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(54) Title: SURFACE-ENHANCED LASER DESORPTION/IONIZATION-BASED DISEASE DIAGNOSIS

(57) Abstract: The present invention provides methods of aiding in a disease diagnosis or prognosis. The methods include cap-
turing antibodies in samples derived from subjects on probes with antigens that are indicative of particular diseases. The methods
also include detecting the captured antibodies by at least one version of surface enhanced laser desorption/ionization gas phase ion
spectrometry. The invention further provides probes and kits for performing the methods of the invention.

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5 **SURFACE-ENHANCED LASER DESORPTION/IONIZATION-BASED
DISEASE DIAGNOSIS**

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CROSS-REFERENCES TO RELATED APPLICATIONS

[0002] This application claims the benefit of U.S. Provisional Application No. 15 60/471,229, filed May 16, 2003, the disclosure of which is incorporated by reference in its entirety for all purposes.

FIELD OF THE INVENTION

[0003] The present invention relates generally to disease diagnosis or prognosis, and more particularly, to methods, probes, and kits for surface-enhanced laser 20 desorption/ionization-based disease diagnosis or prognosis.

BACKGROUND OF THE INVENTION

[0004] Many immunoassay procedures for detecting antibodies in body fluids are known in the immunoassay art and are commonly used in the diagnosis of disease. For example, radio-immunoassay (RIA) techniques are numerous and have been shown 25 to be sensitive. In addition, numerous enzyme-immunoassay (EIA) techniques are also known, including competitive, double antibody solid phase, and sandwich procedures. EIAs typically include color formation as an indicator of a result and the degree of color formation has been used for quantitative determinations. Colorimeters have been used for automatic quantitative determinations based on color gradations of analytical 30 solutions. RIAs and EIAs have both been performed using various solid supports, including finely divided cellulose, solid beads or discs, polystyrene tubes, and microtiter plates.

[0005] Dot enzyme-linked immunosorbent assays using nitrocellulose filters as the solid phase have also been described, *e.g.*, by Hawkes et al. (1982) "A Dot-Immunobinding Assay for Mono-clonal and Other Antibodies," Anal. Biochem. 119:142-147. More specifically, Hawkes et al. described putting antigen spots on
5 nitrocellulose filters, cutting out areas containing spots, and either placing the cut-out portions in the wells of microtiter plates for enzyme immunoassay or, where a range of different antigens are to be screened, using nitrocellulose strips. Hawkes et al. also described a quantitative procedure in which reflectance of spots was determined by a thin layer scanner. Similarly, Pappas et al. (1983) "Dot Enzyme-Linked
10 Immunosorbent Assay (Dot-ELISA): a Micro Technique for the Rapid Diagnosis of Visceral Leishmaniasis," J. Imm. Meth. 64:205-214, have described a dot assay for parasites.

[0006] The many known immunoassays, while providing in some instances high sensitivity, suffer from a variety of drawbacks. For example, radio-immunoassay
15 procedures require the handling and disposal of radioactive materials. Many enzyme-immunoassay procedures produce soluble color solutions and typically require long incubation periods that limit assay throughput. In addition, the nitrocellulose-based dot tests utilize solid supports that can absorb reactants, which often leads to background color and reduced sensitivity. Moreover, to provide quantitative information for
20 samples many preexisting immunoassays require multiple dilutions, which are labor intensive, time-consuming, and expensive to perform.

[0007] From the foregoing, it is apparent that additional immunoassays that do not suffer from the limitations of preexisting techniques are desirable, particularly for the diagnosis or prognosis of disease. These and a variety of additional features of the
25 present invention will become evident upon a complete review of the following.

SUMMARY OF THE INVENTION

[0008] The present invention generally relates to methods of aiding in disease diagnosis or prognosis that can be performed with greater sensitivity and higher throughput than preexisting protocols. The methods include profiling antibodies and/or
30 other disease indicia in samples derived from subjects. The profiling described herein includes detecting, and in preferred embodiments, quantifying specifically captured analyte using surface enhanced laser desorption/ionization (SELDI) gas phase ion

spectrometry. The invention further provides probes and kits for performing these methods.

[0009] In one aspect, the present invention relates to a method of aiding in a disease diagnosis. The method includes (a) capturing one or more antibodies (*e.g.*,
5 autoantibodies, *etc.*), if any, present in at least one sample derived from a subject (*e.g.*, a mammalian subject, such as a human) on a probe (*e.g.*, a biochip or the like) with one or more antigens indicative of the disease (*e.g.*, an autoimmune disease, an infectious disease, prion diseases, *etc.*), which antigens specifically bind the antibodies. The sample generally includes a tissue extract or a biological fluid derived from the subject.
10 In some embodiments, the probe includes at least two different antigens that are each indicative of the disease. The method also includes (b) detecting the captured antibodies, if any, by at least one version of surface enhanced laser desorption/ionization gas phase ion spectrometry to provide antibody capture data. In addition, the method also includes (c) correlating the antibody capture data with a
15 probable diagnosis of the disease or a negative diagnosis for the subject. In preferred embodiments, the version of surface enhanced laser desorption/ionization gas phase ion spectrometry comprises surface enhanced laser desorption/ionization mass spectrometry.

[0010] In certain embodiments, a matrix material is applied to the probe before
20 laser desorption/ionization. Optionally, the probe comprises a surface-enhanced neat desorption probe or another type of surface enhanced laser desorption/ionization probe. The probe is generally derivatized with one or more capture molecules that capture the antigens, which antigens are captured by the capture molecules before or after capturing the antibodies in the sample. To illustrate, the capture molecules optionally
25 include, *e.g.*, linker molecules, receptors, linker antibodies, Protein A, Protein G, mercaptoheterocyclic ligands, or the like.

[0011] Essentially any antigen that is indicative of the disease under consideration can be utilized to perform this method. To illustrate, the antigens are optionally selected from, *e.g.*, organic molecules, inorganic molecules, allergens,
30 biomolecules, nucleic acids, proteins, peptides, peptide nucleic acids, prions, haptens, hapten-carrier conjugates, carbohydrates, lipids, and the like. In certain embodiments, the antigens are covalently attached to the probe, whereas in others the antigens are non-covalently attached to the probe.

[0012] In some embodiments of the invention, the probe further includes one or more affinity reagents that specifically bind one or more inflammation markers. In these embodiments, the method further includes capturing and detecting the inflammation markers, if any, present in the sample to provide inflammation marker capture data, and correlating the inflammation marker capture data with the probable diagnosis of the disease or the negative diagnosis for the subject. The inflammation markers typically comprise cytokines, leukotrienes, and/or the like.

[0013] In preferred embodiments, detecting further includes quantifying the captured antibodies such that the antibody capture data comprises quantified antibody data. In these embodiments, the captured antibodies are optionally quantified by a quantitative surface scanning technique among other approaches. The correlating typically includes comparing the quantified antibody data to at least one control. For example, an amount of antibody above the control correlates with a positive diagnosis of the disease.

[0014] In another aspect, the invention provides a method of aiding in a disease prognosis. The method includes (a) profiling at least a first sample derived from a subject diagnosed with a disease in which profiling comprises detecting antibodies in the first sample using the method described above. The method further includes (b) profiling at least a second sample derived from the subject diagnosed with the disease in which profiling also includes detecting the antibodies in the second sample using the method described above. In addition, the method also includes (c) comparing the relative amounts of the antibodies in the first and second samples detected by profiling, thereby aiding in the prognosis of the disease for the subject.

[0015] In still other aspects, the invention also provides probes and kits that can be used to perform the methods described herein. More specifically, the invention provides a probe that includes a biochip derivatized with one or more antigens indicative of a disease, which antigens specifically bind antibodies present in a sample. The kit includes (a) a probe that includes a biochip derivatized with one or more capture molecules that specifically bind at least one antigen indicative of a disease. In addition, the kit also includes (b) instructions to capture antibodies from a sample with the antigen, which antigen specifically binds the antibodies, and to capture the captured antibodies on the probe.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] Figure 1 schematically shows one embodiment of a surface enhanced laser desorption/ionization assay to determine the presence of antibodies in a sample.

[0017] Figure 2 schematically shows another embodiment of a surface

5 enhanced laser desorption/ionization assay to determine the presence of antibodies in a sample.

[0018] Figure 3 schematically illustrates a surface enhanced laser desorption/ionization time-of-flight mass spectrometry system according to one embodiment of the invention.

10 DETAILED DISCUSSION OF THE INVENTION

I. DEFINITIONS

[0019] As used herein, the terms set forth with particularity below and grammatical variations used herein have the following definitions. If not otherwise defined, all terms used herein have the meaning commonly understood by a person
15 skilled in the art to which this invention pertains.

[0020] "Gas phase ion spectrometer" refers to an apparatus that detects gas phase ions. Gas phase ion spectrometers include an ion source that supplies gas phase ions. Gas phase ion spectrometers include, for example, mass spectrometers, ion mobility spectrometers, and total ion current measuring devices. "Gas phase ion
20 spectrometry" refers to the use of a gas phase ion spectrometer to detect gas phase ions.

[0021] "Mass spectrometer" refers to a gas phase ion spectrometer that measures a parameter that can be translated into mass-to-charge ratios of gas phase ions. Mass spectrometers generally include an ion source and a mass analyzer. Examples of mass spectrometers are time-of-flight, magnetic sector, quadrupole filter,
25 ion trap, ion cyclotron resonance, electrostatic sector analyzer and hybrids of these.

