(12) UK Patent Application (19) GB (11) 2 361 883 (13) A

(43) Date of A Publication 07.11.2001

(21) Application No 0010518.9

(22) Date of Filing 03.05.2000

(71) Applicant(s)
Cell Analysis Limited
(Incorporated in the United Kingdom)
The Old Stable, Farnham Lane, Farnham Royal,
SLOUGH, SL2 3SE, United Kingdom

(72) Inventor(s)
Walter Bernard Betts
Andrew Paul Brown

(74) Agent and/or Address for Service
Saunders & Dolleymore
9 Rickmansworth Road, WATFORD, Herts, WD18 0JU,
United Kingdom

(51) INT CL[7]
B03C 5/02

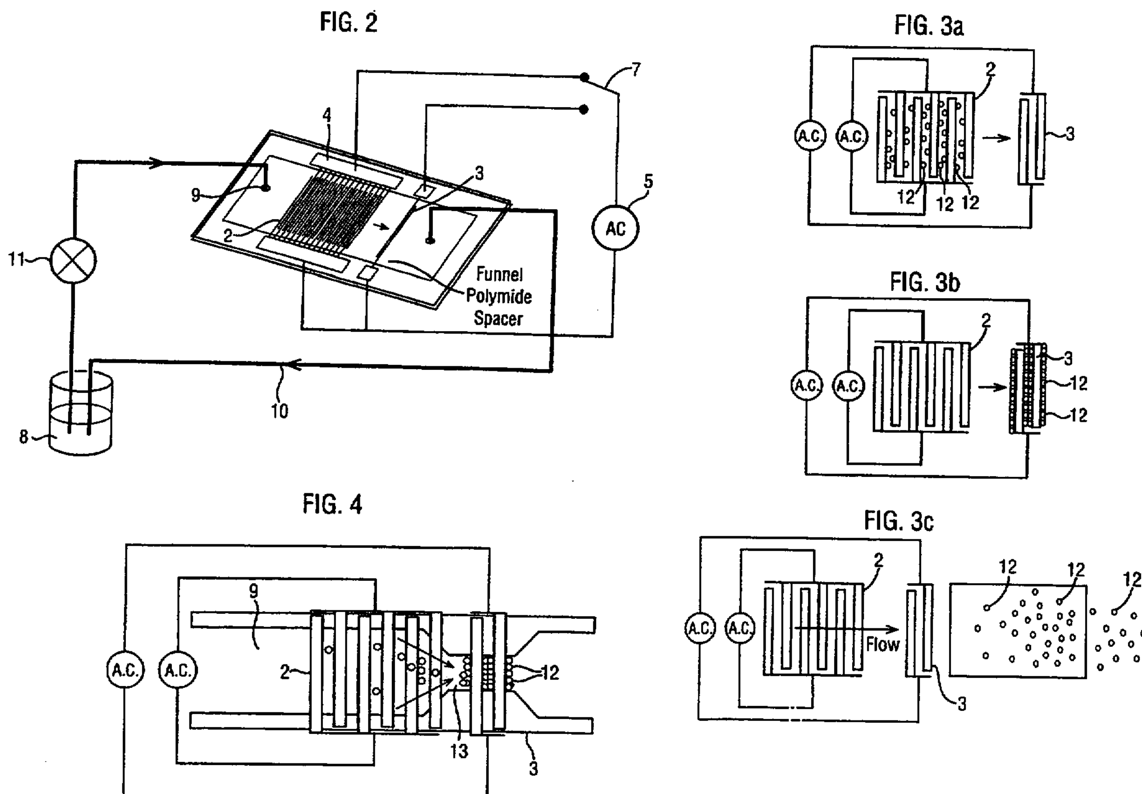
(52) UK CL (Edition S)
B2J J101 J207 J212

(56) Documents Cited
US 5569367 A

(58) Field of Search
UK CL (Edition R) B2J JB1 JB2 JD2
INT CL[7] B03C 5/00 5/02
ONLINE:WPI,JAPIO,INSPEC,EPODOC

(54) Abstract Title
Method and apparatus for analysing low concentrations of particles

(57) The apparatus comprises a support (1) defining a fluid flow channel (9) and a dual electrode array comprising a first electrode means (2) and a second electrode means (3). The first electrode means (2) is energised with an AC voltage of predetermined frequency to attract a predetermined type of particle to the electrode. The voltage is then switched off after a period of time and the second electrode means (3) is energised with an AC voltage of predetermined frequency. After a period of time the voltage is switched off, releasing the particles for subsequent collection and/or enumeration.



GB 2 361 883 A

At least one drawing originally filed was informal and the print reproduced here is taken from a later filed formal copy.

