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(54) **Dielectrophoretic device and method for cell membrane studies**

(57) A dielectrophoretic device and method is described for manipulating one or more particles, e.g. living cells. The device comprises a plurality of electrodes in the form of a DEP cage, and a particle movement detection system for detecting movement of the one or more particles within the cage. Particle movement is indicative of passing through the cross-over frequency. The particle movement detection system includes a particle presence sensor and this can be located eccentrically with respect to a trapping point of the DEP cage.
The device and method may be used for classification, identification, of quantification of diseased versus healthy cells, for diagnosis, for medical research and development and in therapy. They are particularly useful for investigating living cells whose cell membrane alters in conductivity or permeability.

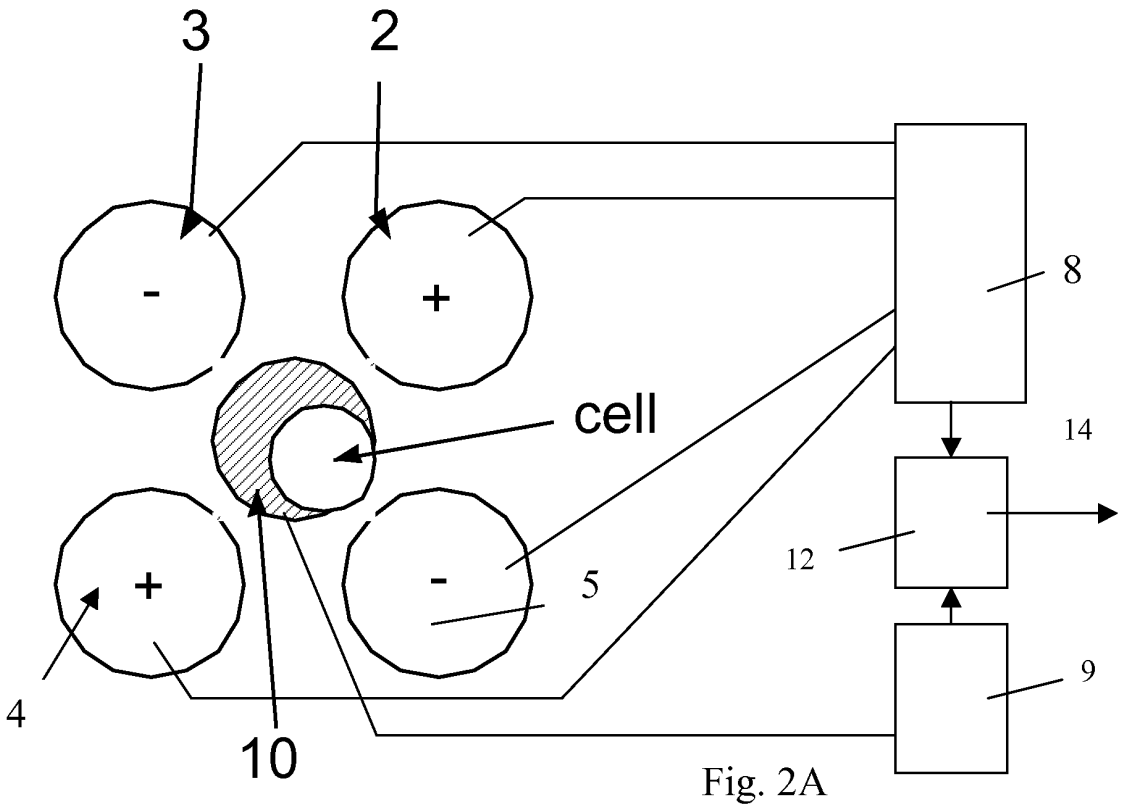

Fig. 2A

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Description

[0001] The present invention relates to dielectrophoretic devices. More particularly the present invention relates to a dielectrophoretic device for manipulating particles, to a method for forming such a device and to a method for manipulating particles using such a device. In particular the present invention relates to a dielectrophoresis method and a dielectrophoresis device for determining and/or controlling permeability of the cell membrane of cells. The methods and devices according to embodiments of the invention may, for example, be used for identifying and/or separating and/or sorting particles or for use in lysis or electroporation of cells.

[0002] The ability to identify and/or separate cell sub-populations from a heterogeneous cell mixture is essential in many biomedical applications. The simplest methods known for such purposes are based on filtration and centrifugation and rely on differences in cell size or density. More advanced methods exploit specific binding of antibodies to antigens on a cell surface to target a particular cell population. Examples of such methods are magnetically activated cell sorting (MACS), where antibody-functionalized magnetic beads are attached to the cells and sorted in a magnetic field, or fluorescence-activated cell sorting (FACS), where cells are labeled with fluorescent antibodies and separated by electrostatically deflecting charged liquid droplets containing the cells. Current FACS analyzers are very versatile instruments and allow cell separation on the basis of multiple simultaneous markers, cell size, and scattering properties. However, they are large and expensive instruments and can only be operated by trained personnel.

[0003] Recently, considerable effort has been put into transferring cell analysis to microfabricated systems. The advantages of lab-on-a-chip devices include ease of use and low fabrication costs (ultimately leading to disposable chips), low fluid volumes and reagents consumption, large integration of functionalities, high-throughput analysis via massive parallellization and increased process control due to the faster response of the system. Electric field based approaches are particularly suited for miniaturization because micropattemed electrodes are easy to fabricate and result in high electric fields at modest voltages.

[0004] One of the most promising methods to separate and manipulate cells in microsystems is dielectrophoresis (DEP), i.e. the movement of dielectric particles in a non-uniform, usually AC, electric field. Unlike electrophoresis, DEP relies on field-induced polarization effects and is independent of the net charge of the particle. The DEP force depends on the electrical properties of the particle and of the surrounding medium, on the size and shape of the particle and on the spatial distribution and frequency of the applied field. Depending on these factors, the particle can be attracted to either high-field (positive DEP) or low-field (negative DEP) regions. By using proper electrode configurations and multiphase fields, DEP can be used to levitate particles, trap them in a field cage, rotate them (electro-rotation) or transport them over relatively long distances (traveling wave DEP).

[0005] DEP has been applied to manipulate and separate a variety of cells including bacteria, yeast, and mammalian cells in microsystems. In particular, DEP has been used to separate cancer cells from blood, isolate CD34+ stem cells from blood, bacteria from blood and to separate various cell sub-populations of blood.

[0006] Most reported experiments are proof-of-principle applications of DEP, in which cells that undergo positive DEP are separated from those experiencing negative DEP on the microscopic level. Practical applications, however, require cell separation on a macroscopic scale. This is usually achieved by combining DEP with liquid flow. The particles that are attracted by the electrodes are retained in the device, while the others are washed away. Such devices are, however, unable to separate cells with different degrees of positive or negative DEP, unless the experiment is repeated several times in succession, e.g. by varying the frequency of the applied field.

[0007] Hyperlayer dielectrophoretic field-flow fractionation (DEP-FFF) is a significant step towards a more refined separation of cell populations. In this method, a linear array of microelectrodes is used to levitate cells by negative DEP. A non-constant flow profile causes cells levitated at different heights to emerge from the separation channel at different times. A similar method, which is referred to as electrosmear, has been developed to allow collection of cells onto characteristic zones on a substrate. Similarly to DEP-FFF, this method is also based on the combination of liquid flow and cell levitation by negative DEP. The cells are introduced at one end of an electrode array, which provides a levitation force that opposes cell sedimentation and prevents cells from adhering to the substrate. The voltage applied to the electrodes varies along the array. The cells flow along the channel until the DEP forces are no longer sufficient to levitate them, at which point they touch down and adhere to the substrate, which is coated with a binding agent.

[0008] The DEP methods described above do not say much about the nature of the cells, e.g. important properties of the cells.

[0009] For example, traditional methods for determining the permeability state of the cell membrane usually rely upon evaluating the uptake of colorimetric or fluorescent dyes by the cells. However, these methods cannot be used for real-time monitoring of variations in the membrane integrity. Also, the addition of a dye (which stays in the cell after transfection) may not be desirable. A recent new method of measuring membrane integrity in real-time was proposed by Huang and Rubinski [Huang, Y. and Rubinsky, B. Microfabricated electroporation chip for single cell membrane permeabilization. Sensors and Actuators A 89 (2001) 242-249; Huang, Y. et al. Instantaneous, quantitative single-cell viability assessment by electrical evaluation of cell membrane integrity with Microfabricated devices. Sensors and Actuators A 105 (2003)

31-39]. Their approach is based on direct measurement of the conductivity of the cell membrane. In this device, a single cell is positioned such that it blocks a micro hole in a non-conducting membrane, and the conductivity between the opposite sides of the membrane is subsequently measured.

[0010] US 2007/010367 discloses an array of DEP cages with optical sensors underneath the device, e.g. in the form of an array of sensors. The content of this document is incorporated by reference in its entirety. This particularly relates to the formation of arrays of electrodes for DEP.

[0011] It is an object of embodiments of the present invention to provide a good dielectrophoretic device for manipulating particles, a good method for forming such dielectrophoretic device and a good method for manipulating particles using such a dielectrophoretic device. The above objective is accomplished by a method and device according to the present invention.

[0012] In one aspect the present invention provides a dielectrophoretic device for manipulating one or more particles, comprising:

a plurality of electrodes in the form of a DEP cage, and
a particle movement detection system for detecting movement of the one or more particles within the cage.

[0013] The particle movement detection system may be a cell movement detection system. The advantage of this device is that the cross-over frequency can be investigated easily and automatically.

[0014] The particle movement detection system may include one or more particle presence sensors whereby the particle presence sensor may be an optical sensor.

[0015] The particle presence sensor is a segmented sensor. By analysis of the outputs of the segments of the sensor, movement of the particles, e.g. cells can be determined.

[0016] The cage will have a trapping point for nDEP and the particle presence sensor can be adapted to detect the presence of the one or more particles located eccentrically with respect to the trapping point. An eccentric location is indicative of a change in the cross-over frequency or a change of the field frequency through the cross-over point.

[0017] The particle presence sensor can be adapted to detect the presence of the one or more particles located at one of the plurality of the electrodes. This allows detection of a pDEP condition easily.

[0018] The device may include or be used with an AC generator for generating AC fields of different phase by supplying electric power to the plurality of electrodes in the form of a DEP cage. The AC generator is preferably adapted for generating AC fields of varying frequency and phase by supplying electric power to the plurality of electrodes in the form of a DEP cage. It is preferred if both cross-over determination and trapping of the cells can be done with the same device.

[0019] The device may have or may be used with means for detecting a DEP crossover, the means for detecting a DEP crossover being adapted to receive an output from the particle movement detection system. The means for detecting a DEP crossover is preferably adapted to receive an output from AC generator to thereby determine a DEP crossover frequency. This provides an efficient and compact device.

[0020] The particle presence sensor may be adapted to output a value indicating the location of one or more particles. The location may be important in some embodiments for determining characteristics of the cross-over frequency behavior of cells near the cros-over point.

[0021] In a second aspect the present invention provides a method of dielectrophoretic manipulation of particles using a DEP cage having electrodes which can be used to generate AC fields of differing frequency and phase, comprising: trapping one or more particles at a position in a DEP cage by means of negative dielectrophoresis, lowering or raising the frequency of the AC field, and recording the frequency of the AC field when movement of the at least one particle automatically is detected as the at least one particle moves away from the position in a DEP cage, the recorded frequency being a DEP crossover frequency. The position in the DEP cage can be a nDEP trapping point or a pDEP trapping point.

[0022] The particles can be cells, e.g. living cells having cell membranes, further comprising altering the conditions so that the permeability of the cell membranes is altered and recording the crossover frequency again. This can be useful in diagnosis, therapy or fundamental medical research. For example, the altered conditions may include addition of a chemical agent that permeabilizes the cells, or changing a temperature.

[0023] The method may include additional electrical signals such as an electropulse. The method is particularly useful when the particles are cells and the method includes determining cell viability or permeability. The method is of advantage because the determining of cell viability or permeability can be done in real-time, e.g. for classification, identification, of quantification of diseased versus healthy cells.

[0024] The present invention provides in a third aspect a dielectrophoretic device for manipulating one or more particles, comprising: a plurality of electrodes in the form of a DEP cage, the DEP cage having a central point, and a particle presence sensor for detecting a presence the one or more particles within the cage, the particle presence sensor being located eccentrically with respect to the central point of the DEP cage.

[0025] In fourth aspect the present invention provides a controller is for controlled driving of electrodes of an array of DEP cages. The controller comprises a control unit for controlling a driving means for applying electric power to the

electrodes of a DEP cage and /or other electrodes for use in a method according to the present invention. The present invention provides acontroller for controlling a system for dielectrophoretic manipulation of particles using a DEP cage having electrodes which can be used to generate AC fields of differing frequency and phase, the controller comprising:

means for controlling the trapping of one or more particles at a position in a DEP cage by means of negative dielectrophoresis,
means for controlling the raising or lowering of the frequency of the AC field, and means for controlling the recording of the frequency of the AC field when movement of the at least one particle automatically is detected as the at least one particle moves away from the position in a DEP cage, the recorded frequency being a DEP crossover frequency.

[0026] The present invention also provides the use of the method according to embodiments of the invention in molecular diagnostics or biological sample analysis or chemical sample analysis.

[0027] The present invention also provides the use of the method according to embodiments of the invention for cell lysis or cell electroporation.

[0028] The present invention furthermore provides a computer program product for performing, when executed on a computing means, a method according to embodiments of the invention.

[0029] The present invention also provides a machine readable data storage device for storing the computer program product according to embodiments of the invention.

[0030] The present invention also provides a transmission of the computer program product according to embodiments of the invention over a local or wide area telecommunications network.

[0031] The dielectrophoretic device according to embodiments of the invention is efficient and sensitive.

[0032] The dielectrophoretic device according to embodiments of the invention can be used for performing manipulation of particles in small, non-flowing volumes of particle suspensions.

[0033] Particular and preferred aspects of the invention are set out in the accompanying independent and dependent claims. Features from the dependent claims may be combined with features of the independent claims and with features of other dependent claims as appropriate and not merely as explicitly set out in the claims.

