

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
20 September 2001 (20.09.2001)

PCT

(10) International Publication Number
WO 01/69202 A2

(51) International Patent Classification⁷: **G01N 15/00**

(21) International Application Number: PCT/EP01/02855

(22) International Filing Date: 14 March 2001 (14.03.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
2000-00425 16 March 2000 (16.03.2000) DK

(71) Applicant and

(72) Inventor: **LARSEN, Ulrik, Darling** [DK/DK]; Kongeve-
jen 191A, DK-2840 Holte (DK).

(74) Agent: **SMART, Peter, J.**; W.H. Beck, Greener & Co., 7
Stone Buildings, Lincoln's Inn, London WC2A 3SZ (GB).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

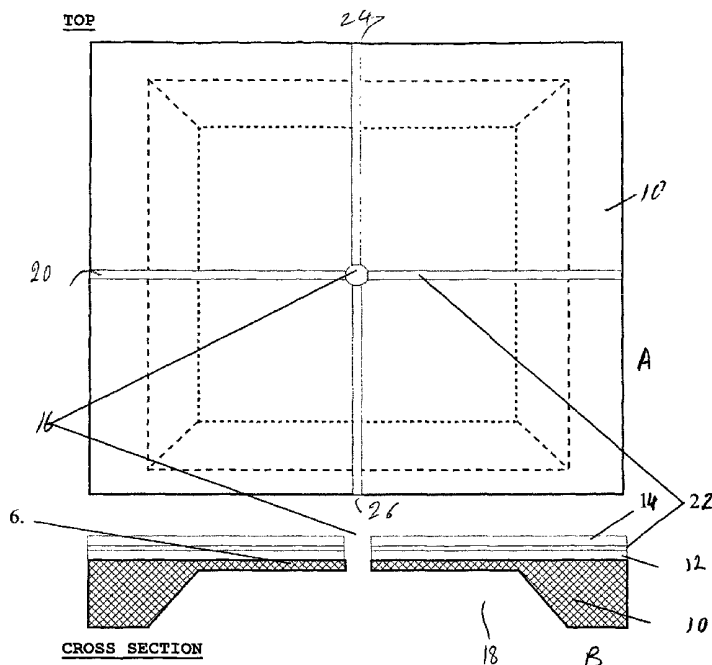
(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— without international search report and to be republished upon receipt of that report

[Continued on next page]

(54) Title: SENSOR UNITS FOR PARTICLE CHARACTERISATION APPARATUS



(57) Abstract: A sensor unit for a particle characterisation apparatus such as a flow cytometer comprises a membrane (10) having an orifice defining a passageway (16) through the thickness of the membrane and at least two optical waveguides (20, 22) within the thickness of the membrane and extending radially from said orifice.



WO 01/69202 A2



For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

Sensor Units for Particle Characterisation Apparatus

The present invention relates to sensor units for particle characterisation apparatus in which particles suspended in a liquid are passed through an orifice, in principle in single file, to enable the characterisation of the particles, for instance by optical detection and to particle characterisation apparatus incorporating such sensor units.

Detection of particles in a liquid stream is known as 'flow cytometry' (see Practical Flow Cytometry, Shapiro, Wiley-Liss, 1995) and is a widely used method of detecting, sorting, separating and analysing biological particles such as cells, cell parts, bacteria, viruses, DNA etc. The use of such apparatus is not, despite the name, restricted to measurements on cells or to materials of biological origin.

One of the most widely used principles in 'flow cytometry' is based on the detection of light emitted or scattered from the particles. This is a method with a large potential in very different applications, but unfortunately it has proved to be quite complicated and laborious. By focusing a beam of light into a sensing zone, the cells are illuminated and the response in terms of absorption, scattering or fluorescence is recorded by optical detectors. Since the optical response from the cells depends on accurately aligned illumination- and collection optics, the cells must be centred in the flow stream i.e. hydrodynamically focused, which is done by establishing a coaxial flow with a sheath fluid. Most optical flow cytometers are based on a precision-machined flow cell

containing an optical cuvette, which is a quartz block with a thin channel for the detection and a broader section for the inlet of sample and sheath fluid.

It is possible to measure structural as well as functional cell parameters with the optical method, since
5 fluorescent labelled reagents or probes can be applied in order to stain specific parts of the cells. Today several thousand probes and reagents are available for staining specific parts of the cell such as the cell membrane, DNA,
10 antibodies, protein etc. The quality and alignment of the optics is a critical parameter if a high sensitivity of the system is necessary.

Both requirements for accurate optics and accurate liquid handling, makes the flow cytometer a very complex
15 combination of pumps, electronics, lasers, detectors and bulk optical devices.

Due to the complexity of flow cytometers they have become too expensive for an ordinary medical or biochemical laboratory. Such places have either established laboratories
20 in collaboration with other laboratories or have outsourced these measurements to companies specialised in flow cytometry measurements. However. This creates excessive processing times and bottleneck situations, and many tasks are performed manually, or are not performed at all, due to the
25 inaccessibility of such measurements.

In order to simplify the current flow cytometers, the present invention demonstrates a novel way of establishing a good alignment between the core flow of particles and the optical elements, by the use of waveguide technology.

In a first aspect, the invention is concerned with providing optical waveguides to convey light to and from a sensing location in particle characterisation apparatus.

In a particle characterisation apparatus of a kind
5 commonly referred to as a Coulter Counter, suspended particles are drawn through an axially short passageway formed as an orifice in a membrane. The change in the electrical impedance of the current path provided by the liquid in the orifice produced by the momentary presence of a
10 particle passing through the orifice can provide information on particle size and numbers. For proper operation, it is necessary that the orifice be large enough to allow the passage of the particles but narrow enough to prevent so far as possible the simultaneous presence of more than one
15 particle therein. It is usual therefore for the operator to select and fit an orifice of a diameter suitable for the sample under investigation, according to the expected particle size. The inconvenience of this procedure is addressed according to a second aspect of the present
20 invention in which a variable effective or virtual orifice diameter is obtained by the use of a sheath fluid.

Advantageously, the two aspects of the invention are combined in a sensor unit which provides a membrane containing a Coulter Counter type orifice with optical
25 waveguides optically addressing the orifice for interrogating particles as they pass through and provision for a sheath flow to produce an adjustable virtual orifice diameter.

