Apparatuses and Methods for Detecting Molecules and Binding Energy

Abstract: The present disclosure provides apparatuses and methods for analyzing the presence of charged analytes and/or the binding force between charged analytes and a capture probe. The apparatuses and methods of the present disclosure can be operated in a multiplexed format to perform various assays of clinical significance for example.
— before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))
APPARATUSES AND METHODS FOR DETECTING MOLECULES AND BINDING ENERGY

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Patent Application Serial Number 62/14,474, filed February 10, 2015, which is incorporated herein by reference in its entirety.

BACKGROUND

[0002] Various types of molecules can recognize other molecules by the formation of multiple (numbers and/or types of) non-covalent bonds (e.g., van der Waals, hydrogen bonds, coulombic attractions and hydrophobic bonds), which can be combined in a particular spatial orientation. These molecular recognition interactions can be reversible and of moderate to high specificity. Examples of such molecular interactions include antibody-antigen interactions and nucleic acid hybridizations. Such interactions are important for biology and several mature and developing industries, such as medical diagnostics, therapeutics, biotechnology, agriculture, fuel and chemical production, defense, environmental monitoring, food and food safety to name a few.

SUMMARY

[0003] Recognized herein is the need for new and improved sensors and methods for detecting the presence of molecular interactions and for measuring the force associated with molecular interactions. The present disclosure provides such sensors and methods for using the sensors. Without limitation, the apparatus and methods of the present disclosure have advantages in differentiating between specific (e.g., strong) and non-specific (e.g., weak) molecular interactions and therefore, can distinguish between false positive and true positive signals. The present apparatuses and methods may be suitable for multiplexing (e.g., performing 5, 10, 10², 10³, 10⁴, 10⁵, 10⁶ or more measurements in parallel) and directly using complex mixtures having two or more analytes such as biological fluids (e.g., blood) or environmental samples. In some embodiments, the apparatuses and methods described herein can be used to measure the force associated with molecular interactions, spanning multiple orders of magnitude in strength (e.g., between about 1 piconewton (pN) and about 400 pN).

[0004] In an aspect, a method for detecting a presence of a target analyte comprises (a) activating at least one sensor comprising one or more surfaces between two or more electrodes, wherein the one or more surfaces comprise two or more immobilized capture probes; (b) bringing the one or more surfaces in contact with a solution containing or suspected of containing a target analyte and a non-target analyte, under conditions sufficient to permit the target analyte and non-
target analyte to each bind to a given one of the two or more immobilized capture probes; (c) applying a voltage across the two or more electrodes that is sufficient to release the non-target analyte, but not the target analyte, from a given one of the two or more immobilized capture probes; and (d) detecting a signal indicative of a presence and/or binding energy of the target analyte on the one or more surfaces. In some embodiments, the target analyte and/or the non-target analyte is a charged analyte. In some embodiments, the voltage is sufficient to exert an applied force of at least about 1 piconewton (pN) on the non-target analyte. In some embodiments, the applied force is at least about 10 pN. In some embodiments, the voltage is sufficient to generate an electric field having a strength of at least about $10^3$ volts per meter.

[0005] In some embodiments, the method further comprises applying an additional voltage across the two or more electrodes that is sufficient to release the target analyte from a given one of the two or more immobilized capture probes. In some embodiments, the additional voltage is sufficient to exert an applied force of at least about 1 pN on the target analyte. In some embodiments, the applied force is at least about 10 pN. In some embodiments, the method further comprises detecting the presence of the target analyte on the one or more surfaces subsequent to application of the additional voltage. In some embodiments, the voltage is less than the additional voltage. In some embodiments, the voltage and the additional voltage are individual voltages of a continuously applied voltage that is changed over time. In some embodiments, the additional voltage is applied in the absence of washing the one or more surfaces.

[0006] In some embodiments, the applied voltage is sufficient to generate an electric field having a strength of less than about $10^9$ volts per meter (V/m) and the additional applied voltage is sufficient to generate an additional electric field having a strength of less than about $10^9$ V/m.

[0007] In some embodiments, each of the target analyte and non-target analyte non-covalently binds to a given one of the two or more immobilized capture probes to form a target probe-analyte complex immobilized and a non-target probe-analyte complex immobilized on the one or more surfaces, respectively.

[0008] In some embodiments, the applied voltage is increased from a first voltage to a second voltage over time. In some embodiments, the applied voltage is increased from the first voltage to the second voltage at a rate of at least about 1 millivolt per second. In some embodiments, the applied voltage is increased from the first voltage to the second voltage over a period of time greater than about 10 microseconds. In some embodiments, the applied voltage is increased from the first voltage to the second voltage over a period of time less than about 1 second. In some embodiments, the periodic voltage waveform has a frequency of less than about 1 gigahertz. In some embodiments, the periodic voltage waveform has a frequency of greater than about 1 millihertz.
In some embodiments, the method further comprises monitoring a signal indicative of the presence of the target analyte on the one or more surfaces as the applied voltage is varied over time. In some embodiments, the method further comprises determining a binding force of the target analyte bound to a given one of the two or more immobilized capture probes.

In some embodiments, the applied force is less than a binding force of the target analyte bound to a given one of the two or more immobilized capture probes.

In some embodiments, the solution comprises charged molecules that bind with the target analyte and/or the non-target analyte, to provide a change in charge on the target analyte and/or non-target analyte. In some embodiments, the charged molecules are antibodies. In some embodiments, (c) and/or (d) is performed in the absence of washing the one or more surfaces.

In some embodiments, the presence of the target analyte on the one or more surfaces is detected by measuring a signal associated with the target analyte. In some embodiments, the signal is a charge signal. In some embodiments, the charge signal is detected using a sensing electrode and a reference electrode. In some embodiments, the charge signal is a voltage. In some embodiments, the signal indicative of the presence and/or binding energy of the target analyte on the one or more surfaces is detected by measuring an optical signal associated with the target analyte. In some embodiments, the optical signal is a fluorescence signal. In some embodiments, the fluorescence signal is provided by a fluorescent probe covalently or non-covalently attached to the target analyte. In some embodiments, the signal indicative of the presence and/or binding energy of the target analyte on the one or more surfaces is detected using surface plasmon resonance.

In some embodiments, the one or more surfaces is substantially planar. In some embodiments, the one or more surfaces comprise an electrically insulating layer and an electrically conducting layer. In some embodiments, the electrically conducting layer is in communication with the solution. In some embodiments, the electrically conducting layer is electrically isolated from the solution and the electrically conducting layer is electrically biased. In some embodiments, the electrically conducting layer comprises platinum. In some embodiments, the one or more surfaces comprise a membrane having a thickness of between about 0.08 nanometers and 1 millimeter. In some embodiments, the one or more surfaces comprise a field confining feature that concentrates an electric field to a strength of at least about $10^3$ volts per meter. In some embodiments, the field confining feature comprises an orifice in the one or more surfaces, which one or more surfaces comprise an electrically insulating layer and an electrically conducting layer.

In some embodiments, the two or more immobilized capture probes are nucleic acid molecules that hybridize with the target analyte. In some embodiments, the target analyte is a
nucleic acid molecule. In some embodiments, the two or more immobilized capture probes comprise antibodies that bind the target analyte. In some embodiments, the target analyte is a protein.

[0015] In some embodiments, the one or more surfaces include a plurality of surfaces. In some embodiments, each of the plurality of surfaces comprises a subset of the two or more immobilized capture probes.

[0016] In some embodiments, the voltage is a direct current voltage. In some embodiments, the voltage is an alternating current voltage.

[0017] In another aspect, a system for detecting a presence of a target analyte comprises at least one sensor comprising one or more surfaces between two or more electrodes, wherein the one or more surfaces comprise two or more immobilized capture probes; a solution chamber in fluid communication with the one or more surfaces, wherein the solution chamber is configured to retain a solution containing or suspected of containing the target analyte and a non-target analyte, under conditions sufficient to permit the target analyte and non-target analyte to each bind to a given one of the two or more immobilized capture probes; and a controller that is operably coupled to the two or more electrodes, wherein the controller is programmed to (i) apply a voltage across the two or more electrodes that is sufficient to release the non-target analyte, but not the target analyte, from a given one of the two or more immobilized capture probes, and (ii) detect a signal indicative of a presence and/or binding energy of the target analyte on the one or more surfaces. In some embodiments, the target analyte and/or the non-target analyte is a charged analyte. In some embodiments, the voltage across the two or more electrodes is sufficient to exert an applied force of at least 1 piconewton on the non-target analyte.

[0018] In some embodiments, the at least one sensor comprises an array of sensors. In some embodiments, the array of sensors comprises at least about 10 sensors. In some embodiments, each sensor of the array of sensors is independently addressable.

[0019] In another aspect, an apparatus for detecting a presence of an analyte comprises a fluidic chamber adapted to contain an electrolyte; two or more electrodes capable of generating an electric field within the fluidic chamber; at least one surface comprising an immobilized capture probe capable of binding the analyte, wherein the at least one surface is between the two or more electrodes and in contact with the fluidic chamber, and wherein the at least one surface comprises an electrically conducting layer and an electrically insulating layer; and at least one field confining feature proximal to the at least one surface, which at least one field confining feature is capable of concentrating an electric field surrounding the at least one field confining feature to a strength of at least about $10^3$ volts per meter (V/m). In some embodiments, the analyte is a charged analyte. In some embodiments, the at least one field confining feature is
capable of concentrating the electric field surrounding the at least one field confining feature to a strength of less than about 10^5 V/m. In some embodiments, the apparatus further comprises a controller operably coupled to the two or more electrodes, wherein the controller is programmed to apply a voltage across the two or more electrodes that is sufficient to generate an applied force on the analyte. In some embodiments, the voltage applied is sufficient to generate an applied force of at least about 1 piconewton (pN) on the analyte. In some embodiments, the voltage is sufficient to generate an applied force of at least about 20 pN on the analyte.

[0020] In some embodiments, the immobilized capture probe is capable of binding to the analyte to form a probe-analyte complex on the at least one surface. In some embodiments, the immobilized capture probe is capable of binding to the analyte via non-covalent interaction(s). In some embodiments, the non-covalent interaction(s) are disruptable through an applied force generated upon the application of a voltage across the two or more electrodes. In some embodiments, the immobilized capture probe is proximal to the at least one field confining feature. In some embodiments, the at least one field confining feature is a plurality of field confining features.

[0021] In some embodiments, the immobilized capture probe is an antibody. In some embodiments, the analyte is an antigen. In some embodiments, the immobilized capture probe is a nucleic acid molecule. In some embodiments, the analyte is a nucleic acid molecule.

[0022] In some embodiments, the analyte has a charge of less than about 10^4 e^−. In some embodiments, the analyte has a charge of greater than about 10 e^−. In some embodiments, the apparatus further comprises a controller operably coupled to the two or more electrodes, wherein the controller is programmed to apply a potential difference of less than about 100 volts (V) between the two or more electrodes.

[0023] In some embodiments, the at least one surface provides a wall of the fluidic chamber and the field confining features is an orifice in the surface. In some embodiments, the field confining feature comprises an orifice in the at least one surface. In some embodiments, the fluidic chamber comprises a top portion and a bottom portion partitioned by the at least one surface, and wherein the field confining feature is an orifice in the at least one surface. In some embodiments, the orifice extends through the surface. In some embodiments, the orifice is an indentation, a well, a pore, a channel, a gap, or a slit. In some embodiments, the at least one field confining feature has a diameter of less than about 50 micrometers. In some embodiments, the at least one field confining feature has a diameter of less than about 50 nanometers. In some embodiments, the field confining feature has an aspect ratio of at least about 0.1. In some embodiments, the aspect ratio is the ratio of the longest dimension of the field confining feature to the shortest dimension of the field confining feature. In some embodiments, the aspect ratio is
the ratio of the width of the field confining feature to the depth of the field confining feature. In some embodiments, the field confining feature has a sharp edge. In some embodiments, the field confining feature is an elevated portion of the at least one surface.