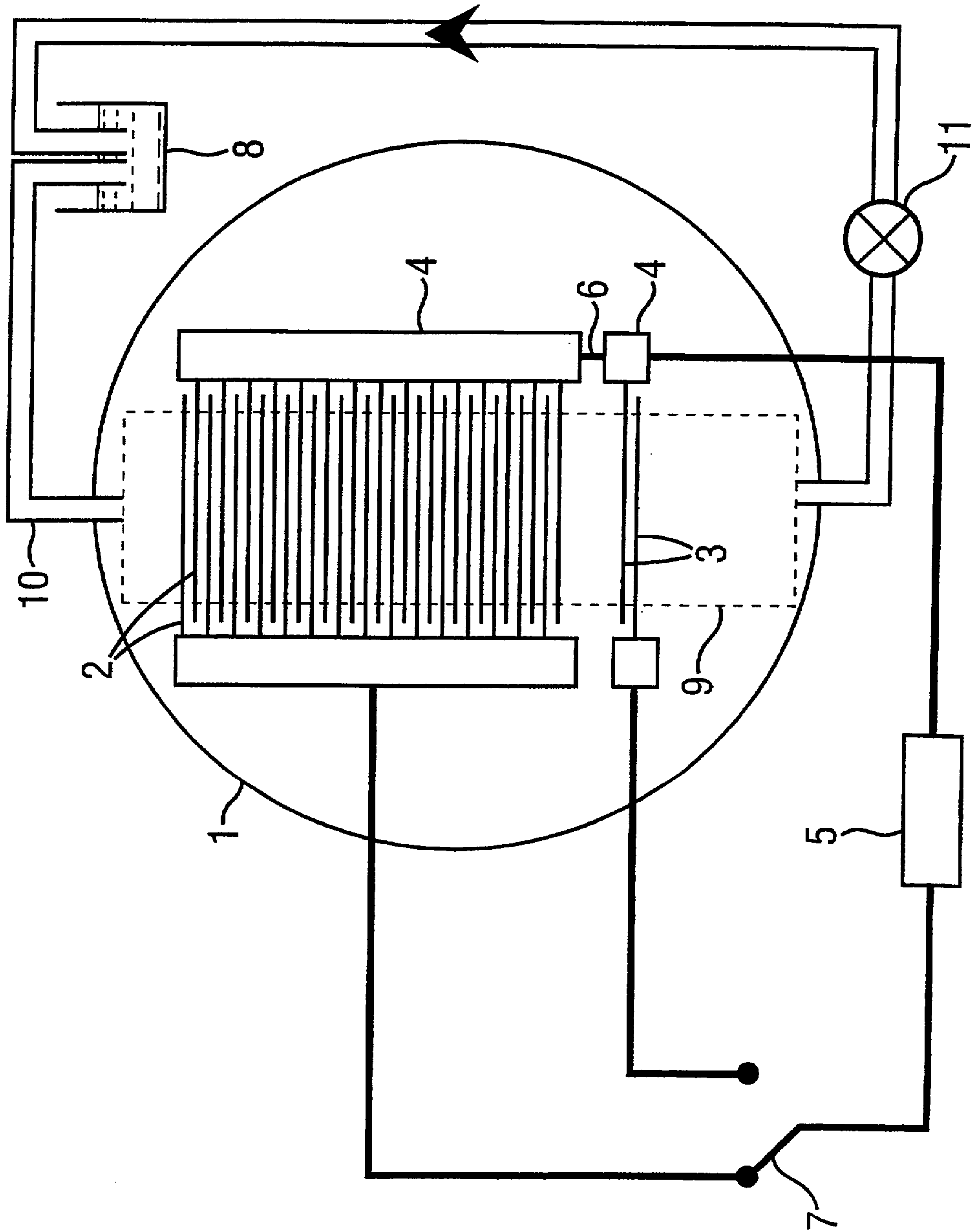


FIG. 1

FIG. 2

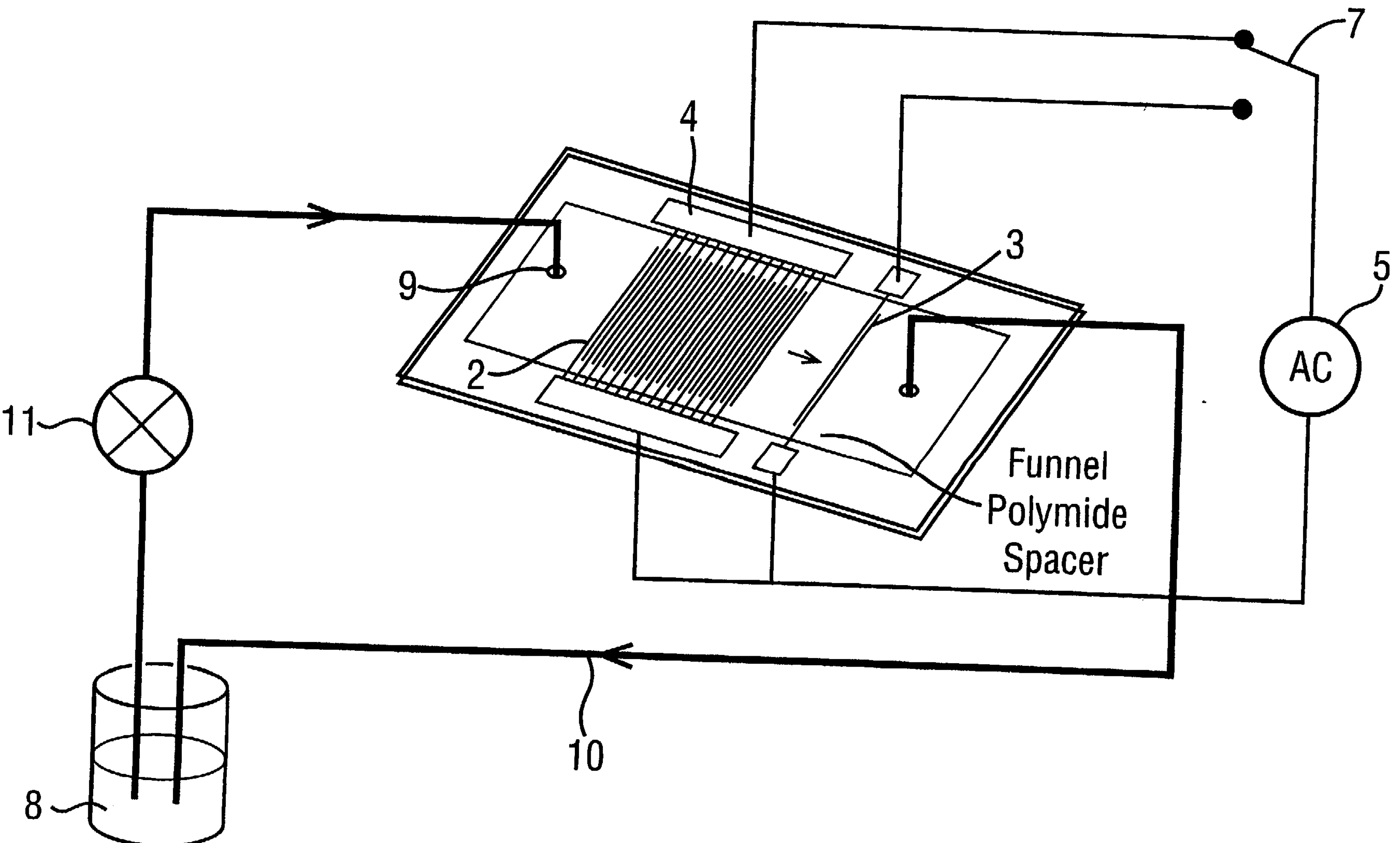


