

**MICROFLUIDIC CHIP DEVICE FOR OPTICAL FORCE MEASUREMENTS
AND CELL IMAGING USING MICROFLUIDIC CHIP CONFIGURATION AND
DYNAMICS**

BACKGROUND

Field of the Invention:

[0001] This invention relates in general to a device and method for particle analysis and imaging for particles or cells in fluids, and in particular to a device and method for particle imaging for fluids using pressure, fluid dynamics, electrokinetic, and optical forces.

[0002] The present invention is directed to a microfluidic chip wherein injection occurs in an upwards vertical direction, and fluid vials are located below the chip in order to minimize particle settling before and at the analysis portion of the chip's channels.

[0003] Changes had to be made to existing microfluidic chip designs to implement the invention herein. For example, to keep the vials vertically in-line with the chip, a different interface with the chip had to be established as compared to the prior art. Specifically, instead of the input tube interfacing with the chip via a port attached to the largest face of the chip (as is typically done in microfluidic lab-on-a-chip systems) in an orthogonal manner and then pumping fluid first across and then up the chip, the current invention has the input and fluid coming up through the bottom of the chip, in one aspect using a manifold, which avoids horizontal re-orientation of fluid/fluid dynamics.

[0004] According to the invention herein, the contents of the vial are located below the chip and pumped upwards and vertically directly into the first channel of the chip. A long channel extends from the bottom of the chip to near the top of the chip. Then the channel takes a short horizontal turn but the new channel is so short as to almost negate

any influence of cell settling due to gravity and zero flow velocity at the walls. Then, contrary to the prior art, the fluid is pumped up to the analysis portion. The horizontal analysis portion is therefore the highest channel/fluidic point in the chip and thus close to the top of the chip, which results in less chip material (e.g., glass) between the microscope/camera and the sample than the prior art and therefore clearer imaging. The laser also suspends cells in this channel during analysis which prevents them from settling.

Description of the Related Art:

[0005] According to the prior art, microfluidic chip vials containing cells or particles to be separated and/or analyzed are located to the side and horizontally pumped into the channels in the microfluidic chip. First the contents of the vials (e.g., particles or cells) were pumped in the upwards vertical direction, then make a u-turn to travel down, and are then pumped horizontally into the chip. (See, e.g., U.S. Patent No. 9,594,071.)

[0006] The connection to the chip is horizontal, which, combined with the dead volume (empty space in fluidic connections that is to some extent unavoidable) in the connection, led to significant additional settling due to gravity. Such a configuration also necessitates a relatively large diameter channel required by the connection, which, in addition to the dead volume, creates an area of relatively low velocity, further increasing the problem of particle settling. The current chip according to the present invention eliminates the need for a large horizontal input channel and a rather abrupt change from a large horizontal input channel to the relatively thin upward flow in the first vertical chip channel. Such a configuration eliminates the horizontal settling and an unnecessary change in direction which causes settling. Having the cells enter the bottom edge of the chip also solves the issue of settling in the dead volume by orienting it vertically with respect to gravity so that the cells or particles cannot settle in the bottom of a horizontal channel, but rather they are constantly guided upwards by the flow. This is not intuitive and required much experimentation to realize the problem before designing the current embodied solution. Currently available microfluidic devices incorporate custom or commercially available

connections on the polished surface and larger area of the glass, in contrast to the present invention, which generally forces any particles, such as cells, contained within the sample stream to take an immediate turn and travel horizontally upon entering the chip.

[0007] Also in the prior art, the cells or particles have several horizontal runs on a microfluidic chip before reaching the analysis channel, which leads to settling. At the point the vial contents enter the chip and the channels in the chip, the contents are pumped horizontally compared to the vertical in-chip channel. The channel then flows upwards and takes a long horizontal turn at which point the cells tend to settle at the bottom of the channel due to gravity, as well as experience lower velocity at the wall due to laminar flow conditions. In essence, due to parabolic velocity profile, the flow is highest in the middle of a channel and decreases to zero at or near the channel wall. After the first horizontal in-chip channel, the fluid takes a downward turn before the analysis channel where the particles are imaged or separated. Due to this configuration, there exists a relatively large distance between the microscope/camera and the analysis channel. In this typical prior art configuration, the particles are forced downward and ultimately exit the bottom of the chip.

[0008] Moreover, due to constraints in the prior art, multiple horizontal runs are required, causing cells to settle in multiple places in the channels. This, in turn, causes a decrease in image quality because of the need to image through additional material at the edge of the chip. The prior art channels in the chip had to be pumped in the upwards vertical direction, then horizontal, then in a zigzag nature for adequate cell or particle suspension, then down and out the chip. Zigzag channels are obviated by the current invention.

[0009] Prior art also exists regarding rendering 3D images of cells or particles in fluid. For example, M. Habaza, M. Kirschbaum, C. Guernth-Marschner, G. Dardikman, I. Barnea, R. Korenstein, C. Duschl, N. T. Shaked, Adv. Sci. 2017, 4, 1600205, teaches trapping a cell, rotating it at high speeds, and using interferometry to measure refractive index distribution within a cell. Interferometry has also been used to analyze cells in microfluidic channels (see e.g., Y. Sung et al., Phys. Rev. Appl. 2014 Feb. 27; 1:

014002). The current invention, however, claims taking multiple images of a cell or particle as it travels in the fluid flow and passes through the focal planes of the imaging device(s), thereby negating any need to trap the cell in order to render a 3D image. Other techniques have been taught, such as moving a cell or particle using a mechanical translation stage (e.g., N. Lue et al., Opt. Express 2008 Sep. 29; 16(20): 16240-6), none of which use bright field imaging as described herein and do not utilize fluid flow to provide cell positioning relative to the image focal plane.

[0010] All prior art references cited herein are incorporated by reference in their entirety.

SUMMARY

[0011] The present invention is directed to a microfluidic chip wherein injection occurs and sample vials are located below the chip in order to minimize particle settling. Therefore, the contents of the vial are located below the chip and pumped upwards and vertically directly into the channel of the chip. A long channel extends from the bottom of the chip to near the top of the chip. Then the channel takes a short horizontal turn but the new channel is sufficiently short as to be insignificant with respect to any influence of cell settling due to zero flow velocity at the walls of the channel. Then, contrary to the prior art, the sample is pumped up to the analysis portion. The horizontal analysis portion is therefore the highest channel/fluidic point in the chip and thus close to the top of the chip, which results in less glass between microscope/camera than the prior art and therefore clearer imaging. Distance of the analysis channel from the top of the chip may be from 100 microns to 2 mm, but as great as 100 mm such as from 100 microns to 200 microns, from 200 microns to 300 microns, from 300 microns to 400 microns, and so on. In one embodiment, after the analysis portion of the chip, the sample, e.g., fluid, cells, and/or particles, are pumped downwards to the bottom of the chip and forced outwards.