In a first aspect therefore, the invention provides a sensor unit for optical characterisation of particles in
30 liquid suspension as they are passing through a passageway,

through a membrane, said unit comprising a membrane having a said passageway extending through the thickness of the membrane for the passage of said suspension, wherein at least two wave guides are formed in said membrane, which waveguides
5 are each in optical communication with a common location in said passageway. This fixes the optics in relation to the measurement or sensing location so that no setting up of the alignment of the optics is required.

The sensor unit may therefore resemble the orifice
10 membrane normally used in a Coulter Counter, but with the addition of waveguides built into the membrane structure.

Preferably, the length of said passageway is from 1 to 100 μm , for instance about 50 μm . Desirably the axial length of the passageway is chosen such that only one particle will
15 be present in the passageway at a time in use when detecting particles of from 0.1 to 100 μm diameter. The aspect ratio of said passageway (axial length divided by diameter) is preferably from 0.5:1 to 5:1, more preferably from 1:1 to 3:1.

20 Preferably, the largest breadth dimension of said passageway at said common location is from 5 to 200 μm , for instance 10 to 50 μm .

The preferred transverse dimensions of the waveguides are related to the width of the passageway and to the
25 diameter of the particles to be measured. Suitably, each said waveguide adjacent said common location has a maximum breadth dimension of from 1 to 200 μm more preferably 5 to 200 μm , e.g. 5 to 50 μm , but preferably, the width waveguide is similar to the width of the orifice so as respectively to
30 radiate to and collect from the entire aperture through which

particles may pass. The thickness of the waveguide (i.e. the transverse dimension in the axial direction of the passageway) is preferably similar to the particles' diameter and preferably does not exceed this.

5 Said waveguides preferably, extend from said common location in said passageway radially of the passageway. They may be angularly separated from one another at said common location by intervening angles of for instance 45, 90, 135 or 180°. Preferably, there are at least four said waveguides,
10 which are angularly separated from one another by intervening angles of 90°. Adjacent waveguides can be used also in setting up the optimal alignment of the optical fibre or other optical interfacing at the edge of the sensor unit in that such an optical fibre or other optics can be moved with
15 relation to its respective waveguide horizontally and vertically until a maximum signal is obtained through the adjacent waveguide.

 The waveguides will comprise a core material of higher refractive index surrounded by a cladding of lower refractive
20 index. Said core material and cladding material are preferably formed in situ by a process comprising deposition of layers of material, photolithographic masking and etching.

 This may comprise forming a first layer of cladding material, optionally on a substrate, forming over this a
25 layer of core material, masking waveguide tracks in said core material and etching away non-masked areas, demasking the remaining core material and forming a second layer of cladding material between and over said core material.

 Alternatively, the core material may be formed from the
30 cladding material by exposing the cladding material to light

such as locally to alter the refractive index of the cladding material.

When operating in this manner to form the waveguides the cladding material is preferably a Ge doped silica.

5 When practising the method of layer deposition or the method of refractive index alteration the membrane is preferably constructed by depositing a glass or a glass-like material on the surface of a silicon wafer. Glass or glass like materials that are suitable include silicon-compound
10 glasses, e.g. doped or non-doped silica glass, silicon nitrate and silicon dioxide. Other possible substrates include GeAs, Kovar, Invar or other materials having a similar thermal expansion coefficient, which should be almost identical to glass over the range of 0 to 400°C.

15 After deposition of the glass layer(s) the silicon wafer may be selectively etched to form a said membrane comprised of said glass or glass like material and a reduced thickness of the silicon layer or only of glass or glass like material.

20 More preferably, waveguides can be fabricated by deposition of a polymer such as polyamide that can be structured by lithographical means (or by other means such as micromachining and embossing) on a substrate. Requirements to the substrate are less restrictive, but the surface should
25 be able to bind the polymer.

Incorporating the characterising feature of the second aspect of the invention, said membrane may comprise an annular manifold within the thickness of the membrane, an outlet or series of outlets from said manifold annularly
30 surrounding said orifice on one surface of said membrane, and

a flow path within the thickness of the membrane communicating between an inlet to said flow path and said annular manifold. For this purpose, the membrane may comprise a silicon layer and a glass or glass like material layer overlying the silicon layer and containing said waveguides, and said manifold and flow path may lie between the silicon layer and the glass or glass-like material layer.

In accordance with the second aspect of the invention there is provided a sensor unit for a particle characterisation apparatus comprising a membrane having an orifice extending through the thickness of the membrane for the one at a time passage of particles suspended in a liquid, an annular manifold within the thickness of the membrane, an outlet or series of outlets from said manifold annularly surrounding said orifice on one surface of said membrane, and a flow path within the thickness of the membrane communicating between an inlet to said flow path and said annular manifold. As described above the membrane may comprise a silicon layer and a glass or glass like material layer overlying the silicon layer, and said manifold and flow path may lie between the silicon layer and the glass or glass-like material layer.

The sensor units described herein may be incorporated into particle characterisation apparatus.

Such particle characterisation apparatus may comprising a sensor unit as described, a light source optically coupled to a first of said waveguides and an optical detector optically coupled to a second of said waveguides. The detector may be for detecting adsorption, scattering or fluorescence.

The apparatus may further comprise a pump connected to pass a liquid sample containing suspended particles through said passageway. This may operate to feed liquid from upstream to said passageway or to aspirate liquid from downstream through said passageway.

There may also be a pump connected to pass a flow of sheath liquid through said manifold when this is present to form an annular sheath flow surrounding said liquid sample as the liquid sample passes through said membrane orifice, and a flow rate controller controlling the flow rate of said sheath flow through said orifice to variably restrict the diameter of the flow of liquid sample through said orifice.

The apparatus may further comprise a first electrode positioned at a location upstream of said passageway and a second electrode positioned downstream of said passageway, a source of electrical voltage connected across said first and second electrodes, and an impedance change detector measuring changes in the impedance of the current path between said electrodes through said passageway. For this purpose any of the electrode and controlling electronics and sensing electronics known in connection with Coulter Counters may be used.

The invention includes particle characterisation apparatus comprising a sensor unit with a manifold as described, with or without optical waveguides, a pump connected to pass a liquid sample containing suspended particles through said passageway, and further comprising a pump connected to pass a flow of sheath liquid through said manifold to form an annular sheath flow surrounding said liquid sample as the liquid sample passes through said

membrane orifice, and a flow rate controller controlling the flow rate of said sheath flow through said orifice to variably restrict the diameter of the flow of liquid sample through said orifice.

