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(54) **Microfluidic filter and fix-bed reactor for superparamagnetic nano-particles**

(57) A particle handler (100) for handling at least sub-micron-sized particles, the particle handler (100) comprising: a main body (110) that comprises a precipitation cavity (130); at least one particle attractor (140) comprising permanent magnets; at least one magnetizer (120, 121) for selectively magnetizing the particle attractor into an attraction mode and a non-attraction mode, wherein in the attraction mode, the at least one particle attractor

(140) exhibits regions of increased magnetic field gradients capable or operative to attract superparamagnetic particles (190) suspended in carrier fluid received by the precipitation cavity (130) via at least one of a plurality of openings (131, 132). In embodiments, the at least one particle attractor (140) extends over a given area and engages with at least a corresponding portion of the inner wall of the precipitation cavity (130).

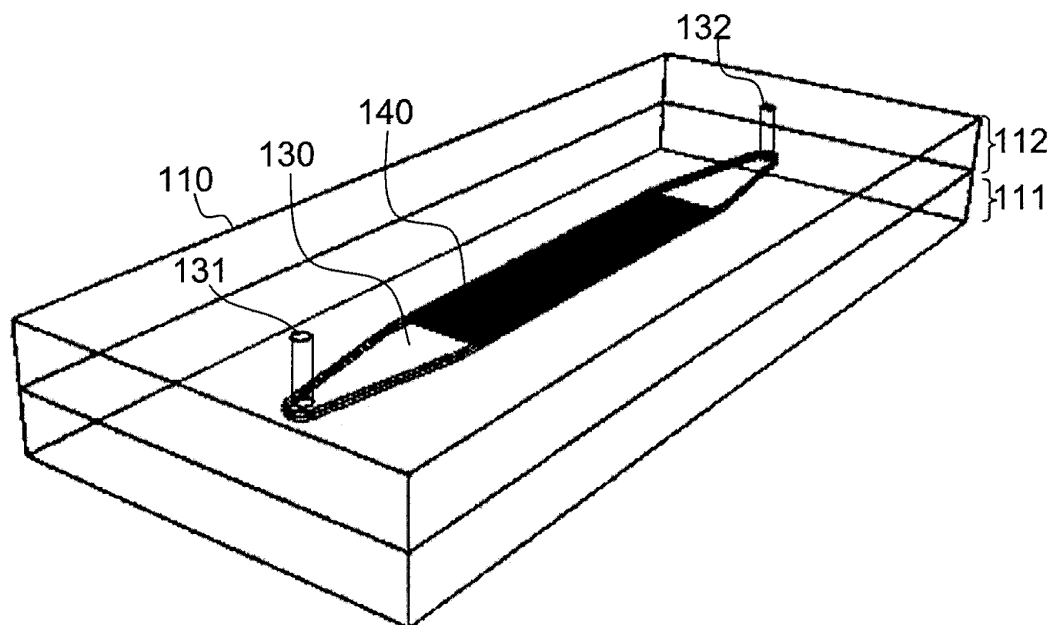


FIGURE 1

Description**FIELD OF THE INVENTION**

5 **[0001]** Generally, the invention relates to the handling of micro- and nano-sized particles and, more particularly, to devices, systems and methods according to the preambles of the independent claims.

BACKGROUND OF THE INVENTION

10 **[0002]** Magnetic phenomena are used in the field of microfluidics for a variety of applications, including particle handling, micropumping, realization of active valves and switches, mixing, steering, particle self-assembling and surface patterning. The usage of magnetic phenomena for manipulating superparamagnetic particles has been regarded with particular interest due to their numerous possible applications in biomedical sciences. Superparamagnetic particles, which usually consist of 1-100 μm polymer or magnetic-nanoparticles aggregates, can be employed for bonding thereto chemical
15 and/or biological species (e.g., biomolecules), and are capable of binding specific targets in biological (e.g., cellular) samples. Due to the superparamagnetic properties of such particles, namely their lack of magnetic memory (i.e., at room temperature, the superparamagnetic particles lose their magnetization in the absence of a magnetic field), their properties include relatively fast magnetic precipitation and redispersion in carrier fluid. Once the superparamagnetic particles have bonded with the targeted biomaterial in a carrier fluid, they can be assembled by employing a suitably strong magnetic
20 field for realizing bioseparation and filtering.

[0003] The employment of individual superparamagnetic particles (like, e.g., beads) has been considered in applications that include diagnosis and therapies. The small size of these functional particles, having submicron diameters of, e.g., 10 nm to 100 nm, allows their usage in living organisms, where they can diffuse across tissues and penetrate into cells, enabling a variety of applications including drug delivery, gene delivery, treatment of hyperthermia and contrast
25 enhancement in magnetic resonance imaging (MRI). Other important applications concern the study of molecular interactions in cellular biology, as is outlined in C.C. Berry, A.S.G. Curtis, "Functionalisation of magnetic nanoparticles for applications in biomedicine", J. Phys. D: Appl. Phys. 36 (2003) R198-R206.

[0004] In many applications related to superparamagnetic particles, including synthesis, surface modification and handling thereof, the superparamagnetic particles have to be precipitated from a solution. More specifically, precipitation
30 may be performed, for example, in bioseparation protocols; and as a purification step during functionalization. If the particles are micron-sized magnetic beads, precipitation can be quickly obtained by approaching a standard permanent magnet close enough to the sample containing the beads. This method is widely used in biological assays, and has also been applied to demonstrate microfluidic sorting of magnetic beads. Nevertheless, efforts have been made to improve and accelerate the collection and sorting of magnetic particles contained in carrier fluids; particularly by employing
35 microfluidic devices.

[0005] Some microfluidic devices integrate current lines or microelectromagnets to trap and handle magnetic beads. More commonly, microscopic magnetic structures are integrated in the devices, and magnetized by an external source which can be either a permanent or a powered electromagnet. By employing such magnetic microstructures, a locally enhanced gradient of the magnetic field can be obtained, which increases the attraction on the particles to the respective
40 location and which is sometimes referred to as "high gradient magnetic filtration".

[0006] Patent application US 2002/0166760 and WO0293125 to Prentiss et al. teaches magnetic microstructures for manipulating biological or chemical species. The microstructures include current carrying wires that are patterned on a substrate. By applying magnetic fields, channels are defined on the surface of the substrate in which the magnetic particles and attached species may be transported, positioned and stored. In other cases, the magnetic fields are
45 generated by magnetic features located within the channels on the surface of the substrate.

[0007] In the following two publications, high gradient magnetic filters are fabricated from commercially available ferromagnetic grids:

[0008] C. Hoffmann, M. Franzreb, W. H. Höll, (hereinafter: Hoffman et al.) disclose in "A Novel High-Gradient Magnetic Separator (HGMS) Design for Biotech Applications", IEEE Transactions on Applied Superconductivity, VOL. 12, NO. 1,
50 (2002) 963-966, a high-gradient magnetic separator (HGMS) which uses a rotary permanent magnet leads to an "on-off" characteristic of the field in the separation zone. This combines the permanent magnet's advantage of low running costs with the solenoid's advantage to flush the filter in place. The utilization of NdFeB magnets into the yoke allows high magnetic inductions leading to efficient and fast separation of magnetic supports used in biotech processes. The bioprocess requirement of complete particle recovery and efficient matrix cleaning after separation was achieved by the
55 integration of an ultrasonication device.

[0009] N.A. Ebner, C.S.G. Gomes, T.J. Hobley, O.R.T. Thomas, M. Franzreb (Ebner et al.) disclose in "Filter Capacity Predictions for the Capture of Magnetic Microparticles by High-Gradient Magnetic Separation", IEEE TRANSACTIONS ON MAGNETICS, VOL. 43, NO. 5, (2007) 1941-1949, disclose experimental and theoretical methods to predict maximum

and working filter capacities for the capture of superparamagnetic microparticles through high-gradient magnetic separation (HGMS). For this, various combinations of nine different HGMS filter matrices and two types of superparamagnetic microparticles were considered. By calculating the separated particle mass per filter mesh area, the influences of wire diameter and wire mesh spacing on the particle build-up density was demonstrated. Together with known physical parameters of the filter matrix and the background field, such average build-up density allows predictions of the operational working filter capacities.

[0010] With respect to Ebner et al. and Hoffmann et al., the employed magnetic meshes and filters may become fully exposed to or immersed within the fluid suspension. In aqueous environment, oxidation of the magnetic meshes may occur. In turn, metallic ions may be released into the aqueous solution which may interfere with biological species or assays. Moreover, the meshes and filters are stacked on one another within the volume where particles may be collected and thus increasingly inhibiting flow in the volume the more particles are collected.

