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(54) METHOD AND APPARATUS FOR IDENTIFYING OBJECTS IN A PLURALITY OF OBJECTS USING DIELECTROPHORESIS

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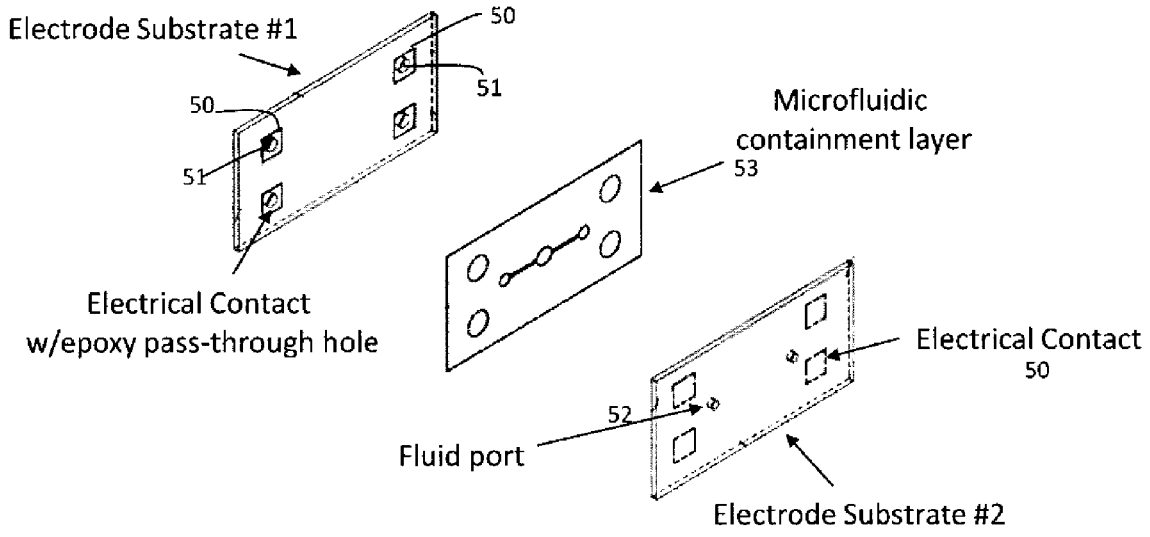
USPC **204/547**; 204/643

(57) **ABSTRACT**

An apparatus for identifying objects in a plurality of objects includes a portion which applies dielectrophoresis to the plurality of objects. The apparatus includes a portion which tracks the plurality of objects' reaction to the dielectrophoresis over time and extracts visible features about the plurality objects being tracked. The apparatus includes a portion which automatically identifies the objects from the plurality of objects based on the objects' reaction to the dielectrophoresis over time and the visible features of the objects. A method for identifying objects in a plurality of objects. A dielectrophoresis cartridge.



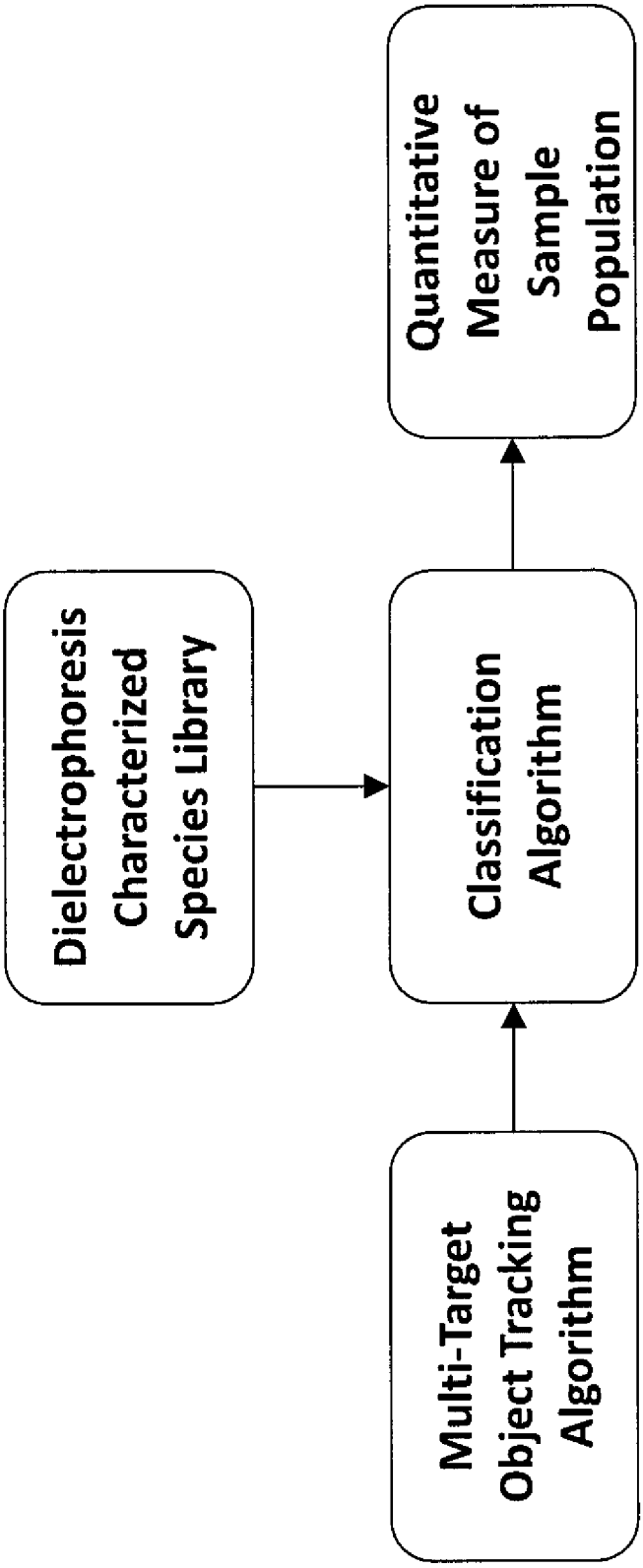

Quantitative Measure of Sample Population
Dielectrophoresis Characterized Species Library
Classification Algorithm
Multi-Target Object Tracking Algorithm

Figure 1A

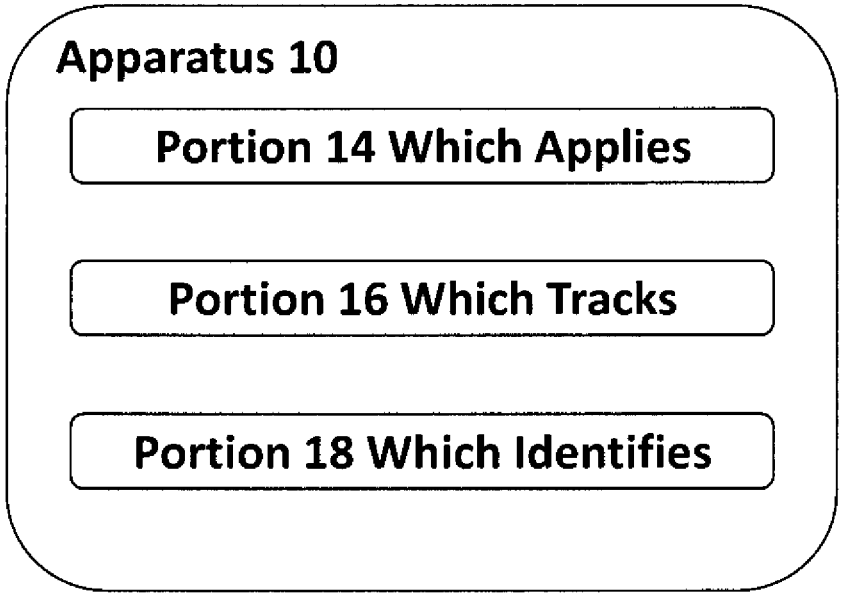

Apparatus 10
Portion 14 Which Applies
Portion 16 Which Tracks
Portion 18 Which Identifies

Figure 1B

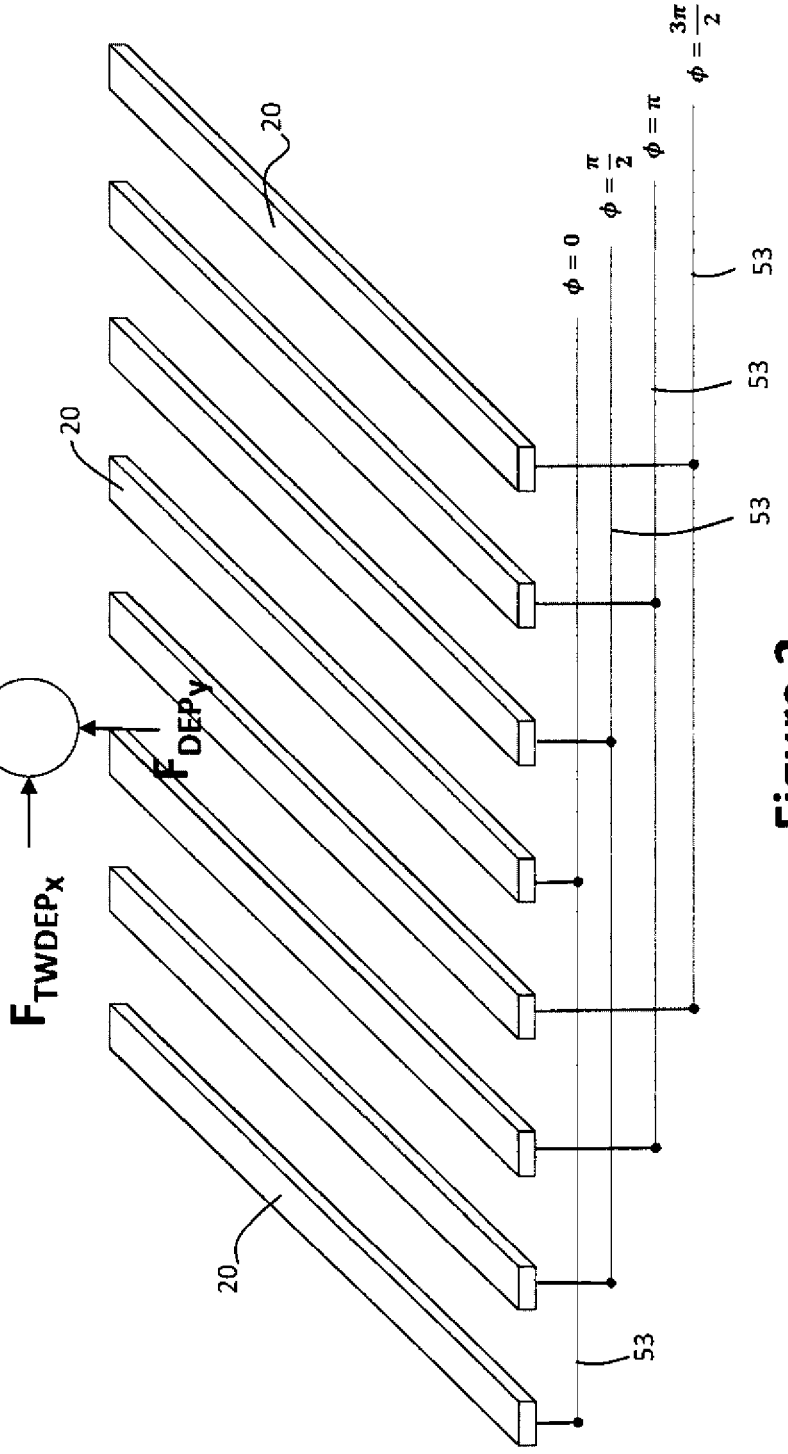

F_{TWDEP_x}
F_{DEP_y}
20
20
20
53
53
53
53
$\phi = 0$
$\phi = \frac{\pi}{2}$
$\phi = \pi$
$\phi = \frac{3\pi}{2}$