"Mass spectrometry" refers to the use of a mass spectrometer to detect gas phase ions.

[0022] "Laser desorption mass spectrometer" refers to a mass spectrometer that uses laser energy as a means to desorb, volatilize, and ionize an analyte.

[0023] "Tandem mass spectrometer" refers to any mass spectrometer that is

30 capable of performing two successive stages of m/z -based discrimination or measurement of ions, including ions in an ion mixture. The phrase includes mass spectrometers having two mass analyzers that are capable of performing two successive

stages of m/z -based discrimination or measurement of ions tandem-in-space. The phrase further includes mass spectrometers having a single mass analyzer that is capable of performing two successive stages of m/z -based discrimination or measurement of ions tandem-in-time. The phrase thus explicitly includes Qq-TOF mass spectrometers, ion trap mass spectrometers, ion trap-TOF mass spectrometers, TOF-TOF mass spectrometers, Fourier transform ion cyclotron resonance mass spectrometers, electrostatic sector – magnetic sector mass spectrometers, and combinations thereof.

[0024] “Mass analyzer” refers to a sub-assembly of a mass spectrometer that comprises means for measuring a parameter that can be translated into mass-to-charge ratios of gas phase ions. In a time-of-flight mass spectrometer the mass analyzer comprises an ion optic assembly, a flight tube and an ion detector.

[0025] “Ion source” refers to a sub-assembly of a gas phase ion spectrometer that provides gas phase ions. In one embodiment, the ion source provides ions through a desorption/ionization process. Such embodiments generally comprise a probe interface that positionally engages a probe in an interrogatable relationship to a source of ionizing energy (*e.g.*, a laser desorption/ionization source) and in concurrent communication at atmospheric or subatmospheric pressure with a detector of a gas phase ion spectrometer.

[0026] Forms of ionizing energy for desorbing/ionizing an analyte from a solid phase include, for example: (1) laser energy; (2) fast atoms (used in fast atom bombardment); (3) high energy particles generated via beta decay of radionuclides (used in plasma desorption); and (4) primary ions generating secondary ions (used in secondary ion mass spectrometry). The preferred form of ionizing energy for solid phase analytes is a laser (used in laser desorption/ionization), in particular, nitrogen lasers, Nd-Yag lasers and other pulsed laser sources. “Fluence” refers to the energy delivered per unit area of interrogated image. A high fluence source, such as a laser, will deliver about $1 \text{ mJ} / \text{mm}^2$ to $50 \text{ mJ} / \text{mm}^2$. Typically, a sample is placed on the surface of a probe, the probe is engaged with the probe interface and the probe surface is struck with the ionizing energy. The energy desorbs analyte molecules from the surface into the gas phase and ionizes them.

[0027] Other forms of ionizing energy for analytes include, for example: (1) electrons that ionize gas phase neutrals; (2) strong electric field to induce ionization

from gas phase, solid phase, or liquid phase neutrals; and (3) a source that applies a combination of ionization particles or electric fields with neutral chemicals to induce chemical ionization of solid phase, gas phase, and liquid phase neutrals.

[0028] “Probe” in the context of this invention refers to a device adapted to engage a probe interface of a gas phase ion spectrometer (*e.g.*, a mass spectrometer) and to present an analyte to ionizing energy for ionization and introduction into a gas phase ion spectrometer, such as a mass spectrometer. A “probe” will generally comprise a solid substrate (either flexible or rigid) comprising a sample presenting surface on which an analyte is presented to the source of ionizing energy.

[0029] “Surface-enhanced laser desorption/ionization” or “SELDI” refers to a method of desorption/ionization gas phase ion spectrometry (*e.g.*, mass spectrometry) in which the analyte is captured on the surface of a SELDI probe that engages the probe interface of the gas phase ion spectrometer. In “SELDI MS,” the gas phase ion spectrometer is a mass spectrometer. SELDI technology is described in, *e.g.*, U.S. patent 5,719,060 (Hutchens and Yip) and U.S. patent 6,225,047 (Hutchens and Yip).

[0030] “Surface-Enhanced Affinity Capture” or “SEAC” is a version of SELDI that involves the use of probes comprising an absorbent surface (a “SEAC probe”).

“Adsorbent surface” refers to a surface to which is bound an adsorbent (also called a “capture reagent” or an “affinity reagent”). An adsorbent is any material capable of binding an analyte (*e.g.*, a target polypeptide or nucleic acid). “Chromatographic adsorbent” refers to a material typically used in chromatography. Chromatographic adsorbents include, for example, ion exchange materials, metal chelators (*e.g.*, nitriloacetic acid or iminodiacetic acid), immobilized metal chelates, hydrophobic interaction adsorbents, hydrophilic interaction adsorbents, dyes, simple biomolecules (*e.g.*, nucleotides, amino acids, simple sugars and fatty acids) and mixed mode adsorbents (*e.g.*, hydrophobic attraction/electrostatic repulsion adsorbents).

“Biospecific adsorbent” refers an adsorbent comprising a biomolecule, *e.g.*, a nucleic acid molecule (*e.g.*, an aptamer), a polypeptide, a polysaccharide, a lipid, a steroid or a conjugate of these (*e.g.*, a glycoprotein, a lipoprotein, a glycolipid, a nucleic acid (*e.g.*, DNA)-protein conjugate). In certain instances the biospecific adsorbent can be a macromolecular structure such as a multiprotein complex, a biological membrane or a virus. Examples of biospecific adsorbents are antibodies, receptor proteins and nucleic acids. Biospecific adsorbents typically have higher specificity for a target analyte than

chromatographic adsorbents. Further examples of adsorbents for use in SELDI can be found in U.S. Patent 6,225,047 (Hutchens and Yip, "Use of retentate chromatography to generate difference maps," May 1, 2001).

[0031] In some embodiments, a SEAC probe is provided as a pre-activated surface which can be modified to provide an adsorbent of choice. For example, certain probes are provided with a reactive moiety that is capable of binding a biological molecule through a covalent bond. Epoxide and carbodiimidazole are useful reactive moieties to covalently bind biospecific adsorbents such as antibodies or cellular receptors.

[0032] "Adsorption" refers to detectable non-covalent binding of an analyte to an adsorbent or capture reagent.

[0033] "Surface-Enhanced Neat Desorption" or "SEND" is a version of SELDI that involves the use of probes comprising energy absorbing molecules chemically bound to the probe surface. ("SEND probe.") "Energy absorbing molecules" ("EAM") refer to molecules that are capable of absorbing energy from a laser desorption/ionization source and thereafter contributing to desorption and ionization of analyte molecules in contact therewith. The phrase includes molecules used in MALDI, frequently referred to as "matrix", and explicitly includes cinnamic acid derivatives, sinapinic acid ("SPA"), cyano-hydroxy-cinnamic acid ("CHCA") and dihydroxybenzoic acid, ferulic acid, hydroxyacetophenone derivatives, as well as others. It also includes EAMs used in SELDI. SEND is further described in United States patent 5,719,060 and United States patent application 60/408,255, filed September 4, 2002 (Kitagawa, "Monomers And Polymers Having Energy Absorbing Moieties Of Use In Desorption/Ionization Of Analytes").

[0034] "Surface-Enhanced Photolabile Attachment and Release" or "SEPAR" is a version of SELDI that involves the use of probes having moieties attached to the surface that can covalently bind an analyte, and then release the analyte through breaking a photolabile bond in the moiety after exposure to light, *e.g.*, laser light. SEPAR is further described in United States patent 5,719,060.

[0035] "Eluant" or "wash solution" refers to an agent, typically a solution, which is used to affect or modify adsorption of an analyte to an adsorbent surface and/or remove unbound materials from the surface. The elution characteristics of an

eluant can depend, for example, on pH, ionic strength, hydrophobicity, degree of chaotropism, detergent strength and temperature.

[0036] "Analyte" refers to any component of a sample that is desired to be detected. The term can refer to a single component or a plurality of components in the sample.

[0037] The "complexity" of a sample adsorbed to an adsorption surface of an affinity capture probe means the number of different protein species that are adsorbed.

[0038] "Molecular binding partners" and "specific binding partners" refer to pairs of molecules, typically pairs of biomolecules that exhibit specific binding.

10 Molecular binding partners include, without limitation, receptor and ligand, antibody and antigen, biotin and avidin, and biotin and streptavidin.

[0039] "Monitoring" refers to recording changes in a continuously varying parameter.

[0040] "Solid support" refers to a solid material which can be derivatized with, or otherwise attached to, a chemical moiety, such as a capture reagent, a reactive moiety or an energy absorbing species. Exemplary solid supports include chips (*e.g.*, probes), microtiter plates and chromatographic resins.

[0041] "Chip" refers to a solid support having a generally planar surface to which a chemical moiety may be attached. Chips that are adapted to engage a probe interface are also called "probes."

[0042] "Biochip" refers to a chip to which a chemical moiety is attached. Frequently, the surface of the biochip comprises a plurality of addressable locations, each of which location has the chemical moiety attached there.

[0043] "Protein biochip" refers to a biochip adapted for the capture of polypeptides. Many protein biochips are described in the art. These include, for example, protein biochips produced by CIPHERGEN Biosystems (Fremont, CA), Packard BioScience Company (Meriden CT), Zyomyx (Hayward, CA) and Phyllos (Lexington, MA). Examples of such protein biochips are described in the following patents or patent applications: U.S. patent 6,225,047 (Hutchens and Yip, "Use of retentate chromatography to generate difference maps," May 1, 2001); International publication WO 99/51773 (Kuimelis and Wagner, "Addressable protein arrays," October 14, 1999); U.S. patent 6,329,209 (Wagner et al., "Arrays of protein-capture agents and

methods of use thereof,” December 11, 2001) and International publication WO 00/56934 (Englert et al., “Continuous porous matrix arrays,” September 28, 2000).