5 Here again, there may be a first electrode positioned at a location upstream of said orifice and a second electrode positioned downstream of said orifice, a source of electrical voltage connected across said first and second electrodes, and an impedance change detector measuring changes in the
10 impedance of the current path between said electrodes through said orifice.

As explained above, the present invention provides in preferred aspects a sensor based on a membrane essentially fabricated by micromachining. The membrane has an orifice
15 placed relatively in the centre of the membrane, which can be used for aspiration of particles suspended in a liquid, as the sensor is submerged into the liquid. This way of transporting particles into a measuring region is known for electrical characterisation of particles by the Coulter
20 principle (V. Kachel, "Electrical Resistance Pulse Sizing: Coulter Sizing", Flow Cytometry and Sorting, 2. ed., pp.45-80, 1990 Wiley-Liss, Inc.). In order to illuminate the particle or detect light from the particles a number of light guiding structures can be placed on the surface of the
25 membrane. Such light guiding structures can be formed when a material of relatively high transmission coefficient has a refractive index relatively higher than the surrounding material, (which also has relatively high transmission coefficient). These structures are also known as Waveguides
30 (see K. Hoppe, M. Svalgaard, M. Kristensen, "Integrated

Optical Waveguides in Fluidic Microsystems", Analytical methods and Instrumentation, Special issue μ TAS '96, side 164-166). Excitation and detection of the particles can both be performed with the waveguides. The formation of the waveguides aligned to the orifice can easily be established by the use of lithography or by laser writing the regions of higher refractive index directly on the material. The sensor can easily be coupled to optical fibres connected to the light source and detectors, and by placing tubes on either end of the sensor, particles can be transported through the orifice and detected without the use of precision optics and sheath fluid. In addition the measurements can also be combined with measurements of impedance for Coulter-sizing.

The invention can be build into a container that is disposable so that all material that has been into contact with the biological sample will be discarded.

The invention will be further described and illustrated in the following description of preferred specific embodiments with reference being made to the accompanying drawings, in which:

Figure 1 shows (A) in plan view and (B) in section on the line B-B an example of a sensor unit in accordance with only the first aspect of the invention;

Figure 2 shows in longitudinal cross section a sensor unit according to Figure 1 mounted into a tubular support;

Figure 3 shows schematically the arrangement of the sensor unit of Figures 1 and 2 in particle characterisation apparatus according to the first aspect of the invention;

Figure 4 shows a cross sectional view taken as in Figure 2, but of a sensor unit incorporating features

according to both the first and second aspects of the invention; and

Figure 5 shows a computer simulation of the flow of liquids obtained using the sensor unit of Figure 4.

5 Figure 1 shows two views of a sensor unit according to the first aspect of the invention. The sensor unit illustrated comprises a rectangular wafer of silicon 10 over which is formed a bottom layer of cladding 12. A top layer of cladding 14 is provided over the bottom layer 12. A
10 central orifice 16 passes through the whole thickness of the illustrated sensor unit but annularly surrounding the orifice 16, the thickness of the silicon wafer 10 is reduced in a zone 18 so that the orifice 16 is formed through a membrane composed of the residual thickness of the silicon and the top
15 and bottom cladding layers. Four optical waveguides 20, 22, 24 and 26 are formed between the top and bottom cladding layers 12, 14 and extend radially with respect to the orifice 16. One end of each waveguide is formed at the wall of the orifice and each waveguide has a second end at the periphery
20 of the illustrated sensor unit. The waveguides 20-26 are formed of a transparent material having a refractive index higher than the refractive index of the top and bottom cladding materials.

 The illustrated structure may be produced in a number
25 of ways. A preferred procedure is as follows. A silicon wafer of uniform thickness is first provided with a bottom cladding layer of glass by deposition by plasma enhanced chemical vapour deposition (PECVD). Suitably the silicon may be of approximately 500 μm thickness. The area of the
30 silicon may be as convenient but the wafer may suitably be

approximately 10 cm per side. The cladding glass layer is suitably of a approximately 15 μm .

A layer of higher refractive index material to serve as the core for the waveguides is then deposited on top of the
5 bottom cladding layer by PECVD. The core layer is suitably of approximately 15 μm in thickness.

A masking photoresist is then spun on top of the core layer and is exposed and developed to reveal all of the core layer except those portions which will form the waveguides
10 20-26. The unmasked portions of the core layer are then removed, suitably by dry etching in a reactive ion etcher (RIE). The top cladding layer is then deposited by PECVD to cover the exposed bottom cladding layer and the tracks of core layer after removal of the protective photoresist from
15 the core layer tracks.

More preferably, the same procedure as described could be used with deposition of two polyamide substances with different refractive index instead of glass. Instead of PECVD-deposition and subsequent etching, the polyamide is
20 simply dispensed on a rotating disc to form a uniform layer of defined thickness and subsequently exposed through a mask and developed in order to fabricate the desired topologies.

The orifice itself may then be formed by protection of the upper cladding layer by the application of a photoresist
25 and exposure of the photoresist to reveal the location in which the orifice is to be formed followed by etching of the orifice by RIE (Reactive ion etching).

Lastly, the silicon in the area 18 may be thinned or entirely removed by etching to leave the glass cladding

layers as a membrane with or without a thin silicon backing as illustrated.

Alternatively, similar structure may be fabricated in plastics materials of selected refractive index.

5 Polymer films can be applied by spin coating techniques, particularly when using pre-polymer solutions of polycyanurate copolymers selected to provided desired refractive index contrast between the cladding and waveguide layers. Waveguide structures have also been obtained by
10 photolithography and subsequent reactive ion etching (RIE) as described in W. Wirges, N. Keil, H.H. Yao, S. Yilmaz, C. Zawadzki, M. Bauer, J. Bauer, C. Dreyer, "Low-loss Polymer Waveguide with High Thermal Stability", Microsystem Technologies, 98, 6th Int. Conf. Micro. Electro. Opto.,
15 Mechanical Systems and Components, Potstan, Dec. 1-3, 1998, H. Reichl, E. Obermeier (Eds.), VDE-Verlag, P.71.

In a further alternative manufacturing technique, a silicon wafer as illustrated may be coated with a cladding layer of a germanium (Ge) doped silica film. Waveguides can
20 be fabricated in such a cladding layer utilising photo-induced refractive index changes. The waveguides may therefore be directly UV-written into the cladding layer as described in M. Svalgaard. C.V. Poulsen, A. Bjärkelev and O. Poulsen, "Direct UV-Writing of Buried Single-Mode Channel
25 Waveguides in Ge-Dopped Silica Films" Electric Letters, 30:1401-1402, 1994. The main advantage of this technique is that the core structures do not have to be etched into a glass layer but can be formed directly by UV exposure.

