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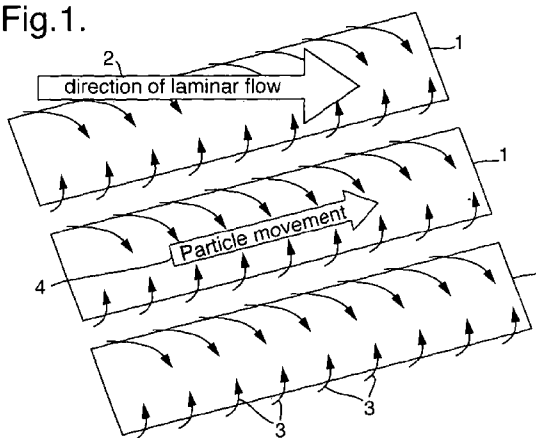
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(54) Title: PARTICLE CONCENTRATION IN A LIQUID FLOW

Fig.1.



(57) Abstract: The present invention is concerned with concentrating particles in a liquid flow through inducing electro-osmotic vortices in the liquid flow, and particularly apparatus comprising a planar interdigitated electrode pair, which electrodes are aligned at an angle to the direction of laminar liquid flow and are driven by an alternating potential; thereby said interdigitated electrode pair being capable of inducing electro-osmotic vortices to alter the direction of particles in the liquid flow, and thereby enable concentration of the particles in the liquid flow.

Particle Concentration in a Liquid Flow

The present invention is generally concerned with apparatus for concentrating and collecting particles in a particle suspension flow, and particularly apparatus comprising a planar interdigitated electrode pair capable of inducing electro-osmotic vortices to alter the direction of particles in the flow and facilitate collection of a concentrated particle suspension.

It is well-known that a particle can become polarised in an inhomogeneous electric field and that the interaction of the induced dipole with the field leads to the movement of the particle towards or away from the area of field inhomogeneity. These effects, termed positive or negative dielectrophoresis respectively, depend on the properties of the particle and the liquid. Dielectrophoresis is the basis for separation and concentration of particles in a large number of apparatus. For example, US Patent Application publication number US 2006/0177815 describes apparatus comprising a fluid flow channel and an electrode pair capable of concentrating particles in a liquid flow through directing particles towards the centre of the fluid flow channel. Concentrating particles in a liquid flow has particular applicability to the fields of detection and identification of particles, and especially in systems whereby a detection means (such as a surface plasmon resonance biosensor) is in liquid flow communication with the particle concentration means. Manipulation of particles in a liquid flow by dielectrophoresis is however inefficient due to the shallow depth of penetration of the dielectrophoretic field. A requirement thus exists for identifying alternative means for concentrating particles in a liquid flow.

Induced electro-osmotic flow, which is movement of a liquid between an electrode pair induced by applying an alternating potential to the electrode pair, is an alternative phenomenon to dielectrophoresis for moving particles in a liquid. Movement of the liquid is driven by a combination of the alternating potential and a diffuse double layer of charge present at each electrode surface, with electro-osmotic vortices originating in the gap between the electrodes. For a detailed explanation, see N.G. Green et al., J. Colloid Interface Science, 1999, 217, 420-422 and N.G. Green et al., J. Am. Phys. Soc., 2000, Physical Review E, 61, 4011-4018 and 4019-4028.

Induced electro-osmotic flow can be used for focussing and collecting particles at a surface, such as described in International Patent Application publication number WO 2004/071668, and for mixing and pumping small volumes of liquid, such as described in US Patent Application publication number US 2008/000772.

The present invention generally aims to provide apparatus utilising induced electro-osmotic flow to manipulate particles in a liquid flow, and especially to provide apparatus for concentrating and collecting particles in a particle suspension flow. The present invention particularly aims to provide apparatus capable of manipulating and concentrating particles within a continuous liquid flow, without removing the particles from the liquid flow. Such an apparatus may have utilisation in a system wherein a detection means is in liquid communication with the apparatus, enabling a continuous flow of liquid through the apparatus to the detection means, and thus delivering a concentrated suspension of particles to the detection means.

In a first aspect the present invention provides apparatus for concentrating particles in a particle suspension flow and collecting the resulting concentrated particle suspension comprising a liquid flow chamber defining a liquid inlet and a liquid outlet arranged so as to provide laminar liquid flow therebetween, a pair of parallel planar electrodes separated by a gap of predetermined distance arranged in the liquid flow chamber to contact the laminar liquid flow, and means for applying an alternating potential difference to the electrode pair such to induce an electro-osmotic vortex in the laminar liquid flow, wherein the liquid outlet comprises a first and second outlet port and the electrode elements are aligned at an angle to the direction of laminar liquid flow such that the induced electro-osmotic vortex drives the particles in the particle suspension flow out of the direction of laminar liquid flow and towards the first outlet port facilitating collection of the concentrated particle suspension at the first outlet port.

The apparatus is capable of recovering a high concentration of particles at the first outlet port, and consequentially a low concentration of particles, or clear liquid, at the second outlet port, whilst providing a continuous flow.

In a preferred embodiment the apparatus comprises multiple pairs of parallel planar electrodes arranged parallel with respect to each other. Multiple pairs of electrodes provide a plurality of gaps, and thus an apparatus capable of providing a plurality of electro-osmotic vortices. Since the pairs of electrodes are parallel and aligned the plurality of electro-osmotic vortices will drive particles to a greater extent out of the direction of laminar flow, and moreover the particles will be driven out of the direction of laminar flow in substantially the same manner. This movement of

particles results in concentration of the particles within the liquid flow. The multiple pairs of parallel planar electrodes may in particular be provided by an interdigitated electrode pair. The parallel planar electrodes would in this embodiment correspond to the interconnected digits of the interdigitated electrode pair.

As used herein, an interdigitated electrode pair has a periodic pattern of interconnected parallel in-plane digit-like or finger-like electrode elements, otherwise known as interconnected digits. Interdigitated electrodes are commonly used electrode configurations. They have also been described as interconnected comb-like electrodes.

In a preferred embodiment, the multiple pairs of parallel planar electrodes are separated by the gap of predetermined distance. Each electrode being separated from its neighbouring electrodes by the same distance provides an apparatus capable of providing electro-osmotic vortices of substantially uniform magnitude throughout the flow chamber.

The electrodes are preferably aligned at a single angle to the direction of laminar liquid flow, which may be provided by the electrodes being linear. Such an arrangement will provide electro-osmotic vortices that drive the particles out of the direction of laminar liquid flow in substantially the same direction (e.g. towards the same wall of a liquid flow chamber). Linear electrode elements provide better control of the particle movement, especially the direction of movement, and thereby concentration of the particles.

The electrodes may be displayed on one face of the flow chamber, and are preferably at, on or adjacent the base of the flow chamber. The electrodes are preferably substantially arranged throughout the length and width of the flow chamber such that the majority, if not the entirety, of the laminar liquid flow may be affected by electro-osmotic vortices. Such an arrangement throughout the flow chamber will provide efficient movement and concentration of the particles.

The electrodes are preferably not aligned in a direction perpendicular to the direction of the laminar liquid flow, and are preferably arranged such that the electrodes align in a direction between about 5° and 85° from the direction of the laminar flow, more preferably between about 5° and 25° , such as 8° , 12° , 16° , 20° and 24° , and most preferably about 16° . In general, better efficiency of concentration is provided by shallower angles to the direction of laminar flow, possibly as a particle will encounter more vortices, originating at each gap between electrodes, on its journey from the bulk laminar flow towards the first outlet port if a shallower angle is used.

The predetermined distance of the gap between the electrodes is a distance suitable for providing an electro-osmotic vortex. The distance of the gap influences the magnitude of the electric field across the gap, and thereby influences the magnitude of the vortex at a given voltage. A distance of between about $50\text{ }\mu\text{m}$ to $200\text{ }\mu\text{m}$ has been shown to be particularly effective in the apparatus of the first aspect, with an optimum gap of about $100\text{ }\mu\text{m}$.

Previous work of the inventors has shown that in a static liquid vortices created by a pair of electrodes separated by a gap of predetermined distance are substantially

circular, and optimally penetrate into the liquid to a distance about half the width of the electrode. The inventors have now shown that the presence of a liquid flow strongly distorts these vortices, and that penetration in this situation is closer to a distance equivalent to the width of the electrodes. Particles are therefore more affected by vortices in a flowing system than in a static system, and that penetration of the vortices can be controlled and/or optimised by manipulation of the width of the electrode and the distance of the gap therebetween.

In embodiments wherein the electrodes are at, on or adjacent the base of the flow chamber, the depth of the flow chamber should preferably be more than a distance equivalent to half the width of the electrodes to accommodate and utilise fully the induced electro-osmotic vortices. The depth of the flow chamber should preferably be a distance less than about five times, and more preferably a distance less than about double, the width of the electrodes to fully utilise the effect of the electro-osmotic vortices to drive the particles. Electrodes with a width of 200 μm perform particularly well in a flow chamber of depth 175 μm . The width of the electrodes is preferably between about 50 μm and 600 μm , more preferably between 100 to 300 μm , and most preferably 200 μm . Particularly efficient movement and concentration of particles is achieved with an interdigitated electrode pair providing linear electrodes (digits) of width 200 μm , with a gap of 100 μm , and aligned at an angle of 15° to the direction of laminar liquid flow. This was achieved in a flow chamber of depth 175 μm or 300 μm , and with a flow rate of laminar liquid flow of either 4.1 or 6.2 $\mu\text{l/min}$. The flow rate of the laminar liquid flow is preferably between 4 $\mu\text{l/min}$ and 25 $\mu\text{l/min}$, and more preferably between 4 $\mu\text{l/min}$ and 7 $\mu\text{l/min}$.

The apparatus preferably further comprises a pump system connected to the liquid inlet and both first and second outlet port of the liquid outlet of the flow chamber to control the flow rate of the laminar liquid flow. The pump system comprises pumps as known in the art, and preferably one pump controlling the flow rate at the inlet, and a pump at each outlet port externally stabilising the flow rate at both outlets, enabling production of a reproducible split, thus avoiding widely fluctuating flow ratios between the two outlets.

The apparatus of the first aspect is preferably for concentrating and collecting biological particles, such as spores, cells and viruses.

In a second aspect the present invention provides use of an apparatus comprising a liquid flow chamber arranged so as to provide laminar liquid flow, a pair of parallel planar electrodes separated by a gap of predetermined distance arranged in the liquid flow chamber to contact the laminar liquid flow, and means for applying an alternating potential difference to the electrode pair, wherein the electrodes are aligned at an angle to the direction of laminar liquid flow such to induce an electro-osmotic vortex in the laminar liquid flow for concentrating particles in a particle suspension flow, and optionally collecting the resulting concentrated particle suspension.