[0034] The above and other characteristics, features and advantages of the present invention will become apparent from the following detailed description, taken in conjunction with the accompanying drawings, which illustrate, by way of example, the principles of the invention. This description is given for the sake of example only, without limiting the scope of the invention. The reference figures quoted below refer to the attached drawings.

Fig. 1	shows video frames of a dielectrophoretic device according to an embodiment of the present invention. The dielectrophoretic device has additional electrodes.
Fig. 2a	illustrates schematically a dielectrophoretic device according to an embodiment of the invention.
Figs. 2b and c	illustrate schematically the use of a dielectrophoretic device according to an embodiment of the invention with a "virtual photodiode.
Fig. 2D	illustrates schematically a dielectrophoretic device according to an embodiment of the invention with additional electrodes.
Fig. 3	illustrates differing crossover frequencies to be used with embodiments of the invention.
Figs. 4 and 5	illustrate schematically dielectrophoretic devices according to embodiments of the present invention.
Fig. 6a and 7a	illustrate driving schemes for dielectrophoretic devices according to embodiments of the present invention.
Figs. 6b and 6c	illustrate ths driving scheme of Fig. 6a.
Figs. 7b and c	illustrate ths driving scheme of Fig. 7a.
Fig. 8	illustrates how a parameter important for coronary artery disease, such as LDL uptake, can be monitored via the cross-over frequency according to an embodiment of the present invention.
Fig. 9	illustrates how a crossover frequency can change with time to be used with embodiments of the invention.
Fig. 10	illustrates how a crossover frequency can change with time for different cells to be used with embodiments of the invention.
Fig. 11	illustrates how a crossover frequency can change with time with cells at different temperatures to be used with embodiments of the invention.
Fig. 12	schematically illustrates a system controller for use with a dielectrophoretic device according to embodiments of the present invention.

[0035] In the different figures, the same reference signs refer to the same or analogous elements.

[0036] The present invention will be described with respect to particular embodiments and with reference to certain drawings but the invention is not limited thereto but only by the claims. Any reference signs in the claims shall not be

construed as limiting the scope. The drawings described are only schematic and are non-limiting. In the drawings, the size of some of the elements may be exaggerated and not drawn on scale for illustrative purposes.

[0037] Where the term "comprising" is used in the present description and claims, it does not exclude other elements or steps. Where an indefinite or definite article is used when referring to a singular noun e.g. "a" or "an", "the", this includes a plural of that noun unless something else is specifically stated.

[0038] The terms top, bottom and the like in the description and the claims are used for descriptive purposes and not necessarily for describing relative positions. It is to be understood that the terms so used are interchangeable under appropriate circumstances and that the embodiments of the invention described herein are capable of operation in other orientations than described or illustrated herein.

[0039] Reference throughout this specification to "one embodiment" or "an embodiment" means that a particular feature, structure or characteristic described in connection with the embodiment is included in at least one embodiment of the present invention. Thus, appearances of the phrases "in one embodiment" or "in an embodiment" in various places throughout this specification are not necessarily all referring to the same embodiment, but may. Furthermore, the particular features, structures or characteristics may be combined in any suitable manner, as would be apparent to one of ordinary skill in the art from this disclosure, in one or more embodiments.

[0040] Similarly it should be appreciated that in the description of exemplary embodiments of the invention, various features of the invention are sometimes grouped together in a single embodiment, figure, or description thereof for the purpose of streamlining the disclosure and aiding in the understanding of one or more of the various inventive aspects. This method of disclosure, however, is not to be interpreted as reflecting an intention that the claimed invention requires more features than are expressly recited in each claim. Rather, as the following claims reflect, inventive aspects lie in less than all features of a single foregoing disclosed embodiment. Thus, the claims following the detailed description are hereby expressly incorporated into this detailed description, with each claim standing on its own as a separate embodiment of this invention.

[0041] Furthermore, while some embodiments described herein include some but not other features included in other embodiments, combinations of features of different embodiments are meant to be within the scope of the invention, and form different embodiments, as would be understood by those in the art. For example, in the following claims, any of the claimed embodiments can be used in any combination.

[0042] Furthermore, some of the embodiments are described herein as a method or combination of elements of a method that can be implemented by a processor of a computer system or by other means of carrying out the function. Thus, a processor with the necessary instructions for carrying out such a method or element of a method forms a means for carrying out the method or element of a method. Furthermore, an element described herein of an apparatus embodiment is an example of a means for carrying out the function performed by the element for the purpose of carrying out the invention.

[0043] In the description provided herein, numerous specific details are set forth. However, it is understood that embodiments of the invention may be practised without these specific details. In other instances, well-known methods, structures and techniques have not been shown in detail in order not to obscure an understanding of this description.

[0044] The present invention provides a dielectrophoretic device for identification and/or manipulation of particles, a method for manufacturing such a dielectrophoretic device and a method for manipulating, e.g. identifying, sorting, separating, lysis or electroporation, of particles, e.g. cells using such a dielectrophoretic device.

[0045] The device and methods according to embodiments of the invention may be used for manipulation of dielectric particles such as microparticles, nanoparticles, cells, or to any other suitable particles having dielectrophoretic properties. Examples of suitable particles which may be used with embodiments of the present invention may be solid dielectric particles such as e.g. polystyrene or latex beads or carrier beads (beads to which molecules or cells can be bound), engineered particles such as e.g. particles with a conductive core and an insulating shell, or vice versa, biological particles such as cells, bacteria, viruses, large molecules e.g. large proteins, complexes of molecules.

[0046] The device and method for manipulation of particles according to embodiments of the present invention need only rely on electric field induced effects to achieve manipulation of particles, e.g. separation of particles In the device according to some embodiments of the invention, particles, e.g. cells are transported electrically through a stationary fluid. Embodiments of the present invention will mainly be described with respect to cells having a membrane by way of example only.

[0047] In accordance with embodiments of the present invention a device or a method is provided to measure or detect the structural changes of (biological) cell membranes by means of the dielectrophoretic (DEP) response of that cell.

[0048] Dielectrophoresis (abbreviated as DEP) is a method of manipulating /moving cells by application of a non-uniform AC electric field. When an electrically uncharged particle, such as a cell, is placed in such a field, it will become polarized, and it will experience a net driving force due to the non-uniformity of the field. This force can either be in the direction of increasing field strength (so-called positive DEP) or decreasing field strength (negative DEP). In general, under suitable experimental conditions, a cell will experience negative DEP at low frequencies (up to the 100 kHz range), and positive DEP at higher frequencies (above several 100 kHz). The frequency at which negative DEP changes to

positive DEP is called the crossover frequency and it is specific for the type of cell under consideration and the experimental conditions, e.g. medium conductivity.

[0049] According to some embodiments of the present invention a dielectrophoresis (DEP) crossover frequency is used as a measure to determine the extent of the permeability of a cell membrane of a cell under investigation. This information can for instance be used in an electroporation device in which the amount of electric field that is applied to the cell must be carefully controlled to prevent lysis/death of the cell, while ensuring that the cell is sufficiently permeabilized, in order to allow transfection. Furthermore, method or device are provided to diagnose and monitor disease states that translate into structural and morphological rearrangements of cell membranes and membrane associated proteins, including; various cancers, malaria (and other viral infections), cellular senescence and apoptosis, atherosclerosis, as well as other cell differentiation and migration processes.

[0050] The structure of a cell membrane is subject to changes, e.g. due to the formation of pores in the lipid bilayer in response to electric fields (electroporation) or chemical substances (such as detergents or alcohols) or sound waves (sonication). The present invention is useful for detecting the state of permeability of a cell membrane, for example in the case where one wants to transfect the cell with (foreign) molecules such as DNA. Transfection can be done by artificially creating pores (holes) in the membrane, e.g. by electroporation. Large molecules in the cell suspension can then diffuse through the pores into the cell. This can for instance be used for testing the toxicity of new drugs, or for inducing genetic modifications (gene therapy).

[0051] It has now been observed in experiments that the crossover frequency, e.g. the frequency at which the DEP response changes from negative (repulsive) to positive (attractive) or vice versa, is highly influenced by the condition of the cell membrane. For example, just prior to rupture of a cell membrane it has been found that the DEP crossover frequency of that cell increases significantly. The cross-over frequency or a change therein may be used for a variety of tasks, e.g. related to diagnosis.

[0052] Figure 1 shows six frames of video captured in an experiment with cells in a DEP quadrupole trap 1. A cage is formed by four electrodes 2, 3, 4, 5. Between these electrodes are channels along which cells may travel. The cells may be moved by flow of a medium or may be moved by other means, e.g. by waveDEP. Other electrodes 6, 7 and other channels therebetween can be provided. A plurality of cages 1 and electrodes may be provided in the form of array. Not shown in the images are drive circuits for generating an AC field by applying AC currents of different phases to the electrodes in accordance with conventional DEP systems, e.g. driving circuits able to generate variable frequencies for nDEP or pDEP. The present invention includes such a variable frequency and phase AC field generator. The connections to the electrodes from the variable frequency and phase AC field generator are also not shown. Also not shown in Figure 1 is a cell presence and/or a cell movement detection system, e.g. a video camera system and image analysis system able to detect the position of cells and optionally their movement.

[0053] In the first frame it is seen that a cell (located in a circle) is attracted by the influence of positive DEP towards the high electric field near an electrode (2,5). In the next frames, it is seen that the same cell after rupture of the membrane moves away from the electrode to the center of the trap, indicating that it underwent a negative DEP response. Without being limited by theory the dependency of crossover frequency on the condition of the cell may be at least partly determined by the surface conductivity of the cell. Accordingly, this cell membrane parameter changes, and hence also the DEP response changes, not only with cell rupture but also as the cell membrane becomes more or less porous or changes its conductivity for another reason. Again this effect can be used in a variety of diagnostic applications.

[0054] The crossover frequency of the DEP force that is experienced by the cell depends (among other things) on the conductivity of the suspending medium. It is known from the literature that the crossover frequency increases linearly with increasing medium conductivity. In order for the crossover frequency to occur in a detectable range (e.g. between 100 kHz and several MHz) the conductivity of the medium is preferably limited to values between 100 and 1000 micro-Siemens/cm. Some usual culturing media for cells (such as PBS) have conductivities of about 10-15 milliSiemens/cm. Hence, to get good experimental conditions, it is advantageous to resuspend the cells in an iso-osmotic buffer with a lower conductivity. For instance, it is possible to use an iso-osmotic suspension of sucrose or mannitol in water. The conductivity of the eventual medium can be set to a desired value by adding a pH buffer (e.g. HEPES) and/or serum proteins (e.g. BSA).

[0055] Washing steps may be needed to completely remove the original culturing medium from the cells in order to ensure a low enough and known conductivity.

[0056] A basic building block of a device that can be used with the present invention is a quadrupole electrode configuration as shown schematically in Figure 2a. Such a quadrupole electrode arrangement may be part of an array of such quadrupoles, e.g. in rows and columns and each individually addressable.. As well as a central arrangement of four electrodes 2, 3, 4, 5 forming a "cage", additional electrodes may be provided (see Figure 1). These additional electrodes provide additional channels which may provide additional locations where cells can collect or be trapped (see Figure 1). Any suitable conductive material may be used for the electrodes. For example, the electrodes may comprise a thin conductive polymer film or a thin metal film, for instance a layer of Platinum on Titanium, deposited on an insulating substrate, e.g. a transparent substrate such as glass. The electric connections to the electrodes are indicated schemat-

ically in Figure 2a to a drive circuit 8. The drive circuit 8 is provided for supplying electric power to the electrodes 2-5 to thereby generate AC fields of different phase. The drive circuit 8 may be part of a device according to the present invention or it can be an external device with which the device according to the present invention co-operates. The drive circuit 8 supplies AC currents differing in phase to the electrodes 2-5 in accordance with conventional DEP systems. The drive circuit 8 is adapted to generate selectably variable frequencies for nDEP or pDEP. In Fig. 2a the drive circuit 8 is a variable frequency and phase AC field generator. A + sign and a - sign on the electrodes 2-5 indicate that the fields generated from these electrodes are out of phase by 180°. Single cells or clusters of cells, in a suspending medium, can be trapped in this dielectrophoretic field cage by means of negative DEP. When experiencing positive DEP the cells will move away from the trapping point to a high field region, e.g. close to one of the electrodes.

[0057] The size of the cage is preferably such that a single cell or a small cluster of cells can be accommodated within the cage. A suitable the gap between the electrodes can be in the order of 15-100 micrometers.

[0058] In an embodiment of the present invention a cell presence sensing device is incorporated in the DEP device in order to determine the position of cells or a cell within the trap. This is shown schematically as item 10 in Figure 2a. The cell presence sensing device may be an optical sensing device such as a photodiode, PIN diode, phototransistor, photoresistor, etc. A light source (which includes ambient light) may pass through the cage and strike the optical sensing device. In such a case, the optical sensing device is adapted to measure the reduction in received light due to the 'shadow' of a cell or cells when this or these are present in the cage. For this purpose a circuit 9 is provided for driving and controlling the cell presence sensing device as well as to receive signals from the cell presence sensing device indicative of the presence or absence of a cell in the cage. Alternatively, a light source may be provided on the same side of the cage as the optical sensing device and light reflected from the cell or cells may be recorded by the sensing device. In yet another embodiment, the cell or cells may be phosphorescent or chemiluminescent and no additional light source is required to detect the presence of the cell or cells.

[0059] Although an optical sensor is a preferred embodiment, other sensors can be used. For example magnetic field sensors may be used when the cell or cells have a magnetic property, e.g. they are tagged with magnetic beads. Other suitable sensors can be sensors that detect the heat output of viable cells in the cage such as microcalorimetric measuring devices. Electrical activity of cells may be detected by means of an adapted Field Effect Transistor, e.g. by exposing a gate of an FET below the trap position such that when a cell rests on the exposed gate, the FET gives an output. Other types of sensor may be used. For example a microvideo camera may be used at each quadrupole position or having a field of view of a plurality of quadrupoles to obtain images (see Figure 1) which can then be processed by image processing, e.g. object recognition algorithms to detect the presence of a cell. The microvideo camera may be mounted on an X-Y tracking platform thus allowing the video camera to be moved to specific locations, e.g. sequential observation of DEP cages of an array of cages.

