Microelectrode substrates for DEP analysis are disclosed having radially spaced curved electrode elements.
ELECTRODES ARRANGEMENT FOR ANALYSING LOW CONCENTRATIONS OF PARTICLES USING DIELECTROPHORESIS

This invention relates to analysing low concentrations of particles, particularly biological particles, such as biological cells, bacteria, virusus and other chemicals, biochemicals or molecules using dielectrophoresis.

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, depending on the relative polarisabilities of the particles and the suspending medium. This phenomenon is known as dielectrophoresis and is commonly used for detecting and counting the concentration of bacteria in samples, such as foodstuffs, medical samples and so on.

Figure 1 shows schematically the concept. A sample A is passed across a substrate having printed upon it, or otherwise formed upon it, a microelectrode structure comprising interdigitated electrodes 2 and 3. AC current 4 is generated and applied to the electrode via connections 5 and 6 to the respective electrodes 2 and 3. The electrodes are of micron dimensions and are energised with the voltage of a predetermined frequency using AC generator 4. The relevant particles (such as bacteria, biological cells and so on) collect on the electrode array and then, after the deposition process, the substrate can be analysed by visual inspection using microscopes or otherwise to count the number of particles and therefore information about the type and/or concentration of particles can be determined.

The term DEP will be used in this specification to mean dielectrophoresis. DEP methods used in this field are known from, for example, GB 2 358 361, GB 2 358 473 and GB 2 361 883.

The microelectrodes are formed on suitable substrates and may be placed into sample wells.
The present invention arose in an attempt to provide an improved electrode structure for DEP analysis.

According to the present invention there is provided an electrode structure for dielectrophoresis analysis of particles, comprising two conductive paths, at least part of which serve as microelectrodes and are spaced apart by a distance suitable for DEP, the two paths each having a curved portion.

In some embodiments, a first electrode comprises a radially inner generally annular part, a plurality of spoke portions extending outwards from the annular part and at least one distal portion extending from each spoke across each plurality of sampling areas, a different sampling area being associated with each spoke, the second electrode comprising a radially outer generally annular part, a plurality of spokes extending radially inwardly from the generally annular part and at least one distal portion extending from each spoke across each respective sampling area, generally towards the associated spoke of the first electrode, wherein the distal portions of the first and second electrodes are arranged in pairs which are spaced apart from each other at such a spacing that DEP can occur between them in the sampling area.

In some alternative embodiments, the structure comprises a first electrode having a plurality of curved electrode portions extending at spaced intervals along it, a second electrode having a second plurality of curved portions extending at spaced intervals along it, the curved portions of the first and second electrodes being interdigitated such that when a voltage is applied between the electrodes, an electric field is applied between the interdigitated arcuate members, which form an interdigitated electrode structure.

The radially inner and radially outer electrodes are preferably annular. The inner electrode is preferably continuous and the outer electrode is preferably discontinuous.

Preferably, two pairs of spaced electrodes are positioned across each sampling area, which may be a sampling well.
In a yet further aspect, the present invention provides an electrode structure for DEP, comprising a first electrode having a plurality of curved electrode portions extending at spaced intervals along it, a second electrode having a second plurality of curved portions extending at spaced intervals along it, the curved portions of the first and second electrodes being interdigitated such that when a voltage is applied between the electrodes, an electric field is applied between the interdigitated curved members, which form an interdigitated electrode structure.

Embodiments of the invention will now be described, by way of example only, with reference to the accompanying drawings, in which:

Figure 1 shows schematically a DEP arrangement;
Figure 2 shows an electrode;
Figure 3 shows an enlarged detail of part of the electrode; and
Figure 4 shows an alternative electrode.

As discussed above, DEP forces are produced across microelectrode arrays by an alternating current (AC) of fixed amplitude and frequency. DEP collects and concentrates microorganisms, suspended in a fluid in contact with the microelectrodes, onto the edge of the electrode. Typically, the microorganisms are labelled with a fluorescent dye to enable detection and enumeration. In the method described in a co-pending patent application, this can be done by laser scanning cytometry.