[0024] In some embodiments, the electrically conducting layer is in communication with the electrolyte. In some embodiments, the electrically conducting layer is electrically isolated from the electrolyte, and wherein, during use, the electrically conducting layer is electrically biased. In some embodiments, the electrically conducting layer comprises platinum. In some embodiments, the apparatus further comprises a sensing electrode configured to measure a charge signal associated with the presence of the analyte.

[0025] In some embodiments, the sensing electrode is proximal to the at least one field confining feature. In some embodiments, the apparatus further comprises a light source and a detector configured to detect an optical signal associated with the presence of the analyte. In some embodiments, the optical signal is a fluorescence signal. In some embodiments, the fluorescence signal is provided by a fluorescent probe covalently or non-covalently attached to the analyte. In some embodiments, the apparatus further comprises a detector configured to detect a surface plasmon resonance signal associated with the presence of the analyte.

[0026] In some embodiments, the at least one surface is substantially planar.

[0027] In some embodiments, the at least one surface is part of an array of sensors, wherein each sensor of the array comprises a field confining feature and a plurality of immobilized capture probes. In some embodiments, the plurality of immobilized capture probes of a given sensor of the array are proximal to the field confining feature for the given sensor of the array. In some embodiments, the plurality of immobilized capture probes of a given sensor of the array is clonal. In some embodiments, a given sensor of the array comprises a plurality of immobilized capture probes that is unique relative to another sensor of the array. In some embodiments, a distance between a given sensor of the array and a nearest neighbor sensor is at least about 50 nanometers (nm). In some embodiments, the distance between the given sensor of the array and the nearest neighbor sensor is at least about 150 nm. In some embodiments, a distance between a given sensor of the array and a nearest neighbor sensor is less than about 50 micrometers. In some embodiments, the array comprises at least about 10 sensors.

[0028] In another aspect, a method for analyzing a binding energy between a target analyte and a capture probe comprises (a) activating at least one sensor comprising one or more surfaces between two or more electrodes, wherein the one or more surfaces comprises an immobilized capture probe; (b) bringing the one or more surfaces in contact with a solution containing or suspected of containing a target analyte under conditions sufficient to permit the target analyte to bind to the immobilized capture probe; (c) applying a voltage across the two or more electrodes
that is sufficient to exert an applied force of at least about 1 piconewton (pN) on the target analyte to release the target analyte form the immobilized capture probe; and (d) detecting a signal indicative of the binding energy between the target analyte and the capture probe. In some embodiments, the applied force is at least about 10 pN. In some embodiments, the voltage is sufficient to release the target analyte, but not a non-target analyte, from the one or more surfaces. In some embodiments, the voltage is sufficient to generate an electric field having a strength of at least about $10^3$ volts per meter.

[0029] Another aspect of the present disclosure provides a computer-readable medium comprising machine executable code that, upon execution by one or more computer processors, implements any of the methods above or elsewhere herein.

[0030] Another aspect of the present disclosure provides a computer system comprising one or more computer processors and computer memory coupled thereto. The computer memory comprises machine executable code that, upon execution by the one or more computer processors, implements any of the methods above or elsewhere herein.

[0031] Additional aspects and advantages of the present disclosure will become readily apparent to those skilled in this art from the following detailed description, wherein only illustrative embodiments of the present disclosure are shown and described. As will be realized, the present disclosure is capable of other and different embodiments, and its several details are capable of modifications in various obvious respects, all without departing from the disclosure. Accordingly, the drawings and description are to be regarded as illustrative in nature, and not as restrictive.

**INCORPORATION BY REFERENCE**

[0032] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

**BRIEF DESCRIPTION OF THE DRAWINGS**

[0033] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings or figures (also "FIG." and "FIGs." herein), of which:

[0034] **FIG. 1A** shows an example of a cross-sectional profile view of an apparatus of the present disclosure; **FIG. 1B** shows an example of a cross-sectional profile view of an apparatus of the present disclosure;
FIG. 2A shows an example of the effect of sensor array size on electric field strength; FIG. 2B shows an example of the effect of aspect ratio on the inter-sensor variation in electric field strength;

FIG. 3 shows an example of the electric field produced without an electrically conducting layer;

FIG. 4 shows an example of the electric field produced with an electrically conducting layer (e.g., field terminating plane);

FIG. 5 shows an example of an apparatus of the present disclosure adapted for optical detection of charged analytes;

FIG. 6A shows an example of the magnitude of the fluorescent signal as a function of applied voltage for operation of the apparatus of FIG. 5; FIG. 6B shows an example of the operation of the apparatus of FIG. 5 at a first voltage; FIG. 6C shows an example of the operation of the apparatus of FIG. 5 at a second voltage; FIG. 6D shows an example of the operation of the apparatus of FIG. 5 at a third voltage; FIG. 6E shows an example of the operation of the apparatus of FIG. 5 at a fourth voltage;

FIG. 7 shows an example of an apparatus of the present disclosure adapted for electrical detection of charged analytes;

FIG. 8A shows an example of the magnitude of sensor voltage as a function of applied voltage for operation of the apparatus of FIG. 7; FIG. 8B shows an example of the operation of the apparatus of FIG. 7 at a first voltage; FIG. 8C shows an example of the operation of the apparatus of FIG. 7 at a second voltage; FIG. 8D shows an example of the operation of the apparatus of FIG. 7 at a third voltage; FIG. 8E shows an example of the operation of the apparatus of FIG. 7 at a fourth voltage;

FIG. 9 shows an example of an apparatus of the present disclosure adapted for plasmonic detection of charged analytes;

FIG. 10 shows an example of an array of the present disclosure for sensing the presence and binding force of nucleic acid molecules;

FIG. 11A shows an example of the method of the present disclosure being used to measure the binding force of a complimentary nucleic acid molecule; FIG. 11B continues from FIG. 11A and shows an example of the method of the present disclosure being used to measure the binding force of a complimentary nucleic acid molecule;

FIG. 12A shows an example of the method of the present disclosure being used to measure the binding force of a nucleic acid molecule having a single base pair mismatch to the capture probe; FIG. 12B continues from FIG. 12A and shows an example of the method of the
present disclosure being used to measure the binding force of a nucleic acid molecule having a
single base pair mismatch to the capture probe;

[0046] FIG. 13A shows an example of the method of the present disclosure being used to
measure the binding force of a mixture of nucleic acid molecules having various amounts of
complimentanty to the capture probe; FIG. 13B continues from FIG. 13A and shows an example
of the method of the present disclosure being used to measure the binding force of a mixture of
nucleic acid molecules having various amounts of complimentanty to the capture probe;

[0047] FIG. 14 shows an example of a multiplexed immunoassay where secondary antibodies
have an attached nucleic acid to increase and control the amount of charge associated with the
analyte;

[0048] FIG. 15 shows an example of performing the method of the present disclosure using
the apparatus shown in FIG. 14;

[0049] FIG. 16 shows an example of the attachment of a nucleic acid molecule to a surface of
the present disclosure;

[0050] FIG. 17 shows an example of an apparatus of the present disclosure; and

[0051] FIG. 18 shows an example of a computer system for operation of the apparatus of the
present disclosure.

DETAILED DESCRIPTION

[0052] While various embodiments of the invention have been shown and described herein, it
will be obvious to those skilled in the art that such embodiments are provided by way of example
only. Numerous variations, changes, and substitutions may occur to those skilled in the art
without departing from the invention. It should be understood that various alternatives to the
embodiments of the invention described herein may be employed.

[0053] An externally applied electric field in an aqueous solution can apply a force on a
charged particle (e.g., molecule or analyte) in the solution. Such force on the charged particle \( F \)
can be proportional to the applied electric field \( E \) and the net electric charge of the particle \( q \)
according to the equation \( F = qE \).

[0054] Using the linear relation among these parameters, the present method correlates the
applied force to the input parameters (e.g., applied external biases). Provided herein is an
apparatus that may utilize such force to perform a force spectroscopy and force differentiation
assay. The assay can be performed on antigen-antibody bonds or used for nucleic acid analysis
(e.g., single nucleotide polymorphism (SNP) analysis). In some cases, the binding force between
the analyte and a capture probe can be from about 1 piconewton (pN) to about 400 pN.
Given an example of a charged particle having a charge of 100e- (e.g., equivalent to a nucleic acid with 100 bases), the external electric field strength used to disrupt some bonds of interest are shown as follows in Table 1.

**Table 1: Electric field needed to disrupt bonds of interest (assuming 100e- of charge on the labeling molecule).**

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Force [pN]</th>
<th>Efield needed [V/m]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptavidin Biotin</td>
<td>141</td>
<td>8.8E+06</td>
</tr>
<tr>
<td>14mer SNP (CCAAACCAACCACC)</td>
<td>178</td>
<td>1.1E+07</td>
</tr>
<tr>
<td>14mer SNP 1 base change 8th C to G</td>
<td>130</td>
<td>8.1E+06</td>
</tr>
<tr>
<td>14mer SNP 1 base change 8th C to A</td>
<td>133</td>
<td>8.3E+06</td>
</tr>
<tr>
<td>Biotin-Antibiotin</td>
<td>112</td>
<td>7.0E+06</td>
</tr>
<tr>
<td>Ferritin-Antiferritin</td>
<td>49</td>
<td>3.1E+06</td>
</tr>
</tbody>
</table>

The field strengths needed may be relatively large, which may be difficult to generate in an open structure. Therefore, the present apparatus employs (physical) confinement using (micro or nano-scale) structures to significantly boost the applied electric field strength. These structures are referred to herein as "field confinement features" and can include pores, channels, gaps and slits (e.g., orifices), as well as raised structures associated with a sensing surface. Nanopores are a type of field confinement feature which can physically confine translocation of molecules, enabling single molecule detection via blocking of the nanopore.

In some cases, an advantage of such physical confinement is the electrical confinement that comes with it. When designed with the orifice being the smallest feature, the orifice can dominate the electrical characteristics of the entire system. The potential drop (thus the electric field) can occur primarily near the orifice and less (to very little) elsewhere. When the orifice is small (e.g., micro or nano scale) such confinement can generate a large electric field with relatively low applied bias. For example, a synthetic nanopore on a 100 nm thick SiNx membrane can generate about 10,000,000 V/m of electric field across it with application of 1 V. The present disclosure encompasses any size, shape or arrangement of field confining features (e.g., orifices), including the embodiments shown in FIG. 1A and FIG. 1B.

By preparing a group of recognition molecules (e.g., capture probes) in advance, the present method can look for the presence of their respective target molecules (e.g., analytes) in a sample by observing the quantity and force by which the analytes bind to the capture probes. The present method of analyzing such molecular recognition (e.g., binding) events can be referred to as a binding assay. Labeled (or labeling) agents can be used for detection of such molecular recognition events. Non-specific binding may present a challenge in existing binding assays.
Other limitations of existing binding assays include an inability to multiplex the assay, inability to perform the assay on a micro or nano-scale (e.g., be densely integrated), and inability to generate sufficient forces.


Various types of cross-reactivity may occur in binding assays, FIG. 14 shows some examples. Given a variety of labels or detection probes, an incorrect label or detection probe can bind to the target in addition to a correct label or detection probe, 1410. An incorrect label or
detection probe can bind to the capture probe, 1413. An incorrect label or detection probe can bind to the correct label or detection probe bound to the correct target, 1411. An incorrect label or detection probe-target compound can bind to the correct probe, 1414. An incorrect target can bind to the correct capture probe which has the correct target and correct label or detection probe bound, 1412. An incorrect label or detection probe can be gravitationally or coulombically adsorbed to the detecting surface in absence of any probes. Each of these events can cause an excess of labels or probes to remain on an assay surface resulting in false positives results. Such false positive results can be reduced by the apparatus and methods of the present disclosure, e.g., by applying a force to a surface that is sufficient to remove undesirable labels or probes or targets and/or by having an apriori expectation of the binding force of the capture probe-target-label/detection probe or probe-target complex and correspondingly measuring the force required to break the complex in comparison with the expected binding force.

