Office de la Propriété Intellectuelle du Canada
Un organisme d'Industrie Canada

Canadian Intellectual Property Office
An agency of Industry Canada

CA 2710024 C 2016/08/23

(11)(21) **2 710 024**

(12) **BREVET CANADIEN**
CANADIAN PATENT

(13) **C**

(86) **Date de dépôt PCT/PCT Filing Date:** 2009/01/15
(87) **Date publication PCT/PCT Publication Date:** 2009/07/23
(45) **Date de délivrance/Issue Date:** 2016/08/23
(85) **Entrée phase nationale/National Entry:** 2010/06/17
(86) **N° demande PCT/PCT Application No.:** US 2009/031154
(87) **N° publication PCT/PCT Publication No.:** 2009/091925
(30) **Priorités/Priorities:** 2008/01/16 (US61/021,443); 2008/09/11 (US12/209,084)

(51) **Cl.Int./Int.Cl.** ***G10K 11/28*** (2006.01), ***G01N 15/14*** (2006.01)

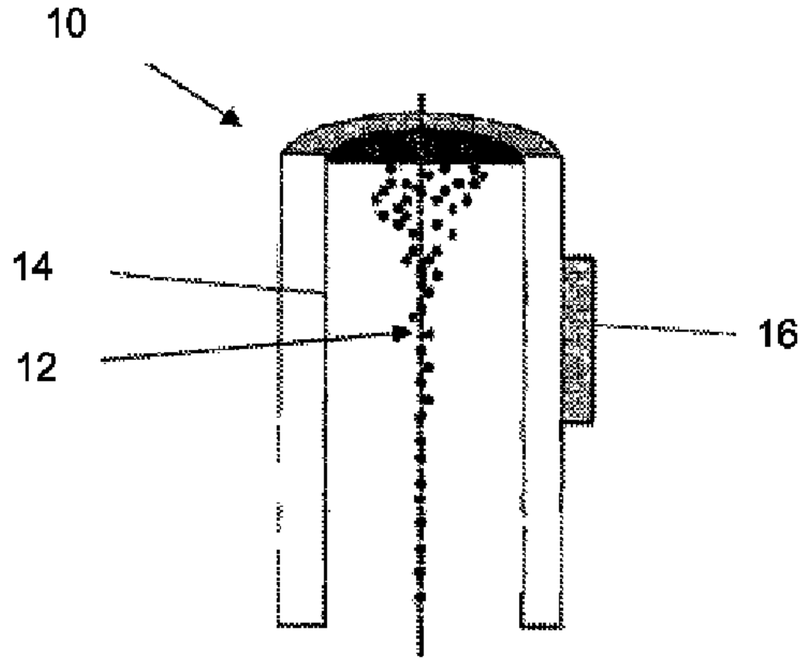
(72) **Inventeurs/Inventors:**
KADUCHAK, GREGORY, US;
WARD, MICHAEL, US

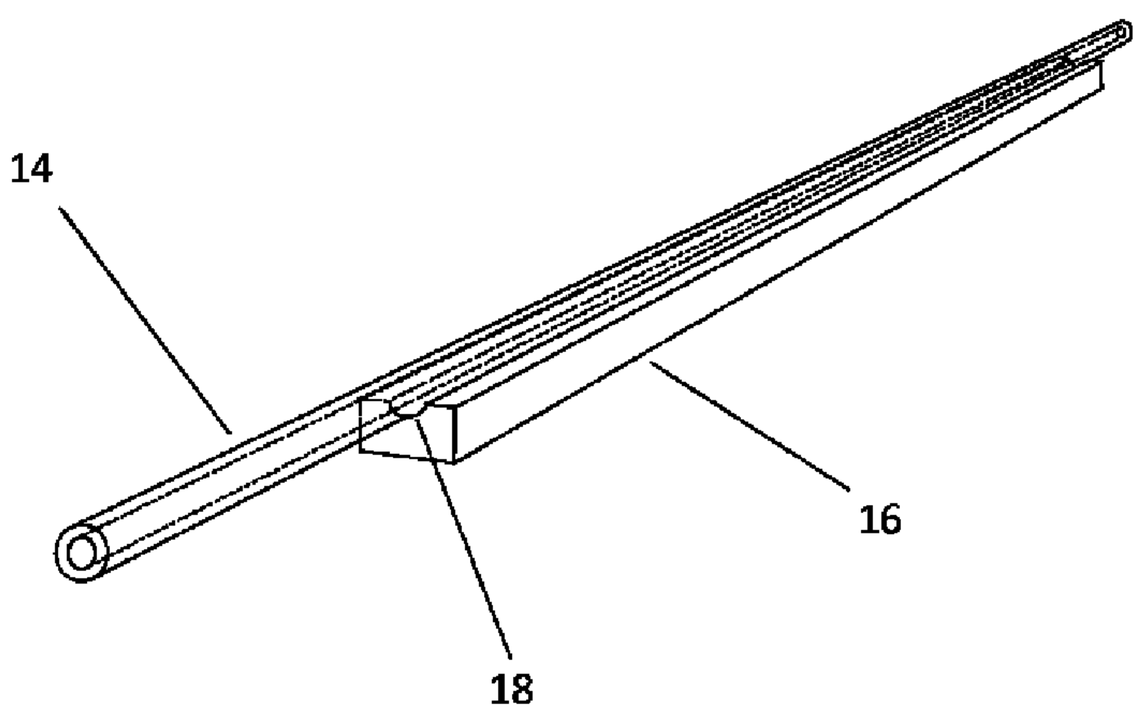
(73) **Propriétaire/Owner:**
LIFE TECHNOLOGIES CORPORATION, US

(74) **Agent:** MBM INTELLECTUAL PROPERTY LAW LLP

(54) **Titre : UN SYSTEME DE CONCENTRATION ACOUSTIQUE DE PARTICULES COMPORTANT UN CAPILLAIRE ET UNE SEULE SOURCE DE VIBRATION**

(54) **Title: A SYSTEM FOR ACOUSTICALLY CONCENTRATING PARTICLES COMPRISING A CAPILLARY AND A SINGLE VIBRATION SOURCE**





(57) **Abrégé/Abstract:**
The present invention provides a system for acoustically concentrating particles comprising a capillary and a single vibration source. The capillary has an elliptical cross-section and an outer wall. The capillary is configured to flow particles in a stream and is coupled to the vibration source. The single vibration source includes a groove and is configured to acoustically focus a plurality of particles to a plane within the capillary. The groove is contoured to the shape of the outer wall of the capillary.

Abstract:

The present invention provides a system for acoustically concentrating particles comprising a capillary and a single vibration source. The capillary has an elliptical cross-section and an outer wall. The capillary is configured to flow particles in a stream and is coupled to the vibration source. The single vibration source includes a groove and is configured to acoustically focus a plurality of particles to a plane within the capillary. The groove is contoured to the shape of the outer wall of the capillary.

A SYSTEM FOR ACOUSTICALLY CONCENTRATING PARTICLES COMPRISING A CAPILLARY AND A SINGLE VIBRATION SOURCE

[0001] Deleted.

BACKGROUND OF THE INVENTION

Field of the Invention (Technical Field):

[0002] Embodiments of the present invention relate to acoustic cytometry and more specifically to acoustic focusing hardware and implementations.

Background

[0003] Note that the following discussion refers to a number of publications by author(s) and year of publication, and that due to recent publication dates certain publications are not to be considered as prior art vis-à-vis the present invention. Discussion of such publications herein is given for more complete background and is not to be construed as an admission that such publications are prior art for patentability determination purposes.

[0004] Flow cytometry is a powerful tool used for analysis of particles and cells in a myriad of applications primarily in bioscience research and medicine. The analytical strength of the technique lies in its ability to parade single particles (including bioparticles such as cells, bacteria and viruses) through the focused spot of light sources, typically a laser or lasers, in rapid succession, at rates exceeding thousands of particles per second. The high photon flux at this focal spot produces scatter of light by a particle and/or emission of light from the particle or labels attached to the particle that can be collected and analyzed. This gives the user a wealth of information about individual particles that can be quickly parleyed into statistical information about populations of particles or cells.

[0005] In traditional flow cytometry, particles are flowed through the focused interrogation point where a laser directs a laser beam to a focused point that includes the core diameter within the channel. The sample fluid containing particles is hydrodynamically focused to a very small core diameter of around 10-50 microns by flowing sheath fluid around the sample stream at a very high volumetric rate on the order of 100-1000 times the volumetric rate of the sample. This results in very fast linear velocities for the focused particles on the order of meters per second. This in turn means that each particle spends a very limited time in the excitation spot, often only 1-10 microseconds. When the linear flow of the hydrodynamic sheath fluid is stopped, the particles are no longer focused. Only resuming the hydrodynamic sheath fluid flow will refocus the particles. Further, once the particle passes the interrogation point the particle cannot be redirected to the interrogation point again because the linear flow velocity cannot be reversed. Still further, a particle cannot be held at the interrogation point for a user defined period of time for further interrogation because focusing is lost without the flow of the hydrodynamic sheath fluid. Because of the very high photon flux at the excitation point, flow cytometry is still a very sensitive technique, but this fast transit time limits the sensitivity and resolution that can be achieved. Often, greater laser power is used to increase the photon flux in an effort to extract more signal but this approach is limiting in that too much light can often photobleach (or excite to non-radiative states) the fluorophores being used to generate the signal and can increase background Rayleigh scatter, Raman scatter and fluorescence as well.

[0006] Slower flowing cytometry systems have been developed to push the limits of sensitivity and have shown detection limits down to the single molecule level. In one of these systems, it was shown that lower laser power (<1 mW) was actually preferable for single molecule detection of double stranded DNA fragments intercalated with fluorescent dyes. Because of the slow transit times (hundreds of microseconds to milliseconds), it was possible to get maximum fluorescence yield out of the dyes while reducing background, photobleaching and non-radiative triplet states with the lower laser power.

[0007] Slow flow hydrodynamic systems, while incredibly sensitive, are not in widespread use because fluidic dimensions are generally very small, which results in easy clogging and very limited sample throughput. In order to focus the sample stream to a core diameter small enough to maintain the uniform illumination and flow velocity required for precision particle measurement, the sheath must still be supplied in a very high volumetric ratio to the sample. In order to achieve a slow linear velocity, the volumetric sample rate must be extremely small. Therefore, to process appreciable numbers of events, the sample must be highly concentrated. If for example a relatively slow linear velocity of 1 centimeter per second is desired with a typical core diameter of about 10 microns, the sample must be

delivered at about 0.05 microliters per minute. To process just 100 cells per second, the cell concentration must be 120,000 per microliter or 120 million per milliliter. This concentration requirement in turn makes clogging even more likely. The problem is further compounded by the tendency of many types of cells to clump in high concentration and to settle out and stick to surfaces when sample delivery rates are slow. The system created by Doornbos, circumvents the clogging problem by using a conventional flow cell with flow resistors to slow the flow, but he found it very difficult to control precise focused delivery of the sample. This method also does not eliminate the need for slow volumetric delivery and highly concentrated samples.

