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(54) Title: A MICROFLUIDIC SYSTEM COMPRISING A HOMOGENIZING COMPONENT

(57) Abstract: The invention relates to a system comprising: - at least one microfluidic device (1), - at least one duct (3) in fluidic connection with the microfluidic device (1), and - at least one vibrating element (8) connected to the duct (3) and adapted to transmit mechanical vibrations to the duct (3). The invention also relates to a method of performing an assay which be implemented using this system.

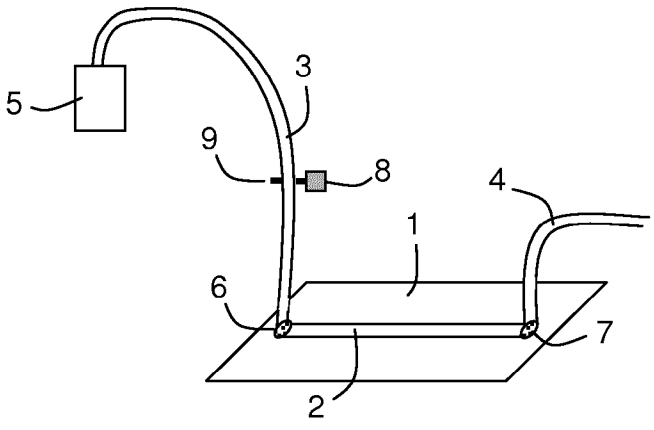


Fig. 1

WO 2014/037508 A1

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A MICROFLUIDIC SYSTEM COMPRISING A HOMOGENIZING COMPONENT

TECHNICAL FIELD

The present invention relates to a microfluidic system comprising a homogenizing component in the form of a vibrating element connected to a duct. The invention also relates to the use of this microfluidic system for performing various assays.

TECHNICAL BACKGROUND

Heterogeneity of fluids in microfluidic systems is a known problem, particularly in connection with fluids containing colloid suspensions. Heterogeneity can notably arise as a result of sedimentation, interaction between colloidal particles, flow variations, etc.

Several suggestions have been made in the prior art to add vibrating elements to microfluidic systems, without however properly addressing the above problem in a satisfactory manner.

For instance, document TW 200914831 discloses a multifunctional unsteady flow microfluidic device suitable for mixing and separating two-phase suspension fluids. A vibrating element is placed on the surface of the body of the microfluidic device. This document therefore describes a direct and intimate transduction of mechanical vibrations between the vibrating element and the microfluidic device.

Document WO 96/06158 relates to a cylindrical device comprising a chamber having a membrane for separating species at one of its ends, as well as a device for generating mechanical vibrations directly affixed to said end.

Document US 2009/0252658 is directed to a method of increasing the packing density of particulates loaded in microchannels. The method makes use of an ultrasound-producing head placed in sonic contact with the microchannels, for providing ultrasonic energy to said microchannels.

In some cases, vibrating elements have been used in the context of cell-lysing apparatuses.

For instance, document WO 2008/104916 relates to a cell lysis or mixing device, comprising a chamber filled with 20-75 vol.% of microparticles, as well as means to put said chamber under vibrations. The vibrations are more particularly generated by a membrane or elastic part of the chamber which is displaced by a stamp or piston. Agitation of the microparticles within the chamber results in shear forces and impact forces which are sufficient to disrupt cells loaded therein.

Document WO 99/33559 is directed to an integrated fluid manipulation cartridge. Said cartridge is adapted for separating a desired analyte from a fluid sample. Cell lysing means can be provided in this cartridge, and in particular ultrasonic lysing means.

In yet other cases, oscillating devices have been used for dispensing fluids or objects on demand.

In particular, document WO 2008/156837 discloses a microfluidic chip comprising a microchannel and a reagent chamber in fluid communication with said microchannel, the reagent chamber having a compliant wall and an actuator coupled therewith. In this case, the function of the actuator is to produce a volume change in the reagent chamber in response to a control signal, so as to controllably dispense particles on-demand from the reagent chamber to the microchannel. Typically, this technique requires two immiscible fluids, a timing accuracy in the order of nanoseconds and an operation frequency above 1 kHz.

Document WO 98/15825 provides a platform for performing synthetic chemistry, analysis and high throughput screening. In some embodiments, use is made of vibratory feeders, which are known in the art for dispensing fluids.

Finally, it is also known to use vibrations to keep a sample homogenous within a container. For example, document WO 2005/084380 teaches a method of delivering analytes to an analytical device, comprising pumping a fluid medium from a container while agitating the container to maintain homogeneity. This method is in particular intended for the transfer of blood from storage containers to analytical devices.

In the prior art, care is generally taken to create a full sonic connection between the vibrating element or ultrasonic transducer and the analytical or preparation device which is used. Therefore, the vibrating element is generally placed in direct sonic contact with the chamber of interest. This is in fact necessary in some instances, such as when sonic energy is used for lysing cells, or for packing beads, in the device. However, this also entails serious drawbacks in the context of microfluidic devices.

First, directly coupling a vibrating element to a microfluidic device results in numerous constraints in the microfluidic device itself, be it in terms of shape and size of microfluidic chambers, or of selection of materials. For example, soft materials such as polydimethylsiloxane (PDMS), which are very popular for microfluidic devices, may be excluded because of excessive vibration damping and of inefficient sonic transmission.

Second, generating vibrations directly in a microfluidic device, or alternatively in a sample container (such as in WO 2005/084380), may not be entirely efficient for achieving the required homogeneity in the microfluidic device itself.

