **500** to accomplish the sort into sort channel 1. As described above, the sort gate waveform has a relatively short duration, between about 15 to about 40 microseconds. This duration is insufficient to cause the particle to overcome the fluidic resistance of sort channel 2, and instead it flows into sort channel 1 at the end of the 15-40 microsecond pulse.

[0110] FIG. **20** shows a first embodiment of a system using the multisort valve **100'**. This figure is schematic only, and lacks many of the details illustrated in FIGS. **14-17**. FIG. **20** is intended to illustrate, in general, the distinguishing concepts in this invention, without the details ascribed to a particular embodiment. In particular, sort valve **100'** may have a plurality of sort output channels, or at least two sort output channels **122'** and **123'**. Which of the plurality of sort output channels is invoked may depend on the features of the waveform driving the sort gate or sort valve **100'**.

[0111] In this schematic illustration, as before, the sample stream is input to the multisort valve **100'** by the sample input channel **120**. From the sample channel, the target particle **5** may flow into either the sort channel 2, **122'** or sort channel 1, **123'**. Which of the paths it takes may depend on the results of the laser interrogation and the shape and/or duration of the pulse delivered to the electromagnet **500**. One type of pulse shape, for example, is a long pulse is likely to send the particle **5** into sort channel 2 **122'**. Another, different shape of pulse, for example, is a shorter duration pulse is more likely to send the target particle into sort channel 1, **123'**.

[0112] In another embodiment shown in FIG. 21, the multisort valve **100'** is combined with a focusing element which tends to urge the particle into a particular streamline of the flow channel. One such focusing element may be the variable cross section focusing element **600**, discussed above. However, the focusing element may alternatively be any other sort, such as the z-focus channel. Alternatively, the focusing element may be an acoustic focusing structure. But in any case, because the focusing element tends to herd the particles into a well-defined portion of the sample stream, the uncertainty in gate timing and particle trajectory may be reduced. Accordingly, a multisort system such as described above may be an ideal application for the particle focusing structures described above, because it can make use of the predictable fluid trajectory of the target particles.

[0113] Another embodiment of a multi-output particle sorting device is illustrated schematically in FIGS. 22 and 23. In contrast to the system described above and illustrated by FIGS. 16 and 17, the additional output channels may be formed in the interposer rather than in the silicon chip that the valve is microfabricated in. Thus, in the embodiment, the features may be larger, and easier to interface with larger system elements. In these embodiments, the same microfabricated chip may be used as was illustrated in FIGS. 1-7. Accordingly, this embodiment may not require laborious and expensive changes to mask design and photolithographic processing. Instead, the changes may be made in the "interposer," a general term intended to mean the non-chip-based technology used to hold and plumb to the chip. Interposers may be plastic for example, and made using injection molding techniques, for example. Interposers are discussed in detail in U.S. patent application Ser. No. 14/638,495, filed Mar. 4, 2015. In other words, an interposer may be any structure that holds the fabrication substrate and is plumbed to the fabrication substrate, that is, it is in fluid communication with the fabrication substrate.

[0114] The design may be used to sort a plurality of target particles, stem cells or lymphocytes for example, from non-target material such as red blood cells and lymph. Two target particles are described herein by way of example, but it should be understood that the techniques can be extended to larger numbers of particles. The two target particles are referred to here as **S1** and **S2**.

[0115] The initial situation is illustrated by FIG. 22a. In this figure, a sample input chamber or receptacle is filled with a mixture of **S1**, **S2** and non target material. The situation is now as shown in FIG. 22a.

[0116] In this first pass, the microfabricated sorter or valve, is directed to sort either **S1** or **S2**. So all instances of **S1** or **S2** are sent to the sort receptacle **210**. At this point, the contents of sort receptacle **210** (**S1+S2**) may be withdrawn and reinserted into the now empty sort chamber **200**. The situation is now as shown in FIG. 22b.

[0117] The material may then be resorted, but with the microfabricated valve or actuator controlled on only divert **S1** into the sort chamber **210**, whereas all the remaining particles may either be directed into a second sort receptacle **220**, or simply allowed to pass to the waste chamber "W". The situation is now as shown in FIG. 22c.