For use in a particle characterisation apparatus, the
30 sensor unit shown in Figure 1 may conveniently be

incorporated into a tubular structure through which liquid can be passed as in a Coulter Counter. The orifice of the membrane is preferably disposed approximately in the centre of the flow path so produced. As shown in Figure 2, the sensor unit of Figure 1 is positioned joining two tubes 30, 32. Liquid containing suspended particles may either be aspirated up through the membrane or may be drained or pumped through the membrane from the upper tube 30 to the lower one 32. Methods of transporting particles into a measuring region in this way are known in connection with electrical characterisation of particles by the Coulter principle, see for instance V. Kachel, "Electrical Resistance Pulsising: Coultersizing", Flow Cytometry and Sorting, Second Edition, pp 45-80, 1990, Wiley-Liss, Inc.

In order to illuminate the particles as they pass through the orifice or to detect light from the particles, the waveguides illustrated are readily coupled to one or more light sources and one or more detectors. Suitably, the optical coupling is via optical fibres connected to the light source and detector and to the end of the waveguides illustrated. A suitable general arrangement for such particle characterisation apparatus is shown in Figure 3 where a laser 34 is coupled to a first optical fibre 36 which is coupled also to an outside end of the waveguide 20. A second optical fibre 38 is coupled to the outside end of the waveguide 22 and to an optical detector 40 which is connected to display apparatus 42 which is illustrated showing a pulse representing the output obtained as a particle bearing a fluorescent label passes through the orifice 16 when a sample 44 is poured into the upper tube 30 and aspirated through a

tube 46 by a suitable pump to draw the liquid through the orifice 16.

The particles are not necessarily labelled. The obstruction of light from the light source caused by the presence of the particle in the orifice may be detected as a decrease in light transmission across the orifice. Alternatively, they may be labelled for optical detection in any one of the many known ways, for instance by the attachment of a fluorescent label. The waveguides 24 and 25 positioned at 90° to the light path may be used for detecting side scatter or for measuring fluorescence. Whilst fluorescence is in theory radiated from the entire surface of the detected particle, and thus in all directions, in order to achieve the highest signal to noise ratio it is preferable to pick up the fluorescence at 90° to the exciting light.

It is also preferably to apply optical filters to eliminate the scattered excitation light before the fluorescence detection. Fluorescence will have larger wavelength than the excitation light due to loss of energy. It is also possible to distinguish different fluorescent probes by successively filtering out light of smaller wavelengths.

Although four waveguides staggered at 90° angles have been shown, a greater or lesser number of waveguides may be used staggered at equal or unequal angles.

As is generally known in the art of flow cytometry, detected particles may be sorted according to the output of the detector 40, for instance by positioning charge plates below the outlet of the orifice 16 which are activated by the

application of a voltage to deflect drops containing detected particles electrostatically into suitable receptacles.

The orifice size will be chosen to be somewhat larger than the diameter of the particles to be measured. Depending
5 on the nature of the particles the orifice may need to be substantially greater in diameter, possibly a number of magnitudes greater than the diameter of the particles. The waveguides desirably have a cross-sectional dimension which is essentially the same as the diameter of the orifice so as
10 to radiate and collect from the entire aperture through which the particles may pass. Preferably, the cross-sectional dimensions of the waveguides do not exceed the size of the particles in question.

As shown in Figure 3, the second sensor unit according
15 to the invention comprises a silicon wafer bearing top and bottom cladding layers incorporating waveguides as previously described. However, surrounding the orifice 16 the silicon wafer 10 is provided with an annular recess. The recess is covered by the top and bottom cladding layers 12, 14, so
20 defining a manifold 50 annular surrounding the orifice 16. A plurality of outlet channels from the manifold so formed pass upwardly through the top and bottom cladding layers 12, 14 to emerge on the upper surface of the top cladding layer 14 at outlets 52 which are radially spaced around the orifice 16.
25 The outlets 52 are spaced angularly from the positions at which the waveguides 20, 22 are formed so that the waveguides are not broken by these outlets.

An inlet 54 is provided connecting the manifold/recess
50 with a source of a sheath fluid. The inlet 54 is also
30 angularly spaced from the position of the waveguides so that

these are not broken. The manifold can be fabricated by etching a matrix of closely spaced holes ($2\text{ }\mu\text{m}$) together with the inlet and outlet of the manifold/recess into the first cladding layer, before deposition of core and second
5 cladding. By selectively isotropically etching of the underlying material the buried channel forms. After deposition of the core and second cladding layer the small holes will have been filled up with the deposited material, however, the bigger inlet and outlet will still be open
10 (although narrowed somewhat). The process illustrated in Figure 6 in which the steps shown are as follows:

1. The orifice is etched.
2. The first cladding layer is deposited.
3. The small holes, inlet and outlet of the manifold
15 are etched into the cladding glass.
4. The underlying substrate (Silicon) is etched isotropically.
5. Core and cladding layer are deposited.
6. The cladding is selectively etched in the orifice
20 and the silicon is etched from the backside to form the membrane.

In use, a sheath liquid may be introduced through the inlet 54 to pass into the manifold 50 and through the outlets 52 on to the surface of the sensor unit. Combined with a
25 downward flow of liquid sample containing particles through the orifice 16, this provides a flow of sheath liquid through the orifice surrounding and constraining the flow of sample liquid as shown in Figure 5 where the sheath liquid is shown in a lighter shade. By adjusting the flow rates of the
30 sheath liquid and the sample flow through the orifice, one

can restrict the diameter of the sample flow to any desired degree, thus preventing more than one particle proceeding side by side through the orifice.