FIG. 3a

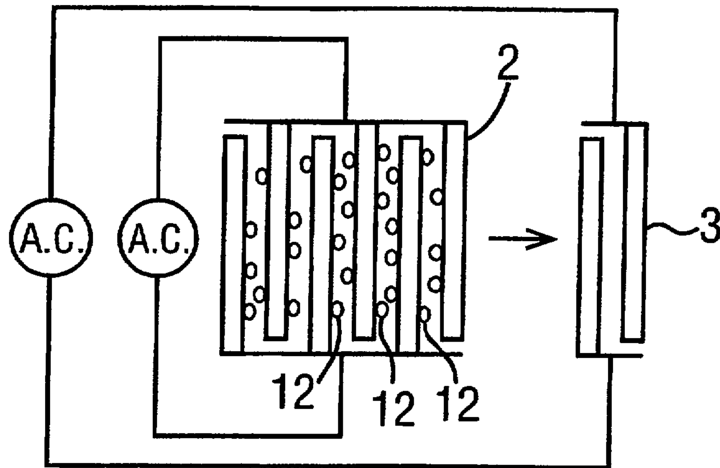


FIG. 3b

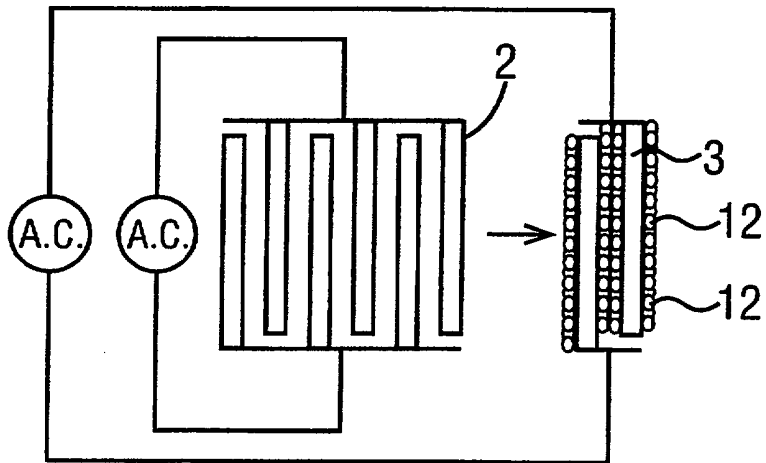