[0061] In an aspect, the present disclosure describes a method for measuring intermolecular interactions, including but not limited to receptor/ligand, protein/protein, nucleic acid/protein and nucleic acid/nucleic acid interactions. A method for measuring intermolecular interactions can involve the simultaneous use of (1) a sensor for detecting the attachment of target molecules in solution to their specific, complementary or near complementary capture molecule bound to a surface (e.g., the hybridization of a nucleic acid in solution to a complementary or near complementary nucleic acid capture probe on the surface) and (2) the use an electric field to apply an electrokinetic force on the bound molecule complex (e.g., a target molecule attached or bound to a capture molecule). When the electrokinetic force exceeds the attachment force of the bound molecule complex, the target molecules can become detached from the capture molecule. This can occur because the attachment force of the capture molecule to the surface can far exceed the attachment force of the target molecule to the capture probe. The electrokinetic force applied by the electric field is such that molecules can be pulled away from the sensor. An electric field may be generated by applying a voltage on two opposing electrodes on the surface of the sensor and device or in solution or a combination. This target molecule may have sufficient charge to create a high enough electrokinetic force. In some cases, the target molecule can be modified with additional charge to create a higher electrokinetic force on the target molecule. As an example, target nucleic acid molecules can be modified during polymerase chain reaction (PCR) using primers comprising a polyanionic polymer. In the case of immunoassays, a secondary antibody or detection antibody can be labeled with nucleic acid or a polyanionic polymer to enable the application of an electrokinetic force on the antibody in the case that the target molecule has insufficient charge.
The present disclosure also provides several apparatuses capable of employing this method, including an all-electronic platform. In an aspect, the present disclosure provides an apparatus for detecting a charged analyte. The apparatus can comprise a fluidic chamber adapted to contain an electrolyte. The apparatus can further include two or more electrodes, such as a first electrode and a second electrode, capable of providing an electric field within the electrolyte and a surface located between the first electrode and the second electrode and in contact with the fluidic chamber. The surface can include an electrically conducting layer and an electrically insulating layer. There can be a field confining feature located in proximity to the surface. The field confining feature can concentrate the electric field to a strength of at least about $10^3$ volts per meter (V/m), at least about $10^4$ V/m, at least about $10^5$ V/m, at least about $10^6$ V/m, at least about $10^7$ V/m, at least about $10^8$ V/m, at least about $10^9$ V/m or greater in proximity to the field confining feature.

In another aspect, the present disclosure provides a method for detecting binding of a charged analyte. The method can include providing a device having two or more electrodes, such as a pair of electrodes, and a surface between the pair of electrodes. The surface can have a capture probe tethered to a surface at a sensing location. The method can further include contacting the surface with a mixture of charged analytes, where the mixture comprises a target charged analyte and a non-target charged analyte. The charged analytes can form non-covalent bonds with the capture probe. The method can further comprise applying a first voltage across the pair of electrodes, which first voltage results in a first electric field that exerts a first applied force of at least 1 piconewton (pN) on the charged analytes that are bound to the capture probe, thereby breaking non-covalent bonds between the capture probe and the non-target charged analyte. The method can then include detecting the presence of the target charged analyte at the sensing location. In some cases, the first electric field has a strength of at least about $10^3$ volts per meter (V/m), at least about $10^4$ V/m, at least about $10^5$ V/m, at least about $10^6$ V/m, at least about $10^7$ V/m, at least about $10^8$ V/m, at least about $10^9$ V/m or greater in proximity to the sensing location.

In some cases, the method can further comprise applying a second voltage across the pair of electrodes, which second voltage results in a second electric field that exerts a second applied force of greater than 1 pN on the charged analytes that are bound to the capture probe, thereby breaking non-covalent bonds between the capture probe and the target charged analyte. The method can include detecting the absence of the target charged analyte at the sensing location.

Turning now to the figures, FIG. 1A shows an example of a cross-sectional profile view of an apparatus of the present disclosure. The apparatus can include a fluidic chamber...
adapted to contain an electrolyte 101. A first electrode 102 can be located in the electrolyte and form a circuit with a second electrode 103, across which electrodes a voltage can be applied by a voltage source 104. In this case, the second electrode forms one of the walls of the fluidic chamber. In some cases (e.g., see FIG. 1B), the fluidic chamber is bisected by a membrane and the first and second electrodes are on opposing sides of the membrane. A surface is laid in proximity to or upon the second electrode in this embodiment. The surface can include an electrically insulating layer 105 and an electrically conducting layer 106. In some cases, the electrically conducting layer is exposed to the electrolyte 101. The surface can have field confinement features 107 in proximity to the surface (e.g., within or upon the surface). The field confinement features can increase the magnitude of an applied electric field in proximity to the field confinement features.

[0066] The apparatus can use an electric field to draw an anchored capture probe and its attached target into a pore to detect the total charge using an auxiliary sensor. The difference in charge between the capture molecules in their non-hybridized (e.g., not bound to target) and hybridized states (e.g., bound to target) can be used to determine the presence of bound molecules. This architecture can be well suited for probing the binding energy of a molecule pair. While measuring the presence of the molecule pair under the influence of an electric field, the electric field can be gradually increased until the electrokinetic force exceeds the binding energy of the molecule pair. Once the target molecule detaches, a change in charge in the sensing area can be detected and that change can be used to determine the binding voltage of the pair.

[0067] FIG. 1B shows an example of a cross-sectional profile view of an apparatus of the present disclosure where the sensor surface bisects the fluidic chamber. The fluidic chamber of FIG. 1A and FIG. 1B can be a microfluidic chamber (e.g., having a smallest dimension of less than about 1 millimeter). As with FIG. 1A, the apparatus can include an electrolyte 101, a first electrode 102, a second electrode 103, a voltage source 104, an electrically insulating layer 105, an electrically conducting layer 106 and a field confinement feature 107.

[0068] Various factors may affect the degree of confinement of a confinement feature (e.g., orifice). The design of the confinement features, such as aspect ratio and density of features, may affect the degree of confinement. Higher aspect ratios may help to reduce the effects of a fringing field. For dense packing of the confining features, the overlap in fringing field can reduce the effect of field confinement. In some cases, the overlapping of fringing field between features can be reduced by providing a region for the fields to terminate before overlapping, such as a conducting surface near the confinement feature. Such a surface can effectively pin the potential near the orifice to allow for further enhancement of the electric field and thus the generated electrokinetic force. An in silico study of the effect of such a field terminating location along
with an investigation into the effects of feature aspect ratio is shown herein. The presence of a conductive surface layer may both enhance single feature's ability to confine the electric field and be very effective against countering the reduction from integration of confinement features into an array.

[0069] As described herein, the size, shape, arrangement and number of field confinement features can have an effect on the magnitude of the electric field created in proximity to the field confinement features and the amount of force that can be applied to the charged analyte. FIG. 2A shows an example of the effect of sensor array size on electric field strength. As seen here, the electrically conductive layer (also referred to as the potential pinning or field terminating layer/plane) may help to create a stronger electric field, particularly as the number of sensing locations (e.g., wells) increases. The electric field in this case may be relatively constant at about 1.5 x 10^6 V/m when an electrically conducting layer is used 201, while the electric field in the absence of the conducting layer 202 may be both lower in magnitude and decreases as the well array size increases. The horizontal axis is number of sensing locations on a side of a square array (e.g., a single location, a 3X3 array, a 5X5 array and a 7X7 array). The electric fields depicted in FIG. 2A, FIG. 2B, FIG. 3 and FIG. 4 are achieved with confinement features having a diameter of 1 µm and spaced 4 µm apart.

[0070] FIG. 2B shows an example of the effect of aspect ratio on the inter-sensor variation in electric field strength. The system architecture is an array of wells as shown in FIG. 1A, where the aspect ratio is defined as the width of the well (e.g., along the surface) divided by the depth of the well (e.g., into the surface). As shown, the electric field strength variation (as measured in %) 203 increases with increasing aspect ratio. The electric fields at the bottoms of the microwells are compared to determine the variation. In this example, the variation in electric field approaches 0.0% (e.g., all sensing locations have the same field) at an aspect ratio of 1:1. In some embodiments, the aspect ratio is less than about 5:1, less than about 4:1, less than about 3:1, less than about 2:1, less than about 1:1, or less than about 1:2.

[0071] The effect of the field terminating layer is shown graphically in FIG. 3 and FIG. 4. Referring to FIG. 3, the system includes an insulating layer 301, an electrolyte 302, and a first electrode 303. A voltage can be applied between the first electrode and second electrode (not shown) to create an electric field that is concentrated in the field confinement features (wells in this case). FIG. 3 shows a series of three profile views of sensor arrays. The magnitude of the electric field decreases as the number of wells increases from a single well 304, to a 3X3 array of 9 wells 305, to a 7X7 array of 49 wells 306.

[0072] FIG. 4 shows a system similar to FIG. 3 including an insulating layer 301, an electrolyte 302, and a first electrode 303. The system also includes an electrically conducting
layer 400 disposed upon the insulating layer. In comparison to FIG. 3 without the electrically conducting layer, the magnitude of the electric field is both stronger and does not diminish with increasing array size. The magnitude of the electric field is shown with shading (darker being stronger) for a single sensor 401, a 3X3 array 402 and a 7X7 array 403.

[0073] In some embodiments, the applied electrokinetic force on the charged analyte ranges from 0 pN to 5000 pN. In some instances, the electric field confining feature (e.g., an orifice) and/or conducting potential pinning plane can be used to enhance the electrokinetic force. In some cases, the applied force is sufficient to dissociate the analytes from the capture probes (e.g., receptor/ligand, protein/protein, nucleic acid/protein or nucleic acid/nucleic acid interactions) sequentially (e.g., in the order of their binding strengths). The method can be able to judge whether molecules are correctly (specifically) or incorrectly (non-specifically) bound to their intended targets.

[0074] In some embodiments, the apparatus and methods of the present disclosure are used to detect and probe the binding force of a single nucleotide polymorphism (SNP) or to analyze immunoassays (e.g., sandwich immunoassays). In some cases, more than one sensor can be integrated in a single chip to allow for parallel analysis of SNPs, immunoassay and sandwich immunoassays by themselves or simultaneously.

[0075] The methods of the present disclosure can involve detecting charged analytes by any suitable approach, such as, for example, optical detection, electrical or electrostatic detection, or plasmonics detection.

[0076] FIG. 5 shows an example of an apparatus of the present disclosure adapted for optical detection of charged analytes (e.g., using a modified fluorescence based DNA microarray). The apparatus can include a top substrate 501 and a bottom substrate 515 (e.g., which can be an optically transparent material), as well as fluidic walls 518 that define a fluidic chamber. In some cases, the chamber is microfluidic (e.g., having micrometer dimensions) and contains an electrolyte 502. A voltage source 523 can be connected by electrical connectors 520 to a first electrode 522 and a second electrode 506. The electrode can be overlaid by a surface having an insulating layer 516 and a conducting layer 517. The surface can be interrupted by a plurality of field confinement features, which in this embodiment are clustered into a first sensing location 507 and a second sensing location 514, which sensing locations are pixels in the optical detection system shown here. The optical detection system can include a light source 512, an excitation filter 511, a dichroic mirror 509 and an emission filter 508. The light can pass through these components as well as an optics module 513 (e.g., containing lenses) that directs light to the sensing locations at an excitation wavelength and receives light from the fluorescent label 503 at an emission wavelength. The emission radiation can be imaged using an imaging system 510.
Capture probes (e.g., antibodies) can be tethered in proximity to the sensing locations. In this case, a first antibody 505 is tethered to the surface (e.g., to the electrode) at the first sensing location 507 and a second antibody 519 is tethered at the second sensing location 514. A first target antigen 504 binds to the first antibody 505 (e.g., capture probe) and a secondary antibody 521 having a fluorescent probe 503 can bind to the target antigen in order to concentrate a fluorescent signal at the sensing locations.