The provision of the buried channels as described above
5 enables one to perform liquid handling functions that would not otherwise be possible. By having a buried channel leading to an opening around the orifice as illustrated, it is possible to inject a sheath fluid in a very controlled way. This can be used to allow a Coulter sizing of even sub-
10 micron particles by using a non-conducting fluid as the sheath. The Coulter sizing of sub-micron particles using a very small orifice of 2 μm has been demonstrated (see for instance B. Roebuck and E.A. Almond, "Measurement of Particles Size of Ultrafine (sub-mircrometer) WC Powders",
15 Powder Metallurgy, Vol.29, No.2, pp 119-124, 1986). However, such a small orifice is very prone to clogging by particles larger than the orifice. By using a non-conducting sheath fluid to narrow the sensing region of the Coulter orifice it is possible to prevent clogging. This has been demonstrated
20 in a different type of apparatus in US-A-5150037 but the arrangement used there is not optimal because of the long narrowed path of the conducting liquid, which introduces a long sensing zone. This increased the frequency into which multiple particles are detected at the same instant occupying
25 different parts of the length of the sensing zone. The introduction of a non-conducting sheath fluid just beside a short orifice removes the need for a long sensing zone on the front side. Furthermore, on the downstream side almost all of the non-conducting fluid will have become conducting
30 through fast diffusion of ions in the conductive liquid

leading to a shortening of the virtual orifice as well as a narrowing of it.

Although the sensor unit shown in Figure 4 incorporates features of both aspects of the invention, it will be appreciated that a similar sensor unit can be formed without
5 optical waveguides and used in a Coulter Counter for purely electrical detection of particles.

Although the invention has been described with reference to specific embodiments by way of illustration, it
10 should be appreciated that many modifications and variations of the invention as so described are possible within the scope of the invention.

CLAIMS

1. A sensor unit for optical characterisation of particles
5 in liquid suspension as they are passing through a
passageway through a membrane, said unit comprising a
membrane having a said passageway extending through the
thickness of the membrane for the passage of said
suspension, wherein at least two wave guides are formed
10 in said membrane, which waveguides are each in optical
communication with a common location in said passageway.
2. A sensor unit as claimed in claim 1, wherein the length
of said passageway is from 1 to 100 μm .
- 15 3. A sensor unit as claimed in claim 2, wherein the length
of said passageway is from about 50 μm .
4. A sensor unit as claimed in claim 2 or claim 3, wherein
20 the aspect ratio of said passageway (axial length
divided by diameter) is from 0.5:1 to 5:1.
5. A sensor unit as claimed in any one of claims 1 to 4,
wherein the largest breadth dimension of said passageway
25 at said common location is from 5 to 200 μm .
6. A sensor unit as claimed in any preceding claim, wherein
each said waveguide adjacent said common location has a
maximum breadth dimension of from 1 to 200 μm .

7. A sensor unit as claimed in any preceding claim, wherein said waveguides extend from said common location in said passageway radially of the passageway.
- 5 8. A sensor unit as claimed in claim 7, wherein said waveguides are angularly separated from one another at said common location by intervening angles of 45, 90, 135 or 180°.
- 10 9. A sensor unit as claimed in claim 8, wherein there are at least four said waveguides which are angularly separated from one another by intervening angles of 90°.
- 15 10. A sensor unit as claimed in any one of the preceding claims, wherein the waveguides comprise a core material of higher refractive index surrounded by a cladding of lower refractive index and wherein said core material and cladding material have been formed in situ by a process comprising deposition of layers of material,
20 photolithographic masking and etching.
- 25 11. A sensor unit as claimed in any preceding claim wherein the waveguides comprise a core material of higher refractive index surrounded by a cladding of lower refractive index and the core material has been formed from the cladding material by exposing the cladding material to light such as locally to alter the refractive index of the cladding material.

12. A sensor unit as claimed in claim 11, wherein the cladding material is a Ge doped silica.
13. A sensor unit as claimed in any preceding claim when
5 directly or indirectly dependent on claim 2, wherein said membrane is constructed by depositing a glass or a glass-like material on the surface of a silicon wafer.
14. A sensor unit as claimed in claim 13, wherein said
10 silicon wafer is selectively etched to form a said membrane comprised of said glass or glass like material and a reduced thickness of the silicon layer or only of glass or glass like material.
15. 15. A sensor unit as claimed in any preceding claim when
15 directly or indirectly dependent on claim 2, wherein said membrane comprises an annular manifold within the thickness of the membrane, an outlet or series of outlets from said manifold annularly surrounding said
20 orifice on one surface of said membrane, and a flow path within the thickness of the membrane communicating between an inlet to said flow path and said annular manifold.
- 25 16. A sensor unit as claimed in claim 15, wherein said membrane comprises a silicon layer and a glass or glass like material layer overlying the silicon layer and containing said waveguides, and said manifold and flow path lie between the silicon layer and the glass or
30 glass-like material layer.

17. A sensor unit for a particle characterisation apparatus comprising a membrane having an orifice extending through the thickness of the membrane for the one at a time passage of particles suspended in a liquid, an annular manifold within the thickness of the membrane, an outlet or series of outlets from said manifold annularly surrounding said orifice on one surface of said membrane, and a flow path within the thickness of the membrane communicating between an inlet to said flow path and said annular manifold.
18. A sensor unit as claimed in claim 17, wherein said membrane comprises a silicon layer and a glass or glass like material layer overlying the silicon layer, and said manifold and flow path lie between the silicon layer and the glass or glass-like material layer.
19. Particle characterisation apparatus comprising a sensor unit as claimed in any preceding claim.
20. Particle characterisation apparatus comprising a sensor unit as claimed in any one of claims 1 to 16, a light source optically coupled to a first of said waveguides and a detector optically coupled to a second of said waveguides.
21. Apparatus as claimed in claim 20, further comprising a pump connected to pass a liquid sample containing suspended particles through said passageway.

22. Apparatus as claimed in claim 20 or claim 21, when directly or indirectly dependent on any one of claims 15 or 16, further comprising a pump connected to pass a flow of sheath liquid through said manifold to form an annular sheath flow surrounding said liquid sample as the liquid sample passes through said membrane orifice, and a flow rate controller controlling the flow rate of said sheath flow through said orifice to variably restrict the diameter of the flow of liquid sample through said orifice.
23. Apparatus as claimed in claim 20 or claim 21 further comprising a first electrode positioned at a location upstream of said passageway and a second electrode positioned downstream of said passageway, a source of electrical voltage connected across said first and second electrodes, and an impedance change detector measuring changes in the impedance of the current path between said electrodes through said passageway.
24. Particle characterisation apparatus comprising a sensor unit as claimed in claim 17 or claim 18 a pump connected to pass a liquid sample containing suspended particles through said passageway and further comprising a pump connected to pass a flow of sheath liquid through said manifold to form an annular sheath flow surrounding said liquid sample as the liquid sample passes through said membrane orifice, and a flow rate controller controlling the flow rate of said sheath flow through said orifice

to variably restrict the diameter of the flow of liquid sample through said orifice.