[0012] The present invention is further directed to a microfluidic chip wherein horizontal runs are minimized, especially at the point fluid enters in the chip channels. Prior art

chips contain about 13 mm in horizontal channels (non-analysis portions), around 2 mm of which is of the much larger diameter injection port, exacerbating settling due to the low velocity. The chip described herein has, in a preferred embodiment, about 0.2 to 3.0 mm in horizontal channels (non-analysis), although the horizontal channels may range in length from 0.01 to 100.0 mm, such as from 0.01 mm to 0.02 mm, from 0.02 mm to 0.03 mm, from 0.03 mm to 0.04 mm, and so on. This is a result of the invented channel system and is an order of magnitude different from the prior art, which improves flow and elimination of cell/particle settling.

[0013] Another aspect of the invention is directed to a microfluidic chip wherein imaging occurs and analysis is based at or near the corner of the chip, whereby imaging from multiple viewpoints is improved because less glass and distance exists between the camera and analysis channel. This also allows a higher numerical objective lens to be used to improve detailed imaging by increasing magnification. Such a design improvement decreases the distortion of the image caused by glass (or other substance comprising the chip, such as plastic or any transparent or semi-transparent material) and distance (e.g., due to imperfections in glass). The distance between the imaging device and analysis channel may be from 100 microns to 2 mm, but as great as 100 mm, such as from 100 microns to 200 microns, from 200 microns to 300 microns, from 300 microns to 400 microns, and so on.

[0014] Further, the present invention is directed to a microfluidic sorting chip with separation downstream from an analysis channel allowing for using both pressure and/or a laser (or other optical force) simultaneously or sequentially separately to activate a sorting function. In one aspect, flow would continue from the analysis channel for the sorting function. For example, the particles would be directed into a vertical channel and then to a horizontal sorting channel. In one embodiment, an optical force and/or pressure would be applied in the direction of the flow with the sorting channel to push particles through the channel. Particles not directly acted upon by the optical force would divert to an alternate channel due to, for example, gravity, electrokinetic forces, magnetic forces, laminar flow lines, stream lines, decreased flow rate, an orthogonal optical force, or a

vacuum being applied to suck the particles into the alternate channel. In another aspect related to the sorting post-analysis channel, the present invention allows for directing an optical force from a back side of the chip (the laser or optical force being oriented in the same direction of flow) and, in some aspects, splitting this primary laser. In embodiments, the optical force and/or pressure may be applied in the opposite direction of the movement of the substance through the channel, e.g., against the flow, or may be applied in the same direction as the movement of the substance in the channel, e.g., with the flow. Cell or particle sorting could occur on a single device or a separate chip. For example, in FIG. 1B, the fifth channel prior to the outlet tubing **145**, contains one or more bifurcations to enable single or multiple sorting regions.

[0015] In another aspect of the current invention, a manifold is connected to a vial in a way such that tubing goes through the manifold and connects to a vial or other vessel on the other side making contact with the substance (e.g., fluid) in the vial. The manifold allows for vials to be connected to the microfluidic chip but stored under the chip, and/or the manifold allows for contents of the vials to be injected from the bottom of the chip, alleviating several problems experienced by the prior art, such as cell or particle settlement where the vial or tubing from the vial connects to or communicates with the microfluidic chip.

[0016] In another aspect, the present invention is directed to a microfluidic chip holder, this chip holder comprising a structure to guide a light source including an integrated prism cavity, which when fitted with a prism causes light to exit at an angle relative to the chip and is a preferred method for illuminating constrained geometry. In embodiments, the light source includes but is not limited to fiber optics or a collimated or focused light source. This light source is precisely directed, or oriented, or directed into the analysis channel, in particular.

[0017] In another aspect of the current invention, the device includes a second imaging device oriented orthogonally to the first camera and channel view. Reasons for the second camera vary. In one aspect, the reason for a second imaging device is to aid in

visual alignment of the laser or optical force in the analysis channel. In another aspect, using the method described herein, data from the first camera can be recorded. With the second camera, data can be combined with data from the first camera, resulting in additional data that can be used to more accurately extrapolate cell position, size, shape, volume, etc. This additional information about the same cell (or particle) increases the accuracy and range of measurements and analysis. In a further aspect, the second camera, combined with the first camera, allows for a 3D reconstruction of a cell or particle, or group of cells or particles, imaged by the orthogonal camera and a camera in the flow or a camera located towards the side of the chip. Using the algorithm described herein or others, including reversing or slowing the flow and taking one or more images of a particular cell or particle, or group of cells or particles, the invention allows for multiple images to be analyzed and processed thereby allowing for determination of characteristics/attributes/quantitative measurements, such as the cell volume, cell shape, nucleus location, nucleus volume, organelle or inclusion body location, etc. In another aspect of the current invention a camera is oriented to image in the axis that is in the direction of flow.

[0018] In one aspect, the current invention does not require a serpentine or zigzag channel, as preferred according to the prior art, to keep particles properly suspended. Because of the vertical nature of fluid and particles or cells being injected in the chip, the zigzag channel is obviated by the vertically integrated pumped particles that flow directly up through the channel to the first horizontal channel (referred to herein as the second channel).

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] The accompanying drawings illustrate certain aspects of some of the embodiments of the present invention, and should not be used to limit or define the invention. Together with the written description the drawings serve to explain certain principles of the invention.

[0020] Figure 1A is a diagram depicting a global view of the device configuration, including the chip, manifold, and vials. Figure 1B is a diagram which shows certain depictions of the chip channel orientations and locations.

[0021] Figures 2A and B contain diagrams depicting the microfluidic chip holder according to the present invention.

[0022] Figures 3A and 3B are a diagram depicting angles and aspects of the chip holder according to the present invention.

[0023] Figures 4A and 4B are diagrams showing examples of the cell path and imaging configuration related to the chip. Figure 4C is a diagram which shows an alternative cell path.

