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(71) Applicant: **Koninklijke Philips Electronics N.V.**
5621 BA Eindhoven (NL)

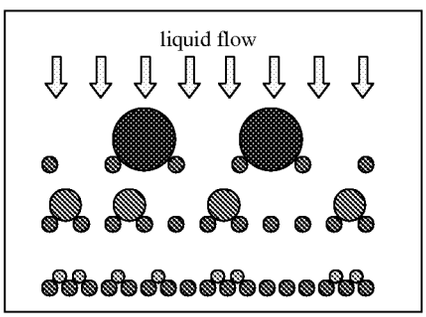
(72) Inventor: **The designation of the inventor has not yet been filed**

(74) Representative: **Rüber, Bernhard Jakob**
Philips Intellectual Property & Standards GmbH
Postfach 50 04 42
52088 Aachen (DE)

(54) A reconfigurable microfluidic filter based on eletric fields

(57) A filter for filtering particles in a fluid, suitable for lab- on-chip microsystems, involves replacing traditional microfluidic filters and traps by reconfigurable devices based on e.g. dielectrophoresis. The filter has a channel (40) for the fluid, and electrodes (50, 60) and drive circuitry (70) arranged to create an electric field pattern in the fluid in the channel, to attract or repel the particles so as to selectively block particles of a given size, and allow particles of another size to pass. By forming the filter using electric fields, rather than using physical structures, it can be much easier to control or move or change the pattern of the filter. The filter can be used for sample preparation, isolation of cells for example from blood, analyte concentration and sorting of cells or functionalized microspheres on the basis of their size.

FIG 7



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Description

[0001] This invention relates to filters for filtering particles in a fluid, to methods of using such devices for manipulating particles, and to methods of manufacturing and operating such devices.

[0002] Lab-on-a-chip systems show great promise for a variety of medical, life-science, forensic and environmental applications. Their advantages include ease of use and low fabrication costs (ultimately leading to disposable chips), low fluid volumes and reagents consumption, large integration of functionalities, high-throughput analysis via massive parallelization and increased process control due to the faster response of the system.

[0003] A key aspect when performing analysis on lab-on-a-chip devices is sample preparation. Ideally, all preparation steps should be done on-chip in order to allow the use of raw, unprocessed samples as input. Essential preparation steps are filtration of the sample prior to processing and concentration of the analyte to increase the sensitivity of the device. In some cases, a specific target cell population has to be extracted from a heterogeneous cell mixture. Examples are the isolation of hematopoietic stem cells, antigen-specific lymphocytes and circulating tumor cells from peripheral blood or the isolation of fetal cells from maternal blood. For nucleic acids or protein analysis, purification and concentration of the analyte is critical. The desired species are often extracted from a biological sample with the aid of micrometer-sized beads. Such beads can be designed to bind to specific cells or macromolecules (DNA, RNA, or proteins) and greatly simplifies the recovery of material for further studies.

[0004] Applications of microfluidic filters have included creation of flow restrictions, such as porous membranes, mesh structures or arrays of cylindrical pillars with high aspect ratio. Such structures can be realized, e.g., by deep reactive ion etching in silicon or by lithography in polymeric materials such as SU8 or PDMS. In the following, some applications of these structures are explained.

A) Microfilters for trapping cells or microspheres

[0005] Cells in suspension are usually transported with flow, but can be physically retained in a lab-on-a-chip device by filters or traps. The separation can be made selective by carefully designing the filter. For example, white blood cells have been separated from other components of whole blood via size exclusion using a row of micropillars inside a chamber on a microchip (see N. J. Panaro et al., Biomolecular Engineering 21, 157-162, 2005). The authors of this study determined 3.5 μm to be the optimal sieve size to capture most white blood cells while allowing blood serum, red blood cells and platelets to pass through the sieve.

[0006] In another example, three-dimensional micromesh structures were fabricated using conventional photolithography (see H. Sato et al., Sensors and Actuators A 111, 87-92, 2004). In this publication the structures were used to trap 20μm beads, while smaller beads could freely pass through the microfilter.

B) Size-sorting of particles

[0007] Recently, a device based on a micropillar array was shown to allow size-sorting of microspheres and bacterial artificial chromosomes with high resolution (see L. R. Huang et al., Science 304, 987-990, 2004, hereafter Huang et al). The separation process makes use of laminar flow through a periodic array of micrometer-scale obstacles. Each row of obstacles is shifted horizontally with respect to the previous row by a fraction of the gap between the obstacles. A particle chooses its path deterministically on the basis of its size. All particles of a given size follow equivalent migration paths, eliminating multipath zone broadening. Microspheres of 0.8, 0.9, and 1.0 μm were sorted in 40sec with a resolution of ~10nm in a device containing nine sections, each of which had a different gap width. Bacterial artificial chromosomes could be separated with the same method in 10min with a resolution of~12%.

C) Microreactors for bead-based processes

[0008] Functionalized microspheres can also be used as a solid substrate for biochemical reactions (see Microsystem Technology Lab, KTH, Stockholm (www.s3.kth.se/mst/)). Micropillar cages can in this case be used to confine the beads to a specific location on the device, thereby defining a micro reaction chamber. The mechanical barrier holds the microspheres within the chamber but allows liquid, reagents and smaller particles to flow freely through the chamber. The shape of the microreactor can be designed to be compatible with the used detection system, as for example a square camera pixel.

[0009] An object of the invention is to provide improved apparatus or methods for filtering particles in a fluid.

[0010] According to a first aspect, the invention provides:

[0011] A filter for filtering particles in a fluid, the filter having a channel for the fluid, and electrodes arranged to create an electric field pattern in the fluid in the channel, to repel the particles at locations spaced apart in the channel, so as to selectively block particles of a one size, and allow particles of another size to pass. The electric field pattern generates

field-induced obstacles.

[0012] All dielectric particles or microspheres behave similarly and cannot be separated by conventional DEP or twDEP methods. The device of the present invention does not rely on differences in the dielectric properties of the particles and therefore shares the robustness and reliability of conventional methods based on arrays of solid obstacles, i.e. conventional filters. The electric field pattern is in the form of field-induced obstacles. The field-induced obstacles can be implemented by equipping the device with micropatterned electrodes, which result in a high electric field at specific locations. The device can also include drive circuitry for generating the field pattern. The drive circuitry can be integrated in the device or may be separate from it.

[0013] By forming the filter using electric field barriers, rather than using physical structures, it can be much easier to control or move or change the pattern of the filter. An advantage of the present invention, at least for the embodiments where the filtering and size-sorting is based on negative DEP, is that it can be applied to highly conductive media such as PBS or blood serum.