25. Particle characterisation apparatus as claimed in claim
5 24, further comprising a first electrode positioned at a location upstream of said orifice and a second electrode positioned downstream of said orifice, a source of electrical voltage connected across said first and second electrodes, and an impedance change detector
10 measuring changes in the impedance of the current path between said electrodes through said orifice.

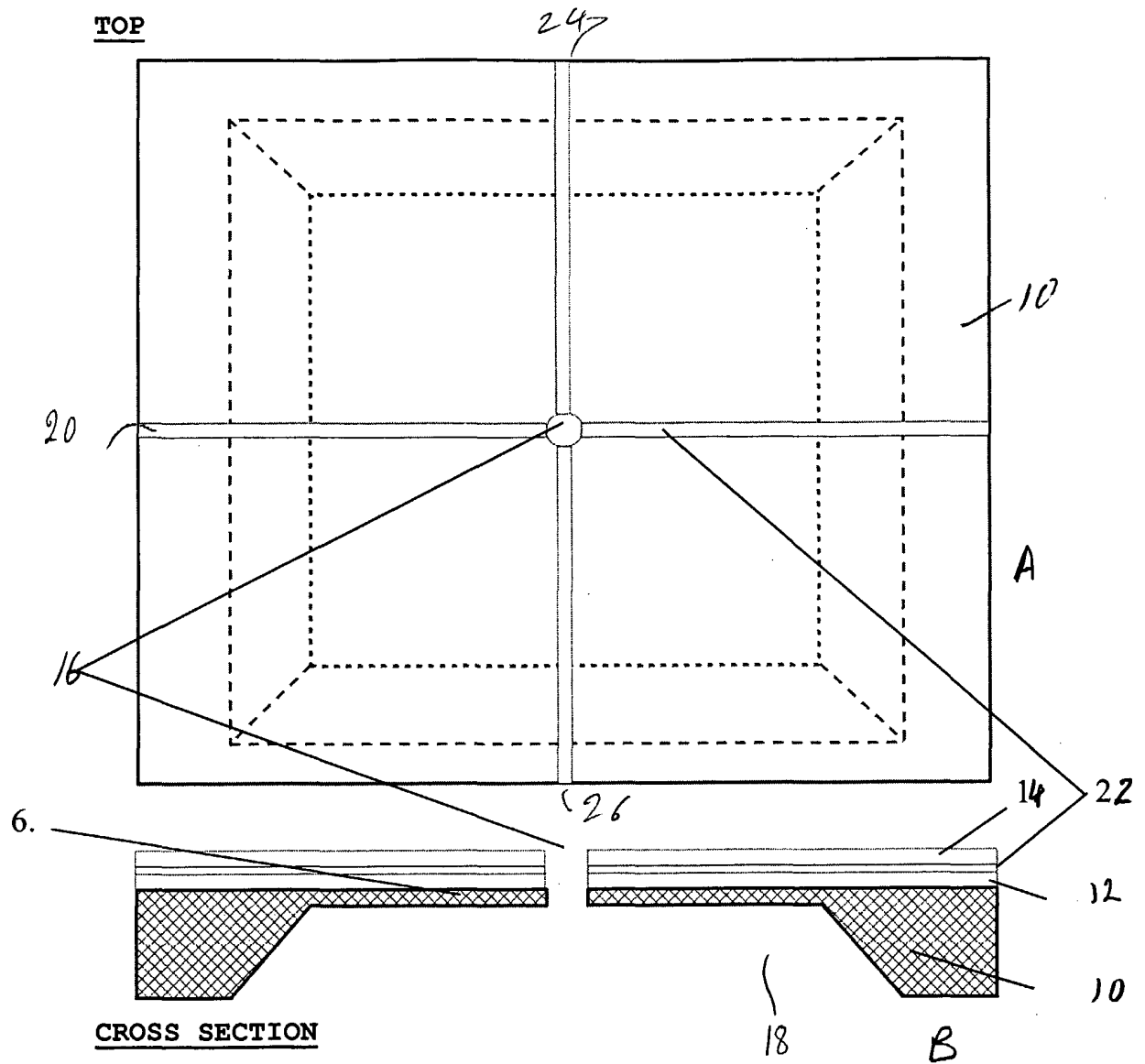


Fig. 1

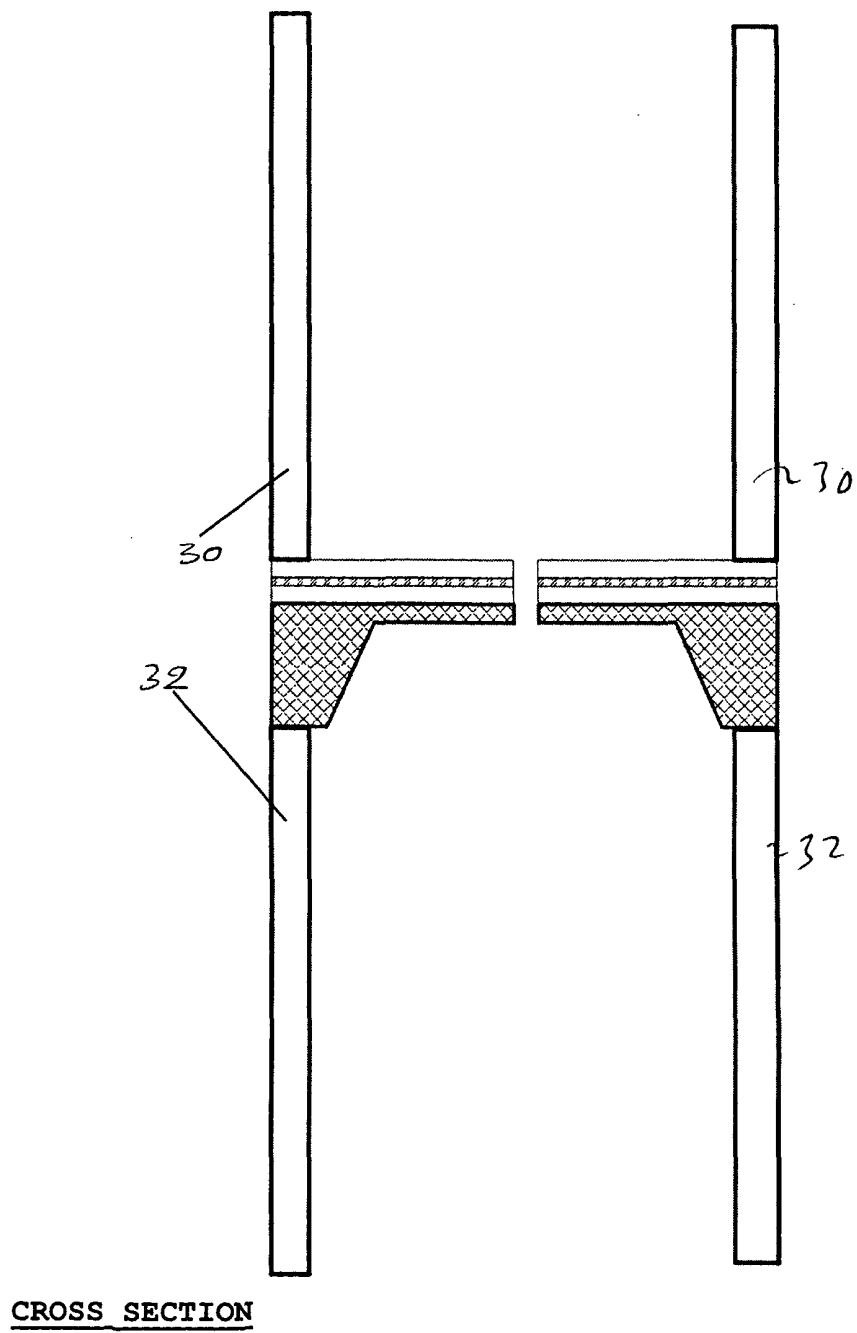


Fig. 2

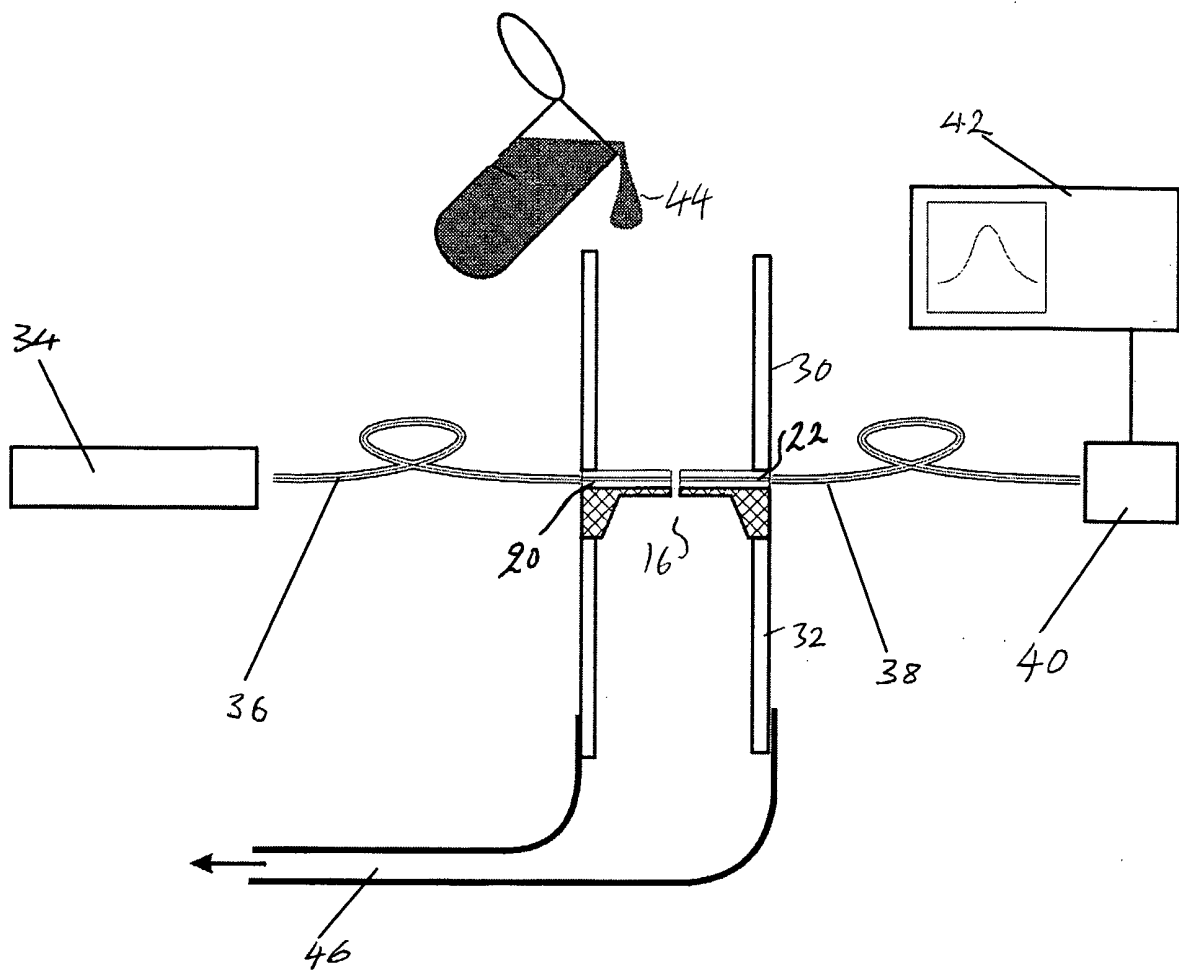


Fig. 3

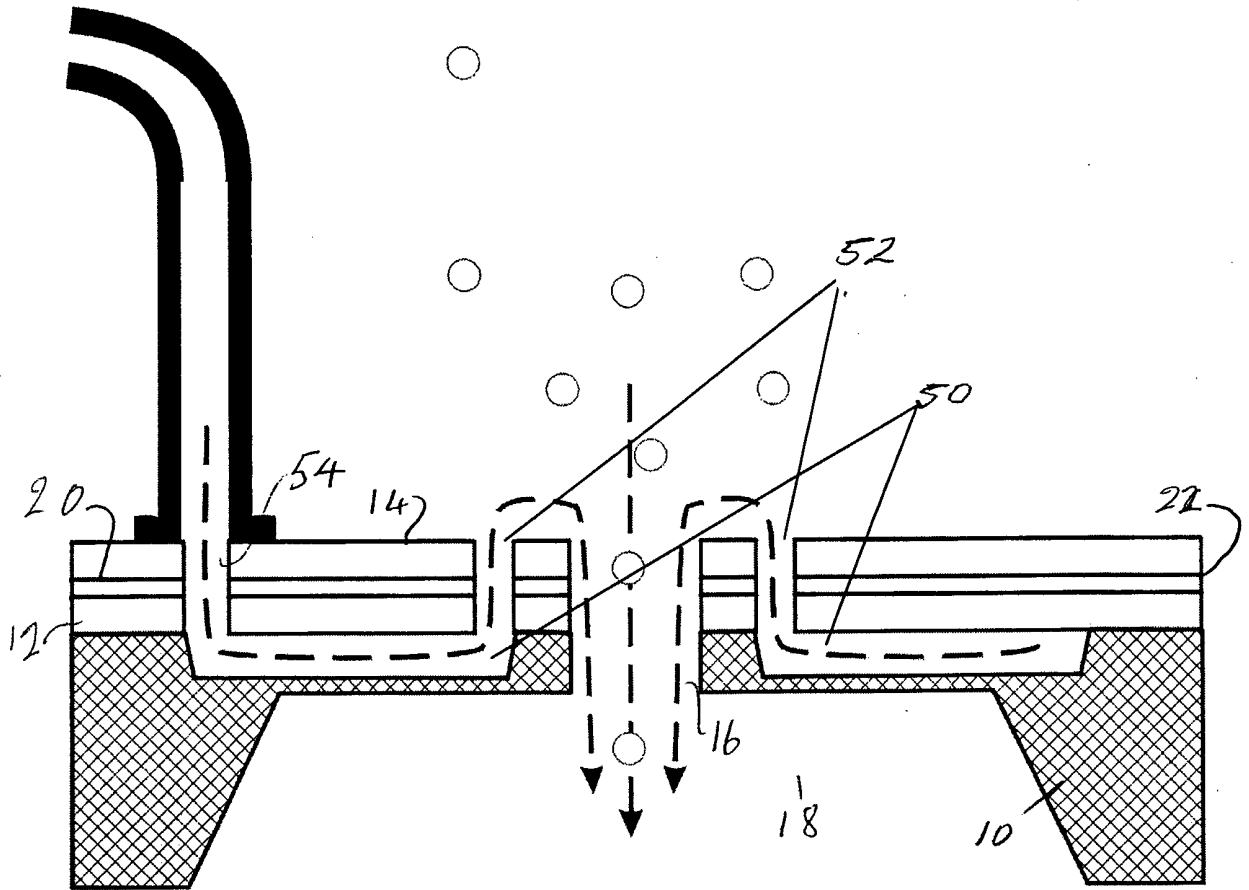