[0060] It is particularly preferred if the cell presence sensor is adapted to detect the movement of the cell or cells within the cage. Hence the present invention provides a cell movement detection system located at each cage position, e.g. within or close to the quadrupole electrodes. A cell movement detection system may comprise one or more cell presence sensors 10 at each cage as well as a detection circuitry 9 which receives the outputs from the cell presence sensors and determines from these that the cell has moved. Depending upon the way the cell presence sensors are arranged, a movement can be detected, a movement in a certain direction can be detected and/or a movement to a certain position can be detected by the cell movement detection system according to the present invention. For example, this may be achieved by a plurality of individual sensors in a pattern, e.g. a segmented sensor such as a plurality of individual sensors in a sectored pattern. Different segmentations of the photodiode are included within the scope of the present invention, e.g. four or six or eight sectors. The plurality of sensors may be arranged symmetrically, e.g. four in a square or in any other suitable pattern, e.g. four sensors in a line. By examining and comparing the outputs of the segment sensors, a change in position of the cell or cells can be detected. Accordingly, the cell movement detection system includes detection circuitry 9 that can process the outputs of the sensors from within a location, e.g. at the quadrupole electrodes or near to them. The detection circuitry 9 may be part of a device according to the present invention or it can be an external device with which the device according to the present invention co-operates. The processing of the outputs can be done by any suitable means. For example, the outputs may be processed as analog signals or digital signals may be converted into digital signals before processing. For example by arithmetic processing, e.g. adding the outputs from an adjacent pair of sensors in a pattern of four sensors in a square and comparing this value with the value formed by addition of the outputs from a neighbouring pair, it can be determined if a cell has moved such as to block or cover some of the sensors more than others. Similarly, a value from adding a pair of diagonally located sensors may be compared with that obtained from the opposite diagonal pair of sensors. Outputs may be subtracted from each other to obtain further information. Any of the sensors mentioned above can be used in the cell movement detection system. For example magnetic field sensors may be used when the cell or cells have a magnetic property, e.g. they are tagged with magnetic beads. Other suitable sensors can be sensors that detect the heat output of viable cells in the cage such as microcalorimetric measuring devices. Electrical activity of cells may be detected by means of an adapted Field Effect Transistor, e.g. by exposing a gate of an FET below the trap position such that when a cell rests on the exposed gate, the FET

gives an output. Other types of sensor may be used. For example a microvideo camera may be used at each quadrupole position or having a field of view of a plurality of quadrupoles to obtain images (see Figure 1) which can then be processed by image processing, e.g. object recognition algorithms to detect not only the presence of a cell but also its movement with time (see Figure 1).

[0061] An example of a cell movement detection system according to an embodiment of the present invention will be explained with reference to Figs. 2b and 2c. In Fig. 2b three areas in a video image (0, 1, 2) are analysed for pixel values (see Fig. 1 for video images of a cage). A photodetector is 'simulated' from a camera image by adding the intensities of the pixels in the areas of the segments 0,1,2 representative of shadow of a cell. High values indicate no shadow, i.e. no cell presence. These areas represent "virtual photodiodes" wherein the pixel values are equivalent to a photodiode at the same position. The positions of the video image 1,0,2 are arranged in a line. The "output" of the "virtual photodiodes is given in Fig. 2C. Shown in the graphs are the levels of incident light for the three segments (0, 1 and 2) versus time. With increasing time (section on the right of the image) the value from position "0" drops indicating shadow from a cell, i.e. cell presence while the values for 1 and 2 stay high, meaning no cell. The change in values can be associated with a movement of the cell. This may be due to a change in permeability of the cell membrane, or its conductivity, e.g. as a result of cell death, thus altering the cross-over frequency and hence the position in the cage. Other sensors such as photodiodes would give a similar output. This provides experimental verification of the fact that cell position and/or cell movement can be detected by measuring an image or a shadow of the cell on a suitable sensor such as a photosensitive detector. It is clear that the presence of a cell over a causes a significant change in the measured signals.

[0062] Detection of the movement of the cell or cells from the trap position towards a position of higher field is indicative of a change from negative DEP to positive DEP. Consequently a device in accordance with this embodiment can also determine the sign change of the DEP force (e.g. from negative to positive or vice versa). This is indicative of a DEP crossover.

[0063] In accordance with an embodiment of the present invention measuring the crossover frequency proceeds as follows:

1. The cell(s) under investigation are trapped at the center of the DEP cage by means of negative dielectrophoresis. For this purpose an AC field is applied with a low frequency (e.g. 50 to 100 kHz). The voltage level can be in the order of 1 Volt but can be increased or decreased until a sufficient trapping force is generated.
2. The frequency of the AC voltage is now gradually increased (e.g. beyond 100 kHz) until a movement of the cell (s) away from the center of the cage, towards the electrodes, is observed. The frequency at which this occurs, is the crossover frequency.
3. This process can now be repeated for different experimental conditions. For instance a chemical agent that permeabilizes the cell to a certain (known or unknown) extent can be added to the medium. Or an electropulse can be applied, either using the already present electrodes, or using additional electrodes. A simple way of doing this, using the existing quadrupole electrodes, is to briefly attract the cell(s) toward a higher field region in the cage by positive DEP using a high frequency (> 1 MHz). The cell can be held at a certain pre-determined position in the cage by rapidly switching between low and high frequency fields using the cell presence sensing device such as a photodiode for position feedback.

[0064] An example of additional electrodes has been given in Fig. 1 but Fig. 2D shows other additional electrodes. A quadrupole cage is shown with additional electrodes that can be used for electroporation. Two electrodes forming a ring-dot structure are placed at the center of the quadrupole electrodes. These electrodes can be used to apply an electropulse to a cell that is trapped in the quadrupole, in order to create temporary pores in the cell membrane.

[0065] Hereafter, a possible change in crossover frequency can be detected by repeating steps 1 and 2.

[0066] To assist in the above embodiment a readout circuit 12 may be provided that receives an output from the detection circuitry 9 and also from the AC field generator 8. The output from the detection circuit 9 can be an indication of the detection of movement, i.e. that the crossover has been reached. The output from the AC field generator 8 can be the frequency at that moment. These outputs may be processed as analog signals or may be provide as digital signals or may be converted into digital signals before processing by the readout circuit 12. An output 14 from the readout circuit 12 can then be the crossover frequency. This may be displayed in any suitable manner, e.g. a print out, display on a video display unit, transmission of a message over a network, etc. The readout circuit 12 may be part of a device according to the present invention or it can be an external device with which the device according to the present invention co-operates.

[0067] Embodiments of the present invention make use of an additional characteristic of the dielectrophoretic (DEP) forces, e.g. in a quadrupole trap. Specifically, dipolar and quadrupolar force terms can be used to determine and control the movement of cells near the crossover frequency. This leads to a more precise method to correlate the DEP response of the cell to the permeability of the cell membrane.

[0068] In an additional embodiment a device and an associated method is provided for measuring the crossover

frequency in a quadrupole trap. It comprises a quadrupole with a cell presence sensing device 10 such as a photodiode placed at an eccentric position with respect to the trap point of the cage as well as the other drive circuits and readout circuits of Fig. 2a. This embodiment can be used for measuring cell position and includes a method for driving the electrodes so as to obtain a good starting position (e.g. close to an electrode) for the crossover frequency measurement. The cell presence sensing device 10 may be any sensing device described above for previous embodiments. An optical sensing device such as a photodiode, PIN diode, phototransistor, photoresistor, etc. is preferred.

[0069] An explanation follows of dielectrophoresis in quadrupole traps based on the so-called effective moment method as described by Jones T.B., "Basic theory of dielectrophoresis and electrorotation", IEEE Engineering in Medicine and Biology Magazine, November/December 2003, pp 33-42.

[0070] The DEP force on a particle can be expressed as the sum of the gradients of a set of electromechanical potentials U_n , where n = 1, 2, 3, etc., corresponding respectively to the dipole, quadrupolar, octopolar etc. components of the induced multipole potential:

$$F = -\nabla\left(U_1 + U_2 + \cdots\right).$$

[0071] In the remainder we will only consider the first two terms (dipole and quadrupole) as the subsequent terms contribute only very little to the resulting force. The quadrupolar term, however, is of significant importance in the quadrupole electrode configuration. For instance, it is this term that causes levitation of particles along the centreline of the trap. The terms U_1 and U_2 are given by Jones T.B., "Basic theory of dielectrophoresis and electrorotation", IEEE Engineering in Medicine and Biology Magazine, November/December 2003, pp 33-42:

$$U_1 = -\pi R^3 \varepsilon_1 \,\mathrm{Re}\left(K_1\right)\left[\left(\frac{\partial \Phi}{\partial x}\right)^2 + \left(\frac{\partial \Phi}{\partial y}\right)^2 + \left(\frac{\partial \Phi}{\partial z}\right)^2\right],$$

$$U_2 = -\frac{2}{3}\pi R^5 \varepsilon_1 \,\mathrm{Re}\left(K_2\right)\left\{\frac{1}{2}\left[\left(\frac{\partial^2 \Phi}{\partial x^2}\right)^2 + \left(\frac{\partial^2 \Phi}{\partial y^2}\right)^2 + \left(\frac{\partial^2 \Phi}{\partial z^2}\right)^2\right] + \left(\frac{\partial^2 \Phi}{\partial y \partial z}\right)^2 + \left(\frac{\partial^2 \Phi}{\partial z \partial x}\right)^2 + \left(\frac{\partial^2 \Phi}{\partial x \partial y}\right)^2\right\}$$

[0072] Here Φ is the electric potential (more precisely: the RMS value of the AC potential), R the radius of the (assumedly spherical) particle and ε_1 the permittivity of the suspending medium. The terms K_1 and K_2 are the so-called Clausius-Mossotti factors. These are complex, frequency dependent terms, and they determine the sign of the dielectrophoretic force on the particles under study. These terms depend on the size of the particle, and on the electric properties (permittivity and conductivity) of the (constituents of the) particle and the surrounding medium. For example in Figure 3 a graph is shown of a typical variation of Re(K_1) and Re(K_2) with frequency for the case where the cell is modelled as a single-shell particle, consisting of a non-conductive membrane encapsulating a conductive cytoplasm.

[0073] It can be observed from Figure 3 that the crossover frequencies of Re(K_1) and Re(K_2) (i.e. the frequencies where the graphs intersect the horizontal axis) are different. This fact influences the general crossover behaviour of cells in the trap, i.e., the transition from negative DEP (nDEP) to positive DEP (pDEP) or vice versa. If the quadrupolar term were absent, the crossover behavior would be binary. That is, for all locations in the trap, there would either be nDEP or pDEP, depending on the frequency. In this case, the cell would either move to the center of the trap (nDEP) or to the position with the highest electric field (at or near the electrodes). However, because the crossover frequencies of Re (K_1) and Re(K_2) are different, it will occur that in a narrow frequency band (for example in a band of about 5 to 10 kHz) between the crossover frequencies, the dipolar and quadrupolar terms oppose each other. This will result in the occurrence of stable equilibrium positions in the region between the electrode edges and the center of the trap. Therefore, instead of binary, the transition between nDEP and pDEP can be expected to be more gradual. Hence the exact position of a cell or cells within the cage is controllable. The exact location of the equilibrium points depends on the electric potential distribution in the trap, the applied frequency and the (di)electric properties of the particle and the medium under study.

[0074] To quantify this effect, it is convenient to model the field in the quadrupole trap using the parametric model for azimuthally periodic electrodes proposed by Jones T.B., "Basic theory of dielectrophoresis and electrorotation", IEEE Engineering in Medicine and Biology Magazine, November/December 2003, pp 33-42. In this approximation the potential is given as:

$$\Phi = V\left\{a + bz + c\left[6z^2 - \rho^2\right] + d\left[6z^3 - 3z\rho^2\right]\right\}\rho^2 \cos 2\theta \ ,$$

where the position in the trap is given in cylindrical coordinates (ρ, θ, z): (radius, azimuth, height).

[0075] For a given electrode configuration, and given electric properties of the medium, the parameters *a*, *b*, *c*, and *d* can be estimated or calculated. Subsequently, the electromechanical potentials U_1 and U_2, and the associated DEP force terms can be computed. The equilibrium points are given by the local minima in the total electromechanical potential $U = U_1 + U_2$.

[0076] The effect of the opposing dipolar and quadrupolar force terms can be used as a refined way to visualize and/or measure the effect of the permeability state of the cell membrane. A change in the porosity of the membrane will change the factors Re(K_1) and Re(K_2) and cause a detectable/measurable shift of the DEP equilibrium position. This shift can be less than the complete shift from the center of the cage to an electrode, hence a finer determination of a change in permeability may be detected.

[0077] In the embodiment described above the state of a cell membrane can be determined by detecting a change in the crossover frequency. The basic method consists of a procedure in which the applied frequency is varied, and a cell presence sensor such as an optical sensor (e.g. an integrated PIN diode) as part of a cell movement detection system according to the present invention is used to detect movement of the cell. In this way it can be determined whether the cell is undergoing nDEP or pDEP. The frequency at which the transition from nDEP to pDEP occurs is the crossover frequency. However, the analysis involving the quadrupolar term, as given in the previous section, implies allows this method can be refined. Basically, the precise movement of the particle at frequencies close to the crossover range can be predicted on the basis of the model given above. Deviations from the predicted behavior can be related to changes in the electric properties of the cell under study, which in turn are due to changes in the integrity of the cell membrane. To achieve the small changes in frequency at the crossover point, the AC field generator 8 may be adapted to a have a fine frequency control that allows small changes of frequency when investigating the characteristics of cells concerned.

[0078] For example, the equilibrium points of the cell position in the frequency range at which the transition from nDEP to pDEP occurs can be related to specific (di)electric properties of the cell. For a given, fixed, frequency, a displacement in the equilibrium position of the cell indicates a change in the integrity of the cell membrane.