[0044] Protein biochips produced by CIPHERGEN Biosystems comprise surfaces having chromatographic or biospecific adsorbents attached thereto at addressable
5 locations. CIPHERGEN ProteinChip® arrays include NP20, H4, H50, SAX-2, Q-10, WCX-2, CM-10, IMAC-3, IMAC-30, LSAX-30, LWCX-30, IMAC-40, PS-10, PS-20 and PG-20. These protein biochips comprise an aluminum substrate in the form of a strip. The surface of the strip is coated with silicon dioxide.

[0045] In the case of the NP-20 biochip, silicon oxide functions as a hydrophilic
10 adsorbent to capture hydrophilic proteins.

[0046] H4, H50, SAX-2, Q-10, WCX-2, CM-10, IMAC-3, IMAC-30, PS-10 and PS-20 biochips further comprise a functionalized, cross-linked polymer in the form of a hydrogel physically attached to the surface of the biochip or covalently attached through a silane to the surface of the biochip. The H4 biochip has isopropyl
15 functionalities for hydrophobic binding. The H50 biochip has nonylphenoxy-poly(ethylene glycol)methacrylate for hydrophobic binding. The SAX-2 and Q-10 biochips have quaternary ammonium functionalities for anion exchange. The WCX-2 and CM-10 biochips have carboxylate functionalities for cation exchange. The IMAC-3 and IMAC-30 biochips have nitriloacetic acid functionalities that adsorb transition
20 metal ions, such as Cu^{++} and Ni^{++} , by chelation. These immobilized metal ions allow adsorption of peptide and proteins by coordinate bonding. The PS-10 biochip has carboimidazole functional groups that can react with groups on proteins for covalent binding. The PS-20 biochip has epoxide functional groups for covalent binding with proteins. The PS-series biochips are useful for binding biospecific adsorbents, such as
25 antibodies, receptors, lectins, heparin, Protein A, biotin/streptavidin and the like, to chip surfaces where they function to specifically capture analytes from a sample. The PG-20 biochip is a PS-20 chip to which Protein G is attached. The LSAX-30 (anion exchange), LWCX-30 (cation exchange) and IMAC-40 (metal chelate) biochips have functionalized latex beads on their surfaces. Such biochips are further described in:
30 WO 00/66265 (Rich et al., “Probes for a Gas Phase Ion Spectrometer,” November 9, 2000); WO 00/67293 (Beecher et al., “Sample Holder with Hydrophobic Coating for Gas Phase Mass Spectrometer,” November 9, 2000); U.S. patent application US 2003 0032043 A1 (Pohl and Papanu, “Latex Based Adsorbent Chip,” July 16, 2002) and U.S.

patent application 60/350,110 (Um et al., "Hydrophobic Surface Chip," November 8, 2001); U.S. patent application 60/367,837, (Boschetti et al., "Biochips With Surfaces Coated With Polysaccharide-Based Hydrogels," May 5, 2002) and U.S. patent application entitled "Photocrosslinked Hydrogel Surface Coatings" (Huang et al., filed
5 February 21, 2003).

[0047] Upon capture on a biochip, analytes can be detected by a variety of detection methods selected from, for example, a gas phase ion spectrometry method, an optical method, an electrochemical method, atomic force microscopy and a radio frequency method. Gas phase ion spectrometry methods are described herein. Of
10 particular interest is the use of mass spectrometry and, in particular, SELDI. Optical methods include, for example, detection of fluorescence, luminescence, chemiluminescence, absorbance, reflectance, transmittance, birefringence or refractive index (*e.g.*, surface plasmon resonance, ellipsometry, a resonant mirror method, a grating coupler waveguide method or interferometry). Optical methods include
15 microscopy (both confocal and non-confocal), imaging methods and non-imaging methods. Immunoassays in various formats (*e.g.*, ELISA) are popular methods for detection of analytes captured on a solid phase. Electrochemical methods include voltametry and amperometry methods. Radio frequency methods include multipolar resonance spectroscopy.

20 [0048] The "sensitivity" of a device or method is a measure of its ability to discriminate between small differences in analyte concentrations.

[0049] "Biological sample" and "sample" identically refer to a sample derived from at least a portion of a multicellular eukaryotic subject. The biological sample can derive from the entirety of the organism or a portion thereof, including from a cultured
25 portion thereof. Biological samples can be in any physical form appropriate to the context, including homogenate, subcellular fractionate, lysate and fluid.

[0050] "Biomolecule" refers to a molecule that can be found in, but need not necessarily have been derived from, a biological sample. This includes molecules, such as nucleotides, amino acids, sugars, fatty acids, steroids, nucleic acids, polypeptides,
30 peptides, peptide fragments, carbohydrates, lipids, and combinations of these (*e.g.*, glycoproteins, ribonucleoproteins, lipoproteins, or the like).

[0051] The terms "polypeptide", "peptide", and "protein" are used interchangeably herein to refer to a naturally-occurring or synthetic polymer

comprising amino acid monomers (residues), where amino acid monomer here includes naturally-occurring amino acids, naturally-occurring amino acid structural variants, and synthetic non-naturally occurring analogs that are capable of participating in peptide bonds. Polypeptides can be modified, *e.g.*, by the addition of carbohydrate residues to form glycoproteins. The terms “polypeptide”, “peptide”, and “protein” include glycoproteins as well as non-glycoproteins.

[0052] “Polynucleotide” and “nucleic acid” equivalently refer to a naturally-occurring or synthetic polymer comprising nucleotide monomers (bases).

Polynucleotides include naturally-occurring nucleic acids, such as deoxyribonucleic acid (“DNA”) and ribonucleic acid (“RNA”), as well as nucleic acid analogs. Nucleic acid analogs include those which include non-naturally occurring bases, and those in which nucleotide monomers are linked other than by the naturally-occurring phosphodiester bond. Nucleotide analogs include, for example and without limitation, phosphorothioates, phosphorodithioates, phosphorotriesters, phosphoramidates, boranophosphates, methylphosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, peptide-nucleic acids (PNAs), and the like.

[0053] “Receptor” refers to a molecule, typically a macromolecule, that can be found in, but need not necessarily have been derived from, a biological sample, and that can participate in specific binding with a ligand. The term further includes fragments and derivatives that remain capable of specific ligand binding.

[0054] “Ligand” refers to any compound that can participate in specific binding with a designated receptor or antibody.

[0055] “Antibody” refers to a polypeptide substantially encoded by at least one immunoglobulin gene or fragments of at least one immunoglobulin gene, that can participate in specific binding with a ligand. The term includes naturally-occurring forms, as well as fragments and derivatives. Fragments within the scope of the term as used herein include those produced by digestion with various peptidases, such as Fab, Fab' and F(ab)'₂ fragments, those produced by chemical dissociation, by chemical cleavage, so long as the fragment remains capable of specific binding to a target molecule, such as an antigen indicative of a disease.

[0056] “Autoantibody” refers to an antibody that specifically binds to self-antigens.

[0057] “Antigen” refers to a ligand that can be bound by an antibody. An antigen need not be immunogenic. For example, haptens are typically not immunogenic in the absence of a coupled carrier. The portions of the antigen that make contact with the antibody are denominated “epitopes”.

5 [0058] The phrase “antigen indicative of a disease” refers to an antigen that relates to the cause or source of the disease. To illustrate, an antigen indicative of a disease can be, *e.g.*, derived from a pathogen that causes an infectious disease, a self-antigen related to an autoimmune disease, an allergen that induces a hypersensitivity reaction or allergic response, a prion that causes a prion disease, or the like.

10 [0059] “Specific binding” refers to the ability of at least two molecular species simultaneously present in a heterogeneous (inhomogeneous) sample to bind to one another preferentially over binding to other molecular species in the sample. For example, an antibody specifically binds to one or more antigens (*e.g.*, antigens indicative of a disease, *etc.*) bearing the epitope for which the antibody has antigenic
15 specificity. Typically, a specific binding interaction will discriminate over adventitious binding interactions in the reaction by at least two-fold, more typically more than 10- to 100-fold. When used to detect analyte, specific binding is sufficiently discriminatory when determinative of the presence of the analyte in a heterogeneous (inhomogeneous) sample. Typically, the affinity or avidity of a specific binding reaction is least about
20 10^{-7} M, with specific binding reactions of greater specificity typically having affinity or avidity of at least 10^{-8} M to at least about 10^{-9} M.

[0060] The term “attached,” as used herein, encompasses interactions including, but not limited to, covalent bonding, ionic bonding, chemisorption, physisorption, and combinations thereof.

25 [0061] The term “disease” refers to an impairment of the normal state of a living organism, such as an animal, or one of its parts that interrupts or modifies the performance of vital functions and is a response, *e.g.*, to environmental factors (*e.g.*, as allergens, malnutrition, industrial hazards, climate, *etc.*), to specific infectious agents (*e.g.*, bacteria, viruses, *etc.*), to inherited defects (*e.g.*, genetic anomalies, *etc.*), or to
30 combinations of these factors.