There is thus a need for systems and methods achieving improved homogeneity within microfluidic devices, without adding any design constraint to said microfluidic devices.

SUMMARY OF THE INVENTION

Therefore, it is a first object of the invention to provide a system comprising:

- at least one microfluidic device,
- at least one duct in fluidic connection with the microfluidic device, and
- at least one vibrating element connected to the duct and adapted, or configured, to transmit mechanical vibrations to the duct.

According to one embodiment, the mechanical vibrations have a frequency ranging from 1 Hz to 10 MHz, preferably from 10 Hz to 1 kHz.

According to one embodiment, the duct is detachably connected to the microfluidic device.

According to one embodiment, the duct is made of a flexible material, which is preferably selected from glassy materials, resin materials, thermoplastic materials, elastomeric materials, and combinations thereof, and is more preferably selected from polyethylene, polypropylene, fluoropolymers, polyether ether ketone, polyimide, silicone and polyurethane.

According to one embodiment:

- the microfluidic device comprises one or more microchannels, the microchannels having a maximum dimension; and
- the distance between the connection of the duct to the microfluidic device and the area of the duct in which the mechanical vibrations are transmitted to the duct is at least 1 time, preferably at least 5

times, more preferably at least 10 times the maximum dimension of the microchannels.

According to one embodiment, the distance between the connection of the duct to the microfluidic device and the area of the duct in which the mechanical vibrations are transmitted to the duct is at least 1 cm, preferably at least 2 cm, more preferably at least 5 cm.

According to one embodiment, the system comprises a plurality of vibrating elements connected to the duct and adapted to transmit mechanical vibrations to the duct, said plurality of vibrating elements being spaced along the duct.

It is a second object of the invention to provide a method of performing an assay, comprising the steps of:

- transmitting mechanical vibrations to at least one duct in fluidic connection with a microfluidic device, by at least one vibrating element connected to said duct; and
- transferring a fluid through the duct towards or from the microfluidic device.

According to one embodiment, the microfluidic device and the duct are part of the above-described system.

According to one embodiment, the fluid comprises a colloidal suspension, said colloidal suspension preferably comprising particles, vesicles, beads, macromolecules, supramolecular assemblies, cells, viruses, aggregates and/or organisms.

According to one embodiment, the assay is performed at least in part in the microfluidic device, said assay being selected from the group consisting of sorting assays, screening assays, analysis assays, culture assays, catalysis assays, hybridization assays, electrochemical reaction assays, enzymatic reaction assays, immunoassays, chromatographic separation assays, chemoluminescent reaction assays, immunocapture assays, affinity capture assays, elution assays, diagnosis assays and combinations thereof; and/or the method comprises a detection step and/or a step of collecting a sample from the microfluidic device.

It is a third object of the invention to provide a method of modifying a system comprising at least one microfluidic device and at least one duct in fluidic connection with the microfluidic device, said method comprising a step of connecting at least one vibrating element to said duct, so that the vibrating element is able to transmit mechanical vibrations to the duct.

According to one embodiment, this method comprises the step of directly attaching the vibrating element to an external surface of the duct; or of attaching the vibrating element to a connecting support and attaching the connecting support to an external surface of the duct.

According to one embodiment, the modified system is the system according to the first object of the invention.

It is a fourth object of the invention to provide a vibrating system comprising:

- a vibrating element;
- a connecting support;

wherein the vibrating element is attached to the connecting support, and the connecting support is adapted for being attached to an external surface of a duct feeding or collecting fluid from or to a microfluidic device.

According to one embodiment, the surface area of the inner cross section of the duct relative to the direction of flow in the duct is from 0.0001 to 100 mm^2, preferably from 0.01 to 1 mm^2.

According to one embodiment, the connecting support is adapted for being detachably attached to the external surface of the duct.

According to one embodiment, the connecting support comprises a first through-hole and a second through-hole, wherein the first through-hole is adapted for lodging the duct and the vibrating element comprises a mobile mass which is fixedly inserted in the second through-hole.

According to one embodiment, the maximum dimension of the vibrating system is less than 5 cm, preferably less than 2 cm.

The present invention makes it possible to overcome the drawbacks of the prior art. In particular the invention provides systems and methods achieving improved homogeneity within microfluidic devices, without adding any design constraint to said microfluidic devices.

The invention is believed to be effective irrespective of the shape and size of the microchannels or microfluidic chambers which are used, and irrespective of the material in which the microfluidic devices are built. The invention can notably be used in connection with microfluidic devices comprising soft materials such as silicone elastomers.

The invention is based on the realization by the inventors that, in a system comprising a microfluidic device, the volume and travel length of fluids in the tubing bringing said fluids to, and / or taking said fluids away from, said microfluidic device, are often larger than the volume and travel length in the actual microfluidic part of said system. Thus, numerous adverse effects can

occur within the tubing which may affect the proper functioning of the system as a whole. Said adverse effects can comprise, for instance, aggregation, sedimentation and / or dispersion. They are particularly prone to occur when the fluids to be transported to or from the microfluidic device are colloidal fluids.

The invention relies on the unexpected finding that vibrating elements placed not in direct mechanical contact with the chambers, microchannels or reservoirs of the microfluidic device, but rather in contact with the connecting elements leading fluids from or to the microfluidic part of the system (preferably at a distance from said microfluidic part and / or at a distance from the fluid-containing reservoirs), can be more useful and effective.