Figure 2

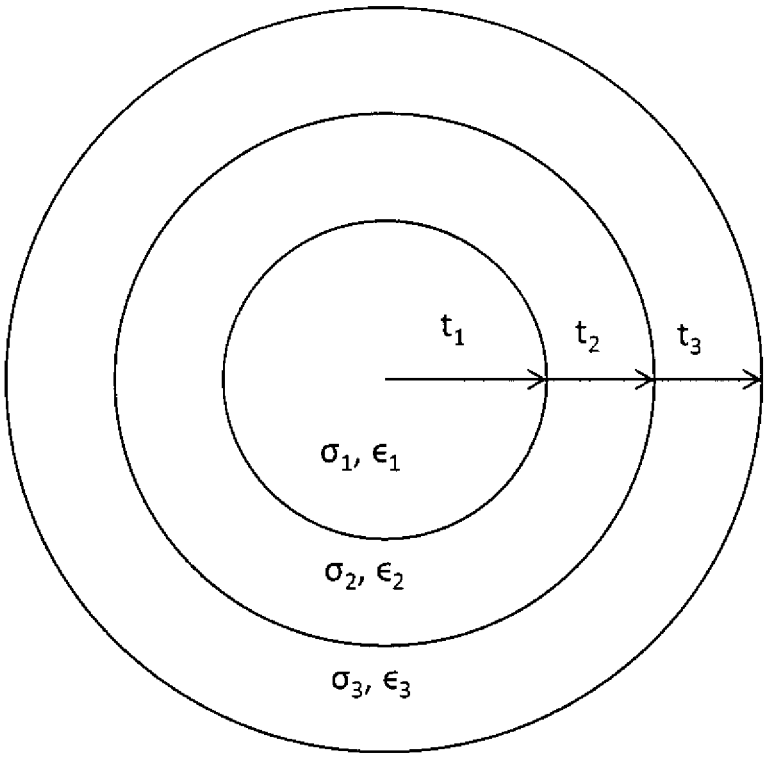


Figure 3

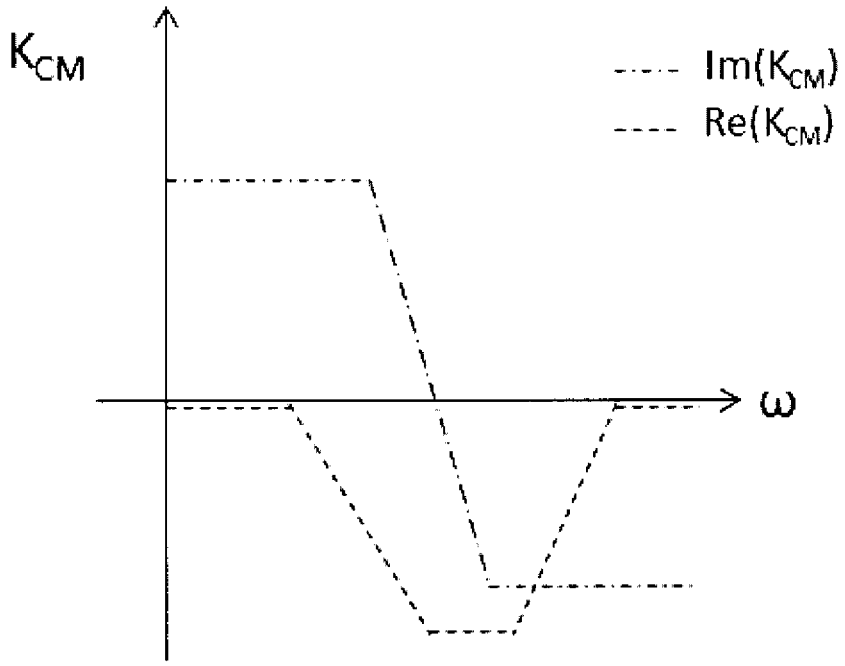


Figure 4

ID	Diameter	Red	Green	Blue	X Vel @ F1	Y Vel @ F1	X Vel @ F2	Y Vel @ F2
vec1	4.9	129	127	20	50	15	92	15
vec2	4.7	63	111	254	38	17	54	11
vec3	4.6	127	129	20	50	14	92	20
vec4	5	174	20	54	33	18	81	15
vec5	5.1	102	186	115	33	16	88	15

Figure 5

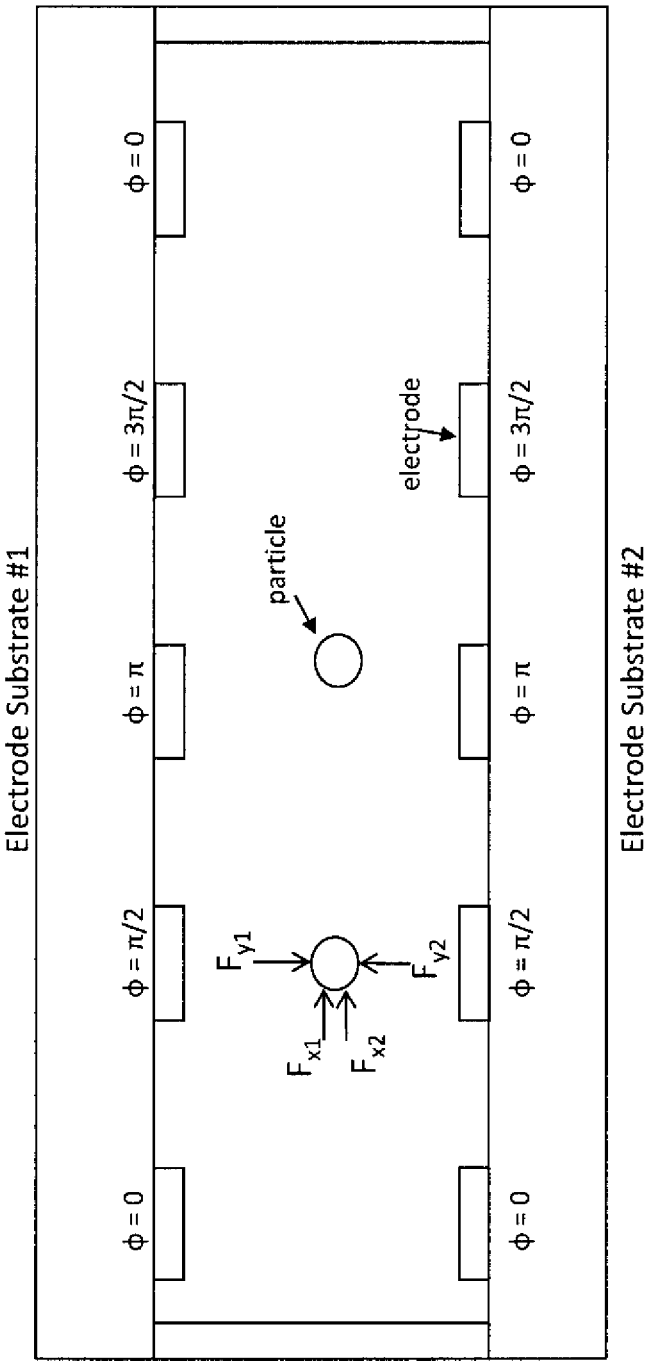

Electrode Substrate #1
Electrode Substrate #2
ϕ = 0
ϕ = π/2
ϕ = π
ϕ = 3π/2
ϕ = 0
ϕ = π/2
ϕ = π
ϕ = 3π/2
ϕ = 0
electrode
particle
F_{y1}
F_{y2}
F_{x1}
F_{x2}

Figure 6

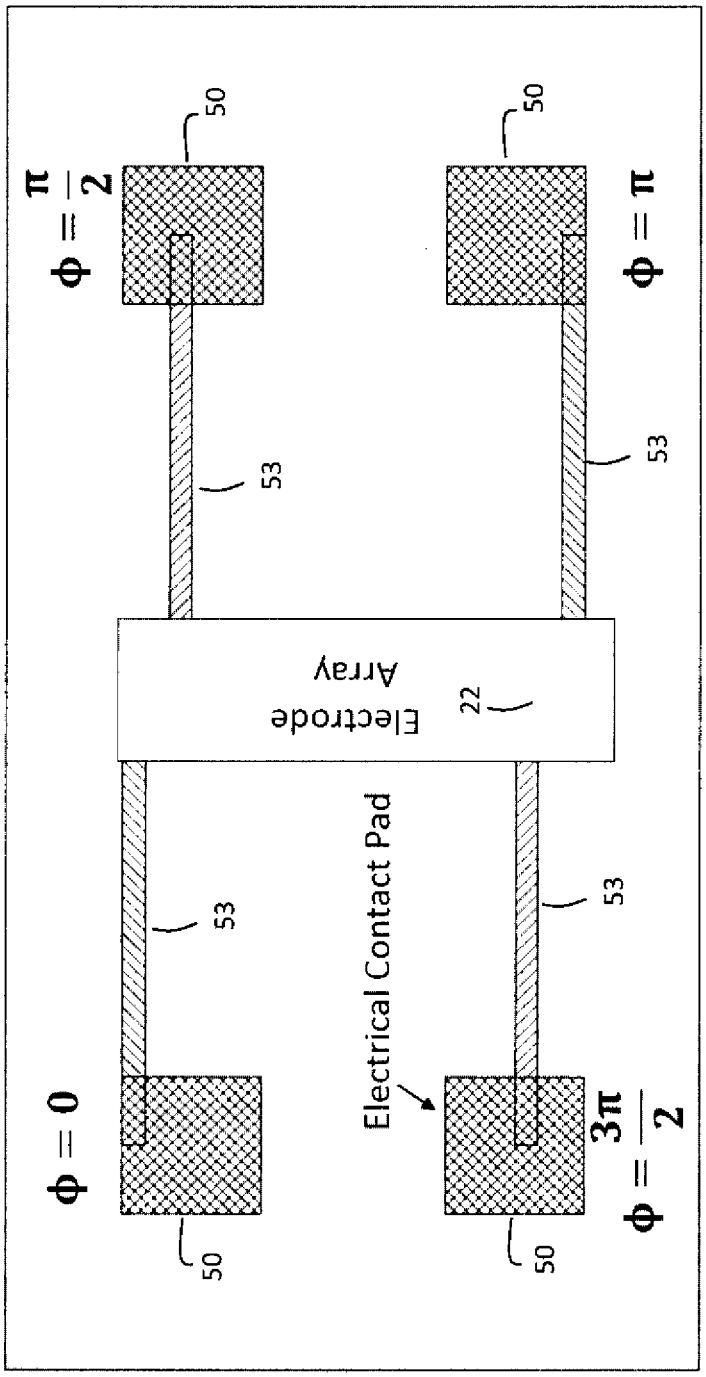

$\phi = \frac{\pi}{2}$
$\phi = \pi$
$\phi = 0$
$\phi = \frac{3\pi}{2}$
50
53
Electrode Array
22
Electrical Contact Pad

