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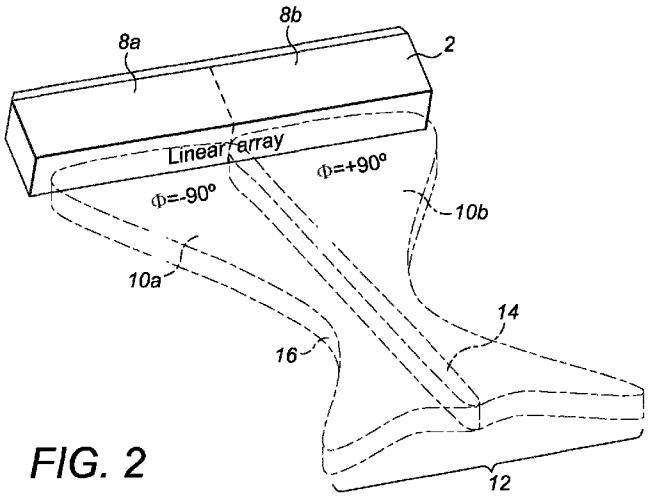

FIG. 2

(57) **Abstract**: Apparatus and method for manipulating particles entrained in a fluid. The apparatus comprises a signal generator (4) and an ultrasound transmitter. The signal generator is arranged to generate driving signals. The ultrasound transmitter comprises at least first and second groups of transmitter elements and is arranged to transmit respective first and second ultrasound beams in response to respective first and second driving signals received from the signal generator to form a composite ultrasound beam. The signal generator is arranged to generate first and second driving signals that are out of phase, the first driving signal being arranged such that the first ultrasound beam produces a lower time averaged acoustic radiation force than the second ultrasound beam, and such that at least part of region of the composite ultrasound beam corresponding to the interface between the first and second ultrasound beams exhibits a pressure minimum.

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Apparatus and Method for Manipulating Entrained Particles

[0001] This invention relates to an apparatus and method for manipulating particles entrained in a fluid flowing through a conduit. In particular, the present invention relates to manipulating entrained particles where the particles and the fluid have different acoustic impedances. In particularly preferred embodiments the particles may comprise microbubbles.

BACKGROUND

[0002] Microbubbles are generally considered to be an encapsulated gas core with a diameter in the order of a micrometre. Commercially produced microbubbles typically range between 0.1 μm and 10 μm, though smaller and larger microbubbles may be produced. The present invention is not limited to any particular size of particle or microbubble. Typically, microbubbles are at least partially filled with a gas such as air or perfluorocarbon and are suspended or entrained with in a liquid. Usually microbubbles are encapsulated with a shell to reduce the rate of gas dissolution into the surrounding fluid. The shell may be formed from a viscoelastic material, or a more rigid material. The present invention is not limited to any particular material. For medical applications, shells from a biocompatible material including lipids such as phospoholipid or proteins such as serum albumin are used. Microbubbles are alternatively referred to in the art as microcapsules, in particular where the microbubble has a shell.

[0003] Microbubbles are widely used in medicine as a contrast agent in ultrasound imaging. This is because the acoustic impedance mismatch between the microbubble and the surrounding body fluids causes a reflection of ultrasound. For medical applications microbubbles are arranged to be more compressible that blood or surrounding tissue by several orders of magnitude, such that the backscatter of ultrasound is significantly higher. Microbubbles for use as contrast agents are selected to provide a large impedance mismatch at an ultrasound imaging frequency. Additionally, microbubbles oscillate when exposed to ultrasound, which increases the reflection of ultrasound. The microbubbles can therefore be clearly distinguished from surrounding tissue. The largest reflections are achieved by exciting the microbubbles near to their resonant frequency.

[0004] Recently the potential for using microbubbles in targeted drug or gene delivery and in other medical has been explored. Microbubbles are used as a vector for delivering the drug or gene material to a target location within the patient's body. The microbubbles may be supplied directly to the target location or indirectly, for instance by being introduced into the blood stream. The use of microbubbles in a drug or gene delivery system is

particularly attractive for microbubbles of a similar diameter to red blood cells as they may therefore pass readily through lung capillaries and enter the circulatory system. The rheological behaviour of microbubbles is similar to that of red blood vessels. This results in microbubbles preferentially travelling along the centre of blood vessels which can reduce targeting efficiency.

[0005] Targeting is used to retain microbubbles at a target location within a blood vessel through the formation of bonds between ligands attached to the microbubbles and specific receptors expressed on the endothelium. Targeted microbubbles travel through blood vessels and selectively adhere to the target location by forming a molecular bond with the target receptor on the vessel wall. Once adhered to a vessel wall, microbubbles can withstand significantly higher shear rates caused by the flow of blood. The longer a microbubble can be retained at the target location, the greater the proportion of the drug or gene which will be delivered to the target location, either discharged from the microbubble or when the microbubble eventually dissolves. Targeting can increase the induction efficiency of the drug or gene, allowing for both the use of more potent drugs (which may prove disadvantageous in other parts of the body) and a reduction in the total amount of drug or gene introduced into the body.

[0006] Ultrasound may be used to release a drug or gene coupled to the microbubbles in a desired location. For instance, PCT patent publication WO-2010/057045-A2 (Baylor Research Institute) discloses techniques using microbubbles for the treatment of diabetes. Microbubbles are used to transport a pre-assembled liposome-nucleic acid complex including a suitable gene known to be beneficial in the treatment of diabetes. The nucleic acid is in contact with, within, and/or about the microbubble. The microbubbles are introduced into the pancreas. An ultrasound beam focussed on a target location within the pancreas disrupts the microbubbles delivering the nucleic acid into surrounding pancreas cells. The disruption of the microbubbles may be either by the ultrasound beam causing oscillation of the microbubbles or by a higher power ultrasound beam causing destruction of the microbubbles such that substantially all of the nucleic acid is released. The ultrasound beam may be generated by an ultrasound probe applied to the exterior of the patient. Prior to using higher power ultrasound to destroy microbubbles conventional ultrasound imaging may be used to identify the location of the microbubbles before the microbubbles are agitated or destroyed.

[0007] Targeting efficiency can be increased by pushing microbubbles away from circulation and towards the endothelium of a blood vessel, thereby reducing the ligand-receptor gap. Additionally, microbubbles can be retained in place and withstand significantly higher shear rates when exposed to a force pushing the microbubbles towards

a vessel wall. There exist a number of different approaches to apply sufficient force to microbubbles to promote a controllable and repeatable translation. Optical tweezers may be used. Alternatively, ultrasound can be used by using two opposing transmitters to generate a standing wave such that the microbubbles move towards acoustic force nulls. However, owing to the need for either high frequencies or small working distances neither approach can be applied in vivo.

[0008] In an alternative approach, a travelling wave ultrasound beam may be used to influence the flow of microbubbles loaded with drugs or genes within the blood stream to increase targeting efficiency. Specifically, the acoustic radiation force can be used to guide microbubbles towards the vessel wall. US-2011/0208113-A1 (University of Pittsburgh – of the Commonwealth System of Higher Education) discloses a technique for directed cell-based therapy using microbubble-tagged cells. Microbubbles are secured to progenitor cells which may then be directed using the acoustic radiation force resulting from an ultrasound beam towards injured tissue. The microbubble-tagged cells are introduced into the blood stream upstream of the injured tissue. An intravascular ultrasound transmitter located proximate to the injured tissue is used to preferentially direct the microbubble-tagged cells towards the injured tissue.

[0009] K. Masuda, "Active Control of Microcapsules in Vivo with Mechatronics and Informatics", International Journal of Applied Biomedical Engineering, Vol. 4, No. 1, 2011 reports a technique for propelling microbubbles in an artificial blood vessel. The ability to retard microbubbles against the flow of a fluid in the artificial blood vessel, to form aggregations of the microbubbles and to select a desired path in a vessel with a bifurcation is reported. Microbubbles aggregate in a fluid owing to Bjerknes forces that are produced by an ultrasound pressure gradient and oscillation of the diameter of the microbubbles. Microbubbles are propelled in the direction of acoustic propagation by the application of the acoustic radiation force (alternatively referred to herein as the Primary Radiation Force, PRF). When microbubbles pass through a sound field where the sound pressure is higher than in other areas, the microbubbles are propelled away from their original course. Masuda reports the ability to influence the flow path of microbubbles using a single element ultrasound transducer external to a vessel (though acoustically coupled to the artificial blood vessel by both being placed in a water bath). The transducer element transmits a travelling wave ultrasound beam. The axis of the transducer is set transverse to the flow of fluid within the vessel with the focal point of the transducer intersecting the interior of the vessel. The effect is a change in the flow of microbubbles within the vessel under the combined action of the fluid flow and the acoustic radiation force, and aggregation of the microbubbles. Two transducer elements having different axes focussed

on the same point and driven in tandem may be used to increase the acoustic radiation force. The technique taught in Masuda for retarding microbubbles is limited by requiring that the transducer can be positioned such that the ultrasound beam axis substantially opposes the flow direction within the vessel.