FIG. 3c

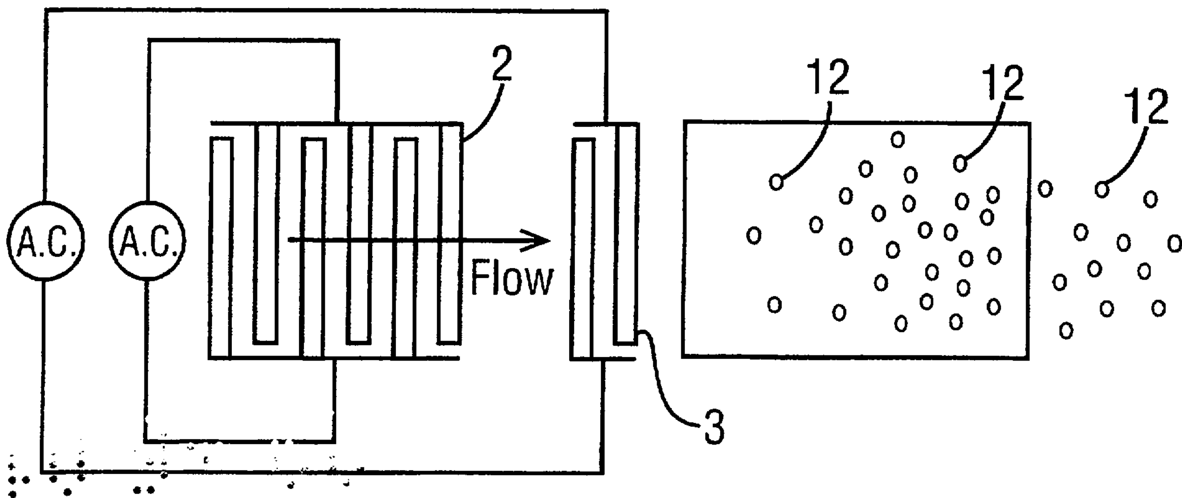
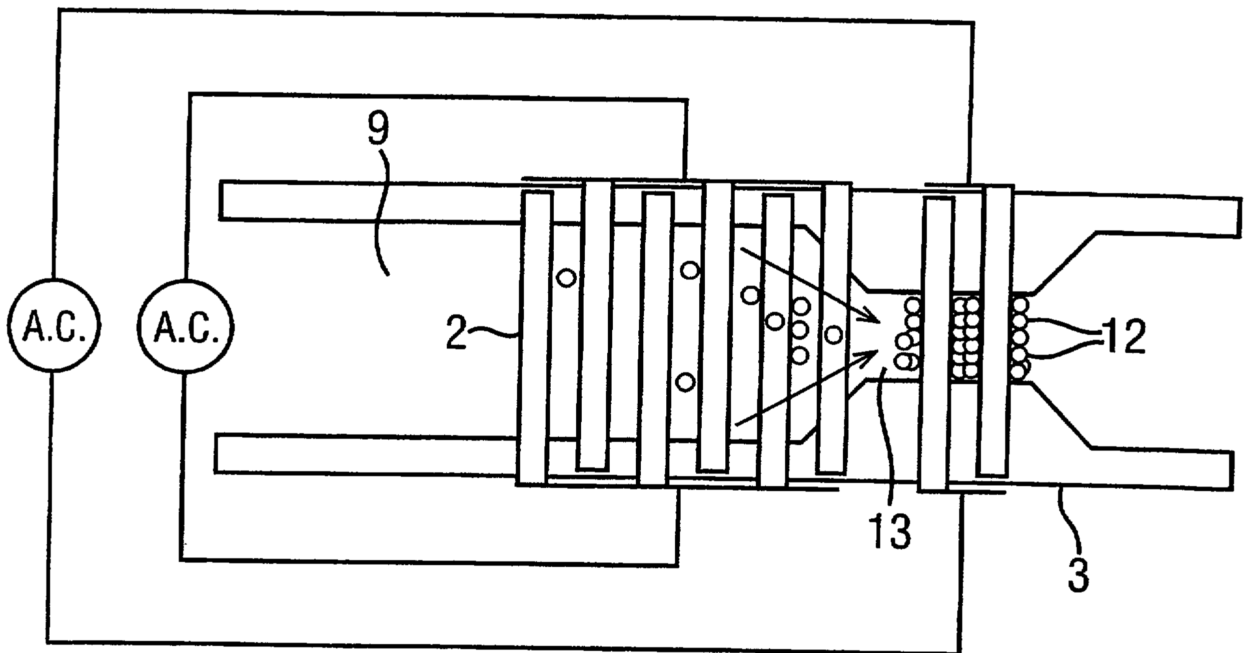