[0024] Figure 5 is a diagram showing on chip multi-plane imaging and how it may be used to render a 3D images and information.

[0025] Figures 6A and 6B are diagrams showing on chip multi-plane imaging in fluid flow and how it may be used to render a 3D image.

[0026] Figure 7 is a diagram showing how a cell may be trapped and/or balanced within the analysis portion of the chip and imaged from multiple angles.

[0027] Figure 8 is a diagram showing how a camera and illumination source can be placed in line with the laser and direction of flow, such that the particles move away from the camera.

[0028] Figure 9 is a diagram showing how a camera and illumination source can be placed in line with the laser and direction of flow, such that the particles move toward the camera.

DETAILED DESCRIPTION

[0029] The present invention has been described with reference to particular embodiments having various features. It will be apparent to those skilled in the art that various modifications and variations can be made in the practice of the present invention without departing from the scope or spirit of the invention. One skilled in the art will recognize that these features may be used singularly or in any combination based on the requirements and specifications of a given application or design. Embodiments comprising various features may also consist of or consist essentially of those various features. Other embodiments of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention. The description of the invention provided is merely exemplary or explanatory in nature and, thus, variations that do not depart from the essence of the invention are intended to be within the scope of the invention.

[0030] Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not limited in its application to the details of construction and the arrangement of the components set forth in the following description or illustrated in the drawings. The invention is capable of other embodiments or of being practiced or carried out in various ways. Also, it is to be understood that the phraseology and terminology employed herein is for the purpose of description and should not be regarded as limiting.

[0031] Turning now to the figures, FIG. 1A shows a global view of the device taught herein. The sample vials **130** are located below the microfluidic chip **100** (which may also be referred to generically herein as a substrate) and are not limited in terms of number of vials or sizes. The vials are configured to hold any type of sample that can be moved through the device, such as one or more substance, including but not limited to one or more of fluids, liquids, gas, plasma, serum, blood, cells, platelets, particles, etc. or combinations thereof. In the context of this specification, the term fluid or sample may be used generically to refer to such one or more substances. The vials are connected

either directly or indirectly, such as indirectly using air tubing **110** in operable communication with the underside of the chip. They may also be connected by way of a manifold **120** as further shown in FIG. 1A. FIG. 1B shows a preferred embodiment of the microfluidic chip **100** whereby the substance, fluid, particles, and/or cells is/are injected at one exterior surface, such as an edge, of the microfluidic chip (e.g., the XZ plane in FIG. 1B). Injection is shown in FIG. 1B along one of the faces shared by the smallest distance, such as the XZ face or the XY face. In this particular embodiment, the length of side X **160** is less than the length of side Y **170** and the length of side Z **180**. Such a configuration allows a sample to have minimal deviation upon entering channels on the chip (e.g., no right turn is necessary as is generally the case in current state of the art).

[0032] In FIG. 1A, a manifold **120** is shown connecting the vial or vials **130** to the microfluidic chip **100** so that the substance, fluid, particles, and/or cells may be injected or pumped into the microfluidic chip in the vertical and upwards direction. The manifold allows injection of substances from the bottom of the chip, while also positioning the electronics, flow sensors, and tubing (both liquid and air) in such a way as to minimize cell settling, which optimizes throughput. Air tubing provides pressure or vacuum, which, when sealed, provides for a closed system within the confines of the manifold apparatus. In one embodiment there is a distance between the vial(s) and the manifold indicating that there is no seal and the pressure of the internal volume is atmospheric. This allows for pumping substances from or to a container open to the atmosphere (vacuum is required to pump from an open container). The manifold positions the electronics, flow sensors, and tubing (both liquid and air) in such a way as to minimize cell settling which optimizes throughput.

[0033] By injecting the contents from the bottom of the chip, the invention minimizes horizontal movement in inlet tubing **140** and outlet tubing **145**, which causes such problems as settlement of the particles or cells in the channel(s) **150**. The outlet tubing is offset from the inlet tubing, in this example, in the Z and X dimensions (FIG. 1B). In embodiments, the outlet tubing is offset, not offset, straight, in-line, or angled in relation

to the inlet tubing. Such a configuration obviates the need for serpentine or zigzag vertical channels because the current configuration resolves problems with settlement that occur in the prior art, such as in the case in which fluid combined with cells and/or particles is injected or pumped into the chip from the side in a horizontal direction that then must change direction and fluid dynamics to be pushed upwards. The manifold and injection from the bottom of the chip also allow for additional elements, components, machinery, or hardware to be placed below the chip. (See FIG. 1A.)

[0034] The manifold **120** works by adjusting the air pressure above the contents in the vial and providing correct geometry for flow sensors and electronics. Tubing, such as fluid or air tubing, passes through the manifold and connects to a vial on the other side. Pressurized air passes through one side of manifold and creates a closed pressurized system within the manifold. By adjusting pressure in the enclosed region, the system allows for changes to parameters such as flow speed and fluid dynamics. The pressurized region is in both the vial(s) **130** and the manifold **120**. In another aspect, no air connection is needed for the vial, because it is open to the ambient atmosphere. This enables sampling of a larger variety of containers and sources. In such an embodiment, a vacuum is applied to the other one or more vials such that a pressure differential is created to drive fluid flow from the open container.

[0035] In one embodiment, pressure-based sample injection is used. The vials are filled with a sample in a fluid and sealed either with a lid or tubing connected to the chip. Prior to attachment to the lid, the vial can be either open to the air or sealed with a septum or other gas-tight device. In one aspect, the lid may contain two connections, one for a fluid such as a gas and one for a liquid. Optionally, the method embodiment further includes providing a sample inlet line tip communicating with a sample inlet line, which communicates with the first channel.

[0036] In another embodiment, a vacuum based sample injection is used. The vials are filled with a sample in a fluid and sealed either with a lid or tubing connected to the chip. Prior to attachment to the lid, the vial can be either open to the air or sealed with a

septum. In one aspect, the lid may contain two connections, one for a fluid such as a gas and one for a liquid. Optionally, fluid can be aspirated from a vial open to the atmosphere by applying vacuum pressure to one or more of the other vials. Optionally, the method embodiment further includes providing a sample inlet line tip communicating with a sample inlet line, which communicates with the first channel.