[0008] Sheathless, non-focusing flow cytometers have been developed but these instruments suffer from low sensitivity due to the need for a focal spot size that will excite particles throughout the channel. The spot size is reduced by using very small capillary channels but particles flow within the channel at variable rates according to the laminar flow profile that develops in the channel. This results in different transit times and coincidence of particles in the laser spot which both make analysis more difficult. Also, background cannot be reduced by spatially filtering optics that are designed to collect light from a tightly focused core stream. This limits sensitivity and resolution.

[0009] Other approaches have been demonstrated to manipulate particles using acoustic radiation pressure in a laboratory setting. These devices are planar devices modeled in Cartesian coordinates. Applying an acoustic field generates a quasi-one-dimensional force field that focuses particles into a ribbon in a rectangular chamber. For laminar flow, the resulting distribution of particles across the chamber places the particles in different velocity stream lines. Particles in different stream lines will not only be in different locations but they will also flow at different velocities. This in turn results in different residence times for particles at a location within the device. Planar focusing does not align particles in a manner suitable for use with flow cytometers.

BRIEF SUMMARY OF THE INVENTION

[0010] An embodiment of the present invention comprises an acoustic focusing capillary further comprising a capillary coupled to at least one vibration source and the at least one vibration source possessing a groove. The capillary of this embodiment is preferably coupled to the vibration source at the groove. The groove preferably has an approximately same cross-sectional geometry as the capillary. The capillary can be circular, elliptical, oblate, or rectangular. The vibration source preferably comprises a piezoelectric material. In this embodiment, the groove preferably increases an acoustic source aperture of the capillary.

[0011] Another embodiment of the present invention comprises a method of manufacturing an acoustic focusing capillary. This embodiment further comprises providing a capillary and at least one vibration source, machining a groove into the vibration source, and coupling the at least one vibration source to the capillary at the groove. The groove preferably has an approximately same cross-sectional geometry as the capillary. The capillary can be circular, elliptical, oblate, or rectangular. The at least one vibration source preferably comprises a piezoelectric material. This embodiment can optionally comprise increasing the acoustic source aperture of the capillary.

[0012] In yet another embodiment of the present invention, an apparatus that hydrodynamically and acoustically focuses particles in a particle stream comprises a flow chamber, an outer confine of the flow chamber for flowing a hydrodynamic fluid therethrough, a central core of the flow chamber for flowing the particle sample stream therethrough, and at least one transducer coupled to the chamber producing acoustic radiation pressure. The transducer of this embodiment is preferably coupled to an outer wall of the flow chamber. The transducer can alternatively form a wall of the flow chamber.

[0013] A further embodiment of the present invention comprises a method of hydrodynamically and acoustically focusing a particle stream. This method preferably comprises flowing a sheath fluid into outer confines of a capillary, flowing a particle stream into a central core of the capillary, and applying acoustic radiation pressure to the particle stream within the sheath fluid. The particle stream of this method can be hydrodynamically focused and subsequently acoustically focused. Alternatively, the particle stream is acoustically focused and hydrodynamically focused simultaneously.

[0014] Another method of hydrodynamically and acoustically focusing particles is still a further embodiment of the present invention. This embodiment preferably comprises providing a fluid comprising particles therein, flowing a sheath fluid into outer confines of a flow chamber, flowing the fluid containing the particles into a central core of the flow chamber, and applying acoustic radiation pressure to the fluid comprising the particles. This embodiment can also comprise analyzing the particles.

[0015] One embodiment of the present invention comprises a method of aligning particles using acoustic radiation pressure. This embodiment preferably includes providing a fluid comprising particles therein, subjecting the fluid to acoustic radiation pressure, rotating the fluid 90 degrees, and subjecting the fluid to acoustic radiation pressure a second time to align the particles. This embodiment can also comprise analyzing the aligned particles.

[0016] Another embodiment of the present invention comprises a method of hydrodynamically and acoustically focusing particles in a fluid. This embodiment includes flowing a fluid comprising particles therein, subjecting the fluid to acoustic radiation pressure in one planar direction to acoustically focus the particles, and flowing a sheath fluid in a second planar direction thereby hydrodynamically focusing the fluid in the second planar direction to further focus the particles.

[0017] The present invention further includes methods for dislodging bubbles in a fluidic system. These methods comprise providing a fluid stream through a channel and resonating the channel at an acoustic frequency. These methods also include providing a fluid stream through a channel and vibrating the channel walls at a low frequency.

[0018] An embodiment of the present invention is an apparatus that acoustically focuses particles into a quasi-planar arrangement in a fluid. This embodiment preferably comprises a capillary with an oblate cross-sectional geometry and at least one transducer coupled to the capillary. The capillary is preferably elliptical. This embodiment can further comprise an imager for imaging the particles.

[0019] Another embodiment of the present invention comprises a method for acoustically focusing particles into a quasi-planar arrangement in a fluid comprising particles. The method preferably comprises flowing the fluid comprising particles therein through a flow chamber comprising an oblate cross-sectional geometry and subjecting the fluid to acoustic radiation pressure. The cross-sectional geometry of the flow chamber is preferably elliptical. This embodiment can also include imaging the particles.

[0020] Objects, advantages and novel features, and further scope of applicability of the present invention will be set forth in part in the detailed description to follow, taken in conjunction with the accompanying drawings, and in part will become apparent to those skilled in the art upon examination of the following, or may be learned by practice of the invention. The objects and advantages of the invention may be realized and attained by means of the instrumentalities and combinations particularly pointed out in the appended claims.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

[0021] The accompanying drawings, which are incorporated into and form a part of the specification, illustrate one or more embodiments of the present invention and, together with the

description, serve to explain the principles of the invention. The drawings are only for the purpose of illustrating one or more preferred embodiments of the invention and are not to be construed as limiting the invention. In the drawings:

[0022] Fig. 1 is an embodiment of the present invention illustrating a line drive capillary where particles are acoustically focused to the central axis of the capillary;

[0023] Fig. 2 illustrates construction of line-drive capillary with grooved piezoelectric transducer (PZT) according to one embodiment of the present invention;

[0024] Fig. 3 illustrates a diagram of a line driven capillary with an elliptical cross section according to one embodiment of the present invention;

[0025] Fig. 4 illustrate force potential U in a line-driven capillary with elliptical cross section according to one embodiment of the present invention;

[0026] Fig. 5 illustrate force potential for different aspect ratios for a spherical latex particle in an elliptical cross section, line-driven capillary according to one embodiment of the present invention;

[0027] Figs. 6A and 6B illustrate focused particle stream flowing through an elliptical cross section line driven capillary according to one embodiment of the present invention;

[0028] Figs. 7A and 7B illustrates hydrodynamically focused particles distributed in a central core stream according to one embodiment of the present invention;

[0029] Fig. 8 illustrates acoustic focusing of particles in combination with hydrodynamic focusing according to one embodiment of the present invention.

[0030] Figs. 9A and 9B illustrates acoustically assisted hydrodynamic focusing according to one embodiment of the present invention; and

[0031] Fig 10 illustrates a combination of acoustic and hydrodynamic focusing in a microfluidic channel according to one embodiment of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

[0032] As used herein “a” means one or more.

[0033] As used herein “flow chamber” means a channel or capillary having a shape selected from rectangular, square, elliptical, oblate circular, round, octagonal, heptagonal, hexagonal, pentagonal, and triagonal. It is not necessary for the shape of the interior walls of the flow chamber be the same as the shape of the exterior walls. As a non-limiting example, a flow chamber can have an interior wall defined by a circular shape and the exterior wall defined by a rectangular shape. Additionally, the flow chamber can be part of a composite construction of materials and geometries wherein one of the above shapes defines the interior shape of the flow chamber.

[0034] As used herein “capillary” means a channel or chamber having a shape selected from rectangular, square, elliptical, oblate circular, round, octagonal, heptagonal, hexagonal, pentagonal, and triagonal. It is not necessary for the shape of the interior walls of the capillary be the same as the shape of the exterior walls. As a non-limiting example, a capillary can have an interior wall defined by a circular shape and the exterior wall defined by a rectangular shape.

[0035] One aspect of one embodiment of the present invention provides for ease in alignment during device fabrication and for larger acoustic source apertures. Another aspect of one embodiment of the present invention provides for a line-driven capillary with oblate cross-section to obtain quasi-planar particle concentration. Another aspect provides for planar particle concentration without particles contacting and/or staying in contact with the inner capillary wall. Another aspect provides for imaging applications where particles spread over a plane in a narrow depth of field. Another aspect provides for applying acoustic radiation pressure forces to assist in stabilizing standard hydrodynamic particle focusing systems. Yet another aspect provides for reduced sheath consumption in slow-flow hydrodynamic systems and to assist in particle focusing in planar systems (e.g. chip based systems). Still another aspect provides a method to dislodge bubbles from fluidic systems.

Construction of Line-Driven Capillaries w/Grooved Source

[0036] Line-driven capillaries are used to acoustically concentrate particles in a flowing stream within a capillary. Particles experience a time-averaged acoustic force resulting from acoustic radiation pressure. Fig. 1 illustrates line-driven capillary **10** operating in a dipole mode where particles **12** are acoustically focused to the central axis of capillary **14** to the position of an acoustically formed particle

trap according to one embodiment of the present invention. (The embodiment illustrated in Fig. 1 is applicable to any vibrational mode of the system whether it be monopole, dipole, quadrapole, etc., or a combination of modes.) It is possible to drive different mode configurations with different spatial configurations of sources attached to the capillary.

[0037] Another aspect of one embodiment of the present invention provides a line-driven capillary system that both delivers stable acoustic signals within the capillary and possess consistent, repeatable electrical properties of the electromechanical circuit that drives the system.

[0038] In one embodiment of the present invention, line-driven capillary **10** is comprised of capillary **14** coupled to vibration source **16**. Capillary **14** can be made from, but are not limited to glass, metal, plastic, or any combination thereof. Low loss materials are preferably particle concentrators and stainless steel is one of the better capillary materials. Vibration source **16** is preferably comprised of a piezoelectric material. Examples of piezoelectric materials include but are not limited to PZT, lithium niobate, quartz, and combinations thereof. Vibration source **16** can also be a vibration generator such as a Langevin transducer or any other material or device that is capable of generating a vibration or surface displacement of the capillary. Another aspect of the embodiment of the present invention comprises an acoustically focused line drive capillary that yields a larger acoustic source aperture than a standard line contact.