[0062] The term “autoimmune disease” refers to a disease produced by an abnormal immune response against self-antigens of a subject.

[0063] The term “infectious disease” refers to a disease resulting from a pathogen, or a portion thereof, that infects or is otherwise introduced into a subject.

[0064] The term “prion disease” refers to a disease caused by a prion.

II. DESCRIPTION OF THE PREFERRED EMBODIMENTS

5 [0065] The present invention relates to the qualitative and quantitative detection of antibodies in samples derived from subjects to aid in the diagnosis and prognosis of disease. More specifically, the multiplexed detection methods of the invention combine the specificity of, e.g., antigen-antibody interaction assays with the resolving power and sensitivity of surface enhanced laser desorption/ionization (SELDI) gas
10 phase ion spectrometry, including SELDI-mass spectrometry. In addition to greater sensitivity, the methods of the present invention can be performed with significantly higher throughput than preexisting approaches, such as RIAs, EIAs, and other immunoassays.

[0066] In overview, the methods of the present invention include a method of
15 aiding in a disease diagnosis. The method includes (a) capturing one or more antibodies, if any, present in at least one sample derived from a subject on a probe with one or more antigens indicative of the disease, which antigens specifically bind the antibodies. In preferred embodiments, a plurality of different antigens, each indicative of the disease, are utilized, e.g., to provide added diagnostic assurance or verification.
20 The method also includes (b) detecting the captured antibodies, if any, by at least one version of SELDI gas phase ion spectrometry to provide antibody capture data. In addition, the method also includes (c) correlating the antibody capture data with a probable diagnosis of the disease or a negative diagnosis for the subject.

[0067] The invention also provides a method of aiding in a disease prognosis,
25 which includes (a) profiling at least a first sample derived from a subject diagnosed with a disease and (b) profiling at least a second sample derived from the subject diagnosed with the disease. The profiling of (a) and (b) includes detecting antibodies in the first and second samples using the method described above. The second sample is typically derived from the subject at a time that is subsequent to the first sample being
30 derived from the subject, e.g., during course of treatment to monitor the efficacy of the treatment. Accordingly, the method also includes (c) comparing the relative amounts

of the antibodies in the first and second samples detected by profiling to aid in the prognosis of the disease for the subject.

[0068] Essentially any disease can be diagnosed or prognosticated using essentially any antigen that is indicative of the particular disease under consideration with the methods described herein. Accordingly, no attempt is made in this disclosure to describe all of the possible diseases, and antigens indicative thereof, that are the subject of the present invention. Based upon the description provided herein, one skilled in the art will readily appreciate how the methods, probes, and kits of the invention can be adapted to the specific disease to be diagnosed or prognosticated.

10 Nonetheless, certain diseases and indicative antigens are described or referred to for purposes of illustration, but not to limit the present invention. In particular, certain general disease classifications that can be analyzed according to the methods of the invention include, *e.g.*, autoimmune diseases, infectious diseases, prion diseases, and the like. In certain cases, there may be overlap among these general classifications. In addition, the antigens used in the methods of the invention are generally selected from, *e.g.*, organic molecules, inorganic molecules, allergens, biomolecules, nucleic acids, proteins, peptides, peptide nucleic acids, prions, haptens, hapten-carrier conjugates, carbohydrates, lipids, and the like.

[0069] Exemplary autoimmune diseases that can be diagnosed or prognosticated using the methods of the invention include, *e.g.*, Systemic Lupus Erythematosus (SLE), Systemic Rheumatic Disease, rheumatoid arthritis, diabetes, Sjögren's Syndrome (SS), Progressive Systemic Sclerosis (PSS), Subacute Erythematosus, congenital complete heart block, neonatal complete heart block, Neonatal Lupus Dermatitis, Polymyositis, thyroid autoimmune disorders, mixed connective tissue disease (MCTD), Multiple Sclerosis (MS), and the like.

[0070] To further illustrate, the presence of human autoantibodies to nuclear antigens, for example, antibodies against Smith (Sm), ribosomal nuclear protein (nRNP), RNP/Sm complex, Ro (SS-A), La (SS-B), double-stranded (native) deoxyribonucleic acid (dsDNA), and Scl-70 antigens have been diagnostic when evaluating patients with SLE. In addition, antibodies to dsDNA, single-stranded DNA (ssDNA), Fc portions of human antibodies (rheumatoid factor), whole histones, and histone subclasses (*e.g.*, distinct molecular fractions) have been used for detecting or evaluating systemic rheumatic disease. Antibodies to the Sm antigen are typically less

commonly found in patients with other rheumatic diseases. Antibodies to ribosomal nuclear proteins (nRNP) have also been found in patients with rheumatoid arthritis, SS, PSS, and MCTD. Twenty to thirty percent of the patients with antibodies to Scl-70 antigen have progressive Systemic Sclerosis. Antibodies to Scl-70 are rarely found in patients with other systemic rheumatic diseases. Antibodies to Ro (SS-A) antigen are found in half of Systemic Lupus Erythematosus patients, most patients with SS or Subacute Lupus Erythematosus and nearly all mothers of infants with congenital complete heart block or Neonatal Lupus Dermatitis. Antibodies to the La (SS-B) antigen usually occur in twenty to thirty percent of SS patients and with five to ten percent of SLE patients. Antibodies to Jo-1 antigen are usually found in patients with polymyositis. Antibodies to Ribosomal P antigens are found to occur in five to ten percent of systemic Lupus Erythematosus patients and ninety percent of those patients typically demonstrate signs of lupus psychosis. Antibodies to mitochondrial antigens are typically found in all primary biliary cirrhosis patients. Antibodies to histone antigens (H1, H2A, H2B, H3, H4) are found in ninety-five to one hundred percent of drug-induced Lupus Erythematosus, fifteen to twenty percent rheumatoid arthritis, and thirty percent of all patients with Systemic Lupus Erythematosus. Antibodies to cytoplasmic components of neutrophil granulocytes are present in the serum of patients with acute Wegener's granulomatosis and microscopic polyarteritis. Myeloperoxidase and proteinase 3 are the two major antigens present. Autoantibodies against human thyroglobulin and thyroid peroxidase (microsome) are typically present in subjects with thyroid autoimmune disease. Furthermore, antibodies against glialfibrillary acidic protein (GFAP) and S100beta are generally present in subjects with diabetes, whereas myelin basic protein is typically present in patients with MS. Additional details related to many of these diseases and indicative antigens are found in, *e.g.*, U.S. Pat. Publ. No. 2003/0008410, entitled "IMMUNOASSAY APPARATUS, KIT AND METHODS," published January 9, 2003 by Hechinger and in, *e.g.*, Harley et al. (1988) "Systemic Lupus Erythematosus-Autoantibodies," Rheumatoid Disease Clinics of N. America, 14(1):43, Nakamura et al. (1983) "Autoantibodies to nonhistone nuclear antigens and their clinical significance," Human Pathology 14(5):392, Tan (1983) "Antinuclear antibodies in diagnosis and management," Hospital Practice 18(1):79, and Czarnocka et al. (1985) "Purification of the human thyroid peroxidase and its identification as the microsomal antigen involved in autoimmune thyroid diseases," FEBS 190:147.

[0071] Exemplary infectious diseases that are optionally diagnosed or prognosticated using the methods of the present invention include those caused by bacterial pathogens, such as Systematic Lyme disease caused by an infection with *Borrelia burgdorferi* transmitted by the bite of an infected tick of the genus *Ixodes*,
5 Leptospirosis caused by spirochetes of the genus *Leptospira*, and the like. Antibodies to HMW (P83-100), Flagellin (P41), BmpA (P39), and OspC antigens are typically found in subjects with Systematic Lyme disease, whereas antibodies to serovar patoc 1 strain antigens are generally found in patients with Leptospirosis. Many infectious diseases are also caused by viral sources including, Human Immunodeficiency Virus
10 (HIV), Severe Acute Respiratory Syndrome (SARS) viral strains, influenza virus, herpes simplex virus, CMV, EBV, HbsAg, Hbc, HTLV, HCV, and the like. Antigens indicative of certain of these viral pathogens include, *e.g.*, HTLV, HCV, EBV, HIV, CMV HbsAg, Hbc, Hepatitis Surface, core, HIV/I, HTL VI/II, Hepatitis C, and HIV-1p24 antigens. Many other bacterial, viral, and other infectious diseases and the related
15 antigens are known in the art. Additional details relating to infectious diseases are found in, *e.g.*, U.S. Pat. No. 6,432,633, entitled "IMMUNOASSAY METHOD OF HIV-1P24 ANTIGEN AND REAGENT THEREFOR," issued August 13, 2002 to Yamamoto et al., U.S. Pat. No. 6,265,176, entitled "DOT IMMUNOASSAY ON PLASTIC SHEETS," issued July 24, 2001 to Lin et al., U.S. Pat. Publ. No.
20 2003/0008410, entitled "IMMUNOASSAY APPARATUS, KIT AND METHODS," published January 9, 2003 by Hechinger, and in, *e.g.*, Simpson et al. (1990) "Reactivity of human Lyme borreliosis sera with a 39-kilodalton antigen specific to *Borrelia burgdorferi*," J. Clin. Micro. 28(6):1329, Levett et al. (1998) "Evaluation of the indirect hemagglutination assay for the diagnosis of acute leptospirosis," J. Clin. Microbio. 36:11, and Normile (2003) "Up close and personal with SARS," Science 300:886.