[0037] In FIGS. 2A-B and 3A-B, a chip holder **200, 300** is shown. The chip holder comprises a structure to guide a light source **210**, such as a fiber optic light source, light emitting diode, or laser, and an integrated prism cavity **220, 320** that can be fitted with a prism. The light source is guided or aligned by an integrated structure or channel **240** within the chip holder to the desired location. The built-in space for the fiber optic light source allows for illumination, such as a cone of illumination **250**, even in constrained geometric environments, such as on a microfluidic chip **230, 330** or a channel in a microfluidic chip. This light source is precisely directed, or oriented, or focused on the analysis channel **260** in particular, in a preferred aspect. In a preferred embodiment, the chip holder includes a built-in space for a prism **220, 320** and fiber optic light source **210** allowing for illumination with constrained geometry. The chip further includes holes or openings in the bottom of the holder **350** to precisely align fluidic tubing as described herein. In one embodiment, adjustable screws are integrated into threaded holes **360** on one or more faces for proper alignment.

[0038] FIG. 4A is a preferred embodiment of the microfluidic chip **400** described herein. As shown, fluid travels first upwards in a vertical direction through a first channel **410** then communicates with a second horizontal channel **420**. Another vertical channel **430** takes the fluid even closer to the top of the chip at which point a fourth channel **440** is horizontal and, as show in the FIG. 4, comprises the analysis channel. The channels are in operable communication with one another to allow for a sample to be moved through the system from one channel to another. In embodiments the sample can flow from the first channel to the second channel to the third channel to the fourth channel, or in the reverse, or combinations thereof. A pump and/or vacuum apparatus can be provided to provide positive and/or negative pressure at either or both the opening and the exit of the

channels to enable movement of the substances through the channels. The analysis channel is close to one or more exterior surface of the substrate, such as the faces, edges, or sides of the chip. For example, the analysis channel, according to this configuration, is close to the top and side of the chip, thereby improving imaging and analysis through the substance of the microfluidic chip. In preferred embodiments, the analysis channel is from around 1 mm to around 2 mm from the top and side of the chip. However, the distance of the analysis channel from the top of the chip may be from 0.1 mm to 100 mm, such as from 0.1 mm to 0.2 mm, from 0.2 mm to 0.3 mm, from 0.3 mm to 0.4 mm, and so on. Expressed another way, the analysis channel can be disposed within the top 50%, 33%, 25%, 10%, or 5% of the substrate. The length of the horizontal analysis channel according the present invention may be from around 250 microns to around 10 mm. However, the length of the analysis channel may be from 100 microns to 100 mm, such as from 0.1 mm to 0.2 mm, from 0.2 mm to 0.3 mm, from 0.3 mm to 0.4 mm, and so on. Expressed another way, the length of the analysis channel can be about 75% or less of the height, width or length of the substrate/chip, such as 50% or less, 33% or less, 25% or less, 10%, or 5% or less of the height, width or length of the substrate/chip.

[0039] In FIG. 4B, two imaging devices **450**, such as machine vision cameras, are shown. In one embodiment, a camera may be located above the chip and be oriented orthogonally to the analysis channel. In another embodiment, a camera may be located to the side of the chip and be oriented orthogonal to the direction of the flow or diagonal to the direction of the flow (such as above the channel, below the channel, or at angles to the side of the channel). In embodiments, the camera can be positioned such that the imaging is performed at any angle relative to the flow of the one or more substance, such as orthogonal or 90 degrees relative to the flow of the one or more substance, or such as from 0 to 90 degrees, or from 10 to 80 degrees, or from 30 to 60 degrees and so on. In another embodiment, two or more cameras may be used to image cells or particles in the analysis channel. For example, a camera may be above the chip and be oriented orthogonally to the analysis channel. A second camera may be located to the side of the chip and be oriented orthogonally to the direction of the flow or diagonal to the direction of the flow (such as above the channel, below the channel, or angles to the side of the

channel). As pictured in FIG. 4B, one or more light sources **460** may be used to illuminate the analysis channel **440**, and such light sources may be located under the chip and shining up, to the side of the chip and shining in the flow direction or opposite the flow direction, above the chip and shining down, or diagonally to the analysis channel.

[0040] Alternatively, a dichroic mirror **840** or other appropriate optical element can be used to selectively divert a specific wavelength range of light while allowing others to pass, such as shown in FIG. 8. This would allow for cameras **810** to be placed in line with the analysis channel. As pictured in FIGS. 8 and 9, several embodiments of this occur, including placing the optical force laser **830** and camera **810** at the same or opposite ends of the analysis region. An illumination source **860** for the camera might also be required and can be oriented in several ways, such as what is shown in FIGS. 8 and 9. The light source could be a broad spectrum source, such as one or more LEDs or a narrow source such as a laser. The camera could be used as a single camera or as part of a multi-camera system in combination with other viewpoints as described herein.

[0041] Also pictured in FIG. 4A, a light source **480**, such as a laser, may be used to affect cell flow. The laser may be placed in line with the cell flow, or opposing the cell flow. The laser may also be placed and/or oriented orthogonally or diagonally to the cell flow.

[0042] An embodiment of the invention includes a device for particle analysis. (See, e.g., FIGS. 4-9.) The embodiment of the invention includes at least one camera **450** for capturing images of particles or cells in the microfluidic channels (e.g., **440**). In one embodiment, a laser or other optical force **480**, such as a collimated light source operable to generate at least one collimated light source beam, is included. The at least one collimated light source beam includes at least one beam cross-section. The embodiment of the invention includes a substrate with a first channel **410** extending in a vertical direction in the substrate such that a first plane traverses first channel **410** substantially along its length and whereby the fluid sample is injected into the substrate/chip from the bottom of the chip and is forced by positive or negative pressure upwards. The embodiment of the invention includes a second channel **420** orthogonal to the first

channel and thus disposed horizontally in the substrate such that a second plane traverses second channel **420** substantially along its length and the second plane is disposed orthogonal to the first plane. This second channel is in the horizontal direction of the chip. The second channel communicates directly or indirectly with the first channel. The second channel communicates directly or indirectly with a short upward vertical third channel **430** that takes the channel network closer to the top of the chip. The third channel communicates directly or indirectly with a fourth horizontal channel **440** that is located near the top and/or corner of the chip. In a preferred embodiment, the fourth channel is the channel closest to the top of the chip. In an embodiment, the fourth channel is an analysis channel. In one aspect, a camera **450** is oriented orthogonally to the flow direction in the fourth channel. The embodiment of the invention includes a focused particle stream nozzle operably connected to the first channel. In another aspect of the current invention, the second channel undergoes a size change and passes through a nozzle before communicating with the third channel.