[0039] According to one embodiment of the present invention, groove **18** is machined into vibration source **16** into which capillary **14** is cradled, as illustrated in Fig. 2. A diagram is shown in Fig. 2, which comprises line-drive capillary **10** with grooved vibration source **16**, a small PZT slab with machined circular groove **18** is adhered to capillary **14** to improve manufacturability and acoustic performance. Groove **18** is circular with a radius that matches the outer radius of capillary **14** plus a small glue layer. The number of grooved vibration sources attached to capillary **14** is not limited to one. Using more than one grooved vibration source is advantageous in driving different acoustic modes that require specific spatial dependence. For example, a dipole mode is driven with a single source or with two sources attached to opposite walls of capillary **14** and driven 180 degrees out of phase. A quadrapole mode is driven by attaching sources at orthogonal positions (90 degree offset from one another) and driven out of phase. For capillaries of non-circular cross section, groove **18** will typically take on the cross sectional geometry of capillary **14**. For example, an elliptical cross section capillary would require an elliptical cross section groove. Capillary **14** is preferably held to vibration source **16** with a small glue layer. When using a piezoelectric crystal as vibration source **16**, it is not necessary to

have an electrical conducting layer inside groove **18** that is cut into the crystal. Construction with and without conductors in groove **18** have been demonstrated.

[0040] Another aspect of one embodiment of the present invention provides ease of device construction.

[0041] Yet another aspect of one embodiment of the present invention provides for larger acoustic source aperture as compared to a true line-driven device.

[0042] Still another aspect of one embodiment of the present invention provides for repeatable acoustic/electrical performance.

[0043] Another aspect of one embodiment of the present invention provides for ease in alignment of capillary **14** with vibration source **16**.

[0044] Still another aspect of one embodiment of the present invention provides for a larger glue surface upon which to attach a transducer.

[0045] Additionally, it is not necessary for the capillary to have a circular cross section. In one embodiment of the present invention, a square cross section groove in PZT is used. Capillaries can be constructed with many geometries including but not limited to elliptical, square, rectangular, general oblate shapes, as well as any cross sectional geometry.

Quasi-Planar Focusing Of Particles In Line-Driven Oblate Capillaries

[0046] Referring now to Fig. 3, line-driven capillaries with circular cross sections can be driven to align particles along the axis of the cylindrical capillary when driven in a dipole mode in one embodiment of the present invention. In this embodiment, it may be desirable in certain applications to localize the particles only to a specific plane within a capillary, not to a point or line. This is the case for imaging applications where particles need to be distributed in a plane within a narrow depth of field of the imaging optics. A method to spatially distribute the particles is to break the circular symmetry of the system. By making the cross section of the capillary more oblate (e.g. elliptical), it is possible to keep tight spatial localization in one dimension while allowing the particles to be spatially distributed in another dimension. This method is advantageous for systems requiring particles placed in a planar (or quasi-planar) arrangement.

[0047] For example, an acoustically driven capillary **10** with an elliptical cross section is illustrated in Fig. 3. In this embodiment, acoustic source **16** spatially distributes particles in a plane along the major axis and tightly confines particles along the minor axis. The aspect ratio A of the ellipse is given by the ratio of the minor axis ay to major axis ax: A = ay/ax. To calculate the acoustic force on particles within the capillary, the acoustic radiation pressure force on a compressible, spherical particle of volume *V* in an arbitrary acoustic field can be written in terms of an acoustic radiation pressure force potential *U* (Gor'kov 1962):

[0048] $$U = \frac{4}{3}\pi a^3 \left[\left(\beta_0 \frac{\langle p^2 \rangle}{2}\right) f_1 - \frac{3}{2}\left(\rho_0 \frac{\langle v^2 \rangle}{2}\right) f_2\right]$$

[0049] Here, *a* is the particle radius, β_0 is the compressibility of the surrounding fluid, and ρ_0 is the density of the surrounding fluid. The pressure and velocity of the acoustic field in the absence of the particle are described by p and v, respectively, and the brackets correspond to a time-averaged quantity. The terms f_1 and f_2 are the contrast terms that determine how the mechanical properties of the particle differ from the background medium. They are given by:

[0050] $$f_1 = 1 - \frac{\beta_p}{\beta_0}$$

[0051] $$f_2 = \frac{2(\rho_p - \rho_0)}{(2\rho_p - \rho_0)}$$

[0052] The subscript *p* corresponds to intrinsic properties of the particle. The force F acting on a particle is related to the gradient of the force potential by:

[0053] $$F = -\nabla U$$

[0054] Particles are localized at positions where the potential U displays a minimum. (For a circular cross section capillary, a potential minimum is coincident with the axis of the capillary forming the particle trap in Fig. 1.)

[0055] The force potential U for an elliptical cross section capillary line-driven in a dipole type mode is illustrated in Fig. 4. The potential is calculated for latex spheres in water. In this configuration,

the particles experience a force that transports them to a potential well that appears to stretch between the foci of the ellipse. The particles are also more tightly focused in the direction of the minor axis and are more “spread out” in the direction of the major axis.

[0056] Depending upon the aspect ratio of the ellipse, the force potential in orthogonal directions can be dramatically different. This is illustrated in Fig. 5. In Fig. 5, force potential for different aspect ratios for a spherical latex particle in an elliptical cross section, line driven capillary is shown. For this configuration, the particles are more localized along the minor axis and less localized along the major axis. A change in frequency can cause greater localization along the major axis and less localization along the minor axis. For aspect ratios closer to unity, the potential well is more pronounced than for aspect ratios further from unity. Note the gradient of the potential is smaller in the direction of the major axis. The reduced gradient implies less localization of the particles along this direction. As the aspect ratio of the ellipse decreases, the potential well depth decreases resulting in milder gradients and less localization. Therefore, with decreasing aspect ratio, particles experience a greater spread along the major axis of the ellipse (reduced force due to reduced gradient). (There is also a spreading of particles along the minor axis, but to much less of an extent than in the direction of the major axis.)

[0057] Results showing this effect are given in Fig. 6. Fig. 6A displays an example of particles flowing through an elliptic cross section capillary. In this example, the particles are approximately 5.6 mm diameter fluorescent latex spheres (more specifically, polystyrene beads) and appear as horizontal streaks in the image. Flow is from left to right. The image plane contains the major axis of the ellipse and the central axis of the capillary. The particles are spread across approximately half the width of the capillary forming a ribbon of particles. In this example, there is enough force on the particles directed toward the axis of the capillary to keep them off the walls. In Fig. 6B, the image plane has been rotated 90 degrees to include the minor axis of the ellipse and the central axis of the capillary. In this direction, the gradient along the potential well is greater leading to a greater confinement of the particles along the capillary axis. Here they are confined to a single line coincident with the central axis of the capillary.

[0058] Several characteristics of these types of modes exist:

[0059] Particles can be tightly focused either along major or minor axis of ellipse with ‘loose’ focusing along the orthogonal direction (selection of which axis is the weak focusing direction is mode dependent).

[0060] In the weakly focused dimension, enough force can exist to keep particles off the wall of the device.

[0061] Particles are confined to a plane which is conducive to imaging applications where it is necessary to place particles in a common plane especially when depth of focus is small.

Acoustically Assisted Hydrodynamic Focusing of Particles

[0062] Hydrodynamically-focused particle streams are used in flow cytometry as well as other areas where precisely aligned particles in a flowing core stream are required. Hydrodynamic focusing is traditionally employed in flow cytometry to focus particles into a tight stream for laser interrogation. A diagram of a hydrodynamically focused particle stream is illustrated in Fig. 7A. In this example, a sample is injected into a central core stream contained within a coaxial sheath flow. The sheath fluid is typically a clean buffer solution traveling at many times the velocity of the sample input in order to hydrodynamically confine the central sample stream into a smaller cross sectional area. This action confines the particles in a cylindrical core stream of very narrow width. The hydrodynamically focused core stream radius r is given approximately as

[0063] 

where Q is the volumetric flow rate of the core stream and v is the velocity of the core stream. Note that larger volumetric sample delivery and/or lower velocities yield larger diameters of the core stream.

[0064] Hydrodynamically focused sample streams can suffer from instabilities of the central core stream position as a function of many factors. These can include but are not limited to nucleation of bubbles on the cell walls that alter stream lines, turbulence, and combinations thereof. It is advantageous to assist hydrodynamically focused systems with an external force that stabilizes the spatial position of the central core stream. An embodiment of the present invention comprises a device that uses multiple fluidic streams to steer the central core stream.

[0065] Fig. 7B illustrates an embodiment of the present invention comprising an acoustically assisted hydrodynamically focused sample stream. In Fig. 7B, an outer coaxial flow of sheath fluid confines the central core stream containing the sample. By applying acoustic forces to the particles in the hydrodynamically focused core stream, the particles are preferably focused further within the stream.

[0066] One embodiment of the present invention combines acoustic focusing of particles with hydrodynamic focusing. Acoustic focusing assists hydrodynamic focusing systems by stabilizing the absolute location of the particle stream against external forces. Acoustic focusing is also used to further tighten the focus of the particle stream within a hydrodynamically focused system where reduction in sheath fluid consumption or increase in sample throughput is desired without the loss of particle focus quality within the stream. This is particularly important for applications where the sample is dilute. A prime example is high speed sorting of "sticky" cells that must be kept at lower concentration to prevent aggregation. Another example is where reduction of sheath fluid is a priority without sacrificing particle focus. Furthermore, some systems that employ acoustic focusing may not want the sample to contact the walls. (For example, this will keep the build-up of proteins and small particles that are unaffected by acoustic radiation pressure off the capillary walls. These systems can use a slow, low-volume sheath to entrain the sample. Acoustic focusing can then be used to tightly focus the particles within the sample stream.)

[0067] An example of acoustically assisted hydrodynamic focusing is shown in Fig. 7B. In this example, a standard hydrodynamically-focused system is outfitted with ultrasonic transducers to set up a standing wave in the fluid cavity. The particles are initially hydrodynamically focused. Ultrasonic radiation pressure then forces the particles to a force potential minimum located along the axis of the central core stream where they are further aligned within the central core stream.

[0068] Referring now to Fig. 8, a schematic of a device capable of applying acoustic focusing prior to, during, or both prior to and during hydrodynamic focus as illustrated according to one embodiment of the present invention. This embodiment comprises sample **20** flowing through capillary **22**. Sheath fluid **24** hydrodynamically focusing particles **26**. Transducers **28** and **30** acoustically focus particles **26** along the axis of the central core prior to hydrodynamic focusing while transducers **32** and **34** acoustically focus particles **26** during hydrodynamically focusing.