[0014] In particular the present invention provides:

[0015] A reconfigurable filter for filtering particles in a fluid, the filter having a channel for the fluid, and electrodes arranged to create a selectable electric field pattern in the fluid in the channel, to repel the particles at locations spaced apart in the channel, so as to selectively block particles of a one size, and allow particles of another size to pass.

[0016] Embodiments within this aspect of the invention can have any additional features, and some such additional features are set out in dependent claims, and some are set out in the examples in the detailed description.

[0017] One such additional feature is the filter being arranged to use dielectrophoresis to repel or attract the particles. In principle other effects can be exploited to cause blocking. In case of charged particles, electrophoresis can be used.

[0018] One such additional feature is control circuitry arranged to vary the field, e.g. reduce the electric field to allow blocked particles to pass. This can be useful to prevent clogging of the filter, or allow the blocked particles to be further processed for example. The control circuitry can be integrated in the device or separate from it.

[0019] Another such additional feature is the electric field pattern to be arranged to use negative DEP. This ensures, in some embodiments, that particles are kept within zones of low field strength. In turn such lower field strengths can reduce the risk of damage to the particles, and can help to enable lower power consumption and greater integration for example.

[0020] Another such additional feature is the drive circuitry being arranged to drive the electrodes with an AC signal, with substantially 180 degree phase difference between opposing electrodes. This can create an area of relatively high electric field to act as an obstacle to the particles.

[0021] Another such additional feature is the electrodes being arranged on opposing sides of channel for the fluid.

[0022] Another such additional feature is the channel having at least one branch, arranged so as to provide different exit paths for different sized particles

[0023] Another such additional feature is control circuitry arranged to reconfigure the filter by changing the pattern of the electric field in the channel so as to change a size of particles which are blocked or passed. This can enable more sophisticated filtering of selected sizes in sequence, or unclogging of the filter for example.

[0024] Another such additional feature is the electrodes being arranged in a line across the channel, and the reconfiguring involving altering the relative drive levels of different ones of the electrodes in the line.

[0025] Another such feature is the electrodes comprising at least one two dimensional array of separately drivable electrodes on at least one side of the channel. This can enable a wide variety of patterns of electric field to be generated.

[0026] Another such feature is the drive circuitry for the electrodes in the two dimensional array being integrated together with the respective electrodes.

[0027] Another such feature is the electrodes and drive circuitry being arranged to enable a cage of obstacles to the particles of the given size, for use as a micro reactor.

[0028] Another such feature is the device being an integrated micro fluidic device having integrated fluid handling components.

[0029] Another aspect provides a method of using a filter to filter particles in a fluid, the filter having a channel for the fluid, and electrodes, the method having the steps of introducing the particles to the filter, and creating an electric field pattern to repel the particles at locations spaced apart in the channel, so as to selectively block particles of a given size, and allow particles of another size to pass.

[0030] An additional feature of some embodiments of such a method is using the method for any one or more of the following: isolation of cells from blood or other substance, sorting of cells, or for filtering micro carrier beads loaded with cells.

[0031] One such additional feature is using dielectrophoresis to repel or attract the particles.

[0032] One such additional feature is the step of reducing the electric field to allow blocked particles to pass. Another such additional feature is arranging the electric field pattern to use negative DEP. Another such additional feature is driving the electrodes with an AC signal, with substantially 180 degree phase difference between opposing electrodes. Another such additional feature is the channel having at least one branch, and the step of using different exit paths for

different sized particles.

[0033] Another such additional feature is the step of reconfiguring the filter by changing the pattern of the electric field in the channel so as to change a size of particles which are blocked or passed.

[0034] Another such additional feature is the electrodes being arranged in a line across the channel, and the reconfiguring step involving altering the relative drive levels of different ones of the electrodes in the line.

[0035] Another such feature is the electrodes comprising at least one two dimensional array of separately drivable electrodes on at least one side of the channel and the reconfiguring step involving altering the relative drive levels of different ones of the electrodes in the array. Another such feature is the step of generating the electric field pattern to form a ring of obstacles to the particles of the given size, for use as a micro reactor.

[0036] Some of the embodiments are described herein as a method or combination of elements of a method that can be implemented by a processor of a computer system or by other means of carrying out the function. Thus, a processor with and memory and the necessary instructions for carrying out such a method or element of a method forms a means for carrying out the method or element of a method. Furthermore, an element described herein of an apparatus embodiment is an example of a means for carrying out the function performed by the element for the purpose of carrying out the invention.

[0037] Another aspect provides software on a computer readable medium for controlling the driving of different ones of the electrodes to generate the electric field pattern for use in any of the above methods.

[0038] Any of the additional features can be combined together and combined with any of the aspects. Other advantages will be apparent to those skilled in the art, especially over other prior art. Numerous variations and modifications can be made without departing from the claims of the present invention. Therefore, it should be clearly understood that the form of the present invention is illustrative only and is not intended to limit the scope of the present invention.

[0039] How the present invention may be put into effect will now be described by way of example with reference to the appended drawings, in which:

FIG. 1 shows a graph of the frequency dependence of the Clausius-Mossotti factor for 1 μm latex beads,

FIG. 2 shows the electric field between 10 μm top and bottom electrodes in a channel containing PBS,

FIG. 3 shows the electric field between a 10 μm top electrode and an unstructured bottom electrode in a channel containing PBS,

FIG. 4 shows the electric field between two 2x30 μm rectangular electrodes on the same side of the channel containing PBS,

FIG. 5 shows the finite element simulation of the electric field strength produced by a row of electrodes, when all electrodes are connected,

FIG. 6 shows the finite element simulation of the electric field strength produced by a row of electrodes, when the electrodes in the middle are grounded,

FIG. 7 shows an example of an electrode configuration having rows perpendicular to the flow, and with decreasing gaps,

FIG. 8 shows another example with rows angled to the flow to lead towards different exits for different sizes of particles,

FIG. 9 shows two dimensional electrode arrays based on active matrix (AM) electronics (CMOS or LTPS), both above and below the channel, and

FIG. 10 shows a two dimensional electrode array based on active matrix (AM) electronics (CMOS or LTPS) above the channel, with a common electrode below the channel.

[0040] The present invention will be described with respect to particular embodiments and with reference to certain drawings but the invention is not limited thereto but only by the claims. The drawings described are only schematic and are non-limiting. In the drawings, the size of some of the elements may be exaggerated and not drawn on scale for illustrative purposes. Where the term "comprising" is used in the present description and claims, it does not exclude other elements or steps. Where an indefinite or definite article is used when referring to a singular noun e.g. "a" or "an", "the", this includes a plural of that noun unless something else is specifically stated.