[0010] R. Gessner et al., "High-Resolution, High-Contrast Ultrasound Imaging using a Prototype Dual-Frequency Transducer: In Vitro and In Vivo Studies", IEEE Transactions on Ultrasonics, Ferroelectronics, and Frequency Control, Vol. 57, No. 8, August 2010 report the use of a dual-frequency confocal transducer probe to apply acoustic radiation force to microbubbles to alter movement of the microbubbles. The probe is acoustically coupled to a vessel in a water bath such that the acoustic radiation force is transverse to the flow of microbubbles through the vessel. A stream of microbubbles within the vessel is visibly diverted towards the far side of the vessel away from the probe.

[0011] A. Patil et al., "Dual Frequency Method for Simultaneous Translation and Real-Time Imaging of Ultrasound Contrast Agents Within Large Blood Vessels", Ultrasound Med Biol., 35, 2021, 2009 report the use of a linear ultrasound transducer array extending parallel to a vessel. Long acoustic radiation force pulses act to propel the microbubbles to the far wall of the vessel. Intervening short pulse inversion pulses push and image the microbubbles. Microbubbles travel along a trajectory towards the opposite vessel wall before contacting the vessel wall.

[0012] Y. Yamakoshi et al., "Micro Particle Trapping by Opposite Phases Ultrasonic Travelling Waves", Ultrasonics, 36, 873-878, 1998 report the use of two single ultrasonic transducer elements places side by side and driven by continuous sinusoidal signals with opposite phases to generate an acoustic black line extending from the boundary between the transducer elements. The acoustic black line has zero acoustic power. Micro particles flowing into the acoustic black spot entrained in a fluid can be trapped if the force applied to the micro particles acting towards the centre of the acoustic black line by surrounding regions of the acoustic field exceeds the force applied to the micro particles by the flowing fluid. A pair of side-by-side focussed concave ultrasound transducer elements is preferably used, resulting in a narrowed acoustic black line. The acoustic black line is narrowest at the focal point of the two concave transducers though the acoustic black line extends in front of and behind the focal point through the region where the ultrasound beams merge, such that the maximum localisation of micro particles the transducers should be positioned so that the focal point intersects the centre of the fluid flow path. Micro particles within the acoustic black line aggregate forming large masses of particles.

[0013] As noted above, the use of microbubbles in drug and gene delivery systems, as well as other medical applications such as molecular imaging and sonothrombolysis can

be improved through the use of travelling acoustic ultrasound waves to control movement of the microbubbles. There remains a need to improve the retention and accumulation of microbubbles at target locations, in particular to improve the targeting efficiency when microbubbles are used as drug or gene vectors.

BRIEF SUMMARY OF THE DISCLOSURE

[0014] It is an aim of embodiments of the present invention to obviate or mitigate one or more of the problems associated with the prior art, whether identified herein or elsewhere. In particular, it is an aim of embodiments of the present invention to provide an apparatus to create an acoustic radiation force trap to non-invasively trap and hold flowing particles such as microbubbles flowing in a liquid such as blood within a patient's blood vessel. Such an acoustic radiation force trap is adapted to trap microbubbles flowing in a liquid, for instance microbubbles flowing entrained in blood within a blood vessel. Trapped microbubbles increase the induction efficiency of drugs when the microbubbles are used as drug and gene delivery vectors by allowing the microbubbles to be trapped and to accumulate at a target location.

[0015] According to a first aspect of the present invention there is provided an apparatus for manipulating particles entrained in a fluid, the particles and the fluid having different acoustic impedances, the apparatus comprising: a signal generator arranged to generate driving signals; and an ultrasound transmitter comprising at least first and second groups of transmitter elements arranged to transmit respective first and second ultrasound beams in response to respective first and second driving signals received from the signal generator to form a composite ultrasound beam; wherein the signal generator is arranged to generate first and second driving signals that are out of phase, the first driving signal being arranged such that the first ultrasound beam produces a lower time averaged acoustic radiation force than the second ultrasound beam, and such that at least part of region of the composite ultrasound beam corresponding to the interface between the first and second ultrasound beams exhibits a pressure minimum.

[0016] The present invention is used to manipulate particles. In particular, the present invention can be used to influence the movement of particles flowing in a fluid such that the time-averaged primary radiation force PRF exerts a force on the particles that can oppose the force on the particles from the fluid. In this way, particles can be trapped in a target location against the flow of the fluid.

[0017] An advantage of the present invention is that when particles such as microbubbles used as drug or gene delivery vectors, trapping the particles at target locations allows the particles to accumulate at the target locations thereby increasing the drug or gene induction efficiency. When used in this way, the first group of elements are

positioned closer to an upstream portion of the blood vessel than the second group of elements. Typically this may be by arranging the apparatus such that the elements of the array extend parallel to the vessel longitudinal axis. The region of minimum pressure extends from the centre point between the first and second groups of elements outwardly from the array. The array can be positioned such that the region of minimum pressure intersects the target location in the blood vessel and the focal point of the array is positioned at the target location (either by moving the array or by controlling the focal depth of the array). In addition to the trapping force acting against the flow of blood, the primary radiation force generated by the composite beam acts to push accumulated clusters of microbubbles towards the opposite vessel wall, which can also increase the effectiveness of targeting.

[0018] Additionally, when the present invention is used in a medical context the present invention is non-invasive and adapted to operate at power levels comparable to conventional medicinal ultrasound techniques. The present invention may also be used in other non-medical applications where it is desirable to control the movement of microbubbles or similar entrained particles in a flowing liquid.

[0019] Certain embodiments of the present invention are implemented using a phased array to generate the first and second ultrasound beams. While the region of minimum pressure extends outward from the array, the pressure minimum is narrowest at the focal depth of the phased array. Advantageously, using conventional phased array techniques the focal depth can be adjusted. This results in a more flexible apparatus which can be used to control the movement of microbubbles at varying depths below the surface of an object, including at varying depths within a patient's body when the present invention is used in a non-invasive medical technique.

[0020] The signal generator may be arranged to generate first and second driving signals comprising time varying signals having substantially the same frequency. The signal generator may be further arranged to generate the first and second driving signals with a relative phase shift of approximately 180º. The signal generator may also be arranged to generate the first and second driving signals such that the first and second driving signals are modulated with a pulsed signal such that the first and second ultrasound beams are transmitted for a proportion of each pulse cycle to reduce the time averaged acoustic radiation force of each ultrasound beam.

[0021] Preferably, the signals are pulse modulated by pulse signals having substantially the same frequency as one another, the pulse signal frequency being lower than a frequency of the driving signals. Preferably, the pulse signals are substantially in phase, but the first driving signal is modulated with a pulse signal having a lower duty factor than

the pulse signal used to modulate the second driving signal such that the time averaged acoustic radiation force of the first ultrasound beam is lower than that of the second ultrasound beam.

[0022] Alternatively, the amplitude of the first driving signal may be lower than the amplitude of the second driving signal such that the time averaged acoustic radiation force of the first ultrasound beam is lower than that of the second ultrasound beam.

[0023] The ultrasound transmitter may comprise at least one array of transmitter elements which are separately controllable or are controllable in groups, the first and second groups of transmitter elements being included in the at least one array. The array may comprise a linear array of transmitter elements, the first and second groups of transmitter elements being spatially separated along the array. Alternatively, the array may comprise a two-dimensional array of transmitter elements, the first and second groups of transmitter elements being spatially separated across the array. The first and second groups of elements may be adjacent to one another within the array.