[0069] Measurements demonstrating acoustically assisted hydrodynamic focusing are illustrated in Fig. 9. The image on the left (Fig. 9A) demonstrates hydrodynamic focusing in a cylindrical channel of width 500 microns. The central core stream comprises approximately 5.6 μm diameter polystyrene particles in solution (approx. 0.0025% by volume). The central core stream is surrounded by a coaxial sheath flow that contains a phosphate buffer solution. The image on the right (Fig. 9B) shows particles in the central core stream during hydrodynamic focusing. The sheath fluid is introduced at a volumetric flow rate of between approximately 100 to 1,000 microL/min and preferably at a rate of

approximately 400 microL/min, and the sample core stream is introduced at a volumetric flow rate of between 50 to 500 microL/min and preferably at a rate of approximately 100 microL/min. The frequency of the acoustic excitation is 2.1 MHz. In the image on the right, an acoustic field is activated that is designed to produce a particle trap along the axis of the core sample stream. Particles within the core sample stream are further isolated within the core stream by the acoustic field.

[0070] Aspects of acoustically assisting hydrodynamically focused sample streams include but are not limited to:

- Repeatable location of focused particle stream
- Increased particle focusing in lower velocity hydrodynamically focused streams thereby reducing the sheath fluid requirements (particles spatially confined to a stream smaller in diameter than core stream)
- Increased sample throughput of dilute samples while maintaining tight spatial positioning
- Less effect of turbulence and other exterior influences on the exact location of the focused particles
- Method to isolate sample stream from capillary walls in a system where predominant particle focusing is conducted by acoustic radiation pressure (e.g. line-driven capillary)

[0071] There are many different arrangements where both acoustically focusing and hydrodynamically focusing can be advantageous. Fig. 7B displays a device with two transducers attached to a hydrodynamic focusing cell. The acoustic field can be used in a cell that is circular, square, or any other geometry. The number of transducers shown in Fig. 7B is two. The minimum number of transducers is one. Using more than one transducer provides feedback to monitor the acoustic field within the chamber. Additionally transducers may be attached in orthogonal directions to create force fields that are optimized for a given application. In an embodiment of the present invention, it is advantageous to focus the particles single file into a line within the core sample stream. In another embodiment of the present invention, it is advantageous to focus particles in one dimension and allow them to spread out in an orthogonal direction.

[0072] The ability to use acoustically assisted hydrodynamic focusing of particles is also advantageous for applications in microfluidics. Hydrodynamic focused particle streams in microchannels, microfluidic chips, or other rectangular (or quasi-rectangular) channel geometrics can be enhanced by combining acoustic focusing and hydrodynamic focusing. In one embodiment of the present invention and to reduce sheath fluid consumption, one dimensional hydrodynamic focusing can be used, as illustrated in Fig. 10. A combination acoustic and hydrodynamic focusing in a microfluidic

channel is shown. Hydrodynamic focusing localizes particles in the horizontal direction and acoustic focusing localizes particles in the vertical direction. Ease of implementation for both acoustic and hydrodynamic focusing in planar devices is accomplished in this embodiment. (This can also work if the force diagram in Fig. 10 is rotated 90 degrees.) Using both acoustic focusing and hydrodynamic focusing also keeps small particles and molecular species from contacting the channel walls.

[0073] Another embodiment of the present invention allows for serial acoustic focusing in microfluidic applications. Acoustic focusing applied to a flowing stream of particles in a quasi-rectangular cross section chamber focuses particles into a ribbon-like structure. In order to preserve the layered construction used in many microfluidic assemblies, it is advantageous to preserve the placement of the transducers in parallel planes. Thus, a method to focus particles into a narrow spatial configuration involves acoustically focusing particles into a plane, rotating the flow by 90 degrees, and then acoustically focusing again into the new orthogonal plane. The net result is a narrow spatial distribution of particles. When the transducer is used to excite a dipole type mode within the flow chamber, the result is particles narrowly focused about the central axis of the flow chamber.

Bubble Dislodging from Fluidic Systems

[0074] In traditional flow cytometry, bubbles that adhere to walls of a fluidic system are problematic. They can interfere by moving laminar flow lines, affecting local reactions, deviating focused particle streams. For example, in flow cytometers, bubbles in a fluidic system can have the effect of moving the position of the hydrodynamically-focused sample stream. This movement appears as a misalignment of the optical system to the user and a recalibration is required. A technique to dislodge bubbles from nucleation sites in fluidic systems, especially microfluidic systems, is very desirable.

[0075] Acoustic radiation pressure has been shown to have a large effect on bubbles in fluids due to the large mismatch in density and compressibility between liquids and gases. Acoustic energy can be used to dislodge bubbles from fluidic system in several different ways.

[0076] In one embodiment of the present invention, a fluidic system is engineered such that when resonated at an appropriate acoustic frequency, bubbles experience a force that pulls them away from the wall and stabilizes their equilibrium position within the fluid stream that exists at the location of a pressure node (for bubbles driven at frequencies below their monopole resonance). The chamber is

preferably driven acoustically with either an internal acoustic source or noninvasively with a source attached to an outer wall of the chamber. This is a robust method to dislodge bubbles from walls.

[0077] In another embodiment of the present invention, vibration of the channel walls at low frequencies is preferably used to dislodge the bubbles. By vibrating the wall as part of a structural resonance of the system, large surface displacements are achieved. (These displacements are typically larger at lower frequencies.) Large forces coupled with large displacements are preferably used to break the bond between the bubble and the chamber surface. The inertial forces coupled with localized fluid flows at the chamber wall surface are effective at bubble dislodgement.

[0078] The preceding examples can be repeated with similar success by substituting the generically or specifically described reactants and/or operating conditions of this invention for those used in the preceding examples.

[0079] Although the invention has been described in detail with particular reference to these preferred embodiments, other embodiments can achieve the same results. Variations and modifications of the present invention will be obvious to those skilled in the art and it is intended to cover in the appended claims all such modifications and equivalents.

THE EMBODIMENTS OF THE INVENTION FOR WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A system for acoustically concentrating particles comprising:

a capillary, wherein the capillary has an elliptical cross-section and an outer wall, wherein the capillary is configured to flow particles in a stream; and

a single vibration source, including a groove, configured to acoustically focus a plurality of particles to a plane within the capillary, wherein the capillary is coupled to the vibration source, and wherein the groove is contoured to the shape of the outer wall of the capillary.

2. The system for acoustically concentrating particles of claim 1 wherein said capillary is coupled to said vibration source at said groove.

3. The system for acoustically concentrating particles of claim 1 wherein said groove has an approximately same cross-sectional geometry as said capillary.

4. The system for acoustically concentrating particles of claim 1 wherein said capillary is oblate.

5. The system for acoustically concentrating particles of claim 1 wherein said vibration source comprises a piezoelectric material.

6. The system for acoustically concentrating particles of claim 1 where said groove increases an acoustic source aperture of said capillary.