[0041] The term "comprising", used in the claims, should not be interpreted as being restricted to the means listed thereafter; it does not exclude other elements or steps. Thus, the scope of the expression "a device comprising means A and B" should not be limited to devices consisting only of components A and B. It means that with respect to the present invention, the only relevant components of the device are A and B.

[0042] Furthermore, the terms first, second, third and the like in the description and in the claims, are used for distinguishing between similar elements and not necessarily for describing a sequential or chronological order. It is to be understood that the terms so used are interchangeable under appropriate circumstances and that the embodiments of the invention described herein are capable of operation in other sequences than described or illustrated herein.

[0043] Moreover, the terms top, bottom, over, under and the like in the description and the claims are used for

descriptive purposes and not necessarily for describing relative positions. It is to be understood that the terms so used are interchangeable under appropriate circumstances and that the embodiments of the invention described herein are capable of operation in other orientations than described or illustrated herein.

[0044] Reference throughout this specification to "one embodiment" or "an embodiment" means that a particular feature, structure or characteristic described in connection with the embodiment is included in at least one embodiment of the present invention. Thus, appearances of the phrases "in one embodiment" or "in an embodiment" in various places throughout this specification are not necessarily all referring to the same embodiment, but may. Furthermore, the particular features, structures or characteristics may be combined in any suitable manner, as would be apparent to one of ordinary skill in the art from this disclosure, in one or more embodiments.

[0045] Similarly it should be appreciated that in the description of exemplary embodiments of the invention, various features of the invention are sometimes grouped together in a single embodiment, figure, or description thereof for the purpose of streamlining the disclosure and aiding in the understanding of one or more of the various inventive aspects. This method of disclosure, however, is not to be interpreted as reflecting an intention that the claimed invention requires more features than are expressly recited in each claim. Rather, as the following claims reflect, inventive aspects lie in less than all features of a single foregoing disclosed embodiment. Thus, the claims following the detailed description are hereby expressly incorporated into this detailed description, with each claim standing on its own as a separate embodiment of this invention.

[0046] Furthermore, while some embodiments described herein include some but not other features included in other embodiments, combinations of features of different embodiments are meant to be within the scope of the invention, and form different embodiments, as would be understood by those in the art. For example, in the following claims, any of the claimed embodiments can be used in any combination.

[0047] Furthermore, some of the embodiments are described herein as a method or combination of elements of a method that can be implemented by a processor of a computer system or by other means of carrying out the function. Thus, a processor with the necessary instructions for carrying out such a method or element of a method forms a means for carrying out the method or element of a method. Furthermore, an element described herein of an apparatus embodiment is an example of a means for carrying out the function performed by the element for the purpose of carrying out the invention.

[0048] In the description provided herein, numerous specific details are set forth. However, it is understood that embodiments of the invention may be practiced without these specific details. In other instances, well-known methods, structures and techniques have not been shown in detail in order not to obscure an understanding of this description.

[0049] By way of introduction to the embodiments, some problems or disadvantages addressed by some of the embodiments will be discussed. DEP has been applied to manipulate and separate a variety of cells including bacteria, yeast, and mammalian cells in microsystems. In particular, DEP has been used to separate cancer cells from blood, isolate CD34+ stem cells from blood, bacteria from blood and to separate various cell sub-populations of blood. US patent 5,814,200 shows separation by DEP forces which are different on different types of particle, such as proteins and chromosomes.

[0050] Most reported experiments are proof-of-principle applications of DEP, in which cells that undergo positive DEP are separated from those experiencing negative DEP on the microscopic level. Practical applications, however, require cell separation on a macroscopic scale. This is usually achieved by combining DEP with liquid flow.

[0051] Traditional micro fluidic filter devices as the ones described above have the advantage of being robust and reliable. Their performance is essentially independent of the detailed composition of the suspension medium and only relies on differences in size between particles. The main disadvantage of such devices is however their lack of flexibility. Essentially, the devices operate in a digital way: they retain particles larger than the apertures in the structure and let smaller particles flow through. As a consequence, a structure has to be carefully designed to perform a specific task, and new structures have to be fabricated for each application. In addition, particles captured in such structures cannot be easily released for downstream processing, but can only, for example, be redistributed into the section preceding the filter by reversing the flow direction. Another major problem with traditional devices is the fact that they become easily clogged with cells or microspheres. These issues can be addressed by a filter device that can be reconfigured, for example, to change the sieve size, vary the distributions of pillars in an array or remove the obstacles completely to release the captured particles or prevent clogging. However, to achieve reconfiguration of physical obstacles is typically difficult, particularly for integrated microsystems.

[0052] Accordingly, to achieve reconfigurable filter structures, according to embodiments of the invention, an electric field pattern is used. This can be useful for integration onto lab-on-a-chip platforms. Some embodiments are based on dielectrophoresis, though in principle other methods can be used, based on charged particles for example. The embodiments based on dielectrophoresis at least can be useful for a wide variety of applications such as for sample preparation, isolation of cells for example from blood, analyte concentration and sorting of cells or functionalized microspheres on the basis of their size. The methods described are especially suited for high conductivity liquids such as blood, but can also be used with fluids of lower conductivity.

[0053] Some of the embodiments involve replacing traditional micro fluidic filters and traps by reconfigurable devices based on dielectrophoresis (DEP). DEP is a phenomenon in which a force is exerted on a dielectric particle when it is subjected to a nonuniform (usually AC) electric field. Unlike electrophoresis, DEP relies on field-induced polarization effects and is independent of the net charge of the particle. In general, the DEP force depends on the dielectric properties of the particle and of the surrounding medium, on the size (and shape) of the particle and on the spatial distribution and frequency of the applied field. Depending on these factors, the particle can be attracted to either high-field (positive DEP) or low-field (negative DEP) regions.

[0054] In the case of media with high conductivities (>1 S/m) such as blood serum or phosphate buffered saline (PBS), cells and dielectric microspheres only undergo negative DEP and are therefore strongly repelled by high-field regions. By equipping the lab-on-a-chip device with carefully designed electrodes, the solid obstacles of traditional devices and traps can be replaced by "virtual" obstacles (e.g. pillars or arches) created by the electric field. The method enables filtering and trapping devices that can be easily reconfigured, for example by varying the gap between the obstacles, changing the configuration of an array of obstacles, or removing the obstacles to release the captured particles and prevent clogging. As is explained below, the method also opens the door to new capabilities, which are not possible with traditional devices.