According to some embodiments, the present invention can also have one or more of the following advantages:

- The invention can be implemented by using low-cost and easy-to-manufacture elements, such as cell phone-type vibrators (as opposed to expensive and complex ultrasonic transducers).
- Non-ultrasonic vibrations are easier to transmit than ultrasonic vibrations.
- A strong mechanical coupling between the vibrating element and some fluid-containing parts of the system, such as e.g. the microfluidic chip itself, or the sample-containing reservoir, is not necessary.
- The design of the microfluidic device is not constrained by the presence of the vibrating elements. It should be emphasized that microfluidic devices are very precise and demanding structures, the fabrication of which generally entails numerous constraints. Therefore, it is often difficult to design a microfluidic device so that a vibration can be transmitted to it at the right place, efficiently and without damaging the device.
- The invention can be used to transport colloidal fluids from or to a microfluidic device, with better efficiency and with less dispersion than in the prior art. This is advantageous in many applications, for instance in analytical sciences, when dispersion is detrimental to resolution. For instance, the invention can be used for searching for rare elements such as cells, cancer cells, bacteria, nanoparticles, contaminating particles, or more generally any analyte of interest, in a fluid.

- In the context of an automated implementation, the invention makes it possible to sequentially perform operations on a fluid, comprising transporting the fluid in ducts, with less carryover and in less time than in the prior art.
- The invention makes it possible to improve the efficiency of operations performed inside a microfluidic device, as compared to the same operations performed in a similar device according to the prior art, be it with or without vibrating elements directly affixed onto said microfluidic device.
- The invention makes it possible to reduce the size of the diffusion layer in the vicinity of the walls of a microfluidic device, which may be advantageous to increase the kinetics and efficiency of processes involving interactions between a fluid contained in the device and the surface of said device.
- The invention makes it possible to increase the efficiency (or yield) of chemical, biological or biochemical assays, such as those involving catalysis, hybridization, an electrochemical reaction, an enzymatic reaction, an immunoassay, a chromatographic separation, a chemiluminescent reaction, an immunocapture, an affinity capture and / or an elution.
- The invention makes it possible to make fluid transport easier and / or more regular.
- The invention makes it possible to prevent aggregation or sedimentation of particles and / or to facilitate the sorting of particles contained in said fluid and / or to facilitate the manipulation of biphasic or multiphasic fluids, such as by fragmentation, coalescence of droplets, liquid-liquid extraction.
- According to some embodiments, and opposite to what has been disclosed in some prior art documents where vibrating elements are directly affixed to microfluidic devices, the invention can be used to decrease the density of suspensions of particles, and thus facilitate the permeation and exchange of ligands between said particles and the suspending fluid.
- The invention makes it possible to homogenize beads or particles contained in a microfluidic device.
- The invention is cheap and simple to implement, while at the same time performing better than several prior art systems.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic drawing showing one embodiment of a system according to the invention.

Figure 2 is a schematic drawing showing one embodiment of a connection between a duct and a vibrating element.

Figure 3 is a graph showing the average light intensity (Y-axis, arbitrary units) measured in a microchannel over time (X-axis, number of frames recorded) according to one embodiment of the invention (see the example section below).

Figure 4 is a graph showing the standard deviation of the light intensity (Y-axis, arbitrary units) measured in the microchannel over time (X-axis, number of frames recorded) according to the same embodiment.

DESCRIPTION OF EMBODIMENTS

The invention will now be described in more detail without limitation in the following description.

As stated above, and making reference to **Fig. 1**, the system of the invention comprises at least one microfluidic device 1 and at least one duct 3, 4 in fluidic connection with the microfluidic device 1, and preferably at least two or more ducts in fluidic connection with the microfluidic device. In the illustrated embodiment, an inlet duct 3 and an outlet duct 4 are respectively connected to the microfluidic device 1, *via* respective connecting elements 6, 7. The other end of the inlet duct 3 is connected to a fluid container 5.

By "*microfluidic device*" is hereby meant a device comprising one or more microstructures on the surface of a substrate, which are features adapted for containing and / or directing fluids. These microstructures have at least one dimension which is less than 5 mm, preferably, less than 1 mm, and most preferably less than 500 µm. In some cases, the microstructures have at least one dimension which is less than 200 µm, or less than 100 µm, or less than 50 µm, or less than 20 µm, or less than 10 µm, or less than 5 µm, or less than 2 µm, or less than 1 µm.

Said microstructures can include enclosed volumes (in which case they can be referred to as "*microchannels*") or otherwise lay on an open surface.

In the illustrated embodiment, the microfluidic device 1 comprises at least one microchannel 2 in fluidic connection with the inlet duct 3 and the outlet duct 4.

The above microfluidic device preferably comprises at least one microchannel, and more preferably a plurality of microchannels which are in

fluidic connection. Each microchannel has at least one dimension of less than 5 mm, preferably, less than 1 mm, and most preferably less than 500 µm; and in some cases, of less than 200 µm, or less than 100 µm, or less than 50 µm, or less than 20 µm, or less than 10 µm, or less than 5 µm, or less than 2 µm, or less than 1 µm.

Each microchannel can have an elongated configuration comprising one longitudinal dimension (the "*maximum dimension*") exceeding the cross-sectional dimensions (by a factor of at least 10, or at least 20, or at least 50, or at least 100, or at least 500, or at least 1000, or at least 10000). But a microchannel can also be any other kind of three-dimensional structure on or within the substrate, such as a chamber for instance. The microchannels may have any cross-sectional shape (relative to the direction of flow), but the latter is preferably rectangular or square, or, in some specific embodiments, for instance involving biphasic fluids, circular or ovoid.