[0072] Prion caused diseases, or transmissible spongiform encephalopathies (TSE), are neurodegenerative disorders that affect both humans and animals. Prion diseases are referred to as sponiform encephalopathies due to the characteristic of
30 forming holes or pores in cranial tissue. Development of prion disease may be the result of mutations in the PrP gene. Inherited prion diseases include Creutzfeldt-Jakob disease (CJD), fatal familial insomnia (FFI) and Gerstmann-Straussler-Scheinker syndrome or disease (GSS) in humans. Prion diseases can also be contracted by an

infectious mechanism. This group of diseases includes iatrogenic CJD and a new variant of CJD, which may be the result of transmission of bovine spongiform encephalopathy (BSE, also referred to as "Mad Cow" disease) from cattle to humans.

Prion diseases are described further in, *e.g.*, U.S. Pat. No. 6,528,269, entitled

5 "IMMUNOLOGICAL AGENTS SPECIFIC FOR PRION PROTEIN (PRP)," issued March 4, 2003 to Sy et al.

[0073] The antigens used in the diagnostic and prognostic methods of the invention are obtained from essentially any source. For example, antigens are optionally chemically synthesized, obtained via recombinant DNA methods, purified
10 from naturally-occurring sources, or the like. To illustrate, samples comprising, *e.g.*, antigens for analysis according to the methods described herein are optionally recovered and purified by any of a number of methods well known in the art, including electrophoresis, chromatography, precipitation, dialysis, filtration, centrifugation, crystallization and/or precipitation. More specifically, purification techniques, such as
15 ultra-centrifugation, ammonium sulfate or ethanol precipitation, acid extraction, ion exchange chromatography, high performance liquid chromatography, size exclusion chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography (*e.g.*, as with C₁-C₁₈ resins), affinity chromatography (*e.g.*, as with immunoaffinity, immobilized metals, dyes, or other tagging systems), hydroxylapatite
20 chromatography, and/or lectin chromatography are optionally used.

[0074] In addition to the references noted herein, a variety of purification methods are well known in the art, including, *e.g.*, those set forth in Sandana, Bioseparation of Proteins, Academic Press, Inc. (1997), Bollag et al., Protein Methods, 2nd Ed., Wiley-Liss (1996), Walker, The Protein Protocols Handbook, Humana Press
25 (1996), Harris and Angal, Protein Purification Applications: A Practical Approach, IRL Press (1990), Harris and Angal (Eds.), Protein Purification Methods: A Practical Approach, IRL Press (1989), Scopes, Protein Purification: Principles and Practice, 3rd Ed., Springer Verlag (1993), Janson and Ryden, Protein Purification: Principles, High Resolution Methods and Applications, 2nd Ed., Wiley-VCH (1998), Walker, Protein
30 Protocols on CD-ROM, Humana Press (1998), Satinder Ahuja ed., Handbook of Bioseparations, Academic Press (2000), and the references cited therein.

[0075] General texts describing additional molecular biological techniques useful herein, including recombinant methods useful in producing certain antigens

include Berger and Kimmel, Guide to Molecular Cloning Techniques, Methods in Enzymology, Vol. 152, Academic Press, Inc. (Berger), Sambrook et al., Molecular Cloning - A Laboratory Manual (2nd Ed.), Vol. 1-3, Cold Spring Harbor Laboratory (1989) (Sambrook), and Current Protocols in Molecular Biology, F.M. Ausubel et al. (Eds.), Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc. (supplemented through 2000) (Ausubel). Methods of transducing cells, including animal cells, with nucleic acids are generally available, as are methods of expressing proteins encoded by such nucleic acids. In addition to Berger, Ausubel and Sambrook, useful general references for culturing animal cells include Freshney, Culture of Animal Cells, a Manual of Basic Technique, 3rd Ed., Wiley-Liss (1994)(Freshney), Humason, Animal Tissue Techniques, 4th Ed., W.H. Freeman and Company (1979), and Ricciardelli et al., In Vitro Cell Dev. Biol. 25:1016-1024 (1989).

[0076] Antibodies and other chemical species can be qualitatively and/or quantitatively detected in essentially any sample derived from a subject using the methods of the present invention. In preferred embodiments, samples are derived from mammalian subjects, particularly human subjects. The samples typically include biological fluids such as blood, serum, plasma, saliva, urine, prostatic fluid, seminal fluid, seminal plasma, lymph, lung/bronchial washes, mucus, feces, nipple secretions, sputum, tears, or the like. Samples also optionally include extracts from biological sources, such as organ extracts, *etc.* In addition, biological samples such as these are optionally collected according to any known technique, such as venipuncture, biopsy, or the like. The specific exemplary sample sources listed herein are offered to illustrate but not to limit the present invention. Additional sources of samples are known in the art and are readily obtainable.

[0077] While a sample can be analyzed directly, in certain embodiments, the methods include fractionating biomolecules in an initial sample by one or a combination of fractionation techniques described above or otherwise known in the art to be useful for separating biomolecules to collect a sample fraction that may include the antibodies prior to mass profiling. Fractionation is typically utilized to decrease the complexity of analytes in the sample to assist detection and characterization of antibodies and/or inflammation markers, such as cytokines, leukotrienes, and the like. Moreover, fractionation protocols can provide additional information regarding

physical and chemical characteristics of biomolecular components in a sample. For example, if a sample is fractionated using an anion-exchange spin column, and if an antibody is eluted at a certain pH, this elution characteristic provides information regarding binding properties of the antibody. In another example, a sample can be
5 fractionated to remove proteins or other molecules in the sample that are present in a high quantity and/or which would otherwise interfere with the detection of a particular antibody, if present (*e.g.*, antibodies that are expressed only at low levels in the subject, such as at the outset of a particular infection).

[0078] Prior to profiling antibody and/or inflammation marker masses in a
10 sample by gas phase ion spectroscopy, for example, proteins in the samples of the invention are optionally fragmented or digested. This approach is particularly useful when components (*e.g.*, antibodies, *etc.*) of a sample are to be identified.

Fragmentation is optionally effected using any technique that produces peptide fragments from proteins in a sample. Many of these techniques are generally known in
15 the art. For example, proteins are optionally fragmented enzymatically, chemically, or physically. In certain embodiments of the invention, antibodies, inflammation markers, and/or peptide fragments resulting from fragmentation are optionally modified to improve resolution upon detection. In other embodiments, the fragmentation of biomolecular components of a sample can be performed "on chip" in a SELDI
20 environment by placing an aliquot of the sample on an adsorbent spot and adding, *e.g.*, the proteolytic agent to the material on the spot. Additional details relating to the identification of biomolecules via fragmentation are described in, *e.g.*, International Publication No. WO 02/074927 entitled "High accuracy protein identification" by Pham.

[0079] The antigens of the invention are optionally bound to or otherwise
25 immobilized on probes (*e.g.*, biochips, *etc.*) either before or after antibodies are captured with those affinity reagents. In certain embodiments, for example, the analysis of antibodies present in a given biological sample according to the present invention includes attaching or immobilizing selected antigens indicative of the disease
30 to be diagnosed and/or prognosticated on the surface of a probe, *e.g.*, a reactive surface (*e.g.*, a biochip such as a ProteinChip[®] Array, *etc.*). Probes suitable for use in the invention are described further herein, *e.g.*, in the definitions provided above. Antigens are optionally covalently or non-covalently attached to the probe. Non-covalent

attachment methods include electrostatic attachment (*e.g.*, using poly-lysine, aminosilane, *etc.*), molecular recognition (*e.g.*, streptavidin-biotin, *etc.*), hydrophobic attachment, and the like. Examples of covalent attachment methods include those using moieties, such as aldehyde, epoxide, thiol, carbodiimide, or other groups. Other

5 covalent and non-covalent attachment methods are described herein or are otherwise known in the art. In certain embodiments, the probe further includes affinity reagents that specifically bind inflammation markers, such as cytokines, leukotrienes, and/or the like. In these embodiments, the methods further include capturing and detecting the inflammation markers, if any, present in the sample to provide inflammation marker

10 capture data. This data is also correlated with, *e.g.*, the probable diagnosis or prognosis of the disease or the negative diagnosis for the subject. The immobilized antigen is typically contacted with the biological sample to be analyzed to effect capture of the antibodies and/or inflammation markers, if any, present in the sample prior to analyte detection. In other embodiments, the antigens and/or other affinity reagents are

15 contacted with the biological sample to effect capture of the antibodies and/or inflammation markers, if any, present in the sample prior to immobilizing the antigens and/or affinity reagents on the probe.

[0080] To further illustrate, probe surfaces (*e.g.*, the surfaces of ProteinChip[®] Arrays or other chips) are optionally directly analyzed by, *e.g.*, SELDI TOF-MS. As

20 described herein, this process generally includes loading energy adsorbing molecules (EAM) on the probe surface, followed by a drying operation, and analyzing the analyte mixture by laser desorption/ionization mass spectrometry. In some embodiments, antigens and/or other affinity reagents disposed on the surfaces of other solid supports, *e.g.*, chromatography beads are used to capture antibodies in samples. In these

25 embodiments, the captured antibodies are typically desorbed and collected from the beads by an acidic or other treatment. The collected antibodies fraction is then typically analyzed using various detection methods, including mass spectrometry (*e.g.*, SELDI, MALDI, electrospray, *etc.*). Analyte detection is described further below.