Figure 7

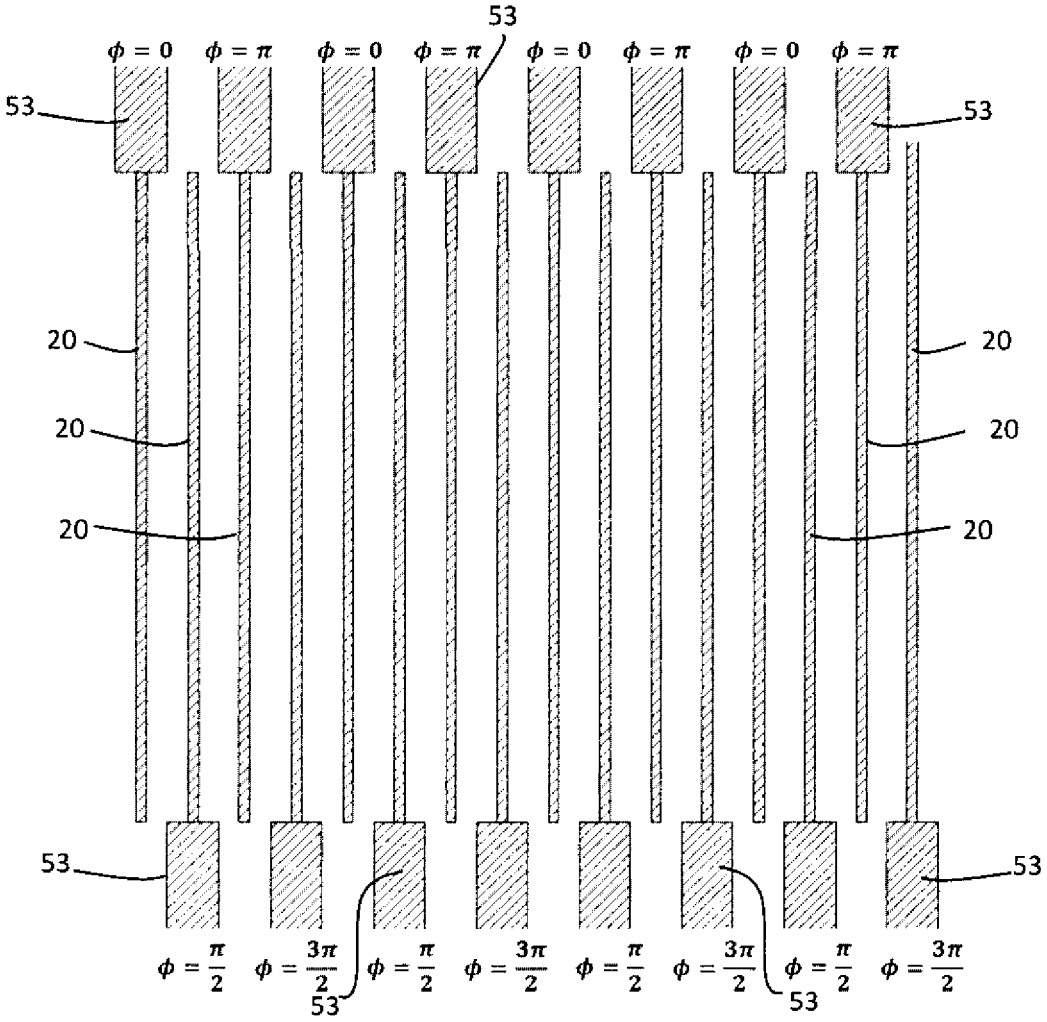

53
$\phi = 0$
$\phi = \pi$
$\phi = 0$
$\phi = \pi$
$\phi = 0$
$\phi = \pi$
$\phi = 0$
$\phi = \pi$
53
53
20
20
20
20
20
20
53
53
$\phi = \frac{\pi}{2}$
$\phi = \frac{3\pi}{2}$
$\phi = \frac{\pi}{2}$
$\phi = \frac{3\pi}{2}$
$\phi = \frac{\pi}{2}$
$\phi = \frac{3\pi}{2}$
$\phi = \frac{\pi}{2}$
$\phi = \frac{3\pi}{2}$
53
53

Figure 8

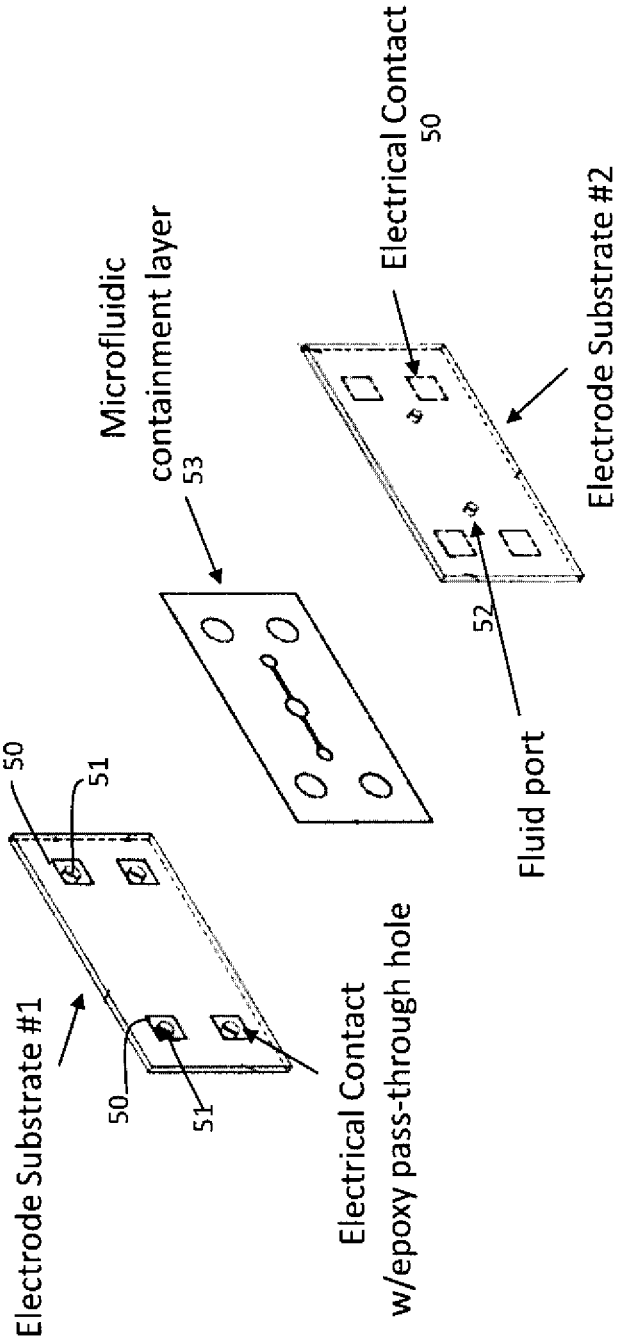
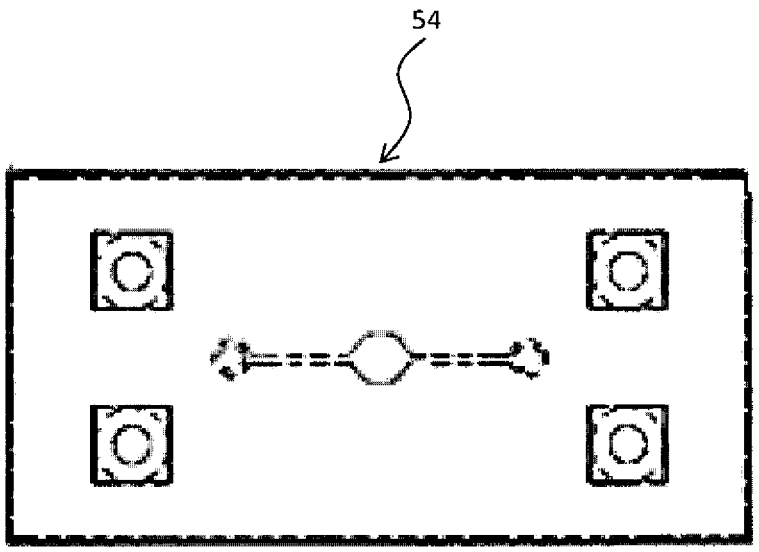

Electrode Substrate #1
50
51
50
51
Electrical Contact
w/epoxy pass-through hole
Microfluidic
containment layer
53
Fluid port
52
Electrical Contact
50
Electrode Substrate #2

Figure 9A



Overhead view after assembly

Figure 9B

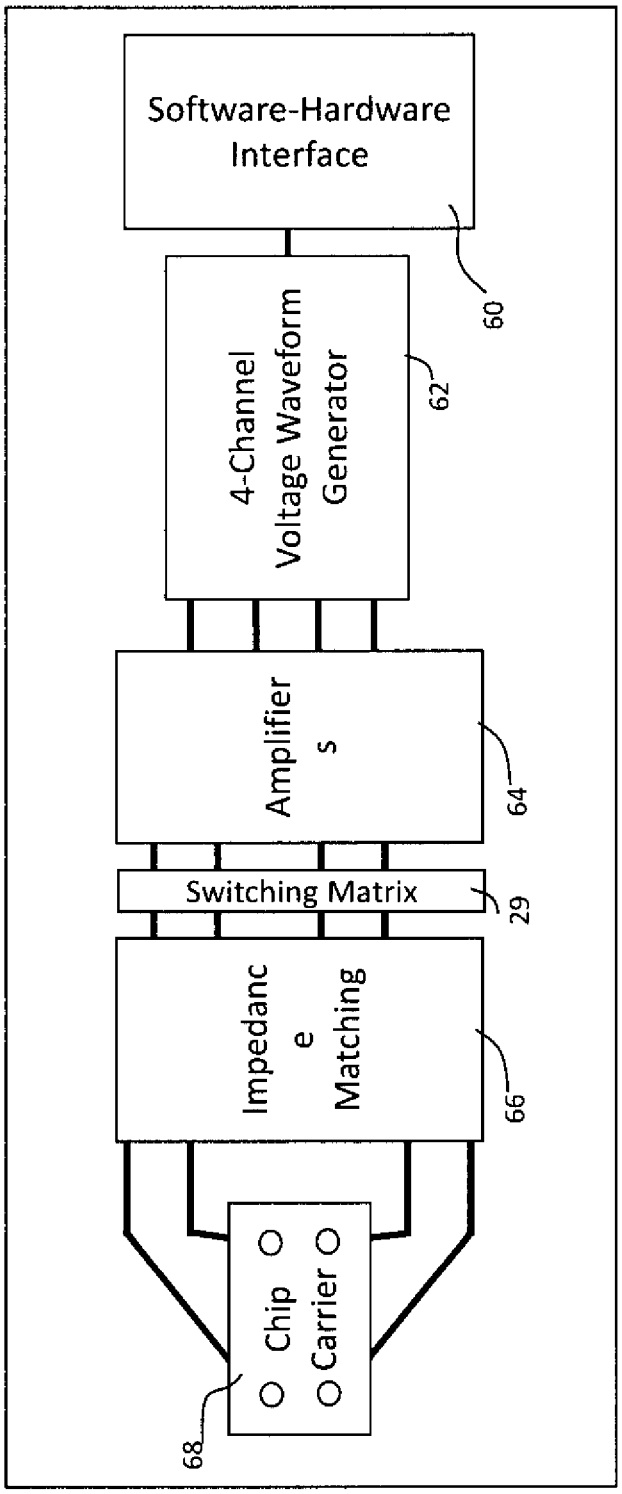

Software-Hardware Interface
60
4-Channel Voltage Waveform Generator
62
Amplifier s
64
Switching Matrix
29
Impedanc e Matching
66
Chip
Carrier
68