The microelectrode can be of various designs. They can be manufactured in various metals, including gold and aluminium, and are generally of dimensions such that the electrode width and gap side are of micron dimensions. The ratio of electrode width to gap size may vary depending upon the nature of the suspending fluid. In embodiments of the invention, the electrodes are formed upon materials which are transparent and of low autofluorescence and may typically be of glass or plastic. The electrodes may be manufactured by micro-fabrication technology employing photolithography or by printing metal ink technology, by print and electrode plating technology or by a combination of these or other methods. The electrode may be placed in the bottom of a well, for example a 96-well plate.
Note, however, that the electrode may have other uses than in laser scanning cytometry and may be used differently.

Figure 2 shows an electrode structure. The figure shows a substrate 1 having a number of wells or sampling areas 7. An AC current generator/controller 4 is used to supply AC current to two electrode arrays 2 and 3.

A first electrode arrangement 2 includes a first conductor 8 which is directly connected to the AC generator 4. This is then connected to a central annular conductor 9 via connections 10. A plurality of 'spokes' 11 extend radially outwards from annular part 9. Note that one of the spokes 11 in effect forms part of connector 10 forming part of the electrical path to the AC generator 4.

A second electrode arrangement 3 includes a part 12 directly connected to AC generator 4 which is connected by connector 13 to a discontinuous annular electrode part 14 which is radially outwards from the radial part 9 of electrode 2. Note that this is discontinuous so electrical connector 10 does not contact it. It may in some embodiments be continuous and insulating bridge means so that connector 10 may pass under or over annular electrode 14 but insulated therefrom.

A plurality of inwardly directed spokes 15 extend from annular part 14 directed towards, but spaced from, inner annular electrode 9. Similarly, the outward spokes 11 from electrode 9 are spaced from electrode 14. Each of the inner inward and outwardly directed spokes extend to the vicinity of ones of these sampling areas/wells 7 and a pair of distal parts of the electrode extend from each of the respective outwardly directed electrode towards, but spaced apart from, the adjacent inwardly directed electrode (across the sampling area). A first one of these such as 6 extends generally from an end or towards one end of a spoke 11 and a second extends from a position between the end of spoke 11 and the annulus 9.

Figure 3 shows an enlarged detail of this structure across a sampling area and shows electrode distal ends 16 and 17 extending from a spoke 11 across a sampling area.
Similarly, electrode distal ends 18 and 19 extend from each inwardly directed spoke 15 towards the adjacent outwardly directed spoke 11 across the sampling area and this is also more clearly shown in Figure 3. The distal parts will generally be parallel to one another and are provided in pairs such, for example, that part 16 is parallel and closely spaced from termination 18 such that an electric field is formed between the pair of them sufficient for DEP to occur. Typically, the spacing S between the electrode pair 16 and 18 (17 and 19) might be 10 µm. Advantageously, it could be less than this or wider eg 5 and 40 µm, or between 5 and 30 µm, or between 5 and 20 µm. It is believed that above about 50 microns the spacing would be too great for effective DEP to occur.

Thus, the two electrode arrangements 2 and 3 are generally interdigitated in three ways. Firstly, the respective annular parts 9 and 14 are coaxial and interdigitated, secondly, the spokes 11 and 15 are, although not parallel, interdigitated across sampling zones since they partially overlap and finally, the electrode termination pair 16, 18; 17, 19 are also interdigitated.

An annular or curved arrangement of electrodes, with an inner electrode being of greater curvature than an outer arrangement of course, leads to variations in electrical field and this affects the force applied to particles such as bacterial or biological cells which are passed across the electrode arrangement, and this helps to circulate the particles which assists in causing deposition of the particles in the sampling areas, and provides an improvement over prior art arrangements.

The term annular is intended to mean generally ring-shaped. It need not be circular and could be elliptical or otherwise.