[0081] Whether immobilized before or after capture, biological samples and

30 antigens or other affinity reagents are contacted or incubated together for a selected period of time (*e.g.*, minutes, hours, days, *etc.*) in order to allow any antibodies or inflammation markers present in the sample to be captured or bound by the antigens or other affinity reagents. Typically, samples and these affinity reagents are contacted for

a period of between about 30 seconds and about 12 hours, and preferably, between about 30 seconds and about 15 minutes. Furthermore, samples are generally contacted with affinity reagents under ambient temperature and pressure conditions. For some samples, however, modified temperature (typically between about 0°C and about 5 100°C and more typically 4°C through 37°C) and pressure conditions can be desirable, which conditions are determinable by those skilled in the art. Generally, a sample volume of about 1 µl to 500 µl is contacted with, *e.g.*, an antigen or other affinity reagent in a particular capture step. For example, the sample volume typically contains from a few attomoles to 100 picomoles of biomolecules (*e.g.*, antibodies, inflammation 10 markers, *etc.*). In embodiments in which samples and affinity reagents are immobilized after the capture step, affinity reagents are also typically provided in volumes of about 1 µl to 500 µl.

[0082] Essentially any method of immobilizing or attaching antigens and other affinity reagents to probes or other solid supports (*e.g.*, to derivatize the probes with the 15 affinity reagents) is optionally utilized. For example, affinity reagents are optionally directly immobilized on a probe surface or via a linker or capture molecule, such as receptors, linker antibodies, Protein A, Protein G, a mercaptoheterocyclic ligand, or the like. Methods of immobilizing affinity reagents to solid supports are generally known in the art and are described further in, *e.g.*, U.S. Patent Application 2003/0017464 20 (Pohl).

[0083] In certain embodiments, samples are analyzed without being fractionated prior to examination by, *e.g.*, SELDI. For example, a sample is optionally analyzed directly from a subject to assess the presence of antibodies and/or inflammation markers. Samples are also optionally analyzed, according to the methods 25 of the present invention, after fractionation of the samples (*e.g.*, before or after being captured by antigens or other affinity reagents, *etc.*). Fractionation of a sample aliquot typically increases the total information content about biomolecules present in the particular sample. For example, fractionation may result in the detection of trace amounts of antibodies that would otherwise be undetectable, or not accurately detected, 30 in an unfractionated sample by eliminating signals attributable to more abundant biomolecules that would otherwise suppress the signals of less abundant components. Further, biomolecules remaining in the sample after fractionation are typically detected

with improved, *e.g.*, mass accuracy as a result of an increased signal:noise ratio. The use of information about sample components from fractionated samples as well as unfractionated samples generally leads to a higher confidence level that a given antibody or inflammation marker has been accurately detected.

5 [0084] The fractionation steps that generate sample fractions can be performed by, *e.g.*, any of the purification/fractionation methods described above. For example, prior to spectrometrically profiling biomolecule masses in a particular sample, biomolecules in the sample are optionally separated into fractions using, *e.g.*, centrifugation, dialysis, HPLC, SEC or the like. Typically, fractionated samples are
10 then analyzed by the methods of detection described herein.

[0085] In some embodiments, fractionating and analyzing the sample is performed by SELDI/retentate chromatography. Retentate chromatography involves directly contacting a sample with adsorbents (*e.g.*, antigens or other affinity reagents) bound to a surface of a probe in which the adsorbents capture one or more antibodies
15 and/or inflammation marker. This embodiment also includes removing non-captured material from the probe, *e.g.*, by one or more washes prior to gas phase ion spectrometric analysis. Optionally, the sample is indirectly contacted with a probe surface after being contacted with, *e.g.*, an affinity reagent bound to a chromatographic resin, which affinity reagent captures one or more components of the sample. In this
20 embodiment, non-captured materials are optionally removed (*e.g.*, by one or more washes) before or after the adsorbent is contacted with the probe surface. Additional details relating to retentate chromatography are provided in, *e.g.*, U.S. Patent Application 20020177242 (Hutchens).

[0086] Washing to remove non-captured materials can be accomplished by,
25 *e.g.*, bathing, soaking, dipping, rinsing, spraying, or washing the surface of the probe or other solid support (*e.g.*, a chromatographic resin, *etc.*) following exposure to the sample with an eluant. A microfluidics process is preferably used when an eluant is introduced to small spots (*e.g.*, surface features) of affinity reagents on the probe. Typically, the eluant can be at a temperature of between 0°C and 100°C, preferably
30 between 4°C and 37°C. Any suitable eluant (*e.g.*, organic or aqueous) can be used to wash the support surface. For example, each of the one or more washes optionally includes an identical or a different elution condition relative to a preceding wash. Elution conditions typically differ according to, *e.g.*, pH, buffering capacity, ionic

strength, a water structure characteristic, detergent type, detergent strength, hydrophobicity, dielectric constant, concentration of at least one solute, or the like. Preferably, an aqueous solution is used. Exemplary aqueous solutions include a HEPES buffer, a Tris buffer, or a phosphate buffered saline, *etc.* To increase the wash stringency of the buffers, additives can be incorporated into the buffers. These include, but are not limited to, ionic interaction modifiers (both ionic strength and pH), water structure modifiers, hydrophobic interaction modifiers, chaotropic reagents, affinity interaction displacers. Specific examples of these additives can be found in, *e.g.*, PCT publication WO 98/59360 (Hutchens and Yip). The selection of a particular eluant or eluant additives is dependent on other experimental conditions (*e.g.*, types of antigens used, or antibodies and/or inflammation markers to be detected), and can be determined by those of skill in the art.

[0087] An option to detect molecules with very large masses, such as IgM antibodies, is to treat them in reducing conditions so as to produce smaller fragments. This results not from digestion, but rather from a dissociation of disulfide bonds (when present). In the case of antibody molecules, this produces heavy and light chains that are both smaller than the whole antibody and more easily detected by, *e.g.*, mass spectrometry.

[0088] In certain embodiments of the invention, captured antibodies and/or inflammation markers are desorbed and ionized from probe surfaces before being detected. Prior to desorption and ionization of biomolecules from a probe surface according to any of the methods described herein, energy absorbing molecules or matrix material is typically applied to a given sample on the substrate surface, usually after allowing the sample to dry. The energy absorbing molecules can assist absorption of energy from an energy source from a gas phase ion spectrometer, and can assist desorption of biomolecules from the probe surface. Exemplary energy absorbing molecules include cinnamic acid derivatives, sinapinic acid ("SPA"), cyano hydroxy cinnamic acid ("CHCA") and dihydroxybenzoic acid. Other suitable energy absorbing molecules are known to those skilled in the art. *See, e.g.*, U.S. Patent 5,719,060 (Hutchens & Yip) for additional description of energy absorbing molecules.

[0089] The energy absorbing molecule and biomolecules in a given sample can be contacted in any suitable manner. For example, an energy absorbing molecule is optionally mixed with a sample and the mixture is placed on the probe surface. In

another example, an energy absorbing molecule can be placed on the probe surface prior to contacting the probe with a sample. As an additional option, a fraction can be placed on the probe surface prior to contacting the probe with an energy absorbing molecule. Then, the biomolecule components in the sample can be desorbed, ionized and detected as described in detail below.

[0090] Optionally, multiple fractions of a given sample are analyzed in parallel. Additional options include analyzing unfractionated and fractionated samples in parallel. However, in other embodiments of the invention, these analyses can be performed in series. For example, an unfractionated sample aliquot can be placed on a spot and allowed to equilibrate. Then, the remaining liquid in the sample can be transferred to an adsorbent spot for fractionation by retentate chromatography.

[0091] In preferred embodiments of the invention, biomolecules, such as antibodies, inflammation markers, or other target molecules in a sample are detected using gas phase ion spectrometry, and more preferably, using mass spectrometry. In certain preferred embodiments, at least one version of SELDI mass spectrometry as described or referred to herein is used to desorb and ionize biomolecules from probe surfaces. SELDI mass spectrometry is typically more sensitive than, *e.g.*, MALDI mass spectrometry. Different versions of SELDI can be utilized to perform the methods of the invention. In general, SELDI includes the use of a probe comprising adsorbents (*e.g.*, antigens or other affinity reagents) to capture antibodies (*e.g.*, autoantibodies, *etc.*) and/or inflammation markers, which are then optionally directly desorbed and ionized from the substrate surface during mass spectrometry. As described above, affinity reagents, such as antigens are optionally immobilized on probe surfaces before or after capturing antibodies. Since the probe surface in surface enhanced laser desorption/ionization captures sample components, a sample need not be quasi-purified as in, *e.g.*, MALDI. However, depending on the complexity of a sample and the type of adsorbents used, it is typically desirable to prepare a sample aliquot with reduced complexity by, *e.g.*, removing non-captured materials prior to surface enhanced laser desorption/ionization analysis.

[0092] To further illustrate aspects of SELDI, Figure 1 schematically shows an assay of an unfractionated sample that includes antigen **106** on biochip **102**. Antigens and other affinity reagents are described further above. As additionally described above, antibodies **104** (*e.g.*, autoantibodies) in the sample are not washed after being

placed on antigen **106** which is bound to surface feature **100** of biochip **102**. Incident photon energy from laser **108** causes the desorption and ionization of antibodies **104**, which are then detected in a mass spectrometer to produce mass spectrum **110**.