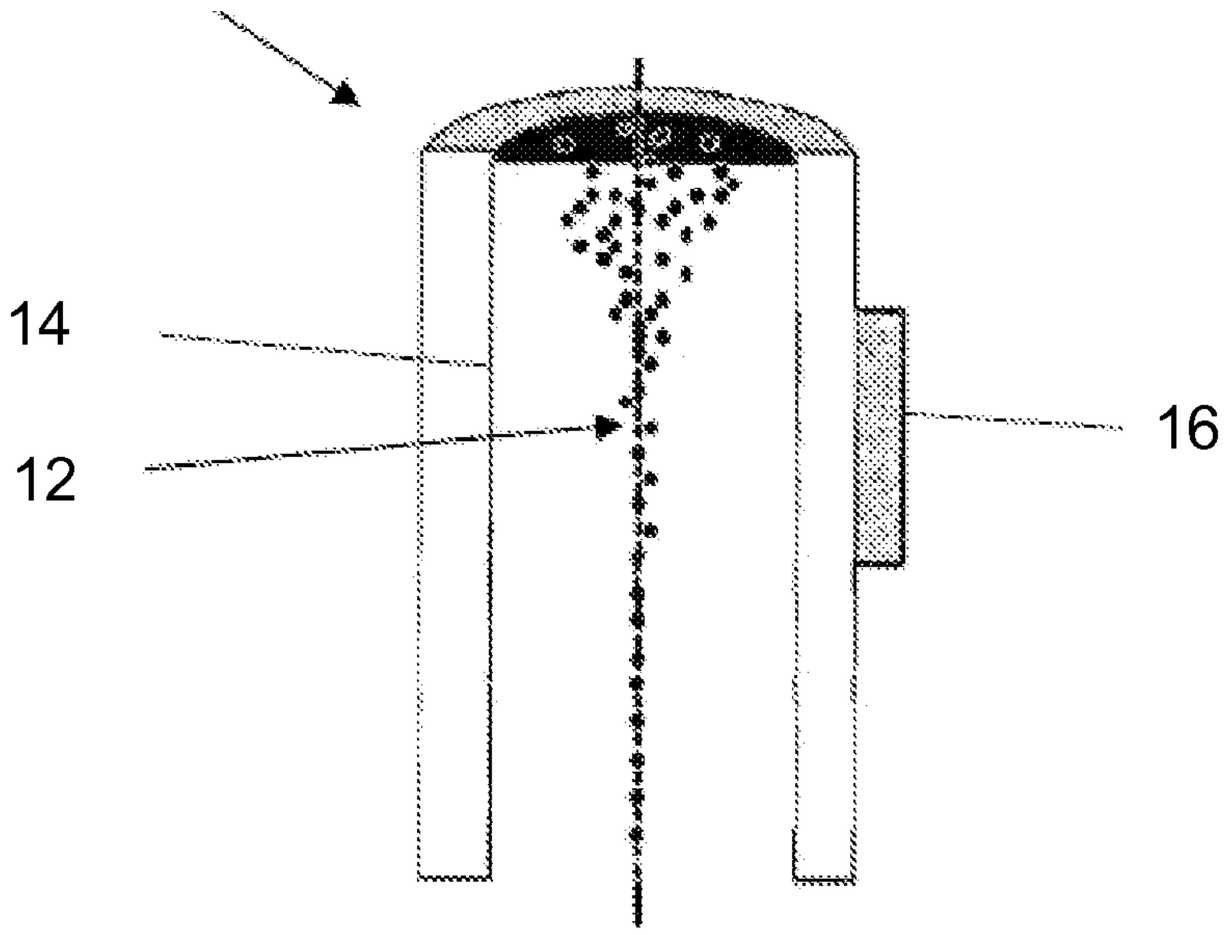


Fig. 1

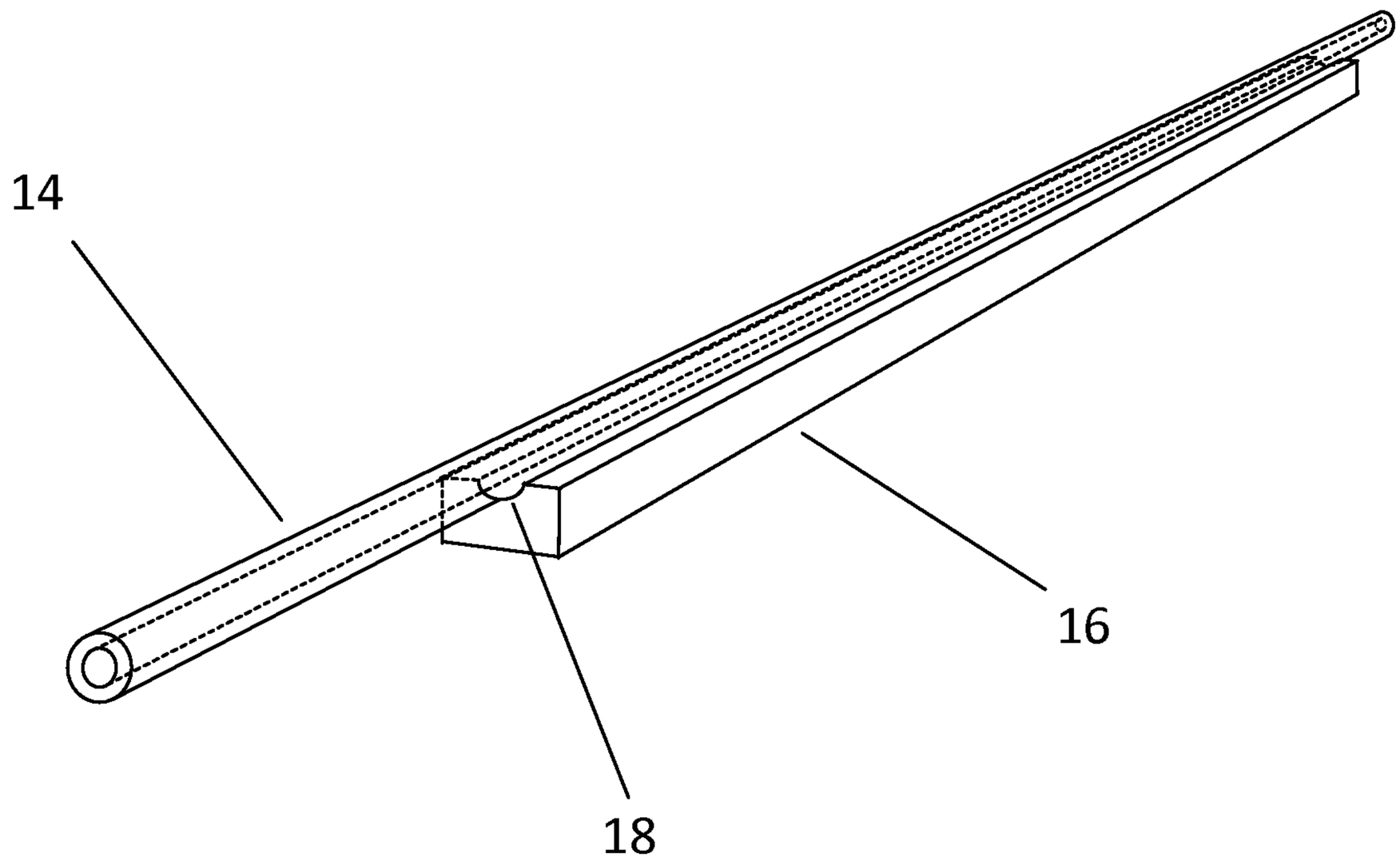


Fig. 2

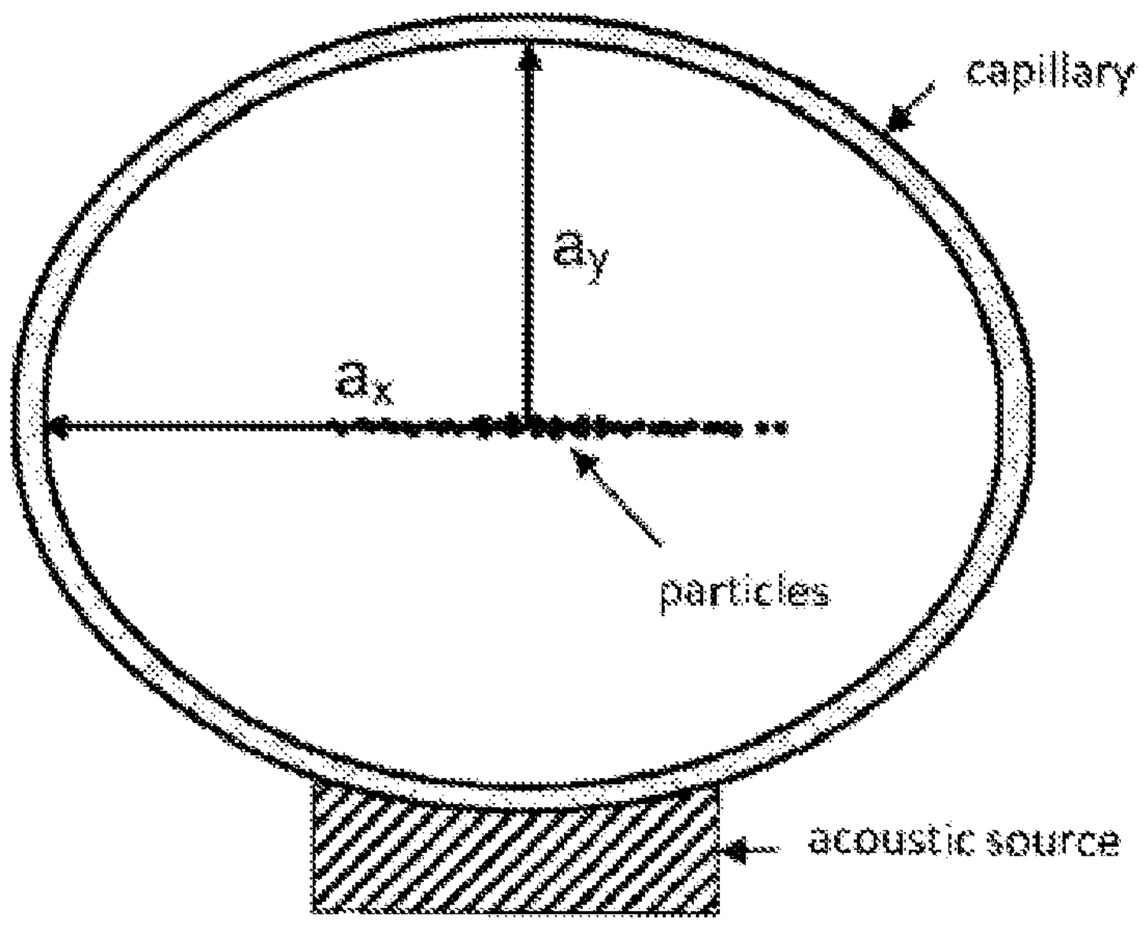


Fig. 3

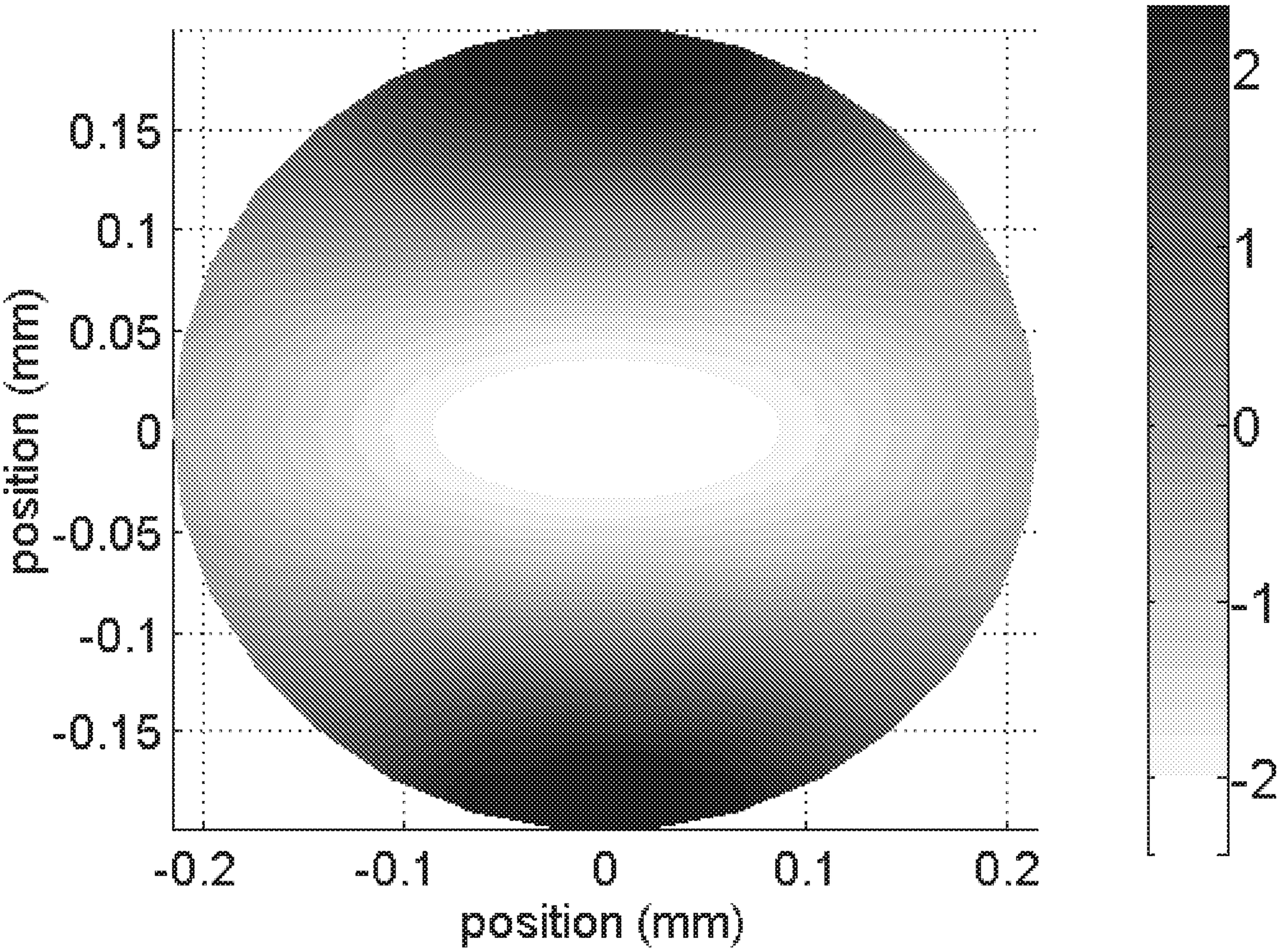

0.15
0.1
0.05
0
-0.05
-0.1
-0.15
position (mm)
-0.2
-0.1
0
0.1
0.2
position (mm)
2
1
0
-1
-2