Such an apparatus has now been found to have utilisation in both concentrating particles in a particle suspension flow and collecting a resulting concentrated particle suspension by inducing electro-osmotic vortices in a laminar liquid flow. Use of electro-osmotic flow for concentrating particles in a particle suspension flow is more

effective and efficient than means currently used in the art, such as use of dielectrophoresis.

Thus, in a third aspect the present invention provides use of electro-osmotic flow for concentrating particles in a suspension flow, and optionally collecting a resulting concentrated particle suspension.

The present invention will now be described by a number of examples and with reference to the following drawings, in which

Figure 1 is a schematic of electrode digits 1 angled to the plane of direction of laminar flow 2, the vortices 3 produced in the flow, and the consequent direction of particle movement 4;

Figure 2 illustrates a test planar interdigitated electrode pair comprising digits arranged at various angles to the direction of laminar flow;

Figure 3 is an image of the electrode pair of Figure 2 having a liquid flow of particles over the surface without an applied electric field. The flow is from right to left at a slight downwards angle;

Figure 4 is an image of the electrode pair of Figure 2 having a liquid flow of particles over the surface with an applied electric field. The flow is from right to left at a slight downwards angle. The vortices distort the flow and particles accumulate at the downstream edge of each digit;

Figure 5 is a magnified trail of a single particle over the electrode pair of Figure 2 as compared to the direction of laminar flow. The data coincides with movement of the particle over the electrode digits which are at angles of 20° and 25° to the direction of laminar flow. The overall diversion from the direction of flow is 11.2° ;

Figure 6 is a schematic of one embodiment of apparatus of the present invention. A computer controlled three-channel syringe pump supplies liquid to the flow chamber at a single inlet and removes liquid from the flow chamber at two outlets. A signal generator supplies the electrode arrangement with an alternating potential, and a microscope camera captures images for processing;

Figure 7 is a plot of light intensity at both outlets of the apparatus of Figure 6, and the ratio of light intensity at the outlets at a laminar flow rate of $8.2 \mu\text{l/min}$. The fluorescence is higher at one outlet with the ratio between outlets stabilising at an average of 2.75;

Figure 8 is a plot of light intensity at outlet 1 and outlet 2 of the apparatus of Figure 6, and the ratio of light intensity at the outlets at a laminar flow rate of $11 \mu\text{l/min}$, with the light intensity adjusted at the start of the experiment. The fluorescence is higher at outlet 1 than outlet 2. The ratio increases as the flow pattern is established, and stabilises at an average of 2.2;

Figure 9 is a schematic of apparatus to investigate movement and concentration of particles in a flow chamber. A computer controlled three-channel syringe pump

supplies liquid to the flow chamber at two inlets and removes liquid from the flow chamber at one outlet. A signal generator supplies the electrode arrangement with an alternating potential, and a microscope camera captures images for processing;

Figure 10 a) and b) are images of liquid comprising fluorescent latex beads flowing through the flow chamber of the apparatus of Figure 9. The beads are the light area in the centre of the images, flanked by buffer on both sides. Figure 10 a) is the liquid flow without applied electric field. Figure 10 b) is the liquid flow with applied electric field;

Figure 11 a) and b) are graphs of fluorescence in the flow chamber of the apparatus of Figure 9 across the width of the flow chamber measured within a distance of 0 to 3 mm of the inlet (Figure 11 a) and a distance within 8.5 to 11.5 mm of the inlet, in the presence of fluorescent latex beads, and both with and without an applied electric field;

Figure 12 is a graph of the outlet concentration ratio for a $\frac{1}{2}$ virtual split against flow cell depth with an electrode digit width of 200 μm , at various flow rates;

Figure 13 is a graph of the outlet concentration ratio for a $\frac{1}{2}$ virtual split against electrode digit width with a flow cell depth of 175 μm , at various flow rates;

Figure 14 is a graph of the outlet concentration ratio for a $\frac{1}{2}$ virtual split against electrode digit angle to the direction of the laminar flow with a flow cell depth of 175 μm , and an electrode digit width of 400 μm , at various flow rates;

Figure 15 is a graph of the outlet concentration ratio for a $\frac{1}{2}$ virtual split against electrode digit gap with a flow cell depth of $175\text{ }\mu\text{m}$, and an electrode digit width of $400\text{ }\mu\text{m}$, at various flow rates;

Figure 16 represents the division of an interdigitated electrode pair into cells for modelling purposes; and

Figure 17 is a model for a 5×21 cell electrode digit system with redistribution factors of $\frac{1}{3}$ and $\frac{2}{3}$.

Example 1

A first prototype apparatus was built and showed promising results. Digits of a planar interdigitated electrode pair were angled approximately 15° to the direction of flow containing 100 nm fluorescent latex beads. The experiment was recorded without and with an electric field applied to the electrodes. With the field applied the particles arranged into clearly visible bands of curved trajectory, illustrating movement of particles out of the direction of the laminar flow in the presence of induced electro-osmotic vortices in the liquid.

Example 2

Having regard to Figure 2, to optimise the angle of the electrode digits to the direction of liquid flow for pushing particles out of the direction of liquid flow and thereby altering their direction, an electrode structure 5 was designed that allowed the testing of multiple angles at the same time. The electrode arrangement consisted of

interdigitated electrodes (dark areas 6) radiating from a central point with an angle of 5° between neighbouring digits and a constant inter-digit gap (light areas 7) of 100 μm . Having regard to Figures 3 and 4, visual inspection showed vortices to distort the flow and particles to clump and travel along the downstream edge of the digits, at an angle to the main liquid flow. A small number of clusters were tracked through the flow chamber using a simple image-processing algorithm to evaluate the cluster trajectories, with and without electric field applied. Considering electrode digit angles of 20° and 25° to the direction of liquid flow, the clusters moved in the direction of the laminar flow without an applied electric field. However, with an alternating electric field applied, the particles clearly diverted from the original flow path. Having regard to Figure 5, numerical analysis showed the direction and movement of a particle 8 to divert from the direction of laminar flow 9 by approximately 11° . The clusters are deflected away from the flow as they encounter the downstream vortex and become trapped briefly before getting pushed along the electrode digit edge.

Example 3

Having regard to Figure 6, apparatus comprised a flow chamber having one inlet 10 and two outlets 11, a planar interdigitated electrode pair at the base of the flow chamber, and a computer controlled three-channel pump system 12. Effects such as surface tension at the outlet of the flow chamber can have a significant effect on the flow ratio between outlets, leading to wildly fluctuating ratios. The three channel pump system provides a reproducible split of the liquid at the outlets of the flow chamber, thus overcoming the problem of fluctuating flow ratios. The computer-controlled three-channel pump system was designed to pump liquid into the inlet of the flow chamber 13 while at the same time removing liquid at controlled flow rates

from the outlets. The set-up also comprises a signal generator 14 supplying the electrode arrangement 15 with an alternating potential, a microscope camera 16 to capture images of the electrode surface, and a computer 17 to control the whole system.

To optimise the angle of the electrode digits to the direction of laminar liquid flow for pushing particles out of the direction of liquid flow, electrode structures were designed with various angles, digit widths and inter-electrode digit gaps, covering angles between 8°, 12°, 16°, 20° and 24°, digit widths of 100 µm, 200 µm, 400 µm, 600 µm and inter-electrode digit gaps of 50 µm, 100 µm and 200 µm. Selected devices were fabricated and tested to identify optimum conditions for concentration. Devices were tested with different flow rates and flow chamber width.

Solutions containing 100 nm latex beads were pumped through the flow chamber and over the planar interdigitated electrode at a range of flow rates (16.5 µl/min, 11.0 µl/min, 8.2 µl/min, 5.5 µl/min and 4.1 µl/min). A fluorescence microscope was focused on the two outlet channels of the flow chamber and monitored the fluorescence intensity in the two outlet streams. Since the fluorescence of the stream is proportional to the concentration of particles within the stream, the ratio between the light intensity in the streams is proportional to the ratio of particle concentration.

The flow chamber has a volume of approximately 80 µL. Considering the range of flow rates analysed a volume of 144 µL per flow rate was chosen to be pumped through the chamber from the syringe pump system. The system was tested without applied field at the highest flow rate to make sure the flow chamber fully filled with

particle suspension, and to measure intensity levels at the outlets. For each successive flow rate, 144 μL of liquid medium was pumped through the chamber with the applied field. The flow rate was decreased to the next flow rate once a volume of 144 μL had passed. To aid in comparison of the data, images were not captured at fixed time points, but instead at fixed volume points. In most experiments, images were captured every 1 μL .

To automatically monitor the movement and separation of particles, a 50-pixel-wide band was extracted from each image spanning across both outlet channels. The pixels in line with the flow were averaged to decrease noise. This resulted in a line giving the relative concentration of particles across the two outlet channels. Lines from different time points were plotted in a contour plot to visualise the change of concentration against time.

To quantify the results, the position of the outlet channels in the image were identified and the whole intensity across each channel was averaged. To correct for background light, the channels were imaged at the beginning of the experiment whilst containing just water. The background intensity was then subtracted from the average intensity of the channel. The corrected average intensity in each channel was plotted against the processed sample volume, and the ratio between the signals was measured to determine by how much the concentration had increased. Experiments were conducted at different flow rates in sequence decreasing the flow rate. Since the flow chamber has a volume of approximately 80 μL , the effect can only be measured after more than 80 μL has passed. The average ratio was calculated for the volume from 80 to 140 μL to measure the effect.

Having regard to Figures 7 and 8, results showed an increase in concentration between the two outlets 18 and 19, and therefore an increase in the ratio 20 of the concentration at the outlets over the period of the experiment. Under appropriate conditions, the concentration in the high-concentration channel 18 was more than three times higher than in the low concentration channel 19.

Example 4

To understand the separation process better a model system was constructed that allowed better tracking of the particle distribution. Having regard to Figure 9, the flow chamber does not separate the flows at the outlet, but has two inlets 21 and only one outlet 22. One of the inlet channels was split within the flow chamber to provide two further channels, allowing a channel containing beads to be pumped into the centre of the flow chamber, where it was sandwiched between two channels of buffer without beads. Comparing images with and without electric field allowed the trajectory of the beads to be traced precisely within the flow chamber. The flow chamber comprises at its base a planar interdigitated electrode. A microscope 16 with video system was setup to monitor the whole width of the flow chamber 13 and images were taken along the length of the flow chamber to monitor how the distribution of beads changed along the flow chamber. To overcome the limited field of view of the microscope (4.5mm) two images were captured at each position and combined to a single image using image processing software after the experiment. Since the flow inside the flow chamber was strictly laminar theoretical output ratios could be assessed and calculated by positioning 'virtual outlets' onto the captured images. This

approach also allowed greater efficiency since a wide range of different output ratios could be evaluated.

