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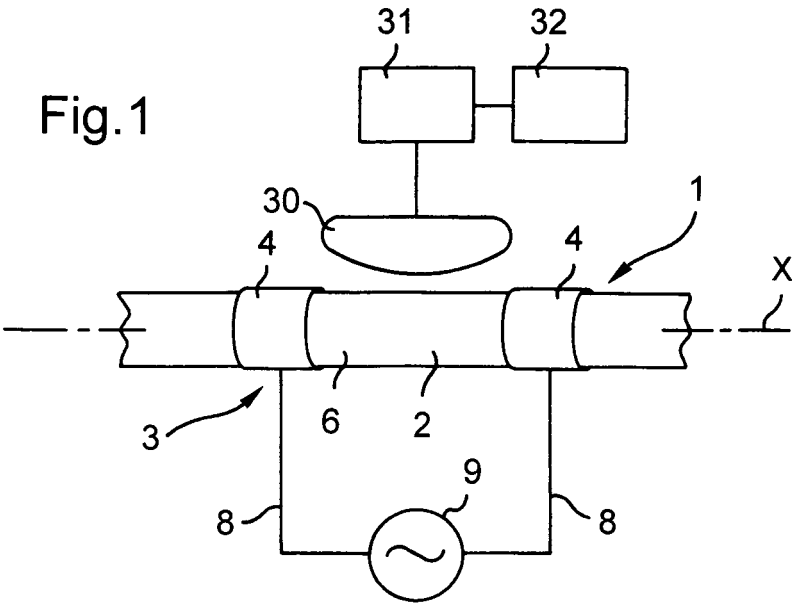
(54) Microfluidic device using a collinear electric field

(57) A microfluidic device (1) for deforming, in particular splitting, at least one packet, or displacing at least two packets towards each other, in particular for collapsing said packets, said device comprising:

- a microchannel (2) having an axis (X),
- packet manipulation means.

Fig.1



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Description

[0001] The present invention relates to a device for manipulation of packets in microchannels.

[0002] As used herein, « packet » refers to compartmentalized matter and may refer to a fluid packet, an encapsulated packet and/or a solid packet.

[0003] A fluid packet refers to one or more packets of liquids or gases. A fluid packet may refer to a droplet or bubble of a liquid or gas. A fluid packet may refer to a droplet of water, a droplet of reagent or sample, a droplet of solvent, a droplet of solution, a particle suspension or cell suspension, a droplet of an intermediate product, a droplet of a final reaction product or a droplet of any material. An example of a fluid packet is a droplet of aqueous solution suspended in oil. In a preferred embodiment, a fluid packet refers to a droplet of water or a droplet of solution.

[0004] An encapsulated packet refers to a packet enclosed by a layer of material. The surface of an encapsulated packet may be coated with a reagent, a sample, a particle or cell, an intermediate product, a final reaction product, or any material. An example of an encapsulated packet is a lipid vesicle containing an aqueous solution of reagent suspended in water.

[0005] A packet may contain for instance a vesicle or other microcapsule of liquid or gas that may contain a reagent, a sample, a particle, a dead cell or alive cell, an intermediate product, a final reaction product, or any material.

[0006] A solid packet refers to a solid material, for example biological material, that may contain, or be covered with, a reagent, a sample, a particle or cell, an intermediate product, a final reaction product, or any material. An example of a solid packet is a latex microsphere with reagent bound to its surface suspended in an aqueous solution. A packet may contain a crystal, a polycrystalline material or a vitreous material.

[0007] Packets may be made to vary greatly in size and shape, and may have a maximum dimension between about 100 nm and about 1 cm.

[0008] Droplet systems, typically consisting of water droplets in oil or a fluorinated solvent, have received much attention in microfluidics as a method for producing precise emulsions, as discrete microreactors for polymerase chain reaction (PCR), for the measurement of fast kinetics, and for the dispersion-free transport and manipulation of sample aliquots. Considerable efforts have thus been developed in the last years to create and/or manipulate microdroplets. Some devices use hydrophobic forces, by moving such droplets in microchannel combining some hydrophilic and some hydrophobic portions. For instance, US patent 6 130 098 discloses a method for moving microdroplets, comprising:

- providing a microdroplet transport channel having one or more hydrophobic regions and in communication with a gas source,
- introducing liquid into said channel under conditions such as the liquid stops at one of the hydrophobic regions,
- separating a microdroplet by increasing the pressure applied by the gas source so as to let such droplet moves over the hydrophobic region.

[0009] This approach imposes that different droplets be in contact with the same solid surface, and is thus prone to contamination.

[0010] Manipulation of droplets on planar arrays of electrodes by electrowetting has also become very popular, since it allows one to address droplets to diverse locations and along complex and programmable paths. For instance, US patent 6 294 063 discloses an apparatus for programmably manipulating a plurality of packets, such packets optionally being droplets, said apparatus comprising a reaction surface configured to provide an interaction site for such packets, an inlet port, means to generate manipulation forces upon said packets, the forces being capable of programmably moving said packets about said reaction surface along arbitrarily chosen paths, and a position sensor.

[0011] US patent 6 565 727 also discloses a device for manipulating a droplet of a polar liquid, comprising an upper and lower surface, defining between them a gap, said upper surface comprising a plurality of interdigitated electrodes, and said lower surface comprising a common counterelectrode. The device further comprises insulating layers between said electrodes and said gap, and a non-polar liquid positioned in the gap. In this device, a droplet can be maintained on top of a first electrode on the upper surface, by applying a potential between said electrode and the counterelectrode on the lower surface, making the upper surface wetting for the droplet in the vicinity of said first electrode. Then, the droplet can be moved to a second electrode on the upper surface interdigitated with said first electrode, by suppressing the potential difference between the first electrode and the counterelectrode, and applying a potential difference between the second electrode and the counterelectrode to make said second electrode wetting to the fluid.

[0012] Electrowetting can also be used to mix two different droplets, as described e.g. in M. Washizu, *IEEE Trans. Ind. Appl.*, 34, 732-737 (1998). Mixing of droplets containing e.g. two reagents or a sample and a reagent is a key technological step for developing microfluidic integrated systems or "lab-on-chips".

[0013] The format of a planar array of electrodes required by electrowetting, however, has severe drawbacks. The fabrication of the array of electrodes is complex, and becomes extremely expensive and technically demanding for surfaces exceeding a few square cm. Therefore, transporting droplets on large distances, e.g. more than 10 cm, is impractical. Also, for liquid droplets the surface should be kept horizontal and relatively vibration free, to avoid unwanted motion of droplets under the action of gravity or acoustic waves. Droplet manipu-

lation in planar format may also be limited by droplet evaporation, the latter being a serious hindrance in quantitative biochemistry applications, since reaction yields are highly sensitive to concentration. Electrowetting may further introduce surface contamination. Another limitation is that electrowetting can only work with liquids, so that it cannot be used to transport solid objects.

[0014] Dielectrophoresis is another way of transporting and mixing droplets or solid objects such as cells or latex particles. For instance, Schwartz *et al.* in Lab Chip, 4, 11-17 (2004) discloses a programmable fluid processor, in which droplets can be moved and mixed on top of an array of electrodes, by energizing sequentially electrodes in the array. This method, however, also requires a complex array of electrodes on a planar surface, and thus shares many of the drawbacks of electrowetting. In another instance, Velev *et al.*, Nature, 426, 515-516 (2003) describes a process for moving and mixing droplets which are floating on a layer of fluorinated oil, where the oil is in contact with a pattern of electrodes. This eliminates the contamination problems inherent in electrowetting, but still requires fabricating a complex array of electrodes.