[0024] The signal generator may be arranged to control the driving signals supplied to array elements such that the focal depth of the composite ultrasound beam is controllable.

[0025] At least some of the array elements may be arranged to receive ultrasound reflections and to supply an imaging signal to an ultrasound imaging device. At least some of the array elements are arranged to transmit imaging ultrasound beams.

[0026] The signal generator may be arranged to generate destruction driving signals supplied to array elements having a higher amplitude than the first and second driving signals so as to generate a composite ultrasound beam having a higher acoustic radiation force.

[0027] According to a second aspect of the present invention there is provided a method for manipulating particles entrained in a fluid, the particles and the fluid having different acoustic impedances, the method comprising: positioning an ultrasound transmitter in proximity to a fluid conduit through which the fluid and entrained particles are flowing, the transmitter comprising at least first and second groups of transmitter elements, the first group of elements being positioned closer to an upstream portion of the conduit than the second group of elements; and generating first and second driving signals at a signal generator and supplying the driving signals to the first and second groups of elements such that they transmit first and second ultrasound beams in response to form a composite ultrasound beam; wherein the first and second driving signals are out of phase, the first driving signal being arranged such that the first ultrasound beam produces a lower time averaged acoustic radiation force than the second ultrasound beam, and such that a

region of the composite ultrasound beam corresponding to the interface between the first and second ultrasound beams exhibits a pressure minimum.

BRIEF DESCRIPTION OF THE DRAWINGS

[0028] Embodiments of the invention are further described hereinafter with reference to the accompanying drawings, in which:

[0029] Figure 1 schematically represents an apparatus for manipulating particles entrained in a fluid in accordance with an embodiment of the present invention;

[0030] Figure 2 schematically represents a composite ultrasound beam emitted from a probe forming part of the apparatus of Figure 1;

[0031] Figure 3 is a graph plotting simulated (dotted line) and measured (continuous line) lateral peak negative pressure (PNP) at the focal depth for a probe forming part of the apparatus of Figure 1;

[0032] Figure 4 schematically illustrates how the primary radiation force (PRF) causes microbubbles aggregation and translation towards an opposite tube wall using the apparatus of Figure 1, in which the engendered trapping force (TF) directly opposes the flow direction thus trapping microbubble clusters showing the face of a linear array forming part of Figure 1 arranged to extend along the length of the tube parallel to the tube axis with the pressure null is aligned to its centre;

[0033] Figure 5 is a micrograph of a cellulose tube showing a microbubble aggregation retained near the pressure null using the apparatus of Figure 1 showing the face of a linear array forming part of Figure 1 arranged to extend along the length of the tube parallel to the tube axis with the pressure null is aligned to its centre;

[0034] Figure 6 schematically represents the arrangement of a probe forming part of the apparatus of Figure 1 positioned with the face the linear array arranged to extend along the length of a tube parallel to the tube axis;

[0035] Figure 7 is a series of time separated micrographs (a)-(f) for experiments in which the flow rate and concentration were 50 ml/hr and 40×10^6 microbubbles/ml respectively, the vertical dotted lines at the centre of each micrograph representing the position of a pressure null generated using the apparatus of Figure 1 (centre frequency was 7 MHz, pulse repetition frequency 10 kHz, PNP of 500 kPa, δ_s = 71% and α = 0.5); and

[0036] Figure 8 is a plot of the largest trapped cluster diameter (dotted line) and position (continuous line) for the micrographs of Figure 7.

DETAILED DESCRIPTION

[0037] In accordance with certain embodiments of the present invention there is provided an acoustic radiation force trap to non-invasively trap and hold flowing microbubbles. A null, or reduction, in the pressure field is created across a tube cross-section by emitting two ultrasound beams of opposite phase. Microbubbles can be subjected to a trapping force directly opposing the fluid flow. For wall shear rates of up to 9000 s^{-1}, microbubble accumulations can be held within a low-pressure region (mechanical index < 0.13) for several seconds, potentially removing the need for molecular targeting when the microbubbles are used as a vector in a medical drug or gene delivery system.

[0038] Referring first to Figure 1, this schematically represents an apparatus for manipulating particles entrained in a fluid in accordance with an embodiment of the present invention. The apparatus comprises an ultrasound transmitter (preferably a transducer) probe 2 coupled to a signal generator 4. The signal generator is powered by a power supply 6. The probe 2 is provided with driving signals by the signal generator 4 in order to transmit a composite ultrasound beam. Specifically, the probe 2 comprises an array of ultrasound transmitter elements which can be individually driven or driven in groups by driving signals generated by the signal generator 4. The composite ultrasound beam is arranged to generate a region of reduced pressure within the composite ultrasound beam along a line extending from the array from a point between two groups of elements which are separately driven to produce first and second ultrasound beams, which together make up the composite beam. In particular, the probe may be a one dimensional medical ultrasound array probe to generate the pressure null using travelling ultrasound waves to induce both an axial acoustic radiation force (the primary radiation force PRF) and a lateral trapping force acting towards a centre point of the composite beam within the pressure null due to the acoustic pressure gradient surrounding the pressure null.

[0039] The apparatus comprises a bespoke Ultrasound Array Research Platform (UARP) designed and built by the University of Leeds and described in greater detail in P. Smith et al., "A PLL-Based Phased Array Method to Minimize Phase Quantization Errors and Reduce Phasing-Lobes", IEEE Ultrasonics Symposium (IUS), 2010, pp. 1837-1840. The probe 2 comprises a one-dimensional medial ultrasound array (LS-8/40EP Prosonic, South Korea) having 96 elements focussed at the same depth z_f and arranged to transmit similar tone bursts. The focal depth z_f can be controlled by controlling the individual elements. In contrast to standard uses of the probe 2, the probe 2 is split into two halves, the driving signals supplied to each half being arranged to be out of phase by 180º. Each half of the probe comprises a 48-element sub-aperture By "similar" tone bursts, it is meant that the driving signals supplied to each half of the array comprise continuous tones having substantially the same centre frequency, but out of phase with one another. It will be

appreciated that in alternative embodiments of the present invention different phase shifts could be used. The result is mixing of the two opposite-phase pressure fields along a line extending away from the centre of the array forming the pressure cancellation. Controlling the position of the array relative to a fluid flow conduit, and controlling the ultrasound beam frequency and amplitude, and the water flow rate, the composite ultrasound beam can be used to manipulate the movement of microbubbles entrained within a fluid flowing through the conduit, as will now be described in greater detail.

[0040] A phase shift of 180º has been found to give the most pronounced null in the acoustic radiation force field. Additionally, while it is generally considered that the probe is driven by continuous, sinusoidal signals, in practice the driving signals supplied to each transducer element or group of elements may be pulsed so as to control the time averaged acoustic radiation force of the composite ultrasound beam.

[0041] The effect of transmitting two ultrasound beams from adjacent sub-apertures driven by identical ultrasound driving signal pulses with a relative phase shift of 180º is schematically illustrated in Figure 2. Figure 2 shows the probe 2 split into a first sub-aperture 8a and a second sub-aperture 8b, each sub-aperture 8a, 8b emitting respective first and second ultrasound beams 10a, 10b to form a composite ultrasound beam 12. At the interface between the first and second beams 10a, 10b there is a pressure null, comprising a region of minimum pressure 14 which extends in a line normally from the centre part of the probe between the two sub-apertures 8a, 8b. The region of minimum pressure is narrowest (and therefore most useful in precisely manipulating particles) at the focal point 16 of the probe 2.