[0093] Figure 2 schematically illustrates another surface enhanced laser
5 desorption/ionization assay of a sample. As depicted, sample **200** is applied to biochip **202** which includes antigen **204** bound to surface feature **206**. Components of sample **200** that are not bound to antigen **204** are washed away (*e.g.*, eluted or the like) from biochip **202** prior to mass analysis, as described above. Following capture and washing of antibodies **208** in sample **200**, energy absorbing molecules **210** (not shown in Figure
10 1) are added to biochip **202** to absorb energy from ionization source **212** (*i.e.*, a laser) to aid desorption of antibodies **208** from the surface of biochip **202**. Mass spectrum **214** is produced by mass spectral analysis of desorbed/ionized antibodies **208**.

[0094] Optionally, any suitable gas phase ion spectrometer is used as long as it allows biomolecular components on the substrate to be resolved and detected. For
15 example, in certain preferred embodiments the gas phase ion spectrometer is a mass spectrometer. In a typical mass spectrometer, a probe comprising biomolecules (*e.g.*, captured antibodies) on its surface is introduced into an inlet system of the mass spectrometer. The biomolecules are then desorbed by a desorption source such as a laser, fast atom bombardment, high energy plasma, electrospray ionization,
20 thermospray ionization, liquid secondary ion MS, field desorption, *etc.* The generated desorbed, volatilized species consist of preformed ions or neutrals which are ionized as a direct consequence of the desorption event. Generated ions are collected by an ion optic assembly, and then a mass analyzer disperses and analyzes the passing ions. The ions exiting the mass analyzer are detected by a detector. The detector then translates
25 information of the detected ions into mass-to-charge ratios. Detection of the presence of biomolecules or other substances will typically involve detection of signal intensity. This, in turn, can reflect the quantity and character of biomolecules bound to the substrate. Any of the components of a mass spectrometer (*e.g.*, a desorption source, a mass analyzer, a detector, *etc.*) can be combined with other suitable components
30 described herein or others known in the art in embodiments of the invention.

[0095] In a preferred aspect, a laser desorption time-of-flight (TOF) mass spectrometer is used in certain embodiments of the invention. In laser desorption mass spectrometry, a substrate or a probe comprising biomolecular components (*e.g.*, bound

antibodies) is introduced into an inlet system. The materials are desorbed and ionized into the gas phase by incident laser energy from the ionization source. The ions generated are collected by an ion optic assembly, and then in a time-of-flight mass analyzer, ions are accelerated through a short high voltage field and let drift into a high vacuum chamber. At the far end of the high vacuum chamber, the accelerated ions strike a sensitive detector surface at a different time. Since the time-of-flight is a function of the mass of the ions, the elapsed time between ion formation and ion detector impact can be used to identify the presence or absence of proteins or protein fragments of specific mass-to-charge ratios.

10 [0096] In another preferred aspect, a tandem mass spectrometer is used in various embodiments of the invention. Tandem mass spectrometers can usefully be selected from the group that includes orthogonal quadrupole time-of-flight (Qq-TOF), ion trap (IT), ion trap time-of-flight (IT-TOF), time-of-flight time-of-flight (TOF-TOF), and ion cyclotron resonance (ICR) varieties. Presently preferred is an orthogonal Qq-TOF MS. Tandem mass spectrometers and associated instrumentation which can be adapted to perform the methods described herein are described further in, *e.g.*, Patent Application Publication No. US 2002/0182649 by Weinberger et al., which published December 5, 2002. Additional details relating to various mass spectrometry techniques and instrumentation are included in, *e.g.*, Skoog et al., Principles of Instrumental
20 Analysis, 5th Ed., Harcourt Brace & Co. (1998), Siuzdak, Mass Spectrometry for Biotechnology, Academic Press (1996), de Hoffmann et al., Mass Spectrometry: Principles and Applications, 2nd, John Wiley & Sons, Inc. (2001), and Chapman, Mass Spectrometry of Proteins and Peptides, Vol. 146, Methods in Molecular Biology Series, Humana Press (2000).

25 [0097] In additionally preferred aspects of the invention, analyte detection also includes quantifying captured antibodies and/or captured inflammation markers in addition to detecting those biomolecules in a sample. In these embodiments, SELDI-based analysis is typically coupled with a quantitative surface scanning technique, such as a method that includes the detection refractive index or diffraction (*e.g.*, surface
30 plasmon resonance, ellipsometry, resonant mirror methods, grating-coupled waveguide methods, interferometry, multi-polar resonance spectroscopy, *etc.*). This significantly enhances the throughput of the diagnostic or prognostic assays of the invention relative to many preexisting approaches, which typically require a series of dilutions to quantify

antibodies present in a sample. Techniques such as surface plasmon resonance are described further in a variety of sources including, *e.g.*, Davies et al. (Eds.) Surface Analytical Techniques for Probing Biomaterial Processes, CRC Press (1996) and Vitrant et al., Electromagnetic Resonances in Nonlinear Optics, Taylor & Francis, Inc.

5 (2000)

[0098] In another embodiment, an ion mobility spectrometer or total ion current measuring device is optionally used to detect biomolecular components.

[0099] The detectors utilized in practicing the invention typically further comprise interfaced digital computers, *e.g.*, to control device operation (*e.g.*, ion generation in a gas phase ion spectrometer, *etc.*) and to participate in data acquisition and analysis. Analysis software can be local to the computer or can be remote, but communicably accessible to the computer. For example, the computer can have a connection to the internet permitting use of analytical packages such as Protein Prospector, PROWL, or the Mascot Search Engine, which are available on the world wide web. The analysis software can also be remotely resident on a LAN or WAN server. Exemplary systems that include digital computers are described further below.

[0100] When the presence of antibodies and/or inflammation markers is detected, for example, using mass spectrometry (*e.g.*, on the surface of a probe, such as a ProteinChip[®] Array), the results would show a pattern of different peaks of given molecular masses. Detected masses typically correspond to single antibodies or inflammation markers (except, *e.g.*, for multiple charged-species, subunits or fragments of the same protein, *etc.*). The size of the signal compared to a standard curve is generally proportional to the amount of the particular antibody or inflammation marker. Pattern analysis software containing data related to these biomolecules typically informs on the identity of detected antibody or inflammation marker and provides quantitative information regarding these contaminating proteins.

[0101] Data generated by desorption and detection of biomolecules, such as antibodies or inflammation markers is optionally analyzed using any suitable method, *e.g.*, to identify and/or quantify detected components and to correlate that data with a diagnosis or prognosis of the disease under consideration. For example, antibody detection and quantification data is typically compared to positive controls (*e.g.*, a SELDI analysis of a sample comprising a known quantity of an identified antibody) and/or negative controls (*e.g.*, a SELDI analysis of a sample that lacks antibodies

and/or inflammation markers related to the disease under consideration). In one embodiment, data is analyzed with the use of a logic device, such as a programmable digital computer that is included, *e.g.*, as part of a system. Systems are described further below. The computer generally includes a computer readable medium that
5 stores logic instructions of the system software. Certain logic instructions are typically devoted to memory that includes the location of each feature on a probe, the identity of the antigen(s) or other adsorbent(s) at that feature, the elution conditions used to wash the adsorbent(s), or the like. The computer also typically includes logic instructions that receives as input, data on the strength of the signal at various molecular masses
10 received from a particular addressable location or surface feature on the probe, and instructions for entering data into a database. This data generally indicates the number and masses of components detected, including the strength of the signal generated by each component.

[0102] To further illustrate, data generation in mass spectrometry typically
15 begins with the detection of ions by an ion detector. A typical laser desorption mass spectrometer can employ a nitrogen laser at 337.1 nm. A useful pulse width is about 4 nanoseconds. Generally, power output of about 1-25 μ J is used. Ions that strike the detector generate an electric potential that is digitized by a high speed time-array recording device that digitally captures the analog signal. Ciphergen's ProteinChip®
20 system employs an analog-to-digital converter (ADC) to accomplish this. The ADC integrates detector output at regularly spaced time intervals into time-dependent bins. The time intervals typically are one to four nanoseconds long. Furthermore, the time-of-flight spectrum ultimately analyzed typically does not represent the signal from a single pulse of ionizing energy against a sample, but rather the sum of signals from a
25 number of pulses. This reduces noise and increases dynamic range. This time-of-flight data is then subject to data processing. In Ciphergen's ProteinChip® software, data processing typically includes TOF-to-M/Z transformation, baseline subtraction, high frequency noise filtering.

[0103] TOF-to-M/Z transformation involves the application of an algorithm that
30 transforms times-of-flight into mass-to-charge ratio (M/Z). In this step, the signals are converted from the time domain to the mass domain. That is, each time-of-flight is converted into mass-to-charge ratio, or M/Z. Calibration can be done internally or externally. In internal calibration, the sample analyzed contains one or more analytes

of known M/Z . Signal peaks at times-of-flight representing these massed analytes are assigned the known M/Z . Based on these assigned M/Z ratios, parameters are calculated for a mathematical function that converts times-of-flight to M/Z . In external calibration, a function that converts times-of-flight to M/Z , such as one created by prior
5 internal calibration, is applied to a time-of-flight spectrum without the use of internal calibrants.

[0104] Baseline subtraction improves data quantification by eliminating artificial, reproducible instrument offsets that perturb the spectrum. It involves calculating a spectrum baseline using an algorithm that incorporates parameters such as
10 peak width, and then subtracting the baseline from the mass spectrum.