Fig. 4

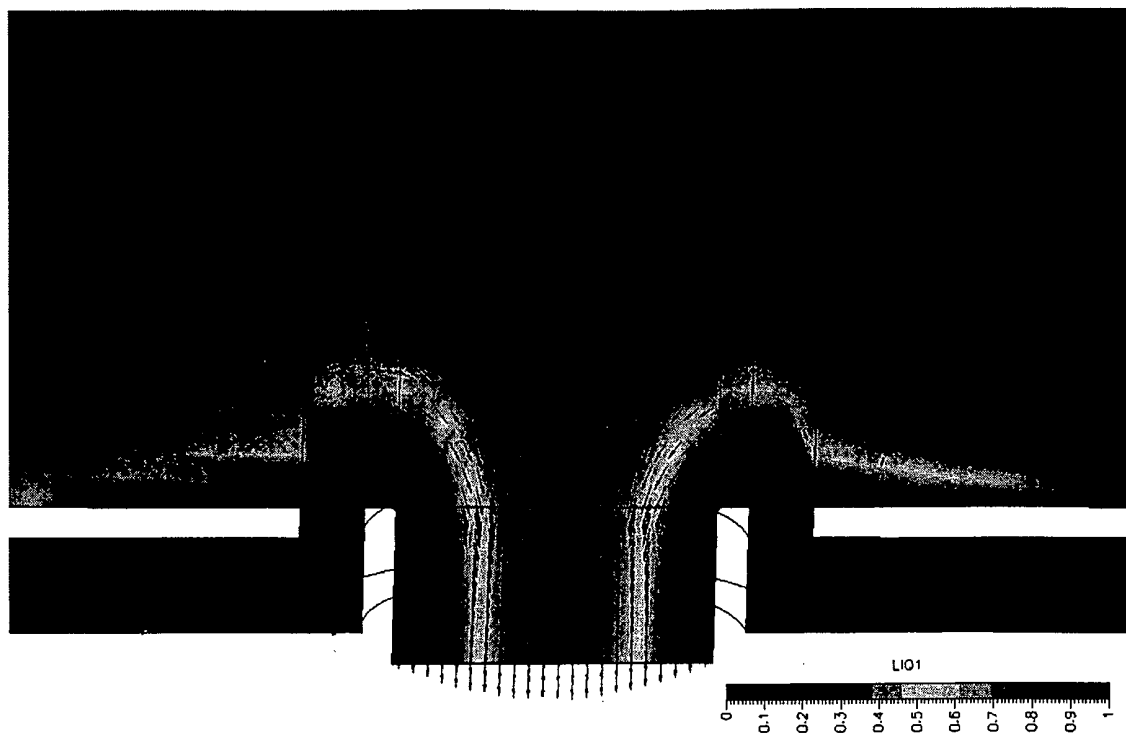
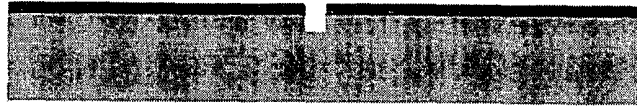


Fig. 5

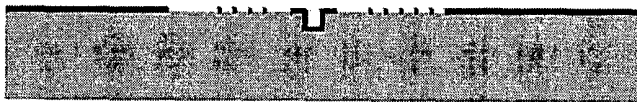
Step 1



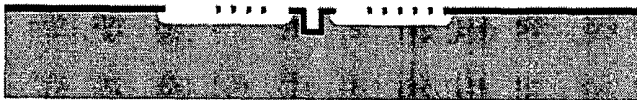
Step 2



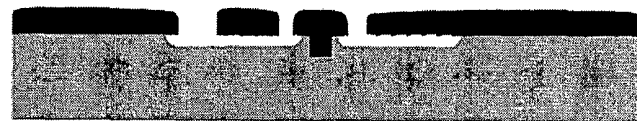
Step 3



Step 4



Step 5



Step 6



Fig 6