[0015] Transporting and mixing droplets in an elongated microchannel, or in a network of connected microchannels is more robust to the above problems. For instance, it avoids evaporation, and allows transport on long distances by simple hydrodynamic mobilization of a carrier fluid surrounding the droplets. Droplets can thus be transported in capillaries several meters long, and used as microreactors, as disclosed e.g. in Curcio and Roeraade, Anal. Chem., 75, 1-7 (2003). When interaction with the walls is well controlled, all droplets move at the same velocity, and very stable trains are achieved.

[0016] It was also proposed to manipulate solid particles or cells in microchannels by dielectrophoresis. Dielectrophoresis uses a force exerted on a particle with a dielectric constant different from that of the surrounding medium in a gradient of electric field. Different types of arrangements were used to date for the application of dielectophoresis in microchannels. In a first one, an array of closely spaced interdigitated electrodes locally creates lines of high field gradient in which particles are attracted. Optionally, these lines can be shifted in time by alternately energizing different series of electrodes, said method being called "*travelling wave dielectrophoresis* ". For instance, Schnelle et al., *Electrophoresis,* 21, 66-73 (2000) discloses a method for sorting particles, in which they are deflected by travelling wave dielectrophoresis between a multiplicity of electrodes in an interdigitated arrangement, and energized sequentially with a four phase alternating electric signal. By using pairs of electrodes of different shapes facing each other across the microchannel, and applying a potential difference between them, it is also possible to create different kinds of dielectrophoretic traps, cages or deflecting electrodes, as described e.g. in Durr *et al.*, *Electrophoresis,* 24, 722-731 (2003).

[0017] These dielectrophoretic devices present some advantages upon planar systems. In particular, they are more robust to tilting or vibration. However, they still require complex microfabrication, and are expensive to fabricate.

[0018] Another key hurdle in the development of microchannel droplet systems, especially for microreactor applications, is the mixing of samples or reagents from different sources. For this, one needs to coalesce two droplets, but Laplace and hydrodynamic forces tend to make this coalescence difficult. When arriving simultaneously at a T-intersection, one drop simply follows the second one into the T without coalescing. Coalescence can be forced by contact charging the droplets, but this could be a major source of contamination in PCR and other biological systems. Once introduced into a channel, a smaller droplet trailing a larger one may eventually coalesce with it, since the smaller droplet moves with a higher average velocity. However, this is not a rationale strategy in microfluidics, since film drainage between the droplets is very slow, leading to coalescence distances between 30-100 tube diameters (Olbricht and Kung, *J. Colloid Interface Sci.,* 120, 229-244 (1987)). Moreover, under certain conditions (relative droplet sizes, viscosities, etc.) no coalescence is achieved, and the coalescence time or position are not very reproducible.

[0019] Consequently, there exists a strong need for a device and method providing reproducible, contamination-free droplet manipulation, in particular coalescence, in microchannels or closed microfluidic systems.

[0020] An object of the present invention among others is to provide such device and method.

[0021] The present invention relates to a microfluidic device for deforming, in particular splitting, at least one packet, or displacing at least two packets towards each other, in particular for collapsing, said device comprising:

- a microchannel having an axis,
- packet manipulation means comprising at least one of:

  - a generator unit, and an electrode assembly coupled to the generator unit and configured for creating inside at least one portion of the microchannel an electric field which is substantially collinear to the axis (X) of the microchannel, wherein the generator unit is capable of generating the electric field with such an amplitude and frequency that the electric field causes the at least one packet to deform, or the at least two packets to displace towards each other in the microchannel,
  - at least one side channel with a first end in connection with a portion of said microchannel and a second end in connection with a delivery system suitable for delivering a solution, with in particular a surfactant, able to alter the interfacial tension between said at least two packets or said

at least one packet and the environment thereof, said delivery system being configured to deliver said solution into said microchannel at least during the passage of said packet(s) in said portion of the microchannel.

[0022] In other words, the manipulation of the packet (s) with the device according to the present invention may be carried out by generating an appropriate electric field, or by modifying the surface tension of a liquid packet, or by combining both techniques.

[0023] The interfacial tension may be modified, preferably decreased, by a factor of at least 20%, and more preferably 50%.

[0024] When two packets are introduced in the portion of the microchannel in which the electric field is applied to cause two packets to collapse, dipoles are created by the electric field in the packets, said dipoles being oriented substantially along the axis of the microchannel such that the packets attract each other and collapse.

[0025] The expression "substantially collinear" means that the average of the direction of the electric field makes with the axis of the microchannel an angle smaller than 45°, for example smaller than 30°, preferably smaller than 20°, and more preferably smaller than 10° or 5°.

[0026] The invention may be used for inducing the collapse of two packets, and for example the coalescence of two droplets inside the microchannel.

[0027] The general physical phenomenon called "electrocoalescence" is disclosed in P. Atten, J. Electrostat. 30, 259 (1993). As electrophoretic forces, and in contrast with dielectrophoresis, electrocoalescence does not require a field gradient.

[0028] When the packet is a cell, or contains several cells, the invention may be used to induce electroporation of such cell or multiplicity of cells.

[0029] The invention may also be used to split a packet into several packets of smaller size, and for example to extract from a droplet one or several droplets of smaller size.

[0030] In the present invention, "microchannel" means a channel that may have a surface/volume ratio substantially greater than 1 mm^{-1}, preferably greater than 4 mm^{-1}, for example greater than 10 mm^{-1}, possibly greater than 1 μm^{-1}. Microchannels also encompass nanochannels.

[0031] The microchannel is preferably elongated, i.e. the dimension along its axis is larger by a factor of 3, preferably by a factor of 10, for example by a factor of 100 or 1000, than along any other direction perpendicular to said axis.

[0032] The axis of the microchannel may be rectilinear or not.

[0033] The microchannel may have a cross-section which is constant or not. The section may be for example circular, ellipsoidal, rectangular, square or with a bowl shape.

[0034] In the present invention, "thickness" means the smallest inner distance in cross section between two opposite sides of the microchannel. As a matter of example, for a cylindrical microchannel having a circular cross section, the thickness is the diameter. For a slit-like microchannel having a rectangular cross section, the thickness is the length of the small side of the rectangle.

[0035] The thickness of the microchannel can take any value between a few nm and a few mm. Preferably, the thickness is comprised between 1 μm and 1mm. Still preferably, the length of the microchannel along its axis, may be chosen to be at least 10 times larger than the thickness. The microchannel may have a length chosen between 10 mm and several meters, for example between 1 cm and 50 cm.

[0036] Preferably, the portion of the microchannel in which the electric field is substantially collinear to the axis of the microchannel has a length along said axis at least as large as the thickness of the microchannel, and smaller than the total length of the microchannel. In a preferred embodiment of the invention, the length of said portion of the microchannel is comprised between about 1 and about 100 times the thickness of said portion, and preferably between about 1 and about 10 times the thickness of said portion.

[0037] The microchannel may be rigid or flexible and comprises for example a tube made of a flexible non-electrically-conducting material. In a variant, the microchannel is made of fused silica glass, polydimethylsiloxane, polymethylmethacrylate, or any kind of elastomer or plastic.

[0038] In an exemplary embodiment of the invention, the microchannel has at least one inlet port, and/or at least one outlet port. Optionally, at least one of said ports can be connected to one or several reservoirs, to one or several pumps, to one or several detectors or sensors or to one or several sampling devices.

[0039] The microchannel may be part of a network of connected microchannels.

[0040] In a preferred embodiment of the invention, said electrode assembly is electrically insulated from an inside surface of the microchannel, for example by an insulating material. The insulating material may have a thickness of at least 1 nm, for example at least 10 nm, preferably at least 100 nm, most preferably of at least 1 μm, for example up to several tens or hundreds μm. Typically, thicker insulating layers may be preferred for larger microchannels. This insulating material may be made out of for example polymeric material, e.g. for example polyethylene, polyimide, epoxy, Teflon®, Parylene®, PMMA, polystyrene, polyethylene terephtalate, fluoropolymer, polyester, cyclic olefin copolymer, PDMS, non-conducting oxide such as, for example, glass, silicon dioxide, diamond, non-conductive ceramics.