[0079] Accordingly a further method according to an embodiment of the present invention includes:

1. The cell(s) under investigation are moved to a location near an electrode means of positive dielectrophoresis. For this purpose an AC field is applied with a high frequency (above 100 kHz, e.g. > 1 MHz). The voltage level can be in the order of 1 Volt but can be increased or decreased until a sufficient trapping force is generated.
2. The frequency of the AC voltage is now gradually reduced (e.g. below 100 kHz) until a movement of the cell(s) towards the center of the cage is observed. The frequency at which this occurs, is the crossover frequency.
3. Hereafter, the crossover frequency can be investigated by altering the frequency slightly in the range where factors Re(K_1) and Re(K_2) cause a detectable/measurable shift of the DEP equilibrium position. Measuring the new stable positions using a cell presence sensor according to the present invention can provide information relating to the permeability state of the cell membrane. This can include a more refined analysis of the crossover behavior between nDEP and pDEP taking into account the contribution of the quadrupolar force term. Specifically, the state of membrane integrity can be determined by detecting displacements of the equilibrium positions of the particles in the frequency range where crossover occurs.
4. This process can now be repeated for different experimental conditions. For instance a chemical agent that permeabilizes the cell to a certain (known or unknown) extent can be added to the medium. Or an electropulse can be applied, either using the already present electrodes, or using additional electrodes. A simple way of doing this, using the existing quadrupole electrodes, is to briefly attract the cell(s) toward a higher field region in the cage by positive DEP using a high frequency (> 1 MHz). The cell can be held at a certain pre-determined position in the cage by rapidly switching between low and high frequency fields using the cell presence sensing device such as a photodiode for position feedback.

[0080] With respect to any of the embodiments of the present invention the cell presence sensing device 19, e.g. a

photodiode can be placed so that measurements of cell presence or cell movement can be made eccentrically in the device, i.e. with respect to the trap position in the cage. This provide two advantages. When the transition from nDEP to pDEP occurs, the particle will move along a radial line running from the center of the quadrupole to an eccentric point on the electrodes, or an eccentric point between the electrodes. In order to study movement of the particle in the trap, one can therefore arrange cell presence sensing device, e.g. place a segmented cell presence sensing device such as a segmented PIN diode along these radial lines, and able to monitor eccentric positions of the cell.

[0081] The DEP force on the particle is proportional to a gradient of the electric field. For the dipolar component of the force term it is proportional to the gradient and for the quadrupolar component it is proportional to the second order gradient of the field. For that reason, also the movement caused by DEP is strongest in the area close to the electrodes, i.e., away from the center. Since the modulation of the DEP effect is strongest in this area, also the influence of noise and disturbing forces will be less. Hence it is advantageous to analyse movement of the particle in an eccentric location of the quadrupole, and also to put the cell presence sensing device such as a PIN diode sensor in this eccentric location.

[0082] In Figure 4 and Figure 5 two configurations according to embodiments of the present invention for eccentric placement of the cell presence sensing device 10 such as a PIN diode are shown. The cell presence sensing devices 10 such as the photodiodes can be segmented, to allow determination of position of the particle along the radius more accurately. The precision, with which the position can be determined, depends on the resolution, i.e. the size and amount of the individual segments of the cell presence sensing device e.g. a PIN diode. The cell presence sensing devices may be part of a cell movement detection system as described above.

[0083] As particles are best analyzed in an eccentric position in the trap, the present invention also proposes embodiments including electrode driving schemes that can be used to bring the particle/cell under study in a well-defined eccentric starting point prior to the analysis of crossover frequency behavior.

[0084] In Fig. 6a, a driving scheme in accordance with an embodiment of the present invention is shown which pushes the particle towards one of the electrodes, by means of nDEP. The phases of the voltage signals applied to the electrodes from the AC field generator 8 (not shown on this figure) are shown in figure 6a, i.e. 180° out of phase, and the electrodes are driven at a frequency where nDEP occurs. One starts from the situation where the electrodes 2-5 are driven in the conventional way, as e.g. shown in Figure 4, with a frequency in the nDEP regime. This will cause the cell to be held at the center of the quadrupole in the "trap". In the next step, one of the four electrodes is set at high impedance. nDEP will then push the particle toward the high impedance electrode where it will be detected by a cell presence detection sensor according to the present invention.

[0085] An example of using this driving scheme is shown in Fig. 6b and Fig. 6c. Fig. 6b shows a cell located at the centre of the quadrupole cage. Fig. 6C shows it moved towards an electrode.

[0086] An alternative driving scheme in accordance with an embodiment of the present invention, corresponding to the diode placement of Figure 5 is shown in Fig. 7a. Again the quadrupole is first driven in the usual way, so as to bring the cell to the center of the trap. Then, in order to bring the particle/cell in a starting position in between two electrodes with nDEP, two electrodes are set to high impedance as is illustrated in the figure. This brings the cell into a different position where it may be detected by a cell presence sensor according to the present invention.

[0087] An example of using this driving scheme is shown in Fig. 7b and Fig. 7c. Fig. 7b shows a cell located at the centre of the quadrupole cage. Fig. 7C shows it moved towards an electrode.

[0088] A primary application of the present invention is real-time monitoring of the state of the membrane of a cell such as the permeability of the cell membrane, e.g. ex vivo, in vitro. This can be used, for instance to track or control cell viability, to monitor or control the up-take of substances that alter the cell membrane permeability or conductivity, e.g. acLDL (of interest for CAD), to monitor or control cell maturity or changes therein, e.g. monocyte differentiation into macrophage, or to monitor or control electroporation, e.g. during transfection. Any of these can be used, for example, in drug development or gene therapy. An example of this use of the present invention in diagnosis, in fundamental medical research, in drug development or gene therapy will be described with reference to Fig. 8. This experiment relates to the uptake in a cell of a certain substance and the change in the cross-over frequency that occurs, e.g. because of changes inn the conductivity or permeability of the cell membrane that can be detected by means of the detection systems and methods of the present invention. The substance could be a toxin, a pharmaceutical, a metabolite, etc. As an example, the take-up of acetylated LDL is an important property of monocytes in the body's response to coronary artery disease. The monocytic cell line U937 was matured by adding 8 nmolar of PMA. The cells were then exposed to ac-LDL in a concentration of about 20 μg/ml for periods of up to 5 days. The cross-over frequency was measured and the average over 10 cells is shown in the Fig. 8. As can be seen in the figure, the cross-over frequency more than doubles after 5 days of exposure.

[0089] The fluorescent images shown above each column illustrate the up-take of ac-LDL by a single cell. This result illustrates how a parameter important for coronary artery disease, such as LDL uptake, can be monitored via the cross-over frequency. This parameter could also be used to estimate a person's risk of coronary artery disease, i.e. in diagnosis.

[0090] Another application is the controlled lysis of single cells. Furthermore, intracellular structural rearrangements lead to global changes in mechanical deformability of the cell. This cellular biomechanical response, in turn, can mediate

cell mobility and thereby facilitates disease progression in situations where the elastic modulus increases or decreases due to membrane or cytoskeleton reorganization. There are many disease states that translate into structural and morphological rearrangements of cell membranes and membrane associated proteins, including; various cancers, malaria, viral infections, cellular senescence and apoptosis, atherosclerosis, as well as other cell differentiation and migration processes. Any such process will have an effect on the membrane permeability of a cell and hence such a device is ideally suited for classification of membrane dynamics within disease states. Upon successful classification, such a device is ideal for the identification and quantification of diseased cells within cell suspensions and hence is suitable for diagnostic and disease monitoring applications.

[0091] An example of real-time monitoring of the state of the membrane of a cell will be described with reference to Figures 9, 10 and 11. Figure 9 shows that the cross-over frequency changes with time due to an event that alters the state of the cell membrane, e.g. its permeability. Fig 9 is only a schematic representation. The frequency units in the graph are arbitrarily chosen and the indicated frequency shift is not representative of actual measurements.

[0092] Figure 10 shows how the crossover frequency alters with time when a cell disintegrates and on cell death. In both cases, the change in crossover frequency with time can be used to indicate such a change. Figure 11 shows how the crossover frequency alters with time when a cell is held at a higher temperature, i.e. one at which the cell is likely to die, e.g. 40°C or above. This can be used for example as a diagnostic in trials of a candidate hyperthermia treatment for cancer. In such a treatment, a higher temperature is used to which the cancer cells are less resistant than healthy cells. By comparing the behaviour with respect to temperature in accordance with a plot such as shown in Fig. 11 using cancer cells and optionally healthy cells from a biopsy, it can be determined whether such a hyperthermia treatment is likely to be successful. Such a procedure can be used to screen a large number of different cancer cells to select those types of cancers for which hyperthermia treatment can be successful. Also in a combined therapy of hyperthermia and a drug such as is used in cancer chemotherapy, the combination of the drug and a hyperthermia therapy can be simulated and, for example optimised ex vivo or in vitro.

[0093] Alternatively, a similar procedure may be used at low temperatures to investigate the capabilities of plant cells to withstand frosts, e.g. to screen a large number of genetically modified plant cells to determine which modification provides a better resistance to cold.

[0094] Instead of cold, other factors may be used in real-time monitoring of the state of the membrane of a cell, e.g. for toxicology, i.e. the effect of toxic or irritant substances on cells with time.

[0095] As indicated above a large array of DEP quadrupoles can be provided, with the electrodes and the necessary connections to the electrodes provided by suitable conductive lines, e.g. deposited by sputtering or as provided by thick film processing. This array allows the simultaneous measurement of many cells. Cell presence sensors and optionally detection electronics may be integrated with the array, e.g. by using Large Area Electronics techniques such as large area (active matrix) electronics technology, e.g. use of low temperature polysilicon (LTPS) substrates, large area amorphous silicon substrates, microcrystalline substrates.

[0096] Additional electrodes for measuring the impedance of the cell can be added to the device. It is known that the impedance of the cell also depends on the condition of the membrane. Hence an impedance measurement can provide additional information about the condition of the cell membrane.

[0097] Since the crossover frequency also depends on the conductivity of the suspending medium, a conductivity measurement unit can be integrated into the device. This would be particularly relevant when conductivity of the medium changes during the experiment, e.g. due to the addition of a transfecting agent.

[0098] Methods described above according to embodiments of the present invention may be implemented in a processing system 40 such as shown in Fig. 12. The processing system of Fig. 12 can be configured to provide the means for driving and controlling the cell presence sensing device shown with reference number 9 in Fig. 2a. Alternatively or additionally, the processing system of Fig. 12 can be configured to provide the readout circuit 12 of Fig. 2a. The processing system of Fig. lé can alternatively or additionally provide a video camera control system and an image analysis system that provides a means for cell movement detection in accordance with embodiments of the present invention.

[0099] Fig. 12 shows one configuration of processing system 40 that includes at least one programmable processor 41 coupled to a memory subsystem 42 that includes at least one form of memory, e.g., RAM, ROM, and so forth. It is to be noted that the processor 41 or processors may be a general purpose, or a special purpose processor, and may be for inclusion in a device, e.g., a chip that has other components that perform other functions. Thus, one or more aspects of the method according to embodiments of the present invention can be implemented in digital electronic circuitry, or in computer hardware, firmware, software, or in combinations of them. The processing system may include a storage subsystem 43 that has at least one disk drive and/or CD-ROM drive and/or DVD drive. In some implementations, a display system, a keyboard, and a pointing device may be included as part of a user interface subsystem 44 to provide for a user to manually input information, such as parameter values. Ports for inputting and outputting data, e.g. desired or obtained flow rate, also may be included. More elements such as network connections, interfaces to various devices, and so forth, may be included, but are not illustrated in Fig. 12. The various elements of the processing system 40 may be coupled in various ways, including via a bus subsystem 45 shown in Fig. 12 for simplicity as a single bus, but will be

understood to those in the art to include a system of at least one bus. The memory of the memory subsystem 42 may at some time hold part or all (in either case shown as 46) of a set of instructions that when executed on the processing system 40 implement the steps of the method embodiments described herein. The system of Fig. 12 may be configured as a microcontroller or embedded with other electronic devices, e.g. on a PC board.

[0100] The present invention also includes a computer program product which provides the functionality of any of the methods according to the present invention when executed on a computing device. Such computer program product can be tangibly embodied in a carrier medium carrying machine-readable code for execution by a programmable processor. The present invention thus relates to a carrier medium carrying a computer program product that, when executed on computing means, provides instructions for executing any of the methods as described above. The term "carrier medium" refers to any medium that participates in providing instructions to a processor for execution. Such a medium may take many forms, including but not limited to, non-volatile media, and transmission media. Non-volatile media includes, for example, optical or magnetic disks, such as a storage device which is part of mass storage. Common forms of computer readable media include, a CD-ROM, a DVD, a flexible disk or floppy disk, a tape, a memory chip or cartridge or any other medium from which a computer can read. Various forms of computer readable media may be involved in carrying one or more sequences of one or more instructions to a processor for execution. The computer program product can also be transmitted via a carrier wave in a network, such as a LAN, a WAN or the Internet. Transmission media can take the form of acoustic or light waves, such as those generated during radio wave and infrared data communications. Transmission media include coaxial cables, copper wire and fibre optics, including the wires that comprise a bus within a computer.

[0101] It is to be understood that although preferred embodiments, specific constructions and configurations, as well as materials, have been discussed herein for devices according to the present invention, various changes or modifications in form and detail may be made without departing from the scope of this invention as defined by the appended claims.

Claims

1. A dielectrophoretic device for manipulating one or more particles, comprising:

a plurality of electrodes in the form of a DEP cage, and
a particle movement detection system for detecting movement of the one or more particles within the cage.

2. The dielectrophoretic device of claim 1, wherein the particle movement detection system is a cell movement detection system.

3. The dielectrophoretic device of claim 1 or 2, wherein the particle movement detection system includes a particle presence sensor.