The microfluidic device may be formed by assembling the substrate with a cover. The substrate and / or the cover may be etched or molded to provide the desired microstructures. The cover may be bonded to the substrate.

Preferably, the substrate is a plate or wafer. The substrate is preferably substantially rigid, which means that it can be manipulated and attached to a holder to be kept globally immobile, with respect *e.g.* to an optical detector. It can be made of glass, silicon, ceramic, metal, or polymeric / plastic material. The cover may be of a similar nature or it may be made of a soft material, such as a silicone elastomer, *e.g.* polydimethylsiloxane (PDMS).

The manufacture of the microfluidic device can be based on a variety of microfabrication techniques such as film deposition, photolithography, wet or dry chemical etching, photoabalation or plasma ablation, air, water or powder abrasion, injection molding, embossing and thermoforming techniques. Film deposition can be performed by spin-coating, thermal oxidization, chemical vapor deposition (CVD), plasma vapor deposition (PVD), low pressure CVD, plasma-enhanced CVD, sputtering…

The microfluidic device can be a laboratory-on-a-chip.

The abovementioned "*ducts*" are any components which may be used to bring a fluid to, or collect a fluid from, the microfluidic device. Generally, ducts are tubes, but they can also be Y-shaped or X-shaped connectors or splitters.

Said ducts are for instance used to convey a fluid (or to convey objects within a fluid) from a reservoir or container to the microfluidic device, or conversely. They can also be used to convey a fluid from the microfluidic

device to a detector, such as an optical detector, or to collecting vials, containers and the like. They can be permanently attached to the microfluidic device, or alternatively be detachably connected to it, so that they can be removed (for instance to perform maintenance or cleansing operations, or to replace the microfluidic device while keeping the same ducts) and then re-connected or re-plugged to it.

The system of the invention may also comprise valves, holders, observation means and any kinds of fittings adapted to keep its different components and devices connected or assembled together.

The system of the invention may also comprise any kind of computerized, electronic, electric or pneumatic controller, in order *e.g.* and non-limitatively, to control the temperature and functioning of the device's components, to automate its operation, to record data.

The system of the invention may also comprise various means for actively transporting fluid (or objects within a fluid), such as pumping means, electrophoretic means, magnetic elements etc.

In some embodiments, the ducts used in the context of the invention are preferably made of flexible tubing.

By "*flexible*", is meant a duct that can be locally bent (transversally to its longitudinal axis), with a radius of curvature smaller than 1000 times, preferably smaller than 100 times, or smaller than 10 times, or even smaller than 5 times, the thickness of the duct (in the plane in which the bending is performed), wherein said bending is reversible, *i.e.* does not result in a breakage of said duct, and does not leave any significant permanent deformation on the duct once the forces exerted to achieve the bending are released.

They are preferably made of a polymeric material, such as a thermoplastic or elastomer material, and more particularly such as *e.g.* polyethylene, polypropylene, fluoropolymers, polyether ether ketone (PEEK), polyimide, polyester, silicone, polyurethanes, and the like. In some cases they may also be made of some metals, *e.g.* stainless steel, or a glassy mineral material, *e.g.* borosilicate glass or fused silica. If made in glass or of a glassy mineral material, the ducts of the invention are preferably covered by some plastic material, which is less brittle than the glassy material itself, for instance Teflon®, polyimide, epoxy, such covering contributing to increase the flexibility of the duct.

The choice of the appropriate tubing depends on the desired application and on the fluid to be used. For instance, fluoropolymers, PEEK, and polyimide

are advantageous for avoiding fouling by biomaterial, and for manipulating chemically aggressive fluids.

In terms of fluid pressure, silicone tubes can typically sustain pressures of up to a few (5 to 10) atmospheres, whereas, depending on the diameter, PEEK or polyimide can sustain pressures of up to 5 to 100 atmospheres. Metal tubing is recommended for even higher pressures.

Polyethylene, polypropylene, silicone and some polyurethanes, but also some fluoropolymers such as polytetrafluoroethylene (PTFE), can be transparent or translucent, which is advantageous when direct visualization of the contents of the tubing is desired.

Silicone tubing is another preferred type of tubing, as it is a biocompatible, transparent material, which can provide very flexible ducts, which can be useful to connect elements that have to be moved with respect to each other without creating mechanical strain.

The ducts used in the present invention preferably have a cylindrical shape. The internal (transverse) cross-section is preferably circular, but can also be square, rectangular, ellipsoidal or elongated.

The cross-section is generally uniform along the ducts, but in some instances it may be non-uniform or comprise some internal structuration, roughness, bumps or inner tortuosity.

The minimum dimension of the cross-section of the ducts (relative to the direction of flow) is generally larger than the minimum dimension of the microstructures (and notably microchannels) of the microfluidic device, preferably by a factor of more than 2, or more than 5, or more than 10, or more than 50 or more than 100.

The surface area of the cross-section of the ducts used in the invention may typically vary from 0.0001 to 100 mm^2, preferably from 0.01 to 1 mm^2.

The length of the ducts used in the invention may vary widely. However, the invention is especially useful when rather long ducts are used. For instance, the length of the ducts can be at least 1 cm, preferably at least 5 cm, or at least 10 cm, or at least 20 cm, possibly at least 30 or 50 cm.