[0041] Preferably, the electrode assembly comprises at least two electrodes axially spaced along the axis of the microchannel by a distance long enough for the electric field between the electrodes to be substantially collinear to the axis of the microchannel. Each electrode may be symmetric relative to the axis of the microchan-

nel. Advantageously, at least one of said electrodes comprises at least two equipotential portions facing each other across the microchannel. At least one of said electrodes may be monolithic having for example a cylindrical surface surrounding the microchannel. In a variant, at least one of said electrodes is composite, i.e. made out of a plurality of pieces, comprising for example at least two substantially parallel equipotential plates sandwiching the microchannel. The electrodes may also have a shape other than those described above.

[0042] This is different from the prior art as disclosed in patent application FR 2 794 039 or in Paik *et al.*, Lab Chip, 3, 28-33 (2003) wherein manipulation of the droplets is obtained by an opposite configuration, i.e. applying a potential difference between two planar electrodes facing each other across the chamber in which the droplet to be manipulated is contained.

[0043] Preferably, the electrodes are spaced by a gap having a length that is greater than the thickness of the microchannel, preferably greater than twice the thickness.

[0044] The generator unit comprises advantageously at least one of a current and a voltage generator, configured to create a difference of potential between said two electrodes, preferably an alternating potential.

[0045] Advantageously, the electrode assembly is configured so that in at least one cross section of the microchannel the amplitude of the electric field varies less than a factor 10, preferably less than a factor 5, better less than a factor 2, and preferably is substantially uniform, in particular in the gap between the electrodes.

[0046] The electric field generated by the generator unit via the electrode assembly may have any temporal profile, for example continuous, variable or alternating (AC), or a combination of such temporal profiles. For instance, the electric field may be an AC field with variable frequency or root mean squared (RMS) amplitude, or a superposition of continuous and AC components.

[0047] By "AC field", we mean any field periodic in time and with a zero time average. Non limiting examples of AC fields according to the invention are sinusoidal, square or sawtooth AC fields.

[0048] The generator unit is preferably capable of generating an AC electric field with a frequency ranging from about 0.01 Hz to about 1 GHz, preferably from about 1 Hz to about 10 MHz.

[0049] The coalescence of droplets is efficiently achieved, for instance, with frequencies between 100 Hz and 10 kHz. The aliquoting of at least one droplet is preferably achieved at frequencies lower than 50 Hz.

[0050] The generator unit may be configured for delivering a RMS voltage ranging between 1V and 30 kV, preferably between 60 V and 2 kV, depending on the nature of the packet, of the fluid surrounding the packet, of the microchannel, and on the size of the device. The voltage may increase as the size of the device increases. The RMS electric field inside the microchannel in the gap between the electrodes may range for instance between 100 V/cm and 100 kV/cm, and preferably between 500 V/cm and 20 kV/cm.

[0051] The generator unit may be configured to deliver a voltage with at least one of the amplitude and the frequency being time-related variable. For example, the amplitude and/or the frequency of the electric field may be modified as two packets come into close contact.

[0052] Advantageously, the electrode assembly is housed in a support, the latter having two separated support members assembled together via a fixing element, each support member carrying one electrode of the electrode assembly. The support may comprise at least one orifice for receiving the microchannel.

[0053] When the microchannel is connected to a side channel, said side channel may have a cross-section with dimensions comparable to those of the microchannel. Preferably, the cross-section of the side channel is smaller than this of the microchannel. In a variant, the cross-section of the side channel is larger than this of the microchannel.

[0054] The delivery system associated with said side channel may comprise pressure control means or flow control means.

[0055] Preferably, said side channel and said delivery system are configured for delivering in the microchannel a solution containing a surfactant.

[0056] The term "surfactant" designs any species, molecules or combination of molecules capable of modifying the interfacial tension between two fluids. A surfactant may be for instance a tensioactive or an amphiphilic species.

[0057] The surfactant may be chosen to favor the formation of oil-in-water emulsions.

[0058] If the packets are aqueous droplets suspended in a non-aqueous liquid, said surfactants are typically surfactants with a high HLB (Hydrophilic/Lipophilic Balance), for example with HLB values larger than 15. Non-limiting examples of such surfactants are Sodium Dodecyl Sulfate (SDS), oleic acid, and CTAB.

[0059] If the packets are oily droplets in an aqueous surrounding fluid, the solution preferably contains at least one surfactant with a low HLB, for example a HLB lower than 15, and preferably lower than 10.

[0060] Numerous surfactants able to reduce the interfacial tension between an oily phase and a water phase, or to favor droplet coalescence, are recited e.g. in *Emulsions, a fundamental and practical approach*, J. S.Sjöblom Ed, Kluwer, Dordrecht (1992), or in P. Becher, *Emulsions, Theory and Practice,* 2nd Ed, R.E. Krieger Pub. Co, Malabar, Fl (1985).

[0061] In an exemplary embodiment of the invention, for coalescing or splitting two droplets, the device comprising a first side channel connected to the delivery system, the microchannel may be connected to a second side channel, preferably connected in regard or in close vicinity of the first side channel and configured for collecting packets formed by the coalescence or splitting of original packets.

[0062] The microchannel may be made out of a wide variety of homogeneous or composite materials. In contrast with prior art disclosed in patent US 6 294 063, in which packets are manipulated onto a reaction surface configured to provide interaction sites for said packets, the wall of the microchannel according to the present invention may be made of a material, or treated with a material, reducing the risk of interaction of the packets with the wall of the microchannel in the presence of the embedding fluid. There may be no chemical interaction at all between the packets and the microchannel.

[0063] In an exemplary embodiment, the packet being a water-based droplet, the interfacial tension between the droplet and the microchannel wall is made larger than the interfacial tension between the water between the droplet and the surrounding fluid, by treating the microchannel wall and/or by including in the droplet and/or in the fluid additives.

[0064] Numerous ways may be used to increase the surface tension between a water-based liquid and a surface. As an example, one may choose a naturally hydrophobic surface, such as fluorocarbon or polyethylene. One may also treat the surface with hydrophobic materials such as Teflon® AF, silane or fluorosilane.

[0065] The surface tension between water and hydrogenated oil-based fluids can be decreased by the presence of surface-active molecules. Numerous such surface active molecules are known in the art, and we list here only a few as a matter of examples: surfactants such as Pluronics® and Symperionics®, Triton®, Tween®, Span® 80, Tergitol®, Sodium Dodecyl Sulfate(SDS), oleic acid, methyl cellulose, hydroxyethyl and hydroxy propyl cellulose, or Coatex®. If the fluid is fluorinated, then fluorinated surfactants, such as 1H,1H,2H,2H perfluorodecan-1-ol or 1H,1H,2H,2H perfluorooctan-1-ol, are particularly suitable.

[0066] In an exemplary embodiment of the invention, the microchannel is filled with a fluid surrounding at least one packet, said fluid may be any liquid or gaseous fluid, provided it is not miscible in the packet or with the wall of the microchannel.

[0067] The fluid surrounding the packet may be a liquid, for example a water-immiscible organic or inorganic liquid.

[0068] The fluid may be a fluorinated liquid or gas, and the droplet may be an organic or hydroorganic liquid, optionally containing species.

[0069] In an exemplary embodiment of the invention, the packet and the surrounding fluid have different conductivities and/or different dielectric constants. For instance, the packet may have a conductivity higher than the surrounding fluid conductivity.

[0070] In an exemplary embodiment of the invention, the packet is a droplet of a first liquid suspended in an immiscible second liquid, said first liquid being more electrically conductive than the first.