4. The dielectrophoretic device of claim 3, wherein the particle presence sensor is an optical sensor.

5. The dielectrophoretic device of claim 3, wherein the particle presence sensor is a segmented sensor.

6. The dielectrophoretic device of any of the claims 3 to 5, wherein the cage has a trapping point for nDEP and the particle presence sensor is adapted to detect the presence of the one or more particles located eccentrically with respect to the trapping point.

7. The dielectrophoretic device of claim 6, wherein the particle presence sensor is adapted to detect the presence of the one or more particles located at one of the plurality of the electrodes.

8. The dielectrophoretic device of any of the previous claims, further comprising an AC generator for generating AC fields of different phase by supplying electric power to the plurality of electrodes in the form of a DEP cage.

9. The dielectrophoretic device of claim 8, wherein the AC generator is adapted for generating AC fields of varying frequency and phase by supplying electric power to the plurality of electrodes in the form of a DEP cage.

10. The dielectrophoretic device of claim 9, further comprising means for detecting a DEP crossover, the means for detecting a DEP crossover being adapted to receive an output from the particle movement detection system.

11. The dielectrophoretic device of claim 9, wherein the means for detecting a DEP crossover is adapted to receive an

output from AC generator to thereby determine a DEP crossover frequency.

12. The dielectrophoretic device of any previous claim, wherein the particle presence sensor is adapted to output a value indicating the location of one or more particles.

13. A method of dielectrophoretic manipulation of particles using a DEP cage having electrodes which can be used to generate AC fields of differing frequency and phase, comprising:

trapping one or more particles at a position in a DEP cage by means of negative dielectrophoresis,
lowering or raising the frequency of the AC field, and
recording the frequency of the AC field when movement of the at least one particle automatically is detected as the at least one particle moves away from the position in a DEP cage, the recorded frequency being a DEP crossover frequency.

14. The method of claim 13, wherein the position in the DEP cage is a nDEP trapping point.

15. The method of claim 13, wherein the position in the DEP cage is a pDEP trapping point.

16. The method of any of the claims 13 to 15, wherein the particles are cells having cell membranes, further comprising altering the conditions so that the permeability of the cell membranes is altered and recording the crossover frequency again.

17. The method of claim 16, wherein the altered conditions include addition of a chemical agent that permeabilizes the cells.

18. The method of claim 16, wherein the altered conditions include changing a temperature.

19. The method according to any of the claims 13 to 18, further comprising addition of an electropulse.

20. The method of any of claims 13 to 19, wherein the particles are cells including determining cell viability or permeability.

21. The method of claim 20, wherein the determining of cell viability or permeability in real-time.

22. The method of any of the claims 13 to 21 for classification, identification, of quantification of diseased versus healthy cells.

23. A dielectrophoretic device for manipulating one or more particles, comprising:

a plurality of electrodes in the form of a DEP cage, the DEP cage having a central point, and
a particle presence sensor for detecting a presence the one or more particles within the cage, the particle presence sensor being located eccentrically with respect to the central point of the DEP cage.

24. The dielectrophoretic device of claim 23, wherein the particle presence sensor is an optical sensor.

25. The dielectrophoretic device of claim 24, wherein the particle presence sensor is a segmented sensor.

26. The dielectrophoretic device of any of claims 23 to 25, wherein the particle presence sensor is adapted to detect the presence of the one or more particles located at one of a plurality of the electrodes forming the DEP cage.

27. A controller for controlling a system for dielectrophoretic manipulation of particles using a DEP cage having electrodes which can be used to generate AC fields of differing frequency and phase, the controller comprising:

means for controlling the trapping of one or more particles at a position in a DEP cage by means of negative dielectrophoresis,
means for controlling the raising or lowering of the frequency of the AC field, and means for controlling the recording of the frequency of the AC field when movement of the at least one particle automatically is detected as the at least one particle moves away from the position in a DEP cage, the recorded frequency being a DEP crossover frequency.

28. The controller of claim 27, wherein the particles are cells having cell membranes, the controller further comprising means for altering the conditions so that the permeability of the cell membranes is altered and recording the crossover frequency again.

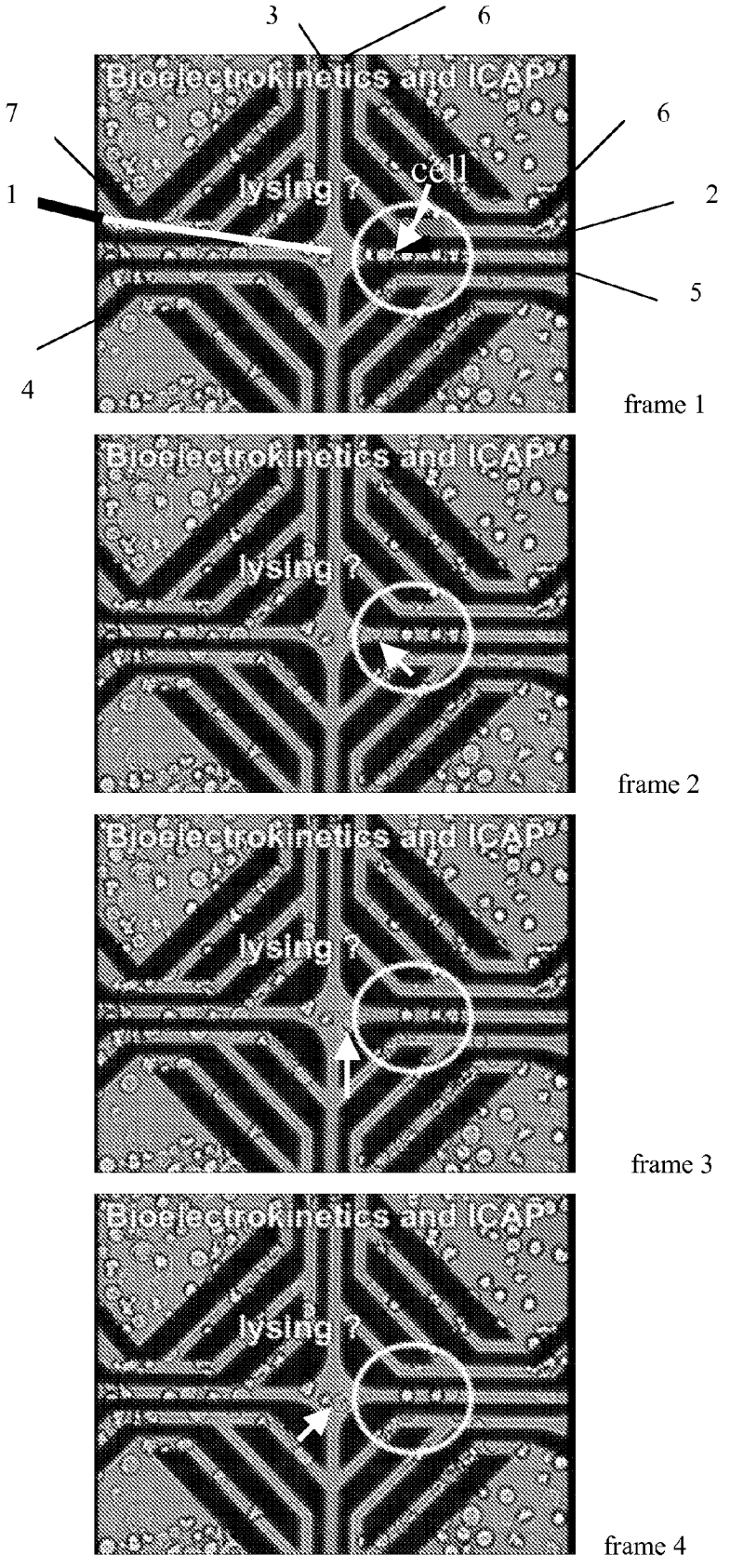
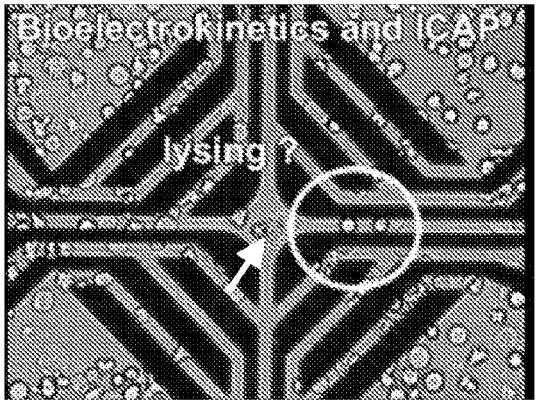
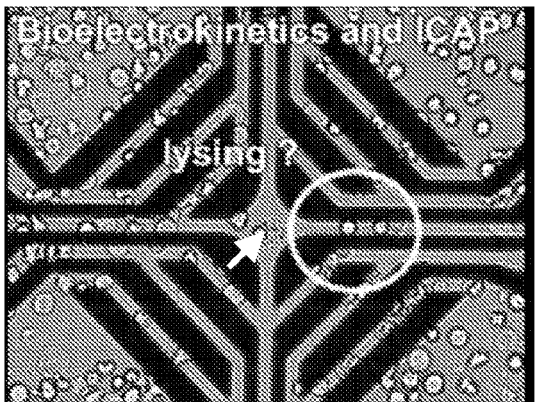

3
6
7
6
1
2
5
4
Bioelectrokinetics and ICAP
lysing ?
cell
frame 1
Bioelectrokinetics and ICAP
lysing ?
frame 2
Bioelectrokinetics and ICAP
lysing ?
frame 3
Bioelectrokinetics and ICAP
lysing ?
frame 4