[0043] Figure 4C shows another embodiment of the sample path of the microfluidic chip. As shown, fluid travels in the chip first upwards in a vertical direction through a first channel **410**, which channel then communicates directly or indirectly with a second horizontal channel **420** at, in this example, the top of the chip and comprises the analysis channel in this embodiment. The analysis channel, according to this configuration, is close to the top of the chip, thereby improving imaging and analysis through the substance of the microfluidic chip. In preferred embodiments, the analysis channel is from around 1 mm to around 2 mm from the top and side of the chip. However, the distance of the analysis channel from the top of the chip may be from 0.1 mm to 100 mm, such as from 0.1 mm to 0.2 mm, from 0.2 mm to 0.3 mm, from 0.3 mm to 0.4 mm, and so on. Expressed another way, the analysis channel can be disposed within the top 50%, 33%, 25%, 10%, or 5% of the substrate. The length of the horizontal analysis channel according the present invention may be from around 250 microns to around 10 mm. However, the length of the analysis channel may be from 100 microns to 100 mm, such as from 0.1 mm to 0.2 mm, from 0.2 mm to 0.3 mm, from 0.3 mm to 0.4 mm, and so on. Expressed another way, the length of the analysis channel can be about 75% or less of the

height, width or length of the substrate/chip, such as 50% or less, 33% or less, 25% or less, 10%, or 5% or less of the height, width or length of the substrate/chip. In embodiments, the substrate can comprise one or more analysis channel, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 analysis channels.

[0044] Also pictured in FIG. 4C, a light source **480**, such as a laser, may be used to affect substance flow, such as cell flow. The laser may be placed in line with the cell flow, or opposing the cell flow. The laser may also be placed and/or oriented orthogonally or diagonally relative to the cell flow.

[0045] In FIGS. 1 and 4, a fluid flow containing cells or particles is directed through a first channel vertically. The one or more substance (fluid, cells, and/or particles) enter through the bottom of the chip at an opening of the first channel and enter the substrate in the vertical direction. The first vertical channel is between 100 microns and 100 mm in length, such as from 0.1 mm to 0.2 mm, from 0.2 mm to 0.3 mm, and so on. The first channel is followed by a second orthogonal/horizontal channel, which, in a preferred embodiment, is shorter than the first channel. The second channel may be 250 microns to 100 mm in length, such as from 0.25 mm to 0.5 mm, from 0.5 mm to 0.75 mm, from 0.75 mm to 1.0 mm, and so on. A third channel runs vertically and parallel to the first channel. The third channel may be 50 microns to 100 mm in length, such as from 0.05 mm to 0.1 mm, from 0.1 mm to 0.15 mm, from 0.15 mm to 0.2 mm, and so on. The channels are disposed in operable communication either directly or indirectly in such a manner as to allow one or more substance to move through multiple channels. The typical direction of the fluid flow is given by the flow arrows in FIG. 4A and 4C, but can be reversed.

[0046] In preferred embodiments, the fourth channel comprises the analysis channel, which is a channel from 250 microns to 100 mm in length, such as from 0.25 mm to 0.5 mm, from 0.5 mm to 0.75 mm, from 0.75 mm to 1.0 mm, and so on. In this embodiment, the fourth channel is the channel closest to the top of the chip. The distance of the fourth channel from the top of the chip, measured vertically, may be from 100 microns to 2 mm,

but as great as 100 mm such as from 100 microns to 200 microns, from 200 microns to 300 microns, from 300 microns to 400 microns, and so on. Expressed another way, the fourth channel can be disposed within the top 50%, 33%, 25%, 10%, or 5% of the chip. The imaging device, such as a camera, can be oriented orthogonal to the fourth channel and around 100 microns to 2 mm from the fourth channel, but as great as 100 mm from the fourth channel, such as from 100 microns to 200 microns, from 200 microns to 300 microns, from 300 microns to 400 microns, and so on.

[0047] In one embodiment, a laser or other optical source is present with a focusing lens element. FIG. 4A depicts the invention with the laser **480** operating, emitting a laser beam, directing the beam through a focusing lens element into the fourth flow channel **440**. The particles are aligned within the laser beam due to the gradient force which draws particles toward the region of highest laser intensity. The laser scatter force propels particles in the direction of laser beam propagation (e.g., left to right in FIG. 4A).

[0048] In another embodiment, a second camera or image capturing device (see **450**), in addition to the camera or image capturing device oriented orthogonally to the fourth channel, is oriented to the side of the analysis (or fourth) channel here and directed orthogonally or at any angle relative to the fourth channel; for example, as pictured in FIG. 4B. Taking one image of each cell at orthogonal views allows for multiple cell properties, for example size and shape, to be calculated in two dimensions, increasing the amount of information that can be captured for each cell. This would also enable the calculation of volumetric properties of the cells, including total volume, shape and give insight into cells that are not symmetric about the axis parallel to the direction of flow (the Z-axis as drawn in Figure 5). Using two or more cameras, a basic 3D model of the cell **550** can be constructed by combining the orthogonal images using, for example, existing 3D reconstruction algorithms, such as diffraction-theory method or illumination rotation-method. A 3D model and analysis of a 3D model will allow for a more accurate analysis of the cell, such as cell size, shape, orientation, and other quantitative and qualitative measurements regarding the particle(s) or cell(s) in the fourth channel of the microfluidic chip.

[0049] In FIG. 5, a portion of the analysis channel **540** is shown from two different planes, such as planes that might be imaged from an imaging device (see, e.g., FIG. 4). In the first plane **520**, one part of the cell or particle **510** is imaged in a certain orientation. In the second plane **530**, another part of the same cell or particle is imaged from a different perspective from another camera. This allows for the calculation of multiple cell properties, creating a matrix of data per cell per camera and a 3D rendition of the cell or particle **550**.