[0011] The documents cited thus far relate to the handling of micron-sized magnetic beads, wherein the standard procedure for magnetic separation applied for these particles is to approach a magnet to the containing vial and to wait several minutes for their precipitation. For superparamagnetic nanoparticles however, the precipitation may take many hours or days. The reason why the sedimentation of superparamagnetic nanoparticles is relatively slow is because the magnetic force is too weak to effectively attract these nanoscopic particles. As long as they are suspended in the carrier fluid, their motion is quasi-chaotic since the magnetic field only exerts a limited influence in their Brownian motion. Particularly for particle sizes below 100 nm the magnetic attraction is typically weaker than the random forces that produce the Brownian motion. Moreover, only in the few microns vicinity of the magnetic trap, the magnetic field gradient may be strong enough as to effectively attract superparamagnetic particles. Only when the superparamagnetic nanoparticles reach a magnetic surface they can be immobilized by the local field gradient. Besides being extremely time-consuming, there is always a fraction of the superparamagnetic particles that is lost in each purification step when employing the abovementioned precipitation method. Clearly, the multi-step process of chemical modification for the functionalization of superparamagnetic nanoparticles is extremely slow and inefficient. Moreover, when the functionalized particles are used in bioassays or cellular biology experiments, the long sedimentation times may render the obtained results non-interpretable and said precipitation procedure thus unsuitable in association with such experiments.

[0012] The publication to B. Steitz, J. Salaklang, A. Finka, C. O'Neil, H. Hofmann, A. Petri-Fink, entitled "Fixed Bed Reactor for Solid-Phase Surface Derivatization of Superparamagnetic Nanoparticles", Bioconjugate Chem. 18 (2007) 1684-1690 (hereinafter: Steitz et al.), relates to the handling, sorting, and sedimentation of single superparamagnetic particles having diameters of about 10 nm by using a fixed bed reactor with a quadrupole repulsive arrangement of permanent magnets that was assayed for functionalized superparamagnetic iron oxide nanoparticles (SPION) surface derivatization. More specifically, in order to circumvent the sedimentation problem during the functionalization, Steitz et al. implemented a magnetic fix bed reactor to keep the particles immobilized on a magnetic surface during the flowing of the desired sequence of chemical solutions. The magnetic trap consisted in this case in a coiled Fe-Ni wire inserted in a flow chamber and surrounded by 4 stacks of permanent magnets. The setup disclosed by Steitz et al. does not significantly increase the speed of collecting of superparamagnetic particles in biological assays. That is, since the internal volume is too large to handle the samples, and secondly, because the sedimentation, which depends on the particles diffusing across this large volume towards the magnetic surface, is still essentially slow.

BRIEF DESCRIPTION OF THE FIGURES

[0013] Features of the invention will become more clearly understood in the light of the ensuing description of a some embodiments thereof, given by way of example only, with reference to the accompanying figures, wherein:

[0014] **Figure 1** is a schematic perspective view of the main body of a particle handler according to an embodiment of the invention;

[0015] **Figure 2A** is a schematic cross-sectional side view illustration of a particle handler, according to an embodiment of the invention;

[0016] **Figure 2B** is a schematic cross-sectional front view illustration of the particle handler, according to the embodiment **Figure 2A**;

[0017] **Figure 3A** is a schematic cross-sectional front view illustration of a particle handler wherein the drift of superparamagnetic particles is schematically illustrated, according to an embodiment of the invention;

[0018] **Figure 3B** is a schematic cross-sectional front view illustration of a particle handler wherein the drift of superparamagnetic particles is schematically illustrated, according to an alternative embodiment of the invention;

[0019] **Figure 4** is a schematic cross-sectional front view illustration of a particle handler wherein the drift of superparamagnetic particles is schematically illustrated, according to a yet other embodiment of the invention;

[0020] **Figure 5A** is a schematic cross-sectional top view illustration of a particle handler and a particle attractor according to an embodiment of the invention;

[0021] **Figure 5B** is a schematic cross-sectional top view illustration of a particle handler and a particle attractor

according to another embodiment of the invention;

[0022] Figure 6A is a schematic cross-sectional top view illustration of a particle handler and a particle attractor according to an alternative embodiment of the invention; and

[0023] Figure 6B is a schematic cross-sectional top view illustration of a particle handler and a particle attractor according to a yet alternative embodiment of the invention.

[0024] It should be noted that for the sake of clarity and simplicity, some elements and/or features may not be illustrated in a correctly sized relation to one another in the figures.

DESCRIPTION OF THE EMBODIMENTS OF THE INVENTION

[0025] Summary of the embodiments of the invention:

[0026] The present invention discloses a particle handler for handling at least submicron-sized particles. The particle handler includes a main body which includes a precipitation cavity; at least one particle attractor comprising permanent magnets; at least one magnetizer for selectively magnetizing the particle attractor into an attraction mode and a non-attraction mode, wherein in the attraction mode, the at least one particle attractor exhibits regions of increased magnetic field gradients capable or operative to attract superparamagnetic particles suspended in carrier fluid received by the precipitation cavity via at least one of a plurality of openings.

[0027] In embodiments, the at least one particle attractor extends over a given area and engages with at least a corresponding portion of the inner wall of the precipitation cavity.

[0028] In embodiments, the at least one particle attractor engages with the inner side wall of the precipitation cavity such to be non-fully exposed to the precipitation cavity.

[0029] In embodiments, the precipitation cavity has a height H_{cavity} ranging from 10 μm to 1 mm, and preferably ranging from 50 μm to 200 μm ; a width W_{cavity} ranging from 200 μm to 10 cm and preferably from 2 mm to 1 cm; and a length L_{cavity} ranging from 1 cm to 1 m and preferably from 1 cm to 10 cm.

[0030] In embodiments, the particle attractor is made of a grid or mesh made of wires including material of a permanent magnet.

[0031] In embodiments, the wires of the particle attractor have a diameter of 0.1 mm and the ratio between the gaps of the mesh and the entire coverage area of the mesh is at least approximately 36%.

[0032] In embodiments, the main body is repetitively disassembleable and reassembleable such that the at least one particle attractor is accessible, exchangeable and disposable.

[0033] The present invention further refers to a method for manufacturing a particle handler operative to handle at least submicron-sized particles.

[0034] In embodiments, the method includes the following procedures: providing a lower body and/or an upper body, the lower body and the upper body forming a main body; providing at least a part of the precipitation cavity in the lower body and/or the upper body, the precipitation cavity having at least two openings; providing at least one particle attractor in the precipitation cavity, and joining the lower body with the upper body to form the main body.

[0035] In embodiments, at least one particle attractor is provided to a side wall of the precipitation cavity such to be non-fully exposed to the precipitation cavity.

[0036] In embodiments, the procedure of providing the at least one particle attractor is accomplished by employing hot press.

[0037] In embodiments, the procedure of joining the lower body with the upper body to form the main body includes at least one of the following manufacturing procedures: thermal bonding, chemical bonding, employing a bonding device, coating, and clamping.

[0038] Detailed description of the embodiments of the invention:

[0039] It is an object of the present invention to provide a combined particle filter and fixed-bed reactor (hereinafter: particle handler) enabling the handling of particles, which may be embodied, for example, by beads, which may be functionalized, e.g., as known in the art, by having attached thereto, for example, biological and/or chemical species. In particular, the particle handler is, inter alia, operative to handle submicron-sized particles, i.e., particles that are smaller than 1 μm (e.g., particles of 140 nm size).

[0040] It should be noted that the terms "handling" or "manipulating" as used herein with reference to particles, as well as grammatical variations of the terms may encompass, inter alia, any of the following meanings: functionalizing, filtering, transporting, steering, directing, storing, trapping, positioning, confining, releasing, separating, mixing, modifying, sorting, receiving, removing, providing, conveying and guiding of particles.

[0041] It should be understood that where the claims or specification refer to "a" or "an" feature, such reference is not to be construed as there being only one of that element. Accordingly, "an" or "a" feature may also encompass the meaning of "at least one" of the feature. For example, "a particle" may also include the meaning of "at least one sample", respectively.

[0042] The terms "front surface", "rear surface" "right", "left", "bottom", "below", "lowered", "low", "top", "above",

"elevated" and "high" as well as grammatical variations thereof as used herein do not necessarily indicate that, for example, a "bottom" component is below a "top" component, or that a component that is "below" is indeed "below" another component or that a component that is "above" is indeed "above" another component as such directions, components or both may be flipped, rotated, moved in space, placed in a diagonal orientation or position, placed horizontally or vertically, or similarly modified. Accordingly, it will be appreciated that the terms "front", "rear", "bottom", "below", "top" and "above" may be used herein for exemplary purposes only, to illustrate the relative positioning or placement of certain components, to indicate a first and a second component or to do both. The particle handler according to embodiments of the invention includes a main body having provided therein a precipitation cavity operative to receive a carrier fluid (gas or liquid, not shown) that includes particles, some of which may have superparamagnetic properties. Such particles are herein referred to as "superparamagnetic particles".