[0071] The droplet may be a water based droplet. Said water based droplet may contain any kind of natural, artificial, organic or inorganic species such as, for example, biological molecules, proteins, protein complexes, enzymes, haptens, antigens, antibodies, aptamers, epitopes, nucleic acids, peptides, polysaccharides, glycopeptides, cells, cell aggregates, drugs, chemicals, latexes, living or dead organisms, viruses, organelles, liposomes, vesicles, micelles, synthetic or natural polymers, nanoparticles, luminescent molecules, quantum dots, chemical reagents, buffers, surfactants, and any combination of such species.

[0072] In another exemplary embodiment of the invention, the packet is a droplet of a water-immiscible liquid, and the surrounding fluid is a water based solution.

[0073] The invention may allow for the manipulation of packets having a size comparable with the section of the microchannel.

[0074] As an exemplary embodiment, the area of the smallest section of said packet is at least equal to one half of the area of the section of the microchannel at the location of a first electrode or at the location of a second electrode, whichever is smaller.

[0075] The packet may be a spherical droplet with a diameter comparable with the diameter of the microchannel, or an elongated droplet spanning the whole section of the microchannel.

[0076] The device according to the present invention may be used to fuse colloids to form a chain, for instance.

[0077] The device may also be used for screening processing.

[0078] The invention also relates to, according to another of its aspects, a method for displacing at least two packets towards each other in a microchannel, in particular in order to collapse the at least two packets, the microchannel having a longitudinal axis, said method comprising:

- introducing the at least two packets in the microchannel,
- generating an electric field within at least one portion of the microchannel, at least when the packets are located within said microchannel portion, said electric field being preferably substantially collinear to the axis of the microchannel in said portion and having an amplitude and a frequency chosen such as to displace the two packets towards each other.

[0079] When the electric field is generated by at least two electrodes axially spaced along the axis of the microchannel, said electrodes being separated by a gap, the method may comprise:

- before generating said electric field, positioning two packets in the gap between the electrodes, said packets being in static equilibrium,
- generating said electric field.

[0080] In a variant, the packets for collapsing may be placed initially in a flowing stream such as to perform an

in-flight operation of collapsing.

[0081] The method may thus comprise:

- positioning two packets in the microchannel, at least one of which being outside the gap between the electrodes,
- displacing the packets towards the gap, for example via a flowing stream in the microchannel,
- generating said electric field at least when the packets are located in the gap.

[0082] In an exemplary embodiment of the invention, at least one of said packets contains biological material, for example a cell or a cytoplasm nucleus. The method for collapsing at least two packets is particularly advantageous when the packets contain a biological membrane.

[0083] Said method may be carried out in order to form hybridoma or to manipulate embryonic founder cells.

[0084] The invention also relates to, according to another of its aspects, a method for displacing at least two packets towards each other in a microchannel, in particular in order to collapse them, or for splitting at least one packet, the microchannel having an axis, said method comprising:

- positioning the at least two packets or the at least one packet in a portion of the microchannel,
- delivering into said portion of the microchannel a solution of a surfactant able to alter the interfacial tension between said at least two packets or said at least one packet and the environment thereof.

[0085] The invention also relates to, according to another of its aspects, a method for splitting at least one packet in a microchannel having a longitudinal axis, said method comprising:

- introducing the at least one packet in the microchannel,
- generating an electric field within at least one portion of the microchannel, at least when the at least one packet is located within said at least one portion, said electric field being preferably substantially collinear to the axis of the microchannel in said portion and having an amplitude and a frequency chosen such as to split the packet.

[0086] The invention also relates to, according to another of its aspects, a method of monitoring the collapsing of at least two packets or splitting of at least one packet, the method comprising:

- causing collapsing or splitting in a microchannel using the microfluidic device as defined above,
- detecting the collapsing or splitting, for example by using a video device or by measuring an electric parameter such as an electric resistance associated for example with at least one substance contained in the microchannel.

[0087] The invention also relates to, according to another of its aspects, a method for displacing at least one packet in a microchannel having an axis, said method comprising:

- introducing at least one packet in the microchannel,
- generating an electric field within at least one portion of the microchannel, at least when the at least one packet is located within said portion, said electric field being preferably collinear to the axis of the microchannel, such as to displace the packet along the microchannel.

[0088] Said electric field may be continuous.

[0089] The operation of displacing at least one packet in the microchannel may be performed independently from an operation of collapsing or splitting.

[0090] In a variant, said operation of displacing at least one packet in the microchannel may be carried out in order to position appropriately said at least one packet in the microchannel before performing the operation of collapsing or splitting.

[0091] The invention may be better understood on reading the following detailed description of non-limiting embodiments, and on examining the accompanying drawings, in which:

- Figure 1 is a diagrammatic partial view of a microfluidic device according to the invention,
- Figure 2 is a diagrammatic view in cross section of the device of Figure 1,
- Figure 3 is a diagrammatic perspective view of a support member of the device of Figure 2,
- Figures 4A-4C and 5A-5C illustrate diagrammatically respectively three steps of two coalescence operations according to the invention,
- Figures 6A-6C illustrate diagrammatically and partially three steps of the aliquoting of a droplet according to the invention,
- Figure 7 is a diagrammatic view of an electric field distribution in a portion of the microchannel,
- Figure 8 is a diagrammatic partial view of a device according to a variant of the invention, and
- Figures 9 and 13 illustrate diagrammatically and partially other variants of the invention.

First exemplary embodiment of the invention

[0092] Figure 1 shows a microfluidic device 1 according to the invention, said device comprising a microchannel 2 and an electrode assembly 3. The microchannel 2 has a longitudinal axis X and an internal cross section that is circular.

[0093] The electrode assembly 3 comprises a pair of electrodes 4, each electrode 4 comprising a metal cylin-

der, for example aluminium. The length of each electrode 4 is for example 4 mm and the inner diameter 1.5 mm and the outer diameter 1.9 mm.

[0094] The electrodes 4 are placed around the microchannel 2 and are spaced along the axis X by a gap 6. The electrodes 4 are connected to a generator unit 9 via connection elements 8 comprising electrical wires.

[0095] The electrodes 4 are housed in a support 10 comprising two support members 11, each being a substantially rectangular parallelepiped made of Plexiglas®, for example with a width of 24 mm, a height of 20 mm and a depth of 20 mm.

[0096] A first cylindrical hole 12, for example of diameter 1.9 mm, is drilled in each support member 11 along the axis X at the center of the support member 11 for holding the electrode 4. The first hole 12 extends from a front face 13 towards a rear face 14 of the support member 11, opposite to the front face 13.

[0097] A second hole 17 is drilled perpendicular to the first hole 12 for receiving a connection element 8 connecting the corresponding electrode 4 to the generator unit 9.

[0098] Each support member 11 further comprises two holes 20 and 21 whose axes are parallel to the axis of hole 12 and configured for receiving respectively a Teflon® screw 22 and a metal rod 23 in order to maintain the support members 11 assembled with the holes 12 being collinear.

[0099] Each electrode 4 is mounted in the corresponding support member 11 such that the electrode 4 is flush with the front face 13, as illustrated in Figure 2.

[0100] The front faces 13 of the support members 11 are spaced for example by a length of 2 mm defining a 2 mm gap 6 between the electrodes 4.

[0101] The generator unit 9 comprises for example a function generator connected to an amplifier such as to deliver sinusoidal voltages up to 2 kV with frequencies up to 1 kHz. The generator unit 9 may also comprise a central processing unit such as a computer to programmably control the voltage delivered to the electrodes 4.

[0102] Figure 3 shows diagrammatically the orientation of electric field given by the arrows together with the equipotential lines.

[0103] As one can see, the electric field is substantially collinear to the axis X of the microchannel 2 and thus favors electrocoalescence and minimizes any effect of dielectrophoresis.