[0050] As different portions of the cell pass through the focal planes (e.g., **630** (the XZ focal plane) and **640** (the YZ focal plane)) of the imaging device(s), different slices of the cell can be imaged as the cell is moved by, for example, the fluid flow **620**. This is shown in Figure 6A. In Figure 6A, parts of the cell or particle are imaged at multiple points in time and/or space in different orientations and from different perspectives. In this example, as the cell moves and rotates through the focal plane, it appears as a different size in the successive images (such as the four example images per plane from two different cameras as shown in Figure 6A). Using 3D reconstruction algorithms, this allows for a more complex 3D rendering **650** of the particle or cell. From such a rendering, certain attributes of the cell or particle may be extrapolated, such as cell size, volume, location and size of the nucleus as well as other organelles, and measurement or outlining of cellular topography. Additionally, cell rotation can be measured as a function of optical force based torque due to changes in biophysical or biochemical properties, including but not limited to, refractive index, birefringence, or cell shape or morphology.

[0051] FIG. 6B depicts on chip multi-plane imaging with multiple images of the same cell being taken over time, as the cell moves through the focal plan, for example. In such cases, a view of the cell is referred to in the art as a “slice” or “image slice.” An image slice is effectively the thickness of the optical plane being imaged. The thickness of the image plane, or slice, is dictated, among other things, by the optical magnification of the imaging system. At higher magnifications the working distance of the objective lens

decreases resulting in the need to have the lens closer to the cell or particle to be imaged. In one embodiment, a laser or other optical force **670** may be used to affect the flow of the cell in the analysis channel. In a preferred embodiment, cells or particles may be purposefully induced into or out of the focal plane of the channel for imaging by either moving the laser and/or camera, or adjusting flow(s), or position using hydrodynamic focusing. For example, the laser source could be moved by a distance **660** using, for example, a piezo electric actuator or linear electric optomechanical stage. This could be performed on a per cell or per population basis. The hydrodynamic focusing of cells could be changed to affect the initial position and trajectory of the cells. For example, particles may be aligned or oriented in the focal plane within a laser beam due to the gradient force which draws particles toward the region of highest laser intensity. The laser scatter force propels particles in the direction of laser beam propagation. See FIG. 6B. Moving the laser, in this case in the X axis, allows for imaging of features in different parts of the cell, illustrated by the disc **680**. This could represent, for example, the nucleus, organelles, inclusion bodies, or other features of the cell or particle. The laser pulls the cell to its center as a result of the gradient force. It is further contemplated that two or more cameras may increase detail and accuracy.

[0052] An embodiment of the invention shown in FIG. 7 is a static mode where the particle or cell **710** is stopped at a specified differential retention location by balancing the optical **730** and fluidic forces **735**. The optical force may be applied by, for example, a laser or collimated light source. Images can be taken in multiple planes such as shown and described for Figures 5 and 6A-B. A flow sensor is used to measure the flow rate at which each particle stops in the flow for a given laser power. Because the optical and fluidic forces are balanced the fluidic drag force (i.e., from flow rate and channel dimension) is equal to the optical force. The properties of each cell can be measured sequentially in this manner. Although not a high throughput measurement system, this embodiment of the invention allows close observation and imaging of the trapped cell and also dynamic changes in optical force resulting from biochemical or biological changes in a cell. Reagent streams containing chemicals, biochemicals, cells, or other standard biological agents can be introduced into the flow channels to interact with the

trapped cell(s). These dynamic processes can be quantitatively monitored by measuring changes in optical force during experiments on a single cell or cells.

[0053] In one embodiment, a camera or other imaging device is oriented and/or focused in flow or opposite the flow of the analysis channel, such that it is in line and parallel to the flow. (See, e.g., FIGS. 8 and 9.) Figure 8 shows a camera **810** in line with the analysis channel **820** and laser or collimated light source **830**. A dichroic mirror or similar device **840** reflects the laser light **835** away from the camera to prevent damage but passes light **865** produced by the illumination source **860** to allow for imaging. The camera is oriented parallel to the fluid flow **870** such that the cells or particles **880** are, in one embodiment, moving away from the camera. The illumination source is oriented orthogonal to the channel and laser. A second dichroic **845** that passes laser light and reflects illuminating light is used to direct both the illuminating light and laser light through the channel. An alternative embodiment of this configuration switches the locations of the laser and illumination source such that the laser is orthogonal to the channel and the illumination source is parallel to the channel. The second dichroic would still direct both the laser light and visible light through the channel.

[0054] An alternative embodiment is shown in FIG. 9. In this case, the camera or imaging device **910** is oriented such that the cells or particles **980** are traveling toward the camera in the fluid flow **970**. Thus, the camera and laser **930** are on the same side of the channel, while the illumination source **960** is on the opposite end of the channel. Two dichroics **940** and **945** are used to direct laser light **935** and illumination light **965** into the channel, direct the illumination light to the camera, and divert the laser light away from the illumination source. An alternative embodiment of this configuration switches the locations of the laser and camera such that the laser is orthogonal to the channel and the illumination source is parallel to the channel. The second dichroic **945** would then direct the laser light through the channel and illuminating light to the camera.

[0055] Optionally, the embodiment of the invention further includes at least one optical element between a source of optical force and said fourth channel, and operable to

produce a standard TEM₀₀ mode beam, a standard TEM₀₁ mode beam, a standard TEM₁₀ mode beam, a standard Hermite-Gaussian beam mode, a standard Laguerre-Gaussian beam mode, Bessel beam, or a standard multimodal beam. Optionally, the at least one optical element includes a standard cylindrical lens, a standard axicon, a standard concave mirror, a standard toroidal mirror, a standard spatial light modulator, a standard acousto-optic modulator, a standard piezoelectric mirror array, a diffractive optical element, a standard quarter-wave plate, and/or a standard half-wave plate. Optionally, the source of optical force may include a standard circularly polarized beam, a standard linearly polarized beam, or a standard elliptically polarized beam.

[0056] Optionally, a device is embodied comprising a microfluidic channel, a source of laser light focused by an optic into the microfluidic channel, and a source of electrical field operationally connected to the microfluidic channel via electrodes; flowing particles in a liquid through the microfluidic channel; and manipulating the laser light and the electrical field to act jointly on the particles in the microfluidic channel, thereby separating the particles based on size, shape, refractive index, electrical charge, electrical charge distribution, charge mobility, permittivity, and/ or deformability. In yet another embodiment, a device comprises a microfluidic channel configured to supply a dielectrophoretic (DEP) field to an interior of the channel via an (1) electrode system or (2) insulator DEP system, and a source of laser light focused by an optic into the microfluidic channel; flowing a plurality of particles in a liquid into the microfluidic channel; and operating the laser light and field jointly on particles in the microfluidic channel to trap the particles or modify their velocity, wherein said DEP field is linear or non-linear. Another possible embodiment of a device includes a microfluidic channel comprising an inlet and a plurality of exits, and a source of laser light focused by an optic to cross the microfluidic channel at a critical angle matched to velocity of flow in the microfluidic channel so as to produce an optical force on the particles while maximizing residence time in the laser light of selected particles, thus separating the particles into the plurality of exits, wherein the laser light is operable to apply forces to particles flowing through the microfluidic channel, thereby separating the particles into the plurality of exits.