Figure 10

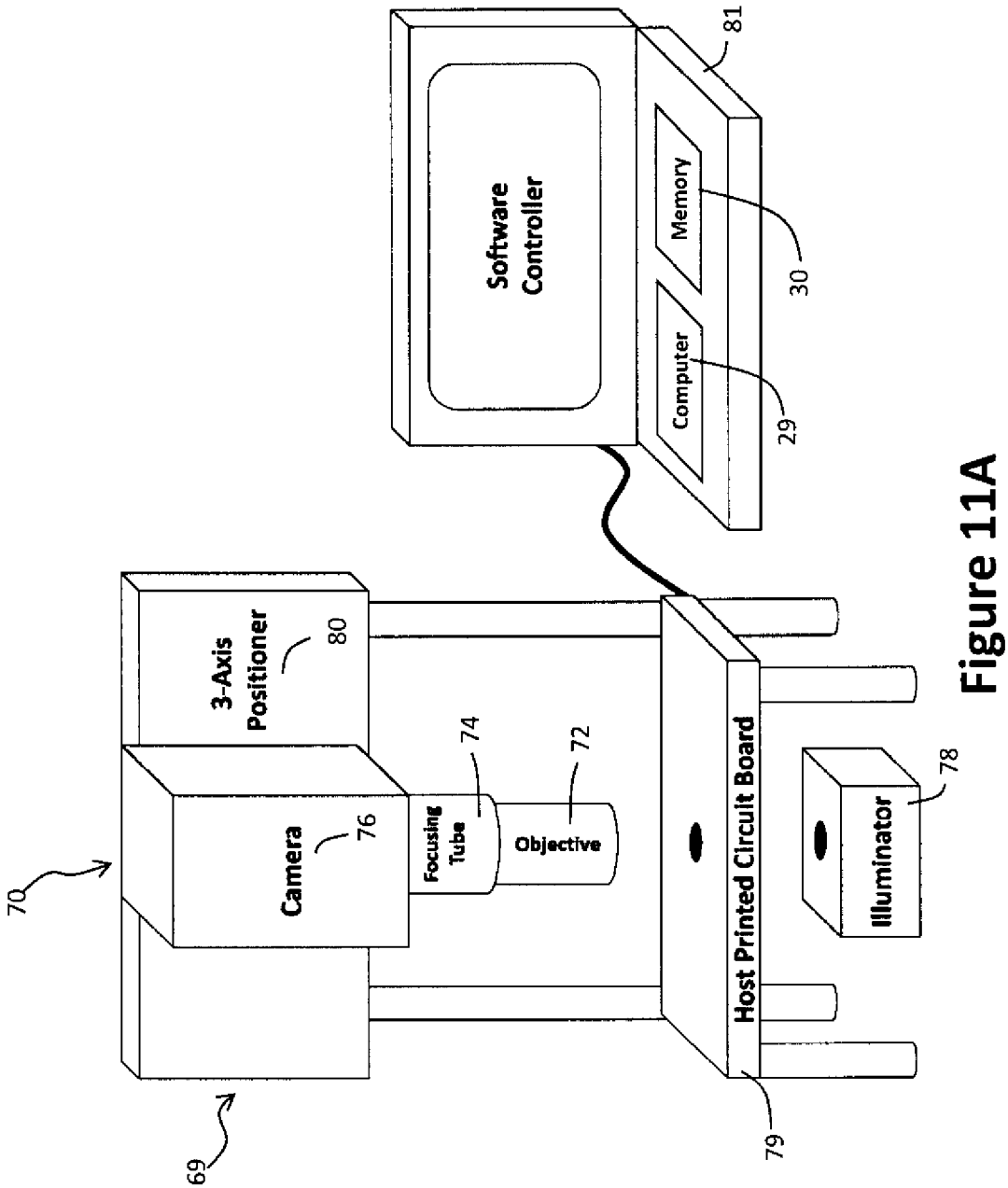

Software Controller
Memory
Computer
81
30
29
3-Axis Positioner
80
Camera
76
Focusing Tube
74
Objective
72
Host Printed Circuit Board
Illuminator
78
79
70
69

Figure 11A

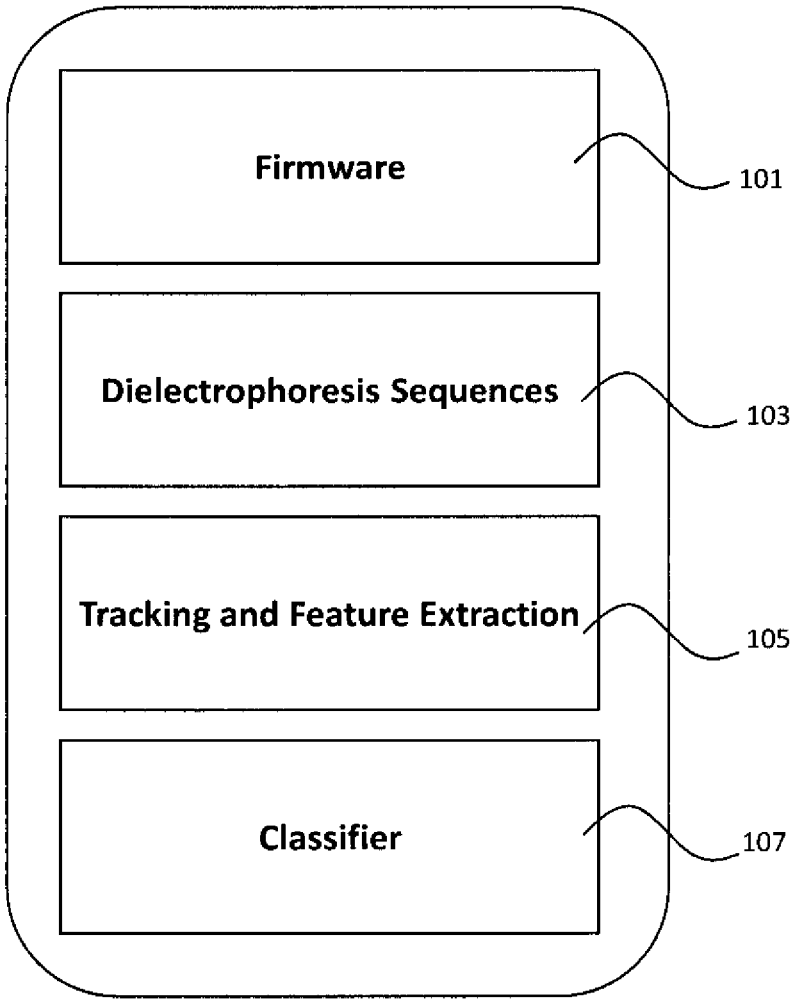

Firmware
101
Dielectrophoresis Sequences
103
Tracking and Feature Extraction
105
Classifier
107