[0055] Electric field-based approaches are particularly suited for miniaturization because micropatterned electrodes are easy to fabricate and result in high electric fields at modest voltages. DEP, in particular, has received considerable attention as a method to manipulate and separate micrometer-sized particles. DEP separation methods have been applied to variety of cells including bacteria, yeast, mammalian, and human cells in lab-on-a-chip systems (see H. Morgan and N. Green, AC Electrokinetics: colloids and nanoparticles, Research Studies Press, (Baldock, UK, 2002), hereafter Morgan et al). In the vast majority of the reported experiments, particles are sorted by exploiting differences in their dielectric properties and, consequently, DEP responses. The classic example is separation based on positive vs. negative DEP: cells undergoing negative DEP are trapped at field minima, while those experiencing positive DEP are collected at the electrodes tips. As the latter are held by a stronger force than the first, a liquid flow of suitable strength can be used to selectively remove the particles undergoing negative DEP. The remaining particles are then released into the flow when the field is switched off. Another common method for separating particles is based on traveling wave dielectrophoresis (twDEP). In this technique, an array of electrodes is driven by multiphase AC voltage signals, creating a traveling wave potential distribution that can levitate particles and simultaneously propel them along the array. As for conventional DEP separation methods, twDEP methods separate particles based on differences in their dielectric properties.

[0056] If one assumes a harmonic electric field of the form $\mathbf{E}(\boldsymbol{x}, t) = \mathrm{Re}[\hat{\mathbf{E}}(\mathbf{x})\exp(-i\omega t)]$, where the complex phasor $\tilde{\mathbf{E}} = \mathbf{E}_R + i\mathbf{E}_I$ allows the possibility of an elliptically or circularly polarized electric field, the time-averaged dipolar DEP force acting on a dielectric sphere of radius r is then given by Morgan as:

$$\left\langle F_{DEP} \right\rangle = \pi \varepsilon_m r^3 \left\{ \mathrm{Re}\left[\tilde{f}_{CM} \right] \nabla \left| \tilde{\mathbf{E}} \right|^2 - 2\,\mathrm{Im}\left[\tilde{f}_{CM} \right] \left[\nabla \times \left(\mathbf{E}_R \times \mathbf{E}_I \right) \right] \right\} , \quad (1)$$

where ε_m is the dielectric permittivity of the medium, and $\tilde{f}_{CM}$ is the complex, frequency dependent Clausius-Mossotti factor:

$$\tilde{f}_{CM} = \frac{\tilde{\varepsilon}_p - \tilde{\varepsilon}_m}{\tilde{\varepsilon}_p + 2\tilde{\varepsilon}_m} . \quad (2)$$

[0057] The subscripts p and m in Eq. (2) refer to the particle and the medium, respectively, and the complex permittivity includes the conductivity σ and is given by $\tilde{\varepsilon}=\varepsilon-i\sigma/\omega$.

[0058] The first term in Eq. (1) is the conventional DEP force, indicating that a particle will be attracted or repelled from regions of stronger electric field depending on whether $\mathrm{Re}[\tilde{f}_{CM}] > 0$ or $\mathrm{Re}[\tilde{f}_{CM}] < 0$, respectively. The second term only occurs when $\mathrm{Im}[\tilde{f}_{CM}] \neq 0$ and when the electric field is rotating ($\mathbf{E}_I \neq 0$) and is responsible for the phenomena of electro-rotation and twDEP.

[0059] Most of the published studies are performed in controlled experimental conditions and in weakly conductive media (10^{-3}-10^{-2} S/m). Although important from the point of view of fundamental research, these methods are very difficult to implement in raw biological samples, which present far more challenging conditions.

Fig 1, graph of frequency dependence of force on particle

[0060] Figure 1 shows a graph showing four lines representing the real and imaginary parts of the Clausius-Mossotti factor $\tilde{f}_{CM}$ for a latex bead with a diameter of 1 μm, in two conditions. A first case is in a low conductivity fluid medium (10^{-3} S/m) and a second case is in a highly conductive medium such as PBS (1.5 S/m). As can be seen, at low conductivity both negative and positive DEP occur, with a crossover frequency around 700 kHz. The imaginary part of $\tilde{f}_{CM}$ is also non-zero over a broad frequency range, indicating that electro-rotation and twDEP also occur. At high conductivity, however, the Clausius-Mossotti factor becomes a real, negative number for all frequencies and the only observable effect is negative dielectrophoresis.

[0061] This indicates that in highly conductive media such as blood serum (~1.2 S/m) essentially all dielectric particles or microspheres behave similarly and cannot be separated by the conventional DEP or twDEP methods described above. In contrast, at least for the embodiments where the filtering and size-sorting is based on negative DEP, they can be applied to highly conductive media such as PBS or blood serum. The method does not rely on differences in the dielectric properties of the particles and therefore shares the robustness and reliability of conventional methods based on arrays of solid obstacles.

[0062] The field-induced obstacles can be implemented by equipping the device with micropatterned electrodes, which result in a high electric field at specific locations. As explained above, cells or microsphere in highly conductive media experience negative DEP, and will therefore be repelled by these regions.

Figs 2, 3, 4 field pattern between electrodes

[0063] Figure 2 shows for example the electric field created by two round electrodes of 10 μm diameter in a 10 μm high microfluidic channel 40 filled with PBS, by assuming a potential difference of 2V between top and bottom electrodes. In practice, the top electrode 50 and bottom electrode 60 will be energized with high frequency AC voltage signals with a phase difference of 180° to prevent electrolysis and gas formation. As can be seen, the magnitude of the electric field is high in the volume enclosed by the two electrodes and is negligibly small outside of this region. Shown schematically in this figure is AC drive circuitry 70 for generating an AC field by applying AC currents of different phases to the electrodes in accordance with conventional DEP systems, e.g. driving circuits able to generate variable frequencies for nDEP or pDEP, and suitable control circuitry to maintain or reconfigure a desired field pattern. In some embodiments of the present invention the drive circuitry can include a variable frequency and phase AC field generator. The connections to the electrodes from the variable frequency and phase AC field generator are shown schematically. Such parts that are not shown in detail can be implemented using conventional hardware or circuitry as would be apparent to those skilled in the art, and so need not be described in more detail. They can be integrated on the same chip or located elsewhere.

[0064] Alternatively, devices can be made with patterned electrodes only on one of the two substrates (top or bottom) and an unstructured electrode on the other substrate (Fig. 3). The substrates are on either side of the channel having the fluid. Although the high-field region is less confined than in the previous case, the field is still seen to decrease strongly in the radial direction. The unstructured electrode can be either a metallic film or an optically transparent layer of indium tin oxide (ITO) for example.

[0065] High-field regions can also be created by using exclusively in-plane electrodes, as illustrated in Fig. 4. In this case there are two elongate electrodes stretching across the channel underneath the channel, with a gap between the electrodes. The field pattern forms an arch between the electrodes and so extends out of the plane of the electrodes into the channel or part of the channel.