[0104] The device 1 may be mounted on an observation stage of a binocular microscope 30 connected to a CCD camera 31 and a video recorder 32, as illustrated on Figure 1, thus enabling the monitoring of the collapsing of two packets or the splitting of a packet in the microchannel.

[0105] The device may be configured such that after the collapsing or splitting, the packet(s) are drained off.

Second exemplary embodiment of the invention

[0106] Figure 8 shows an electrode assembly 3' comprising two composite electrodes 35 each having a pair of substantially parallel equipotential plates 36 facing each other and sandwiching a microchannel 2' which has a rectangular cross-section. Each pair of plates 36 is connected to a respective pole of the generator unit 9.

Example of an oscillation method

[0107] In an embodiment, the droplet fluid is TBE 5x buffer (0.45 M Trisbase®, 0.45 M boric acid and 0.01 M EDTA; Sigma®) dyed with 0.25 wt% bromophenol blue for observation in a carrier fluid of fluorinated oil (FC-40, 3M) with 0.5 wt% 1H,1H,2H,2H perfluorodecan-1-ol (Fluorochem®) added to prevent interactions with the wall of the microchannel 2. The droplet conductivity is 3 mS/cm and the carrier fluid conductivity is $2.5.10^{-13}$ mS/cm. For droplet formation, the two fluids are layered in a 1.5 ml Eppendorf® tube so that the bottom layer consists of approximately 0.6 ml of the carrier fluid (FC-40/1H,1H,2H,2H perfluorodecan-1-ol) and the upper layer consists of approximately 0.6 ml of the droplet fluid (TBE 5x/bromophenol blue).

[0108] The microchannel 2 is filled from a syringe pump (commercialised by KD Scientific) using for example a Hamilton® Gas-Tight 250 μl syringe filled with the carrier fluid. The excess fluid pumped into the microchannel may be collected in a waste reservoir. After completely filling the capillary, the microchannel 2 is placed into the carrier fluid phase of the layered Eppendorf® tube. The pump is then aspirated at a rate of 1 ml/hr. Droplets are formed by oscillating the microchannel between the carrier phase and the droplet phase, either manually or by attaching the microchannel to a mechanical oscillator, at for example approximately 2 Hz. Droplets formed by this method have approximately the same diameter as the channel.

Exemplary method for displacing a droplet in a microchannel

[0109] The method may be carried out with any of the devices defined above.

[0110] A single droplet is formed by the oscillation method described above. Using the syringe pump, the droplet is aspirated into the gap 6 between the electrodes 4. When the droplet has reached the section of the gap just before the upstream electrode, the flow is stopped and the system allowed to settle to equilibrium. A continuous voltage is then applied, with the positive voltage applied to the electrode 4 closest to the droplet and with the farthest electrode grounded. The motion of the droplet towards the grounded electrode can be recorded on video and the time for a given displacement measured. The droplet only moves when it is between the electrodes 4 and stops when it is under the grounded electrode.

Example of static coalescence

[0111] The static coalescence may be performed by any of the devices defined above.

[0112] Droplets are formed by the oscillation method described above such that the spacing between the two droplets is larger than the gap 6 between the electrodes 4. A first droplet is initially brought into the gap 6 between the electrodes 4 using the syringe pump. When the droplet arrives at the upstream electrode, the flow is stopped. The droplet is moved against the direction of the previously applied flow using the electric field actuation described above until it reaches the downstream electrode. The flow is restarted until the first droplet returns to the upstream electrode. This procedure is repeated until a second droplet appears between the electrodes 4. The microchannel position in the electrodes is then adjusted so that the second droplet is outside of the gap 6 between the electrodes. The first droplet is moved against the direction of the previously applied flow until the gap between the two droplets is for example 0.5 mm. The microchannel is then repositioned such that the midpoint between the two closest edges of the droplets is centered between the two electrodes and the system is allowed to settle to equilibrium.

[0113] In the example depicted in Figures 4A to 4C, the first droplet 40 has a diameter of about 540 μm and the second droplet 41 has a diameter of about 560 μm. Upon applying a 2 kV, 1kHz sinusoidal voltage to the electrodes 4, initial droplet motion is steady, with the smaller droplet 40 moving at a slightly higher velocity (Figures 4A and 4B). When the droplets 40 and 41 come into close contact they rapidly accelerate and drain the intervening film (Figure 4C), which indicates that the device 1 should provide essentially instantaneous coalescence of droplets which are initially close together, such as occurs after two droplets arrive simultaneously at a T-junction.

Example of in-flight coalescence

[0114] The in-flight coalescence may be performed by any of the devices defined above.

[0115] The first droplet 43 may have a diameter of about 580 μm and the second droplet may have a diameter of about 560 μm.

[0116] After positioning the droplets, the microchannel is displaced such that both droplets are outside the gap 6 between the electrodes 4. A 2 kV, 1kHz sinusoidal voltage is then applied to the 2 mm-spaced electrodes and kept on throughout the duration of the experiment. After applying the electric field, the flow is started by aspirating with the syringe pump at 50μL/hr.

[0117] The droplet 42 enters the gap 6 between the electrodes 4 and moves at a constant velocity in the absence of the droplet 43 (Figure 5A). The leading interface of the droplet 43 appears after 13 sec, but has no effect on the droplet 42. Only when the trailing droplet is well inside the gap 6 between the electrodes does the dipolar force manifest itself. Thereafter, the coalescence time is essentially the same as in the static case (approximately 8 sec) for these widely separated droplets, but the dynamics are slightly different due to the flow. The dipolar force is sufficiently strong to stop the droplet 42 (Figure 5B), whereupon the droplet 43 moves toward it at a constant rate, closing the distance at essentially the same rate as in the static case. Once the droplets are close together, the strong dipolar force rapidly drains the intervening fluid and coalescence is achieved (Figure 5C).

Example of droplet splitting

[0118] A single large droplet 46 is formed by oscillating the interface as described above but at a lower frequency. The droplet 46 is brought into the gap 6 between the electrodes, by aspirating with the syringe pump (Figure 6A). The drop depicted in the present embodiment is an ellipsoid of revolution with a 2.5 mm long axis. Upon applying a 2 kV, 0.1Hz square tension, the drop 46 splits into two smaller, stable drops 47 (Figures 6B and 6C) that are ejected from the gap 6.

[0119] Typical operating conditions for achieving a clean droplet splitting, that is to say one big droplet splitting into two smaller and stable ones without formation of any satellite drops may consist in a square voltage with a frequency between 0.1 and 1 Hz and an amplitude between 1kV and 2 kV. Under such condition, the droplet may break in less than 1 minute. The lowest the voltage applied, the "cleanest" the splitting but the longer it may take. The droplet length may be about the length of gap 6.

Other exemplary embodiments of the invention

[0120] As illustrated in Figures 9 and 10, the microchannel portion 50 between the electrodes 4 may form a T-intersection with a transverse channel 51. After coalescence of the droplets caused by the electric field between the electrodes 4, the resulting droplet 52 may be driven in the transverse channel 51, for example by using a syringe pump connected to the transverse channel 51.

[0121] As illustrated in Figures 11 and 12, the droplet splitting may be performed by extracting a droplet 53 from a relatively large mass of fluid 54 by applying the electric field between the two electrodes 4.

[0122] Thus droplets 53 can be formed when desired, for example by programmably controlling the electrodes 4.

Another exemplary embodiment of the invention

[0123] Figure 13 shows a device 60 according to the invention, said device 60 comprising a microchannel 61 connected in a portion 62 to first and second side channels 63 and 64.

[0124] Portion 62 may for instance be situated substantially at the middle of the microchannel.

[0125] In the present embodiment, the microchannel 61 may have a thickness of about 100 μm and a width of about 300 μm and the side channel 63 a thickness of about 100 μm and a width of about 50 μm.