[0057] Optionally, the embodiment of the invention further includes at least one particle interrogation unit communicating with one or more of the channels, such as the analysis channel(s) and in particular the fourth channel. The particle interrogation unit includes a standard illuminator, standard optics, and a standard sensor. Optionally, the at least one particle interrogation unit includes a standard bright field imager, a standard light scatter detector, a standard single wavelength fluorescent detector, a standard spectroscopic fluorescent detector, a standard CCD camera, a standard CMOS camera, a standard photodiode, a standard photomultiplier tube, a standard photodiode array, a standard chemiluminescent detector, a standard bioluminescent detector, and/or a standard Raman spectroscopy detector.

[0058] The at least one particle interrogation unit communicating with the fourth channel comprises a laser-force-based apparatus or device that facilitates cell disease identification, selection, and sorting. In one aspect, the unit utilizes inherent differences in optical pressure, which arise from variations in particle size, shape, refractive index, or morphology, as a means of separating and characterizing particles. In one aspect, a near-infrared laser beam exerts a physical force on the cells, which is then measured. Optical force via radiation pressure, when balanced against the fluidic drag on the particles, results in changes in particle velocity that can be used to identify differing particles or changes with populations of particles based on intrinsic differences. The fluidic and optical force balance can also be used to change the relative position of particles to one another based upon their intrinsic properties thus resulting in physical separations. Another embodiment of the interrogation unit includes a device for particle analysis and/or separation, such as at least one collimated light source operable to generate at least one collimated light source beam. The at least one collimated light source beam includes at least one beam cross-section.

[0059] An embodiment of the instant invention involves the combination of several of the above-mentioned design elements discussed above in a unitary device. Embodiments also include methods of using such devices. An example of such a unitary device is

illustrated in FIG. 1. The illustrated embodiment of the invention is a 5-layer structure with all 5 layers bonded to each other to yield a solid microfluidic chip, although the chip may be one structure as opposed to bonded layers. The chip could be constructed using a number of standard materials including, but not limited to, fused silica, crown glass, borosilicate glass, soda lime glass, sapphire glass, cyclic olefin polymer (COP), poly(dimethyl)siloxane (PDMS), OSTF, polystyrene, poly(methyl) methacrylate, polycarbonate, other plastics or polymers. This chip allows for sample input, hydrodynamic focusing, optical interrogation, imaging, analysis, sample exit and clear optical access for the laser light to enter and exit the regions. The chip in embodiments can also be 3D printed, molded, or otherwise shaped.

[0060] Optionally, the at least one particle type includes a plurality of particle types. Each particle type of the plurality of particle types includes respective intrinsic properties and respective induced properties. Optionally, the intrinsic properties include size, shape, refractive index, morphology, intrinsic fluorescence, and/or aspect ratio. Optionally, the induced properties include deformation, angular orientation, rotation, rotation rate, antibody label fluorescence, aptamer label fluorescence, DNA label fluorescence, stain label fluorescence, a differential retention metric, and/or a gradient force metric. This method embodiment further includes identifying and separating the plurality of particles according to the respective particle types based on at least one of the intrinsic properties and the induced properties. Optionally, this method embodiment further includes interrogating or manipulating the sample flow. Optionally, interrogating the sample flow includes determining at least one of the intrinsic properties so and the induced properties of the particle types, and measuring particle velocity of the plurality of particles. Measurement of at least one of the intrinsic properties can be used for a range of applications, including but not limiting to: determining the viral infectivity of a cell sample (the number of functionally infectious virus particles present in a particular cell population, similar to a plaque assay or end point dilution assay) for the purposes of viral quantification, process development and monitoring, sample release assays, adventitious agent testing, clinical diagnostics, biomarker discovery, determining the productivity of a cell in terms of antibody or protein for process development and monitoring, determining

the efficacy, quality, or activation state of cells produced as a cell-based therapy, including CAR T and other oncology applications and stem cells, determining the effect of a chemical, bacteria, virus, antimicrobial or antiviral on a specific cell population, and determining the disease state or potential of a research or clinical cell sample.

Optionally, a source of optical force includes at least one beam axis, and the sample flow includes a sample flow axis. The step of determining at least one of the intrinsic properties and the induced properties of the particle types, and the step of measuring particle velocity of the plurality of particles together comprise offsetting the beam axis from the sample flow axis. Optionally, the step of determining at least one of the intrinsic properties and the induced properties of the particle types, and the step of measuring particle velocity of the plurality of particles together comprise calculating a slope and a trajectory of a particle of the plurality of particles deviating from a sample flow axis toward at least one beam axis.

[0061] One skilled in the art will recognize that the disclosed features may be used singularly, in any combination, or omitted based on the requirements and specifications of a given application or design. When an embodiment refers to “comprising” certain features, it is to be understood that the embodiments can alternatively “consist of” or “consist essentially of” any one or more of the features. Other embodiments of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention.

[0062] It is noted in particular that where a range of values is provided in this specification, each value between the upper and lower limits of that range is also specifically disclosed. The upper and lower limits of these smaller ranges may independently be included or excluded in the range as well. The singular forms “a,” “an,” and “the” include plural referents unless the context clearly dictates otherwise. It is intended that the specification and examples be considered as exemplary or explanatory in nature and that variations that do not depart from the essence of the invention fall within the scope of the invention. Further, all of the references cited in this disclosure are each individually incorporated by reference herein in their entireties and as such are

intended to provide an efficient way of supplementing the enabling disclosure of this invention as well as provide background detailing the level of ordinary skill in the art.