[0066] In addition to the electrode shapes illustrated in the figures, other shapes are possible (e.g., squares, wires, serpentine electrodes, etc.). Microstructured conductive surfaces can be obtained either by lithographically patterning metallic layers into electrodes or by lithographically defining apertures in an insulating layer deposited on top of an unstructured metal layer. In case of a high number of electrodes, active matrix electronics (for example large area electronics based on LTPS technology) could be used to drive the electrodes.

[0067] By arranging electrode as the ones described above in an array, one can fabricate devices that selectively filter or trap micrometer-sized particles in a flow along the channel. The flow can be forced by a pump, or by gravity or by centrifugal force for example. In some cases the fluid will cause the flow of particles, in other cases, the particles can flow through a static fluid. The sieve size is approximately defined by the gap between the electrodes. A straightforward way to change the sieve size and, consequently, the size of the particles to be trapped is to selectively turn on or off electrodes within the array.

Figs 5, 6, 7, 8, reconfiguring the field pattern to alter the size of gap between obstacles

[0068] Figure 5 shows a representation of a field pattern with small gaps, produced by a line of electrodes. Figure 6 shows the field pattern after some of the electrodes are switched off, to increase the size of the gaps. In contrast to

devices based on solid obstacles, devices based on the disclosed method can block or filter particles as long as the negative DEP force experienced by the particles is higher than the drag force caused by the fluid flow. Therefore, the sieve size can be continuously tuned by adjusting the voltage applied on the electrodes or by varying the strength of the fluid flow.

[0069] In addition to changing the sieve size, the method allows reconfiguring the arrangement of the electrodes in an array. For example, rows of obstacles with different gap sizes can be placed in succession. The rows can be perpendicular to the flow direction (Fig. 7), in which case particles of different sizes would be trapped in the different sections of the device. After that, the electrodes can be turned-off (e.g., starting from the bottom) to release the trapped particles selectively. Alternatively, the activated electrode configuration can be changed in order do tilt the filtering rows with respect to the flow direction (as shown in Fig. 8). This would enable, for example, sorting particles of different sizes into separate micro fluidic exit channels.

[0070] Size-sorting of particles can also be obtained with the method described in Huang et al, i.e., by using rows of obstacles with the same gap and shifting each successive row horizontally with respect to the previous one by a fraction of the gap between the obstacles.

Figs 9, 10, reconfigurable array of electrodes

[0071] A particularly attractive way to fabricate a large reconfigurable array is based on active matrix electronics (e.g. CMOS or LTPS). This would allow making devices with two dimensional arrays of microlocations, each consisting of a surface electrode, embedded sensors and logic (Fig. 9). The array can be present on both top and bottom substrates or only on one substrate, in which case the other substrate carries an unpatterned counter electrode (metal or ITO) (Fig. 10).

[0072] Each electrode in the array can be activated individually. When an electrode is energized with an AC voltage signal with a 180° phase difference with respect to the corresponding counter electrode, a high-field region (i.e., an obstacle) is created. Low-field regions can be created by grounding the electrodes or by driving them with in-phase voltage signals. Depending on the relative size of the electrodes in the array compared to the size of the required obstacles, a pillar can be obtained by activating a single electrode or by energizing multiple adjacent "pixels". The last option would allow creating larger obstacles of arbitrary shape. An array based on CMOS technology has been used by the company Silicon Biosystems (see www.siliconbiosystems.com) to trap, levitate and transport individual cells across the array. But there is no suggestion of using the technology for reconfigurable microfluidic filters.

[0073] The method described above according to embodiments of the present invention may be implemented in a processing system, e.g. a micorcontroller. One configuration of such a processing system includes at least one customisable or programmable processor coupled to a memory subsystem that includes at least one form of memory, e.g., RAM, ROM, and so forth. It is to be noted that the processor or processors may be a general purpose, or a special purpose processor, and may be for inclusion in a device, e.g., a chip that has other components that perform other functions. Thus, one or more aspects of the method according to embodiments of the present invention can be implemented in digital electronic circuitry, or in computer hardware, firmware, software, or in combinations of them. The processing system may include a storage subsystem that has at least one disk drive and/or CD-ROM drive and/or DVD drive and/or solid state memory. In some implementations, a display system, a keyboard, and a pointing device may be included as part of a user interface subsystem to provide for a user to manually input information, such as parameter values. Ports for inputting and outputting data, e.g. desired or obtained flow rate, also may be included. More elements such as network connections, interfaces to various devices, and so forth, may be included. The various elements of the processing system may be coupled in various ways, including via a bus subsystem, e.g. for simplicity as a single bus, but will be understood to those in the art to include a system of at least one bus. The memory of the memory subsystem may at some time hold part or all of a set of instructions that when executed on the processing system implement the steps of the method embodiments described herein.

[0074] The present invention also includes a computer program product which provides the functionality of any of the methods according to the present invention when executed on a computing device. Such computer program product can be tangibly embodied in a carrier medium carrying machine-readable code for execution by a programmable processor. The present invention thus relates to a carrier medium carrying a computer program product that, when executed on computing means, provides instructions for executing any of the methods as described above. The term "carrier medium" refers to any medium that participates in providing instructions to a processor for execution. Such a medium may take many forms, including but not limited to, non-volatile media, and transmission media. Non-volatile media includes, for example, optical or magnetic disks, such as a storage device which is part of mass storage. Common forms of computer readable media include, a CD-ROM, a DVD, a flexible disk or floppy disk, a tape, a memory chip or cartridge or any other medium from which a computer can read. Various forms of computer readable media may be involved in carrying one or more sequences of one or more instructions to a processor for execution. The computer program product can also be transmitted via a carrier wave in a network, such as a LAN, a WAN or the Internet. Transmission media can

take the form of acoustic or light waves, such as those generated during radio wave and infrared data communications. Transmission media include coaxial cables, copper wire and fibre optics, including the wires that comprise a bus within a computer.

[0075] Control circuitry to control the individual electrodes according to a desired sequence, can be implemented using conventional digital logic circuits in the form of integrated circuitry, ASICs, microprocessors and so on. Such circuitry can execute instructions coded in conventional software languages. The control can be made dependent on external conditions, or inputs from local sensors as desired.

[0076] The embodiments described above can be applied essentially to any application involving arrays of obstacles to selectively filter or block micrometer-sized particles such as cells or dielectric beads in a liquid flow. It can also be applied to define microreactors for bead-based processes, as described above. The method is expected to have important applications for sample preparation and filtration, isolation of cells for example from blood, extraction and purification of an analyte (e.g., nucleic acids or proteins) and size-sorting of cells or functionalized microspheres. In particular the filtering or trapping of micro-carrier beads loaded with cells.