Electrodes with 400 μm wide digit tracks, 100 μm gaps, 50 mm length and various electrode angles were tested to identify optimum conditions for separation. Devices were tested with different flow rates and flow chamber width. Images were analysed in two ways. Firstly, the distribution of particles against the width of the flow chamber was measured at different points along the length of the flow chamber. Secondly, the boundary between buffer containing beads and buffer without beads was monitored close to the inlet, since it showed the movement of the vortex front.

Having regard to Figures 10a and 10b, the images show the stream of beads (light area in the centre of the image, 23) in the flow chamber, flanked by streams of clear buffer (dark area on both sides of the image, 24). The sides of the flow chamber show as faint lines running close to the edge of the images. Figure 10a shows the stream without a field, and Figure 10b with the field switched on. The flow spreads in both directions with the field on, however the spread is not symmetrical, with more beads being pushed to the left of the image

Having regard to Figures 11a and 11b, distribution of brightness at the inlet (within a distance of 0-3mm; Figure 11a) and further in the flow chamber (within a distance of 8.5-11.5mm, Figure 11b). The distribution of beads can be measured by plotting the brightness of the image against width of the flow chamber. For comparison the distribution of brightness is plotted with 25 and without 26 the field applied. These measurements confirm an asymmetry in the particle distribution. More particles move

to the left of the image and start to form a peak close to the wall after a distance of 10 mm.

These experiments confirmed the ability of apparatus to concentrate particles, but also indicated limitations in the set-up; particles clearly preferentially moved in one direction, but a proportion of particles move in the opposite direction. This limited the overall efficiency of the system, since the concentration effect did not increase linearly with flow chamber length, but reached a stable state in very long flow chambers.

Example 5

The following variation in parameters was investigated to optimise the apparatus: flow chamber height/depth of 75 μm , 120 μm , 175 μm and 300 μm ; electrode digit angle of 15° and 24° to the direction of laminar flow; electrode digit width of 100 μm , 200 μm , 400 μm and 600 μm ; gap between digits of 50 μm , 100 μm and 200 mm; and flow rates of 3.2 $\mu\text{l/min}$, 4.1 $\mu\text{l/min}$, 6.6 $\mu\text{l/min}$, 13.1 $\mu\text{l/min}$ and 25 $\mu\text{l/min}$.

The flow chamber design used two inlets and one outlet, allowing the flow of beads to be “sandwiched” between two flow streams of buffer. This allows better tracking of the particles, but also allows particles to flood the whole width of the cell by disabling the flow of buffer. Since the flow regime inside the flow chamber is strictly laminar, ‘virtual outlets’ at different flow ratios could be extracted from the images. The optical part of the system was designed to work without the use of a microscope, with the introduction of a video camera modified to view fluorescence. A 490 nm blue power LED was used to excite the fluorescent beads, whilst using a colour camera

capable of separating the signal into red, green, and blue channels. However, only the green channel was used as the blue channel recorded reflected light from the excitation source, whilst the red channel recorded only noise. Software was written to control camera, pump and signal generator, and to automate the experiments. Large parts of the data analysis were automated and could run in parallel. A single set of experiments consisted of beads at five flow rates as well as a purge with buffer in order to calibrate the background signal. At each flow rate, the whole volume of the flow chamber was first flushed without an applied electric field to establish distribution of beads, then the field was applied and the change in distribution observed. An image was captured for every 1 μ l passing through the flow chamber. The data was corrected using calibration curves to remove any artefacts as a result of nonlinear response of the camera.

The apparatus achieved good concentration, but also revealed some surprising results since most parameters did not behave linearly. 'Virtual outlet' data was derived from a 1/2 flow-split of the volume of the flow chamber along the length of the flow chamber. The ratio of fluorescence at the 'virtual outlets' provides a useful indication as to the potential performance of an apparatus comprising two outlets, though they are not optimal as particles predominately build up in a narrow band along the edge of the flow chamber. All experiments were performed on 7 mm wide flow chambers with 100 nm fluorescent latex beads.

The concentration ratio achieved between each 'virtual outlet' was calculated as the ratio of fluorescence at the two outlets after the electric field was applied / the ratio of fluorescence at the two outlets before the electric field was applied. This approach

was chosen since the ratio between outlets without an electric field was often more or less than 1, and thus some compensation for this effect was required. This effect is believed to be caused by factors such as uneven excitation illumination, defects on the electrodes, or beads stuck to the surface of the flow chamber.

Flow chamber Depth

Having regard to Figure 12, the flow chamber depth has some influence on the efficiency of concentration with 200um wide digit electrodes performing best in a 175um deep flow chamber. Performance in a deeper 300um flow chamber was also good, but shallower chambers (120um and 75 um) decreased the efficiency of concentration. This would suggest that the vortex extends into the liquid medium a depth almost equivalent to the width of the digit. This is further than expected based on previous results in a static system, in which vortices were shown to extend to a depth of approximately half the electrode width into the liquid medium. Moreover, a flow was expected to flatten rather than improve the depth of the vortex.

Electrode Width

Having regard to Figure 13, best results were achieved with 200um wide digit electrodes, with 100um inter electrode gap, and angled at 15° to the plane of direction of the liquid flow. Electrodes having a width of 100um were largely ineffective, indicating there to be an optimum width for the digit electrode.

Electrode Angle

Having regard to Figure 14, in general, shallower angles provided a better performance. This may be because a particle will encounter more vortices, originating

at each gap between digits, on its journey from the bulk flow towards the first outlet if a shallower angle is used. An angle of 16° to the plane of direction of the liquid flow performed particularly well with $200\text{ }\mu\text{m}$ wide digit electrodes at a flow rate of $6.6\text{ }\mu\text{l/min}$.

Inter-Electrode Gap

Having regard to Figure 15, the distance of the gap between digits influences the strength of the electric field across the gap, and thereby the magnitude of the vortices at a given voltage. The system performed best with a $100\text{ }\mu\text{m}$ gap.

Edge Effects

Better concentration is achieved when a liquid flow of beads is 'sandwiched' between two liquid flows of buffer, rather than a system without these additional buffer flows. This may be as a result of secondary effects re-distributing beads from the flow chamber edge back into the bulk. This effect was particularly prominent at higher flow rates.

Flow Rate

The device performed consistently well at flow rates of 4.1 or $6.2\text{ }\mu\text{l/min}$. Broader flow chambers should sustain higher flow rates.

Modelling of Results

The examples show that redistribution of particles within the flow chamber does not happen continuously, but only at transitions over an electrode digit edge, when a vortex at the trailing edge of the electrode pushes the particle in the desired direction

for concentration, and a vortex at the leading edge of each electrode digit pushes particles away from the desired direction, in fact pushing particles off course. The laminar flow distorts the vortices at the edges, however the particles exhibit a wave-shaped trajectory in the desired direction for concentration as the vortex at the trailing edge is prominent.

Having regard to Figure 16 and Figure 17, the phenomenon providing movement of particles is therefore not continuous but can be broken down into cells containing a single redistribution step comprising one transition in the direction of the flow and one perpendicular to the flow. Each cell passes on a proportion of the particle concentration to neighbouring cells, based on a redistribution factor at both the leading edge and at the trailing edge of an electrode digit. A redistribution factor is a numerical indication for the proportion of particle concentration that is directed towards neighbouring cells. Having regard to Figure 17, the redistribution factor at the trailing edge will be higher (e.g. $2/3$) than that for the leading edge (e.g. $1/3$), since the vortex at the trailing edge is more prominent. The cells at the edges of the flow chamber can only pass in one direction since there is only a single neighbouring cell. In this case a proportion of the particle concentration remains in the cell at the edge of the flow chamber. This results in particles slowly shifting towards one edge of the flow chamber. This model explains the build up of particles along one edge but it also explains why the system reaches equilibrium. After around 20 passes the top cell reaches a particle concentration of 2.6 times the original value while the next lower cell reaches a particle concentration of 1.3. This means the top cell passes $2.6 \times (1/3) = 0.86$ downwards while the lower cell passes $1.3 \times (3/3) = 0.86$ upwards resulting in a stable pattern across the cell. Model and experimental results show a good match,

based on redistribution factors of $1/3$ and $2/3$, even though the experimental results show less linear growth at the high concentration side of the flow chamber and less depletion at the low concentration side.

The maximum concentration ratio that can be achieved depends on the redistribution factors between the cells, however further modelling has confirmed that it also depends on the number of cells along the width of the flow chamber. Increasing this number leads to a higher concentration ratio. Therefore the efficiency can be improved by making the flow chamber wider to fit more electrode digits along the width. It also explains why shallower angles such as 15° work better since at shallower angles more cells can be accommodated across the width of the flow chamber. This also matches experimental data where the 200 μm wide electrodes provide better results than wider electrodes.

The model also predicts that the band containing the highest concentration should be about the width of one cell. In our system with optimum choice of parameters this would equate to about $1/22$ of the flow chamber width. This is confirmed by revisiting the 'virtual outlet' distribution model, where the concentration starts to plateau at approximately a $1/20$ flow split ratio, with a theoretical 7-fold improvement in concentration.

From these examples it is clear that the minimum requirement for driving a particle out of laminar liquid flow is a pair of parallel electrodes separated by a gap of a predetermined distance, wherein the electrodes align at an angle to the direction of laminar liquid flow. The effect is however more pronounced, and more effective,

when multiple pairs of electrodes parallel with respect to each other are provided. The multiple pair of electrodes may conveniently be provided by an interdigitated electrode pair, wherein the digits of the interdigitated electrode pair correspond to the multiple pairs of electrodes.

Claims

1. Apparatus for concentrating particles in a particle suspension flow and collecting the resulting concentrated particle suspension comprising:

a liquid flow chamber defining a liquid inlet and a liquid outlet arranged so as to provide laminar liquid flow therebetween, a pair of parallel planar electrodes separated by a gap of predetermined distance arranged in the liquid flow chamber to contact the laminar liquid flow, and means for applying an alternating potential difference to the electrode pair such to induce an electro-osmotic vortex in the laminar liquid flow, wherein the liquid outlet comprises a first and second outlet port and the electrodes are aligned at an angle to the direction of laminar liquid flow such that the induced electro-osmotic vortex drives the particles in the particle suspension flow out of the direction of laminar liquid flow and towards the first outlet port facilitating collection of the concentrated particle suspension at the first outlet port.

2. Apparatus according to Claim 1, wherein the apparatus comprises multiple pairs of parallel planar electrodes arranged parallel with respect to each other.

3. Apparatus according to Claim 2, wherein the multiple pairs of parallel planar electrodes are provided by an interdigitated electrode pair.

4. Apparatus according to Claim 2 or Claim 3, wherein the pairs of parallel planar electrodes are separated by the gap of predetermined distance.