[0118] Thus the two now separated particle populations, **S1** may be withdrawn from receptacle **210** and **S2** from receptacle **220** or **W**. This two step process may greatly improve both sorting efficiency and yield. Since the cell sorting system uses gently flowing microchannels and capillaries, the damage to the cells from repetitive handling is minor.

[0119] A second similar concept may be applied to the system to perform a serial sort, but again using the same microfabricated valve or sorter **100**. This concept is illustrated in FIG. 23.

[0120] Initially, a sampler receptacle may be filled with a mixture of a single type of target particle, **S**, suspended in a non-target fluid such as saline, blood or plasma. In the first pass, the valve **100** directs all target particle **S** into sort chamber **210**. The contents of **210** are then returned to the sample chamber **200**, and the process is repeated. Serial sorting such as this may yield much improved purity, by providing two opportunities to separate the target species **S** from the non target material.

[0121] The active elements of this design include, in addition to the microfabricated sorter **100**, other fluid control devices such as the valve between sample chamber **200** and sort chamber **210**. This valve may be a simple suction device, which may be applied to the fluid to evacuate it from the chamber. The selector **190** may similarly be a very simple device such as a flap or diverter. Both of these elements may be exceedingly inexpensive.

[0122] Next is described a particle sorting system **1000** which may make use of the multisort valve **100'** and the focusing element.

[0123] The microfabricated particle manipulation device with multisort capability **100'** with focusing element **600** may be used in a particle sorting system **1000** enclosed in a housing containing the components shown in FIG. **24**. The MEMS particle manipulation devices **100'**, or **100** may be enclosed in a plastic, disposable cartridge which is inserted into the system **1000**. The insertion area may be a movable stage with mechanisms available for fine positioning of the particle manipulation device **100'**, or **100** and associated microfluidic channels against one or more data, which orient and position the detection region and particle manipulation device **100'**, or **100** with respect to the collection optics **1100**. If finer positioning is required, the stage may also be a translation stage, which adjusts the positioning based on observation of the location of the movable member **110** relative to a datum.

[0124] The focusing channel **600** may be formed on the chip or on the interposer as described in U.S. patent application Ser. No. 14/638,495, filed Mar. 4, 2015 and incorporated by reference in its entirety. Similarly, the filter structure **1** or **2** may be formed on the valve chip or the interposer. The interposer may be a plastic structure with channels formed therein, which translates the fluidic passages from the very narrow microfluidic passages formed lithographically on the MEMS substrate, to the macroscopic fluid reservoirs formed in the plastic, disposable cartridge.

[0125] It should be understood that although FIG. **24** shows a multisort particle sorting system **1000** which uses a plurality of laser sources **1400** and **1410**, only a single laser may be required depending on the application. For the plurality of lasers shown in FIG. **24**, one of the laser sources **1410** may be used with an associated set of parallel optics (not shown in FIG. **21**) to illuminate the at least one additional laser interrogation region. This setup may be somewhat more complicated and expensive to arrange than a single laser system, but may have advantages in that the optical and detection paths may be separated for the different laser interrogation regions. For this embodiment, it may not be necessary to alter the trajectory, spectral content, timing or duration of the laser **1410** light. Although not shown explicitly in FIG. **24**, it should be understood that the detection path for additional laser(s) **1410** may also be separate from the detection path for laser **1400**. Accordingly, some embodiments of the particle sorting system **1000** may include a plurality of laser sources and a plurality of optical detection paths, whereas other embodiments may only use a single laser source **1400** and collection optics **1100**. In the embodiment described here, a plurality of excitation lasers uses a common optical path, and the optical signals are separated electronically by the system shown in FIG. **24**.

[0126] The embodiment shown in FIG. **24** is based on a FACS-type detection mechanism, wherein one or more lasers **1400**, **1410** excites one or more fluorescent tags affixed to the target particles. The laser excitation may take place in multiple interrogation regions. The fluorescence emitted as a result are detected and the signal is fed to a computer **1900**. The computer then generates a control signal that controls the electromagnet **500**. It should be understood that other detection mechanisms may be used instead, including electrical, mechanical, chemical, or other effects that can distinguish target particles from non-target particles.