[0043] At least some parts of the inner walls of the precipitation cavity feature a magnetizable particle-attractor, which in magnetized state, exhibits over a certain area and into the precipitation cavity spatially distributed separate regions of increased magnetic field gradients. Accordingly, the magnetizable particle-attractor covers at least some area or portion of the inner surface of the wall(s) of the precipitation cavity. The particle-attractor is selectively magnetizable by employing a magnetizer.

[0044] The generated increased magnetic field gradient is sufficiently high in absolute values and the precipitation cavity is shallowly designed such to effect or at least increase the probability that at least some of the superparamagnetic particles conveyed, suspended, carried or disposed in the precipitation cavity become subjected to magnetic attraction forces. In turn, the probability that the superparamagnetic particles drift towards the particle attractor is increased. Thus, the superparamagnetic particles precipitate more rapidly to the particle attractor and the probability that the superparamagnetic particles remain trapped in the precipitation cavity increases accordingly.

[0045] As a consequence, at least some of the superparamagnetic particles that are present in the precipitation cavity may be trapped in the particle attractor, whereas non-superparamagnetic particles remain non-trapped.

[0046] By sufficiently rescinding the increased magnetic field gradients, thus far attracted and accordingly trapped particles may become elutable for further handling.

[0047] Reference is now made to **Figure 1**, **Figure 2A** and **Figure 2B**. According to an embodiment of the invention, a particle handler **100** includes a main body **110** housing a precipitation cavity **130** having a plurality of openings like, e.g., a first opening **131** and a second opening **132**, either one or both which are capable to receive submicron superparamagnetic particles **190**. It should be noted that, inter alia, the superparamagnetic particles **190** and precipitation cavity **130** are not illustrated in the figures in a realistic relation to one another with regard to their sizes.

[0048] It should be noted that in order to simplify the discussion herein, main body **110** is specified herein as having only precipitation cavity **130** with the respective first and second opening **131** and **132**. However, this is not to be construed as limiting. Accordingly, main body **110** may include in embodiments of the invention a plurality of separate and/or combined precipitation cavities each communicating with at least one opening like, e.g., first and second opening **131** and **132**, respectively. In addition, to simplify the discussion herein, the cross-sectional shape of precipitation cavity **130** is herein indicated as being of rectangular shape. Again, this should not be construed as limiting and the precipitation cavity(es) may be of additional or alternative cross-sectional shapes such as, for example, quadratic, circular, oval or amorphous shapes.

[0049] Precipitation cavity **130** may include a section running in at least approximately horizontal plane within main body **110**. The length of the horizontally running section of precipitation cavity **130** is herein referred to as L_{cavity} . It should be noted that in embodiments of the invention, precipitation cavity **130** may include various branches (not shown) and/or turns (not shown) and/or curves (not shown). Such curves and/or turns (which may for example be S-shaped or Z shaped) may introduce secondary flows in carrier fluid which may propel superparamagnetic particles **190** towards the at least one particle attractor **140**, thus increasing the precipitation speed, in addition to the precipitation induced by the at least one particle attractor **140**.

[0050] Submicron particle handler **100** further includes at least one particle attractor like, e.g., upper particle attractor **140** that is provided such to engage with at least some portions of the inner side wall of precipitation cavity **130**. The at least one particle attractor like, e.g., upper particle attractor **140**, is selectively actuatable into an attraction-mode and a non-attraction mode. In the attraction-mode, superparamagnetic particles **190** become attracted and drift towards the at least one particle attractor **140** to become trapped by the latter. Conversely, in the non-attraction mode, particles become elutable from particle handler **100**. The attraction-mode and the non-attraction mode may both be effectively operable when there is flow of carrier fluid and therefore of superparamagnetic particles **190** in precipitation cavity **130** as well as when there is no flow in precipitation cavity **130**. The direction of flow of the carrier fluid in precipitation cavity **130** may be selectively changed from one direction to another such to flow from first opening **131** to second opening **132** and inversely, thus increasing the probability of the exposure of superparamagnetic particles **190** to the at least one particle attractor **140** to correspondingly increase the probability that superparamagnetic particles **190** become attracted and trapped by the at least one particle attractor **140**. The flow rate of the carrier fluid within precipitation cavity **130** may range, for example, from 0.1 ml/hr to 10 ml/hr. The maximal rate at which the direction of the flow of the carrier fluid may

be changed may range, for example, from 10 - 60 times per minute.

[0051] According an embodiment of the invention, selectively setting the at least one particle attractor into the particle-attracting and non-attraction mode is accomplished by selectively subjecting the latter to an external magnetizing field. More specifically, by subjecting for example upper particle attractor **140** to a magnetic field, as will be outlined in more detail below, particle handler 100 exhibits in turn a plurality of regions with increased magnetic field gradients causing attraction and drift of at least some superparamagnetic particles **190** towards upper particle attractor **140** such to become trapped in the latter. Otherwise stated, the magnetic field gradients generate drift forces which may overcome for at least some superparamagnetic particles **190** suspended in the carrier fluid the random forces causing Brownian motion. The attraction of superparamagnetic particles **190** towards a particle attractor like, e.g., upper particle attractor **140** occurs according to stochastically-induced motion. Conversely, in the non-attraction mode, wherein the at least one particle attractor (e.g., upper particle attractor **140**) is not subjected to an external magnetizing field, particle handler **100** is free or substantially free of spatially separated regions of increased magnetic field gradients which may otherwise, when being present, overcome forces, inter alia, respective of Brownian motion. Accordingly, particles become non-trapped from the particle attractor like, e.g., upper particle attractor **140** in precipitation cavity **130** and are thus elutable from precipitation cavity **130**, e.g., for further handling. The term "elutable" also encompasses the meaning of the term "completely elutable" and "substantially completely elutable". To enable the un-trapping of superparamagnetic particles **190**, the particle attractor(s) according to embodiments of the invention should be made of a material having sufficiently low or no residual magnetization for a given working temperature. Such material may be, for example, e.g., Ni, Fe or any suitable combination of materials. The term "sufficiently low residual magnetization" refers to the amount of magnetization which is below a threshold enabling the elution of superparamagnetic particles **190** from the at least one particle attractor **140** by diffusion of superparamagnetic particles **190** back into the carrier fluid and optionally by viscous friction due to the induction of flow of the carrier fluid within precipitation cavity **130**. The working temperature of particle handler **100** may range, for example, from 10°C - 30 °C or room temperature.

[0052] According to an embodiment of the invention, the at least one particle attractor **140** includes a plurality of spatially distinguishable magnetizable structures that are made of at least one permanent magnet(s) such as, for example, rare earth magnets or ferromagnets. The smaller or pointier the magnetizable structures, the higher are the obtainable magnetic gradients. In any event, at the working temperature of particle handler 100, the remanence of the at least one particle attractor **140** is significantly lower than its saturation magnetization. Therefore, as already outlined herein, by sufficiently reducing the increased magnetic field gradients, superparamagnetic particles that are thus far trapped by the at least one particle attractor **140** become non-trapped and hence elutable.

[0053] As already outlined hereinabove, these magnetizable structures are engaging with at least some portions of the inner walls of precipitation cavity **130**. For example, if precipitation cavity **130** is devised such to have an at least approximately rectangular cross sectional shape defining a lower surface **135**, an upper surface **136** as well as a two opposite side walls **133**, then the at least one particle attractor **140** may fixedly engage with upper surface **136** of precipitation cavity **130**. To obtain spatially distributed regions of increased magnetic field gradients on at least some portions of the inner walls of precipitation cavity **130**, the magnetizable structures may include in an embodiment of the invention a plurality of spatially distributed geometric alterations of the permanent magnet(s).

[0054] The length along which particles may be subjected to increased magnetic field gradients and thus by the at least one particle attractor **140** is herein referred to as $L_{\text{diffusion}}$, whereas the length of the at least one particle attractor **140** is herein referred to as $L_{\text{attractor}}$. It should be noted that the lengths $L_{\text{diffusion}}$ and $L_{\text{attractor}}$ as illustrated is for exemplary purposes only and should not be construed as limiting. Accordingly, the at least one particle attractor **140** may be structured such that $L_{\text{diffusion}}$ may in some embodiments of the invention equal or shorter than $L_{\text{attractor}}$. The width of the at least one particle attractor **140** may be at least half of the width W_{cavity} of precipitation cavity **130**. The width of the at least one particle attractor **140** may thus in an embodiment of the invention be equal and thus entirely cover top wall **135**.

[0055] According to an embodiment of the invention, subjecting the at least one particle attractor **140** to the external magnetizing field is accomplished by employing a magnetizer **120**, which may be embodied, for example, by at least one permanent magnet such as, for example, a rare earth magnet or ferromagnetic materials, wherein the at least one permanent magnet may be selectively approached towards and withdrawn from the at least one particle attractor **140** (e.g., automatically or manually) such to respectively increase and decrease the magnetic field gradients exhibited by the at least one particle attractor **140**. The at least one permanent magnet may be approached to the at least one particle attractor **140** such that an area of the least one permanent magnet is at least approximately parallel with the area of the at least one particle attractor **140**. In additional or alternative embodiments of the invention, magnetizer **120** may be embodied by an electromagnet or by any other suitable device enabling selectively subjecting the at least one particle attractor **140** to external magnetizing fields.