5. Apparatus according to Claims 1 to 4, wherein the electrodes are aligned neither parallel nor perpendicular to the direction of laminar liquid flow.
6. Apparatus according to Claims 1 to 5, wherein the electrodes are linear.
7. Apparatus according to Claims 1 to 6, wherein the electrodes are displayed on one face of the flow chamber.
8. Apparatus according to Claims 1 to 7, wherein the electrodes are displayed substantially throughout the length and width of the flow chamber.
9. Apparatus according to Claims 1 to 8, wherein the electrodes are aligned in a direction between about 5° and 85° from the direction of the laminar liquid flow.
10. Apparatus according to Claim 9, wherein the electrodes are aligned in a direction between about 5° and 25° from the direction of the laminar liquid flow.
11. Apparatus according to Claims 1 to 10, wherein the predetermined distance of the gap is between $50\text{ }\mu\text{m}$ and $200\text{ }\mu\text{m}$.
12. Apparatus according to Claims 1 to 11, wherein the electrodes have a width of between $50\text{ }\mu\text{m}$ and $600\text{ }\mu\text{m}$.
13. Apparatus according to Claim 12, wherein the electrodes have a width of between $100\text{ }\mu\text{m}$ and $300\text{ }\mu\text{m}$.

14. Apparatus according to Claims 1 to 13, further comprising a pump system connected to the liquid inlet and both first and second outlet port of the liquid outlet of the flow chamber to control the flow rate of the laminar liquid flow.

15. Use of an apparatus comprising a liquid flow chamber arranged so as to provide laminar liquid flow, a pair of parallel planar electrodes separated by a gap of predetermined distance arranged in the liquid flow chamber to contact the laminar liquid flow, and means for applying an alternating potential difference to the electrode pair, wherein the electrodes are aligned at an angle to the direction of laminar liquid flow such to induce an electro-osmotic vortex in the laminar liquid flow for concentrating particles in a particle suspension flow.

16. Use of an electro-osmotic flow for concentrating particles in a suspension flow, and collecting a resulting concentrated particle suspension.

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Fig.1.

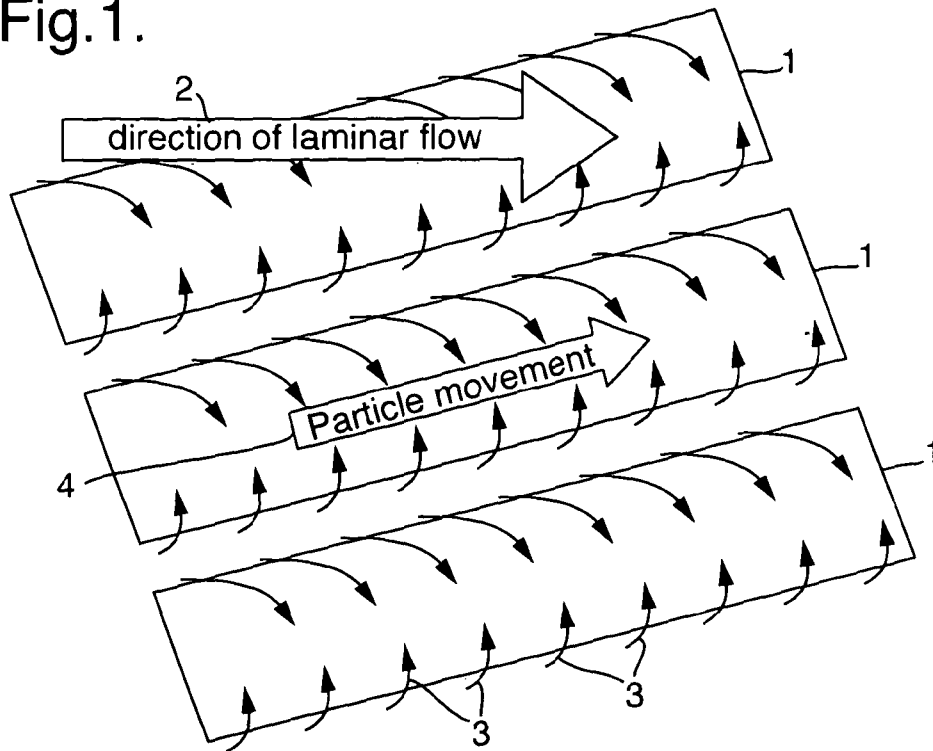
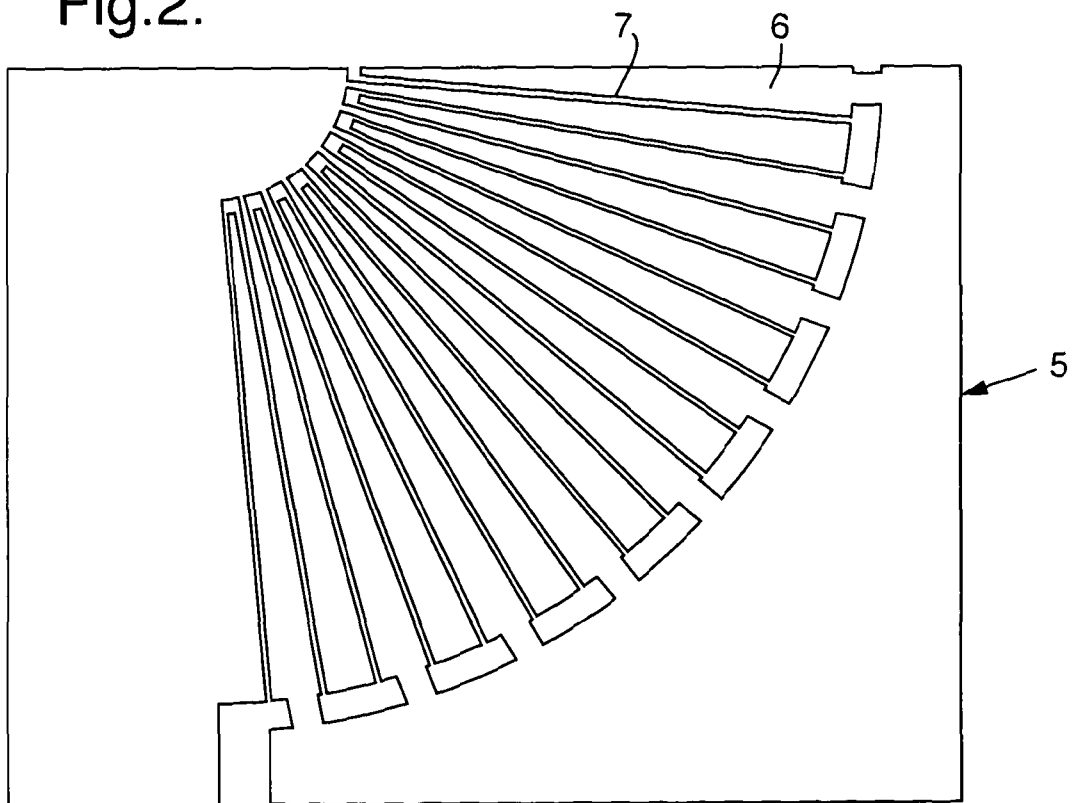


Fig.2.

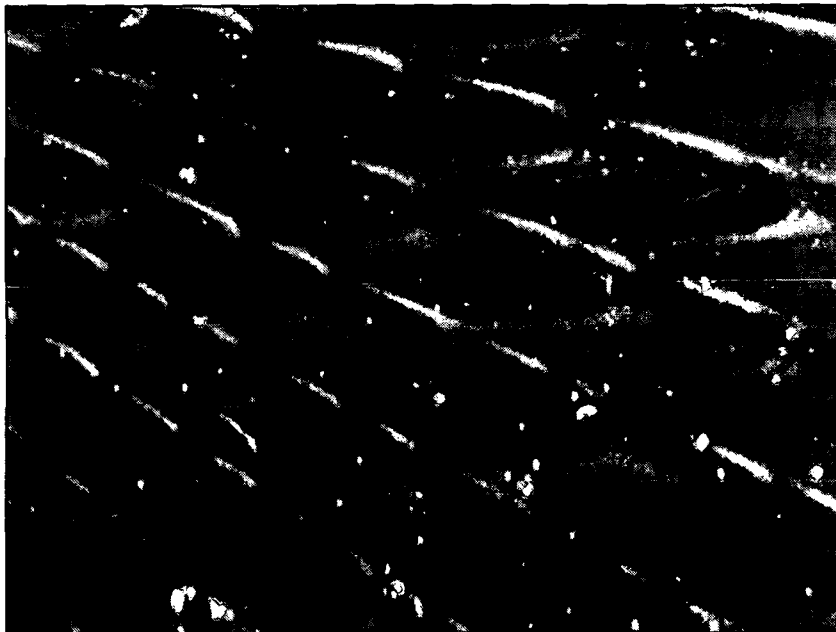


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Fig.3.

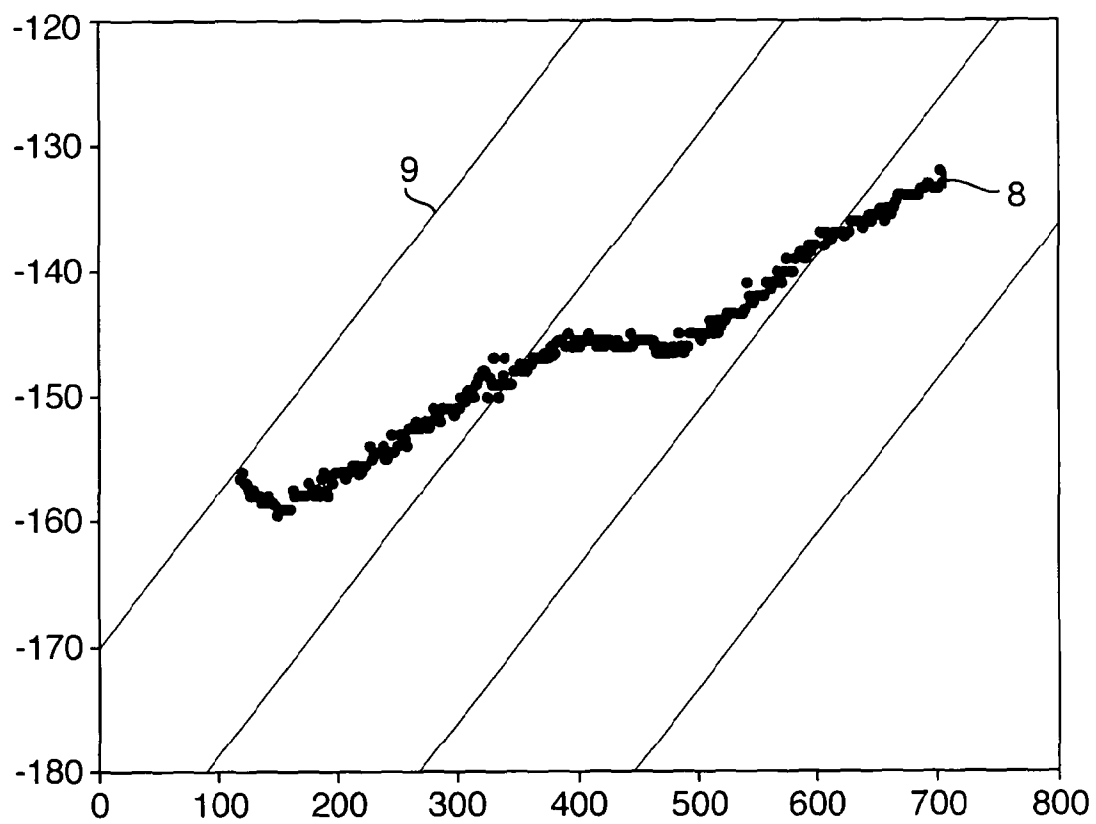


Fig.4.