[0042] The generation of a pressure null through the interference of two ultrasound beams of opposite phase can be verified experimentally. Figure 3 shows a plot of the measured pressure field (solid line) using a 200 μm needle hydrophone (Precision Acoustics Ltd., Dorchester, UK) mounted on a 3-D computer controlled translation system arranged to scan along the length of the probe 2 from -0.5 mm to =0.5 mm in 0.1 mm steps at the focal depth z_f. The signal from the hydrophone is digitised using a LeCroy 64xi digital oscilloscope (LeCroy Corporation, Chestnut Ridge, NY, USA) and processed in Matlab (Mathworks Inc., Natick, MA, USA) on a personal computer. The plot is illustrated relative to the position of the probe 2 and the centre point between the two sub-apertures 8a, 8b (referred to as input and output sub-apertures 8a, 8b, for reasons discussed below). It can be seen that the pressure null is aligned with the junction between the sub-apertures 8a, 8b. The ultrasound probe 2 and hydrophone were submerged within a tank of de-ionized and de-gassed water at 20±1ºC. With the driving voltage set to its minimum and for a centre frequency of 7 MHz, the peak negative pressure (PNP) was measured to be

350 kPa relative to maximum measured pressure at lateral positions of ±200 μm. The width and slope gradient of the low-pressure region approximated the data simulated (dashed line) using the Field II package in Matlab (J. Jensen et al., "Field: A Program for Simulating Ultrasound Systems", 10th Nordicbaltic Conference on Biomedical Imaging, Vol. 4, No. 1, 1996, pp. 351-353). The plot of Figure 3 is presented as a normalised PNP plot relative to the measured maximum pressure. It can be seen that there is close conformance between the simulated results and the measured results.

[0043] Referring now to figure 4, this schematically illustrates the use of the apparatus of Figure 1 to accumulate and retain a microbubble population at a target location. Probe 2 is arranged such that the elements extend along the length of a cellulose tube 26 through which microbubbles are flowing, with the probe long axis being substantially parallel to the tube axis. The flowing microbubbles 20 form clusters 22 as they first enter the ultrasound field emitted from probe 2. The line of minimum pressure emerging from the centre point between the two sub-apertures 8a, 8b is marked as 24. As illustrated, microbubbles 20 and aggregates 22 translate towards the opposite tube wall, in particular when flowing through the portion of the composite ultrasound beam generated by the input sub-aperture where the primary radiation force (PRF) is more significant. The Bjerknes principle predicts that microbubbles excited above resonance are attracted to the pressure minima of an ultrasound field. In the experiment shown in Figure 4 a sufficient trapping force (TF) was generated directly opposing the flow direction to trap and accumulate microbubbles against the fluid flow (from left to right), within the low-pressure region. The excitation frequency was set to 7 MHz; hence not all microbubbles were affected by the trapping force.

[0044] Figure 5 is generally the same as Figure 4, and is a micrograph of the cellulose tube showing a microbubble aggregation retained near the pressure null. The face of the probe linear array is parallel to the tube axis and the pressure null is aligned to its centre.

[0045] Figure 6 is a perspective schematic view of the experimental set up of Figure 4 showing the composite ultrasound beam 12 intersecting tube 26 such that the probe focal point 16 is aligned with the centre of the tube 26. The flow direction is indicated by arrow 30 with microbubbles 20 generally flowing from left to right unless trapped within the pressure null 14.

[0046] Phospholipid microbubbles with a size distribution of 1-10 μm and a mean diameter of 2 μm may be used in an exemplary experiment to measure the effectiveness of the acoustic trap. The measured resonant frequency of this microbubble population can be measured via a separate technique (not described herein) and may be 3.8 MHz. The microbubbles are diluted such that they flow freely, for instance to 40×10^6 microbubbles/ml

in de-gassed and de-ionised water. The tube 26 may be a 200 μm cellulose tube in a water tank that is optically and acoustically transparent to mimic a typical blood vessel. The fluid flow rate through the tube 26 can be regulated with a programmable syringe pump (Aladdin-1000, World Precision Instruments, Sarasota, FL, USA), and an inverted microscope (Eclipse Ti -U, Nikon, Melville, NY, USA) can be used to optically image the tube. The syringe pump and the microscope are not illustrated. The ultrasound probe is aligned orthogonal to the tube and at a 45º angle in relation to the bottom of the tank.

[0047] It is necessary to prevent microbubbles accumulating before the input beam due to being prevented from entering the low-pressure region 14. This tendency to accumulate at the edge of the input (upstream) field in an area of high pressure is undesirable as the effect of the high pressure field is to tend to destroy both aggregates and individual microbubbles. To remedy this, in accordance with the present invention the apparatus is arranged to lower the time averaged primary radiation force PRF generated by the input ultrasound beam 10a relative to the output ultrasound beam 10b. In one embodiment the input and output beams are generated by pulsed driving signals received from the signal generator 4 having a different duty factor (though preferably the same pulse frequency). By setting the input sub-aperture pulse duty factor δ_i to be lower than that of the output sub-aperture, δ_o the time averaged PRF of the input beam 10a can be reduced whilst still ensuring that the input and output sub-apertures 8a, 8b are driven by similar pulsed continuous tones that are out of phase such that a pressure null 14 is generated. When both sub-apertures 8a, 8b are transmitting, preferably both sub-apertures are transmitting identical, though out of phase, continuous tones. The input sub-aperture 8a transmits for a small proportion of the time. The pressure null 14 is symmetrical during those periods of time for which both sub-apertures 8a, 8b are transmitting. For a proportion of the time during each pulse cycle only the output sub-aperture 8b is transmitting due to the reduced duty cycle of the input sub-aperture. Consequently, a time averaged plot of the lateral beam profile is not symmetrical. This is due to the reduced time averaged PRF of the input beam. The composite ultrasound beam illustrated in Figure 3 is symmetrical as this represents the form of the beam during the proportion of each pulse cycle for which both sub-apertures 8a, 8b are transmitting. In accordance with one embodiment of the invention the input and output PRF when both sub-apertures 8a, 8b are transmitting is the same. However, it will be appreciated that in alternative embodiments of the invention the input and output PRF may be varied by further controlling the driving signals supplied to the sub-apertures.

[0048] The ratio of input and output duty factors may be defined as $\alpha = \delta_i/\delta_o$. In one experiment a concentration (C_B) of 1×10^9 microbubbles/ml was flowed at a rate Q = 10

ml/hr. The output sub-aperture duty factor was set to 57 % with a pulse repetition frequency of 10 kHz. The measured mechanical index MI at the focal depth was 0.13 with a centre frequency of 7 MHz. The MI was calculated following MI = PNP/$\sqrt{f}$, with the pressure expressed in MPa and the frequency in MHz. The experiment was repeated for α = 0.25, 0.5, 0.75 and 1. The most efficient accumulation of microbubbles was observed for a ratio α = 0.5. The effect of varying the duty cycle in this way is for microbubbles to be able to enter the low pressure region and accumulate without then being able to escape. Specifically, the reduction in the input pressure field, in combination with the direction of movement into the low pressure region being in the direction of fluid flow is sufficient to permit entry of the microbubbles into the low pressure region. Once in the low pressure region, the pressure gradient in both directions (upstream and downstream) is too high for at least a proportion of the microbubbles to exit the low pressure region.

[0049] For the same ultrasound parameters, the performance of the ultrasonic tap was then evaluated at different flow rates with $C_B = 40\times10^6$ microbubbles/ml. Image processing was performed in Matlab (Mathworks Inc., Natick, MA, USA) on the recorded videos to track the diameter and position of the largest cluster. The cluster accumulation duration t_{acc} and the maximum diameter d_{max} it reached are listed in Table 1 for varying values of Q. The cluster resting position before it exited the trap was always between 70 and 110 µm downstream from the pressure null. The cluster resting position is downstream of the trap due to the force acting upon the cluster by the flow of fluid. For a flow rate of 40 ml/hr a 9.5 µm cluster was retained 70 µm away from the pressure null for 1.4 s. The ultrasound parameters were constant with a centre frequency of 7 MHz, a pulse repetition frequency of 10 kHz, a MI of 0.13, δs = 57% and a = 0.5.

[0050] Table 1:

Q (ml/hr)	14	16	20	30	40
d_{max} (µm)	32	22	19	11.9	9.5
t_{acc} (s)	13	5.3	4.5	2.7	1.4

[0051] The syringe driver was then set to its maximum flow rate of 50 ml/hr and the output duty factor was increased to 71 %. The ratio between input and output duty factor was kept at 0.5. For a MI of 0.13, a 12 µm cluster was retained 95 µm beyond the pressure null for 1.6 s. The cluster diameter then became too great for the trapping force to retain it. To intensify the trapping force, the MI was raised to 0.2. Referring to Figure 7, micrographs (a)-(f) were acquired at different times during the experiment. The tracking of the cluster diameter and position are plotted in Figure 8. Micrograph (a) was taken before the ultrasound source was activated and illustrates only imperfections visible between the

tube walls. Although microbubbles were flowing past the region imaged in micrograph (a), they were not visible due to the high flow rate. As revealed in Figure 8 it took 3 s for a first cluster to enter the low-pressure region. Micrograph (b) shows a cluster of 11.4 μm diameter which continued to accumulate microbubbles for a further 5 s (micrograph (c)). The cluster then stopped accumulating microbubbles, and its diameter and position remained around 19 μm and 90 μm for 7 s. In micrograph (d) the cluster size increased beyond 20 μm and, as the trapping force wasn't sufficient, it started to shift away. It was replaced by a smaller 7 μm cluster that remained held (micrograph (e)) until the end of the ultrasound exposure (micrograph (f)).