FIG. 4



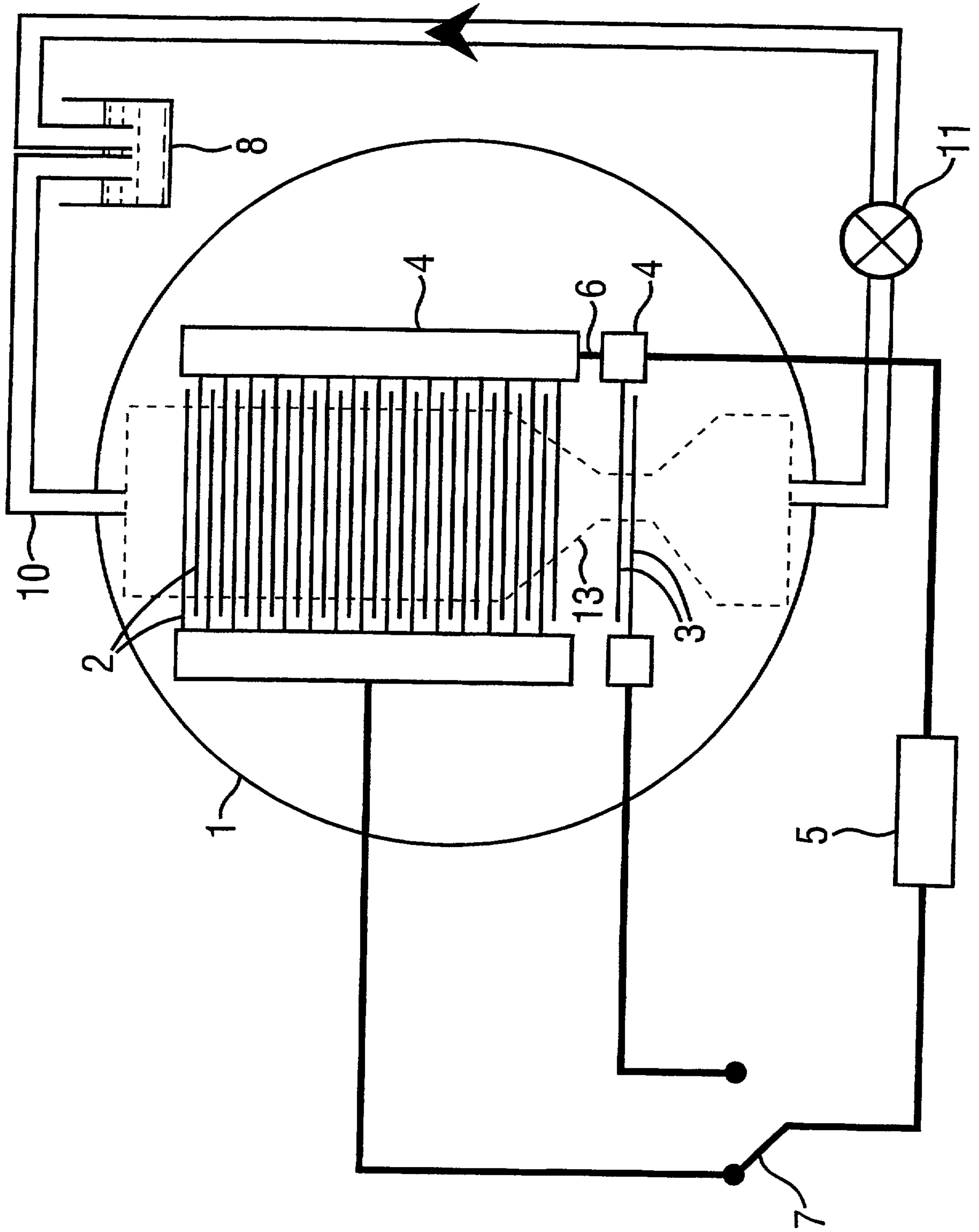


FIG. 5

2361883

METHOD AND APPARATUS FOR ANALYSING LOW CONCENTRATIONS OF PARTICLES

The present invention relates to a method and apparatus for collecting and
5 analysing very low concentrations of abiotic and/or biotic particles, such as biological cells, cell organelles, viruses and prions, and chemicals, biochemicals or macromolecules using dielectrophoresis. It also relates to a method and apparatus for enumerating a particular particles present in a test sample.

10 It is well known that when an AC voltage is applied to a pair of electrodes which have a suspension of particles between them, the particles may polarise and have a force exerted upon them where the electric field is non-uniform. This translational force (the dielectrophoretic force) may cause the particles to aggregate in areas of either high or low electric field gradient, dependent upon the
15 relative polarisabilities of the particles and the suspending medium. The polarisability of the particle is a function of its conductivity and permittivity, and varies with the frequency of the electric field. Measuring the number of particles collected as the frequency of the voltage generating the electric field changes allows a collection spectrum to be plotted. This has been shown to be
20 characteristic for individual species of biological cells and for abiotic particles, since the polarisability of a particle type is dependent upon its individual, unique structure.