[0056] According to embodiments of the invention, precipitation cavity **130** is operative or capable to enable diffusion of most or all superparamagnetic particles that are subjected to increased magnetic field gradients towards the at least one particle attractor **140**. In order to ensure trapping of 50-100% of the superparamagnetic particles **190** introduced by the carrier fluid into precipitation cavity **130**, the length L_{cavity} and $L_{\text{attractor}}$ must be such to enable the generation of a

diffusion length L_{diffusbn} that is sufficiently large. According to an embodiment of the invention, H_{cavity} may be limited such that the maximal possible distance D_{partide} between a given superparamagnetic particle **190** and the at least one particle attractor **140** remains within the distance at which the likelihood that the motion of the given superparamagnetic particle **190** becomes biased towards the at least one particle attractor **140** by drift force F_{drift} is maximal. To simplify the discussion that follows, F_{drift} are herein schematically indicated as being one-sided parallel forces that may act on superparamagnetic particles **190**. By employing the at least one particle attractor **140**, superparamagnetic particles **190** present in precipitation cavity **130** diffuse to the at least one particle attractor **140** within maximal, e.g., 20 sec, 15 sec, 10 sec or 5 sec.

[0057] Figures **3A**, **3B** and **4** schematically illustrate alternative embodiments of particle handler **100**. However, these exemplifications for particle handler **100** should not be construed as limiting.

[0058] As is schematically illustrated in Figure **4**, particle handler **100** may employ a plurality of particle attractors. For example, a lower particle attractor **140** may be employed at lower surface **135**, in addition to upper particle attractor **140** employed at upper surface **136**. Thusly configured, precipitation cavity **130** and therefore superparamagnetic particles **190** are positioned between lower and upper particle attractors **140**. In this embodiment, particle handler **100** is configured such that by setting the latter into the attraction-mode, superparamagnetic particles **190** in the upper and lower half of precipitation cavity **130** are biased to drift towards upper and lower particle attractor **140**, respectively. Correspondingly, by employing in an embodiment of the invention both upper and lower particle attractors **140**, the likelihood that superparamagnetic particles **190** become attracted to and trapped by either one of them is twice as high, in comparison to the likelihood of superparamagnetic particles **190** becoming attracted and trapped in precipitation cavity **130** in the embodiment wherein only one of upper or lower particle attractor **140** is employed.

[0059] Upper and lower particle attractors **140** may be set into the attraction mode such to exhibit a plurality of spatially separated regions with increased magnetic field gradients with the use of one magnetizer **120** only or with a plurality of magnetizers **120**. By employing a plurality of magnetizers **120** (Figure **4**), the magnetic field gradients effected at the particle attractors may be correspondingly increased. It should however be noted that particle handler **100** is configured such that the employment of a single magnetizer **120**, as is exemplified in Figures **2A**, **2B**, **3A** and **3B** may suffice to generate the increased magnetic field gradients for biasing superparamagnetic particles **190** towards either one or both upper or lower particle attractor **140**. Otherwise stated, employing on magnetizer **120** may suffice to saturate both upper and lower particle attractors **140**. It should be noted that in any event, the generated increased magnetic field gradients respective of upper and lower particle attractors **140** vanish at very short distances of e.g., < 100 nm such that these increased field gradients may not cancel each other out. Accordingly, it should be noted that the length of F_{drift} as schematically illustrated in the Figures **3A**, **3B** and **4** is for exemplary purposes only and should not be construed as limiting. For example, F_{drift} may only act on superparamagnetic particles **190** that are located in at a distance of e.g., < 100 nm from a particle attractor.

[0060] According to an embodiment of the invention, H_{cavity} is large enough to allow other macro or micro-sized particles that might be present in the solution to pass precipitation cavity **130** without clogging particle handler **100**. Otherwise, precipitation cavity **130** remains unclogged during the attraction mode and the non-attraction mode. Such macro- and micro-sized particles may be, for example, cellular samples, organic material, beads, biologic debris and the like. Cross-sectional form and dimensions of precipitation cavity **130** may depend of the specific application being envisaged. For example, the larger L_{cavity} and W_{cavity} , the larger is the trapping-surface of a particle attractor (like e.g., lower particle attractor **140**) for trapping superparamagnetic particles **190**, and a corresponding increased quantity of the latter may be trapped. On the other hand, if particle handler **100** is employed as a particle concentrator, then an increase in L_{cavity} and the W_{cavity} causes a corresponding increase in the fluid volume that can be received in precipitation cavity **130**, thus reducing the obtainable concentration for a given number of superparamagnetic particles **190** received in precipitation cavity **130**.

[0061] The characteristic sizes indicated in Figure **2A** to **4** for the embodiments of the invention refer to: the height of the precipitation cavity (H_{cavity}), the length (L_{cavity}) and width (W_{cavity}) of the surface covered with a particle attractor like, e.g., lower particle attractor **140**.

[0062] Table 1 below exemplifies ranges of values for H_{cavity} , L_{cavity} and W_{cavity} , as well as for trapping-area to cavity volume ratios R . The trapping-area is referenced as "A", and the cavity volume V is calculated as follows: $V = L_{\text{cavity}} \times W_{\text{cavity}} \times H_{\text{cavity}}$. Clearly, the trapping-area may significantly vary for different structures of particle attractors. Thus, the ratios R may vary accordingly, even if L_{cavity} , W_{cavity} and H_{cavity} would remain constant. In any event, having a ratio of at least 50 as exemplified in table 1 below enable the processing of large amounts of particles while using a relatively small volume of carrier fluid.

[0063] It should be noted that the values outlined below should not be construed as limiting:

Table 1:

Parameter for Particle handler	First possible range for the parameter:	Second possible range for the parameter
H_{cavity}	10 μm - 1 mm	50 μm - 200 μm
W_{cavity}	200 μm - 10 cm	2 mm - 1 cm
L_{cavity}	1 cm - 1 m	1 cm - 10 cm
R	50 - 200 cm^2 / ml	

Considering the relatively large L_{cavity} -to- H_{cavity} ratio of, e.g., minimal 10, and the maximal H_{cavity} -to- W_{cavity} ratio of, e.g., 5, and a substantially unloaded particle attractor a correspondingly high trapping efficiency ranging, for example, from 95% to at least approximately 100% may be attained. The trapping efficiency is defined as $\eta = N_{\text{trapped}}/N_{\text{introduced}}$, wherein N_{trapped} refers to the number of superparamagnetic particles **190** trapped by particle attractor **140**, and $N_{\text{introduced}}$ to the number of superparamagnetic particles **190** that are introduced into the precipitation cavity **130**.

[0064] It should be noted that in some embodiments of the invention, W_{cavity} may be larger than L_{cavity} .

[0065] Elution of superparamagnetic particles **190** that are trapped by, e.g., upper and/or lower particle attractor **140** may be accomplished by setting particle handler **100** into the non-attraction mode, which renders thus far by particle attractors trapped superparamagnetic particles **190** non-trapped. In addition, the flow rate of the fluid suspension (not shown) carrying superparamagnetic particles **190** is such to enable the elution of the non-trapped superparamagnetic particles **190**. The flow rate of the carrier fluid enabling the elution of superparamagnetic particles **190** (hereinafter: elution flow rate) may be for example, 0.1-10 ml/hr. The flow in particle handler **100** may be attained prior, concurrently or after setting particle handler **100** into the non-attraction mode. The elution speed may be controlled by changing the elution flow rate. For example, an increase in the elution flow rate may result in a corresponding increase in the elution speed. With respect to the number of superparamagnetic particles **190** that may become trapped in particle handler **100**, a particle attractor (e.g., particle attractor **140**) made of, e.g., 20x4 mm Ni mesh and engaging with one side of precipitation cavity **130** may entrap about $10E15$ superparamagnetic particles **190**, given that the size of the superparamagnetic particles **190** is for example about 60 nm.