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Fig.5.



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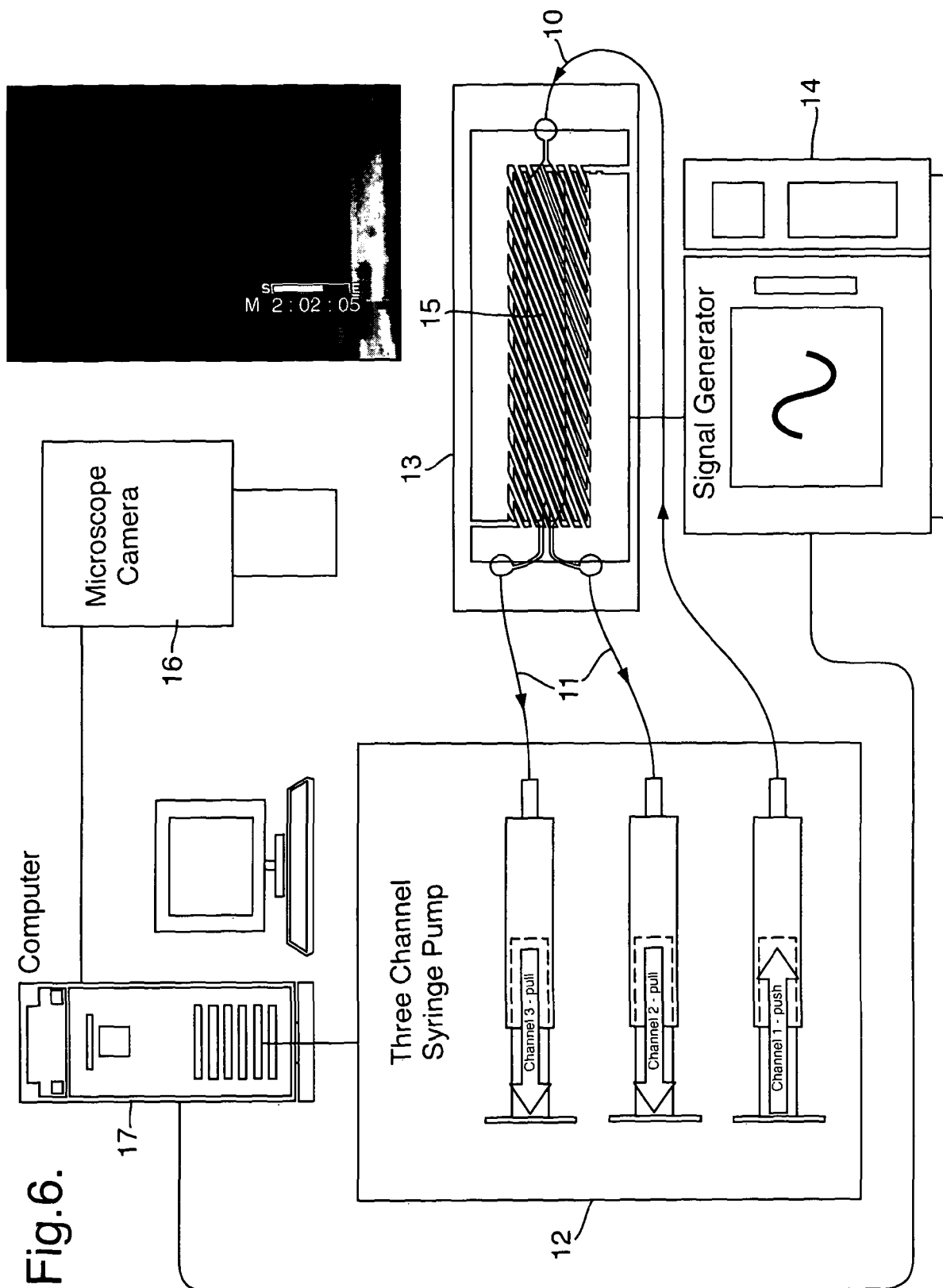


Fig. 6.

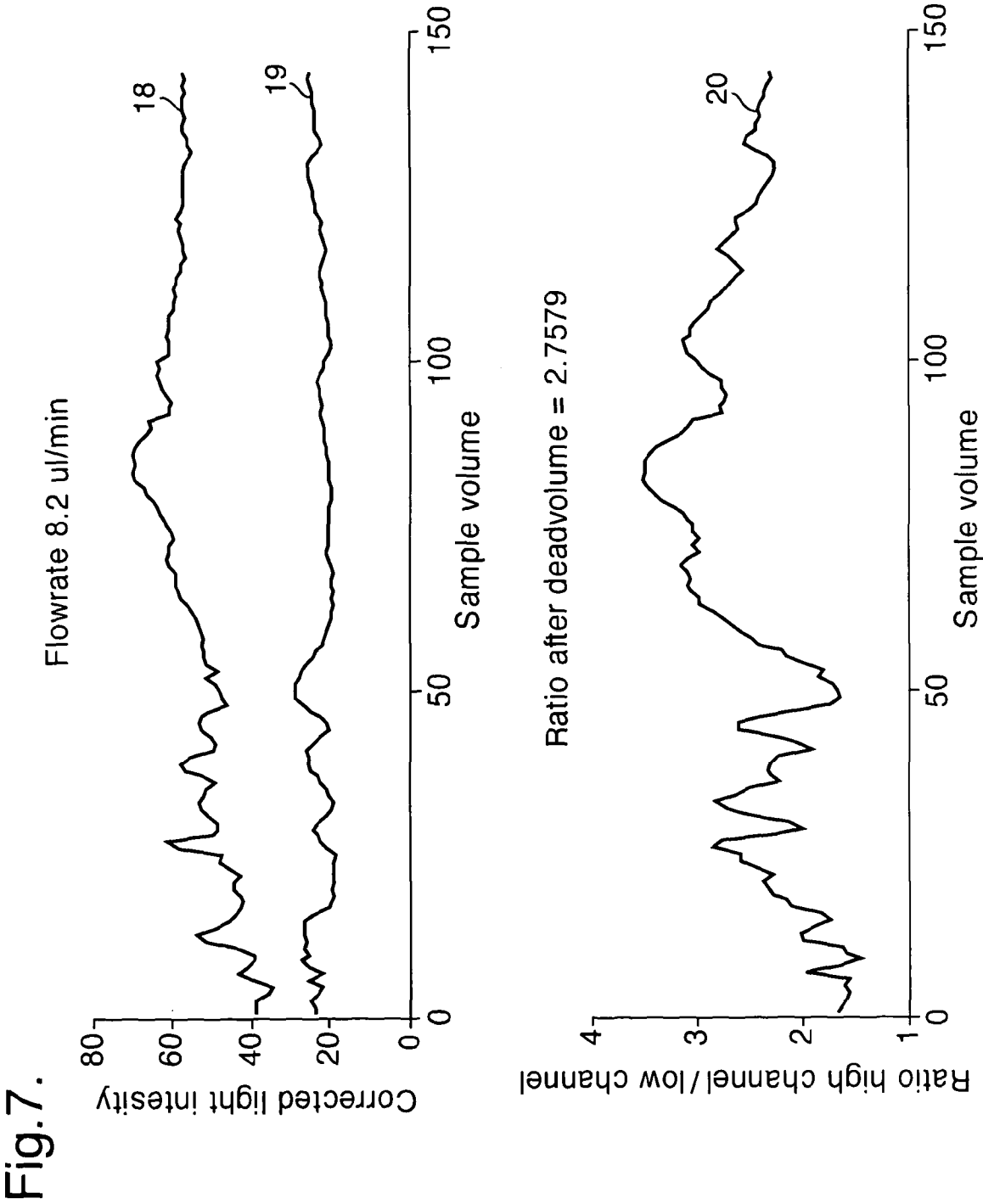
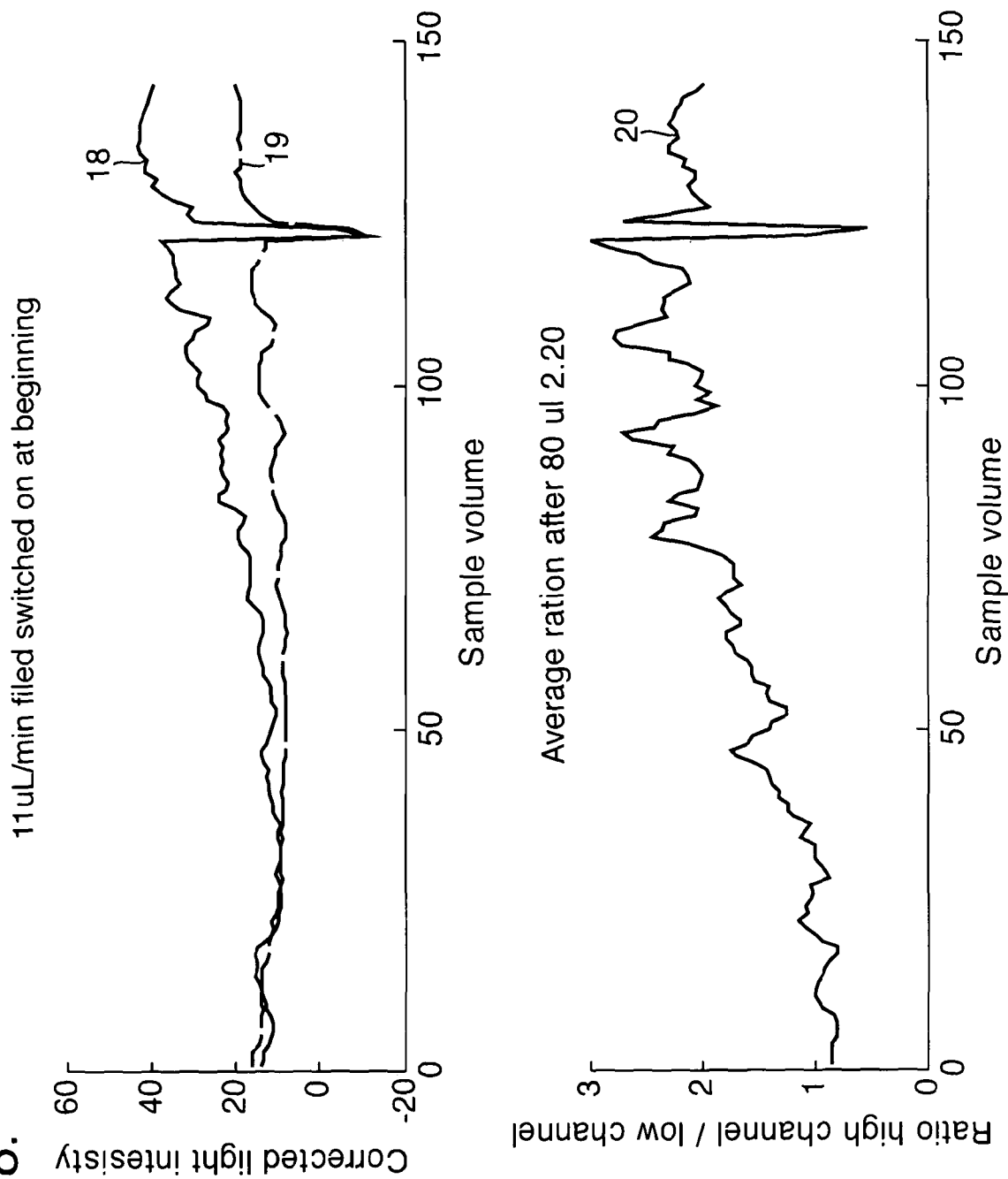


Fig.8.