Fig. 1



frame 5



frame 6 Fig. 1 continued

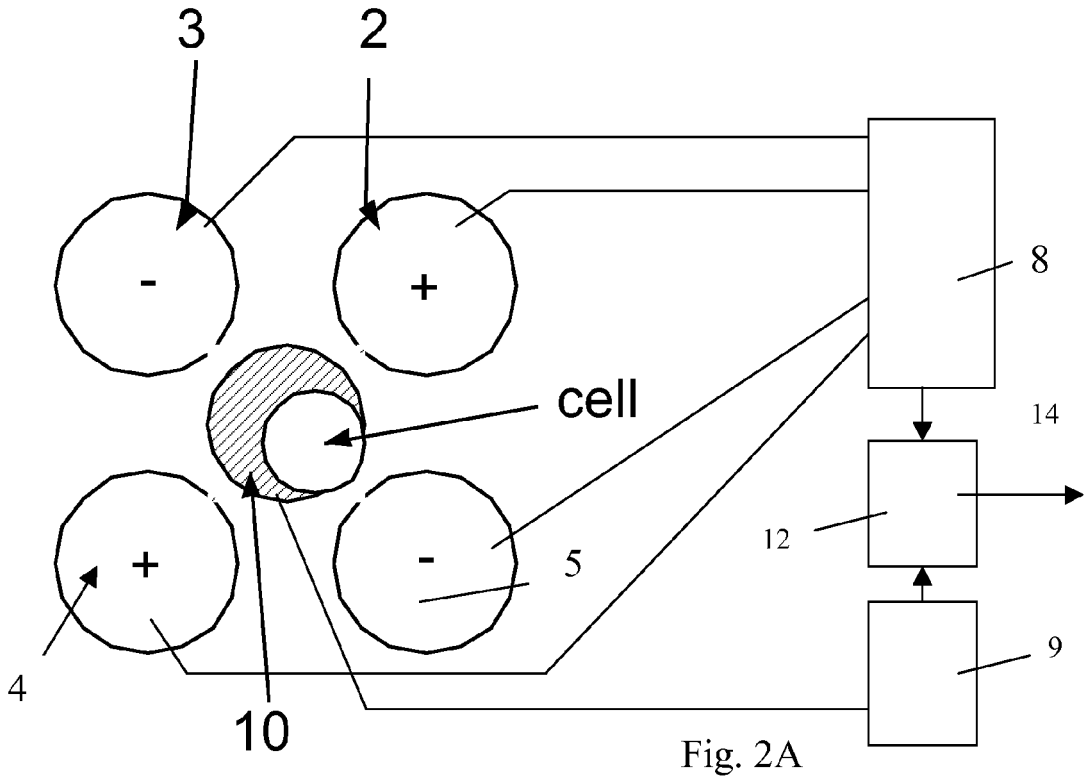


Fig. 2A

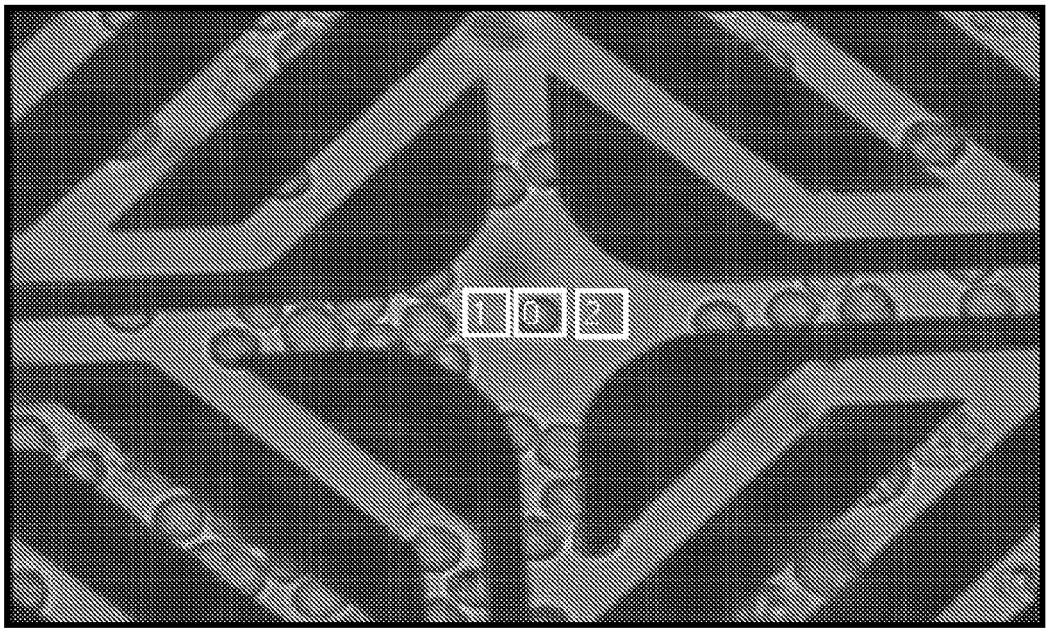

1
0
2

Fig. 2B

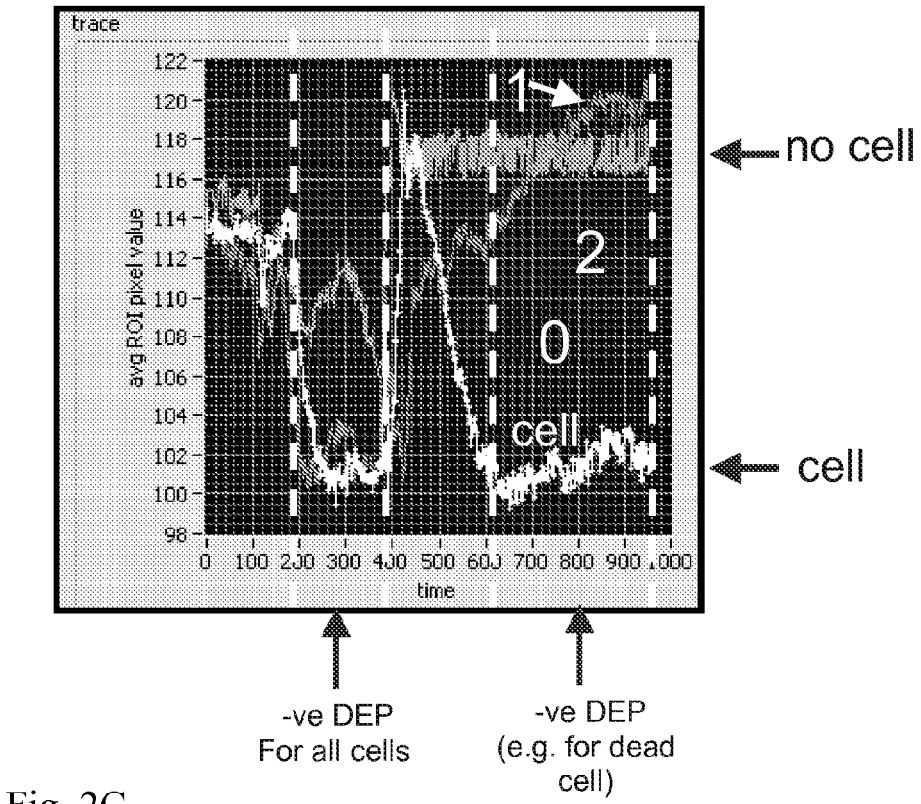

trace
avg ROI pixel value
122
120
118
116
114
112
110
108
106
104
102
100
98
0
100
200
300
400
500
600
700
800
900
1000
time
1
2
0
cell
no cell
cell
-ve DEP
For all cells
-ve DEP
(e.g. for dead
cell)

Fig. 2C

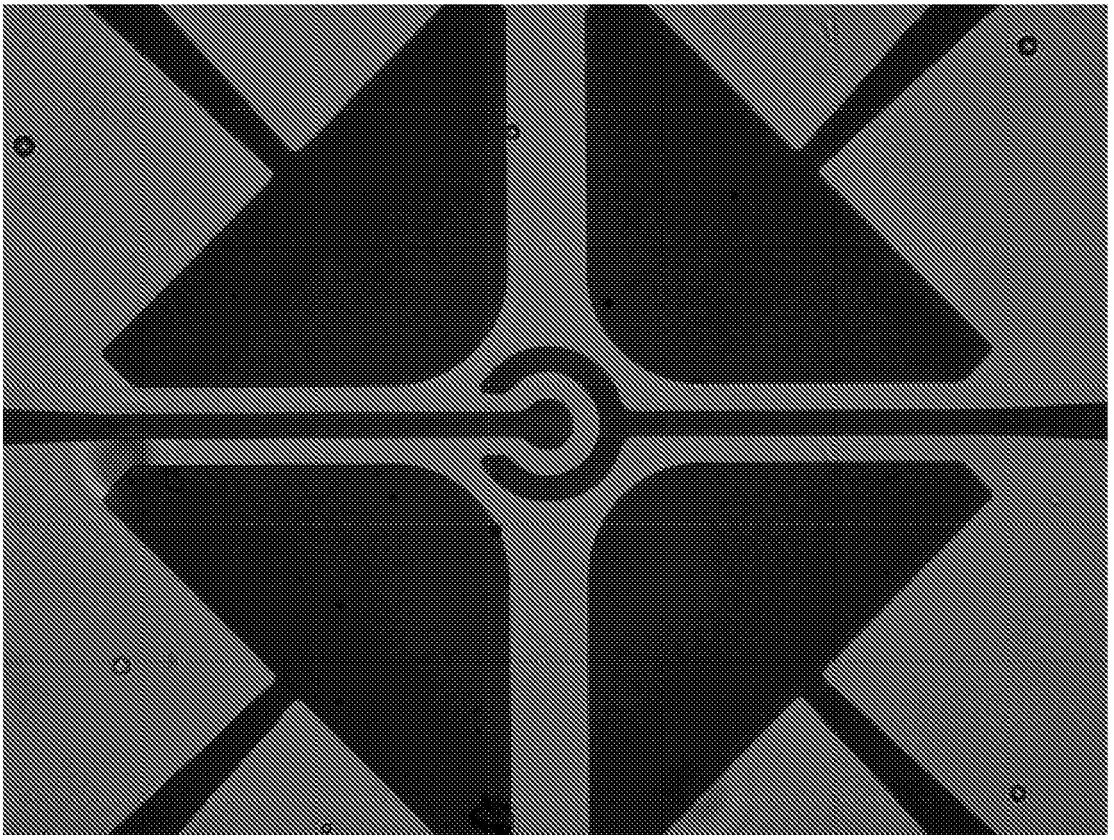


Fig. 2D

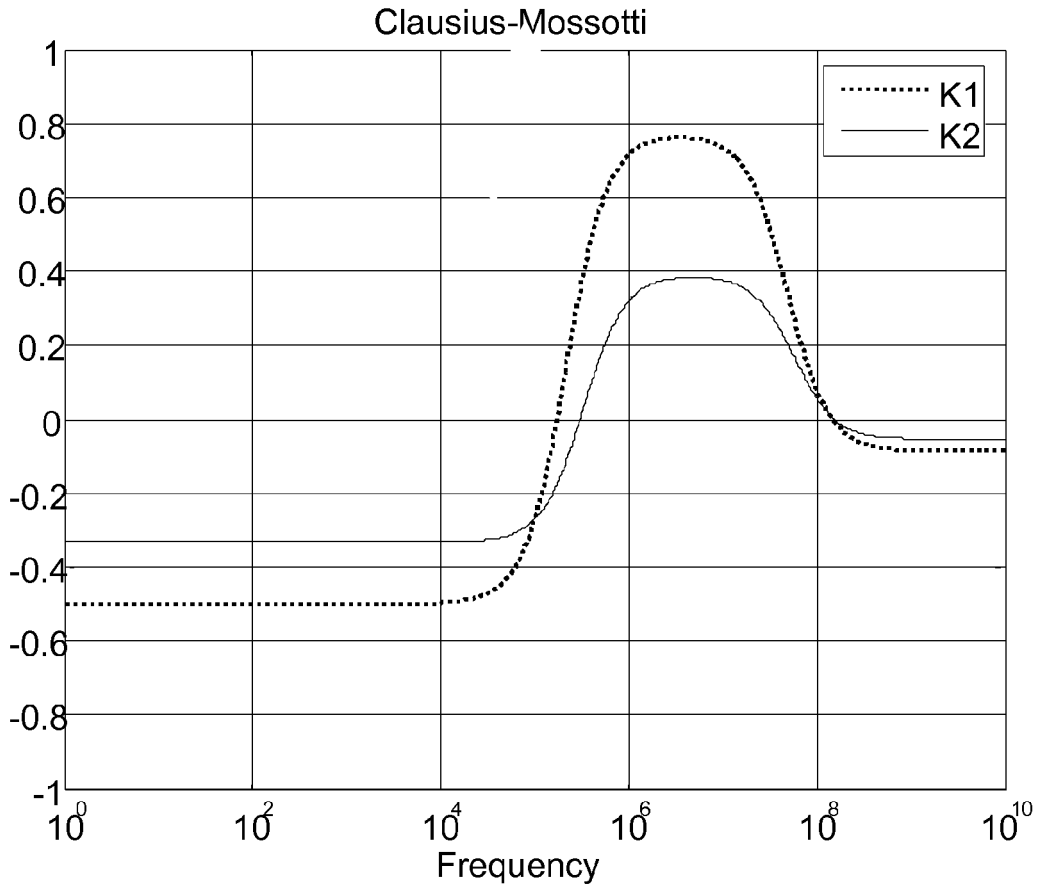

Clausius-Mossotti
K1
K2
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
10^0
10^2
10^4
10^6
10^8
10^10
Frequency