The bottom surface can act like the surface of a traditional nucleic acid microarray and is packaged with a fluidic channel on top. Both the top surface of the microarray and the bottom surface of the fluidic channel can be modified to have an electrode. Either one or both electrode surfaces can be a transparent conducting material, for example indium tin oxide (ITO) to allow for simultaneous imaging of the surface and the application of an electric field. The process of detection is shown in FIG. 6A to FIG. 6E. For clarity, it may be assumed that only events on the surface lead to a fluorescent signal and there is very little background. In practice, this can be accomplished by first hybridizing the target nucleic acid on the microarray slide and washing prior to testing. As the voltage is increased, the electrokinetic force on the hybridized nucleic acid may increase until nucleic acid is pulled off. This pull-off voltage can be related to the binding energy of the target analyte to the capture probe, which is identified as the binding energy voltage ($V_{BE}$). This can be used to discriminate between a target that may be a correct complementary match and a target that has a single or plurality of mutations and is not completely complementary to the capture probe. A target that is not completely complimentary to the capture probe may still hybridize to the probe. The methods of the present disclosure can be used to identify this hybridization between a target that is not completely complementary to the capture probe (e.g., due to the target detaching at a lower voltage). This method can be employed to identify mixed mutation samples on a single capture site.

FIG. 6A shows an example of the magnitude of the fluorescent signal as a function of applied voltage for operation of the apparatus of FIG. 5. The applied voltage can be increased over time and the fluorescence output at the first pixel 601 and the second pixel 602 can be monitored. The signal begins relatively higher at lower applied voltages as analyte is bound both specifically and non-specifically and decreases according to the curves shown in FIG. 6A as the voltage is increased, providing a force that pulls the charged analytes from the capture probes. A series of drawings are provided which clarify the binding of the analytes at various applied voltages; with the lowest voltage at position 603 corresponding to FIG. 6B, the third highest voltage at position 604 corresponding to FIG. 6C, the second highest voltage at position 605 corresponding to FIG. 6D, and the highest voltage at position 606 corresponding to FIG. 6E.
FIG. 6B shows an example of the operation of the apparatus of FIG. 5 at a first voltage where initial binding occurs. The first pixel 611 has a plurality of the first capture antibodies 609 tethered to it and the second pixel 612 has a plurality of the second capture antibodies 610 tethered to it. The charged analyte 608 can bind specifically (e.g., at high strength) to the first capture antibody and can bind non-specifically (e.g., at low strength) to the second capture antibody. As shown in FIG. 6A, a fluorescent signal can be detected at both the first and second pixel, however, since the magnitudes of the signals are similar at the first voltage, specific versus non-specific binding cannot be easily differentiated at the first applied voltage. Therefore, the applied voltage can be increased to a second voltage.

FIG. 6C shows an example of the operation of the apparatus of FIG. 5 at a second voltage where non-specific components detach 613. The detachment from the second capture antibody can be due to the applied force exerted on the charged analyte by the electric field in proximity to the confinement features. In this case, some non-specifically bound analytes 614 remain bound at the second voltage, which can be detached by increasing the applied voltage further.

FIG. 6D shows an example of the operation of the apparatus of FIG. 5 at a third voltage. The remaining non-specific binders detach 614. In some cases, some true positives 615 (e.g., specifically bound analytes) detach as well. As shown in FIG. 6E, the applied voltage can be increased yet further to a fourth voltage where all components detach. The third and/or fourth voltages can be used to determine the binding force between the target antigen and the capture probe.

In some cases, the charged analytes can be electronically detected. FIG. 7 shows an example of an apparatus of the present disclosure adapted for electrical detection of charged analytes. The apparatus can include a fluidic chamber 702 (e.g., microfluidic) that is bisected by a surface. The surface can include several layers including a substrate 707, an insulating layer 704 (e.g., a dielectric membrane) and a field terminating layer 712 (e.g., a conducting metal such as platinum, copper, aluminum or silver). An applied voltage can be applied using a voltage source 708 in electrical communication with a first electrode 701 and a second electrode 706. A sensing electrode can also be located in proximity to the surface. In this case, a first sensing electrode 705 surrounds the first field confinement feature 713 and a second sensing electrode 709 surrounds the second field confinement feature 714. The sensing electrodes can be individually addressable. The binding of charged analytes in proximity to the first field confinement feature can be monitored by a first voltage output 703. The binding of charged analytes in proximity to the second field confinement feature can be monitored by a second voltage output 710.
FIG. 8A shows an example of the magnitude of the electrical signal as a function of applied voltage for operation of the apparatus of FIG. 7. The applied voltage can be increased over time and the voltage output near the first confinement feature 801 and near the second confinement feature 802 can be monitored. The signal begins relatively lower at lower applied voltages as analyte is bound both specifically and non-specifically and pulled into the orifice (e.g., field confinement feature) and eventually detach. A series of drawings are provided which clarify the binding of the analytes at various applied voltages; with the lowest voltage at position 803 corresponding to FIG. 8B, the third highest voltage at position 804 corresponding to FIG. 8C, the second highest voltage at position 805 corresponding to FIG. 8D, and the highest voltage at position 806 corresponding to FIG. 8E.

FIG. 8B shows an example of the operation of the apparatus of FIG. 7. The apparatus has a first orifice 807 and a second orifice 808. A first capture probe 809 is tethered in proximity to the first orifice 807 and a second capture probe 810 is tethered in proximity to the second orifice 808. In this case, the analyte 812 itself is not charged. It can become charged by associating with a charge label 811. The charge label can be covalently or non-covalently attached to the analyte, and the analyte may thereby become a charged analyte. The charge label can be afixed to a secondary antibody that also binds to the analyte. The analyte can bind to the capture probes and the secondary antibody at a low (or zero) applied voltage 803.

As shown in FIG. 8C, the applied voltage can be increased to a second voltage sufficient to pull the charged analytes into the orifice 813. An electrical signal can be detected at both the first and second orifice, however, since the magnitudes of the signals are similar at the second voltage, specific versus non-specific binding cannot be easily differentiated at the second applied voltage. Therefore, the applied voltage can be increased to a third voltage.

FIG. 8D shows an example of the operation of the apparatus of FIG. 7 at a third voltage where non-specific components detach 814. The detachment from the second capture antibody can be due to the applied force exerted on the charged analyte by the electric field in proximity to the confinement feature. As shown in FIG. 8A, there is a difference in sensor voltage signal between the first orifice and the second orifice, therefore the third voltage can distinguish between specific and non-specific binding of the analyte in proximity to the field confining feature.

FIG. 8E shows an example of the operation of the apparatus of FIG. 7 at a fourth voltage. The true positives (e.g., specifically bound analytes) can detach 815. The third and/or fourth voltages can be used to determine the binding force between the target antigen and the capture probe.
In some embodiments, the detection of target molecules is based on plasmonics. An example using a surface plasmon resonance imaging setup (SPRI) is provided herein. The gold film of an SPRI chip can be used for one electrode and a conducting film on the bottom of the fluidic cap can be used as a second. In some cases, an electrode for this system may not be transparent.

The methods disclosed herein can be used for probing cross-reactive and non-specific binding events in immunoassays. Non-specific and cross-reactive antibodies may have much lower binding energies than specific antibodies. By applying an electrokinetic force, the approximate binding energy voltage of the molecular pairs can be determined and non-specific and cross-reactive groups can be identified and removed in order to identify the presence of specific antibodies. In some embodiments, the capture probes are antibodies tethered to a polymer chain such as DNA.

In some embodiments, the target analytes are directly detected by the sensor. In such case, cross-reactivity or non-specific binding can occur when a secondary antigen is bound to the surface. The applied electrokinetic force can then act on the analytes themselves, probing the binding strength between the analytes and the capture probe. As the electrokinetic force is increased, the cross-reactive secondary analyte, more weakly bound to the capture probe than the target analyte, can dissociate at a smaller applied electrokinetic force than the target antigen.

In some cases, a sensing or detection antibody is used to enhance the detectability of the antigens. The sensing antibody can be modified to enhance the detectability by the sensor (e.g., by conjugation to a nucleic acid or protein). In embodiments where a sensing antibody is used, possible reactions include, but are not limited to: (a) correct interaction where the capture antibody is attached to the target antigen and the correct sensing antibody, modified or otherwise, binds to the target antigen; (b) a cross-reaction where a secondary antibody binds to the target antigen; (c) a cross-reaction where a secondary antibody binds to the capture antibody; (d) a cross-reaction where a secondary antigen binds to the capture antibody and a secondary antibody binds to the secondary antigen; (e) a secondary antibody binding to the capture antibody in absence of the target antigen; and (f) a secondary antigen binding to the capture antibody and a secondary antibody binds to the secondary antigen. The increasing electrokinetic force can remove the cross-reactive components, such as described in (b) ~ (f), earlier than the correct interaction, such as described in (a).

FIG. 9 shows an example of an apparatus of the present disclosure adapted for plasmonic detection of charged analytes. The apparatus includes charged analytes 901 to be detected, which in this case are nucleic acid molecules. The plasmonic detection system can include a light source 904, a polarizer 903, a prism 902, a metal (e.g., gold) film 908, an optical
filter 906 and a detector 905. The charged analytes 901 can be suspended in an electrolyte
confined in a fluidic chamber 909. The charged analytes 901 can hybridize to the capture probes
907, thereby becoming detectable by the plasmonic system.

[0093] In some embodiments of the present disclosure, the detection of target molecules is
based on ionic current blockage of a single pore or porous structure. Some instances of the
present disclosure include methods and apparatuses where the applied electrokinetic force is
enhanced by the presence of membranes whose thickness ranges from about 0.08 nanometer
(nm) to about 1 millimeter (mm). These apparatuses also include one or more electrodes which
are embedded within the membrane that are used to sense the presence or absence of the target
molecule. In some cases, the embedded electrodes may be used to control the physical location of
the capture probes and/or the target molecules. These apparatuses can also include various
orifices through which the electrokinetic force is concentrated. These orifices can be any opening
in the membrane including pores, slits or gates of various sizes. Via these orifices, the electric
field (generating the electrokinetic force) may be directed in any desired direction. In some cases,
the surface of the apparatus is coated with material to change its surface charge in solution, such
materials include but are not limited to Silicon Dioxide (Si0₂), Silicon Nitride (SiNx), Hafnium
Dioxide (Hf0₂), Zirconium Dioxide (Zr0₂), Aluminum Oxide (Al₂O₃) and Titanium Dioxide
(Ti0₂).

[0094] FIG. 10 shows an example of an array of the present disclosure for sensing the
presence and binding energy of nucleic acid molecules. The system includes a fluidic chamber
1000 containing a buffer 1002 and opposed by a pair of transparent electrodes 1004 capable of
creating an electric field. In this case, the field confinement features are not shown. The charged
antigens contain fluorescent labels 1006 and can hybridize to the capture probes 1008. Nucleic
acids are typically negatively charged, so no secondary charge label may be needed.

[0095] FIG. 11A shows an example of the method of the present disclosure being used to
measure the binding force of a complimentary nucleic acid molecule. The graphic depicts a series
of three states of the system (top to bottom) having different relative applied voltages (dashed
lines) and fluorescent signals (solid lines). The target analytes are initially not bound to the
capture probes 1100 and a baseline signal is detected 1102. The hybridization of the analytes
1104 to the capture probes increases the signal 1106. Application of a linearly increasing applied
voltage 1108 creates an electric field 1110 and an applied force on the charged analytes, but does
not initially affect the signal. FIG. 11B continues from FIG. 11A and shows the effect of
continuing to increase the applied voltage. As the electrokinetic force reaches a level close to the
binding force between the analyte and the capture probe, some of the target analytes can
dissociate 1112 and the signal can begin dropping 1114. As the voltage continues to increase,
eventually all of the analytes can dissociate and the signal can return to baseline. The binding energy voltage can be defined as the voltage where half of the target analytes are released from the surface.