[0052] In Newtonian flow the wall shear rate is $\gamma_w = 32Q/\pi D^3$ and the corresponding mean velocity is $v_z = D\gamma_w/8$. The tube 26 internal diameter D was observed to expand to 250 μm when filled with water. Thus, a flow rate of 50 ml/hr was expected to induce mean velocities nearing 28 cm/s and wall shear rates of up to 9000 s^{-1}. When exploiting the primary radiation force to translate microbubbles toward the endothelium in large arteries, the acoustic radiation force is applied orthogonally to the flow. Patil et al. (referenced above) reported that, even when excited to near resonance, a mechanical index of 0.15 was required to halt microbubbles for a wall shear rate of 52 s^{-1}. Through the generation of a trapping force opposing the flow, the apparatus in accordance with the present invention is able to halt microbubbles subject to wall shear rates of up to 9000 s^{-1}. Yet the mechanical index of the pressure field at the focal depth was 0.13. The diameter of the tube used in the experiments described above is similar to that of capillaries. However, for the same flow rate, the microbubble accumulation could be more significant in larger vessels such as arteries due to the lower wall shear rates. Table 1 and Figures 7 and 8 demonstrate that, instead of detaching microbubbles as they flow past, clusters could be retained at the target site for several seconds. If the present invention is applied in conjunction with molecular targeting, the facility to hold microbubble clusters could allow the aggregates to form bonds and, due to the secondary radiation force, attract passing microbubbles.

[0053] The use of primary radiation force to enhance targeting presents a challenge in large arteries, where the flow velocity can reach several cm/s. Moreover, flowing microbubble clusters can impede accumulation by engulfing already bound microbubbles. The present invention allows the trapping of populations of microbubbles flowing at up to speeds comparable to those found in the body and at shear rates comparable to those found in the body. Advantageously, the present invention provides the ability to significantly increase the induction efficiency of drugs or genes when microbubbles are used as a vector in a drug or gene delivery system. Indeed, in some medical situations the

targeting efficiency may be sufficiently enhanced that it could potentially negate the need for targeting altogether.

[0054] In accordance with further embodiments of the present invention, the ability to separately control each transducer element in the array can be exploited to achieve further functions. For instance, once a population of microbubbles has been trapped at the target location, the ultrasound beam acoustic radiation force can be significantly increased by increasing the driving signals to some or all of the elements such that some or all of the microbubbles are agitated or destroyed to release their drug or gene loads at the target location. This destruction phase may be applied while simultaneously maintaining the trapping force, either by increasing the power output of the whole array or by driving elements outside of the input and output sub-apertures to generate a destruction pulse. The frequency of the destruction pulse may be adjusted, for instance to correspond to the resonant frequency of the microbubbles.

[0055] In a further embodiment, the ability of the transducer array to perform imaging may be exploited while simultaneously trapping microbubbles at a target location. For instance, the input and output sub-apertures may not comprise the whole of the array, such that unused elements between or to either side of the sub-apertures may be arranged to excite and receive scattered ultrasound signals from the microbubbles. The imaging may be performed continuously and simultaneously with the trapping. Alternatively, or in addition, the imaging may be performed in alternate phases with trapping phases such that a specific imaging ultrasound pulse is transmitted towards the microbubbles while the trapping beam is turned off. The ability to perform trapping and imaging simultaneously provides significant benefits as this allows the microbubbles to be used in a conventional manner as an ultrasound contrast agent to identify a target location. The probe may then be positioned and, if required the trapping driving signals adjusted, such that the focal point of the probe is aligned with the target location. The apparatus can then be used to trap microbubbles at the target location, optionally with microbubble destruction as noted above, while continuously imaging the target location to identify the accumulation of microbubbles and therefore the efficiency of drug or gene induction.

[0056] The present invention may also be applied in non-medical applications. In principle any industrial application including a flowing fluid including entrained particles where it is desirable to be able to manipulate, and particular to trap, the particles at a location of interest may benefit from the present invention. Potential industrial applications including removing pollution or contaminants from water. The only requirement is that the particles are acoustically mismatched to the surrounding fluid.

[0057] Particular embodiments of the present invention described above relate to the control of microbubbles comprising phospholipid encapsulated bubbles between 1 μm and 10 μm, with a typical diameter of 2 μm. However, the present invention is not limited to this. Microbubbles are classified using their shell parameters, gas core and size distribution. Phospholipid encapsulated bubbles are commonly used in medicinal applications. Thicker shell materials such as albumin or polymer may be used. However, a thicker, more rigid shell results in a weaker interaction with the acoustic radiation force. The net radiation force experienced by a bubble or particle within a travelling wave ultrasound field results from the impedance mismatch between the surrounding liquid and the gas core. The present invention is therefore applicable at least to any particle with a core material presenting an acoustic impedance mismatch in relation to the surrounding fluid. The present invention has particular benefits for controlling the movements of particles when the ultrasound frequency is between 1 MHz and 30 MHz. Other particles which can be entrained within a fluid and present an acoustic impedance mismatch include polystyrene beads and biological cells.

[0058] Embodiments of the present invention described above relate specifically to a linear array of ultrasound elements. However, the present invention is not limited to this. For instance, in some applications a two-dimensional array may be appropriate. The only requirement is that the array includes separately controllable elements or groups of elements such that elements or groups of elements corresponding to an upstream or input direction when the array is positioned near a fluid conduit can be controlled to reduce the relative acoustic radiation force so as to permit the particles to enter the trap.

[0059] In certain embodiments the width of the pressure null can be adjusted, either by selection of alternative physical array shapes or by controlling the driving signals applied to the array elements, for instance to adjust the focal depth, in a manner which will be readily apparent to the appropriately skilled person. Additionally, the embodiments of the present invention described above refer specifically to the array being split into input and output sub-apertures which represent respective halves of the array. In alternative embodiments the sub-apertures may represent less than half each of the array, and may be spaced apart, which may vary the width of the pressure null. In further embodiments, in place of a single array being used two separate arrays brought close together may be used to form the respective sub-apertures.

[0060] Throughout the description and claims of this specification, the words “comprise” and “contain” and variations of them mean “including but not limited to”, and they are not intended to (and do not) exclude other moieties, additives, components, integers or steps. Throughout the description and claims of this specification, the singular encompasses the

plural unless the context otherwise requires. In particular, where the indefinite article is used, the specification is to be understood as contemplating plurality as well as singularity, unless the context requires otherwise.

[0061] Features, integers, characteristics, compounds, chemical moieties or groups described in conjunction with a particular aspect, embodiment or example of the invention are to be understood to be applicable to any other aspect, embodiment or example described herein unless incompatible therewith. All of the features disclosed in this specification (including any accompanying claims, abstract and drawings), and/or all of the steps of any method or process so disclosed, may be combined in any combination, except combinations where at least some of such features and/or steps are mutually exclusive. The invention is not restricted to the details of any foregoing embodiments. The invention extends to any novel one, or any novel combination, of the features disclosed in this specification (including any accompanying claims, abstract and drawings), or to any novel one, or any novel combination, of the steps of any method or process so disclosed.

[0062] The reader's attention is directed to all papers and documents which are filed concurrently with or previous to this specification in connection with this application and which are open to public inspection with this specification, and the contents of all such papers and documents are incorporated herein by reference.

[0063] Further modifications to, and applications of, the present invention will be readily apparent to the appropriately skilled person from the teaching herein, without departing from the scope of the independent claims.