[0126] The side channel 63 is connected to a delivery system 66 comprising a syringe pump having a reservoir 67 containing a solution of surfactant of oleic acid and SDS in hexadecane, at a concentration superior to the critical micellar concentration.

[0127] The microchannel 61 is filled with a solution containing hexadecane containing SPAN®80 at a concentration adjusted to avoid interaction of aqueous droplets with the microchannel walls.

[0128] The side channel 64 is connected to a springe pump 68 in aspiration mode configured to aspirate the solution from the microchannel 61.

[0129] Two droplets 70 of a 5X TBE Buffer are introduced and displaced in the microchannel 61 by its both ends. The aspiration of droplets 70 by both ends is synchronized so that the droplets 70 arrive from both sides at the same time at the portion 62. When the droplets 70 are in the portion 62, a solution of surfactant contained in the reservoir 67 is delivered by the delivery system 66 into the portion 62 of the microchannel with a predetermined flow rate such as droplets 70 coalesce. The optimal flow rate may be determined by progressively increasing the flow until droplets coalesce at each collision, which sets the optimal flow rate.

[0130] In another embodiment, the solution of surfactant may be delivered in pulses synchronized with the arrival of the pair of droplets at the connection portion 62.

[0131] The resulting droplet 71 is collected in syringe pump 68 for further use, or e.g. transferred to another microchannel for detection.

Examples of applications

[0132] A device made in accordance with the invention may be used to carry out, for instance:

- mixing,
- DNA screening processing (collapsing or hybridization of a packet comprising a DNA sample and a packet comprising a DNA probe),
- polymerase chain reaction in a microreactor,
- genotyping,
- proteomic analysis (collapsing of a packet comprising a protein and a packet comprising an antibody for instance),
- transcriptome analysis (collapsing of a packet comprising an RNA sample and a packet comprising an RNA probe),
- crystallisation, and in particular protein crystallisation (collapsing of at least two packets comprising proteins),
- searching and evaluation of pharmaceutical targets, pharmaceutical hits or leads, or drugs (collapsing of a packet comprising a receptor and a packet comprising a ligand for instance),
- enzyme-protein reaction (collapsing of a packet comprising an enzyme and a packet comprising a protein),
- antigen-antibody reaction (collapsing of a packet comprising an antigen and a packet comprising an antibody),
- screening of libraries of chemical of biological products,
- high throughput screening,
- drug delivery,
- diagnosis,
- analysis or lysis of at least one living cell or dead cell,
- analysis of microorganisms,
- chemical reaction,
- reactive-catalyzer reaction (collapsing of a packet comprising a reactive and a packet comprising a catalyzer),
- polymerisation reaction,
- fusing particles, for example colloids, to form a chain,
- preparation of colloids, emulsions, vesicles, in particular monodisperse colloidal objects,
- preparation of nanoparticules or microparticles,
- environmental control,
- detection of pollutants,
- control of an industrial process.

Claims

1. A microfluidic device (1) for deforming, in particular splitting, at least one packet, or displacing at least two packets towards each other, in particular for collapsing said packets, said device comprising:

- a microchannel (2) having an axis (X),
- packet manipulation means comprising at least one of:

  • a generator unit (9), and an electrode assembly (3) coupled to the generator unit (9) and configured for creating inside at least one portion of the microchannel an electric field which is substantially collinear to the axis (X) of the microchannel, wherein the generator unit (9) is capable of generating the electric field with such an amplitude and frequency that the electric field causes the at least one packet to deform, or the at least two packets to displace towards each other in the microchannel,
  • at least one side channel with a first end in connection with a portion of said microchannel and a second end in connection with a delivery system suitable for delivering a solution able to alter the interfacial tension between said at least two packets or said at least one packet and the environment

thereof, said delivery system being configured to deliver said solution into said portion of the microchannel at least during the passage of said packet(s) in said portion of the microchannel.

2. A device according to the preceding claim, wherein the at least one portion of the microchannel in which the electric field is substantially collinear to the axis (X) of the microchannel has a length comprised between about 1 and about 100 times the thickness of said portion, and preferably between about 1 to about 10 times the thickness of said portion.

3. A device according to any preceding claim, wherein the electrode assembly (3) is electrically insulated from the inside surface of the microchannel (2).

4. A device according to any preceding claim, wherein the electrode assembly (3) comprises at least two electrodes (4; 35) axially spaced along the axis of the microchannel by a distance long enough for the electric field between the electrodes to be substantially collinear to the axis (X) of the microchannel.

5. A device according to the preceding claim, wherein at least one of said electrodes (4; 35) comprises at least two equipotential portions facing each other across the microchannel (2).

6. A device according to any one of claims 4 and 5, wherein at least one of said electrodes (4) is monolithic.

7. A device according to any one of claims 4 to 6, wherein at least one of said electrodes (4) has a cylindrical surface surrounding the microchannel.

8. A device according to any one of claims 4 and 6, wherein at least one of said electrodes (35) is composite.

9. A device according to the preceding claim, wherein at least one of said electrodes (35) comprises at least two substantially parallel equipotential plates (36) sandwiching the microchannel (2).

10. A device according to any one of claims 4 to 9, wherein the electrodes (4; 35) are spaced by a gap (6) having a length that is greater than the thickness of the microchannel, preferably greater than twice the microchannel thickness.

11. A device according to any preceding claim, wherein the electrode assembly (3; 3') is configured so that in at least one cross section of the microchannel the amplitude of the electric field varies less than a factor 10, better less than 2, and preferably is substantially uniform.

12. A device according to any preceding claim, wherein the generator unit is capable of generating a continuous field.

13. A device according to any of claims 1 to 11, wherein the generator unit is capable of generating a variable field.

14. A device according to the preceding claim, wherein the generator unit is capable of generating an AC field.

15. A device according to the preceding claim, wherein the generator unit (9) is capable of generating the AC electric field with a frequency ranging from about 0,01 Hz to about 1 GHz, preferably from about 1 Hz to about 10 MHz.

16. A device according to the preceding claim, for coalescing droplets, wherein the generator unit (9) is capable of generating the AC electric field with a frequency comprised between 100 Hz and 10 kHz.

17. A device according to claim 15, for aliquoting at least one droplet, wherein the generator unit (9) is capable of generating the AC electric field with a frequency lower than 50 Hz.

18. A device according to claim 1, wherein said solution contains a surfactant.

19. A method for displacing at least one packet in a microchannel having an axis, said method comprising:

- introducing at least one packet in the microchannel,
- generating an electric field within at least one portion of the microchannel, at least when the at least one packet is located within said portion, said electric field being preferably collinear to the axis of the microchannel, such as to displace the packet along the microchannel.

20. Method for collapsing at least two packets in a microchannel (2), the microchannel having a longitudinal axis (X), said method comprising:

- introducing the at least two packets in the microchannel,
- generating an electric field within at least one portion of the microchannel, at least when the packets are located within said microchannel portion, said electric field being preferably substantially collinear to the axis of the microchannel in said portion and having an amplitude and a frequency chosen such as to displace two

packets towards each other.

21. Method according to the preceding claim, the electric field being generated by at least two electrodes (4; 35) axially spaced along the axis (X) of the microchannel, said electrodes being separated by a gap (6), the method comprising:

- before generating said electric field, positioning two packets in the gap between the electrodes, said packets being in static equilibrium,
- generating said electric field.

22. Method according to claim 20, the electric field being generated by at least two electrodes (4; 35) axially spaced along the axis of the microchannel, said electrodes being separated by a gap, the method comprising:

- positioning two packets in the microchannel, at least one of which being outside the gap between the electrodes,
- displacing the packets towards the gap, for example via a flowing stream in the microchannel,
- generating said electric field at least when the packets are located in the gap.