[0096] FIG. 12A and FIG. 12B are similar to FIG. 11A and FIG. 11B, but in this instance show an example of the method of the present disclosure being used to measure the binding force of a nucleic acid molecule having a single base pair mismatch to the capture probe (e.g., a single nucleotide polymorphism (SNP)). The graphic depicts a series of three states of the system (top to bottom) having different relative applied voltages (dashed lines) and fluorescent signals (solid lines). The target analytes are initially not bound to the capture probes and a baseline signal is detected. The hybridization of the analytes to the capture probes increases the signal. Application of a linearly increasing applied voltage creates an electric field and an applied force on the charged analytes, but does not initially affect the signal. With reference to FIG. 12B, the mismatched analyte dissociates from the capture probe at a relatively lower voltage compared with the voltage at which a perfectly complimentary analyte dissociates (see FIG. 11B). Also, all mismatched analytes dissociate at a relatively lower voltage and the binding energy voltage is relatively lower compared with an analyte not having the SNP. The difference in signal versus applied voltage behavior between FIG. 11B and FIG. 12B can be used to identify the presence of the polymorphism. Note that the embodiment described herein is able to distinguish between two or more nucleic acid molecules at a single sensing location using a single capture probe.

[0097] The methods described herein can also be used to identify a plurality of different analytes in a mixture. For example, FIG. 13A and FIG. 13B show the method of the present disclosure being used to measure the presence of and/or binding force of a mixture of nucleic acid molecules having various amounts of complementarity to the capture probe. The graphic depicts a series of three states of the system (top to bottom) having different relative applied voltages (dashed lines) and fluorescent signals (solid lines). The target analytes are initially not bound to the capture probes and a baseline signal is detected. The hybridization of the analytes to the capture probes increases the signal. Application of a linearly increasing applied voltage creates an electric field and an applied force on the charged analytes, but does not initially affect the signal. Turning attention to FIG. 13B, the voltage signal displays two inflection points and two binding energy voltages, corresponding to, first, dissociation of the mismatched nucleic acid analytes and dissociation of the completely complimentary nucleic acid analytes from the capture probes, respectively.

[0098] FIG. 14 shows an example of a multiplexed immunoassay, which depicts the issue of cross reactivity when trying to test for multiple analytes with multiple antibodies. Some instances...
of the assay use secondary antibodies 1400 that have an attached nucleic acid 1402 (e.g., to increase and control the amount of charge associated with the analyte 1404, which can be bound to a capture probe 1406 tethered to a surface 1408). FIG. 14 shows six different types of cross-reactivity. From left to right, (A) the molecules can interact correctly, (B) the secondary antibody can interact with the target analyte (e.g., in a secondary epitope), (C) the secondary antibody can interact with the capture probe, (D) the secondary antibody can interact with the capture probe or other entity through an intermediary molecule, (E) the secondary antibody can interact with the capture probe without the analyte, and (F) the wrong analyte can be bound by the capture probe and/or the secondary antibody. These are non-limiting examples. The methods of the disclosure use an applied electrokinetic force to resolve such issues by pulling apart such non-specific interactions.

[0099] FIG. 15 shows an example of performing the method of the present disclosure using the apparatus shown in FIG. 14. The various types of correct and non-specifically interacting molecules can be pulled into the sensing orifice 1500 by an applied electric field 1502. A plot of sensor output versus applied voltage shows detachment of non-specific binding 1504, followed by an actual signal 1506 associated with the disruption of the binding of the correctly bound target analyte.

[00100] FIG. 16 shows an example of a process of covalently attaching the capture probe (e.g., nucleic acids) to the surface. A SiO$_2$ surface 1600 comprising hydroxide moieties 1601 (and/or that is hydroxylated) can be silanized (e.g., using aminosilane 1602). Silanization can be performed in gas phase. A cross linker (e.g., PMPI 1603) can be used to connect the amine group on the silane and the sulfhydryl group of the 5’ thiol-modified primer 1604. The resulting product of the surface chemistry described herein is shown at 1605.

[00101] In practice, sensors can be plasma cleaned, rehydrated and functionalized with (3-aminopropyl)-trimethoxysilane (APTMS) using a chemical vapor deposition system. The aminofunctionalized surfaces can be subsequently transformed into a thiol-reactive moiety by exposure to a 2.3 mM solution of N-(p-maleimidophenyl) isocyanate (PMPI) in anhydrous toluene at 40°C for 2 hours under an argon atmosphere. The surfaces can be subsequently washed with anhydrous toluene and dried in a stream of argon followed by DNA immobilization using thiolated oligonucleotides. Prior to immobilization, the thiolated oligos can be reduced using tris(2-carboxyethyl)phosphine (TCEP) as a reducing agent and desalted using a spin column (MWCO=3000). Thiolated oligos can be spotted directly onto sensing chips for 6 hours at 10μM concentration in a 1M NaCl buffer solution under a controlled atmosphere, followed by extensive washing. The various surface modification steps can be followed by x-ray photoelectron spectroscopy and the presence of the expected elements and peak shifts confirmed the
transformation of the sensing surface. The presence of immobilized nucleic acid can be verified by fluorescence microscopy with appropriately excited SYBR Gold nucleic acids dye. Subsequently, the immobilized oligonucleotides can be used to further increase the pool of possible surface modifications by introducing the desired probes conjugated to the complementary strand of the immobilized nucleic acid. Additional details pertaining to fabrication or operation of the devices described herein can be found in PCT Patent Application Serial No. PCT/US2015/036800, which is incorporated herein in its entirety for all purposes.

[00102] FIG. 17 shows an example of an apparatus of the present disclosure, including a fluorescence microscopy image 1701 of TAMRA (5-carboxytetramethylrhodamine) labeled oligonucleotides in an array of wells for concentrating electric fields, transparent indium tin oxide electrodes 1702, and a field terminating metal plate of platinum surrounding the array of wells 1703.

[00103] FIG. 18 shows a computer system 1801 that is programmed or otherwise configured to regulate the operation of the apparatus of the present disclosure. The computer system 1801 can regulate, for example, flow rates, temperatures, pressures, mechanical manipulations, applied voltages or other electrical inputs and/or outputs, and the like.

[00104] The computer system 1801 includes a central processing unit (CPU, also "processor" and "computer processor" herein) 1805, which can be a single core or multi core processor, or a plurality of processors for parallel processing. The computer system 1801 also includes memory or memory location 1810 (e.g., random-access memory, read-only memory, flash memory), electronic storage unit 1815 (e.g., hard disk), communication interface 1820 (e.g., network adapter) for communicating with one or more other systems, and peripheral devices 1825, such as cache, other memory, data storage and/or electronic display adapters. The memory 1810, storage unit 1815, interface 1820 and peripheral devices 1825 are in communication with the CPU 1805 through a communication bus, such as a motherboard. The storage unit 1815 can be a data storage unit (or data repository) for storing data.

[00105] The CPU 1805 can execute a sequence of machine-readable instructions, which can be embodied in a program or software. The instructions may be stored in a memory location, such as the memory 1810. Examples of operations performed by the CPU 1805 can include fetch, decode, execute, and writeback.

[00106] The storage unit 1815 can store files, such as drivers, libraries and saved programs. The storage unit 1815 can store programs generated by users and recorded sessions, as well as output(s) associated with the programs. The storage unit 1815 can store user data, e.g., user preferences and user programs. The computer system 1801 in some cases can include one or more additional data storage units that are external to the computer system 1801, such as located
on a remote server that is in communication with the computer system 1801 through an intranet or the Internet.

[00107] The computer system 1801 can be in communication with a system 1830, including a device with integrated fluidics and/or process elements. Such process elements can include sensors, flow regulators (e.g., valves), and pumping systems that are configured to direct a fluid.

[00108] Methods as described herein can be implemented by way of machine (e.g., computer processor) executable code stored on an electronic storage location of the computer system 1801, such as, for example, on the memory 1810 or electronic storage unit 1815. The machine executable or machine readable code can be provided in the form of software. During use, the code can be executed by the processor 1805. In some cases, the code can be retrieved from the storage unit 1815 and stored on the memory 1810 for ready access by the processor 1805. In some situations, the electronic storage unit 1815 can be precluded, and machine-executable instructions are stored on memory 1810.

[00109] The code can be pre-compiled and configured for use with a machine having a processor adapted to execute the code, or can be compiled during runtime. The code can be supplied in a programming language that can be selected to enable the code to execute in a pre-compiled or as-compiled fashion.

[00110] Aspects of the systems and methods provided herein, such as the computer system 1801, can be embodied in programming. Various aspects of the technology may be thought of as "products" or "articles of manufacture" typically in the form of machine (or processor) executable code and/or associated data that is carried on or embodied in a type of machine readable medium. Machine-executable code can be stored on an electronic storage unit, such memory (e.g., read-only memory, random-access memory, flash memory) or a hard disk. "Storage" type media can include any or all of the tangible memory of the computers, processors or the like, or associated modules thereof, such as various semiconductor memories, tape drives, disk drives and the like, which may provide non-transitory storage at any time for the software programming. All or portions of the software may at times be communicated through the Internet or various other telecommunication networks. Such communications, for example, may enable loading of the software from one computer or processor into another, for example, from a management server or host computer into the computer platform of an application server. Thus, another type of media that may bear the software elements includes optical, electrical and electromagnetic waves, such as used across physical interfaces between local devices, through wired and optical landline networks and over various air-links. The physical elements that carry such waves, such as wired or wireless links, optical links or the like, also may be considered as media bearing the software. As used herein, unless restricted to non-transitory, tangible "storage"
media, terms such as computer or machine "readable medium" refer to any medium that participates in providing instructions to a processor for execution.

[00111] Hence, a machine readable medium, such as computer-executable code, may take many forms, including but not limited to, a tangible storage medium, a carrier wave medium or physical transmission medium. Non-volatile storage media include, for example, optical or magnetic disks, such as any of the storage devices in any computer(s) or the like, such as may be used to implement the databases, etc. shown in the drawings. Volatile storage media include dynamic memory, such as main memory of such a computer platform. Tangible transmission media include coaxial cables; copper wire and fiber optics, including the wires that comprise a bus within a computer system. Carrier-wave transmission media may take the form of electric or electromagnetic signals, or acoustic or light waves such as those generated during radio frequency (RF) and infrared (IR) data communications. Common forms of computer-readable media therefore include for example: a floppy disk, a flexible disk, hard disk, magnetic tape, any other magnetic medium, a CD-ROM, DVD or DVD-ROM, any other optical medium, punch cards paper tape, any other physical storage medium with patterns of holes, a RAM, a ROM, a PROM and EPROM, a FLASH-EPROM, any other memory chip or cartridge, a carrier wave transporting data or instructions, cables or links transporting such a carrier wave, or any other medium from which a computer may read programming code and/or data. Many of these forms of computer readable media may be involved in carrying one or more sequences of one or more instructions to a processor for execution.

[00112] An aspect of the present disclosure provides an apparatus for detecting a presence of an analyte, comprising: (a) a fluidic chamber adapted to contain an electrolyte; (b) two or more electrodes capable of generating an electric field within the fluidic chamber; (c) at least one surface comprising an immobilized capture probe capable of binding the analyte, wherein the at least one surface is between the two or more electrodes and in contact with the fluidic chamber, and wherein the at least one surface comprises an electrically conducting layer and an electrically insulating layer; and (d) at least one field confining feature proximal to the at least one surface, which at least one field confining feature is capable of concentrating an electric field surrounding the at least one field confining feature to a strength of at least about $10^3$ volts per meter (V/m). In some cases, the analyte is a charged analyte. The analyte can be charged directly, or indirectly by binding to a charged molecule such as a charged antibody. The apparatus can further comprise a controller operably coupled to the two or more electrodes, where the controller is programmed to apply a voltage across the two or more electrodes that is sufficient to generate an applied force on the analyte.
The at least one field confining feature can be capable of concentrating the electric field surrounding the at least one field confining feature to any suitable strength. In some embodiments, the field strength is at least about $10^3$ volts per meter (V/m), at least about $10^4$ V/m, at least about $10^5$ V/m, at least about $10^6$ V/m or at least about $10^7$ V/m. In some cases, the field strength is at most about $10^3$ V/m, at most about $10^5$ V/m, at most about $10^7$ V/m, or at most about $10^9$ V/m.