CLAIMS:

1. An apparatus for manipulating particles entrained in a fluid, the particles and the fluid having different acoustic impedances, the apparatus comprising:

a signal generator arranged to generate driving signals; and

an ultrasound transmitter comprising at least first and second groups of transmitter elements arranged to transmit respective first and second ultrasound beams in response to respective first and second driving signals received from the signal generator to form a composite ultrasound beam;

wherein the signal generator is arranged to generate first and second driving signals that are out of phase, the first driving signal being arranged such that the first ultrasound beam produces a lower time averaged acoustic radiation force than the second ultrasound beam, and such that at least part of region of the composite ultrasound beam corresponding to the interface between the first and second ultrasound beams exhibits a pressure minimum.

2. An apparatus according to claim 1, wherein the signal generator is arranged to generate first and second driving signals comprising time varying signals having substantially the same frequency.

3. An apparatus according to claim 2, wherein the signal generator is arranged to generate the first and second driving signals with a relative phase shift of approximately 180º.

4. An apparatus according to claim 2 or claim 3, wherein the signal generator is arranged to generate the first and second driving signals such that the first and second driving signals are modulated with a pulsed signal such that the first and second ultrasound beams are transmitted for a proportion of each pulse cycle to reduce the time averaged acoustic radiation force of each ultrasound beam.

5. An apparatus according to claim 4, wherein the signals are pulse modulated by pulse signals having substantially the same frequency as one another, the pulse signal frequency being lower than a frequency of the driving signals.

6. An apparatus according to claim 5, wherein the pulse signals are substantially in phase, but the first driving signal is modulated with a pulse signal having a lower duty factor than the pulse signal used to modulate the second driving signal such that the time averaged acoustic radiation force of the first ultrasound beam is lower than that of the second ultrasound beam.

7. An apparatus according to claim 2 or claim 3, wherein the amplitude of the first driving signal is lower than the amplitude of the second driving signal such that the time averaged acoustic radiation force of the first ultrasound beam is lower than that of the second ultrasound beam.

8. An apparatus according to any one of the preceding claims, wherein the ultrasound transmitter comprises at least one array of transmitter elements which are separately controllable or are controllable in groups, the first and second groups of transmitter elements being included in at least one array.

9. An apparatus according to claim 8, wherein the array comprises a linear array of transmitter elements, the first and second groups of transmitter elements being spatially separated along the array.

10. An apparatus according to claim 8, wherein the array comprises a two-dimensional array of transmitter elements, the first and second groups of transmitter elements being spatially separated across the array.

11. An apparatus according to any one of claims 8 to 10, wherein the first and second groups of elements are adjacent to one another within the array.

12. An apparatus according to any one of claims 8 to 11, wherein the signal generator is arranged to control the driving signals supplied to array elements such that the focal depth of the composite ultrasound beam is controllable.

13. An apparatus according to any one of claims 8 to 12, wherein at least some of the array elements are arranged to receive ultrasound reflections and to supply an imaging signal to an ultrasound imaging device.

14. An apparatus according to claim 13, wherein at least some of the array elements are arranged to transmit imaging ultrasound beams.

15. An apparatus according to any one of claims 8 to 14, wherein the signal generator is arranged to generate destruction driving signals supplied to array elements having a higher amplitude than the first and second driving signals so as to generate a composite ultrasound beam having a higher acoustic radiation force.

16. A method for manipulating particles entrained in a fluid, the particles and the fluid having different acoustic impedances, the method comprising:

positioning an ultrasound transmitter in proximity to a fluid conduit through which the fluid and entrained particles are flowing, the transmitter comprising at least first and second groups of transmitter elements, the first group of elements being positioned closer to an upstream portion of the conduit than the second group of elements; and

generating first and second driving signals at a signal generator and supplying the driving signals to the first and second groups of elements such that they transmit first and second ultrasound beams in response to form a composite ultrasound beam;

wherein the first and second driving signals are out of phase, the first driving signal being arranged such that the first ultrasound beam produces a lower time averaged acoustic radiation force than the second ultrasound beam, and such that a region of the composite ultrasound beam corresponding to the interface between the first and second ultrasound beams exhibits a pressure minimum.

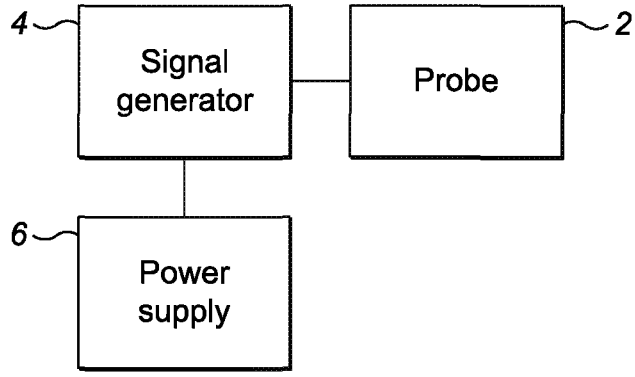
4
Signal generator
Probe
2
6
Power supply

FIG. 1

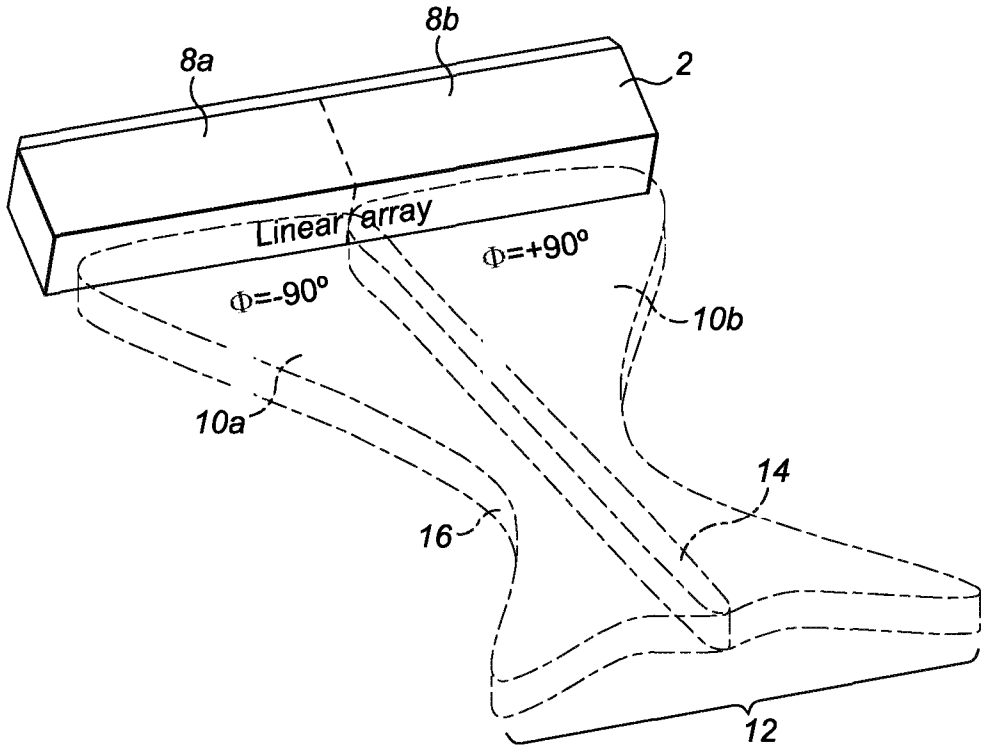
8b
8a
2
Linear array
Φ=+90°
Φ=-90°
10b
10a
14
16
12