It is also preferred if the length of the ducts is at least 5 times the maximum dimension of the microstructures in the microfluidic device, more preferably at least 10 times or 20 times or 50 times or 100 times.

The invention makes use of at least one vibrating element connected to at least one duct.

A "*vibrating element*" is defined as a mechanical, electronic, electric, or preferably electromechanical element comprising a moving part which is

adapted to move or oscillate in a repetitive manner. Said motion can be periodic or aperiodic, provided it involves during a relatively short time, preferably 1 s or less, and possibly down to 1 ns, a multiplicity of changes in motion orientation or direction.

The vibrating element can notably be a piezoelectric transducer or an ultrasonic transducer. Such vibrating elements are generally used when high frequency, typically in the ultrasound range, is needed.

However, for reasons of cost, energy consumption, size, as well as efficiency, it is generally preferred to use a vibrating element comprising a mass attached to a mobile magnetic element driven by an electromagnet or an electromagnetic coil; or a mass attached to a motor driven at a given speed. Loudspeaker-type vibrating elements, which are typically used in the audible range, *e.g.* from 20 Hz to 20 kHz, or cellular phone-type vibrators, are particularly advantageous in the invention.

The vibration generated by the vibrating element may have an amplitude in the range of nanometers to centimeters. It is generally advantageous if the amplitude of the vibration is commensurate with the cross-sectional dimension of the corresponding duct. More advantageously, said amplitude of vibration is from 0.01 to 100 times, preferably from 0.1 to 10 times, the largest (or the smallest) dimension of the internal cross-section of the duct. In some cases, and notably if the duct has a rather thick wall (for instance larger than the internal maximum diameter of the duct), reference may be made to the external cross-section instead of the internal cross-section.

Typically, the amplitude of the vibrations is therefore from 100 nm to 10 cm, preferably from 10 µm to 1 cm, and for instance rom 10 µm to 100 µm or from 100 µm to 1 mm, or from 1 mm to 1 cm.

The vibrations generated by the vibrating element may have various spatial shapes. Preferably, they are translational or rotational vibrations.

The vibration frequency may range from 1 Hz to 10 MHz. Preferably, and in contrast with the prior art, the vibrations used in the present invention are not high frequency ultrasound vibrations, and more preferably are not ultrasound vibrations. Preferred frequency ranges are from 10 Hz to 1 kHz, for instance from 10 to 100 Hz or from 100 Hz to 1 kHz. Lower frequencies of 1 Hz to 10 Hz may be advantageous in connection with systems comprising relatively large ducts and / or microchannels.

The amplitude of acceleration of the vibrations is preferably from 0.01 to 100 G, and more preferably from 0.1 to 10 G.

The vibrating element may be placed in direct contact with the outer surface of the duct. For instance, it may be glued to the outer surface of the duct, or fixed against the duct by means of a wire, or screw, or any other attachment means.

Alternatively, for an easier and more flexible implementation, the vibrating element may be attached to a connecting support, the connecting support being itself attached to the duct.

Making again reference to **Fig. 1**, in the illustrated embodiment, a vibrating element 8 is connected to the inlet duct 3, *via* a connecting support 9.

Fig. 2 provides an example of such an attachment. According to this embodiment, the connecting support 11 (or frame) is essentially a parallelepiped block comprising a first through-hole 12 and a second through-hole 13 which is parallel to the first through-hole 12. The connecting support 11 is preferably made of a rigid material, such as metal or rigid plastic or resin, such as polystyrene, polymethyl methacrylate or more generally acrylate materials, polyolefins, acrylonitrile butadiene styrene (ABS), polyimide, polyester, epoxy, cyclic olefin copolymer, cyclic olefin polymer, polycarbonate, PEEK, Teflon, or alternately composite materials or ceramics.

The duct 14 can be inserted through the first through-hole 12. A part of the vibrating element, such as a mobile mass 15, can be inserted through the second through-hole 13. A tight connection between the connecting support 11 and the duct 14 on the one hand, as well as the mobile mass 15 on the other hand, is achieved by way of respective clamping screws (not shown) which can be inserted into respective threaded holes 18, 19 respectively leading to the first through-hole 12 and the second through-hole 13. Other means of attachment can be contemplated as well, such as the application of glue, or the provision of respective resilient slots in the connecting support 11 on one side of the through-holes 12, 13.

The rest of the vibrating element 16 extends away from the connecting support 11. It *inter alia* comprises an electric power supply 17.

According to a preferred embodiment, the vibrations are transmitted to the duct in a substantially transverse manner relative to the (local) longitudinal axis of the duct. The use of the above-described connecting support ensures such a transverse transmission of vibrations to the duct, *via* the connecting support or frame.

The vibrating element is preferably placed at a distance from the microfluidic device such that the vibrations are substantially not transmitted to

the microfluidic device. Preferably, the attenuation factor of the vibration is better than 10, in some preferred embodiments better than 100.

Accordingly, the area of the duct in which the mechanical vibrations are transmitted to the duct (namely, the area of attachment of the vibrating element, or of the connecting support, to the duct) is at least 1 cm, preferably at least 2 cm, more preferably at least 5 cm away (possibly at least 10 cm away) from the connection of the duct to the microfluidic device (or from the inlet of the microfluidic device). The above distance is measured along the duct.