Claims

1. A filter for filtering particles in a fluid, the filter having a channel (40) for the fluid, and electrodes (50, 60) arranged to create an electric field pattern in the fluid in the channel, to repel the particles at locations spaced apart in the channel so as to selectively block particles above a given size, and allow particles of another size to pass.

2. The filter of claim 1 arranged to use dielectrophoresis to repel or attract the particles.

3. The filter of claim 1 or 2, further comprising drive circuitry (70) to drive the electrodes to create the electric field pattern, the drive circuitry being arranged to automatically reduce the electric field to allow blocked particles to pass.

4. The filter of claim 3, the drive circuitry being arranged to generate an electric field pattern for negative dielectrophoresis.

5. The filter of any of the claims 3 or 4, the drive circuitry being arranged to drive the electrodes with an AC signal, with substantially 180 degree phase difference between opposing ones of the electrodes.

6. The filter of any preceding claim, the electrodes being arranged on opposing sides of channel for the fluid.

7. The filter of any preceding claim, the channel having at least one branch, arranged so as to provide different exit paths for different sized particles.

8. The filter of any of the claims 2 to 7, the drive circuitry being arranged to reconfigure the filter by changing the pattern of the electric field in the channel so as to change a size of particles which are blocked or passed.

9. The filter of claim 8, the electrodes being arranged in a line across the channel, and the reconfiguring involving altering the relative drive levels of different ones of the electrodes in the line.

10. The filter of claim 8, the electrodes comprising at least one two dimensional array of separately drivable electrodes on at least one side of the channel, and the reconfiguring involving altering the relative drive levels of different ones of the electrodes in the line.

11. The filter of claim 10, the drive circuitry for the electrodes in the two dimensional array being integrated together with the respective electrodes.

12. The filter of any preceding claim, the electrodes and drive circuitry being arranged to generate the field pattern in the form of a cage of obstacles to trap the particles of the given size.

13. An integrated micro fluidic device having integrated fluid handling components, and the filter of any preceding claim.

14. A method of using a filter to filter particles in a fluid, the filter having a channel (40) for the fluid, and electrodes (50,60), the method having the steps of introducing the particles to the filter, and creating an lectric field pattern to repel the particles at locations spaced apart in the channel so as to selectively block particles of a given size, and

allow particles of another size to pass.

15. The method of claim 14 used for any one or more of the following: isolation of cells from blood or other substance, sorting of cells, or for filtering micro carrier beads loaded with cells.

16. The method of claim 14 or 15, using dielectrophoresis to repel or attract the particles.

17. The method of any of claims 14 to 16 having the step of reducing the electric field to allow blocked particles to pass.

18. The method of any of claims 14 to 17 having the step of reconfiguring the filter by changing the pattern of the electric field in the channel so as to change a size of particles which are blocked or passed.

19. The method of any of claim 18, the electrodes comprising at least one two dimensional array of separately drivable electrodes on at least one side of the channel and the reconfiguring step involving altering the relative drive levels of different ones of the electrodes in the array.

20. The method of any of claims 14 to 19, having the step of generating the electric field pattern to form a ring of obstacles to trap the particles of the given size.

21. Computer program product stored on a computer readable medium for controlling the driving of different ones of the electrodes to generate the electric field pattern for use in the methods of any of claims 14 to 20.

FIG 1

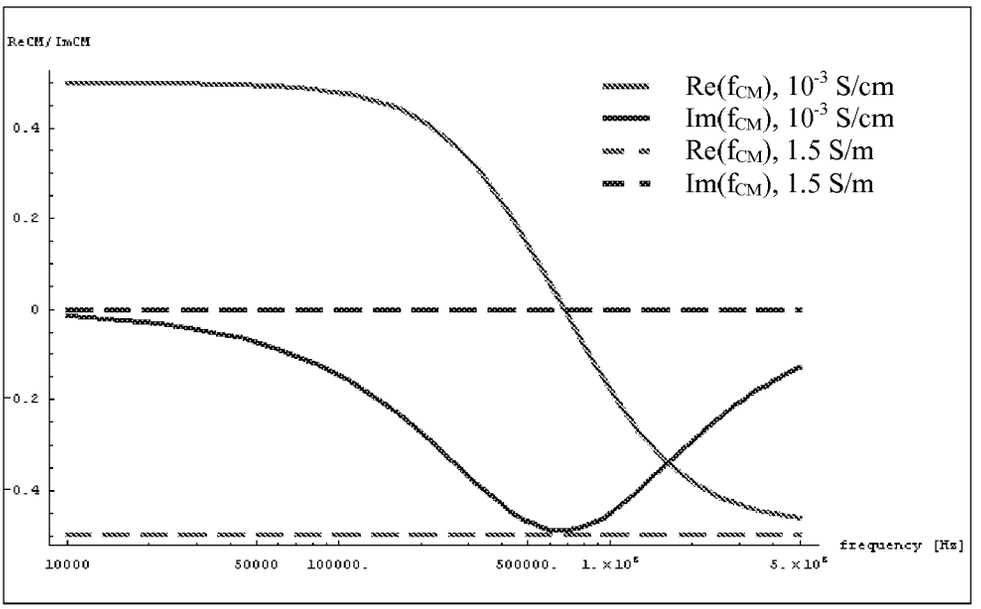
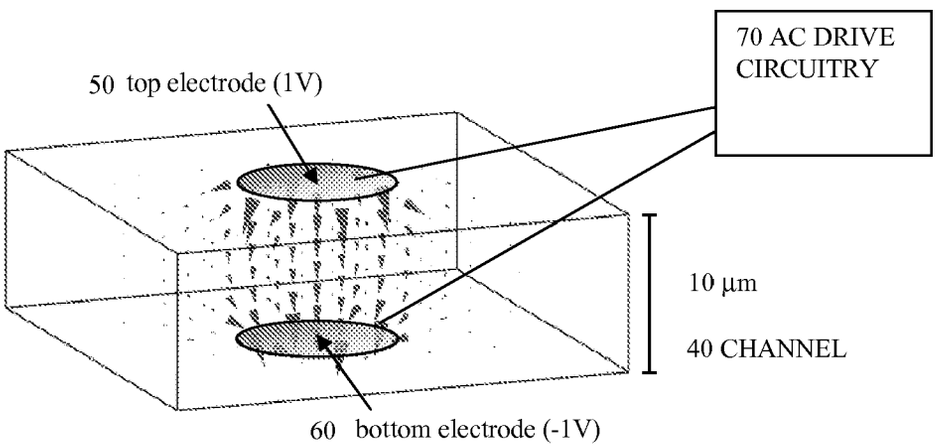