Fig. 4

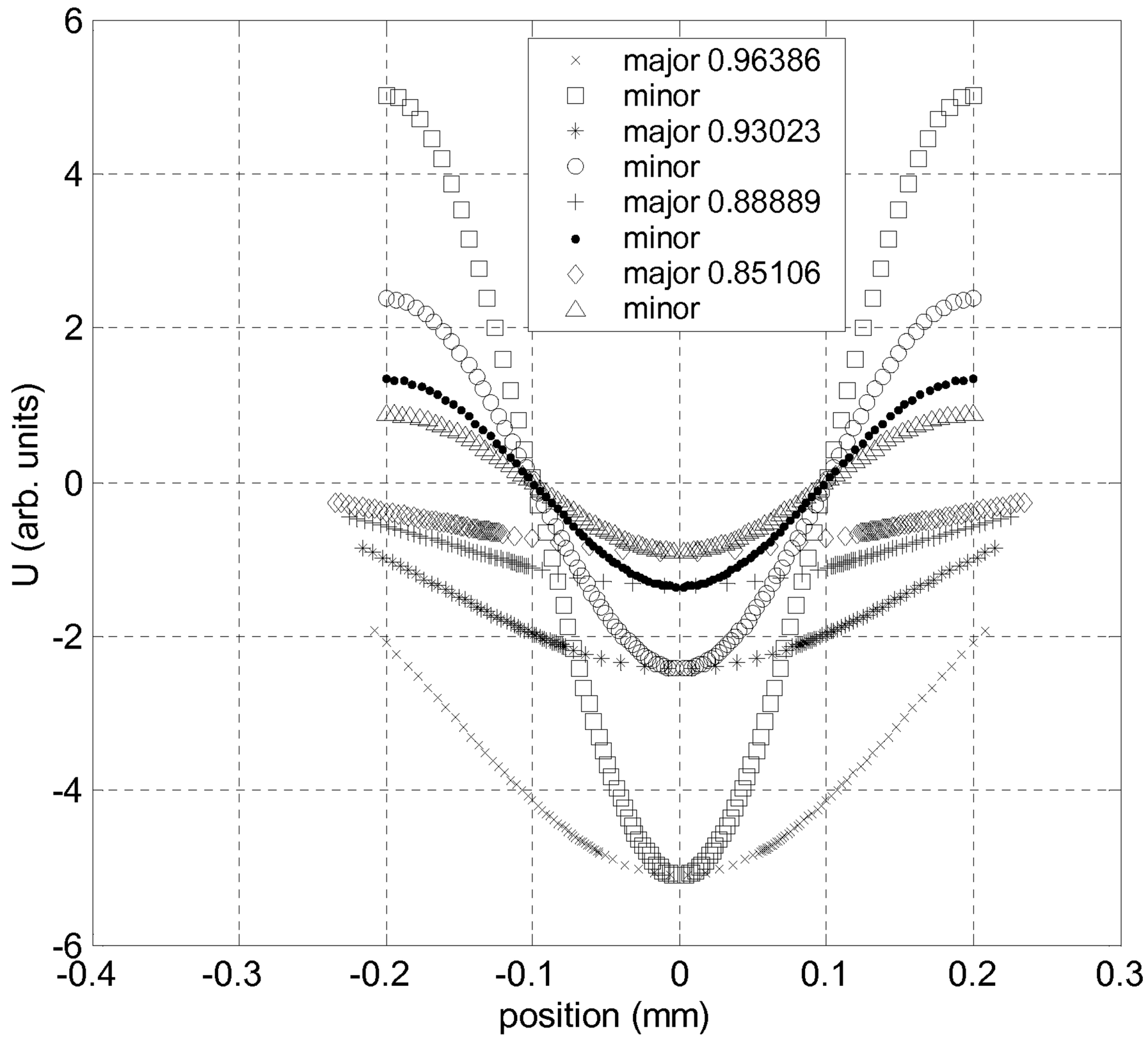

major 0.96386
minor
major 0.93023
minor
major 0.88889
minor
major 0.85106
minor
U (arb. units)
position (mm)
6
4
2
0
-2
-4
-6
-0.4
-0.3
-0.2
-0.1
0
0.1
0.2
0.3