Figure 11B

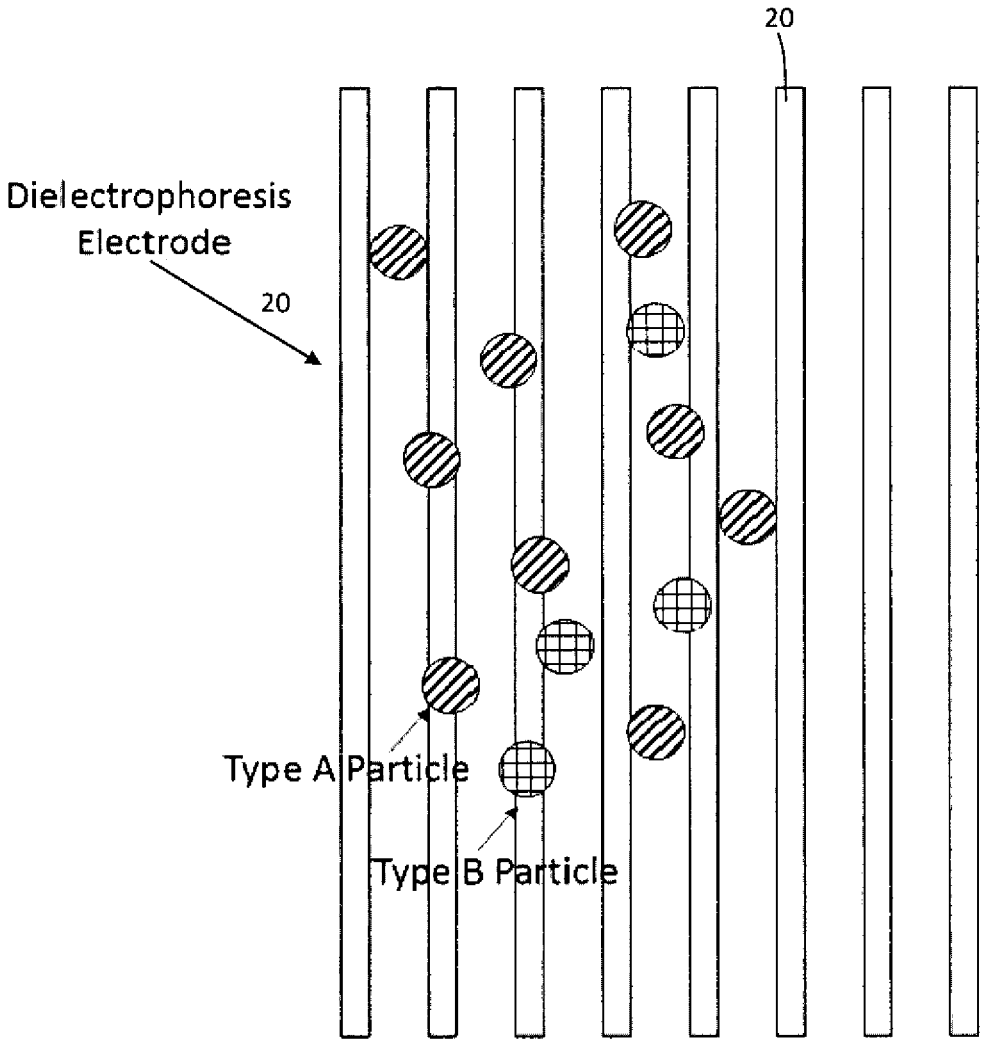

20
Dielectrophoresis
Electrode
20
Type A Particle
Type B Particle

Figure 12

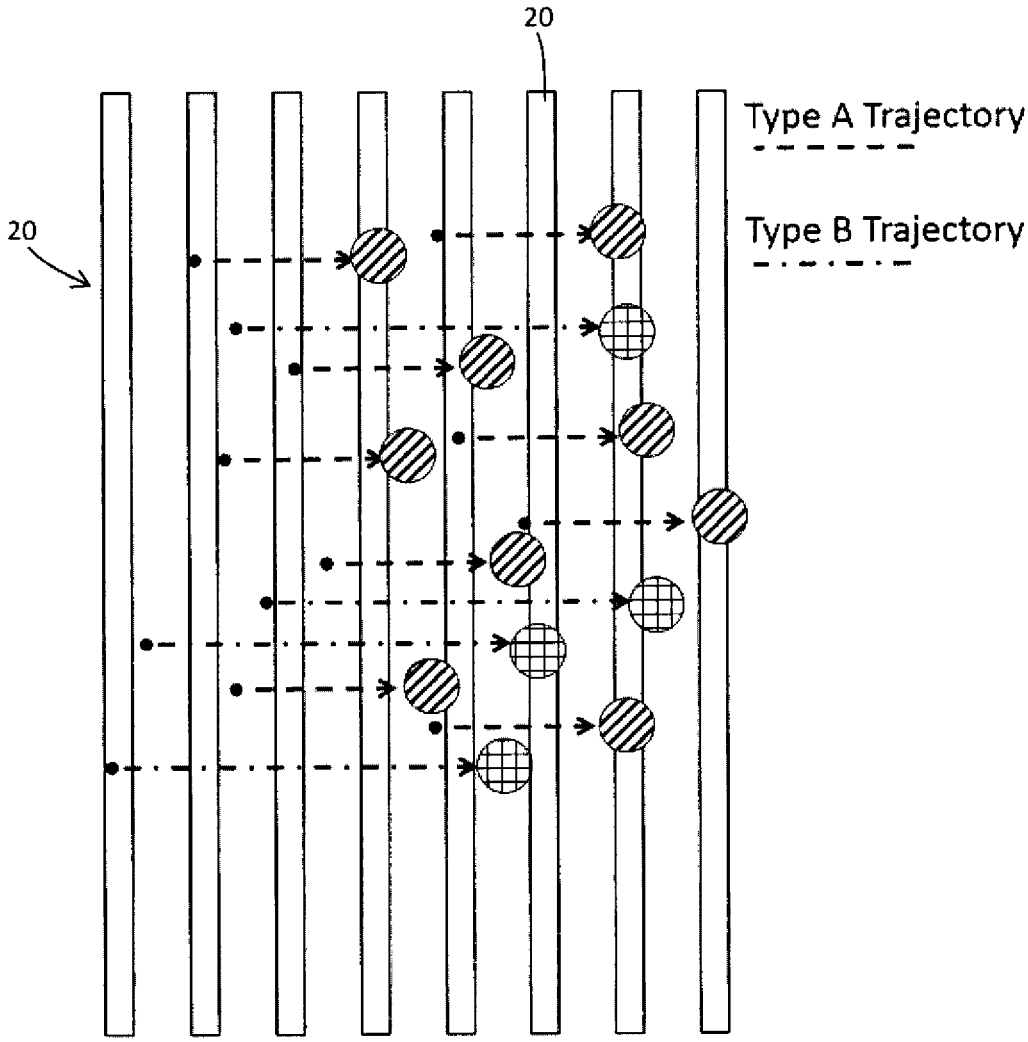


Figure 13

METHOD AND APPARATUS FOR IDENTIFYING OBJECTS IN A PLURALITY OF OBJECTS USING DIELECTROPHORESIS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is related to U.S. provisional patent application Ser. No. 61/835,134 filed Jun. 14, 2013, incorporated by reference herein.

FIELD OF THE INVENTION

[0002] The present invention is related to identifying an object in a plurality of objects automatically based on the use of dielectrophoresis. (As used herein, references to the "present invention" or "invention" relate to exemplary embodiments and not necessarily to every embodiment encompassed by the appended claims.) More specifically, the present invention is related to identifying an object in a plurality of objects automatically based on the use of dielectrophoresis where the object is identified from the plurality of objects based on the objects' reaction to the dielectrophoresis over time and the visible features of the object.

BACKGROUND OF THE INVENTION

[0003] This section is intended to introduce the reader to various aspects of the art that may be related to various aspects of the present invention. The following discussion is intended to provide information to facilitate a better understanding of the present invention. Accordingly, it should be understood that statements in the following discussion are to be read in this light, and not as admissions of prior art.

[0004] In recent years, there has been growing interest in the use of dielectrophoresis as a means to characterize and identify cells. To date, this characterization has remained a manual process where observations of cells undergoing dielectrophoresis are made under a microscope and cell velocities are individually recorded. Such a laborious process becomes impractical once the cell population is large, as is the case with the cell and bacteria samples used in medical diagnostics and has prevented dielectrophoresis from being used in practical applications.

BRIEF SUMMARY OF THE INVENTION

[0005] With the technology described herein, recent advancements made in the area of object tracking are leveraged to be able to automatically collect dielectrophoresis velocity data to overcome this problem. When combined with algorithms for statistically classifying the cell tracks, a unique platform is provided for rapidly identifying cell types in heterogeneous mixtures.

[0006] The present invention pertains to an apparatus for identifying an object in a plurality of objects. The apparatus comprises a portion which applies dielectrophoresis to the plurality of objects. The apparatus comprises a portion which tracks the plurality of objects' reaction to the dielectrophoresis over time and extracts visible features about the plurality of objects being tracked. The apparatus comprises a portion which automatically identifies the object from the plurality of objects based on the object's reaction to the dielectrophoresis over time and the visible features of the objects.

[0007] The present invention pertains to a method for identifying an object in a plurality of objects. The method comprises the steps of applying dielectrophoresis to the plurality of objects. There is the step of tracking the plurality of objects' reaction to the dielectrophoresis over time and extracting visible features about the plurality objects being tracked. There is the step of automatically identifying the objects from the plurality of objects based on the object's reaction to the dielectrophoresis over time and the visible features of the objects.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWING

[0008] In the accompanying drawings, the preferred embodiment of the invention and preferred methods of practicing the invention are illustrated in which:

[0009] FIG. **1**A is a block diagram showing the technologies associated with the claimed invention.

[0010] FIG. **1**B is a block diagram of the apparatus of the present invention.

[0011] FIG. **2** shows Dielectrophoresis electrodes.

[0012] FIG. **3** shows an example concentric shell model that can be used to characterize the electrical features of cells.

[0013] FIG. **4** shows the real and imaginary parts of the Clausius-Mosotti factor for cells based on a multi-layer model and in a conductive medium.

[0014] FIG. **5** shows an example set of feature vectors from a hypothetical data-set for five particles.

[0015] FIG. **6** shows a cross-section of the electrodes of the cassette of the present invention.

[0016] FIG. **7** is an overhead view of a substrate.

[0017] FIG. **8** is a representation of the electrode array.

[0018] FIG. **9**A is an exploded view of the cassette.

[0019] FIG. **9**B is an overhead view of the cassette.

[0020] FIG. **10** is a block diagram showing the base station electronics.

[0021] FIG. **11**A is a representation of the base station and the controller.

[0022] FIG. **11**B is a block diagram of software functionality of the controller.

[0023] FIG. **12** is an overhead view of electrodes with particles where the electrodes are not activated.

[0024] FIG. **13** is an overhead view of electrodes with particles where the electrodes are activated.

DETAILED DESCRIPTION OF THE INVENTION

[0025] Referring now to the drawings wherein like reference numerals refer to similar or identical parts throughout the several views, and more specifically to FIG. **1**B thereof, there is shown an apparatus **10** for identifying an object in a plurality of objects. The apparatus **10** comprises a portion **14** which applies dielectrophoresis to the plurality of objects. The apparatus **10** comprises a portion **16** which tracks the plurality of objects' reaction to the dielectrophoresis over time and extracts visible features about the plurality of objects **12** being tracked. The apparatus **10** comprises a portion **18** which automatically identifies the object **12** from the plurality of objects **12** based on the object's reaction to the dielectrophoresis over time and the visible features of the objects **12**.

[0026] The portion **14** which applies may include a plurality of dielectrophoresis electrodes **20**, as shown in FIG. **6**. The portion **14** which applies may include a controller **24** which causes dielectrophoresis fields to be generated by the electrodes **20**, as shown in FIG. **11**A. The apparatus **10** may include a containment chamber **26** in which the objects **12** are disposed, as shown in FIG. **9**A. The chamber **26** may have

inlet and outlet ports **52** through which the objects **12** are delivered to or removed from the chamber **26**. The portion **16** which tracks may include voltage sources **62** in communication with the controller **24** that drive the electrodes **20**. The chamber **26** may be a sample cartridge **54**.

[0027] Each electrode **20** may be connected to the voltage sources **62** via a programmable switching matrix **28** that allows for any electrode **20** to be connected to any of the voltage sources **62**. The portion which tracks may include a memory **30** and an optical sub-system **70** which takes images of the objects **12** in the chamber **26** over time and stores the images in the memory **30**. The optical sub-system **70** may include a microscope objective **72**, focusing tube **74** and a camera **76**.

[0028] The controller **24** may include a computer **29** programmed to use a multi-target tracking algorithm to track the objects **12** in the chamber **26**. The tracking algorithm may be one that is an implementation of the multi-target multi-hypothesis tracking method. The controller **24** may cause the electrodes **20** to generate dielectrophoresis to induce motion in the objects **12**. The type of dielectrophoresis used may include traveling-wave dielectrophoresis. The computer **29** may be programmed to use a statistical classification algorithm to determine a category or type in regard to the objects **12** based on the images of the objects **12**. The statistical classification algorithm may include a general likelihood ratio test.

[0029] The present invention pertains to a method for identifying objects **12** in a plurality of objects **12**. The method comprises the steps of applying dielectrophoresis to the plurality of objects **12**. There is the step of tracking the plurality of objects' reaction to the dielectrophoresis over time and extracting visible features about the plurality of objects **12** being tracked. There is the step of automatically identifying the objects **12** from the plurality of objects **12** based on the objects' reaction to the dielectrophoresis over time and the visible features of the objects **12**.