[0127] Accordingly, the MEMS particle sorting system **1000** shown in FIG. **24** may include a number of elements that may be helpful in implementing the additional interrogation regions. First, an optical manipulating means **1600** may alter the trajectory, spectral content, timing or duration of the laser radiation from laser **1400** to the second or third interrogation spots. Examples of items that may be included in optical manipulating means **1600** are a birefringent crystal, spinning prism,

mirror, saturable absorber, acousto-optic modulator, harmonic crystal, Q-switch, for example. More generally, optical manipulating means **1600** may include one or more items that alter laser frequency, amplitude, timing or trajectory along one branch of the optical path to an additional interrogation region.

[0128] For example, optical manipulating means **1600** may include a beamsplitter and/or acousto-optic modulator. The beam splitter may separate a portion of the incoming laser beam into a secondary branch or arm, where this secondary branch or arm passes through the modulator which modulates the amplitude of the secondary beam at a high frequency. The modulation frequency may be, for example, about 2 MHz or higher. The light impinging on the first laser interrogation region **101** may, in contrast, be continuous wave (unmodulated). The secondary branch or arm is then directed to the additional laser interrogation region. This excitation will then produce a corresponding fluorescent pattern from an appropriately tagged cell.

[0129] This modulated fluorescent pattern may then be picked up by the detection optics **1100**, which may recombine the detected fluorescence from other interrogation regions with fluorescence from laser interrogation region **101**. The combined radiation may then impinge on the one or more detectors **1300**.

[0130] An additional optical component **1700** may also alter the frequency, amplitude, timing or trajectory of the second beam path, however, it may perform this operation upstream (on the detector side) of the collection optics **1100** rather than downstream (on the sample side) of it, as does optical component **1600**.

[0131] The output of detectors **1300** may be analyzed to separate the content corresponding to laser interrogation region **101** from the content corresponding to other laser interrogation regions. This may be accomplished by applying some electronic distinguishing means **1800** to the signals from detectors **1300**. The details of electronic distinguishing means **1800** may depend on the choice for optical manipulation means **1600**. For example, the distinguishing means **1800** may include a high pass stage and a low pass stage that is consistent with a photoacoustic modulator that was included in optical manipulating means **1600**. Alternatively, electronic distinguishing means **1800** may include a filter (high pass and/or low pass) and/or an envelope detector, for example.

[0132] Therefore, depending on the choice of optical manipulating means **1600**, the unfiltered signal output from detectors **1300** may include a continuous wave, low frequency portion and a modulated, high frequency portion. After filtering through the high pass filter stage, the signal may have substantially only the high frequency portion, and after the low pass stage, only the low frequency portion. These signals may then be easily separated in the logic circuits of computer **1900**. Alternatively, the high pass filter may be an envelope detector, which puts out a signal corresponding to the envelop of the amplitudes of the high frequency pulses.

[0133] Other sorts of components may be included in electronic distinguishing means **1800** to separate the signals. These components may include, for example, a signal filter, mixer, phase locked loop, multiplexer, trigger, or any other similar device that can separate or distinguish the signals. Component **1800** may also include the high pass and/or low pass electronic filter or the envelope detector described previously. The two sets of signals from the electronic distinguishing means **1800** may be handled differently by the logic circuits **1900** in order to separate the signals.

[0134] The description now turns to the fabrication of the devices shown in FIGS. **1-17**.