The voltage applied can sufficient to generate any suitable applied force on the charged analyte. In some cases, the applied force is at least about 1 piconewton (pN), at least about 5 pN, at least about 10 pN, at least about 20 pN, at least about 50 pN, at least about 100 pN, at least about 500 pN, at least about 1000 pN or at least about 5000 pN on the analyte. In some instances, the applied force is at most about 1 piconewton (pN), at most about 5 pN, at most about 10 pN, at most about 20 pN, at most about 50 pN, at most about 100 pN, at most about 500 pN, at most about 1000 pN or at most about 5000 pN on the analyte.

The immobilized capture probe can be capable of binding to the analyte to form a probe-analyte complex on the at least one surface. The immobilized capture probe can be capable of binding to the analyte via non-covalent interaction(s). The non-covalent interaction(s) can be disrupted through an applied force generated upon the application of a voltage across the two or more electrodes.

In some embodiments, the immobilized capture probe is proximal to the at least one field confining feature. For example, the capture probe can be immobilized within about 10 nanometers (nm), within about 20 nm, within about 50 nm, within about 100 nm, within about 500 nm, or within about 1000 nm of the field confining feature.

The at least one field confining feature can be a plurality of field confining features. For example, the surface can have at least about 5, at least about 10, at least about 50, at least about 100, at least about 500, at least about 1000, at least about 5000, at least about 10000, at least about 50000, at least about $10^6$, or at least about $10^7$ field confinement features.

In some cases, the immobilized capture probe is an antibody. In some instances, the analyte is an antigen. In some cases, the immobilized capture probe is a nucleic acid molecule. In some instances, the analyte is a nucleic acid molecule.

Note that the use of the term "immobilized" does not mean that the capture probe cannot move. For example, the capture probe can be tethered to the surface at one or more locations and move within the electrolyte due to fluid flow or other forces.

The analyte can have any suitable charge (e.g., in order to provide an adequate applied force). In some embodiments, the analyte has a charge of at least about $1\ e^-$, at least about $10\ e^-$, at least about $10^2\ e^-$, at least about $10^3\ e^-$, at least about $10^4\ e^-$, at least about $10^5\ e^-$ or at least
about $10^6$ e$^-$. In some embodiments, the analyte has a charge of at most about 1 e$^-$, at most about 10 e$^-$, at most about $10^2$ e$^-$, at most about $10^3$ e$^-$, at most about $10^4$ e$^-$, at most about $10^5$ e$^-$ or at most about $10^6$ e$^-$. Note that the analyte can have a positive charge or a negative charge. Positive

charges of a similar magnitude to those disclosed herein are also encompassed by the present disclosure.

[00121] The apparatus can further include a controller operably coupled to the two or more electrodes, where the controller is programmed to apply a potential difference of less than about 100 volts (V) between the two or more electrodes. In some cases, the applied voltage is less than about 50 V, less than about 10 V, less than about 5 V, or less than about IV. In some instances, the applied voltage is greater than about 50 V, greater than about 10 V, greater than about 5 V, or greater than about IV.

[00122] The at least one surface can provide a wall of the fluidic chamber (as shown in FIG. 1A) and the field confining feature is an orifice in the surface. In some cases, the field confining feature comprises an orifice in the at least one surface. In some cases, the fluidic chamber comprises a top portion and a bottom portion partitioned by the at least one surface, and wherein the field confining feature is an orifice in the at least one surface (as shown in FIG. 1B). In some cases, the orifice extends through the surface. The orifice can be, without limitation, an indentation, a well, a pore, a channel, a gap, or a slit. The field confining feature can be any shape, including circular, oval, square, rectangular, or any polygon.

[00123] The field confining feature can have any suitable diameter. In some cases, the diameter is less than about 50 micrometers, less than about 10 micrometers, less than about 5 micrometers, less than about 1 micrometer, less than about 500 nanometers, less than about 100 nanometers, or less than about 50 nanometers. In some cases, the diameter is greater than about 50 micrometers, greater than about 10 micrometers, greater than about 5 micrometers, greater than about 1 micrometer, greater than about 500 nanometers, greater than about 100 nanometers, or greater than about 50 nanometers.

[00124] The field confining feature can have any suitable aspect ratio. In some cases, the aspect ratio is the ratio of the longest dimension of the field confining feature to the shortest dimension of the field confining feature. In some instances, the aspect ratio is the ratio of the width of the field confining feature to the depth of the field confining feature. Without limitation, the field confining feature can have an aspect ratio of at least about 0.1, at least about 0.5, at least about 1, at least about 2, at least about 3, at least about 5, at least about 10, at least about 50, at least about 100, or more. The field confining feature can have a sharp edge (e.g., having an angle of at least about 60 degrees). The field confining feature can be an elevated portion of the at least one surface.
[00125] The electrically conducting layer can be in communication with the electrolyte, such as physically contacting or in electrical communication. The electrically conducting layer can be electrically isolated from the electrolyte, and wherein, during use, the electrically conducting layer is electrically biased. The electrically conducting layer can comprise any suitable metal, such as platinum, copper, gold or silver.

[00126] The apparatus can further comprise a sensing electrode configured to measure a charge signal associated with the presence of the analyte. The sensing electrode can be proximal to the at least one field confining feature (e.g., within about 10 nanometers (nm), within about 20 nm, within about 50 nm, within about 100 nm, within about 500 nm, or within about 1000 nm of the field confining feature).

[00127] The apparatus can further comprise a light source and a detector configured to detect an optical signal associated with the presence of the analyte. The optical signal can be a fluorescence signal. In some cases, the fluorescence signal is a fluorescence resonance energy transfer, FRET, signal. The fluorescence signal can be provided by a fluorescent probe covalently or non-covalently attached to the analyte.

[00128] The apparatus can further comprise a detector configured to detect a surface plasmon resonance signal associated with the presence of the analyte.

[00129] The at least one surface can be substantially planar. The at least one surface can be part of (or comprise) an array of sensors, wherein each sensor of the array comprises at least one field confining feature and a plurality of immobilized capture probes. The plurality of immobilized capture probes of a given sensor of the array can be proximal to the field confining feature for the given sensor of the array. The plurality of immobilized capture probes of a given sensor of the array can be clonal. In some cases, a given sensor of the array comprises a plurality of immobilized capture probes that is unique relative to another sensor of the array.

[00130] The distance between a given sensor of the array and a nearest neighbor sensor can be any suitable distance. In some cases, the distance is at least about 50 nanometers (nm), at least about 100 nm, at least about 150 nm, at least about 200 nm, at least about 250 nm, at least about 500 nm, at least about 1000 nm, at least about 5000 nm or at least about 10000 nm. In some cases, the distance is at most about 50 nanometers (nm), at most about 100 nm, at most about 150 nm, at most about 200 nm, at most about 250 nm, at most about 500 nm, at most about 1000 nm, at most about 5000 nm or at most about 10000 nm.

[00131] The array can comprise any suitable number of sensors. In some cases, the array comprises at least about 5, at least about 10, at least about 50, at least about 100, at least about 500, at least about 1000, at least about 5000, at least about 10000, at least about 50000, at least about $10^6$, or at least about $10^7$ sensors.
In another aspect, the disclosure provides method for detecting a presence of a target analyte, comprising (a) activating at least one sensor comprising one or more surfaces between two or more electrodes, wherein the one or more surfaces comprise two or more immobilized capture probes; (b) bringing the one or more surfaces in contact with a solution containing or suspected of containing a target analyte and a non-target analyte, under conditions that are sufficient to permit the target analyte and non-target analyte to each bind to a given one of the two or more immobilized capture probes; (c) applying a voltage across the two or more electrodes that is sufficient to release the non-target analyte, but not the target analyte, from a given one of the two or more immobilized capture probes; and (d) detecting a presence of the target analyte and/or the non-target analyte can be a charged analyte. The voltage can be sufficient to exert an applied force of at least about 1 piconewton (pN) on the non-target analyte. Operations (c) and/or (d) can be performed in the absence of washing the one or more surfaces.

The method of can further comprise applying an additional voltage across the two or more electrodes that is sufficient to release the target analyte from a given one of the two or more immobilized capture probes. The additional voltage can be sufficient to exert an applied force of at least about 1 piconewton (pN) on the target analyte.

In various embodiments, the force applied on the target or non-target analytes is at least about 1 piconewton (pN), at least about 5 pN, at least about 10 pN, at least about 20 pN, at least about 50 pN, at least about 100 pN, at least about 500 pN, at least about 1000 pN or at least about 5000 pN. In some instances, the applied force is at most about 1 piconewton (pN), at most about 5 pN, at most about 10 pN, at most about 20 pN, at most about 50 pN, at most about 100 pN, at most about 500 pN, at most about 1000 pN or at most about 5000 pN on the analyte.

The method can further comprise detecting the presence of the target analyte on the one or more surfaces subsequent to application of the additional voltage. The voltage can be less than the additional voltage.

The voltage and the additional voltage can be individual voltages of a continuously applied voltage that is changed over time, as shown in FIG. 6A or FIG. 8A.

The voltage and/or the additional voltage can be applied in the absence of washing the one or more surfaces. For example, the methods of the present disclosure can be performed in complex mixtures such as biological fluids (e.g., blood) or environmental samples.

The applied voltage can be sufficient to generate an electric field having a strength of less than about 10⁹ volts per meter (V/m) and the additional applied voltage can be sufficient to generate an additional electric field having a strength of less than about 10⁹ V/m, less than about
10^8 \text{ V/m}, \text{ less than about } 10^7 \text{ V/m}, \text{ less than about } 10^6 \text{ V/m}, \text{ less than about } 10^5 \text{ V/m}, \text{ less than about } 10^4 \text{ V/m}, \text{ less than about } 10^3 \text{ V/m}, \text{ or lower.}

[00139] In some cases, each of the target analyte and non-target analyte non-covalently binds to a given one of the two or more immobilized capture probes to form a target probe-analyte complex immobilized and a non-target probe-analyte complex immobilized on the one or more surfaces, respectively.

[00140] The applied voltage can be increased from a first voltage to a second voltage over time. The applied voltage can be increased from the first voltage to the second voltage at a rate of at least about 1 millivolt per second (mV/s), at least about 5 mV/s, at least about 10 mV/s, at least about 50 mV/s, at least about 100 mV/s, at least about 500 mV/s, at least about 1000 mV/s, at least about 5000 mV/s, or more.

[00141] In some cases, the applied voltage is increased from the voltage to the additional voltage over a period of time greater than about 10 microseconds, greater than about 50 microseconds, greater than about 100 microseconds, greater than about 500 microseconds, greater than about 1000 microseconds, greater than about 5000 microseconds, greater than about 10000 microseconds or more. In some cases, the applied voltage is increased from the voltage to the additional voltage over a period of time less than about 1 second.

[00142] The applied voltage can be a periodic voltage waveform. The periodic voltage waveform can have any suitable frequency, such as a frequency of less than about 10^9 \text{ Hertz (Hz)}, \text{ less than about } 10^8 \text{ Hz}, \text{ less than about } 10^7 \text{ Hz}, \text{ less than about } 10^6 \text{ Hz}, \text{ less than about } 10^5 \text{ Hz}, \text{ less than about } 10^4 \text{ Hz}, \text{ less than about } 10^3 \text{ Hz}, \text{ less than about } 10^2 \text{ Hz}, \text{ less than about } 10^1 \text{ Hz}, \text{ or less than about } 10^{-3} \text{ Hz}. \text{ The periodic voltage waveform can have a frequency of greater than about } 10^9 \text{ Hertz (Hz)}, \text{ greater than about } 10^8 \text{ Hz}, \text{ greater than about } 10^7 \text{ Hz}, \text{ greater than about } 10^6 \text{ Hz}, \text{ greater than about } 10^5 \text{ Hz}, \text{ greater than about } 10^4 \text{ Hz}, \text{ greater than about } 10^3 \text{ Hz}, \text{ greater than about } 10^2 \text{ Hz}, \text{ greater than about } 10^1 \text{ Hz}, \text{ or greater than about } 10^{-3} \text{ Hz}.