[0066] Additionally referring to **Figures 5A, 5B, 6A and 6B**, the magnetizable structures of particle attractor **140** may include in respective embodiments of the invention permanent magnets formed, for example, to a grid (**Figure 5A**), to a plurality of longitudinal structures running at least approximately parallel to the long portion of precipitation cavity **130** (**Figure 5B**) or at least approximately vertically disposed with respect to the long side of precipitation cavity **130** (**Figure 6A**). In some embodiments of the invention, the magnetizable structures of particle attractor **140** may include a plurality of elevations; depressions; a uniformly or non-uniformly corrugated magnetizable surface; wire arrays, grains, a contoured surface or any other suitable micro-sized magnetizable structures enabling the generation of spatially distributed regions of increased magnetic field gradients for attracting and consequently trapping of particles that may be present in precipitation cavity **130**. The elevations may be, for example, of a plateau-like, pyramid-like, sphere-like or amorphous form. In some embodiments of the invention, the particle attractor(s) (e.g., lower particle attractor **140**) may be provided in precipitation cavity **130** such that the magnetizable structures are embedded (partially or fully) in the inner wall of precipitation cavity **130**. Accordingly, the at least one particle attractor **140** is non-fully exposed to precipitation cavity **130**. Otherwise stated, the at least one particle attractor **140** may become only partially exposed (if partially embedded or flush with the inner wall) or remain non-exposed to the carrier fluid in precipitation cavity **130**, if fully embedded in the inner wall.

[0067] According to some embodiments of the invention, particle attractor **140** may be a plate having elevations and/or depressions formed of the material of a permanent magnet made of or including e.g. Fe-particles, respectively. Additionally or alternatively, the at least one particle attractor **140** may be made of a ferromagnetic grid or gauze covering and engaging, e.g., upper surface **136**. Such a ferromagnetic grid or gauze may be made of, for example, a wired mesh made of, e.g., Nickel wires having a diameter of, e.g., 0.1 mm and wherein the spacing between two neighboring wires is, e.g., at least approximately 250 μm .

[0068] The ratio between the gaps of the mesh and the entire coverage area of the mesh may be, for example, at least approximately 36%.

[0069] In the attraction mode, superparamagnetic particles **190** become by trapped by the at least one particle attractor **140** such to cover the magnetized surface of the latter like a molecular coating. The number of superparamagnetic particles **190** that can be trapped per unit area corresponds to a compact of stack of hundreds to thousands of particle monolayers.

[0070] In embodiments of the invention, the at least one particle attractor **140** engages with the inner wall of precipitation cavity **130**, which may be made of, e.g., Poly methyl methacrylate (PMMA), Polydimethylacrylamide (PDMS), plastic or any other suitable material or combination of materials. It should be noted that the term "engage" as well as grammatical

variations thereof as used herein in connection with the engagement of the at least one particle attractor **140** with an inner wall of precipitation cavity **130** may encompass the meaning of the at least one particle attractor **140** being fully or partially embedded, joined, coupled, integrally formed, adjacent to and the like, to the inner wall. In some embodiments, the at least one particle attractor **140** may be fixedly or removably engaging with the inner wall(s) of precipitation cavity **130**.

[0071] In some embodiments of the invention, the at least one particle attractor **140** may be joined to an inner surface of precipitation cavity **130** such that the at least one particle attractor **140** might become almost entirely exposed to the carrier fluid, except for the part of the at least one particle attractor **140** that is joinedly engaging with the inner surface. Nevertheless, to simplify the discussion herein, in the latter embodiment the at least one particle attractor **140** is referred to as being fully exposed to precipitation cavity **130**.

[0072] Engaging the at least one particle attractor **140** with the inner wall of precipitation cavity **130** may be accomplished, for example, by employing hot press, or any suitable method, e.g., known in the art. Thusly configured, the at least one particle attractor **140** according to an embodiment of the invention remains only partially (i.e., not fully) exposed to the carrier fluid which may be present in precipitation cavity **130**. Thus, the probability of the generation of bubbles in the carrier fluid is reduced, e.g., during the filling of precipitation cavity **130** with the carrier fluid, in comparison to the probability that bubbles are generated if a magnetic mesh, grid or filter, which may retain some air, was entirely exposed to the carrier fluid.

[0073] In addition, embedding the at least one particle attractor **140** in the substrate provides protection against oxidation of metals from the at least one particle attractor **140**. Accordingly, the probability of release of metal ions from the at least one particle attractor **140** into an aqueous carrier fluid is reduced compared to the probability of metal ion release if the at least one particle attractor **140** was fully exposed to an aqueous carrier fluid. It should be noted that the release of metal ion is undesired as they may interfere and/or engage and/or damage biological species or assays of the carrier fluid.

[0074] According to some embodiments of the invention the ratio of the maximal elevation of the at least one particle attractor **140** protruding inward to precipitation cavity **130** and the height H_{cavity} of precipitation cavity **130** may range, for example, from 0 to 0.5. The ratio is zero in the embodiment wherein the at least one particle attractor **140** is fully embedded in or flush with the inner side wall of precipitation cavity **130**. Accordingly, flow of carrier fluid within precipitation cavity **130** remains substantially unobstructed and thus unaffected by the particle attractor(s) both during the attraction mode and the non-attraction mode which is of particular interest if for example cells are present in the carrier fluid. Otherwise stated, both during the attraction mode and the non-attraction mode, free passage of carrier fluid through precipitation cavity **130** is ensured.

[0075] According to an embodiment of the invention, main body **110** may be an assembly of a lower body **111** and an upper body **112**. Main body **110** may be made of, for example, plastic materials like, e.g., thermoplasts (e.g. Polymethyl methacrylate, Propylene Carbonate), thermosets (e.g. Epoxies, SU-8), elastomers (e.g. silicones), thermoplastic elastomers (TPE); non-plastic materials including, for example, glass, metals or alloys, ceramics; or any other suitable material or combination of materials. Providing precipitation cavity **130** in main body **110** can be accomplished, for example, by employing micromachining, injection molding, casting, embossing or by any other suitable manufacturing method or combination of methods, e.g., known in the art. More specifically, precipitation cavity **130** may for example be micromachined into lower body **111** or upper body **112**. Alternatively, respective portions of precipitation cavity **130** may be provided in lower body **111** and upper body **112**, wherein the respective portions may be identical or non-identical. In any event, lower body **111** and upper body **112** may then be joined together, for example, by bonding e.g., thermal bonding and/or by employing a suitable bonding device (e.g., double sided bonding tape) provided that the latter does not dissolve even partially into the carrier fluid. Additionally or alternative, joining lower body **111** with upper body **112** may be accomplished by coating and/or clamping lower body **111** together with upper body **112**, with or without usage of an additional gasket. When clamping lower body **111** to upper body **112**, main body **110** becomes repetitively disassembleable and reassembleable. Therefore, particle attractor(s) may be repetitively accessible and exchangeable.

[0076] According to some embodiments of the invention, a particle handler system (not shown) may employ a plurality of particle handler devices **100** communicating in parallel and/or in series with each other, e.g., via suitably configured channels.

[0077] In embodiments of the invention, particle handler **100** or a particle handler system may be employed as separators or filters for superparamagnetic particle **190** and non-superparamagnetic particles. For example, by functionalizing superparamagnetic particles and/or non-superparamagnetic particles with molecules and by marking molecules either of superparamagnetic particles **190** or of the non-superparamagnetic particles, marked molecules can be separated from unmarked molecules. Complete extraction or elution of superparamagnetic particles **190** may be performed in connection with cleaning of a sample, e.g., upon finalization of magnetic resonance imaging of the sample. Particle handler **100** may also be employed in connection with the treatment of waste, or any other application where superparamagnetic particles **190** the size below, e.g., 1 μm and in particular equal or below 100 nm, may be present or introduced including but not limited to, for example, localized drug delivery, hypothermia treatment and molecular recognition.

[0078] Particle handler **100** or a particle handler system according to an embodiment of the invention may for example

be used as a fixed-bed reactor operative to immobilize superparamagnetic particles **190** on a solid substrate (namely the particle-attractors) for their functionalization and/or any other processing. In the attraction mode, superparamagnetic particles **190** trapped by the at least one particle attractor **140** remain substantially immobilized within precipitation cavity **130** during all steps of rinsing and exposure to the individual reagents. Accordingly, the procedures according to em-
 5 bodiments of the invention are therefore less time-consuming and generate fewer losses of carrier fluid and particles than the procedure employed according to the prior art which includes precipitating and re-dispersing the particles between each functionalization step. In other words, according to the embodiment of the invention, the employment of particle handler **100** enables the functionalization of particles in a single series of different (non-repetitive) steps.

[0079] Particle handler **100** and the particle handler system according to some embodiments of the invention are disposable and sized such to fit on a bench top for example.

[0080] It will be appreciated by persons skilled in the art that the disclosed invention is not limited to what has been particularly shown and described hereinabove.

Claims

1. A particle handler (100) for handling at least submicron-sized particles, said particle handler (100) comprising:

a main body (110) that comprises a precipitation cavity (130);
 at least one particle attractor (140) comprising permanent magnets;
 at least one magnetizer (120, 121) for selectively magnetizing said particle attractor into an attraction mode and a non-attraction mode, wherein in the attraction mode, said at least one particle attractor (140) exhibits regions of increased magnetic field gradients capable or operative to attract superparamagnetic particles (190) sus-
 25 pended in carrier fluid received by said precipitation cavity (130) via at least one of a plurality of openings (131, 132)

characterized in that

said at least one particle attractor (140) extends over a given area and engages with at least a corresponding portion of the inner wall of said precipitation cavity (130).