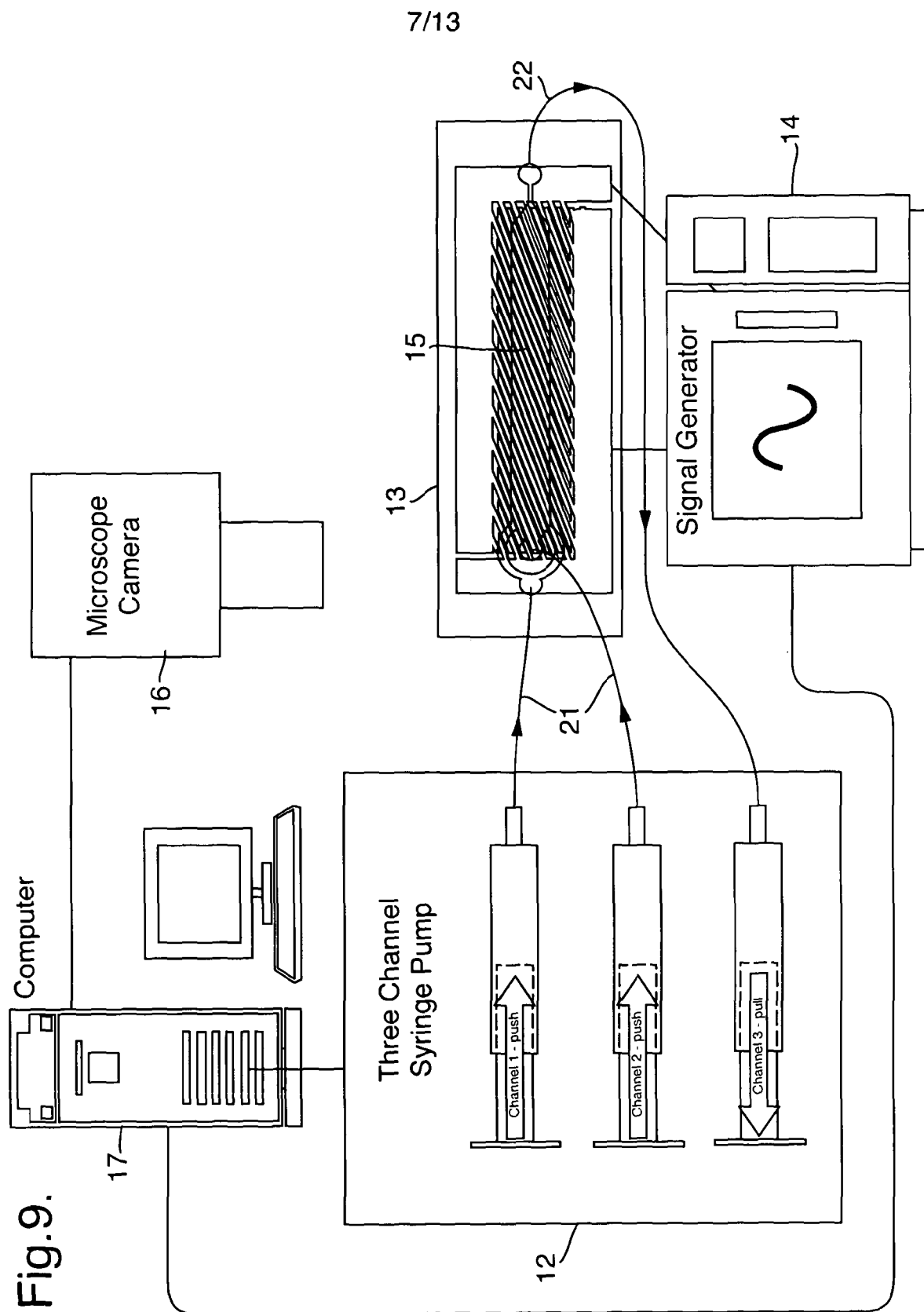


Fig.9.

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Fig.10(a).

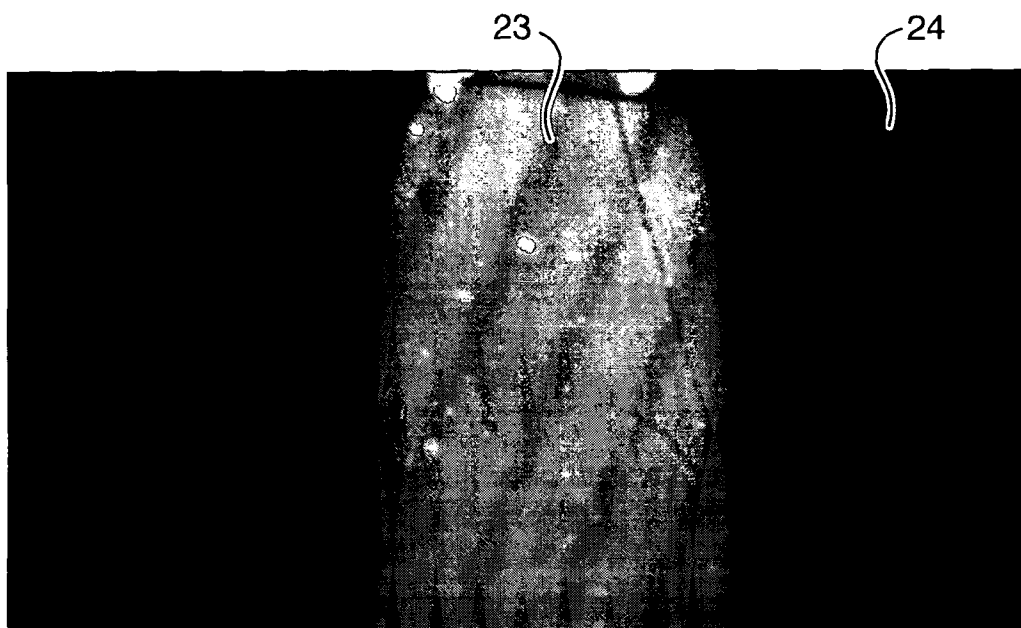
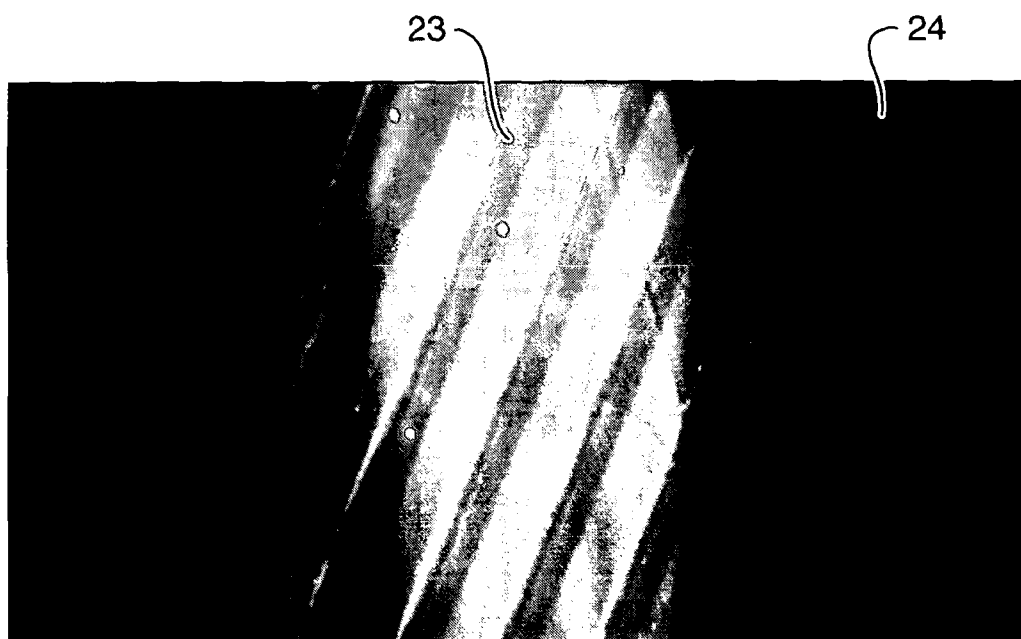


Fig.10(b).



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Fig.11(a).