[00143] The method of can further comprise monitoring a signal indicative of the presence of the target analyte on the one or more surfaces as the applied voltage is varied over time.

[00144] The method can further comprise determining a binding force of the target analyte bound to a given one of the two or more immobilized capture probes (e.g., the force required to dissociate the analyte from the binding probe).

[00145] The applied force can be less than a binding force of the target analyte bound to a given one of the two or more immobilized capture probes.
The electrolyte (solution) can comprise charged molecules that bind with the target analyte and/or the non-target analyte, to provide a change in charge on the target analyte and/or non-target analyte. The charged molecules can be antibodies.

The presence of the target analyte on the one or more surfaces can be detected by measuring a signal associated with the target analyte. The signal can be a charge signal. The charge signal can be detected using a sensing electrode and a reference electrode. The charge signal can be a voltage.

The presence of the target analyte on the one or more surfaces can be detected by measuring an optical signal associated with the target analyte. The optical signal can be a fluorescence signal. The fluorescence signal can be provided by a fluorescent probe covalently or non-covalently attached to the target analyte.

The presence of the target analyte on the one or more surfaces can be detected using surface plasmon resonance.

The one or more surfaces can comprise a membrane having a thickness of between about 0.08 nanometers and 1 millimeter. The one or more surfaces can include a plurality of surfaces. Each of the plurality of surfaces can comprise a subset of the two or more immobilized capture probes.

The voltage can be a direct current voltage or an alternating current voltage.

In another aspect, the present disclosure provides a system for detecting a presence of a target analyte, comprising: (a) at least one sensor comprising one or more surfaces between two or more electrodes, wherein the one or more surfaces comprise two or more immobilized capture probes; (b) a solution chamber in fluid communication with the one or more surfaces, wherein the solution chamber is configured to retain a solution containing or suspected of containing the target analyte and a non-target analyte, under conditions that are sufficient to permit the target analyte and non-target analyte to each bind to a given one of the two or more immobilized capture probes; and (c) a controller that is operably coupled to the two or more electrodes, wherein the controller is programmed to (i) apply a voltage across the two or more electrodes that is sufficient to release the non-target analyte, but not the target analyte, from a given one of the two or more immobilized capture probes, and (ii) detect a presence of the target analyte on the one or more surfaces.

The target analyte and/or the non-target analyte can be a charged analyte. In some cases, the target analyte and/or the non-target analyte can have a first charge (e.g., zero charge) and may obtain a second charge (e.g., +2, -2) upon coupling to a charged molecule.

The voltage across the two or more electrodes can be sufficient to exert an applied force of at least 1 piconewton on the non-target analyte. The at least one sensor can comprise an
array of sensors. Each sensor of the array of sensors can be independently addressable (e.g., have measurements taken from it).

[00155] It should be understood from the foregoing that, while particular implementations have been illustrated and described, various modifications can be made thereto and are contemplated herein. For example, the embodiments described herein can be combined with or modified to yield yet more embodiments of the present invention. It is also not intended that the invention be limited by the specific examples provided within the specification. While the invention has been described with reference to the aforementioned specification, the descriptions and illustrations of the preferable embodiments herein are not meant to be construed in a limiting sense. Furthermore, it shall be understood that all aspects of the invention are not limited to the specific depictions, configurations or relative proportions set forth herein which depend upon a variety of conditions and variables. Various modifications in form and detail of the embodiments of the invention will be apparent to a person skilled in the art. It is therefore contemplated that the invention shall also cover any such modifications, variations and equivalents. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.
CLAIMS

WHAT I CLAIMED IS:

1. A method for detecting a presence of a target analyte, comprising:
   (a) activating at least one sensor comprising one or more surfaces between two or more
       electrodes, wherein the one or more surfaces comprise two or more immobilized
       capture probes;
   (b) bringing the one or more surfaces in contact with a solution containing or suspected of
       containing a target analyte and a non-target analyte, under conditions sufficient to
       permit the target analyte and non-target analyte to each bind to a given one of the two
       or more immobilized capture probes;
   (c) applying a voltage across the two or more electrodes that is sufficient to release the
       non-target analyte, but not the target analyte, from a given one of the two or more
       immobilized capture probes; and
   (d) detecting a signal indicative of a presence and/or binding energy of the target analyte
       on the one or more surfaces.

2. The method of Claim 1, wherein the target analyte and/or the non-target analyte is a
   charged analyte.

3. The method of Claim 1, wherein the voltage is sufficient to exert an applied force of at
   least about 1 piconewton (pN) on the non-target analyte.

4. The method of Claim 3, wherein the applied force is at least about 10 pN.

5. The method of Claim 1, wherein the voltage is sufficient to generate an electric field
   having a strength of at least about 10^3 volts per meter.

6. The method of Claim 1, further comprising applying an additional voltage across the two
   or more electrodes that is sufficient to release the target analyte from a given one of the two or
   more immobilized capture probes.

7. The method of Claim 6, wherein the additional voltage is sufficient to exert an applied
   force of at least about 1 piconewtons (pN) on the target analyte.

8. The method of Claim 7, wherein the applied force is at least about 10 pN.

9. The method of Claim 6, further comprising detecting the presence of the target analyte on
   the one or more surfaces subsequent to application of the additional voltage.

10. The method of Claim 6, wherein the voltage is less than the additional voltage.

11. The method of Claim 6, wherein the voltage and the additional voltage are individual
    voltages of a continuously applied voltage that is changed over time.
12. The method of Claim 6, wherein the additional voltage is applied in the absence of washing the one or more surfaces.

13. The method of Claim 6, wherein the applied voltage is sufficient to generate an electric field having a strength of less than about $10^9$ volts per meter (V/m) and the additional applied voltage is sufficient to generate an additional electric field having a strength of less than about $10^9$ V/m.

14. The method of Claim 1, wherein each of the target analyte and non-target analyte non-covalently binds to a given one of the two or more immobilized capture probes to form a target probe-analyte complex immobilized and a non-target probe-analyte complex immobilized on the one or more surfaces, respectively.

15. The method of Claim 1, wherein the applied voltage is increased from a first voltage to a second voltage over time.

16. The method of Claim 15, wherein the applied voltage is increased from the first voltage to the second voltage at a rate of at least about 1 millivolt per second.

17. The method of Claim 15, wherein the applied voltage is increased from the first voltage to the second voltage over a period of time greater than about 10 microseconds.

18. The method of Claim 15, wherein the applied voltage is increased from the first voltage to the second voltage over a period of time less than about 1 second.

19. The method of Claim 1, wherein the applied voltage is a periodic voltage waveform.

20. The method of Claim 19, wherein the periodic voltage waveform has a frequency of less than about 1 gigahertz.

21. The method of Claim 19, wherein the periodic voltage waveform has a frequency of greater than about 1 millihertz.

22. The method of Claim 1, further comprising monitoring a signal indicative of the presence of the target analyte on the one or more surfaces as the applied voltage is varied over time.

23. The method of Claim 1, further comprising determining a binding force of the target analyte bound to a given one of the two or more immobilized capture probes.

24. The method of Claim 3, wherein the applied force is less than a binding force of the target analyte bound to a given one of the two or more immobilized capture probes.

25. The method of Claim 1, wherein the solution comprises charged molecules that bind with the target analyte and/or the non-target analyte, to provide a change in charge on the target analyte and/or non-target analyte.

26. The method of Claim 25, wherein the charged molecules are antibodies.

27. The method of Claim 1, wherein (c) and/or (d) is performed in the absence of washing the one or more surfaces.
28. The method of Claim 1, wherein the presence of the target analyte on the one or more surfaces is detected by measuring a signal associated with the target analyte.

29. The method of Claim 28, wherein the signal is a charge signal.

30. The method of Claim 29, wherein the charge signal is detected using a sensing electrode and a reference electrode.

31. The method of Claim 29, wherein the charge signal is a voltage.

32. The method of Claim 1, wherein the signal indicative of the presence and/or binding energy of the target analyte on the one or more surfaces is detected by measuring an optical signal associated with the target analyte.

33. The method of Claim 32, wherein the optical signal is a fluorescence signal.

34. The method of Claim 33, wherein the fluorescence signal is provided by a fluorescent probe covalently or non-covalently attached to the target analyte.

35. The method of Claim 1, wherein the signal indicative of the presence and/or binding energy of the target analyte on the one or more surfaces is detected using surface plasmon resonance.

36. The method of Claim 1, wherein the one or more surfaces is substantially planar.

37. The method of Claim 1, wherein the one or more surfaces comprise an electrically insulating layer and an electrically conducting layer.

38. The method of Claim 37, wherein the electrically conducting layer is in communication with the solution.

39. The method of Claim 37, wherein the electrically conducting layer is electrically isolated from the solution and the electrically conducting layer is electrically biased.

40. The method of Claim 37, wherein the electrically conducting layer comprises platinum.

41. The method of Claim 1, wherein the one or more surfaces comprise a membrane having a thickness of between about 0.08 nanometers and 1 millimeter.

42. The method of Claim 1, wherein the one or more surfaces comprise a field confining feature that concentrates an electric field to a strength of at least about $10^3$ volts per meter.

43. The method of Claim 42, wherein the field confining feature comprises an orifice in the one or more surfaces, which one or more surfaces comprise an electrically insulating layer and an electrically conducting layer.

44. The method of Claim 1, wherein the two or more immobilized capture probes are nucleic acid molecules that hybridize with the target analyte.

45. The method of Claim 44, wherein the target analyte is a nucleic acid molecule.

46. The method of Claim 1, wherein the two or more immobilized capture probes comprise antibodies that bind the target analyte.
47. The method of Claim 46, wherein the target analyte is a protein.
48. The method of Claim 1, wherein the one or more surfaces include a plurality of surfaces.
49. The method of Claim 48, wherein each of the plurality of surfaces comprises a subset of the two or more immobilized capture probes.
50. The method of Claim 1, wherein the voltage is a direct current voltage.
51. The method of Claim 1, wherein the voltage is an alternating current voltage.
52. A system for detecting a presence of a target analyte, comprising:
   at least one sensor comprising one or more surfaces between two or more electrodes, wherein the one or more surfaces comprise two or more immobilized capture probes;
   a solution chamber in fluid communication with the one or more surfaces, wherein the solution chamber is configured to retain a solution containing or suspected of containing the target analyte and a non-target analyte, under conditions sufficient to permit the target analyte and non-target analyte to each bind to a given one of the two or more immobilized capture probes; and
   a controller that is operably coupled to the two or more electrodes, wherein the controller is programmed to (i) apply a voltage across the two or more electrodes that is sufficient to release the non-target analyte, but not the target analyte, from a given one of the two or more immobilized capture probes, and (ii) detect a signal indicative of a presence and/or binding energy of the target analyte on the one or more surfaces.
53. The system of Claim 52, wherein the target analyte and/or the non-target analyte is a charged analyte.
54. The system of Claim 52, wherein the voltage across the two or more electrodes is sufficient to exert an applied force of at least 1 piconewton on the non-target analyte.
55. The system of Claim 52, wherein the at least one sensor comprises an array of sensors.
56. The system of Claim 55, wherein the array of sensors comprises at least about 10 sensors.
57. The system of Claim 55, wherein each sensor of the array of sensors is independently addressable.
58. An apparatus for detecting a presence of an analyte, comprising:
   a fluidic chamber adapted to contain an electrolyte;
   two or more electrodes capable of generating an electric field within the fluidic chamber;
   at least one surface comprising an immobilized capture probe capable of binding the analyte, wherein the at least one surface is between the two or more electrodes and in contact with the fluidic chamber, and wherein the at least one surface comprises an electrically conducting layer and an electrically insulating layer; and
at least one field confining feature proximal to the at least one surface, which at least one
field confining feature is capable of concentrating an electric field surrounding the at least one
field confining feature to a strength of at least about $10^3$ volts per meter (V/m).