Preferably, the above distance between the vibrating element and the microfluidic device is selected according to the material of the duct and its dimensions. This can be achieved by trial and error, but typically, longer minimal distances will be used for more rigid and larger ducts, and smaller minimal distances can be accepted for smaller and more flexible ducts. For instance, for a silicone duct with a diameter smaller than 1 mm, a minimal distance as small as 1 cm can be accepted, whereas for a fused silica duct with an outer diameter of 300 µm, or for a PEEK duct with an outer diameter of 1 mm, a distance of at least 5 cm is preferred, for a stainless steel duct with an outer diameter of 1 mm, a distance of at least 10 cm is preferred.

In some circumstances, especially when a long duct is used, it may be advantageous to use two or more than two vibrating elements along the duct, the successive vibrating elements being spaced by at least 1 cm, preferably at least 2 cm, more preferably at least 5 cm, or at least 10 cm, or at least 20 cm.

When the duct is connected to a container, reservoir, chamber or the like at one of its end which is opposite the end connected to the microfluidic device, it is advantageous that the distance between the area of the duct in which the mechanical vibrations are transmitted to the duct and the connection of the duct to the container, reservoir, chamber or the like is at least 1 cm, preferably at least 2 cm, more preferably at least 5 cm and in some cases at least 10 cm.

The same general rules for optimizing the position of the vibrating elements, as described above regarding their distance to the microfluidic device, may also be applied regarding their distance to the containers or reservoirs or chambers, or regarding their mutual distance if several vibrating elements are positioned on a single duct.

The system of the invention can comprise more than one microfluidic device having at least one duct connected thereto. It can also comprise more than one duct per microfluidic device. In such cases, one or more vibrating

elements can be attached to several ducts, depending on where the homogenizing effect of the invention is required.

The present invention being particularly simple to implement, it is possible to adapt and upgrade an existing system comprising at least one microfluidic device and at least one duct connected to or from said microfluidic device, by connecting at least one or more vibrating elements (possibly together with corresponding connecting supports, as described above) to said duct.

The invention can be implemented in the context of any assay which can be performed in the microfluidic device. The invention is particularly advantageous when colloidal objects are transported (in a fluid) to or from the microfluidic device in the context of said assay.

As used here, the term "*colloidal object*" may represent a large variety of natural or artificial, organic, or inorganic, compounds, including cells, organelles, viruses, cell aggregates, cell islets, embryos, pollen grains, artificial or natural organic particles such as latex particles, dendrimers, vesicles, magnetic particles, nanoparticles, quantum dots, metal microparticles, metal nanoparticles, organometallic micro or nanoparticles, nanotubes, artificial or natural macromolecules, microgels, macromolecular aggregates, proteins or protein aggregates, polynucleotides or polynucleotide aggregates, nucleoproteic aggregates, polysaccharides, or supramolecular assemblies, or combinations of the above.

A fluid containing colloidal objects is termed a colloidal suspension.

Generally, colloidal objects have at least one dimension (in the fluid) that is larger than 2 nm, preferably larger than 5 nm, more preferably larger than 10 nm, or larger than 50 nm, or greater than 100 nm. In some embodiments, they have at least one dimension that is larger than 200 nm, or 500 nm, or 1 µm.

The transport of colloidal objects may often lead to heterogeneity problems, which the invention can effectively reduce.

Indeed, it is believed that colloidal objects can easily sediment, or get aggregated, especially in connecting ducts, as will be demonstrated in the example below. The invention prevents such aggregation and sedimentation. This is all the more beneficial as, in most microfluidic systems, the amount of fluid contained in connecting ducts is in fact larger than that contained in the microfluidic device itself, so that the time spent by the colloidal objects in the ducts is in fact longer than the time spent in the microfluidic device itself.

EXAMPLE

The following example illustrates the invention without limiting it.

In this example, magnetic beads were transported through a microchannel in a microfluidic device according to the embodiments illustrated in **Fig.1** and **Fig.2**.

The magnetic beads used were COOH-functionalized Dynabeads® M270. 100 µL of bead solution (at 2×10^9 beads / mL) was diluted in 500 µL of phosphate buffered saline. The dilute bead solution was placed in a 2 mL microtube.

The microchannel was made of PDMS. Its dimensions were 150 µm × 50 µm × 2 cm. A fluoropolymer microtube having an internal diameter of 800 µm was used as the main connecting duct between the fluid reservoir and the microchannel. A short section of a PEEK tube having an internal diameter of 250 µm was used as a diameter adaptor between the inlet of the microfluidic device and the fluoropolymer microtube.

A vibrating element, namely a Precision Drive 4 mm motor (304-101), was connected to the fluoropolymer tube, at a distance of 5 cm from the inlet of the microchannel. The amplitude of acceleration provided by this motor, according to the provider, was 1.2 G. The amplitude of vibration depends on the inertia and resonance modes of the overall system, and therefore depends on the length of the tube and on the details of the positioning of the vibrator on the tube. In this case, it was comprised between 0.5 and 5 mm. The vibration frequency of the vibrator was 280 Hz and the rotation speed was 16,000 turns/min, per provider's specifications.

The diluted bead solution was transported from the microtube to the microchannel, *via* the fluoropolymer tube, at a flow rate of 25 µL/min (using a pressure control device MFCS 4C 1 bar by Fluigent, together with a flowmeter).

The microchannel was subjected to a magnetic field of 30 mT by a copper solenoid having 600 turns (6 A, 31.5 V).

The flow of beads in a detection zone of the microchannel was monitored owing to a camera at 25 frames / s.

The results are shown in **Fig.3** and **4**. The periods marked "ON" are those during which the vibrating element was operated.

It may readily be seen that the intensity fluctuates less, *i.e.* the solution is more homogeneous, when the vibrating element is activated.