Fabrication may begin formation of the valves **100'** or **100**. To make these structures, one may begin with formation of the inlaid permeable features **116** and **130** formed in a first substrate. The substrate may be a single crystal silicon substrate, for example. To form these structures, depressions may be formed in these areas of the substrate surface by etching. First, photoresist may be deposited over the substrate surface and removed over the areas corresponding to **116** and **130**. Then, the trenches may be formed by, for example, etching the substrate in potassium hydroxide (KOH) to form a suitable depression. A seed layer may be deposited conformally over the first substrate surface and patterned to provide the seed layer for plating NiFe into the trenches. The

seed layer may be, for example, Ti/W or Cr/Au and may be deposited by sputtering, CVD or plasma deposition. This layer may be covered with photoresist and patterned according to the desired shape of the areas **116** and **130**. Unwanted areas of photoresist and seed layer may then be removed by chemical etching. The permeable features **116** and **10** may then be deposited over the patterned seed layer by sputtering, plasma deposition or electrochemical plating. It is known that permalloy (80% Ni and 20% Fe), for example, can readily be deposited by electroplating.

[0135] Alternatively, a liftoff method may be used to deposit a sheet of permeable material, most of which is then lifted off areas other than **116** and **130**. Further details into the lithographic formation of inlaid, magnetically permeable materials may be found in, for example, U.S. Pat. No. 7,229,838. U.S. Pat. No. 7,229,838 is hereby incorporated by reference in its entirety. The substrate may then be planarized by chemical mechanical polishing (CMP), leaving a flat surface for the later bonding of a cover plate.

[0136] Having made the permeable features **116** and **130**, the movable member or valve **110** may be formed. The surface may again be covered with photoresist and patterned to protect the inlaid permeable features **116** and **130**. The sample inlet channel **120** and output channels **122** and relieved area **144** may be formed simultaneously with the movable member **110**. With movable member **110**, and other areas whose topography is to be preserved may be covered with photoresist, the features **110**, **120**, **122** and **144** may be formed by deep reactive ion etching (DRIE) for example.

[0137] To form the fluidic channels, a cover plate may be bonded to the surface of the substrate which was previously planarized for this purpose. The cover plate may be optically transparent to allow laser light to be applied to the particles in the fluid stream flowing in the sample inlet channel **120**, and for fluorescence emitted by the fluorescent tags affixed to the particles to be detected by the optical detection system described above. A hole formed in this transparent material may form the waste channel **142**. Alternatively, a waste channel **142** may be formed in a second substrate, such as a second silicon substrate, and bonded to the surface of the first substrate. Alternatively, output channel **142** may be formed on the opposite surface of the first substrate using a silicon-on-insulator (SOI) substrate, with waste channel **142** and orifice **140** formed in the handle layer and dielectric layer of the SOI substrate, and the movable feature formed in the device layer.

[0138] Additional details for carrying out this process outlined above are well known to those skilled in the art, or readily found in numerous lithographic processing references.

[0139] Accordingly, described here is a micromechanical particle manipulation device, formed on a surface of a fabrication substrate. The fabrication substrate may be held in a larger structure which is in fluid communication with the fabrication substrate. The device may include a sample chamber holding a fluid, a microfabricated, movable member formed on the substrate. The substrate may have a plurality of flow channels formed therein, including a input channel, a waste channel and a plurality of different sort channels, and having a microfabricated, movable member formed on the same substrate, wherein the movable member moves to direct at least one target particle from the input channel into at least one of the plurality of different sort channels formed in the plane of the substrate.

[0140] In some embodiments, the movable member may move from a first position to a second position in response to a sort waveform applied sort waveform applied to an actuator, which generates a force to move the movable member, which directs at least one target particle from the sample chamber into at least one sort receptacle, and a means for returning contents of the sort receptacle back to the sample chamber.

[0141] The micromechanical particle manipulation device may further comprise a second sort receptacle. The sample chamber may hold at least two types of target particles, **S1** and **S2**, along with nontarget material. The device may further comprise a waste receptacle that contains primarily nontarget material.

[0142] The means for transferring may be a pneumatic suction device.

[0143] The means for transferring may alternatively be a fluid pump, or a flexible diaphragm, for example.

[0144] A method is also described. The method may be directed to sorting at least one target particle from a sample stream containing target particles and non-target material, wherein the sample is held in a sample chamber. The method may include separating at least one target particle from the sample stream, diverting the target particle to a sort receptacle, and transferring contents of the sort receptacle back to the sample chamber.