Fig. 3

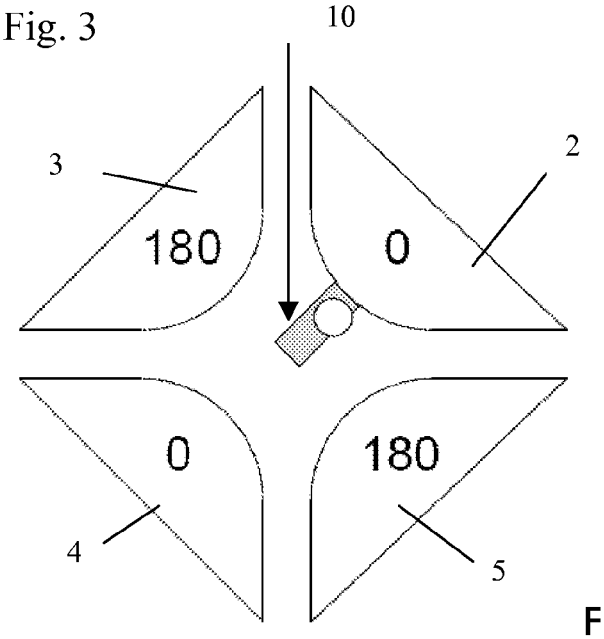

10
3
180
2
0
0
180
4
5

Fig. 4

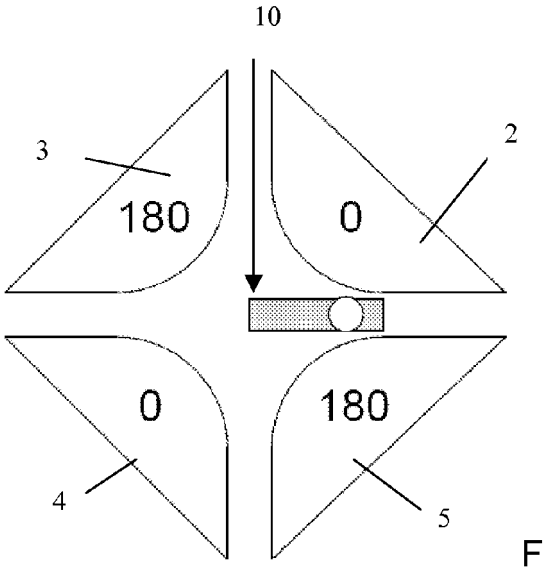


Fig. 5

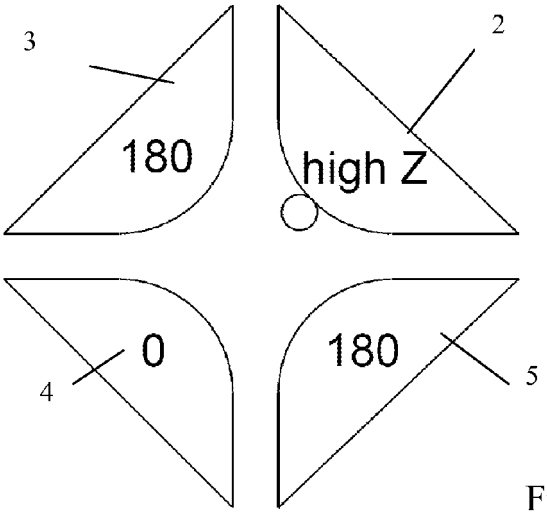


Fig. 6a

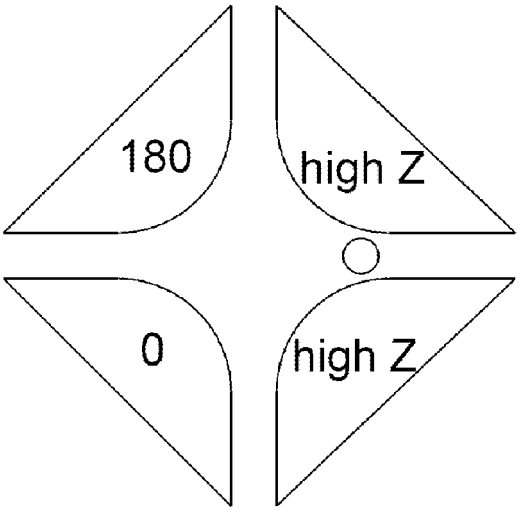


Fig. 7a

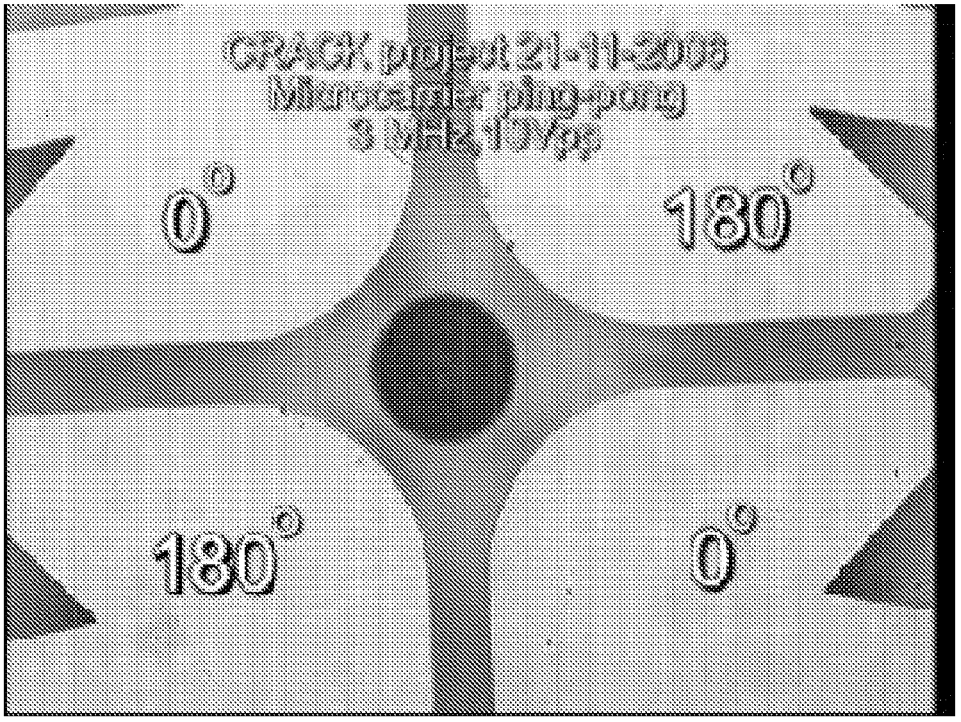


Fig. 6b

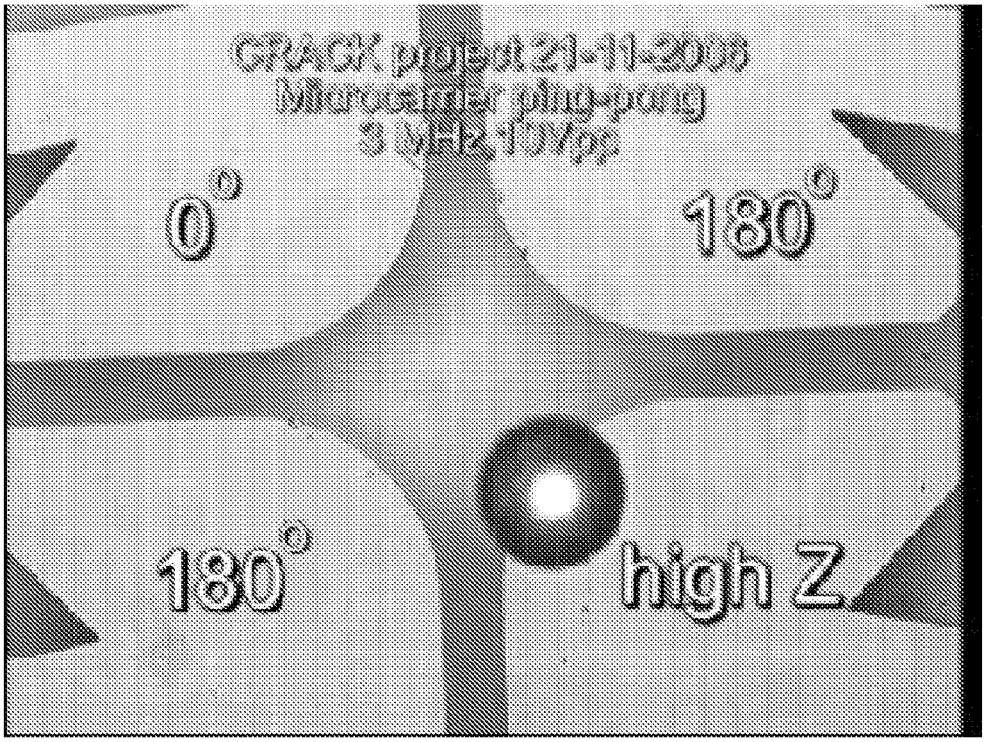


Fig. 6C

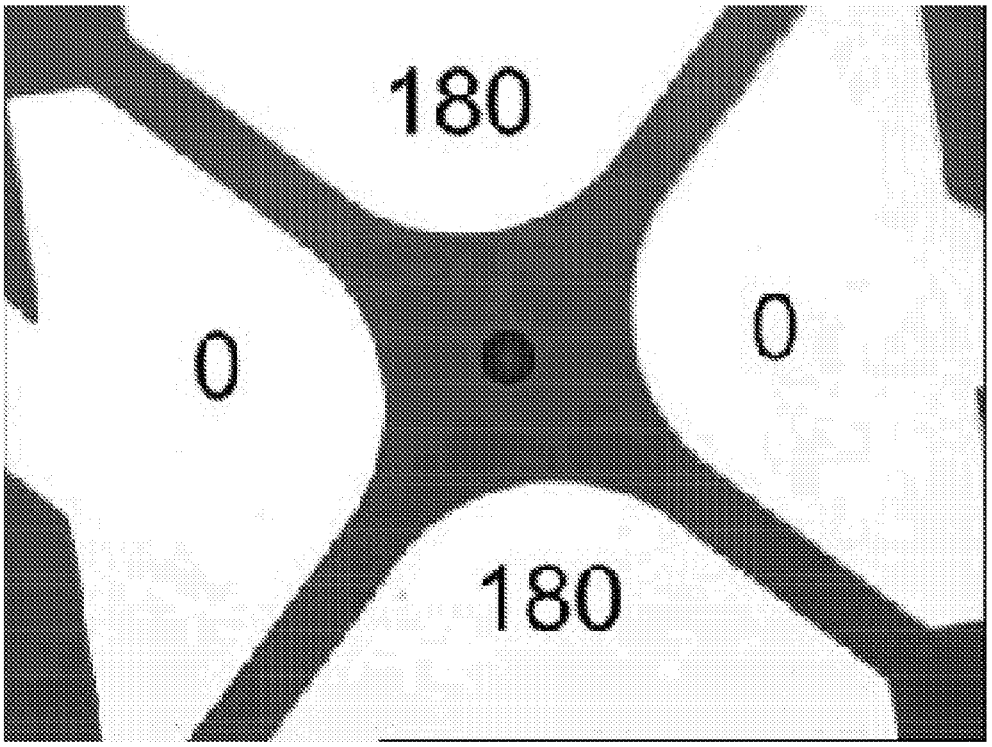


Fig. 7b

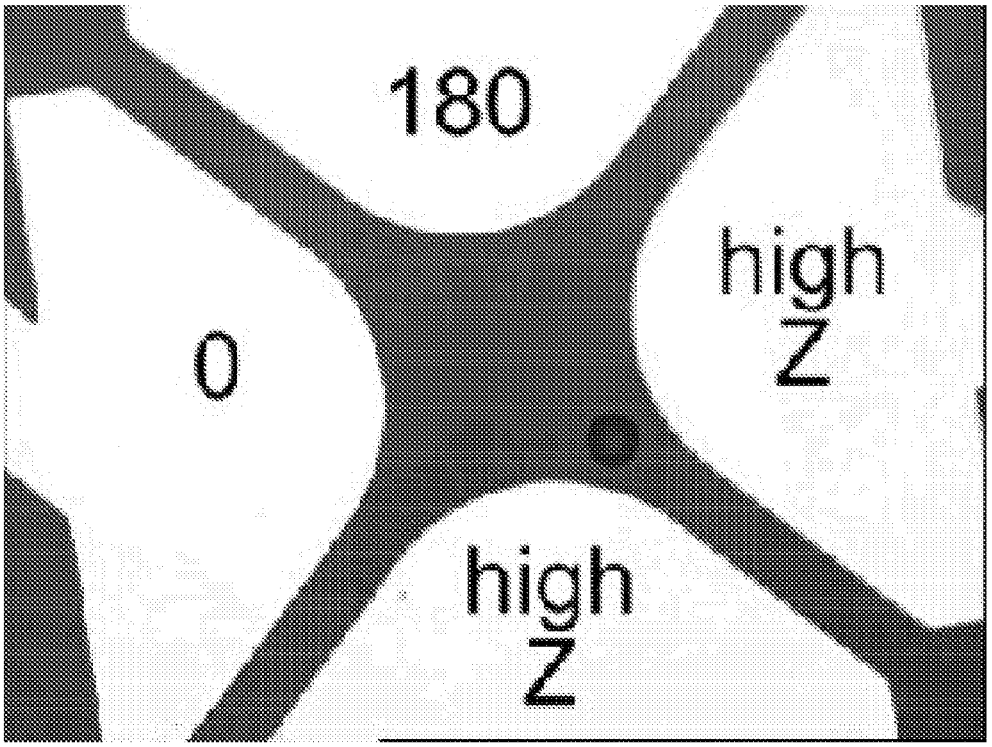


Fig. 7C

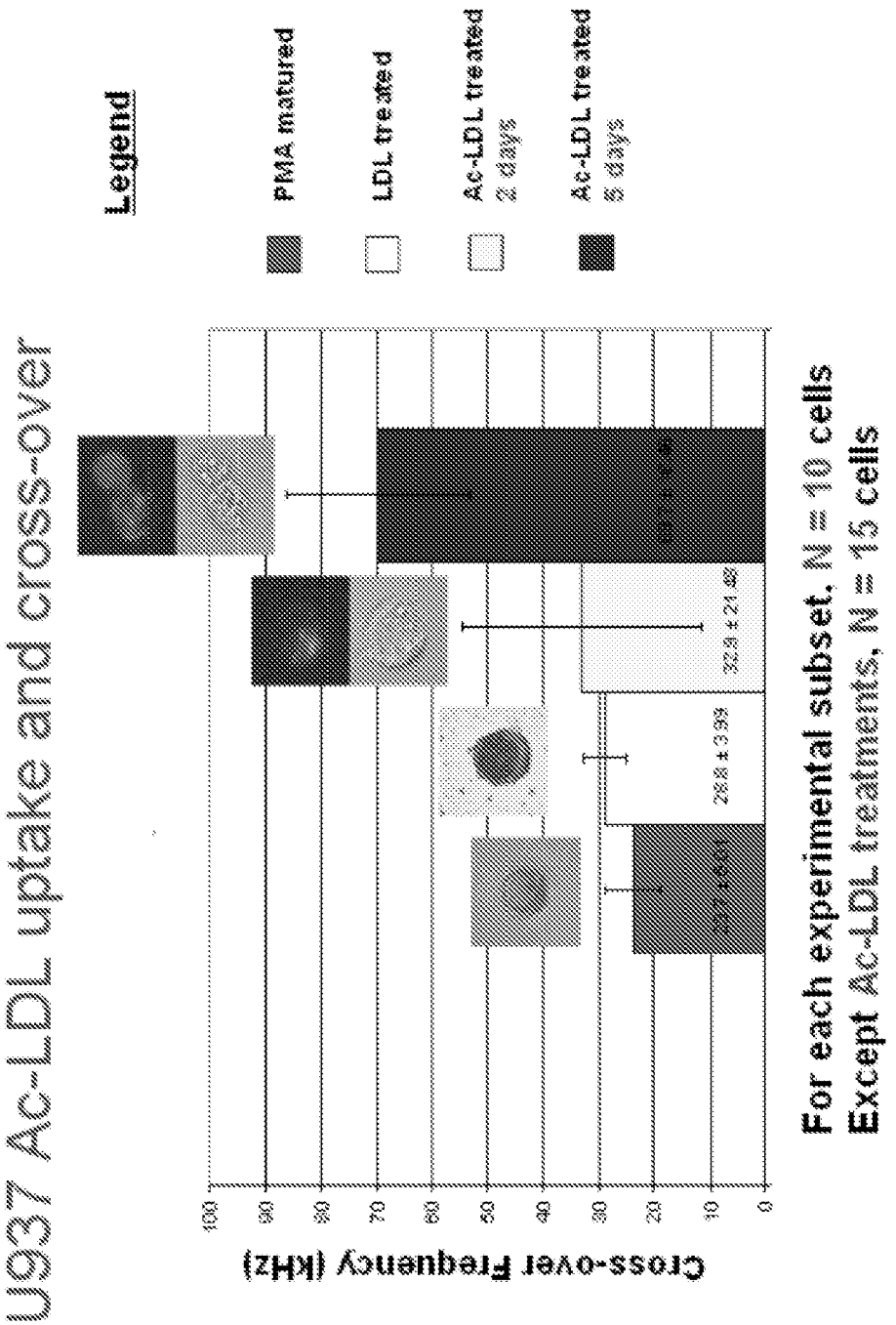

U937 Ac-LDL uptake and cross-over
Legend
PMA matured
LDL treated
Ac-LDL treated 2 days
Ac-LDL treated 5 days
Cross-over Frequency (kHz)
100
90
80
70
60
50
40
30
20
10
0
32.9 ± 21.49
28.8 ± 3.99
For each experimental subset, N = 10 cells
Except Ac-LDL treatments, N = 15 cells