59. The apparatus of Claim 58, wherein the analyte is a charged analyte.

60. The apparatus of Claim 58, wherein the at least one field confining feature is capable of
concentrating the electric field surrounding the at least one field confining feature to a strength of
less than about $10^5$ V/m.

61. The apparatus of Claim 58, further comprising a controller operably coupled to the two or
more electrodes, wherein the controller is programmed to apply a voltage across the two or more
electrodes that is sufficient to generate an applied force on the analyte.

62. The apparatus of Claim 61, wherein the voltage applied is sufficient to generate an
applied force of at least about 1 piconewton (pN) on the analyte.

63. The apparatus of Claim 62, wherein the voltage is sufficient to generate an applied force
of at least about 20 pN on the analyte.

64. The apparatus of Claim 58, wherein the immobilized capture probe is capable of binding
to the analyte to form a probe-analyte complex on the at least one surface.

65. The apparatus of Claim 64, wherein the immobilized capture probe is capable of binding
to the analyte via non-covalent interaction(s).

66. The apparatus of Claim 65, wherein the non-covalent interaction(s) are disruptable
through an applied force generated upon the application of a voltage across the two or more
electrodes.

67. The apparatus of Claim 64, wherein the immobilized capture probe is proximal to the at
least one field confining feature.

68. The apparatus of Claim 58, wherein the at least one field confining feature is a plurality
of field confining features.

69. The apparatus of Claim 58, wherein the immobilized capture probe is an antibody.

70. The apparatus of Claim 69, wherein the analyte is an antigen.

71. The apparatus of Claim 58, wherein the immobilized capture probe is a nucleic acid
molecule.

72. The apparatus of Claim 71, wherein the analyte is a nucleic acid molecule.

73. The apparatus of Claim 58, wherein the analyte has a charge of less than about $10^4$ e$^-$.  

74. The apparatus of Claim 58, wherein the analyte has a charge of greater than about 10 e$^-$.  

75. The apparatus of Claim 58, further comprising a controller operably coupled to the two or
more electrodes, wherein the controller is programmed to apply a potential difference of less than
about 100 volts (V) between the two or more electrodes.
76. The apparatus of Claim 58, wherein the at least one surface provides a wall of the fluidic chamber and the field confining features is an orifice in the surface.

77. The apparatus of Claim 58, wherein the field confining feature comprises an orifice in the at least one surface.

78. The apparatus of Claim 58, wherein the fluidic chamber comprises a top portion and a bottom portion partitioned by the at least one surface, and wherein the field confining feature is an orifice in the at least one surface.

79. The apparatus of Claim 78, wherein the orifice extends through the surface.

80. The apparatus of Claim 77, wherein the orifice is an indentation, a well, a pore, a channel, a gap, or a slit.

81. The apparatus of Claim 58, wherein the at least one field confining feature has a diameter of less than about 50 micrometers.

82. The apparatus of Claim 81, wherein the at least one field confining feature has a diameter of less than about 50 nanometers.

83. The apparatus of Claim 58, wherein the field confining feature has an aspect ratio of at least about 0.1.

84. The apparatus of Claim 83, wherein the aspect ratio is the ratio of the longest dimension of the field confining feature to the shortest dimension of the field confining feature.

85. The apparatus of Claim 83, wherein the aspect ratio is the ratio of the width of the field confining feature to the depth of the field confining feature.

86. The apparatus of Claim 58, wherein the field confining feature has a sharp edge.

87. The apparatus of Claim 58, wherein the field confining feature is an elevated portion of the at least one surface.

88. The apparatus of Claim 58, wherein the electrically conducting layer is in communication with the electrolyte.

89. The apparatus of Claim 58, wherein the electrically conducting layer is electrically isolated from the electrolyte, and wherein, during use, the electrically conducting layer is electrically biased.

90. The apparatus of Claim 58, wherein the electrically conducting layer comprises platinum.

91. The apparatus of Claim 58, further comprising a sensing electrode configured to measure a charge signal associated with the presence of the analyte.

92. The apparatus of Claim 91, wherein the sensing electrode is proximal to the at least one field confining feature.

93. The apparatus of Claim 58, further comprising a light source and a detector configured to detect an optical signal associated with the presence of the analyte.
94. The apparatus of Claim 93, wherein the optical signal is a fluorescence signal.
95. The apparatus of Claim 94, wherein the fluorescence signal is provided by a fluorescent probe covalently or non-covalently attached to the analyte.
96. The apparatus of Claim 58, further comprising a detector configured to detect a surface plasmon resonance signal associated with the presence of the analyte.
97. The apparatus of Claim 58, wherein the at least one surface is substantially planar.
98. The apparatus of Claim 58, wherein the at least one surface is part of an array of sensors, wherein each sensor of the array comprises a field confining feature and a plurality of immobilized capture probes.
99. The apparatus of Claim 98, wherein the plurality of immobilized capture probes of a given sensor of the array are proximal to the field confining feature for the given sensor of the array.
100. The apparatus of Claim 98, wherein the plurality of immobilized capture probes of a given sensor of the array is clonal.
101. The apparatus of Claim 98, wherein a given sensor of the array comprises a plurality of immobilized capture probes that is unique relative to another sensor of the array.
102. The apparatus of Claim 98, wherein a distance between a given sensor of the array and a nearest neighbor sensor is at least about 50 nanometers (nm).
103. The apparatus of Claim 102, wherein the distance between the given sensor of the array and the nearest neighbor sensor is at least about 150 nm.
104. The apparatus of Claim 98, wherein a distance between a given sensor of the array and a nearest neighbor sensor is less than about 50 micrometers.
105. The apparatus of Claim 98, wherein the array comprises at least about 10 sensors.
106. A method for analyzing a binding energy between a target analyte and a capture probe, comprising:

(a) activating at least one sensor comprising one or more surfaces between two or more electrodes, wherein the one or more surfaces comprises an immobilized capture probe;
(b) bringing the one or more surfaces in contact with a solution containing or suspected of containing a target analyte under conditions sufficient to permit the target analyte to bind to the immobilized capture probe;
(c) applying a voltage across the two or more electrodes that is sufficient to exert an applied force of at least about 1 piconewton (pN) on the target analyte, to release the target analyte form the immobilized capture probe; and
(d) detecting a signal indicative of the binding energy between the target analyte and the capture probe.

107. The method of Claim 106, wherein the applied force is at least about 10 pN.

108. The method of Claim 106, wherein the voltage is sufficient to release the target analyte, but not a non-target analyte, from the one or more surfaces.

109. The method of Claim 106, wherein the voltage is sufficient to generate an electric field having a strength of at least about $10^3$ volts per meter.
FIG. 1B
FIG. 2B
FIG. 6C
FIG. 7
FIG. 8C
FIG. 8E
FIG. 12A
FIG. 13A
FIG. 13B
Types of Cross Reactivity possible

**FIG. 14**
FIG. 16
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12M 1/34 (2016.01)

CPC - G01N 33/54366; G01N 33/54373; B01J 2219/00722

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8) - C12M 1/34 (2016.01)

CPC-G01 N 33/54366; G01N 33/54373; B01J 2219/00722

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

USPC-435/287.1 .287.2

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PatBase, Google Patents, Google Scholar (without Patents)

Keywords: detecting target analyte sensor electrodes immobilized capture probes immobilized capture probes applying voltage detecting signal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>US 2011/0031 123 A1 (Schulze et al.) 10 February 2011 (10.02.2011) Abstract; para [0001], para [0018], para [0008], para [0009], para [0025], para [0069], para [0097], entire document</td>
<td>1-57,106-109</td>
</tr>
</tbody>
</table>

Further documents are listed in the continuation of Box C.

* Special categories of cited documents:
  "A" document defining the general state of the art which is not considered to be of particular relevance
  "E" earlier application or patent but published on or after the international filing date
  "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
  "O" document referring to an oral disclosure, use, exhibition or other means
  "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"G" document member of the same patent family

Date of the actual completion of the international search: 05 July 2016

Date of mailing of the international search report: 08 AUG 2016

Name and mailing address of the ISA/US:

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-8300

Authorized officer: Lee W. Young

Form PCT/ISA/210 (second sheet) (January 2015)
This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

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

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

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

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

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

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

4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-57, 106-109

Remark on Protest

- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: Claims 1-57,106-109 is directed towards a method and system for detecting a presence of a target analyte; the method comprising: (a) activating at least one sensor comprising one or more surfaces between two or more electrodes, wherein the one or more surfaces comprise two or more immobilized capture probes; (b) bringing the one or more surfaces in contact with a solution containing or suspected of containing a target analyte and a non-target analyte, under conditions sufficient to permit the target analyte and non-target analyte to each bind to a given one of the two or more immobilized capture probes; (c) applying a voltage across the two or more electrodes that is sufficient to release the non-target analyte, but not the target analyte, from a given one of the two or more immobilized capture probes; and (d) detecting a signal indicative of a presence and/or binding energy of the target analyte on the one or more surfaces; and the system comprising at least one sensor; and a controller that is operably coupled to the two or more electrodes, wherein the controller is programmed to (i) apply a voltage across the two or more electrodes that is sufficient to release the non-target analyte, but not the target analyte, from a given one of the two or more immobilized capture probes, and (ii) detect a signal indicative of a presence and/or binding energy of the target analyte on the one or more surfaces.

Group II: Claims 58-105 is directed towards an apparatus for detecting a presence of an analyte, comprising: a fluidic chamber adapted to contain an electrolyte; two or more electrodes capable of generating an electric field within the fluidic chamber; at least one surface comprising an immobilized capture probe capable of binding the analyte, wherein the at least one surface is between the two or more electrodes and in contact with the fluidic chamber, and wherein the at least one surface comprises an electrically conducting layer and an electrically insulating layer; and at least one field confining feature proximal to the at least one surface, which at least one field confining feature is capable of concentrating an electric field surrounding the at least one field confining feature to a strength of at least about 103 volts per meter (V/m).

The inventions listed as Groups I-II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features:

Group I requires a method and system for detecting a presence of a target analyte comprising activating at least one sensor comprising one or more surfaces between two or more electrodes, wherein the one or more surfaces comprise two or more immobilized capture probes; bringing the one or more surfaces in contact with a solution containing or suspected of containing a target analyte and a non-target analyte, under conditions sufficient to permit the target analyte and non-target analyte to each bind to a given one of the two or more immobilized capture probes; applying a voltage across the two or more electrodes that is sufficient to release the non-target analyte, but not the target analyte, from a given one of the two or more immobilized capture probes; and detecting a signal indicative of a presence and/or binding energy of the target analyte on the one or more surfaces, not required by groups II.

Group II requires an apparatus for detecting a presence of an analyte comprising a fluidic chamber adapted to contain an electrolyte; two or more electrodes capable of generating an electric field within the fluidic chamber; at least one surface comprising an immobilized capture probe capable of binding the analyte, wherein the at least one surface is between the two or more electrodes and in contact with the fluidic chamber, and wherein the at least one surface comprises an electrically conducting layer and an electrically insulating layer; and at least one field confining feature proximal to the at least one surface, which at least one field confining feature is capable of concentrating an electric field surrounding the at least one field confining feature to a strength of at least about 103 volts per meter (V/m), not required by groups I.

Shared Technical Features:

Groups I-II share the common feature of an immobilized capture probe comprising a at least one surface between two or more electrodes that can bind an analyte. However, these shared technical features do not represent a contribution over prior art, because the shared technical feature is anticipated by US 201 1/0031 123 A1 to Schulze et al. (hereinafter Schulze). Schulze discloses an immobilized capture probe to bind an analyte (para [0072]). The analyte may bind directly to the binding phase or the solid phase may comprise a capture probe which is specific for the analyte to be detected and capable of reacting with the analyte to capture it on the solid phase comprising at least one surface (para [0003], improved methods for binding target analytes to a capture probe or solid surface) between two or more electrodes (para [0018], plurality of electrodes and an electric field source for applying at least two alternating fields across the medium and Figures 1 and 11 showing the electrodes having a gap where the binding takes place).

As the shared technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups. Therefore, Groups I-II lack unity under PCT Rule 13