[0030] There may be the step of introducing the objects **12** into a chamber having dielectrophoresis electrodes **20**. There may be the step of initiating a frequency sweep by a controller **24** of voltage sources **62** causing the objects **12** to move according to dielectrophoresis forces exerted on the objects **12** at each frequency.

[0031] There may be the step of capturing image frames of the objects' **12** motion during the frequency sweep with an optical imaging subsystem. There may be the step of processing the image frames by a computer of the controller **24** with tracking software stored in a memory **30** to record in the memory **30** trajectories of each object within the image frames at each time step during the frequency sweep. There may be the step of classifying the objects **12** based on the trajectories of the objects **12** from the images stored in the memory **30**.

[0032] In the operation of the invention, the present invention is a novel methodology and accompanying hardware platform for rapid identification of micro and nano scale objects **12** or "particles". Analyzed samples may contain particles that are biological in nature (e.g., cells, viruses, bacteria, etc.), particles that are non-biological (e.g., plastic, glass, etc.) or any mixture thereof in an aqueous sample. The approach here is based on the combination of three technologies (FIG. **1**A): Dielectrophoresis, where electric fields are used to induce diverse motion in particle populations based on the electrical properties of each particle type. Multi-target tracking algorithms that can simultaneously track the motion of all particles in a sample and create a database of their trajectories and features, and classification algorithms that can classify the database based on learned motion characteristics for a particle type of interest. Together these three technologies provide a unique ability to identify specific particles (from a library of pre-characterized species). The existence of particular particle types (e.g., biological pathogens) can be identified using minute samples even when there are a relatively small number of particles of interest present in the sample. The time to process a sample can be just a few minutes, including the time for sample preparation, electrokinetic manipulation, image processing, tracking and identification.

[0033] In the first step of the technique, a sample containing particles is exposed to a sequence of dielectrophoresis fields specifically selected to differentiate the target particle type. While undergoing dielectrophoresis, each particle type reacts differently to a given field and has a unique velocity profile based on its internal structure, size, shape, and electrical characteristics. Therefore, with a general knowledge of these parameters for a given particle type, electric field frequencies are selected at which particles will be responsive to dielectrophoresis, as further explained below.

[0034] Next, a machine vision system is used to observe the reactions of the bacteria in the sample to the dielectrophoresis fields and their motion is tracked in real time using multi-target tracking software **105**. This tracking software **105** generates a database of the motion of each particle along with its size, shape, and orientation, collectively known as features.

[0035] In the final step, this database is analyzed using a statistical classification algorithm **107** that compares the feature set of each particle with previously recorded and learned features for a bacteria type of interest. If particles exist in the sample with features that are statistically similar to the learned features, they are identified as the target bacteria. This technique provides a unique ability to identify specific bacteria without the need for culturing and population amplification and fluorescent labels not needed to observe the unique motion patterns. Since classification is based on a library of pre-characterized bacterial species, the platform is thus generalizable to many types of biological and non-biological particles. Sample handling and processing for the platform is simple and straightforward. Specimens can be swabbed, transferred to an aqueous transport medium and then delivered into the sample cartridge **54**. Since the field configurations are driven by software, the technology uses one dielectrophoresis cartridge **54** design that is suitable for analyzing many different types of particles.

[0036] Dielectrophoresis Particle Manipulator

[0037] The first key technology is dielectrophoresis, a technique where electric fields can be used to differentiate particles based on their inherent electrical properties. The use of dielectrophoresis was first reported by Pohl in [1]. These electrical properties reflect differences not just in particle mass and volume, but they also capture subtle variations in the internal composition and morphology of the particle. Depending on the field characteristics, particles with variations in composition and morphology can be made to experience different amounts of force, forces in opposite directions, or no force at all. Using this effect we can simultaneously induce diverse motion in large numbers of

particles across a sample in which the direction and velocity of each particle can be directly mapped to particle composition and morphology.

[0038] Dielectrophoresis is unique among electrokinetic techniques in that it operates on neutral particles with no net charge. Thus it can be used on a large class of inert and biological particles ranging from whole cells to viruses. When an electrically neutral particle is placed in the presence of a spatially non-uniform electric field, the particle becomes polarized. The force on the polarized particle is the product of its effective dipole moment, which is a function of the electrical properties of the particle in comparison to the medium, the particle radius, r, and the electric field gradient.

[0039] There are many forms of dielectrophoresis. The form of dielectrophoresis employed here (although not limited to) is Traveling Wave dielectrophoresis (TWDEP). With traveling wave dielectrophoresis the AC electric field is spatially non-uniform in both amplitude and phase. Typically, traveling-wave configurations employ a linear electrode **20** array **22**, as shown in FIG. **2**. There are no hard requirements on the width of the individual electrodes **20** used. However, in order for dielectrophoresis to operate, the gap spacing between electrodes **20** must be 'significantly greater' than the diameter of the particle being manipulated. Some specific examples (but not limited to) are yeast cells (with average diameter on the order of 6 um) can be manipulated with electrode gaps on the order of 10 um-15 um and up. HSV capsids (with average diameter on the order of 60 nm, can be manipulated with electrode gaps on the order of 150 nm and up. As the radius of the particle decreases, so does the amount of force exerted on that particle. Therefore, a reduction in particle size can be compensated for by either increasing the voltage magnitude or decreasing the electrode gap spacing (thereby increasing the field strength).

[0040] In this case, the electrodes **20** are driven by AC voltage signals, with each electrode **20** having a constant shift in phase with respect to its neighbor. In the example of FIG. **2**, each electrode **20** along the array **22** is phase shifted by 90 degrees. This causes the magnitude of the electric field to be uniform along the lateral x-axis. In addition, because of symmetry, the magnitude of the electric field along the z-axis (parallel to the electrodes **20**) is constant, therefore $\nabla E_x^2=\nabla E_z^2=0$. Similarly, the phase of the field about the y and z axes is constant, thus $\nabla\phi_y^2=\nabla\phi_z^2=0$. Therefore the time-averaged force exerted on the particle reduces to:

$$(\vec{F}_{DEP})=2\pi\epsilon_m r^3[Re(K_{CM})\nabla E_y^2+Im(K_{CM})E_x^2\nabla\phi_x]$$

[0041] From this expression, it can be seen that the traveling-wave dielectrophoresis force vector consists of a y-component that levitates the particle vertically with strength proportional to the magnitude gradient of the field and a horizontal force that moves the particle along the x-axis with strength proportional to the product of the electric field intensity and the gradient of the phase of the field. The strength of the force on a particle at any particular AC field frequency depends on a ratio of the complex permittivity of the particle to the complex permittivity of the buffer medium. This ratio is called the Clausius-Mossotti factor (K_{CM}). It is expressed as:

$$K_{CM}=\frac{\varepsilon_p^*-\varepsilon_m^*}{\varepsilon_p^*+2\varepsilon_m^*}$$

[0042] where ϵ^*_m and ϵ^*_p are are the permittivity of the medium and particle respectively and are a function of the frequency of the electric field.

[0043] The Clausius-Mossotti factor, K_{cm}, is the key to the selective manipulation capabilities of dielectrophoresis. Both the real and imaginary parts of the complex K_{cm} are important. The real part acts as a proportionality constant to the vertical component of force exerted on the particle (DEP), while the imaginary part acts on the lateral force component (TWDEP). The appeal of dielectrophoresis in this methodology is that the direction and magnitude of the force exerted on a particle can be controlled externally by certain electric field parameters and the forces can be targeted towards a particle of a particular type. This high level of contactless yet specific control is possible because the inherent electrical characteristics of particles are based on their makeup. The electrical properties of particles not only reflect differences in their size and structure, but also capture subtle variations in their internal composition. Particles of different types have dissimilar electrical conductivities and permittivities that result in unique frequency/phase dependencies. These dependencies translate into distinct responses when introduced into an AC electric field. Previous attempts to characterize these frequency-dependent behaviors typically involve observation of particles dielectrophoretically induced velocities using a microscope and manual collection of data, as was reported by Huang [2].

[0044] The electrical features of the particle are characterized using a concentric shell model to model its effective complex permittivity, which in turn determines the Clausius-Mossotti factor. In the shell model, particles are electrically modeled as concentric spheres of varying thickness, each layer having unique values for electrical conductivity and permittivity. In this model, the effective permittivity of the particle can be approximated by successively combining expressions for each shell to pairs of layers that make up the complete model. While the multi-shell model is simplistic from a biological perspective, it is accurate enough to predict the forces exerted on particles and their resulting motion in fluids.

[0045] FIG. **3** shows an example concentric shell model that can be used to characterize the electrical features of cells. FIG. **4** shows the real and imaginary parts of the Clausius-Mossotti factor for cells based on a multi-layer model and in a conductive medium. The model reveals that cells will exhibit various modes of motion based on frequency. At very low frequencies, the negative real force component ($Re(K_{CM})<0$) exerted on the cells will cause them to levitate, with little to no lateral movement ($Im(K_{CM})<0$). As frequency increases, the particles will transition into a region where negative forces continue to elevate it, and but strong TWDEP force components will push the particle along the direction of the positive phase gradient. In the next band of frequencies where $Re(K_{CM})$ is positive, strong positive DEP forces will pull the particles down towards the electrode **20** edges. Eventually at high frequencies, when $Re(K_{CM})$ goes back to being negative, the cells will once again levitate and move laterally due to TWDEP forces. However, since the sign of $Im(K_{CM})$ is negative in this region, the particles will move in a direction opposite to the phase gradient.

[0046] In practice, the real (DEP) and imaginary (TWDEP) force components can be measured indirectly by configuring a known electric field and observing the particle's velocity. In order to extract the unique responses of the particles within

the population, observations of particle motion are sampled over frequency band that ranges between the DC and the frequency at which the high frequency steady state response is observed. For this technology the extraction of this data takes place automatically by way of advanced multi-target tracking algorithms as the field generator frequency parameter is varied.

[0047] Multi-Target Multi-Hypothesis Object Tracker **105**

[0048] Simultaneously tracking the trajectories of multiple objects **12** (e.g. thousands of bacterial cells moving under the influence of dielectrophoresis) is a difficult, computationally intensive problem to solve and is currently the subject matter of much research for defense surveillance applications. Multi-target tracking (MTT) algorithms are fundamentally different from single-target algorithms in that they must be able to accommodate cases in which there may be an unknown number of targets, targets that are closely spaced together and targets having paths that cross over one another. This presents a data association problem in which one is given a sequence of sets of track measurements, and must determine which measurements to associate with which targets and which to discard.