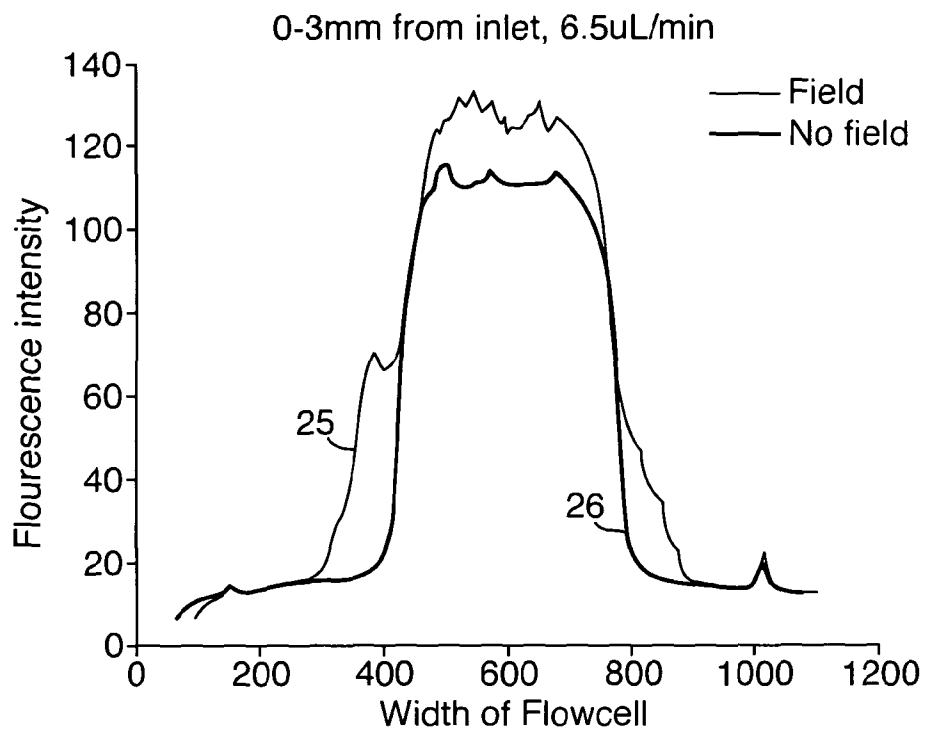
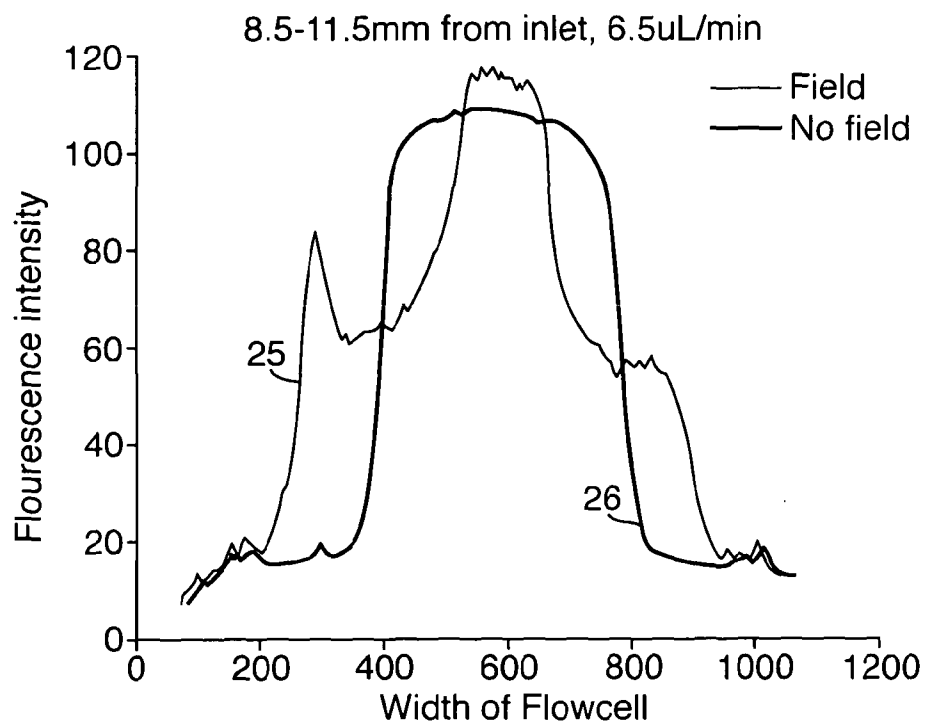


Fig.11(b).



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Fig.12.

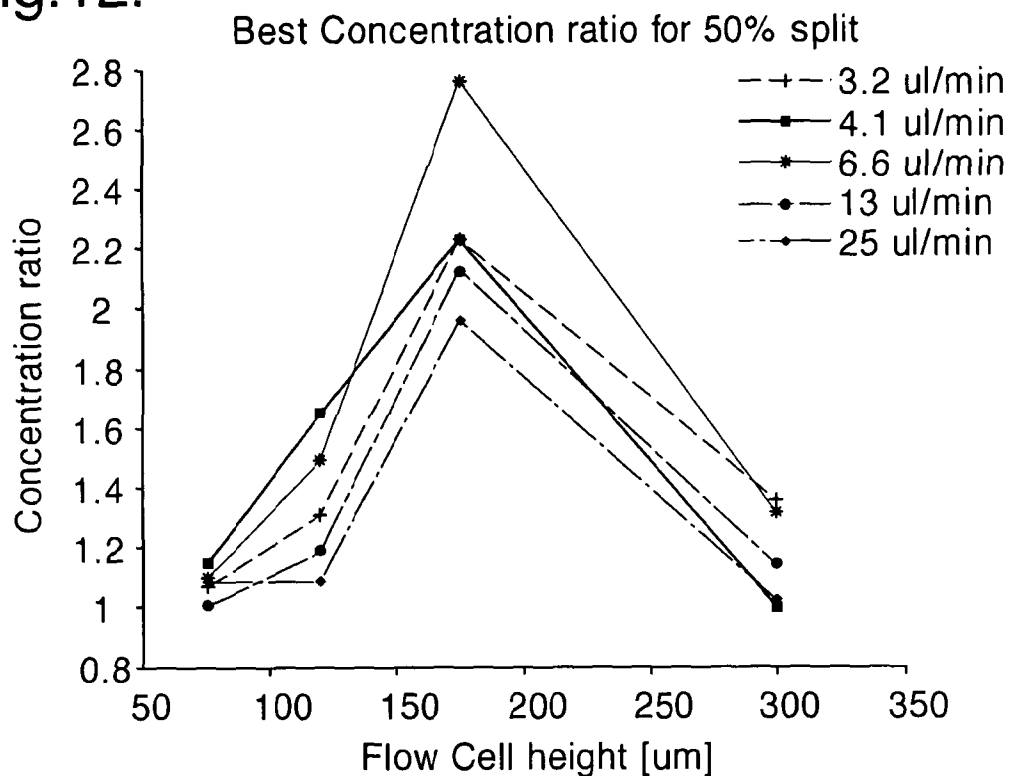
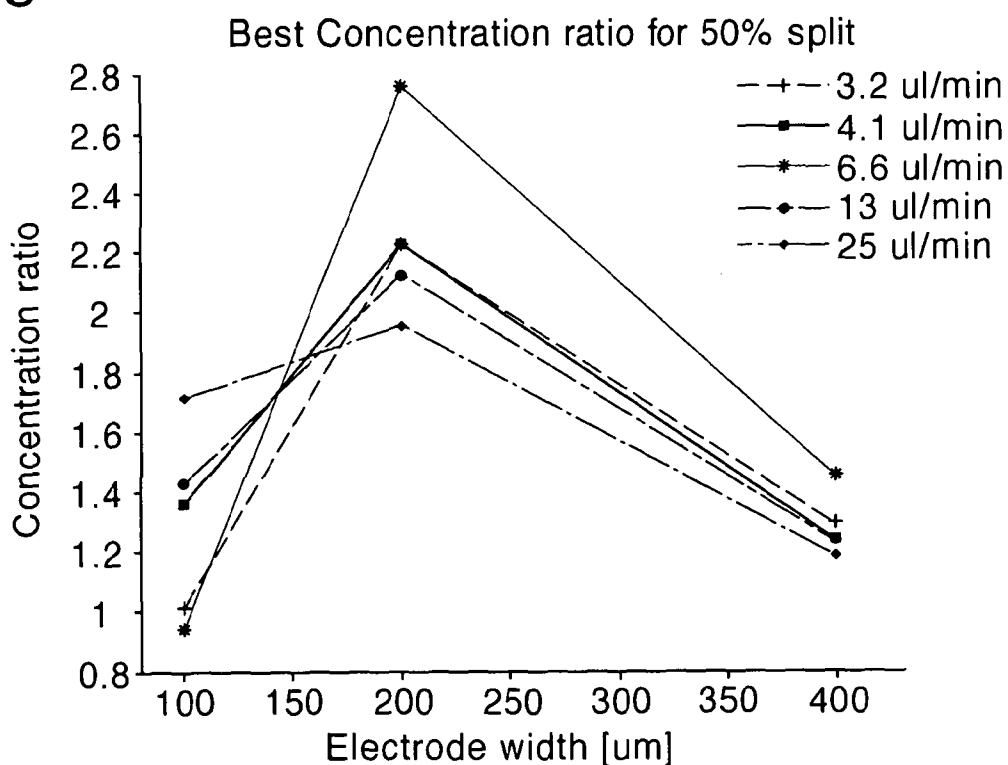


Fig.13.



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Fig.14.