2. The particle handler (100) according to claim 1,

characterized in that

said at least one particle attractor (140) engages with the inner side wall of said precipitation cavity (130) such to be non-fully exposed to said precipitation cavity (130).

3. The particle handler (100) according to claim 1 or 2,

characterized in that

said precipitation cavity (130) has:

a height H_{cavity} ranging from 10 μm to 1 mm, and preferably ranging from 50 μm to 200 μm ;
 a width W_{cavity} ranging from 200 μm to 10 cm and preferably from 2 mm to 1 cm; and
 a length C_{cavity} ranging from 1 cm to 1 m and preferably from 1 cm to 10 cm.

4. The particle handler (100) according to any of the preceding claims,

characterized in that

said particle attractor (140) is made of a grid or mesh made of wires with permanent magnetic characteristics.

5. The particle handler (100) according to claim 4,

characterized in that

the wires of said particle attractor (140) have a diameter of 0.1 mm and the ratio between the gaps of the mesh and the entire coverage area of the mesh is at least approximately 36%.

6. The particle handler (100) according to any of the preceding claims,

characterized in that

said main body (110) is repetitively disassembleable and reassembleable such that said at least one particle attractor (140) is accessible, exchangeable and disposable.

7. A method for manufacturing a particle handler (100) operative to handle at least submicron-sized particles, said

method **characterized by** comprising the following procedures:

providing a lower body (111) and/or an upper body (112), said lower body (111) and said upper body (112) forming a main body (110);

providing at least a part of said precipitation cavity (130) in said lower body (111) and/or said upper body (112), said precipitation cavity (130) having at least two openings (131, 132);

providing at least one particle attractor (140) in said precipitation cavity (130), and joining said lower body (111) with said upper body (112) to form said main body (110).

8. The method for manufacturing the particle handler (100) according to claim 7, wherein said at least one particle attractor (140) is provided to a side wall of said precipitation cavity (130) such to be non-fully exposed to said precipitation cavity (130).

9. The method for manufacturing the particle handler (100) according to any of the claims 7 or 8, **characterized in that** providing said at least one particle attractor (140) is accomplished by employing hot press.

10. The method for manufacturing the particle handler (100) according to any of the claims 7 to 9, **characterized in that** the procedure of joining said lower body (111) with said upper body (112) to form said main body (110) comprises at least one of the following manufacturing procedures: thermal bonding, chemical bonding, by employing a bonding device, coating, and clamping.

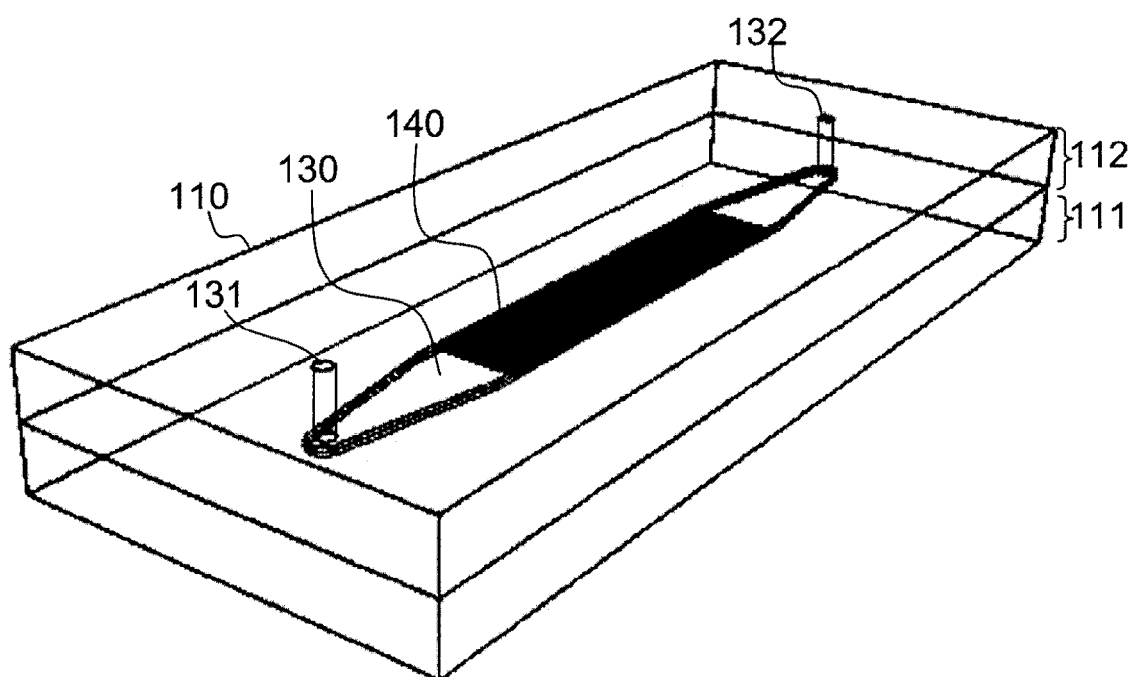
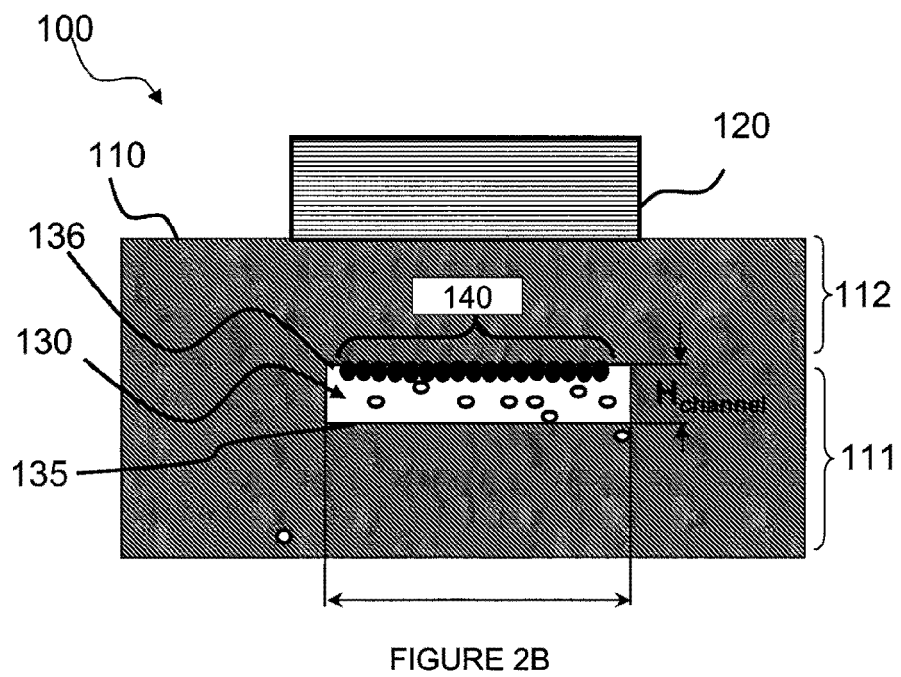
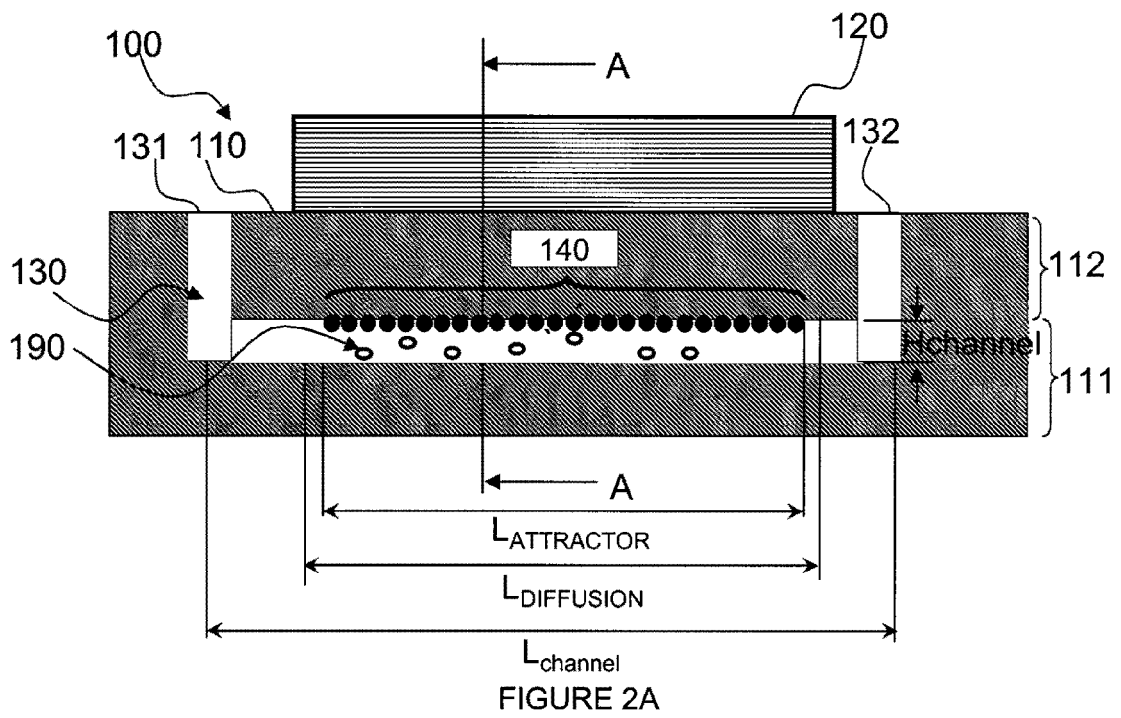