Other arrangements have coaxial annular electrodes and/or spoked or overlapping (interdigitated) radial (spoke) elements may of course of be used, besides the specific one of Figures 2 and 3.

Figure 4 shows an alternative electrode structure.
This is again a generally annularly interdigitated structure. In this case, the electrode arrangement comprises a first electrode 20 and a second electrode 21. Electrode 20 comprises a first spine extending from a connection to an AC generator (not shown) to a central node 20 to generally central in the annular electrode arrangement. At spaced intervals along this a series of arcuate conductive members 23 are connected. These may be connected as shown in pairs, each extending in an opposite direction from a point on the spine. These are all generally annular but discontinuous, having a small gap or discontinuity by where they approach a spine 24 of the other electrode 21. The electrode parts 23 are all coaxial with central node 22. A further series of similar generally annular (but discontinuous) parts 25 extends from spine 24 of second electrode 21. The spines are linear and co-linear with each other. Parts 25 are also arranged to be generally coaxial with node 22 and are spaced between respective pairs of members 23. Thus, if the annular extensions from the two electrodes 20 and 21 are annularly alternated and therefore interdigitated. The spacing between them is in the μm range, allowing DEP to occur when a voltage and a sample is applied across them.

Although, in this embodiment, all of the annular parts are discontinuous, they may whole annuli and insulation bridges be formed such that the annular parts of one electrode do not electrically contact a spine of the other respective electrode.

In effect, the electrode structure of the embodiment of Figure 4 is a series of coaxial interdigitated annular electrodes parts. Note that instead of being annular (ie circular) they may of course be other shapes, such as generally elliptical or have more complex shape. The arcuate nature of the interdigitalation again affects the strength of the electrical field since the field, at a part of an electrode having a greater curvature, will be greater than that of an electrode having a lower curvature (ie radially inward and outward) and so this improves the circulating effect of particle and improves deposition.

There will typically be more than two annular parts for each electrode. The embodiment shown has eight. There may two, three, four, five, six, seven, eight or more.
In use of any of the electrodes, DEP is generated by a signal of fixed wavelength and amplitude. This may vary depending upon the conductivity of the suspending medium and the type of microorganism to be collected. The signal may be a sine wave or a square wave and may, in some embodiments, have a direct current (DC) offset depending upon the conductivity of the sample and the type of microorganism to be collected.

In use any of the electrodes simultaneously generate dielectrophoretic forces and electro-osmotic forces, which circulate the sample within the well and collect the bacteria, or other particles, onto the electrode edge. This removes the requirement to circulate the sample using an external stirrer or fluid displacement device, and improves the collection efficiency. It is particularly advantageous when the well is circular. In comparison, a straight inter-digitated electrode micro-array offers poor circulation and collection.

The embodiment of Figure 2 may be termed a mask.
Claims

1. An electrode structure for dielectrophoresis analysis of particles, comprising two conductive paths, at least part of which serve as microelectrodes and which are spaced apart by a distance suitable for DEP, the two paths each having a curved portion.

2. An electrode structure as claimed in Claim 1, wherein the curved portions are generally annular, one lying radially inward of the other.

3. An electrode structure as claimed in Claim 2, wherein the annular portions are coaxial.

4. An electrode structure as claimed in any preceding claim, wherein a first electrode comprises a radially inner generally annular part, a plurality of spoke portions extending outwards from the annular part and at least one distal portion extending from each spoke across each plurality of sampling areas, a different sampling area being associated with each spoke, the second electrode comprising a radially outer generally annular part, a plurality of spokes extending radially inwardly from the generally annular part and at least one distal portion extending from each spoke across each respective sampling area, generally towards the associated spoke of the first electrode, wherein the distal portions of the first and second electrodes are arranged in pairs which are spaced apart from each other at such a spacing that DEP can occur between them in the sampling area.