This phenomenon, known as Dielectrophoresis (DEP), has been shown to
25 be useful for particle and cell characterisation for the separation of a particle type from a mixed suspension, and also for the manipulation of biomolecules.

The dielectric properties of all materials have characteristic frequency-dependent components and the polarisability of the particle, and therefore the
30 polarity and magnitude of the dielectrophoretic force, will vary as a function of the field frequency. The polarisation mechanisms involved in dielectrophoresis of

particles occur at different rates. Therefore with increasing frequencies successive mechanisms will drop out of the polarisation process as their relaxation can no longer keep pace with the speed of the alternating field. Thus when using AC electric fields, the level of particle collection at electrodes observed over a frequency range will vary. Furthermore, characteristic collection spectra for particular particle types can be produced, since the different types will possess a variety of polarisation mechanisms each with distinct frequency responses and occurring to different extents.

One problem for many potential applications for dielectrophoresis is the requirement to analyse very low concentrations of particles (including biological cells, cell organelles, viruses, prions, macromolecules and abiotic particles). The following examples illustrate the problem: regulations stipulate that concentrations of coliform bacteria present in potable water should be <1 coliform per 100 ml water and thus the organism should be detectable at the level of 1 coliform per 100 ml; hygienically significant concentrations of bacteria within food samples are commonly $<10^4$ cfu/ml and it is accepted that detection of 1 bacterium in 25 g of food is necessary; the presence of bacteria in certain contaminated blood products need to be detectable at the clinically significant level of 10^4-10^5 cfu/ml; and oocysts of the protozoan *Cryptosporidium* should be detectable at the level of 100 oocysts in 1000 litres, or water.

Traditional microbiological methods almost exclusively require enrichment techniques involving incubations of several hours to several days to increase the concentration of cells to detectable levels. Dielectrophoretic techniques offer an alternative procedure, which do not necessitate long incubations or enrichment stages. Instead, native organisms present within the sample can be analysed after abstraction from the sample matrix. However, until now dielectrophoretic techniques have suffered from a lack of sensitivity of the detection system which is used to quantify the number of particles collected upon

the electrodes (or elsewhere). For example, spectrophotometric detection required levels of 10^7-10^8 cfu/ml to give high signal:noise ratio; image analysed microscopical detection similarly requires particle concentration in excess of 10^6 cfu/ml. This is largely due to the nature of the dielectrophoretic electrodes and
5 their containment chambers, which provide poor collection efficiencies, especially when utilising a two bar electrode arrangement with a relatively small edge surface area. Due to: i) relatively deep channels in the chamber; ii) small detection "windows"; iii) low electrode edge length, and iv) the use of slow collection speeds and short pulse lengths (to improve the analysis time), as few as
10 100-200 cells might be detected out of a circulating concentration of 10^6 cfu/ml.

Larger surface area, multiple electrode configurations (referred to as columns) are more efficient at particle collection due to the increased total electrode edge length available for cell collection. However, such columns are
15 disadvantageous for detection when standard techniques such as image analysis microscopy are used. As there may be a time delay before cells released from the columns pass through the detection window, the peak of detection is often indistinct. If there are very low concentrations of cells, then individual cells might be collected on electrodes at relatively large distances from each other and
20 the electrode "window" imaged might not contain any cells at all. Furthermore, over this period, the released particles have time to move out of the plane of focus of the microscope and may not be detected at all. This means that even though low concentrations of particles may be collected using dielectrophoresis, they cannot currently be detected using techniques such as spectrophotometry, image
25 analysis microscopy and others.

It is an object of the invention to provide an accurate dielectrophoretic method and apparatus for rapidly enumerating particular particles present in a test sample.

30

It has now been found that by utilising a novel dual electrode arrangement comprising a first electrode means and a second electrode means, passing or circulating a liquid sample containing a low concentration of particles suspended therein past the electrode arrangement, applying at least one AC voltage of predetermined frequency to the first electrode means, switching off the voltage(s) to the first electrode means and applying the same or a different AC voltage(s) to the second electrode means, then switching off the voltage(s) to the second electrode means, that it is possible to collect and/or analyse very low concentrations of abiotic and/or biotic particle or biomolecules.

According to one aspect of the invention there is provided a method of analysing very low concentrations of particles present in a fluid sample, the method comprising passing or circulating the liquid or gaseous sample through a region of non-uniform electric field density produced by a dual electrode arrangement, said arrangement comprising a first electrode means for producing successive electric fields so as to collect all or most of the particles in the sample and a second electrode means to collect all the particles released from the first electrode means for detection, energising said first electrode means with at least one AC voltage having a predetermined frequency selected to attract a predetermined type of particle in the sample to said array, switching off the voltage(s) to the first electrode means thereby releasing the particles, energising the second electrode means with at least one AC voltage having a predetermined frequency selected to attract particles in the sample to said second electrode means, switching off the voltage(s) to the second electrode means thereby releasing the particles for subsequent separation, collection, identification and/or enumeration.