[0145] The method may further include sorting two types of target particles S1 and S2 into a single sort receptacle, transferring the contents of the single sort receptacle back to the sample chamber, and sorting a single target particle S1 into a first chamber while allowing the second type of target particle S2 to flow into a separate receptacle. The method may include re-sorting contents of the sample chamber to improve purity by excluding non-target particles.

[0146] In other embodiments, the method may include sorting two types of target particles S1 and S2 into a single sort receptacle, transferring the contents of the single sort receptacle back to the sample chamber, sorting a single target particle S1 into a first chamber while allowing the second type of target particle S2 to flow into a separate receptacle.

[0147] The transferring step may use a pneumatic suction device, or a pump, or a flexible membrane, for example.

[0148] The micromechanical particle manipulation device may move between positions, wherein at least one of the first, second and third positions are metastable. The micromechanical particle manipulation device may also include a restoring spring, wherein the restoring spring may be non-linear over its travel.

[0149] While various details have been described in conjunction with the exemplary implementations outlined above, various alternatives, modifications, variations, improvements, and/or substantial equivalents, whether known or that are or may be presently unforeseen, may become apparent upon reviewing the foregoing disclosure. Accordingly, the exemplary implementations set forth above, are intended to be illustrative, not limiting.

Claims

1. A micromechanical particle manipulation device, formed on a surface of a fabrication substrate, wherein the fabrication substrate is held in a larger structure which is in fluid communication with the fabrication substrate, comprising: a sample chamber holding a fluid; a substrate having a plurality of flow channels formed therein, including an input channel, a waste channel and a plurality of different sort channels, and having a microfabricated, movable member formed on the same substrate, wherein the movable member moves to direct at least one target particle from the input channel into at least one of the plurality of different sort channels formed in the plane of the substrate; and a means for returning contents of the sort receptacle back to a sample chamber.
2. The micromechanical particle manipulation device of claim 1, wherein the movable member moves from a first position to a second position in response to a sort waveform applied to the movable member, which generates a force to move the movable member, and wherein this motion directs at least one target particle from the sample chamber into a first sort receptacle, and wherein the movable member moves from a first position to a third position in response to another sort waveform applied to an actuator, which generates a force to move the movable member, which directs at least one target particle from the sample chamber into a second sort receptacle.
3. The micromechanical particle manipulation device of claim 1, wherein the larger structure further comprises a second sort receptacle.
4. The micromechanical particle manipulation device of claim 1, wherein the sample chamber holds at least two types of target particles, S1 and S2, along with nontarget material.
5. The micromechanical particle manipulation device of claim 1, further comprising a waste

receptacle that contains primarily nontarget material.

6. The micromechanical particle manipulation device of claim 1, wherein the means for transferring comprises a pneumatic suction device.

7. The micromechanical particle manipulation device of claim 1, wherein the means for transferring comprises a fluid pump.

8. The micromechanical particle manipulation device of claim 1, wherein the means for transferring comprises a flexible diaphragm.

9. A method for sorting at least one target particle from a sample stream containing target particles and non-target material, wherein the sample is held in a sample chamber, comprising: separating at least one target particle from the sample stream diverting the target particle to a sort receptacle; and transferring contents of the sort receptacle back to the sample chamber.

10. The method of claim 9, further comprising: re-sorting contents of the sample chamber to achieve increase purity.

11. The method of claim 9, further comprising: sorting two types of target particles S1 and S2 into a single sort receptacle; transferring the contents of the single sort receptacle back to the sample chamber; sorting a single target particle S1 into a first chamber while allowing the second type of target particle S2 to flow into a separate receptacle.

12. The method of claim 11, wherein the transferring further comprises uses a pneumatic suction device.

13. The micromechanical particle manipulation device of claim 1, wherein the means for transferring comprises a fluid pump.

14. The micromechanical particle manipulation device of claim 1, wherein the means for transferring comprises a flexible diaphragm.

15. The micromechanical particle manipulation device of claim 1, wherein at least one of the first, second and third positions are metastable.

16. The micromechanical particle manipulation device of claim 1, wherein the restoring spring may be non-linear over its travel.