FIG. 2

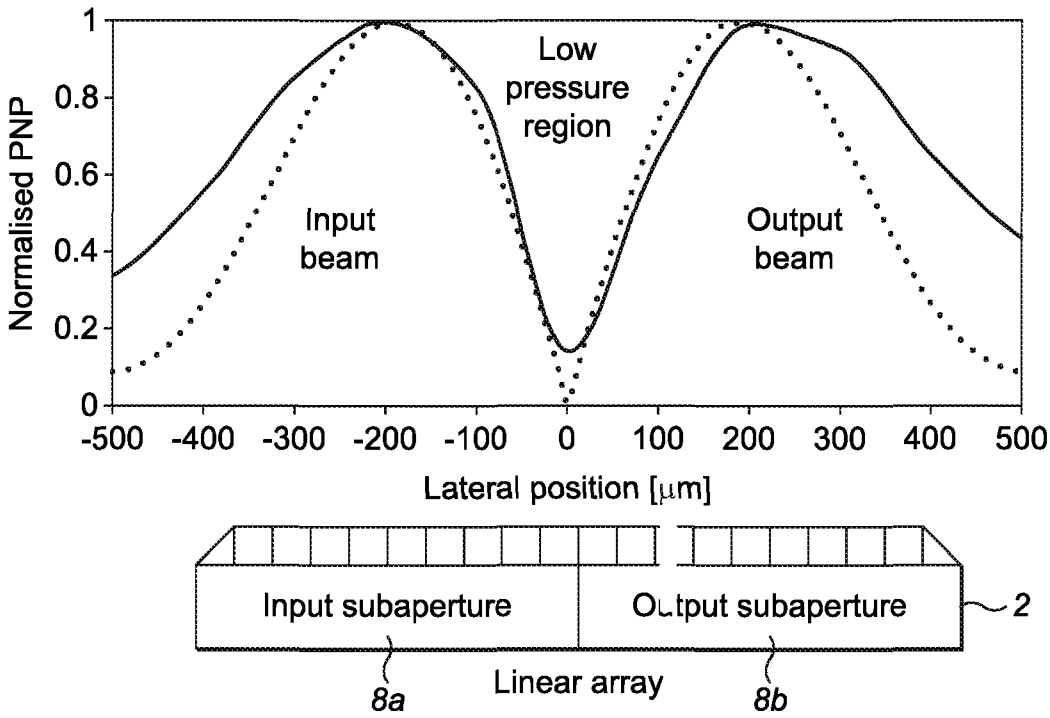
Normalised PNP
1
0.8
0.6
0.4
0.2
0
Low pressure region
Input beam
Output beam
-500 -400 -300 -200 -100 0 100 200 300 400 500
Lateral position [μm]
Input subaperture
Output subaperture
2
8a
Linear array
8b

FIG. 3

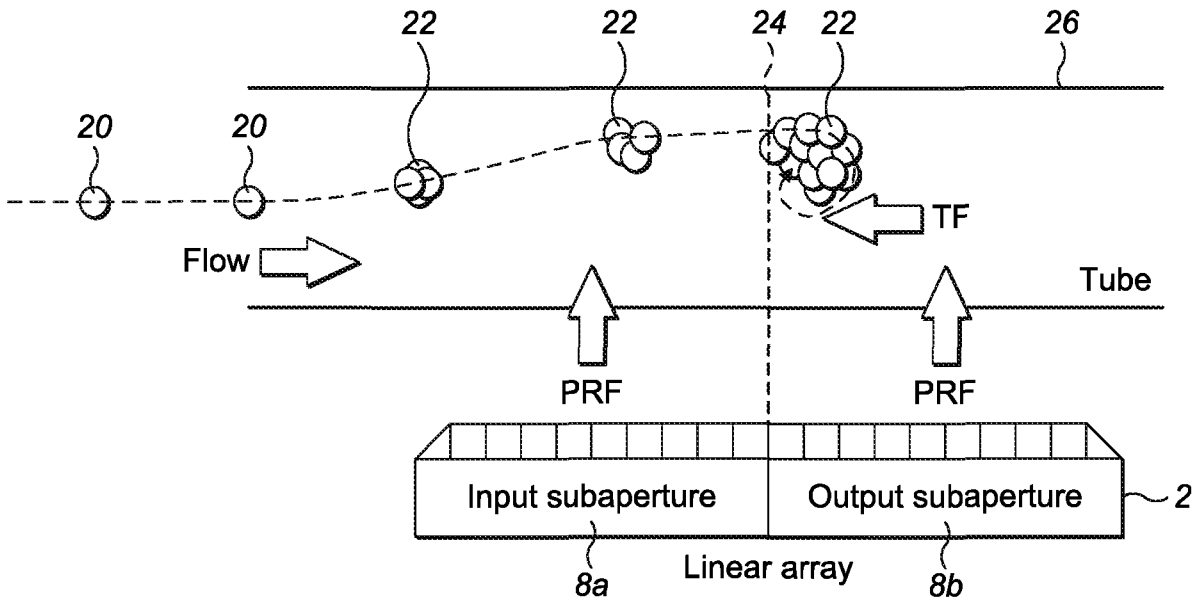
22
22
24
22
26
20
20
TF
Flow
Tube
PRF
PRF
Input subaperture
Output subaperture
2
8a
Linear array
8b

FIG. 4

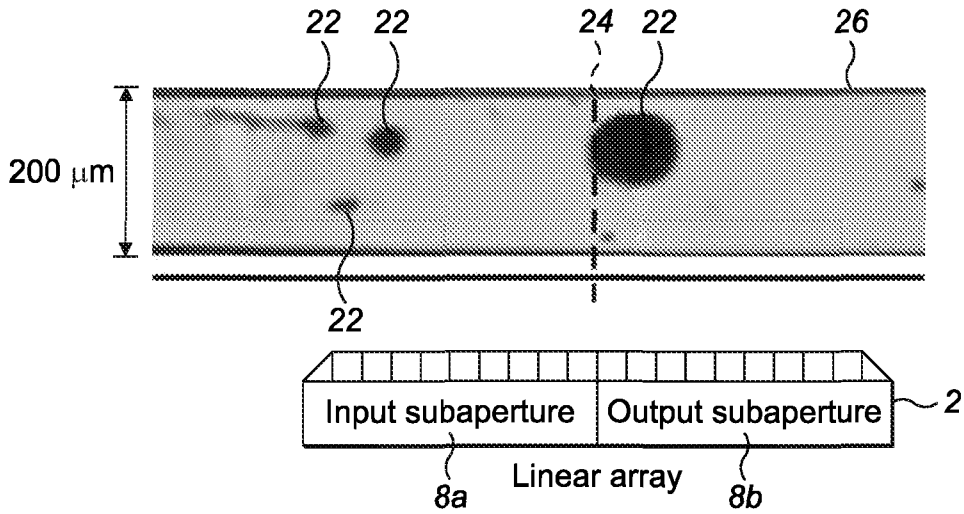
22
22
24
22
26
200 μm
22
Input subaperture
Output subaperture
2
8a
Linear array
8b

FIG. 5

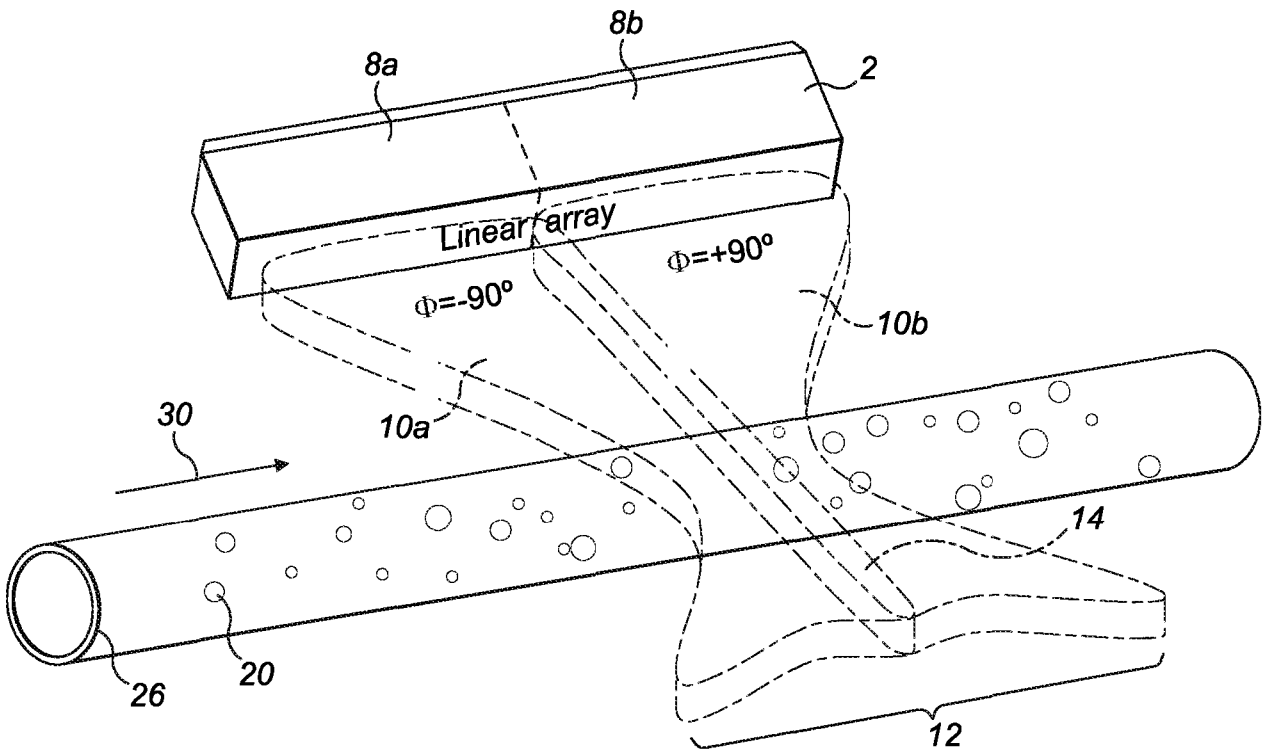
8b
8a
2
Linear array
Φ=+90°
Φ=-90°
10b
30
10a
14
26
20
12