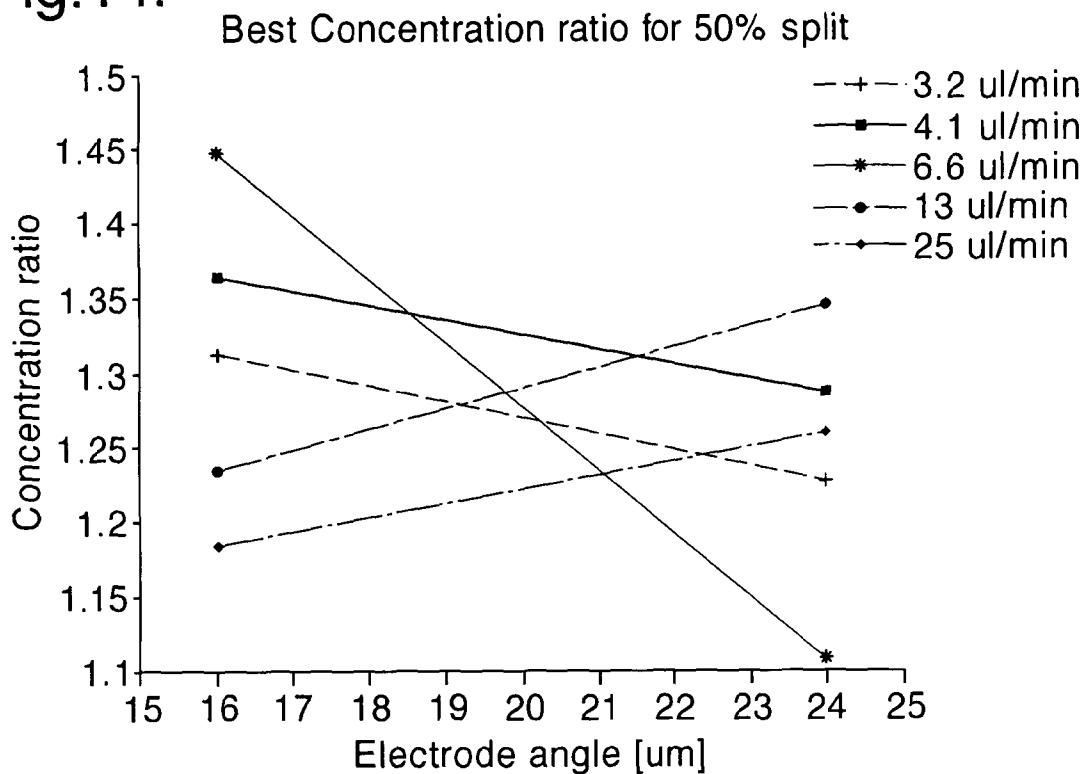
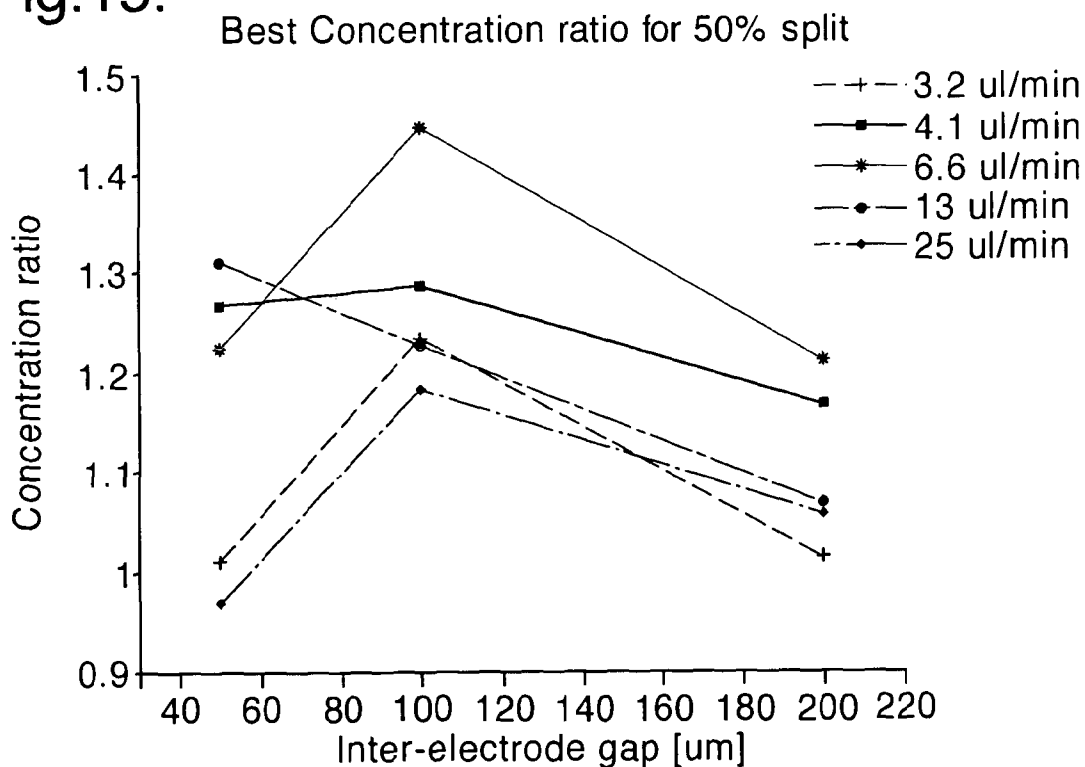


Fig.15.



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Fig.16.

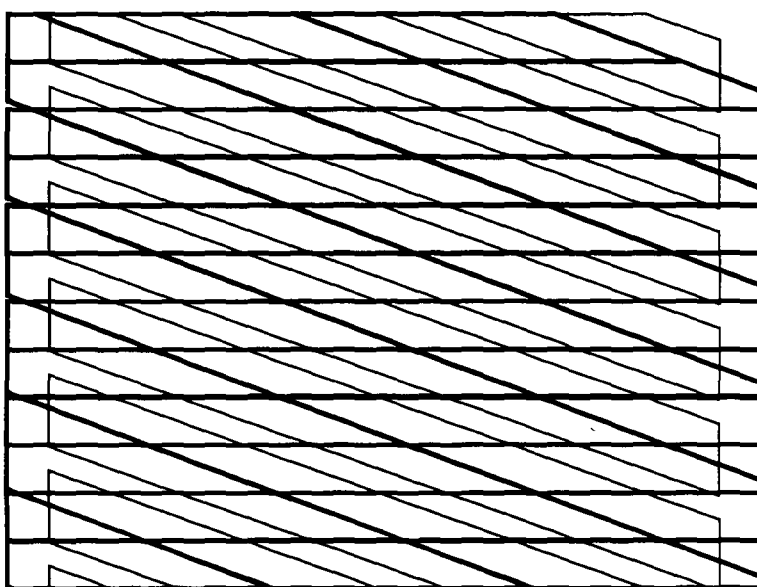
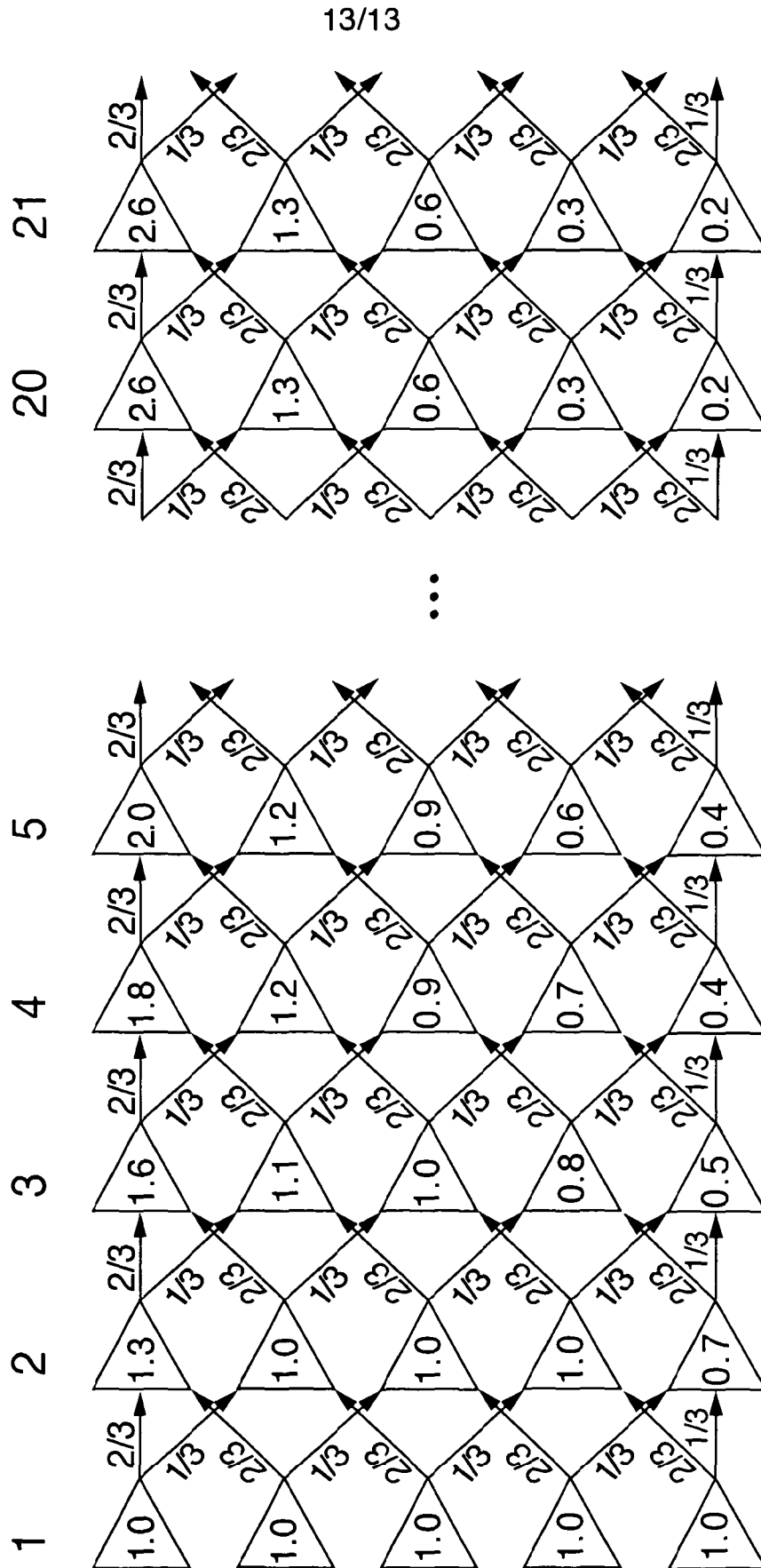


Fig.17.



INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2010/001751

A. CLASSIFICATION OF SUBJECT MATTER
INV. B03C5/02

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)
B03C

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 practical, search terms used)

EPO-Internal, WPI Data, INSPEC

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JINSUN SEO ET AL: "Lateral-Driven Continuous Dielectrophoretic Separation Technology for Blood Cells suspended in a Highly Conductive Medium" NANOTECHNOLOGY 2008: MICROSYSTEMS, PHOTONICS, SENSORS, FLUIDICS, MODELING AND SIMULATION. TECHNICAL PROCEEDINGS OF THE 2008 NSTI NANOTECHNOLOGY CONFERENCE AND TRADE SHOW NANO SCIENCE AND TECHNOLOGY INSTITUTE CAMBRIDGE, MA, USA, vol. 3, 2008, pages 320-323, XP002606584 ISBN: 978-1-4200-8505-1 the whole document ----- -/--	1-16

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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Date of the actual completion of the international search

26 October 2010

Date of mailing of the international search report

29/11/2010

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Volmer, Wilhelm

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2010/001751

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	YUNUS NAM, GREEN NG: "Continuous separation of submicron particles using angled electrodes" JOURNAL OF PHYSICS: CONFERENCE SERIES, vol. 142, 2008, XP002606392 ISSN: 1742-6596 the whole document	1-16
X	----- KI-HO HAN ET AL: "Lateral-driven continuous dielectrophoretic microseparators for blood cells suspended in a highly conductive medium" LAB CHIP, vol. 8, no. 7, July 2008 (2008-07), pages 1079-1086, XP002606393 ISSN: 1473-0197 the whole document	1-16
X	----- KI-HO HAN ET AL: "Lateral displacement as a function of particle size using a piecewise curved planar interdigitated electrode array" LAB ON A CHIP, vol. 9, no. 20, 2 July 2009 (2009-07-02), pages 2958-2964, XP002606391 ISSN: 1473-0197 the online publishing date of this article is 2-7-2009. the whole document	1,15,16
X	----- DURR M ET AL: "Microdevices for manipulation and accumulation of micro- and nanoparticles by dielectrophoresis" ELECTROPHORESIS, vol. 24, no. 4, February 2003 (2003-02), pages 722-731, XP002904334 ISSN: 0173-0835 the whole document	1,15,16
A	----- WO 2007/092253 A2 (MASSACHUSETTS INST TECHNOLOGY [US]) 16 August 2007 (2007-08-16) paragraph [0008] - paragraph [0056]; claims 1-8 figures 1-5	1-16

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/GB2010/001751

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2007092253 A2	16-08-2007	US 2008000772 A1	03-01-2008