Fig. 8

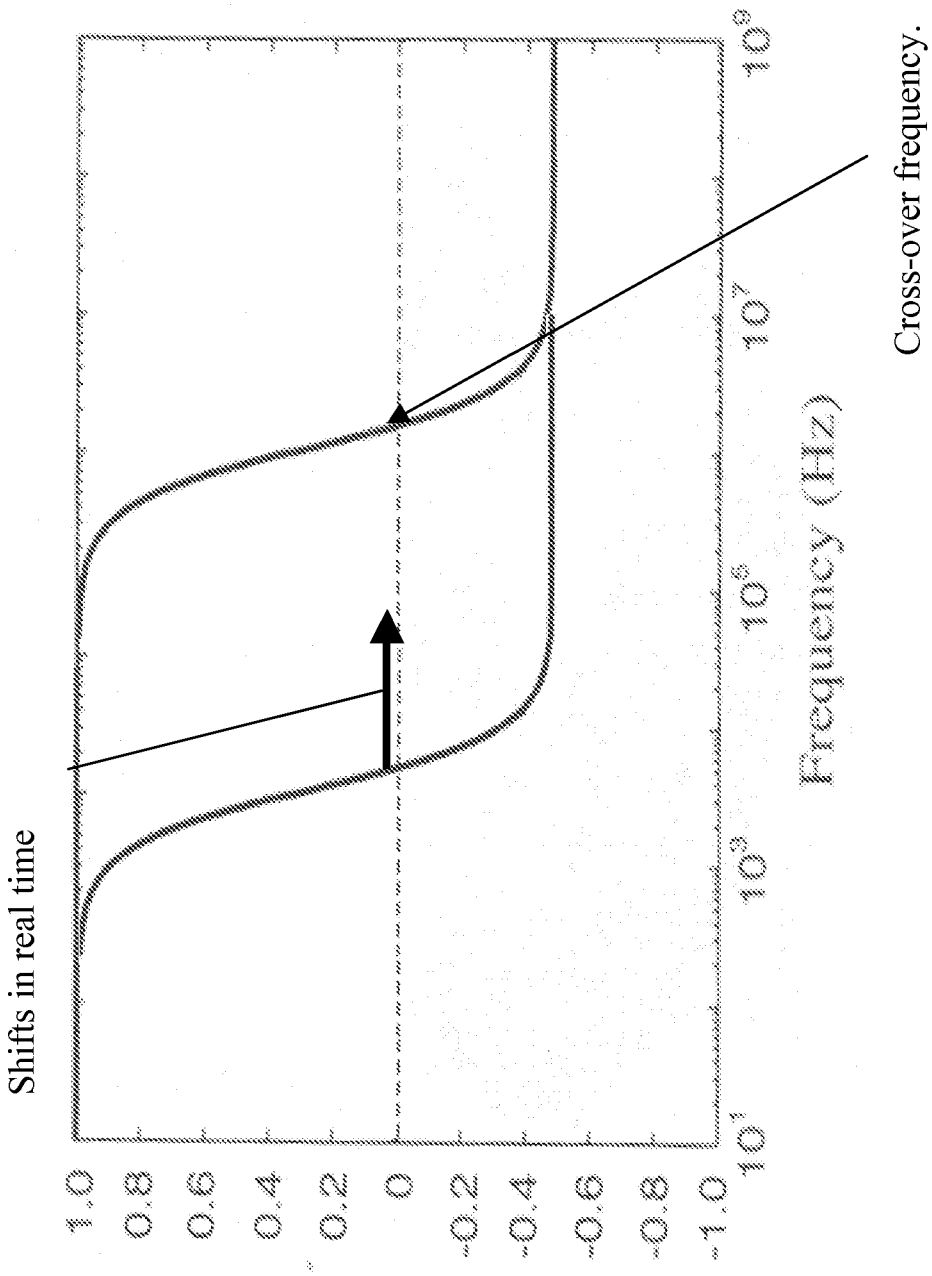

Shifts in real time
1.0
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1.0
10^1
10^3
10^5
10^7
10^9
Frequency (Hz)
Cross-over frequency.

Fig. 9

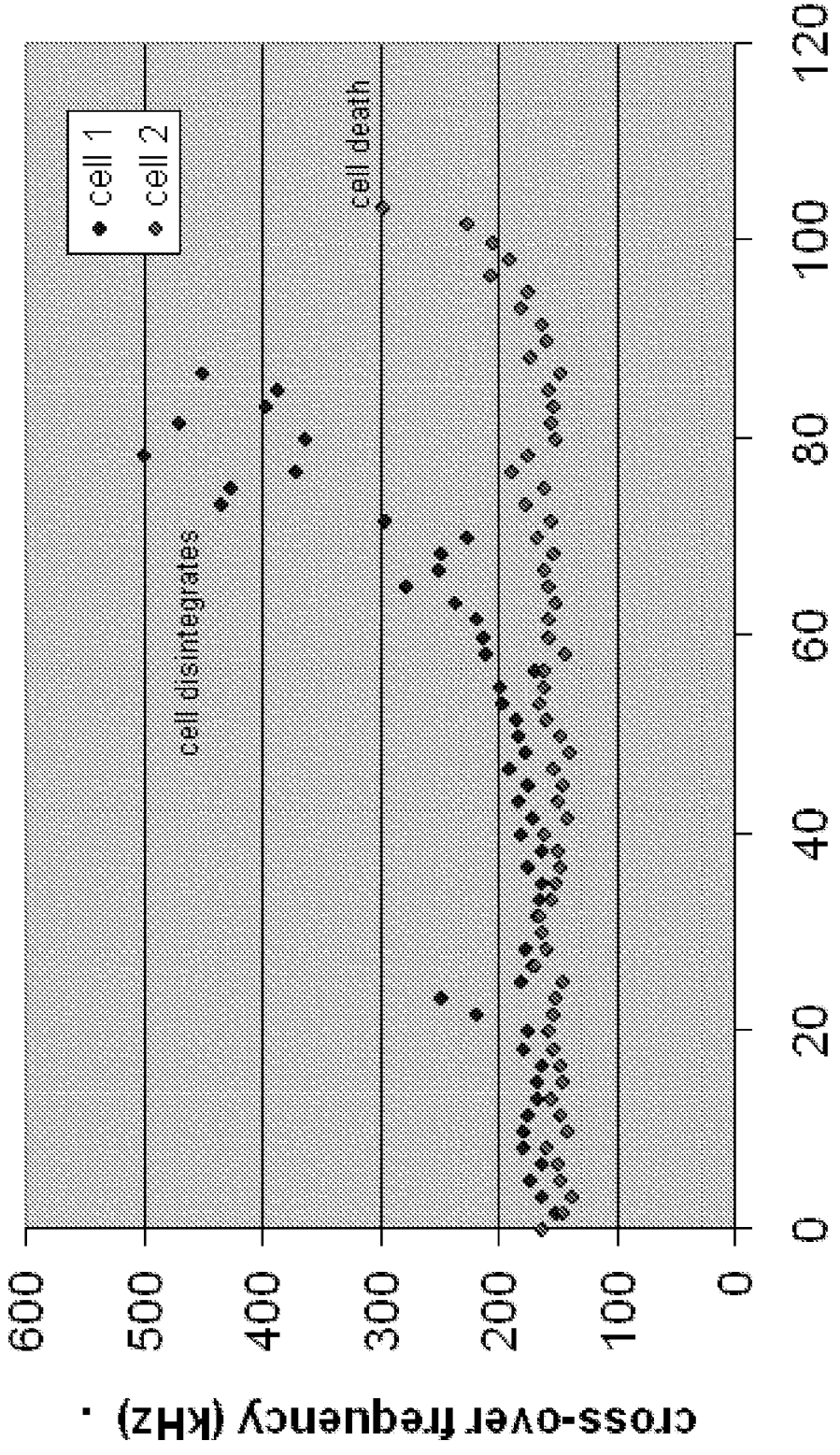

cell 1
cell 2
cell disintegrates
cell death
cross-over frequency (kHz)
time (mins)
600
500
400
300
200
100
0
0
20
40
60
80
100
120

Fig. 10

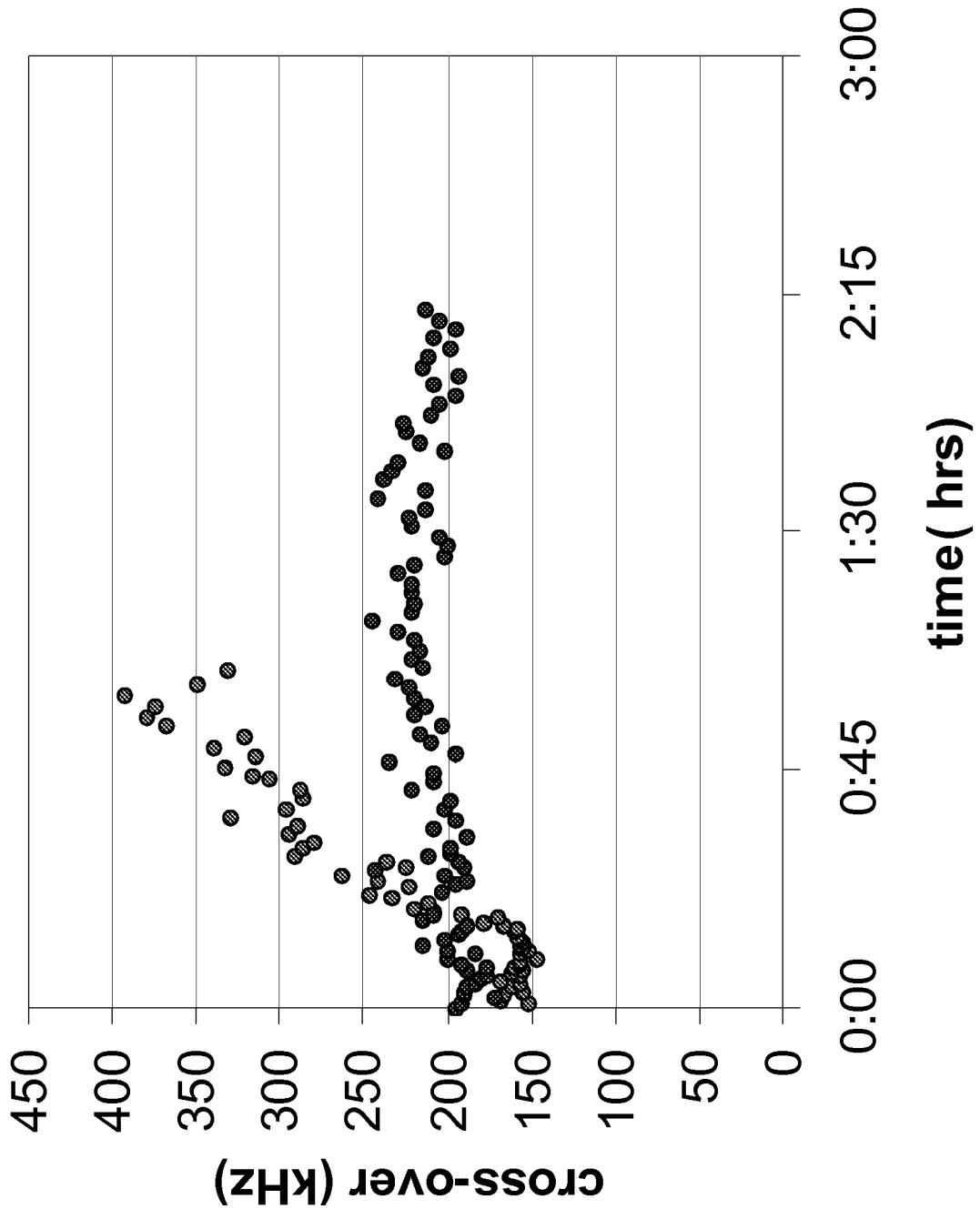

cross-over (kHz)
450
400
350
300
250
200
150
100
50
0
0:00
0:45
1:30
2:15
3:00
time(hrs)

Fig. 11

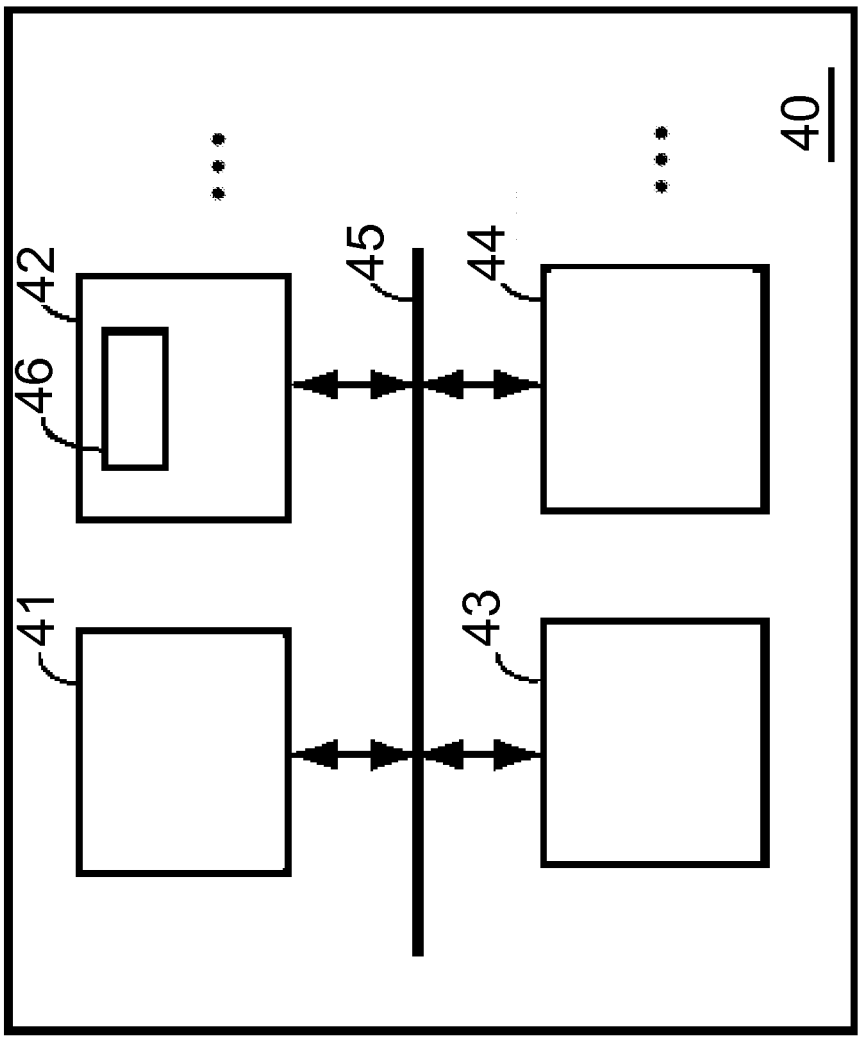


Fig. 12

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 07 11 6010

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Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
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B03C

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
The Hague	29 February 2008	Demol, Stefan

CATEGORY OF CITED DOCUMENTS

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L : document cited for other reasons

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- **JONES T.B.** Basic theory of dielectrophoresis and electrorotation. *IEEE Engineering in Medicine and Biology Magazine,* November 2003, 33-42 **[0069] [0071] [0074]**