FIGURE 1



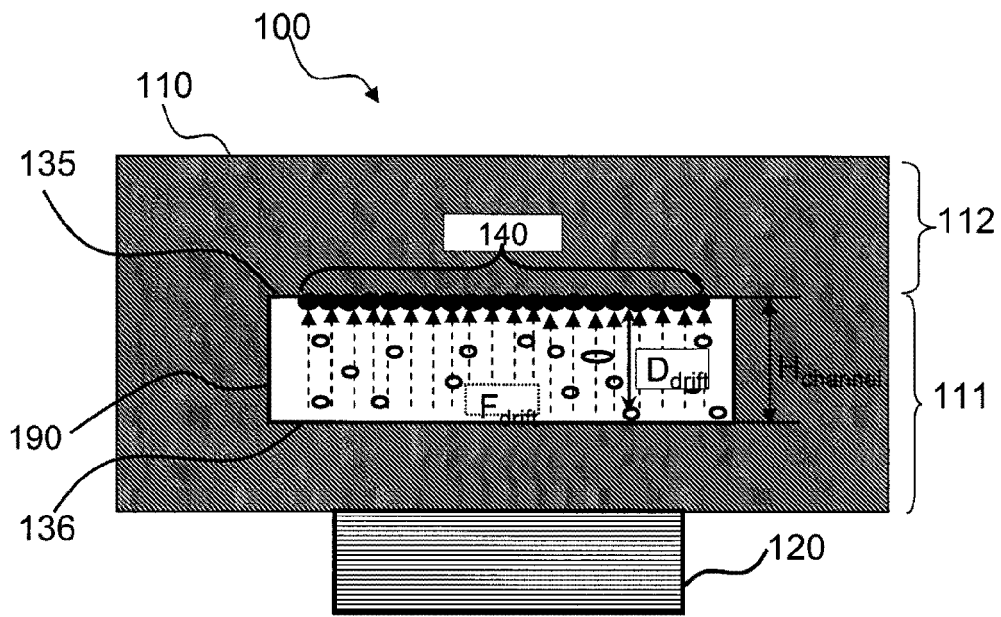


FIGURE 3A

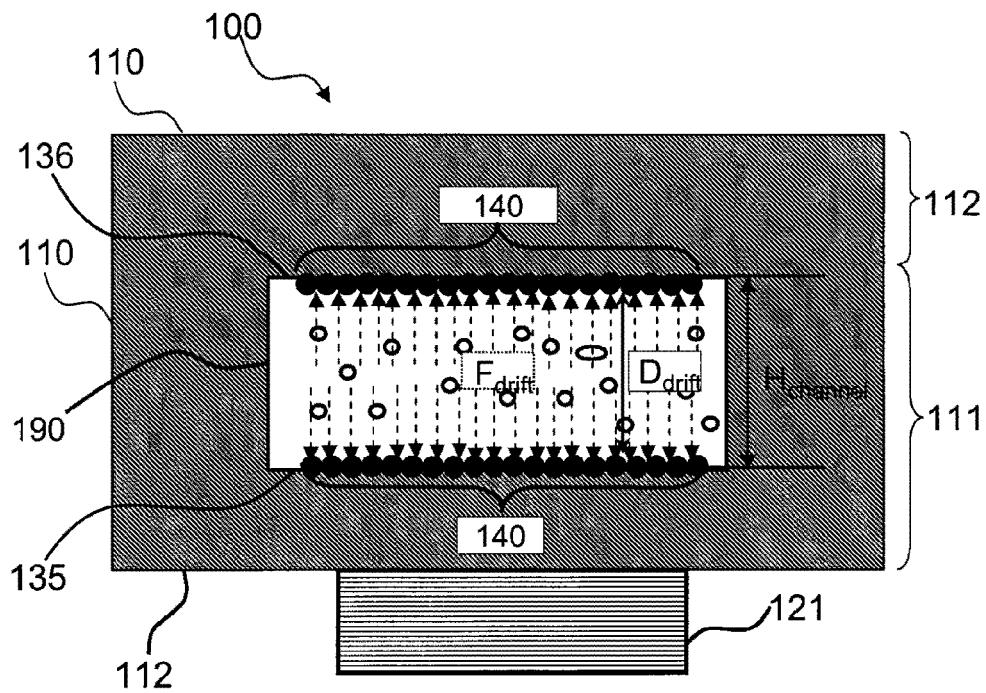


FIGURE 3B

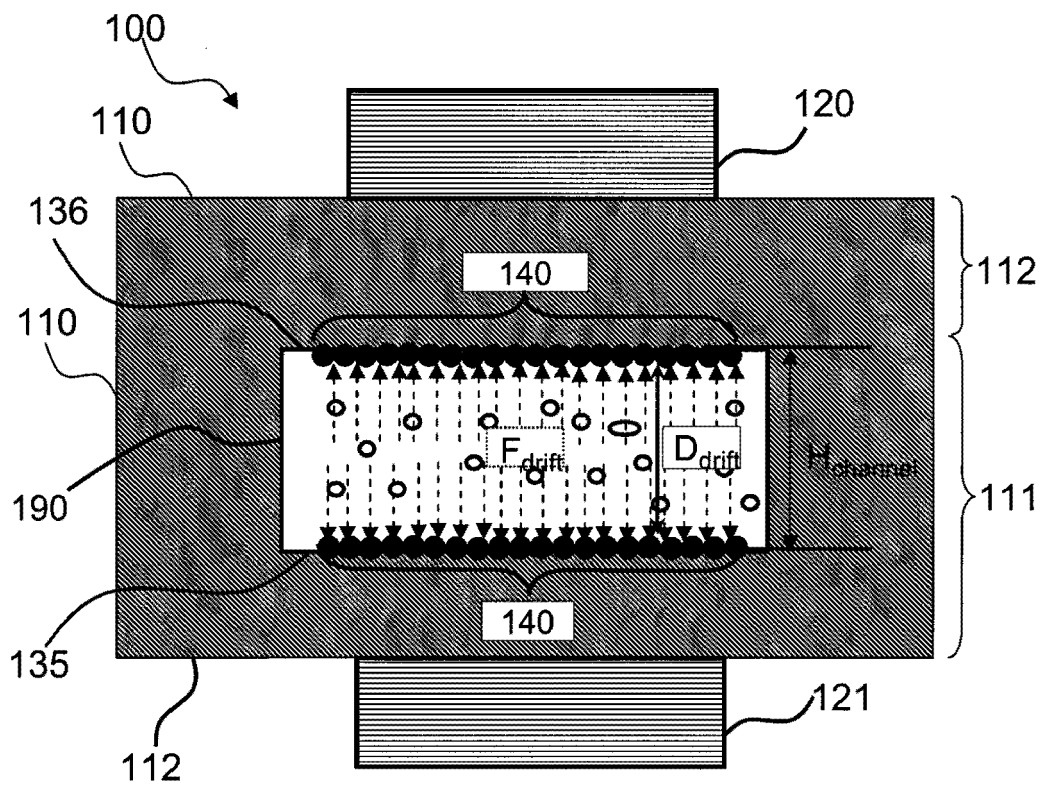


FIGURE 4

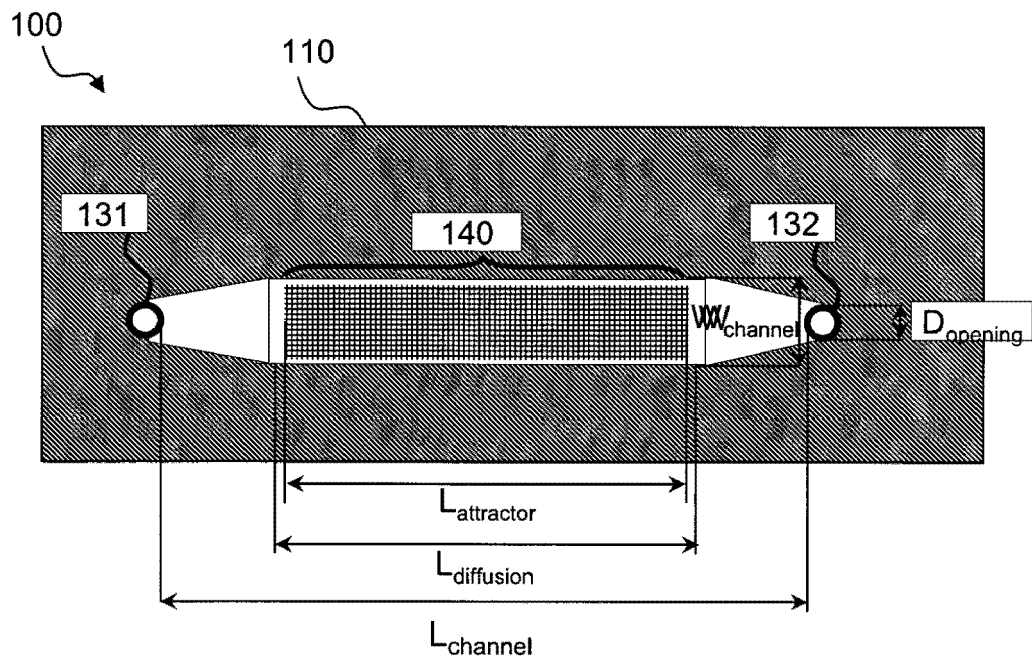


FIGURE 5A

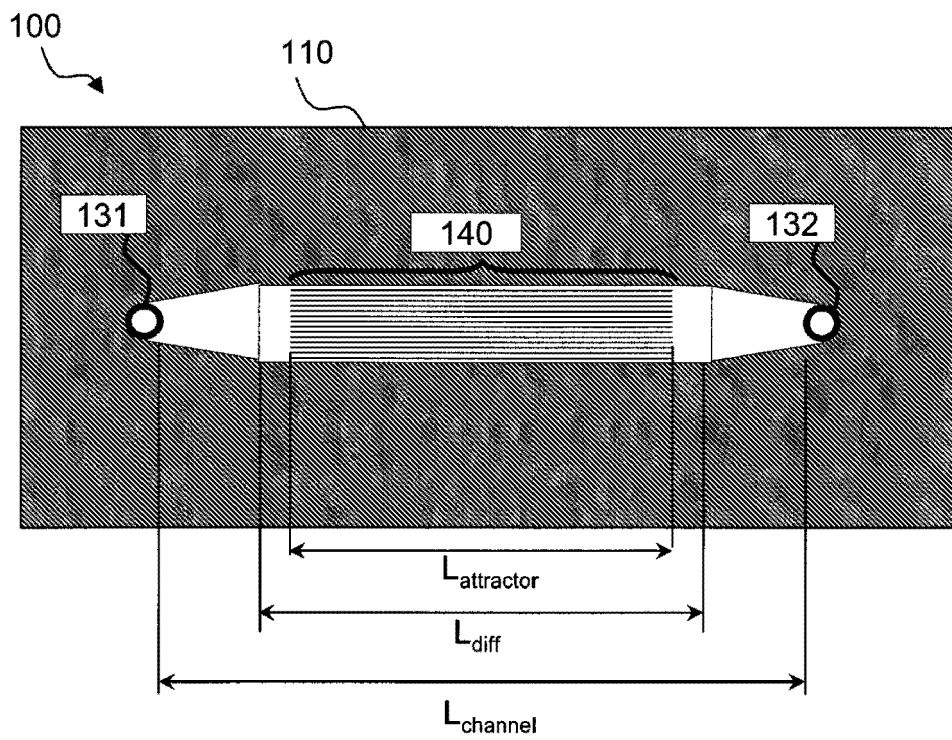
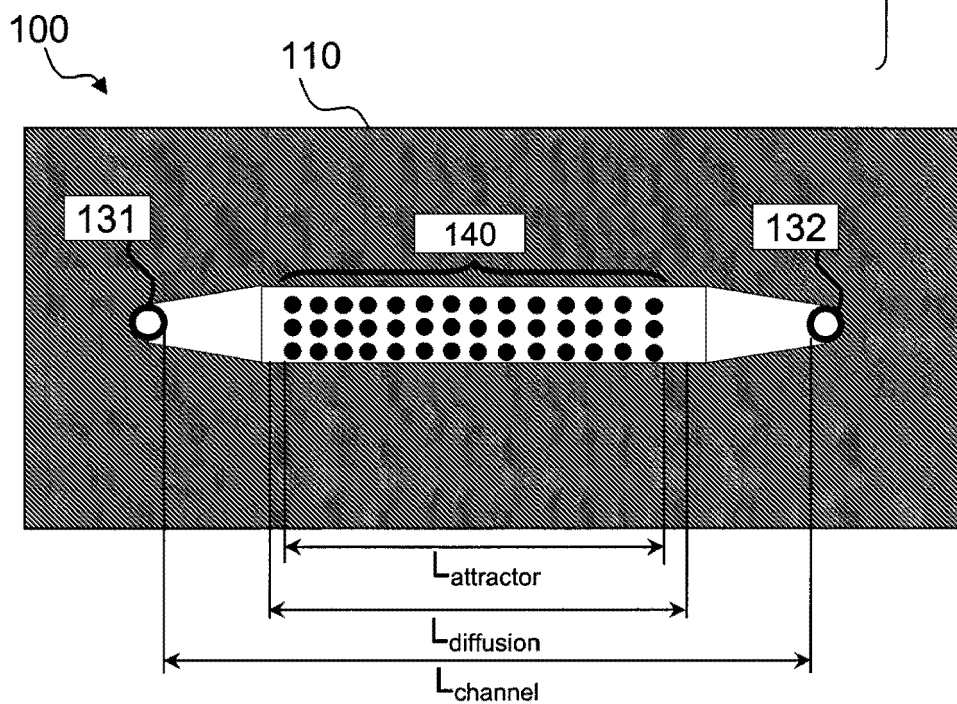
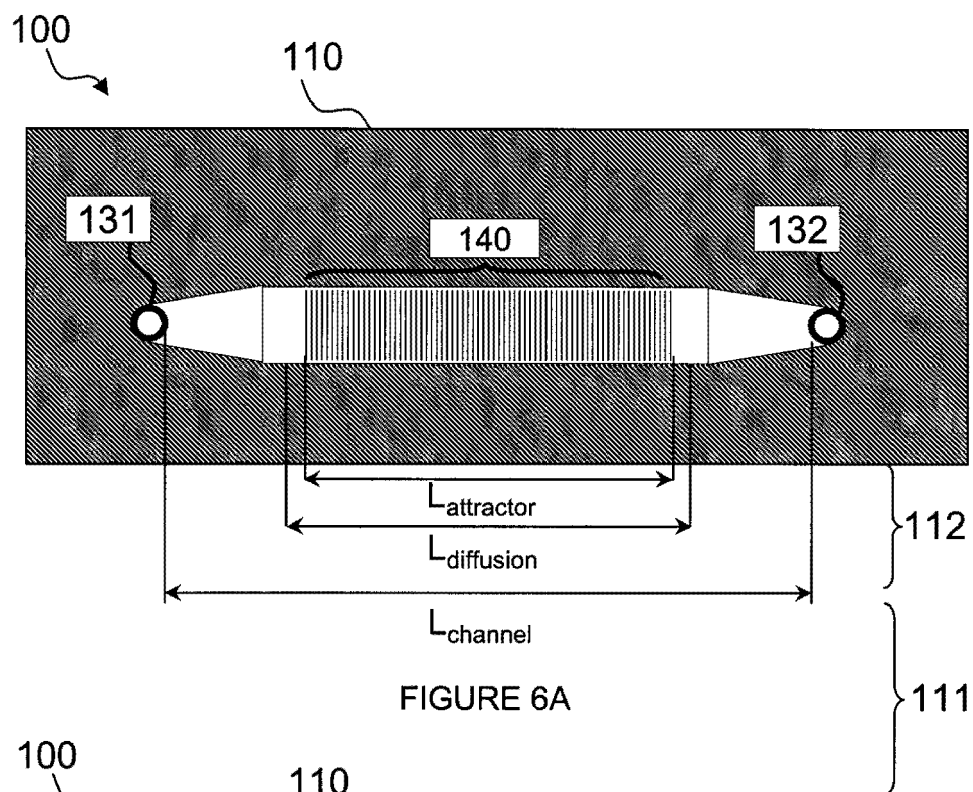


FIGURE 5B





EUROPEAN SEARCH REPORT

Application Number
EP 09 17 4297

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Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
X	US 2004/018611 A1 (WARD MICHAEL DENNIS [US] ET AL) 29 January 2004 (2004-01-29) * paragraphs [0014], [0025] - [0028], [0083] - [0092] *	1,2,7,8	INV. B01L3/00
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			B01L
The present search report has been drawn up for all claims			
Place of search Munich		Date of completion of the search 2 March 2010	Examiner Tragoustis, Marios
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document			

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For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

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