CLAIMS

1. A system comprising:
- at least one microfluidic device (1),
- at least one duct (3) in fluidic connection with the microfluidic device (1), and
- at least one vibrating element (8) connected to the duct (3) and adapted to transmit mechanical vibrations to the duct (3).

2. The system of claim 1, wherein the mechanical vibrations have a frequency ranging from 1 Hz to 10 MHz, preferably from 10 Hz to 1 kHz.

3. The system of claim 1 or 2, wherein the duct (3) is detachably connected to the microfluidic device (1); and/or wherein the duct (3) is made of a flexible material, which is preferably selected from glassy materials, resin materials, thermoplastic materials, elastomeric materials, and combinations thereof, and is more preferably selected from polyethylene, polypropylene, fluoropolymers, polyether ether ketone, polyimide, silicone and polyurethane.

4. The system of one of claims 1 to 3, wherein:
- the microfluidic device (1) comprises one or more microchannels (2), the microchannels (2) having a maximum dimension; and
- the distance between the connection (6) of the duct (3) to the microfluidic device (1) and the area of the duct (3) in which the mechanical vibrations are transmitted to the duct (3) is at least 1 time, preferably at least 5 times, more preferably at least 10 times the maximum dimension of the microchannels (2).

5. The system of one of claims 1 to 4, wherein the distance between the connection (6) of the duct (3) to the microfluidic device (1) and the area of the duct (3) in which the mechanical vibrations are transmitted to the duct (3) is at least 1 cm, preferably at least 2 cm, more preferably at least 5 cm.

6. The system of one of claims 1 to 5, which comprises a plurality of vibrating elements (8) connected to the duct (3) and adapted to transmit mechanical vibrations to the duct (3), said plurality of vibrating elements (8) being spaced along the duct (3).

7. A method of performing an assay, comprising the steps of:
- transmitting mechanical vibrations to at least one duct (3) in fluidic connection with a microfluidic device (1), by at least one vibrating element (8) connected to said duct (3); and
- transferring a fluid through the duct (3) towards or from the microfluidic device (1).

8. The method of claim 7, wherein the microfluidic device (1) and the duct (3) are part of the system of one of claims 1 to 6.

9. The method of claim 7 or 8, wherein the fluid comprises a colloidal suspension, said colloidal suspension preferably comprising particles, vesicles, beads, macromolecules, supramolecular assemblies, cells, viruses, aggregates and/or organisms.

10. The method of one of claims 7 to 9, wherein the assay is performed at least in part in the microfluidic device, said assay being selected from the group consisting of sorting assays, screening assays, analysis assays, culture assays, catalysis assays, hybridization assays, electrochemical reaction assays, enzymatic reaction assays, immunoassays, chromatographic separation assays, chemoluminescent reaction assays, immunocapture assays, affinity capture assays, elution assays, diagnosis assays and combinations thereof; and/or wherein the method comprises a detection step and/or a step of collecting a sample from the microfluidic device.

11. A method of modifying a system comprising at least one microfluidic device (1) and at least one duct (3) in fluidic connection with the microfluidic device (1), said method comprising a step of connecting at least one vibrating element (8)

to said duct (3), so that the vibrating element is able to transmit mechanical vibrations to the duct (3).

12. The method of claim 11, comprising the step of directly attaching the vibrating element (8) to an external surface of the duct (3); or of attaching the vibrating element (8) to a connecting support (9) and attaching the connecting support (9) to an external surface of the duct (3).

13. The method of claim 11 or 12, wherein the modified system is the system of one of claims 1 to 6.

14. A vibrating system comprising:
– a vibrating element (8);
– a connecting support (9);
wherein the vibrating element (8) is attached to the connecting support (9), and the connecting support (9) is adapted for being attached to an external surface of a duct (3) feeding or collecting fluid from or to a microfluidic device (1).

15. The vibrating system of claim 14, wherein the surface area of the inner cross section of the duct (3) relative to the direction of flow in the duct is from 0.0001 to 100 mm^2, preferably from 0.01 to 1 mm^2 ; and/or the connecting support (9) is adapted for being detachably attached to the external surface of the duct (3); and/or the connecting support (9) comprises a first through-hole (12) and a second through-hole (13), wherein the first through-hole (12) is adapted for lodging the duct (3) and the vibrating element (8) comprises a mobile mass (15) which is fixedly inserted in the second through-hole (13); and/or the maximum dimension of the vibrating system is less than 5 cm, preferably less than 2 cm.