The first electrode means of the dual electrode arrangement comprises an electrode with a large surface area to provide for particle collection. Thus, it may comprose a multiple electrode array such as a multiple bar electrode, column array

or other suitable electrode geometry preferably comprises a multiple electrode array such as a multiple bar electrode or column array.

The second electrode means of the dual electrode arrangement which
5 forms the focussing element of the dual electrode arrangement preferably comprises a two bar electrode which enables all the particles released from the column array to be collected and concentrated into a small area for easy detection. The focussing two bar electrodes can be energised separately from the multiple bar electrode thus enabling a different frequency or voltage(s) to be applied,
10 thereby improving selectivity.

More than two different voltages having different predetermined frequencies may be superimposed on and applied to the electrode arrangement in order to attract all the particles in the liquid sample to them. The particles can
15 then be subsequently released en masse by switching off all of the voltages, thus permitting a total particle count to be determined. Alternatively, the particles may be released from the electrodes individually by type by switching off a selected voltage thus facilitating separation of the particles for subsequent collection, identification and/or enumeration. (see copending patent application no
20 0001376.3).

The method may be used for collecting and analysing very low concentrations of different biotic particles such as micro-organisms and/or different cell types and cell organelles including plasmids. The term micro-
25 organism is intended to embrace bacteria, viruses, yeasts, algae, protozoa, fungi and prions. Abiotic particles which may be separated include for example metal particles or any inorganic or organic material. Chemical or biochemical species can also be separated.

30 According to a second aspect of the invention there is provided an apparatus for analysing very low concentrations of particles present in a liquid

sample, the apparatus comprising a support defining a fluid flow channel through a region of non-uniform electric field density, circulating means for circulating said sample containing said particles through said channel and a dual electrode arrangement for providing the non-uniform electric field, said electrode arrangement comprising a first electrode means connected to which is an AC source for applying at least one voltage at a predetermined frequency and downstream of said first electrode means a second electrode means connected to the same or a different AC source for applying the same or a different voltage(s), wherein the frequency of said voltage(s) is selected to cause a predetermined type of particle to be attracted to said electrode arrangement, and means for determining the quantity of particles when the voltage(s) is not applied.

According to yet another aspect of the invention there is provided the use of the method defined above or the use of the apparatus defined above for the detection and enumeration of very low concentrations of eukaryotic cells, bacteria, yeasts, viruses, algae, protozoa, fungi, prions, inorganic or organic abiotic particles, plasmids, cell organelles, chemicals or biochemicals including nucleic acids and chromosomes.

A method and apparatus for collecting and analysing very low concentrations of abiotic and/or biotic particles will now be described, by way of example, with reference to the accompanying diagrammatic drawings in which:

Figure 1 is a diagram of an electrical and fluid circuit of an apparatus in accordance with the invention;

Figure 2 is a perspective view of an apparatus in accordance with the invention;

Figures 3a, b, c are diagrams of the dual electrode arrangement showing the collection and release of particles during the method in accordance with the invention;

Figure 4 is a diagram of an alternative embodiment of part of the apparatus in accordance with the invention;

Figure 5 is a diagram of an electrical and fluid circuit of an apparatus incorporating the embodiment of Figure 4.

The apparatus shown in Figure 1 comprises a silicon wafer substrate 1
5 upon which multiple interdigitating parallel electrode bars forming a column array 2 have been deposited to form the first electrode means as a column array. Spaced from the column array 2 is the second electrode means which comprises a two bar electrode 3 which forms the focussing element of the electrode arrangement. Electrode tabs 4 connect the electrode bars 2 and 3 to a signal generator 5 which
10 supplies an AC voltage(s) to the electrodes 2 and 3. Connector 6 joins the electrode tabs 4 of the column array 2 to the two bar electrode 3. A switch arrangement 7 is provided to facilitate the alternate energising of column array 2 and the two bar electrode 3.

15 A reservoir 8 containing the particle suspension under analysis is connected to a fluid flow channel 9, in which the dual electrode arrangement 2 and 3 is positioned, by tubing 10. A pump 11 is provided to move the particle suspension through the tubing 10.

20 The pump 11 is advantageously a peristaltic pump to prevent any contamination to the sample liquid and particles therein. The fluid in the reservoir 8 may be agitated by bubbling air or other gas therethrough to keep the particles in suspension.

25 In order to collect a particular particle for enumeration, e.g. E.coli bacteria, the liquid sample is placed in the reservoir 8 and pumped by pump 11 via tubing 10 through the fluid flow channel 9 over electrodes 2 and 3.