[0049] Multiple-hypothesis tracking (MHT) is generally acknowledged as the most powerful currently-known paradigm for multi-target tracking. It was first formalized in what is now referred to as hypothesis-oriented form. Unfortunately, the hypothesis-oriented methodology typically leads to an unmanageable number of hypotheses even for small problems. The track-oriented MHT approach was developed in the 1980s by researchers at ALPHATECH. Large-scale track-oriented MHT problems often require distributed, multi-stage solutions. For a sequence of sets of contacts $Z^k = (Z_1, \ldots, Z_k)$, one wishes to estimate the state history X^k for all objects **12** present in the surveillance region that exist over the time sequence $(t_1, \ldots, t_k)$ given the auxiliary discrete state history q^k that represents a full interpretation of all contact data: which contacts are false, how the object-originated ones are to be associated, and when objects **12** are born and die. Of interest here is the probability distribution $p(X^k|Z^k)$ for object state histories given data. This quantity can be obtained by conditioning over all possible auxiliary states histories q^k.

$$p(X^k \mid Z^k) = \sum_{q^k} p(X^k \mid Z^k, q^k) p(q^k \mid Z^k). \tag{1}$$

[0050] The MHT approach seeks to identify the MAP estimate for the auxiliary state history q^k, and identify the corresponding MMSE estimate for the object state history X^k conditioned on the estimate for q^k,

$$\hat{q}^k = \underset{q^k}{\operatorname{argmax}}\, p(q^k \mid Z^k) \tag{2}$$

$$\hat{X} = \hat{X}_{MMSE}(Z^k, \hat{q}^k) \tag{3}$$

[0051] Track-oriented MHT avoids enumeration of all global hypotheses q^k, though these are implicitly defined in the set of track hypotheses trees. With the track-oriented approach, there is selected a set of leaves that identify the MAP solution (2), with the feasibility constraint that all measurements are utilized at most once.

[0052] In practice, computational requirements preclude optimal, batch MHT solutions. For practical MHT solutions, a number of computation simplifications are adopted that include the following:

[0053] Only objects **12** that have been detected are included in the determination of tracks.

[0054] The maximum number of possible hypotheses is limited by disallowing unlikely associations between tracks and objects **12**.

[0055] The tree data structure used to represent the candidate hypotheses for an object's track is pruned to a single global hypothesis with a fixed delay by eliminating the least likely tracks for that object.

[0056] Extraction of the track associated with an object is performed traversing the nodes of the pruned tree using logic-based or statistical tests.

[0057] One of the primary advantages of this tracking algorithm is that it does not require all of the possible global hypotheses for an object to be enumerated, thereby making it computationally-efficient.

[0058] Coraluppi and Carthel [3] have adapted multi-target tracking algorithms to particle tracking problems. For the dielectrophoresis application of interest here, the tracking solution is further modified by adding the following elements: (1) particle detection based on advanced image-segmentation technology; (2) feature-aided multi-target tracking where the augmented state space includes both target kinematics and detection-level features such as particle size.

[0059] In order to condition the recorded tracks, used as input to the classifier, the tracks are filtered using a Kalman smoothing filter. While it is known that track smoothing does not improve data-association performance, it does improve the input for downstream statistical particle classification algorithms. Several versions of the Kalman smoother have been documented in the literature; these include the conceptually-simple forward-backward algorithm and the computationally-efficient Rauch-Tung-Striebel smoother. The Kalman smoother provides the optimal trajectory by reasoning over all measurements in the past as well as the future. Like the Kalman filter, the Kalman smoother relies on a statistical object dynamical model and sensor measurement model; no further assumptions are invoked. The Kalman smoother provides the optimal trajectory by reasoning over all measurements in the past as well as the future. Like the Kalman filter, the Kalman smoother relies on a statistical object dynamical model and sensor measurement model; no further assumptions are invoked.

[0060] Statistical Object Classifier **107**

[0061] In this step of the process, motion tracking and image data for each particle type will be analyzed using one or more algorithms from the general class of "classification" algorithms. Examples may include, but are not limited to, algorithms for statistical classification, linear classifiers, support vector machines, quadratic classifiers, decision trees, supervised machine learning, unsupervised machine learning, or clustering. The outcome of this analysis will be a measurement of the statistical similarity between the tracking and image data for each particle observation from the sample and previously analyzed and stored image and tracking information corresponding to one or more specific particles types of interest when exposed to the same or a similar dielectrophoresis field. Based on the computed level of statistical

similarity, the system will determine if particles of the previously analyzed and stored types are present or not present in the sample.

[0062] The encoding of specific characteristics for particle motion tracking and image data particle type (known and unknown) will be recorded as a set of "features". Features may include, but are not be limited to:

[0063] 1. Morphological data such as size, shape and color of the particle

[0064] 2. Exterior texture or surface features of the particle

[0065] 3. Preferred orientation, rotational direction, and/or rotational speed induced on the particle when exposed to specific dielectrophoresis fields

[0066] 4. Translational motion characteristics including velocity and acceleration, measured and recorded and three dimensions.

[0067] Track classification will be based on analysis of kinematic and feature characteristics of individual tracks, with prior knowledge of the characteristics of all particle types of interest. Sample characterization will be based on aggregation of track classification information. It is important to note that the actual dielectrophoresis field configuration parameters are not explicitly encoded in the feature set. Instead, training data sets for a given cell type are obtained by exposing samples of known composition to a predefined sequence of fields. Subsequently, trajectories from an arbitrary mixture of cell types are scored with respect to each calibrated type.

[0068] FIG. **5** shows an example set of feature vectors from a hypothetical data-set for five particles. In general, there could be hundreds or thousands of particles in each data-set. Here each vector represents the measurements extracted by the image processing and tracking software. Diameter, color (in three primary channels) and X and Y velocity response is shown for two frequencies for five particles. By observation it can be seen that particle **1** and **3** are mostly of the same type since they are very similar for most of their parameter values. For real data sets there are many more particles with more parameters and more variations. Therefore statistical classification methods such as those listed above will be used to perform "best matching" to pre-characterized particle types. Thus the presence and population of various particle populations in a heterogeneous mixture can be identified.

[0069] More generally, the specific algorithm currently employed to statistically classify tracks is the generalized likelihood ratio test (GLRT). The calibration used equations are:

$$\mu_{i,j} = \frac{1}{N} \sum_{N_{tracks}} X_s \tag{4}$$

$$\sum_{i,j}^{2} = \frac{1}{N-1} \sum_{N_{tracks}} (X_s - \mu_{i,j})^2 \tag{5}$$

[0070] First, the hypothesis (H_i) that best explains the observations is generated by determining what the maximum-likelihood cell type is for each cell track. Then, the probability of that hypothesis is tested against the null hypothesis (H_0), that the tracking data could be better explained as resulting from a general type referred to as 'other', a type for which there is no corresponding model or training data available. H_0 must be sufficiently distinct from H_i in order to reasonably determine the cell type. A bounding region is introduced around H_i and the restriction $\Sigma > \Sigma_{min}$ is maintained so as to avoid degeneracy, as more likely hypotheses corresponding to type 'other' can always be generated.

$$p(z|H_i) = N(z; \mu_i, \Sigma_i) \tag{6}$$

$$p(z|H_0) = \max_{\mu \in U_i[\mu_i - \Delta\mu_i, \mu_i + \Delta], \Sigma > \Sigma_{min}} (N(z; \mu, \Sigma)) \tag{7}$$

[0071] Classification based on comparisons to calibrated training data, versus solely on models, is advantageous in real-world experimental settings where there are difficult to predict, but deterministic, factors to contend with such as AC electroosmosis and unknown medium conductivities. These factors cause significant deviations from model predictions. An additional advantage of GLRT in this application is that it provides accurate results without prior knowledge of the distribution of cell types in the mixture, which is precisely the parameter wished to be determined.

[0072] Apparatus Implementation

[0073] There are three major components used to realize the technology: a cartridge **54** that holds the dielectrophoresis electrodes **20** and microfluidics that deliver the sample, a configurable base station unit that contains electronics to generate the dielectrophoresis fields and capture images of the sample under test, and a collection of software modules to handle particle tracking and identification of targeted particles.

[0074] Sample Cartridges **54**

[0075] The electrode **20** arrangement used to exert dielectrophoresis forces is similar to the traveling-wave dielectrophoresis electrodes **20** shown in FIG. **2**. The electrodes **20** are arranged into a linear array **22** of equally spaced conductors. The electrode **20** width and gap spacing is determined by the size of the particles being manipulated. The electrode **20** gap spacing must be larger than the particle.

[0076] In order to manufacture the sample cartridge **54**, arrays of conductive electrodes **20** are photolithographically patterned on substrates. A design is employed in which traveling-wave dielectrophoresis electrodes **20** are positioned both above and below the object being manipulated (as shown in FIG. **6**). The phase gradients of the top and bottom electrode **20** chips are made to be identical. The electrode **20** arrangement of FIG. **6**, where electrodes **20** are positioned above and below the particles, has two effects that enhance particle manipulation:

[0077] 1) It increases the amount of lateral force exerted on a particle in a given direction, as the particle now experiences dielectrophoretic forces from both chips (F_{x1} and F_{x2}).

[0078] 2) This arrangement uses dielectrophoresis to stably position particles in the middle of the sample containment region (with respect to the vertical axis), since the vertical components of force exerted by both chips (F_{y1} and F_{y2}) oppose each other.

[0079] FIG. **7** shows an overhead view of the individual electrode **20** chips. Four electrical contact pads **50** are used to connect the electrodes **20** to four externally generated voltage signals. A two-layer metal process is used in the design so as to simplify the routing of electrodes **20**, making the number of contact pads **50** necessary equal to the number of phases required (in this case four phases, four contact pads **50**). A transparent substrate is used in fabrication so that the overhead view of the image capture portion is not obstructed during operation. The array **22** of dielectrophoresis electrodes

20 are contained in the center of the chip. Individual conductors as electrodes **20** are spaced at a fixed interval and connected in a repeating phase sequence, as shown by the zoomed in view of the electrode **20** array **22** in FIG. **8**. The connections of the conductors **53** from the pads **50** to the electrodes **20** in the array **22** are depicted in FIG. **2**.

[0080] FIG. **9***a* shows how the cartridge **54** is assembled after the fabrication step. Two electrode **20** chips are selected. One is designated as the bottom chip (electrode substrate #**1** of FIG. **9***a*) and the other the top chip (electrode substrate #**2** of FIG. **9***a*). The bottom chip **1** has holes **51** drilled through the center of each electrical contact pad **50**. The top chip **2** has two holes drilled through the top of it that are specifically designated to serve as fluid inlet/outlet ports **52**. These fluid ports **52** can be connected to tubing via syringes, thereby allowing for delivery of the sample containing particles to the region above the electrode array **22**. Placed in between the top and bottom chip is a patterned layer **53** of thin film. This patterned layer **53** acts as a spacer between the top and bottom chips and creates a containment region for the fluidic sample. The thickness of this layer determines the depth of the sample chamber. The shape/pattern of the cutout in this layer determines the overall volume of the sample chamber and also allows fluid flow to be directed to the region in between the top and bottom dielectrophoresis electrodes **20**. The three sample cartridge **54** components (top chip **2**, fluid spacer **53** and bottom chip **1**) are aligned and stacked on top of one another. In the final assembly step, an electrically conductive epoxy is filled into the electrical contact pad **53** holes of the bottom chip **1**. This step creates an electrical connection between the contact pads **50** of the bottom chip **1** and the contact pads **50** of the top chip **2**, ensuring that the voltage signals coming from the control electronics are driving the electrodes **20** on both chips. FIG. **9***b* shows the overhead view of the completed assembly.