23. Method according to any of claims 20 to 22, wherein at least one of said packets contains biological material.

24. Method for collapsing at least two packets in a microchannel, or for splitting at least one packet, the microchannel having an axis, said method comprising:

- positioning the at least two packets or the at least one packet in a portion of the microchannel,
- delivering into said portion of the microchannel a solution of a surfactant able to alter the interfacial tension between said at least two packets or said at least one packet and the environment thereof.

25. A method for splitting at least one packet in a microchannel having a longitudinal axis, said method comprising:

- introducing the at least one packet in the microchannel,
- generating an electric field within at least one portion of the microchannel, at least when the at least one packet is located within said at least one portion, said electric field being preferably substantially collinear to the axis of the microchannel in said portion and having an amplitude and a frequency chosen such as to split the packet.

Fig.1

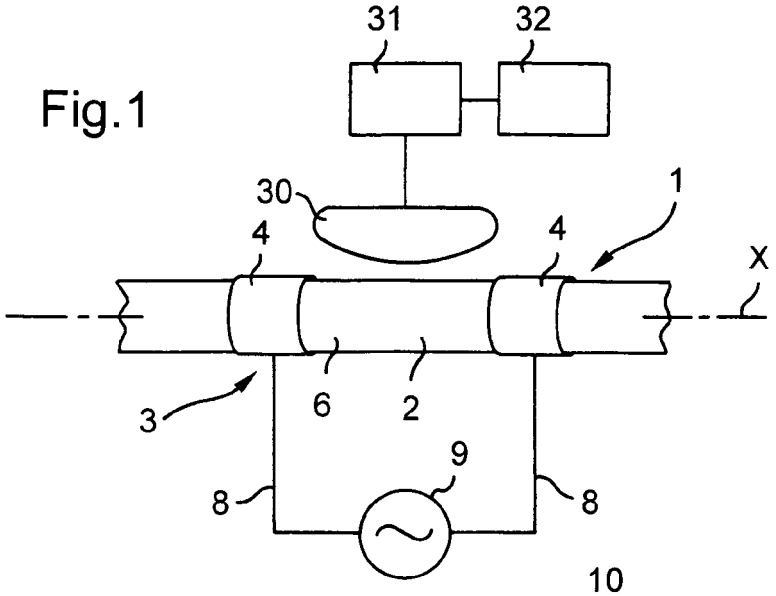


Fig.2

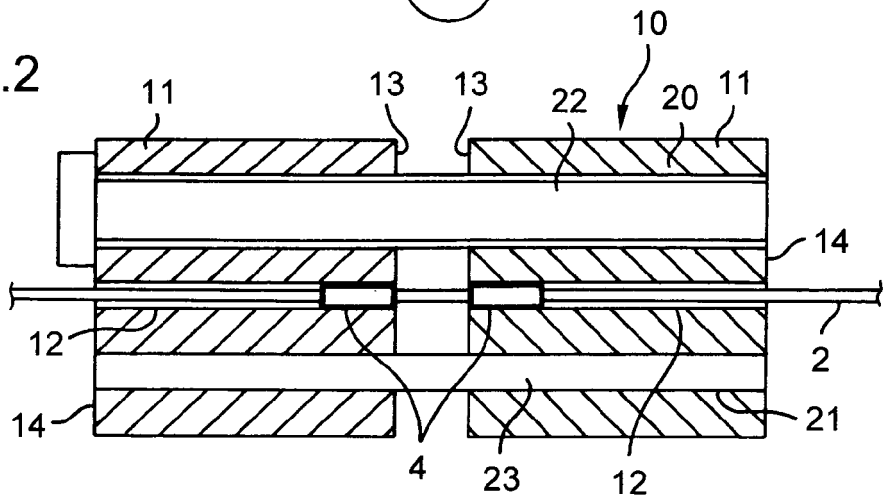
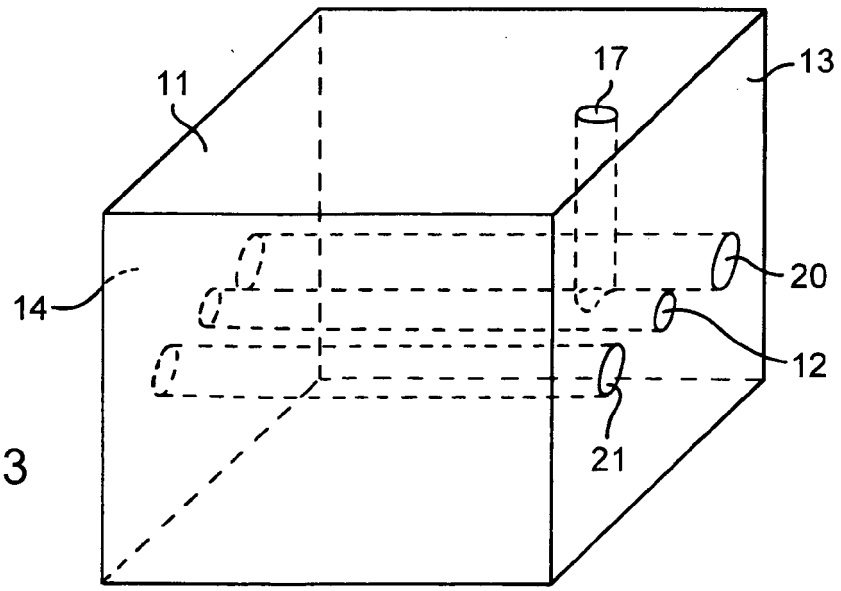