CLAIMS

1. A device comprising:
 - a substrate comprising a plurality of channels configured to transport one or more substances and a collimated light source capable of interacting with the one or more substances, wherein the plurality of channels comprises:
 - a first channel oriented vertically upwards with respect to gravity within the substrate,
 - a second channel in operable communication with the first channel and oriented horizontally within the substrate,
 - a third channel in operable communication with the second channel and oriented vertically upwards with respect to gravity within the substrate, and
 - a fourth channel in operable communication with the third channel and oriented horizontally within the substrate;
 - wherein the first, second, third and fourth channels are oriented in such a manner as to provide a path for movement of the one or more substances through the substrate from the first channel to the second channel to the third channel to the fourth channel,
 - wherein the device is arranged for the one or more substances to be injected into the first channel disposed vertically upwards with respect to gravity within the substrate through a bottom horizontal planar surface having an opening to the first channel, in a vertical direction to maintain directional and volumetric continuity with the first channel.
2. The device of claim 1, , wherein the bottom horizontal planar surface has at least one shorter length from one edge to another edge compared to at least one length from one edge to another edge on a vertical planar surface of the substrate.
3. The device of claim 1, wherein the bottom horizontal planar surface has a smaller or equal surface area compared to a surface area on a vertical planar surface of the substrate.
4. The device of claim 1, wherein the first channel comprises an opening disposed at an exterior surface of the substrate and in such a manner as to provide a path for the one or more substances to enter vertically into the substrate and to move vertically within the first channel.

5. The device of claim 1, wherein a cross section of the first channel and a cross section of the opening have the same area, a smaller area, or a larger area.
6. The device of claim 1, wherein the first channel and the opening are shaped, sized and oriented in a manner to maintain directional and volumetric continuity.
7. The device of claim 1 wherein the collimated light source is oriented to interact with particles or cells in the fourth channel.
8. The device of claim 1, wherein the collimated light source is oriented to propagate in the direction of, opposite the direction of, orthogonal to the direction of, or diagonal to the movement of the one or more substances in the fourth channel.
9. The device of claim 1, wherein the fourth channel allows for imaging and analysis of particles or cells in multiple focal planes.
10. The device of claim 1, wherein the fourth channel allows for imaging and analysis of particles or cells in multiple focal planes during movement of the one or more substances.
11. The device of claim 1, wherein the fourth channel allows for imaging and analysis of particles or cells during movement of the one or more substances.
12. The device of claim 1, wherein the fourth channel allows for imaging and analysis of particles or cells during movement of the one or more substances and from multiple angles and/or orientations.
13. The device of claim 1, wherein the fourth channel allows for imaging and analysis of particles or cells during movement of the one or more substances and from multiple focal planes, angles, and/or orientations by one or more imaging devices.

14. The device of claim 1, arranged for one or more of an electrical force, an optical force, and/or a fluidic force to move cell(s) or particle(s) in one or more channels.
15. The device of claim 1, arranged for one or more of an electrokinetic force, an electrophoretic force, and/or a dielectrophoretic (DEP) force to move cell(s) or particle(s) in one or more channels.
16. The device of claim 1, further comprising one or more imaging device, wherein at least one of the imaging devices is capable of being moved in a manner to change the focal plane being imaged in the fourth channel.
17. The device of claim 1, wherein cell(s) or particle(s) in the fourth channel are capable of being moved by a change in optical and/or fluidic forces, and the region of the cell being imaged in the fourth channel is changed as the particle moves.
18. The device of claim 1, wherein the fourth channel is located at a distance from two or more outer surfaces of the substrate allowing for imaging and analysis of multiple image slices of a cell or a particle as a focal plane moves relative to the cell or particle.
19. The device of claim 1, wherein the fourth channel is located at a distance from two or more outer surfaces of the substrate allowing for imaging and analysis of multiple image slices of a moving cell or a particle as it moves through a focal plane.
20. The device of claim 1, wherein the fourth channel is located at a distance from two or more outer surfaces of the substrate allowing for imaging and analysis of multiple image slices of a suspended or static cell or particle.
21. The device of claim 1, wherein the one or more substances is/are capable of being moved by pressure, vacuum, peristalsis, electrokinetic force, electrophoretic force, magnetic force, optical force, or any combination thereof.

22. The device of claim 1 further comprising a dichroic mirror to direct light to or away from an imaging device imaging and/or analyzing cells or particles in the fourth channel.
23. The device of claim 1 further comprising a dichroic mirror to direct a collimated or focused light source to interact with cells or particles in the fourth channel.
24. The device of claim 1 further comprising a dichroic mirror to direct a collimated or focused light source away from an imaging device, another light source, or another part of the device.
25. The device of claim 1, wherein the plurality of channels comprises a fifth channel disposed horizontally, vertically, or diagonally within the substrate.
26. The device of claim 1, wherein the plurality of channels comprises a fifth channel, which splits into two or more channels or wells to sort cells or particles.
27. The device of claim 1, wherein the fourth channel is located closer to the top of the substrate than either the first channel, the second channel, or the third channel.
28. The device of claim 1, wherein the fourth channel is located from 100 microns to 100 mm from the top of the substrate.
29. The device of claim 1, wherein the first channel ranges from 0.1 mm to 100.0 mm in length.
30. The device of claim 1, wherein the second channel ranges from 0.1 mm to 100.0 mm in length.
31. The device of claim 1, wherein the third channel ranges from 0.05 mm to 100.0 mm in length.

32. The device of claim 1, wherein the fourth channel ranges from 0.1 mm to 100.0 mm in length.
33. The device of claim 1, wherein the first channel is greater in length than the second channel, the third channel, or the fourth channel.
34. The device of claim 1 further comprising a cell or particle interrogation unit.
35. The device of claim 1 further comprising a cell or particle collection channel.
36. The device of claim 1 further comprising an imaging device selected from at least one of a bright field imager, a light scatter detector, a single wavelength fluorescent detector, a spectroscopic fluorescent detector, a CCD camera, a CMOS camera, a photodiode, a photodiode array (PDA), a spectrometer, a photomultiplier tube or tube array, a photodiode array, a chemiluminescent detector, a bioluminescent detector, a standard Raman spectroscopy detection system, surface enhanced Raman spectroscopy (SERS), coherent antistokes Raman spectroscopy (CARS), and/or coherent stokes Raman spectroscopy (CSRS).
37. The device of claim 1 further comprising a tube end arranged for injecting the one or more substances into an opening disposed at an exterior surface of the substrate and in such a manner as to provide a path for the one or more substances to enter into the substrate and to move within the first channel, wherein a cross section of the opening has an equal, larger, or smaller area than a cross section of the tube end.