Fig. 5

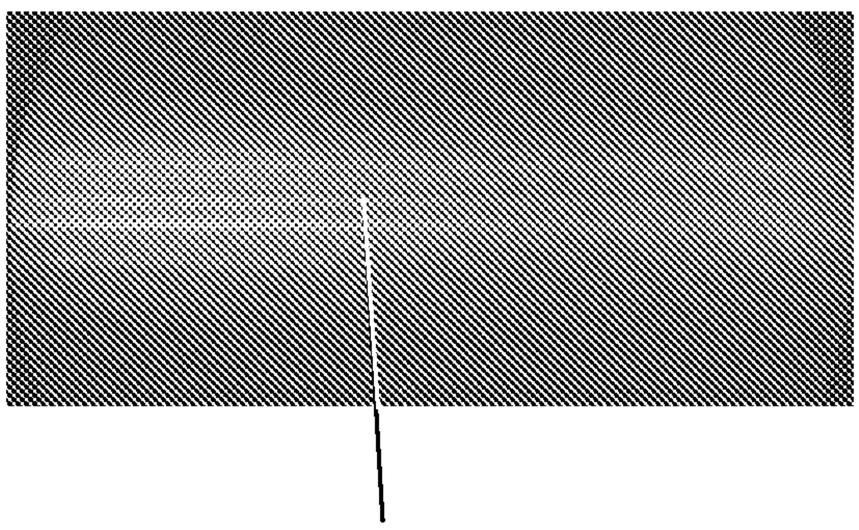

weak focused particle
stream

Fig. 6A

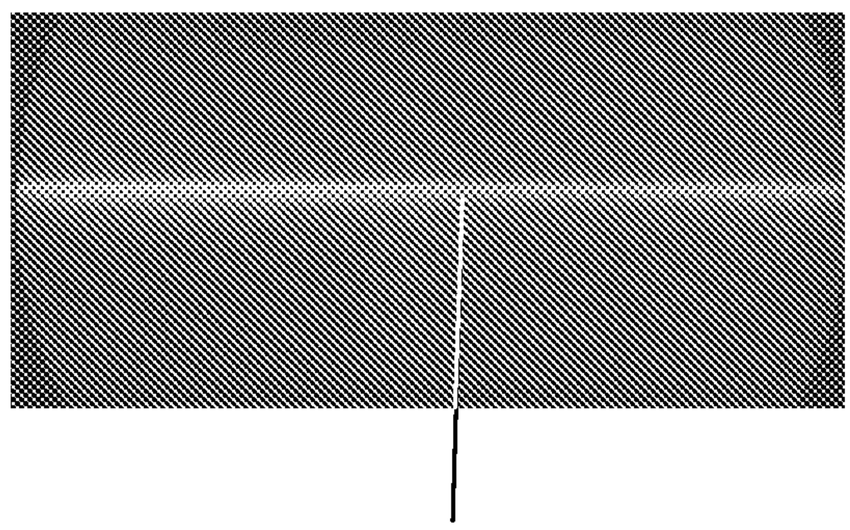

tight focused particle
stream

Fig. 6B

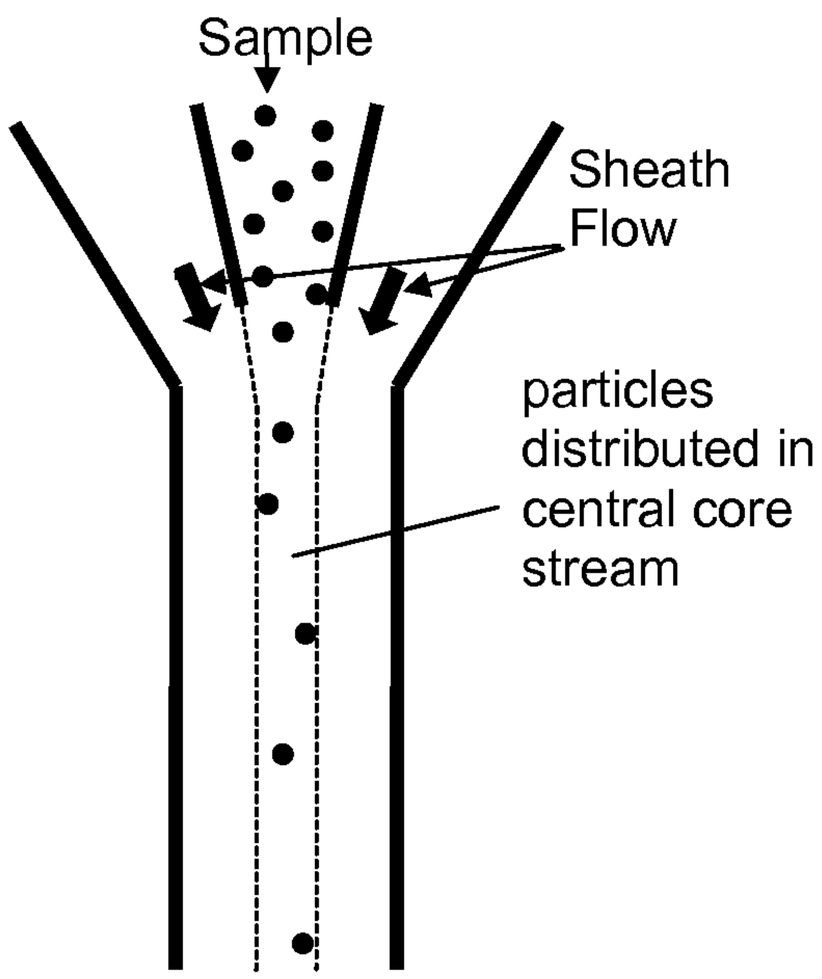

Sample
Sheath Flow
particles distributed in central core stream

Fig. 7A

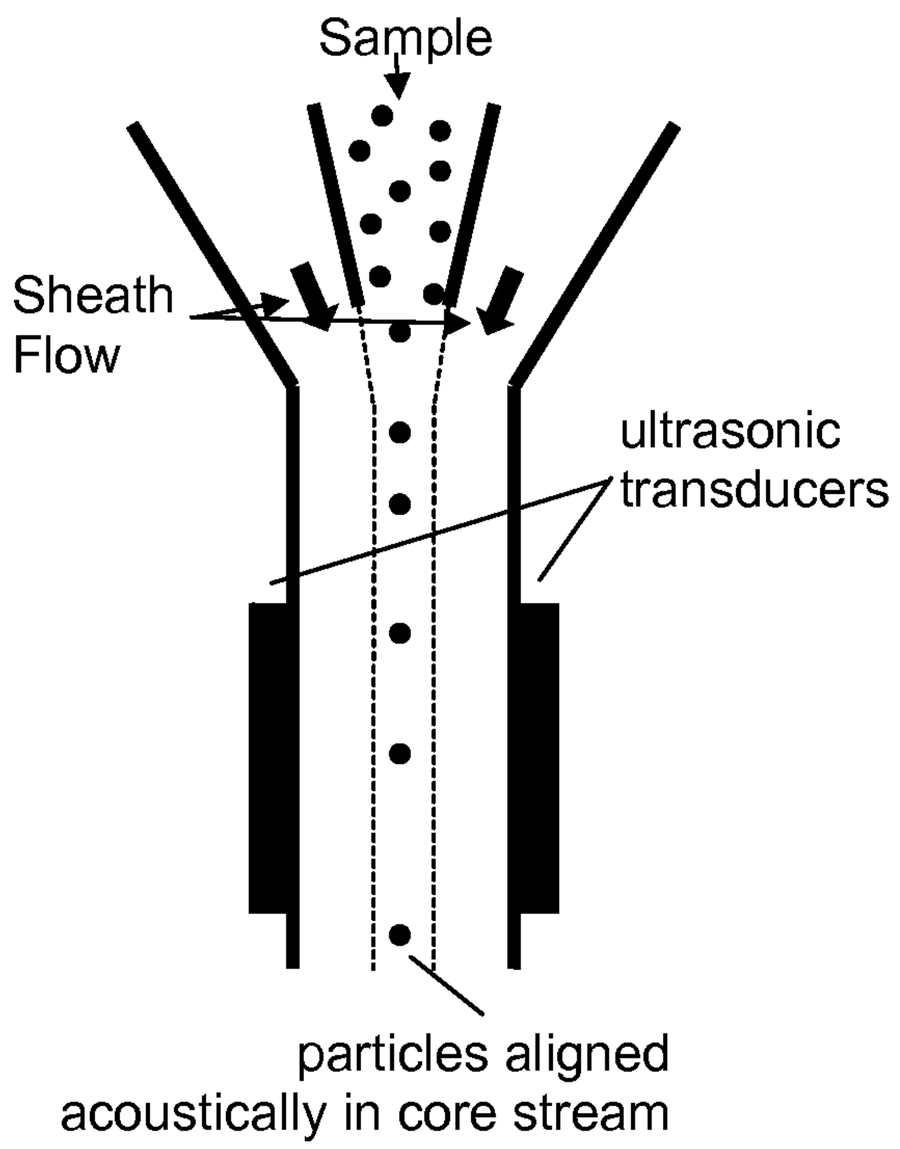

Sample
Sheath Flow
ultrasonic transducers
particles aligned acoustically in core stream

Fig. 7B

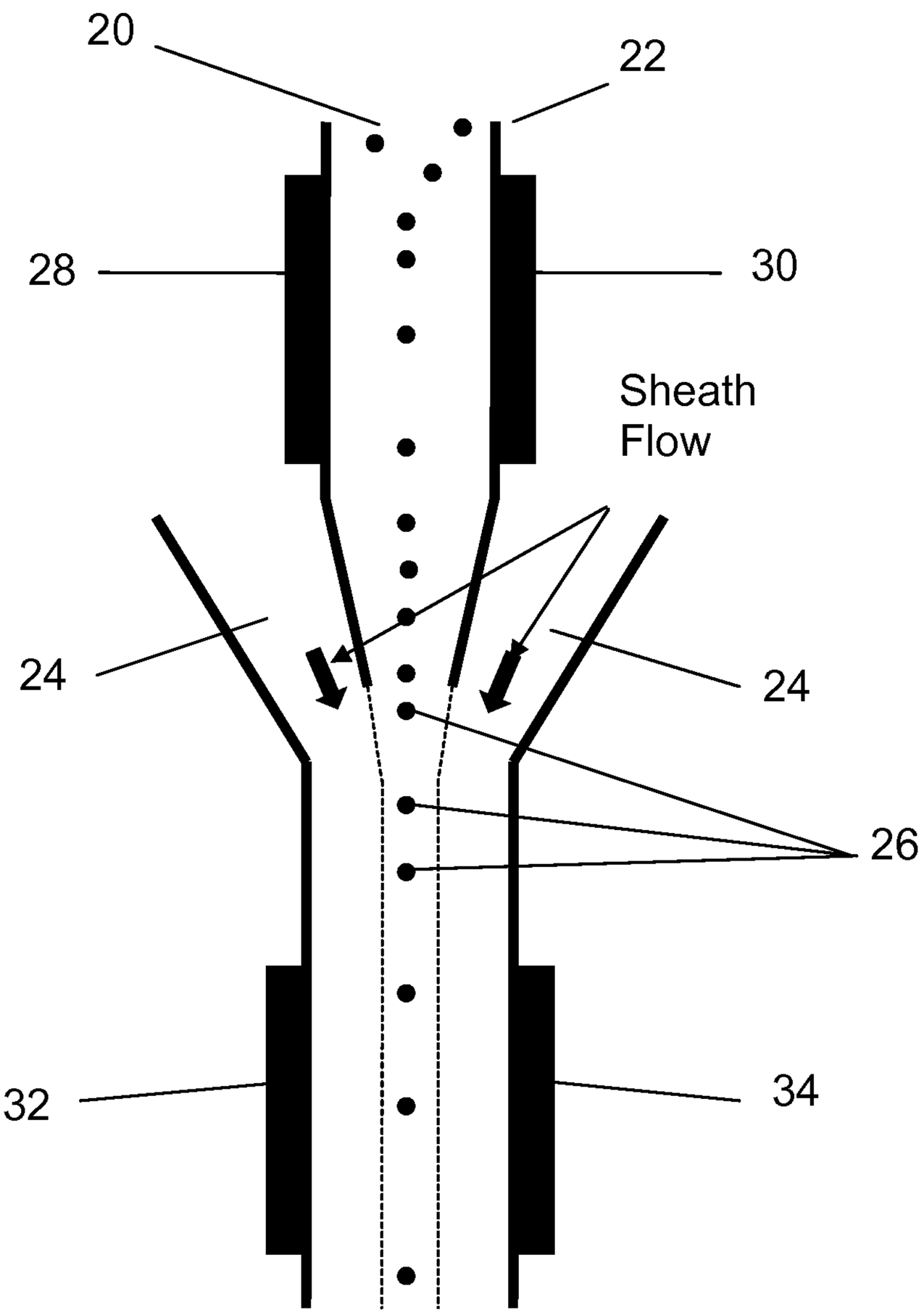


Fig. 8

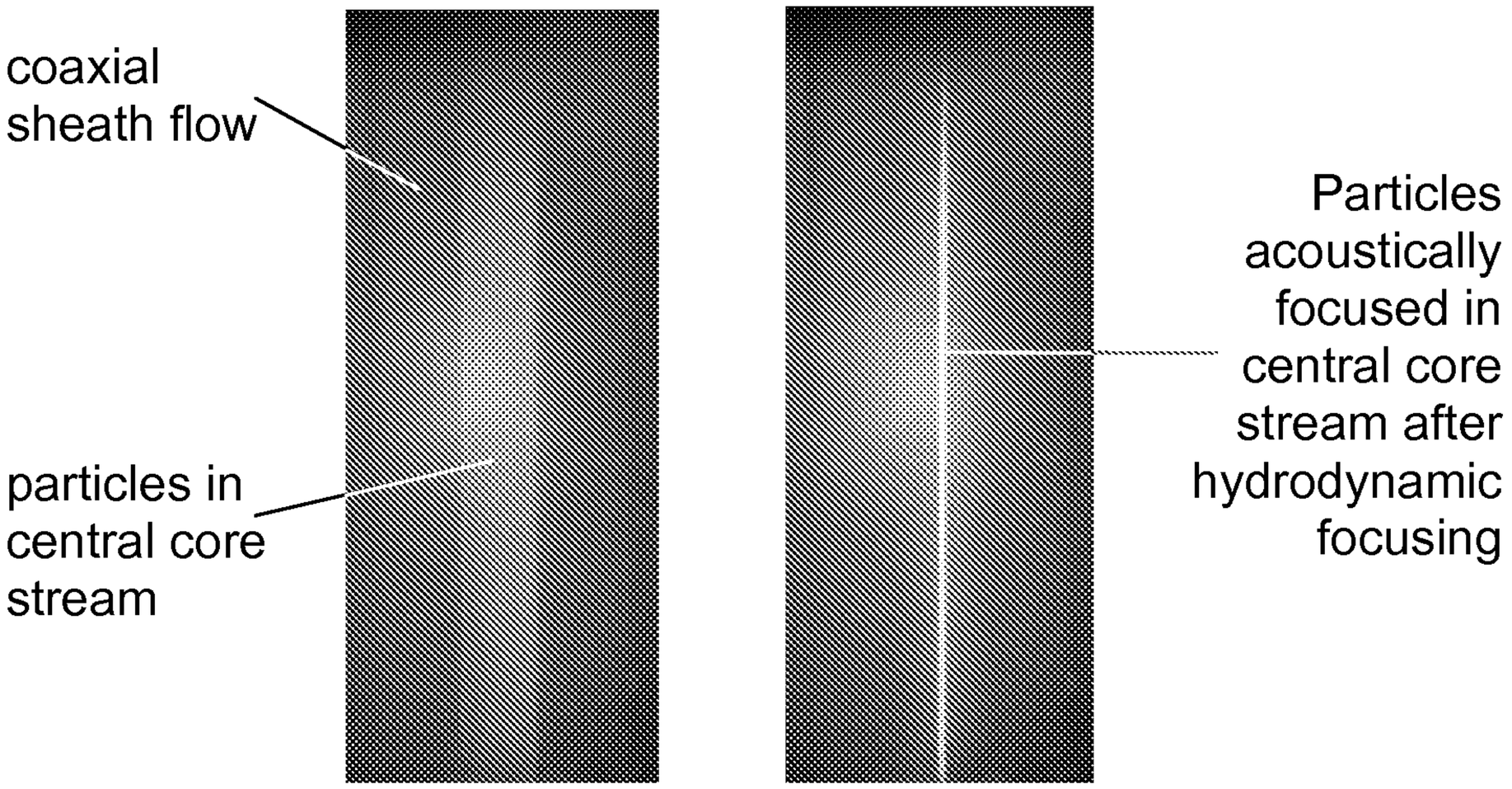

coaxial
sheath flow
particles in
central core
stream
Particles
acoustically
focused in
central core
stream after
hydrodynamic
focusing