Fig.3



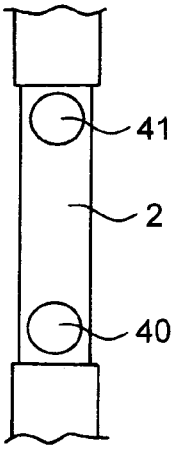


Fig.4A

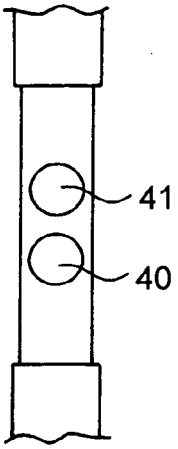


Fig.4B

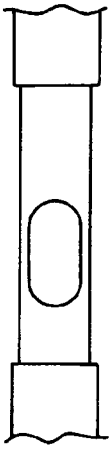


Fig.4C

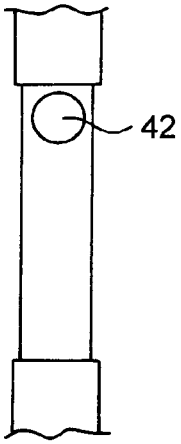


Fig.5A

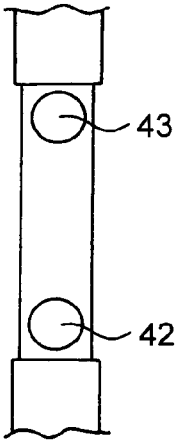


Fig.5B

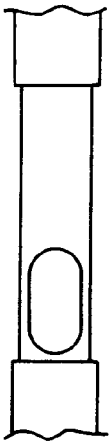


Fig.5C

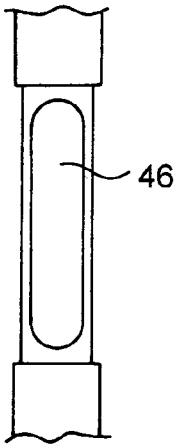


Fig.6A

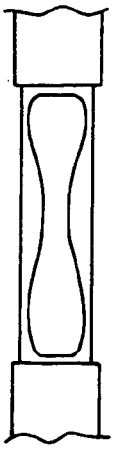


Fig.6B

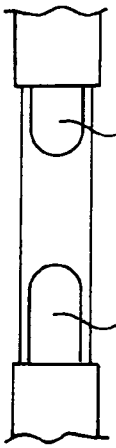


Fig.6C

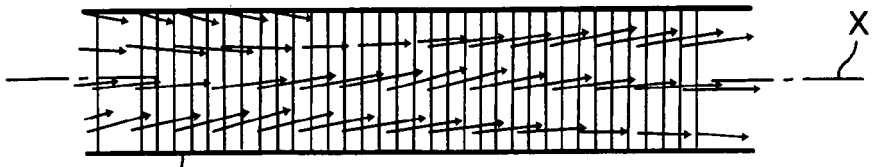


Fig.7

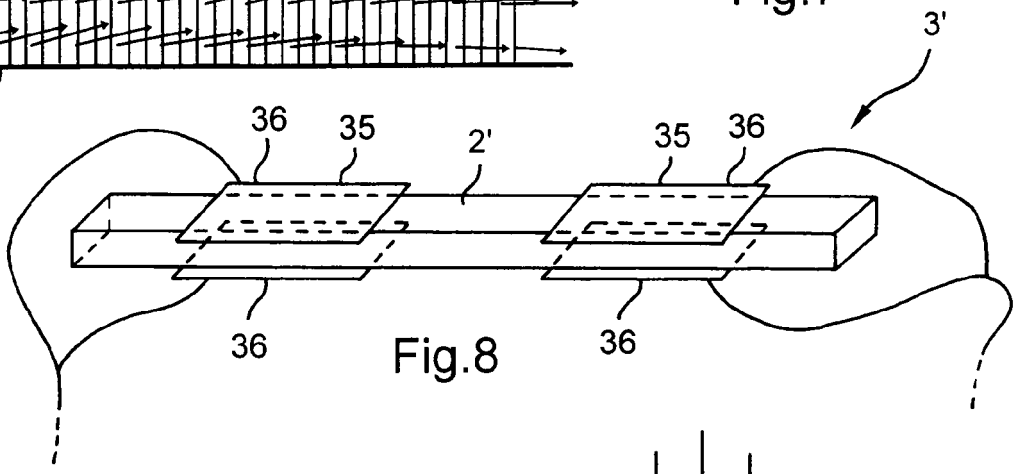


Fig.8

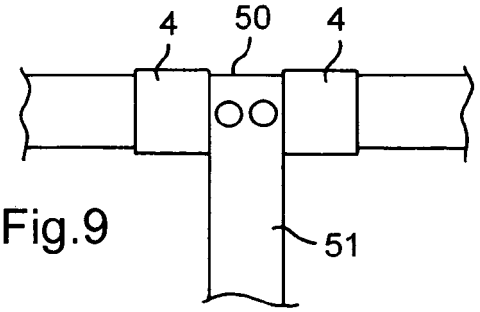


Fig.9

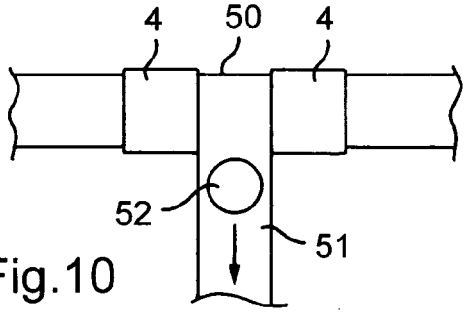


Fig.10

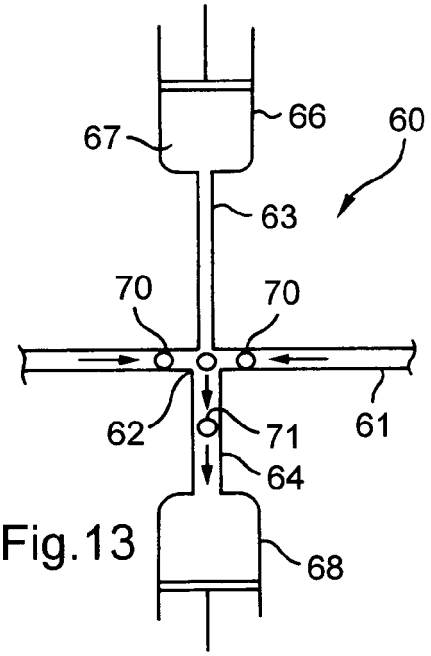


Fig.13

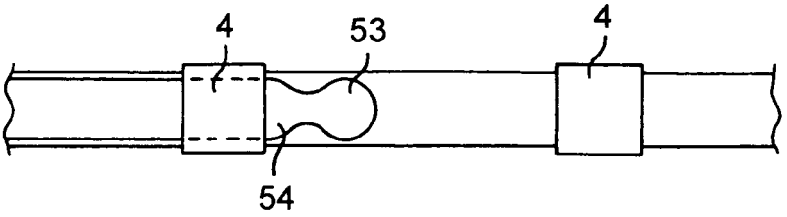


Fig.11

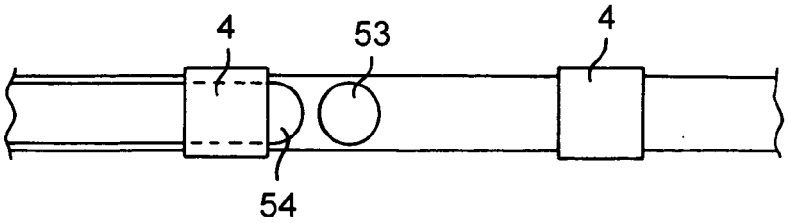


Fig.12

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 04 29 2173

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
A	WO 00/73655 A (OSMOOZE S.A; LE PESANT, JEAN-PIERRE; MILLET, JEAN-CLAUDE) 7 December 2000 (2000-12-07) * the whole document *	1-23,25	B01L3/00 F04B19/00
A	US 6 294 063 B1 (BECKER FREDERICK F ET AL) 25 September 2001 (2001-09-25) * the whole document *	1-23,25	
A	US 6 146 103 A (LEE ET AL) 14 November 2000 (2000-11-14) * the whole document *	1-23,25	
A	ZHANG ET AL: "Theoretical Prediction of Elecric Fields-Enhanced Coalescence of Spherical Drops" AICHE JOURNAL, vol. 41, no. 7, July 1995 (1995-07), pages 1629-1639, XP009043703 * the whole document *	1-23,25	
A	EP 1 348 546 A (TOYO INK MFG. CO., LTD) 1 October 2003 (2003-10-01) * the whole document *	1-23,25	TECHNICAL FIELDS SEARCHED (Int.Cl.7) B01L F04B

~~The present search report has been drawn up for all claims~~

Place of search	Date of completion of the search	Examiner
Munich	11 February 2005	Skowronski, M

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

EPO FORM 1503 03.82 (P04C01)

European Patent Office

Application Number
EP 04 29 2173

CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing more than ten claims.

☐ Only part of the claims have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid, namely claim(s):

☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

see sheet B

☐ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.

☐ As all searchable claims could be searched without effort justifying an additional fee, the Search Division did not invite payment of any additional fee.

☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid, namely claims:

☒ None of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims, namely claims:

1-23, 25

European Patent Office

LACK OF UNITY OF INVENTION SHEET B

Application Number
EP 04 29 2173

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

1. claims: 1-23,25

Microfluidic device and method of using an electrical field which is collinear to the axis of the microchannel. The applicant is informed that in independent claims 19, 20 and 25 the term "preferably" in preferably collinear should be deleted and that indpendent method claims 19, 20 and 25 should indicate the use of the device of claim 1 in order to meet the requirements of Article 82 EPC (unity of invention).

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2. claim: 24

Method for collapsing at least two packs in a microchannel or for splitting at least on packet using a surfactant able to alter the interfacial tension

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ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO. EP 04 29 2173

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

11-02-2005

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