The column array 2 is energised with a voltage of a predetermined
30 frequency using signal generator 5 and cells 12 collect on the column array 2 as shown in Figure 3a. At this time the two bar electrode 3 may be switched on or

off. After a suitable period of time has elapsed, the current to the column array 2 is turned off and simultaneously the two bar electrode 3 is energised. The cells 12 will be released from the column array 2 and will collect in large numbers on the two bar electrode 3 as shown in Figure 3b. Since the cells 12 released from the
5 column array 2 will be maintained close to the plane of the electrodes due to the parabolic velocity profile of the flow, the cells 12 will be collected very easily on the two bar electrode 3 with minimal losses.

Once the cells 12 have been collected on the two bar electrode 3 for a
10 suitable length of time, the current can be turned off thus releasing the cells 12 from the two bar electrode 3, as shown in Figure 3c, allowing the cells to be counted by, for example, image analysis microscopy.

The two bar electrode 3, or focussing electrode, may be energised
15 separately from the column array 2 thus pre-selected voltages of different frequency can be employed with a resultant improvement in selectivity.

Figure 4 illustrates an alternative arrangement of the dual electrode and the fluid flow channel. In this embodiment the fluid flow channel 9 narrows at a
20 point 13 in the vicinity of the two bar electrode 3. The effect of this construction is that cells released from column array 2, once the current to the array is switched off, are funnelled into a small area where the two bar electrode 3 is positioned. This arrangement helps to concentrate the cells further, improving the enumeration of them.

25

Apart from the image analysis technique referred to above, other methods for enumerating the number of particles released from the two bar electrode 3 include spectrophotometric (including fluorescence) laser, impedance analysis and radiometric (see copending patent application 0001374.8).

Any number of signal generators may be inductively coupled to apply several different frequencies of voltage to the dual electrode arrangement. By using an appropriate number of frequencies, it may be possible to collect every type of particle in a suspension. By changing the applied frequencies or
5 voltage(s) different particle types can be released individually for subsequent enumeration.

CLAIMS

1. A method of analysing very low concentrations of particles present in a fluid sample, the method comprising passing or circulating the liquid or gaseous sample through a region of non-uniform electric field density produced by a dual electrode arrangement, said arrangement comprising a first electrode means for producing successive electric fields so as to collect all or most of the particles in the sample and a second electrode means to collect all the particles released from the first electrode means for detection, energising said first electrode means with at least one AC voltage having a predetermined frequency(s) selected to attract a predetermined type of particle(s) in the sample to said array, switching off the voltage to the first electrode thereby releasing the particles, energising the second electrode means with at least one AC voltage(s) having a predetermined frequency(s) selected to attract particles in the sample to said second electrode means, switching off the voltage(s) to the second electrode means thereby releasing the particles for subsequent separation, collection, identification and/or enumeration.

2. The method according to Claim 1, wherein the first electrode means is a multiple bar electrode and the second electrode means is a two bar electrode.

3. An apparatus for analysing very low concentrations of particles present in a fluid sample, the apparatus comprising a support defining a fluid flow channel through a region of non-uniform electric field density, circulating means for circulating said sample containing said particles through said channel and a dual electrode arrangement for providing the non-uniform field, said electrode arrangement comprising a first electrode means connected to which is an AC source for applying at least one voltage at a predetermined frequency(s) and downstream of said first electrode means a second electrode means connected to the same or a different AC source for applying the same or a different voltage(s),

wherein the frequency(s) of said voltage is selected to cause a predetermined type of particle to be attracted to said electrode arrangement, and means for determining the quantity of particles when the voltage(s) is not applied.

5 4. The apparatus according to Claim 3, wherein the first electrode means is a multiple bar electrode and the second electrode means is a two bar electrode.

5. Use of the method according to Claim 1 or Claim 2, or of the apparatus according to Claim 3 or Claim 4 for the detection and enumeration of very low
10 concentrations of eukaryotic cells, bacteria, yeasts, viruses, algae, protozoa, fungi, prions, inorganic or organic abiotic particles, plasmids, cell organelles, chromoscomes, chemicals or biochemicals including nucleic acids and proteins.

6. The method of analysing very low concentrations of particles present in a
15 liquid or gaseous sample substantially as hereinbefore described.

7. Apparatus for analysing very low concentrations of particles present in a liquid sample substantially as hereinbefore described with reference to the accompanying drawings.

Application No: GB 0010518.9 **Examiner:** John Cockitt
Claims searched: 1-7 **Date of search:** 9 November 2000

Patents Act 1977
Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:
UK Cl (Ed.R): B2J [JB1, JB2, JD2]
Int Cl (Ed.7): B03C [5/00; 5/02]
Other: ONLINE: EPODOC, WPI, JAPIO, INSPEC

Documents considered to be relevant:

Category	Identity of document and relevant passage	Relevant to claims
X	US5569367A BRITISH - see fig 2	1,3,5

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
		E	Patent document published on or after, but with priority date earlier than, the filing date of this application.
&	Member of the same patent family		