FIG. 6

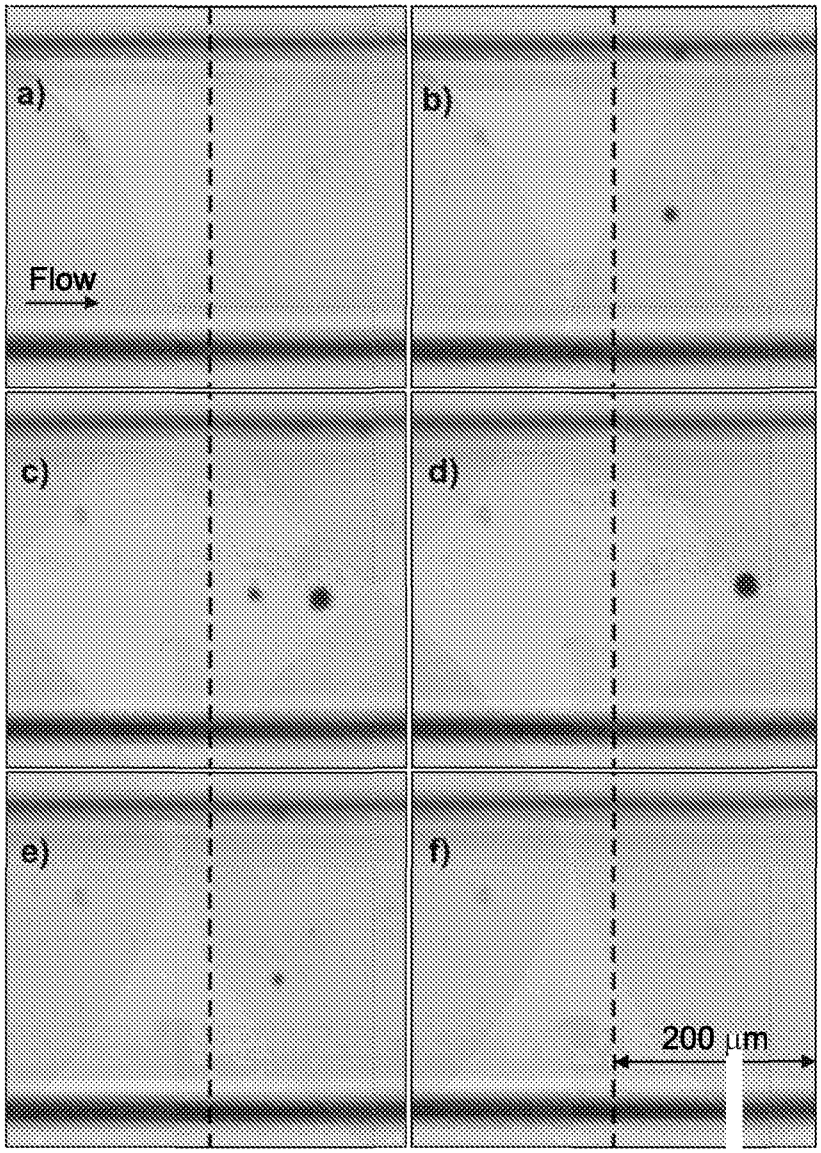
a)
b)
Flow
c)
d)
e)
f)
200 µm

FIG. 7

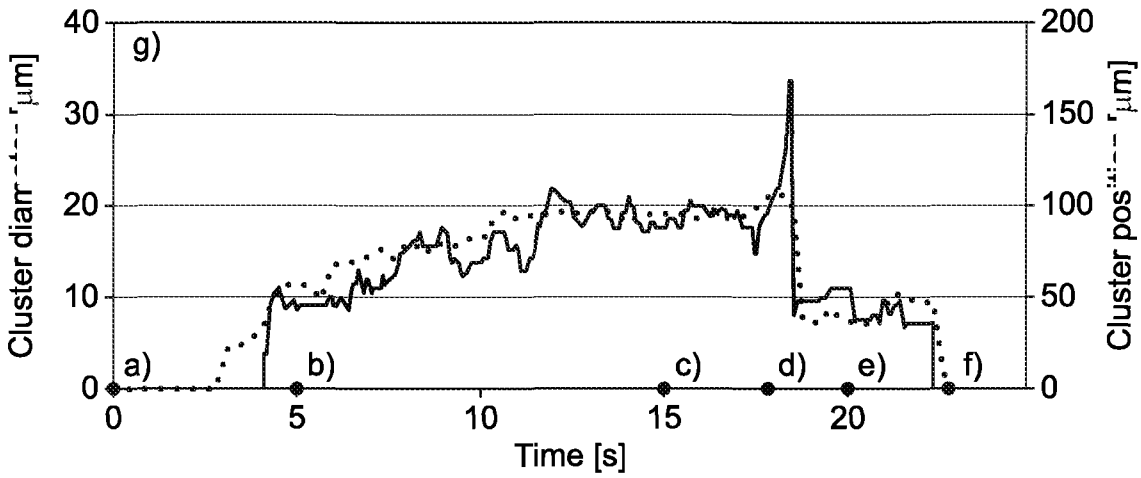
g)
40
30
20
10
0
200
150
100
50
0
a)
b)
c)
d)
e)
f)
0
5
10
15
20
Time [s]

FIG. 8

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2013/050738

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61M37/00 A61B8/08 A61K41/00 A61K49/22
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61M A61K A61B

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2009/297455 A1 (SUIJVER JAN [NL] ET AL) 3 December 2009 (2009-12-03) figures 1, 6, 7 paragraph [0017] paragraph [0044] paragraph [0063] paragraph [0064] paragraph [0065] paragraph [0069] -----	1-16
A	US 4 983 189 A (PETERSON STEPHEN C [US] ET AL) 8 January 1991 (1991-01-08) the whole document -----	1-16
A	US 2006/049114 A1 (HAAKE ALBRECHT [CH] ET AL) 9 March 2006 (2006-03-09) the whole document -----	1-16
	-/--	

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

[X] See patent family annex.

* Special categories of cited documents :

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"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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

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

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

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

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 June 2013	28/06/2013

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Przykutta, Andreas

1

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2013/050738

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6 029 518 A (OEFTERING RICHARD C [US]) 29 February 2000 (2000-02-29) the whole document	1-16
A	WO 2012/028898 A1 (INST NAT SANTE RECH MED [FR]; CHAPELON JEAN-YVES [FR]; LAFON CYRIL [FR) 8 March 2012 (2012-03-08) the whole document	1-16

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2013/050738

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2009297455 A1	03-12-2009	CN 101500649 A	05-08-2009
		EP 2051776 A2	29-04-2009
		JP 2010500091 A	07-01-2010
		US 2009297455 A1	03-12-2009
		WO 2008018019 A2	14-02-2008
US 4983189 A	08-01-1991	NONE	
US 2006049114 A1	09-03-2006	AT 414974 T	15-12-2008
		AU 2003270259 A1	28-09-2004
		DK 1599865 T3	02-03-2009
		EP 1599865 A1	30-11-2005
		US 2006049114 A1	09-03-2006
		WO 2004079716 A1	16-09-2004
US 6029518 A	29-02-2000	NONE	
WO 2012028898 A1	08-03-2012	NONE	