Fig. 9A Fig. 9B

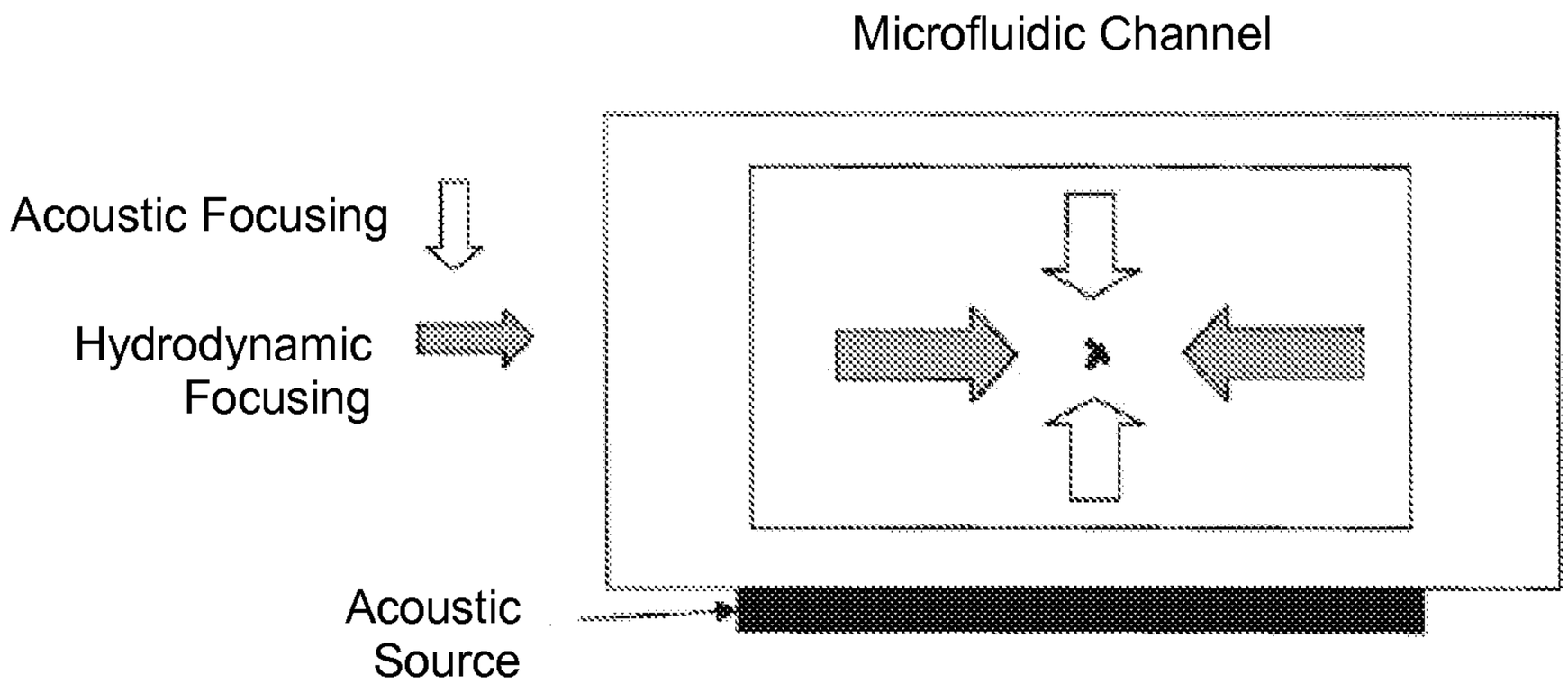
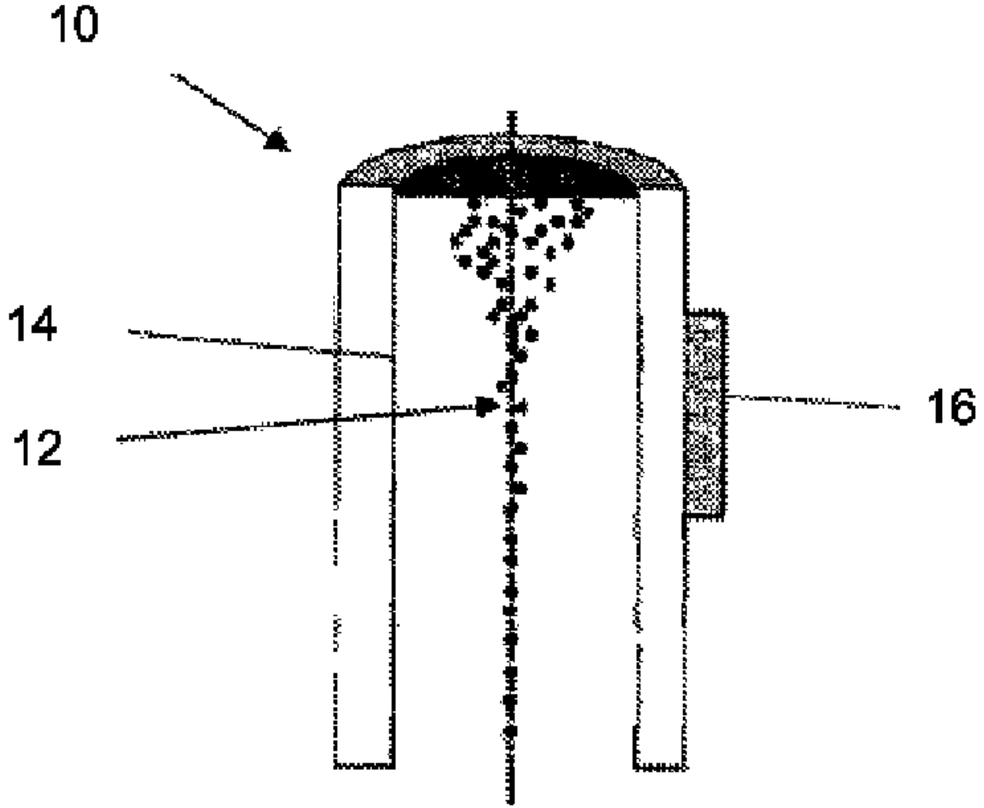
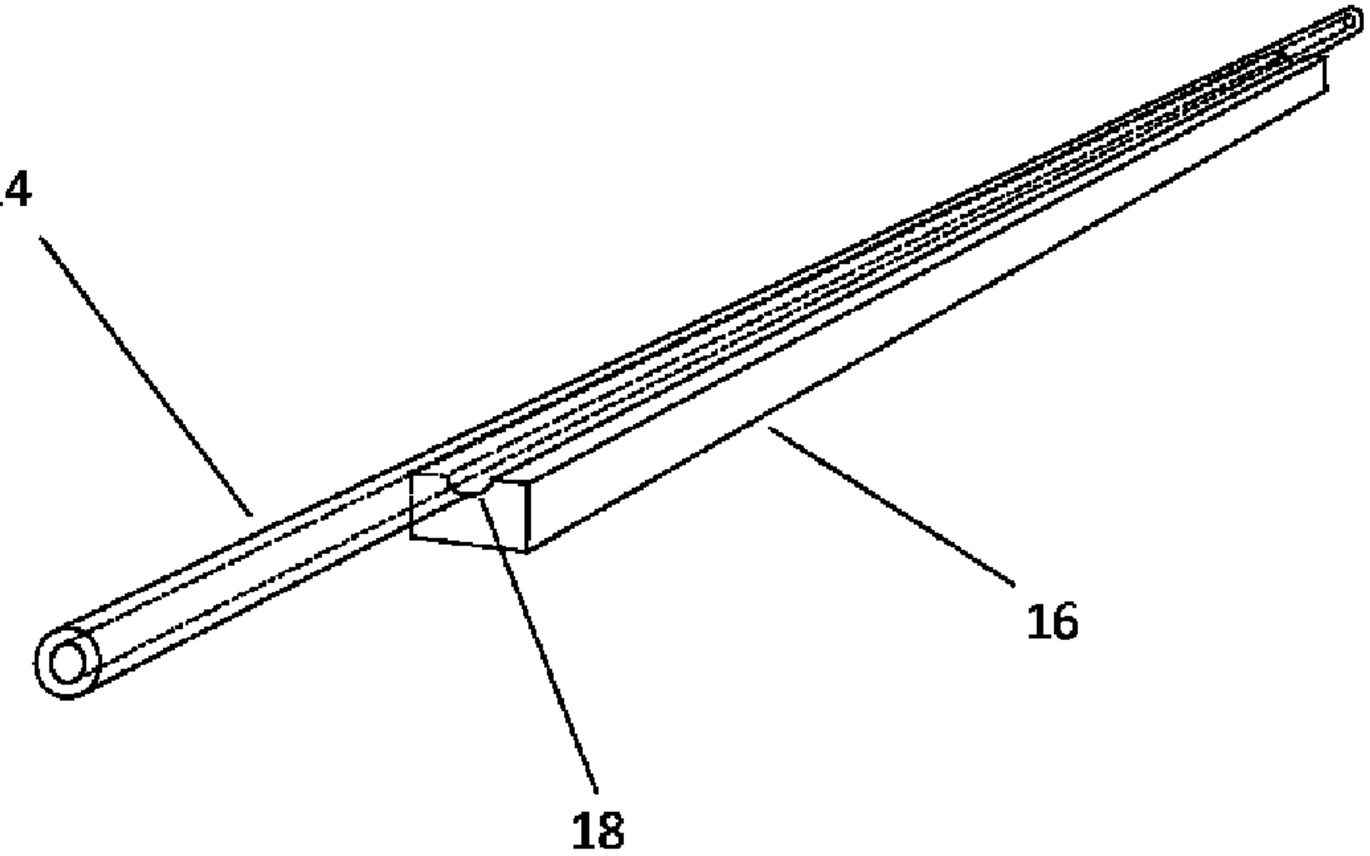

Microfluidic Channel
Acoustic Focusing
Hydrodynamic Focusing
Acoustic Source

Fig. 10


10
14
12
16


14
16
18