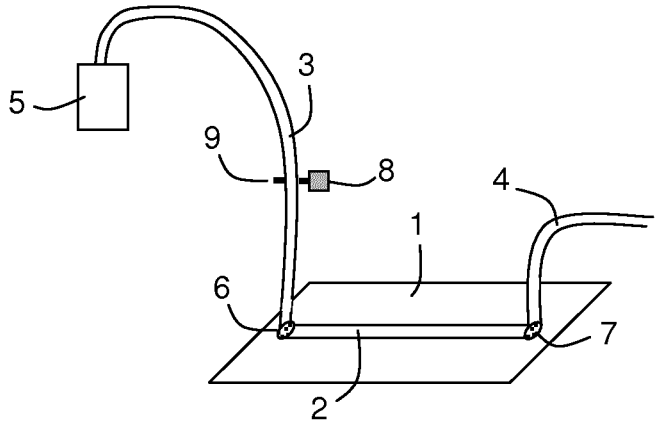


Fig. 1

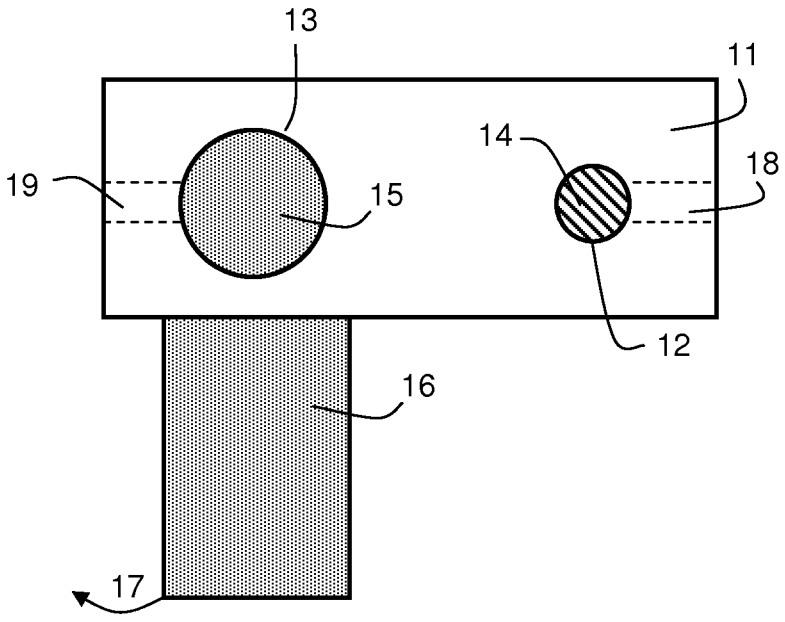


Fig. 2

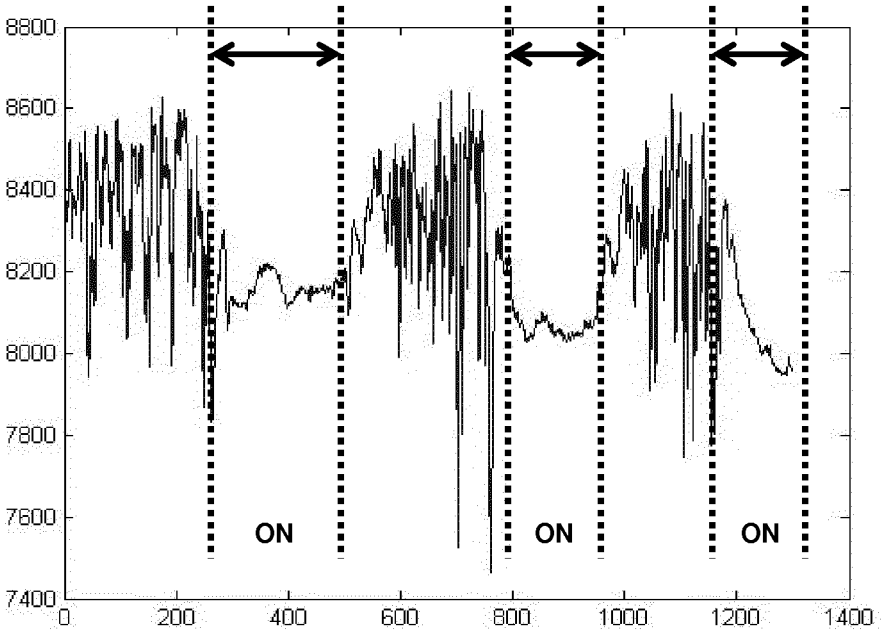

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Fig. 3

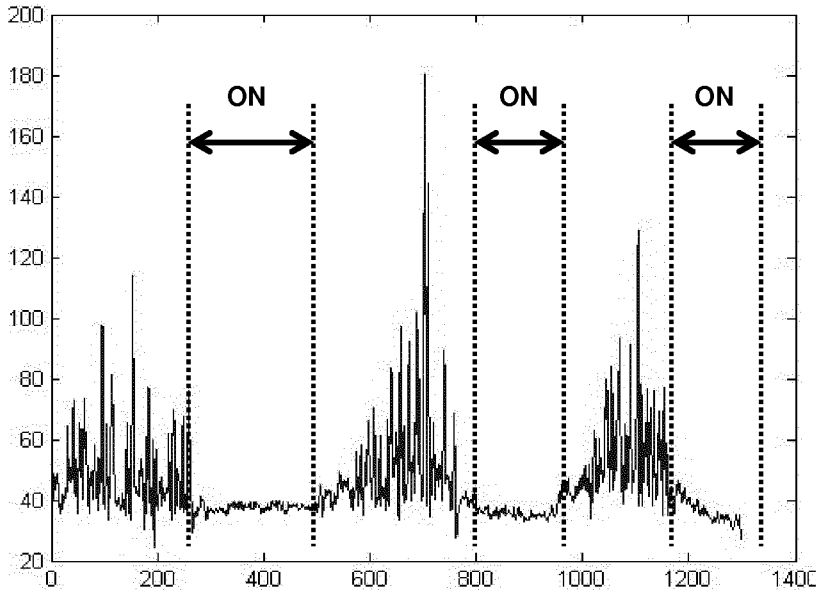

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Fig. 4

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2013/068476

A. CLASSIFICATION OF SUBJECT MATTER
INV. B01F13/00 B01F11/02
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
B01F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 November 2013	22/11/2013

Name and mailing address of the ISA/	Authorized officer
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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2013/068476

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