ReCM/ImCM
0.4
0.2
0
-0.2
-0.4
Re(fCM), 10-3 S/cm
Im(fCM), 10-3 S/cm
Re(fCM), 1.5 S/m
Im(fCM), 1.5 S/m
frequency [Hz]
10000
50000
100000.
500000.
1.×10^6
5.×10^6

FIG 2


70 AC DRIVE CIRCUITRY
50 top electrode (1V)
10 µm
40 CHANNEL
60 bottom electrode (-1V)

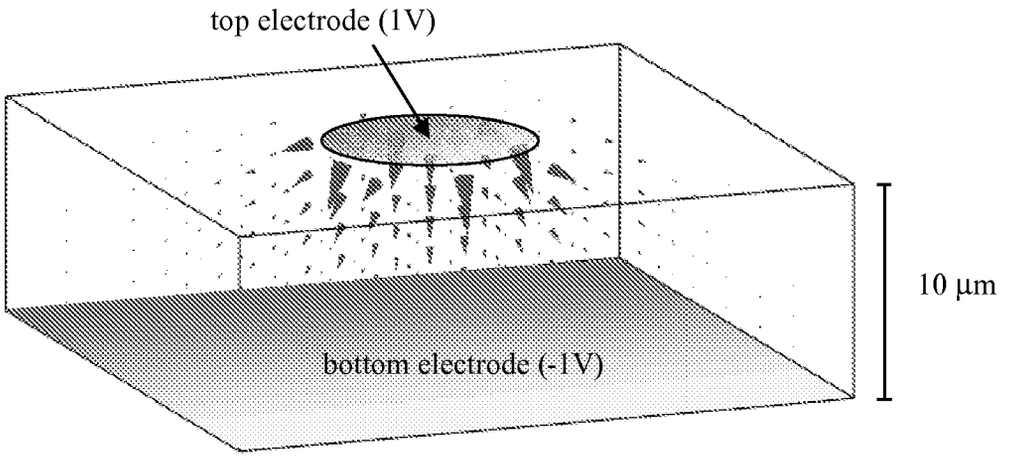

top electrode (1V)
10 µm
bottom electrode (-1V)

FIG 3

FIG 4

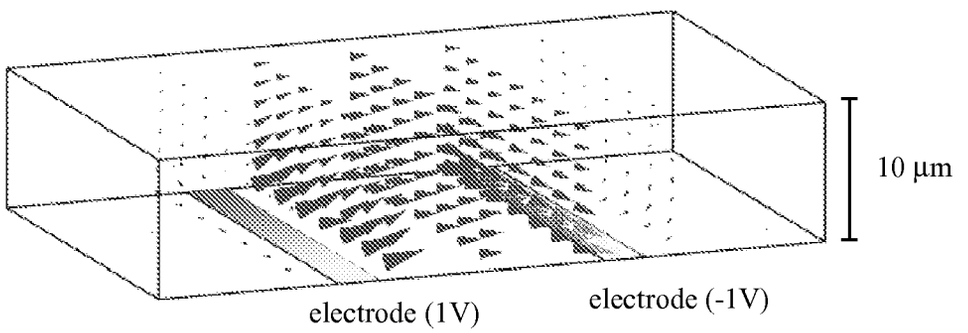

10 µm
electrode (1V)
electrode (-1V)

FIG 5

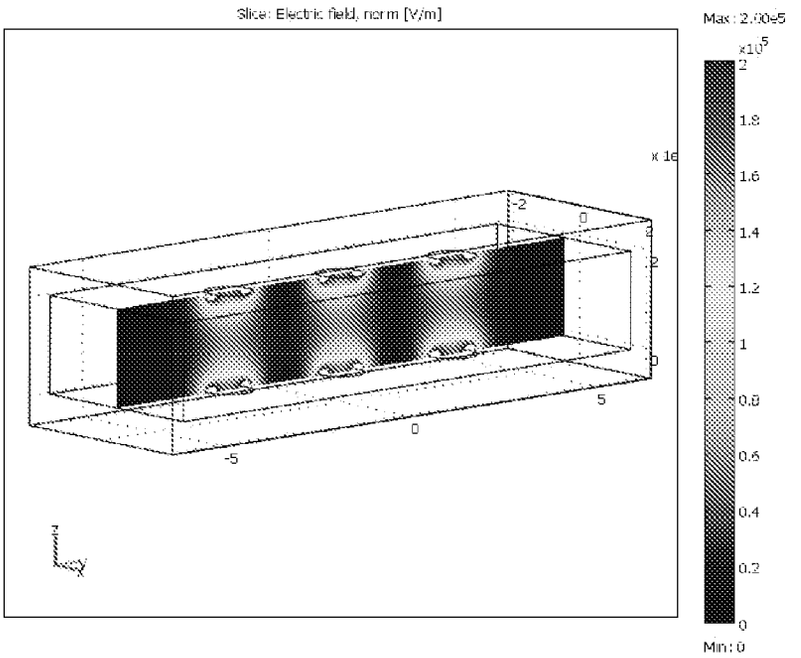

Slice: Electric field, norm [V/m]
Max : 2.00e5
x10^5
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Min : 0
x 1e
-2
0
5
0
-5

FIG 6

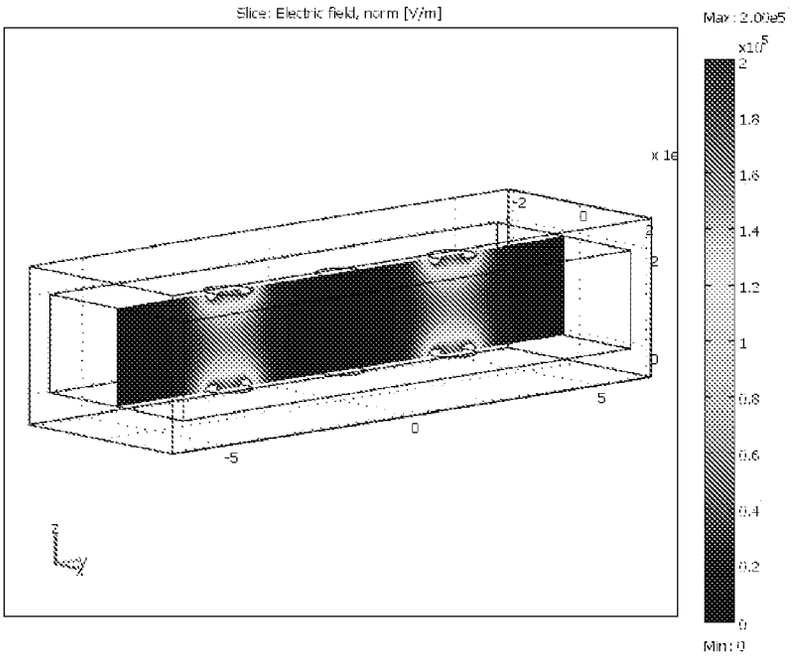

Slice: Electric field, norm [V/m]
Max : 2.00e5
x10^5
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Min : 0
x 1e
-2
0
5
0
-5