[0081] Base Station **69**

[0082] The base station is a bench top or a field portable device that contains the dielectrophoresis field electronics and the optics needed to capture images of the particle motion.

[0083] FIG. **10** shows a block diagram representation of the base station electronics. A software interface **60** is used to program the apparatus **10** and signal generation logic. A multi-channel voltage waveform generator **62**, comprised of programmable logic and digital to analog converters, is used to generate voltage signals at the desired frequency and phase. The use of programmable logic in conjunction with digital to analog converters configuration allows for voltage waveforms of arbitrary shape, frequency and phase to be used as sources. The number of voltage channels required corresponds to the number of phases need (at least 2 phases/channels, typically 4 phases/channels). Next, the generated voltage waveforms are put through amplifiers **64** an amplification stage in order to set the appropriate voltage amplitude such that it can drive the electrode **20** array **22**. After amplification, the voltage sources **62** are put through a stage of impedance matching electronics **66**. The impedance matching stage is used to compensate for the uncertainty in the impedance of the electrode **20** array **22** once a fluidic sample has been injected into the cartridge **54**. When the apparatus **10** is in use, the packaged sample cartridge **54** of FIGS. **9***a* and **9***b* is inserted into the chip carrier **68** of FIG. **10** that is mounted on the host printed circuit board **79**. The carrier **68** makes a connection between the sample cartridge **54** electrical contact pads and the output channels of the impedance matching stage through the host printed circuit board **79**. Inserting the chip into the carrier **68** also by extension connects the individual electrodes **20** within the sample cartridge **54** to the voltage signals generated by the control electronics, thereby enabling dielectrophoresis fields to be created in the region of the sample cartridge **54** where the particle sample is contained.

[0084] The optical sub-system **70** comprises three major parts: a microscope objective **72** **72**, focusing tube **74** and a camera **76**. The optical sub-system **70** can be as simple as a fixed focus system that images an object that is exactly at the fixed working distance of the objective **72**. A small CCD or CMOS imaging chip captures images of the particles in motion. The sample can be illuminated with an illuminator **78** from directly above or underneath using mirrors placed below the assembly. A 3-axis positioner **80** is used to make minor alignment and focusing adjustments. This low cost imaging solution not only makes the entire base station **69** portable, but also minimizes power consumption. The complete base station **69** is shown in FIG. **11***a*.

[0085] Software Modules

[0086] The software analysis modules will run on either an end-user computer laptop **81** or mobile device, depending on the computational capabilities required. The software modules, as shown in FIG. **11***b*, include: 1) firmware **101** for the sensor platform and a user interface, 2) specific field sequences **103** for the target particle, 3) tracking and feature extraction software **105**, and 4) a classifier **107** trained for the target particle, all of which is stored in the memory **30** of the computer **81**.

[0087] Use Case Scenario

[0088] An example of the use of the technology to identify the composition of a sample containing theoretical particle types, particles of 'type A' and 'type B' is now described.

[0089] The user will first inject an aqueous sample containing particles into the sample cartridge **54**. The cartridge **54** is placed into the base station **69** where contact between the electrodes **20** and voltage sources **62** are made and the cartridge **54** is aligned for imaging. Initially particles are at rest between the top and bottom electrodes **20** of the dielectrophoresis electrode array **22** and are located at arbitrary positions (FIG. **12**).

[0090] The user, by way of software control, selects a targeted particle of interest. This selection initiates a frequency sweep of the voltage sources **62**. The sweep starts at a specified minimum frequency and is increased until it reaches a specified maximum frequency. The number of the frequency steps between the minimum and maximum frequency, and the duration each frequency step is applied is determined by the performance of the tracker/classifier software and the granularity desired by the user. For example, for a sample containing a mixture of live and dead yeast cells, applying a sequence of dielectrophoresis fields that ranges from a minimum frequency of 10 kHz to a maximum frequency of 10 MHz and extracting velocity features at the sample frequencies of {1 kHz, 10 kHz, 50 kHz, 100 kHz, 200 kHz, 300 kHz, 400 kHz, 500 kHz, 750 kHz, 1 MHz, 2 MHz, 3 MHz, 4 MHz, 5 MHz, 10 MHz}, enough velocity features will have been recorded so as to be able to distinguish the two particle types and determine their relative concentration.

[0091] During the frequency sweep, the particles in the sample will move according to the dielectrophoresis forces exerted on them at that particular frequency. This motion is

captured by the optical imaging sub-system **70** in the base station **69**. The resulting image frames are processed by the tracking software and the trajectories of each particle within the field of view, at each time step during the frequency sweep is recorded. FIG. **13** depicts the results from multi-target tracking of the imaged sample on the hardware platform. It can be seen in this example, that while particles of type A and type B travel in the same lateral direction, particles of type B on average travel at a much higher speed than those of type A, and can allow the particle types to be distinguished from one another.

[0092] By the completion of the frequency sweep sequence, a large volume of statistics will have been gathered about the behavior of the sample population. These statistics (features) include both motion characteristics and observed physical properties such as size, shape, and color. The software classifier, trained to a library of observed velocity of known particle types, will classify this data based on its correspondence to the trained response. This classification will provide a present/not present indication for the particle type(s) of interest (in this case live and dead yeast cells) and their relative concentration in the sample. For example, live yeast cells in a 5 mS solution, under the influence of dielectrophoresis electrodes in a traveling wave configuration that have an electrode gap size of 15 um, and driven by voltages with amplitudes of 2 Vpp, will have approximate velocities of {10 um/s, 250 um/s, 500 um/s, 1000 um/s, 700 um/s, 600 um/s 500 um/s 400 um/s 300 um/s 200 um/s 100 um/s 10 um/s −10 um/s −100 um/s −200 um/s −300 um/s −500 um/s} at the sampling frequencies listed in [0098]. For that same configuration, and at those same sampling frequencies, dead yeast cells will have approximate velocities of {0 um/s, 0 um/s, 5 um/s, 10 um/s, 5 um/s, −10 um/s −15 um/s −20 um/s −25 um/s −50 um/s −75 um/s −100 um/s −150 um/s −175 um/s −150 um/s −100 um/s −55 um/s}, allowing the two types to and their relative concentrations to be determined.

[0093] References, all of which are incorporated by reference herein.

[0094] 1. Pohl, Herbert A. "The Motion and Precipitation of Suspensoids in Divergent Electric Fields", J. Appl. Phys. 22, 869, 1951.

[0095] 2. Y Huang et al. "Differences in the AC electrodynamics of viable and non-viable yeast cells determined through combined dielectrophoresis and electrorotation studies." Phys. Med. Biol., Vol 37, No 7 1992.

[0096] 3. S. Coraluppi and C. Carthel, Modified Scoring in Multiple-Hypothesis Tracking, ISIF Journal of Advances in Information Fusion, vol. 7(2), pp. 153-164, December 2012.

[0097] 4. C. Carthel and S. Coraluppi, Detection and Multiple-Hypothesis Tracking of Cells and Nuclei, IEEE International Symposium on Biomedical Imaging—Cell Tracking Challenge Workshop, San Francisco Calif., USA, April 2013.

[0098] 5. C. Carthel and S. Coraluppi, Particle Tracking Workshop Methods and Results, IEEE International Symposium on Biomedical Imaging—Particle Tracking Challenge Workshop, Barcelona, Spain, May 2012.

[0099] Although the invention has been described in detail in the foregoing embodiments for the purpose of illustration, it is to be understood that such detail is solely for that purpose and that variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention except as it may be described by the following claims.

1. An apparatus for identifying objects in a plurality of objects comprising:

a portion which applies dielectrophoresis to the plurality of objects;

a portion which tracks the plurality of objects' reaction to the dielectrophoresis over time and extracts visible features about the plurality objects being tracked; and

a portion which automatically identifies the objects from the plurality of objects based on the objects' reaction to the dielectrophoresis over time and the visible features of the objects.

2. The apparatus of claim **1** wherein the portion which applies includes a plurality of dielectrophoresis electrodes.

3. The apparatus of claim **2** wherein the portion which applies includes a controller which causes dielectrophoresis fields to be generated by the electrodes.

4. The apparatus of claim **3** including a containment chamber in which the objects are disposed.

5. The apparatus of claim **4** wherein the chamber has inlet and outlet ports through which the objects are delivered to or removed from the chamber.

6. The apparatus of claim **5** wherein the portion which tracks includes voltage sources in communication with the controller that drive the electrodes.

7. The apparatus of claim **6** wherein each electrode is connected to the voltage sources via a programmable switching matrix that allows for any electrode to be connected to any of the voltage sources.

8. The apparatus of claim **7** wherein the portion which tracks includes a memory and an optical sub-system which takes images of the objects in the chamber over time and stores the images in the memory.

9. The apparatus of claim **8** wherein the optical sub-system includes a microscope objective, focusing tube and a camera.

10. The apparatus of claim **9** wherein the controller includes a computer programmed to use a multi-target multi hypothesis tracking algorithm to check the objects in the chamber.

11. The apparatus of claim **10** wherein the controller causes the electrodes to generate traveling-wave dielectrophoresis to induce motion in the objects.

12. The apparatus of claim **11** wherein the computer is programmed to use a statistical classification algorithm to determine a category or type in regard to the objects based on the images of the objects.

13. The apparatus of claim **12** wherein the statistical classification algorithm includes a general likelihood ratio test.

14. A method for identifying objects in a plurality of objects comprising the steps of:

applying dielectrophoresis to the plurality of objects;

tracking the plurality of objects' reaction to the dielectrophoresis over time and extracting visible features about the plurality objects being tracked; and

automatically identifying the objects from the plurality of objects based on the objects' reaction to the dielectrophoresis over time and the visible features of the objects.

15. The method of claim **14** including the step of introducing the objects into a chamber having dielectrophoresis electrodes.

16. The method of claim **15** including the step of initiating a frequency sweep by a controller of voltage sources causing the objects to move according to dielectrophoresis forces exerted on the objects at each frequency.

17. The method of claim **16** including the step of capturing image frames of the objects' motion during the frequency sweep with an optical imaging sub-system.

18. The method of claim **17** including the step of processing the image frames by a computer of the controller with tracking software stored in a memory to record in the memory trajectories of each object within the image frames at each time step during the frequency sweep.

19. The method of claim **18** including the step of classifying the objects based on the trajectories of the objects from the images stored in the memory.

20. A dielectrophoresis cartridge for holding objects comprising:

a first substrate having dielectrophoresis electrodes;

a microfluidic containment layer in which the objects are disposed; and

a second substrate having dielectrophoresis electrodes, the layer disposed between the first and second substrates.

21. The cartridge of claim **19** wherein the second layer has inlet and outlet ports through which the objects are introduced to and removed from the layer.

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