5. An electrode structure as claimed in Claim 4, wherein two distal portions extend from each spoke, each being spaced from an associated distal portion of the other electrode by an amount suitable for DEP to occur.

6. An electrode structure as claimed in Claim 5, wherein the distal portions from each spoke extends across a sampling area but do not contact the associated spoke of the other electrode.
7. An electrode structure as claimed in Claim 5 or 6, wherein the distal portions are spaced from each associated distal portion of the other electrode by between 0 and 50 \(\mu\)m, preferably between 5 and 40 \(\mu\)m and most preferably at around 10 \(\mu\)m.

8. An electrode structure as claimed in any of Claims 5 to 7, wherein the radially inner annular portion is annular and the radially outer annular portion is discontinuous, whereby an electrical connection is made with the radially inner portion to connect it to an external voltage source, which is insulated from the radially outer portion.

9. An electrode structure as claimed in any of Claims 1 to 3, comprising a first electrode having a plurality of curved electrode portions extending at spaced intervals along it, a second electrode having a second plurality of curved portions extending at spaced intervals along it, the curved portions of the first and second electrodes being interdigitated such that when a voltage is applied between the electrodes, an electric field is applied between the interdigitated arcuate members, which form an interdigitated electrode structure.

10. An electrode structure as claimed in Claim 9, wherein the curved parts are annular.

11. An electrode structure as claimed in Claim 9 or Claim 10, wherein the radial parts are generally annular with a discontinuity.

12. An electrode structure as claimed in any of Claims 9 to 11, wherein each of the first and second electrodes comprises a spine from which the annular part extends at spaced intervals.

13. An electrode structure as claimed in Claim 12, wherein the first electrode includes a node at the centre of the electrode and with which all the radial electrode parts are coaxial.

14. An electrode structure as claimed in any of Claims 9 to 13, wherein more than two radial parts are provided on each electrode.
15. An electrode structure as claimed in Claim 14, wherein the number of annular parts extending from the side of each electrode spine is two, three, four, five, six, seven, eight or more.

16. An electrode structure as claimed in any of Claims 9 to 15, wherein each electrode includes a spine and the annular parts extend in pairs from each spine in opposing circumferential directions.

17. An electrode structure for DEP, comprising a first electrode having a plurality of curved electrode portions extending at spaced intervals along it, a second electrode having a second plurality of curved portions extending at spaced intervals along it, the curved portions of the first and second electrodes being interdigitated such that when a voltage is applied between the electrodes, an electric field is applied between the interdigitated arcuate members, which form an interdigitated electrode structure.

18. An electrode structure as claimed in Claim 17, wherein the members each form an arc of a circle.

19. An electrode structure for DEP, substantially as hereinbefore described with reference to, and as illustrated by, Figure 2 or Figure 4 of the accompanying drawings.
**INTERNATIONAL SEARCH REPORT**

**A. CLASSIFICATION OF SUBJECT MATTER**

| INV. | B03C5/02 |

**INTERNATIONAL APPLICATION**

| No.         | PCT/GB2009/001720 |

**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):

| BO3C |

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

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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<td>WO 96/31282 A (SCIENT GENERICS LTD [GB]); DAMES ANDREW NICHOLAS [GB]; SAFFORD NICHOLAS) 10 October 1996 (1996-10-10) figures 1,2</td>
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Further documents are listed in the continuation of Box C. See patent family annex.

**Date of the actual completion of the international search**

| 14 September 2009 |

**Date of mailing of the international search report**

| 23/09/2009 |

**Name and mailing address of the ISA**

European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk
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**Authorized officer**

Demol, Stefan
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**INTERNATIONAL SEARCH REPORT**

**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [x] Claims Nos.: 19
   because they relate to subject matter not required to be searched by this Authority, namely:
   
   Rule 6.2(a) PCT reference to drawings

2. [ ] Claims Nos.:  
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:  
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.

Form PCT/ISA/21.0 (continuation of first sheet (2)) (April 2005)
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