FIG 7

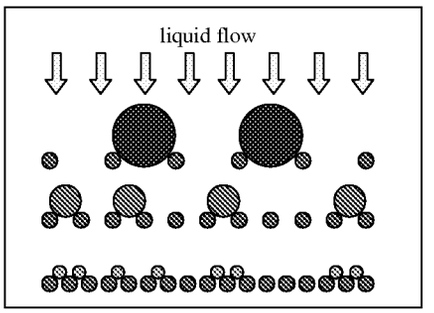


FIG 8

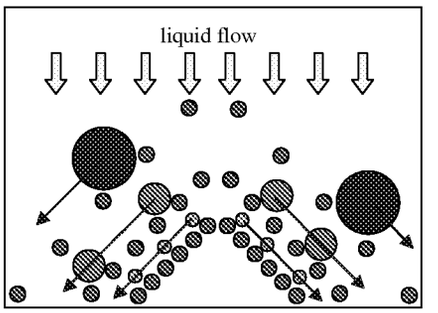


FIG 9

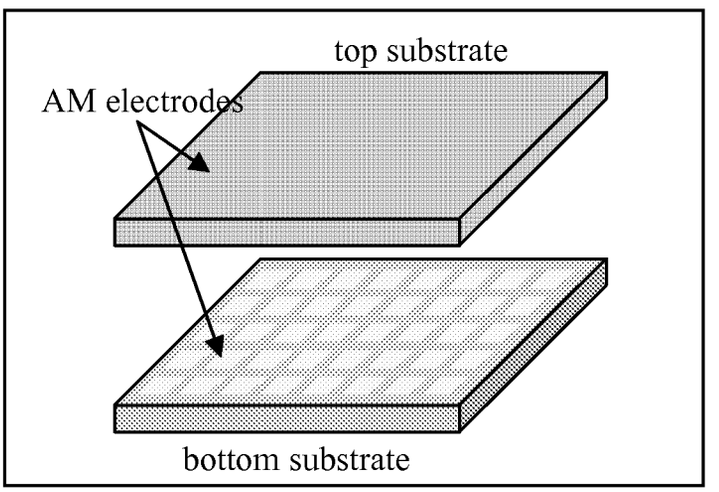
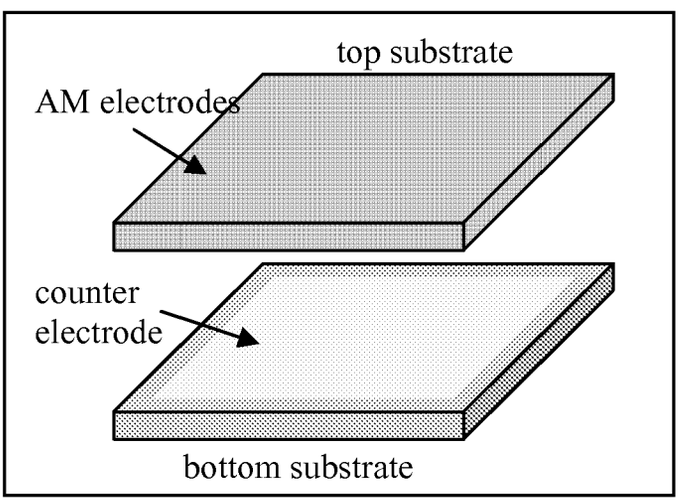


FIG 10



European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 07 11 9287

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
A,D	US 5 814 200 A (PETHIG RONALD [GB] ET AL) 29 September 1998 (1998-09-29) * claims 1,17 *	1-21	INV. G01N27/26 B03C5/02
A	WO 95/08640 A (DU PONT [US]) 30 March 1995 (1995-03-30) * page 3, line 1 - line 24 *	1-21	
A	US 2004/226819 A1 (TALARY MARK STUART [GB] ET AL) 18 November 2004 (2004-11-18) * claim 1 *	1-21	
A	WO 03/001193 A (SANDIA NAT LAB [US]; CUMMINGS ERIC B [US]) 3 January 2003 (2003-01-03) * claims 1,9 *	1-21	
A	US 2007/125941 A1 (LEE ABRAHAM P [US] ET AL) 7 June 2007 (2007-06-07) * paragraph [0038] - paragraph [0043]; figures 2-4 *	1-21	TECHNICAL FIELDS SEARCHED (IPC)
A	US 2004/065599 A1 (LAL AMIT [US] ET AL) 8 April 2004 (2004-04-08) * claim 1 *	1-21	B01L G01N B03C C12M

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
Berlin	14 March 2008	Clement, Jean-Paul

CATEGORY OF CITED DOCUMENTS

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14-03-2008

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5814200 A	29-09-1998	AT 163576 T	15-03-1998
		AU 6382894 A	24-10-1994
		CA 2159342 A1	13-10-1994
		DE 69408830 D1	09-04-1998
		DE 69408830 T2	18-06-1998
		EP 0691891 A1	17-01-1996
		WO 9422583 A1	13-10-1994
		JP 3586279 B2	10-11-2004
		JP 8508205 T	03-09-1996
		ZA 9402320 A	02-10-1995
WO 9508640 A	30-03-1995	EP 0720658 A1	10-07-1996
		US 5578460 A	26-11-1996
US 2004226819 A1	18-11-2004	EP 1623014 A2	08-02-2006
		JP 2007504952 T	08-03-2007
		WO 2005007807 A2	27-01-2005
WO 03001193 A	03-01-2003	US 2003010637 A1	16-01-2003
		US 2004026250 A1	12-02-2004
US 2007125941 A1	07-06-2007	NONE	
US 2004065599 A1	08-04-2004	NONE	

EPO FORM P0459

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

REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- US 5814200 A **[0049]**

Non-patent literature cited in the description

- **N. J. PANARO et al.** *Biomolecular Engineering,* 2005, vol. 21, 157-162 **[0005]**
- **H. SATO et al.** *Sensors and Actuators A,* 2004, vol. 111, 87-92 **[0006]**
- **L. R. HUANG et al.** *Science,* 2004, vol. 304, 987-990 **[0007]**
- **H. MORGAN ; N. GREEN.** AC Electrokinetics: colloids and nanoparticles. Research Studies Press, 2002 **[0055]**