[0105] High frequency noise signals are eliminated by the application of a smoothing function. A typical smoothing function applies a moving average function to each time-dependent bin. In an improved version, the moving average filter is a variable width digital filter in which the bandwidth of the filter varies as a function of,
15 *e.g.*, peak bandwidth, generally becoming broader with increased time-of-flight. *See, e.g.*, WO 00/70648, November 23, 2000 (Gavin et al., "Variable Width Digital Filter for Time-of-flight Mass Spectrometry").

[0106] A computer can transform the resulting spectrum into various formats for displaying. In one format, referred to as "spectrum view or retentate map," a
20 standard spectral view can be displayed, wherein the view depicts the quantity of analyte reaching the detector at each particular molecular weight. In another format, referred to as "peak map," only the peak height and mass information are retained from the spectrum view, yielding a cleaner image and enabling analytes with nearly identical molecular weights to be more easily seen. In yet another format, referred to as "gel
25 view," each mass from the peak view can be converted into a grayscale image based on the height of each peak, resulting in an appearance similar to bands on electrophoretic gels. In yet another format, referred to as "3-D overlays," several spectra can be overlaid to study subtle changes in relative peak heights. In yet another format, referred to as "difference map view," two or more spectra can be compared,
30 conveniently highlighting unique analytes and analytes which are up- or down-regulated between samples.

[0107] Analysis generally involves the identification of peaks in the spectrum that represent signal from an analyte, *e.g.*, captured antibodies. Peak selection can, of

course, be done by eye. However, software is available as part of Ciphergen's ProteinChip® software that can automate the detection of peaks. In general, this software functions by identifying signals having a signal-to-noise ratio above a selected threshold and labeling the mass of the peak at the centroid of the peak signal. In one
5 useful application many spectra are compared to identify identical peaks present in some selected percentage of the mass spectra. One version of this software clusters all peaks appearing in the various spectra within a defined mass range, and assigns a mass (M/Z) to all the peaks that are near the mid-point of the mass (M/Z) cluster.

[0108] Peak data from one or more spectra can be subject to further analysis by,
10 for example, creating a spreadsheet in which each row represents a particular mass spectrum, each column represents a peak in the spectra defined by mass, and each cell includes the intensity of the peak in that particular spectrum. Various statistical or pattern recognition approaches can applied to the data.

[0109] In some embodiments, data derived from the spectra (*e.g.*, mass spectra
15 or time-of-flight spectra) that are generated using samples such as "known samples" can then be used to "train" a classification model. A "known sample" is a sample that is pre-classified. The data that are derived from the spectra and are used to form the classification model can be referred to as a "training data set". Once trained, the classification model can recognize patterns in data derived from spectra generated
20 using unknown samples. The classification model can then be used to classify the unknown samples into classes.

[0110] The training data set that is used to form the classification model may comprise raw data or pre-processed data. In some embodiments, raw data can be obtained directly from time-of-flight spectra or mass spectra, and then may be
25 optionally "pre-processed" as described above.

[0111] Classification models can be formed using any suitable statistical classification (or "learning") method that attempts to segregate bodies of data into classes based on objective parameters present in the data. Classification methods may be either supervised or unsupervised. Examples of supervised and unsupervised
30 classification processes are described in Jain, "Statistical Pattern Recognition: A Review", IEEE Transactions on Pattern Analysis and Machine Intelligence, Vol. 22, No. 1, January 2000, which is herein incorporated by reference in its entirety.

[0112] In supervised classification, training data containing examples of known categories are presented to a learning mechanism, which learns one more sets of relationships that define each of the known classes. New data may then be applied to the learning mechanism, which then classifies the new data using the learned

5 relationships. Examples of supervised classification processes include linear regression processes (*e.g.*, multiple linear regression (MLR), partial least squares (PLS) regression and principal components regression (PCR)), binary decision trees (*e.g.*, recursive partitioning processes such as CART - classification and regression trees), artificial neural networks such as backpropagation networks, discriminant analyses (*e.g.*,

10 Bayesian classifier or Fischer analysis), logistic classifiers, and support vector classifiers (support vector machines).

[0113] A preferred supervised classification method is a recursive partitioning process. Recursive partitioning processes use recursive partitioning trees to classify spectra derived from unknown samples. Further details about recursive partitioning

15 processes are in U.S. Provisional Patent Application Nos. 60/249,835, filed on November 16, 2000, and 60/254,746, filed on December 11, 2000, and U.S. Non-Provisional Patent Application Nos. 09/999,081, filed November 15, 2001, and 10/084,587, filed on February 25, 2002. All of these U.S. Provisional and Non-Provisional Patent Applications are herein incorporated by reference in their entirety

20 for all purposes.

[0114] In other embodiments, the classification models that are created can be formed using unsupervised learning methods. Unsupervised classification attempts to learn classifications based on similarities in the training data set, without pre-classifying the spectra from which the training data set was derived. Unsupervised

25 learning methods include cluster analyses. A cluster analysis attempts to divide the data into "clusters" or groups that ideally should have members that are very similar to each other, and very dissimilar to members of other clusters. Similarity is then measured using some distance metric, which measures the distance between data items, and clusters together data items that are closer to each other. Clustering techniques

30 include the MacQueen's K-means algorithm and the Kohonen's Self-Organizing Map algorithm.

[0115] The classification models can be formed on and used on any suitable digital computer. Suitable digital computers include micro, mini, or large computers

using any standard or specialized operating system such as a Unix, Windows™ or Linux™ based operating system. The digital computer that is used may be physically separate from the mass spectrometer that is used to create the spectra of interest, or it may be coupled to the mass spectrometer.

5 [0116] The training data set and the classification models according to embodiments of the invention can be embodied by computer code that is executed or used by a digital computer. The computer code can be stored on any suitable computer readable media including optical or magnetic disks, sticks, tapes, *etc.*, and can be written in any suitable computer programming language including C, C++, visual basic,
10 *etc.*

[0117] Software included in the systems utilized in performing the methods of the invention typically has logic instructions, *e.g.*, capable of quantifying detected antibodies and/or inflammation markers, capable of determining closeness-of-fit between one or more detected biomolecule masses in sets of mass data and database
15 entries to aid in the diagnosis or prognosis of the particular disease under consideration. Various software packages are currently available for querying databases, improving the speed of mass spectrometric protein identification processes, and otherwise integrating mass spectrometry into bioinformatics. For example, Mascot is a search engine that uses mass spectrometry data to identify proteins from primary sequence
20 databases. *See, e.g.*, Perkins et al. (1999) "Probability-based protein identification by searching sequence databases using mass spectrometry data," Electrophoresis 20(18):3551-3567. Another exemplary software package that is useful for performing the methods of the present invention includes ProFound, which performs rapid database searching combined with Bayesian statistics for protein identification. ProFound is
25 described further in, *e.g.*, Zhang and Chait (2000) "ProFound-An expert system for protein identification using mass spectrometric peptide mapping information," Anal. Chem. 72:2482-8249, Zhang and Chait (1998) "ProFound-An expert system for protein identification," Proceedings of the 46th ASMS Conference on Mass Spectrometry and Allied Topics, Orlando, Florida, p.969, and Zhang and Chait (1995) "Protein
30 identification by database searching: a Bayesian algorithm," Proceedings of the 43rd ASMS Conference on Mass Spectrometry and Allied Topics, Atlanta, Georgia, p. 643.

[0118] The invention also provides for the storage and retrieval of a collection of data in a computer data storage apparatus, which can include magnetic disks, optical

disks, magneto-optical disks, DRAM, SRAM, SGRAM, SDRAM, RDRAM, DDR RAM, magnetic bubble memory devices, and other data storage devices, including CPU registers and on-CPU data storage arrays. Typically, the target data records are stored as a bit pattern in an array of magnetic domains on a magnetizable medium or as
5 an array of charge states or transistor gate states, such as an array of cells in a DRAM device (*e.g.*, each cell comprised of a transistor and a charge storage area, which may be on the transistor).

[0119] The invention also preferably provides a magnetic disk, such as an IBM-compatible (DOS, Windows, Windows95/98/2000, Windows NT, OS/2) or other
10 format (*e.g.*, Linux, SunOS, Solaris, AIX, SCO Unix, VMS, MV, Macintosh, *etc.*) floppy diskette or hard (fixed, Winchester) disk drive, comprising a bit pattern encoding data from an assay of the invention in a file format suitable for retrieval and processing in a computerized sequence analysis, comparison, or relative quantitation method.

15 [0120] The invention also provides a network, comprising a plurality of computing devices linked via a data link, such as an Ethernet cable (coax or 10BaseT), telephone line, ISDN line, wireless network, optical fiber, or other suitable signal transmission medium, whereby at least one network device (*e.g.*, computer, disk array, *etc.*) comprises a pattern of magnetic domains (*e.g.*, magnetic disk) and/or charge
20 domains (*e.g.*, an array of DRAM cells) composing a bit pattern encoding data acquired from an assay of the invention.

[0121] The invention also provides a method for transmitting assay data that includes generating an electronic signal on an electronic communications device, such as a modem, ISDN terminal adapter, DSL, cable modem, ATM switch, or the like in
25 which the signal includes (in native or encrypted format) a bit pattern encoding data from an assay or a database comprising a plurality of assay results obtained by the method of the invention.

[0122] In a preferred embodiment, the invention provides a computer system for comparing a query target to a database containing an array of data structures, such
30 as an assay result obtained by the method of the invention, and ranking database targets based on the degree of identity and gap weight to the target data. A central processor is preferably initialized to load and execute the computer program for alignment and/or comparison of the assay results. Data for a query target is entered into the central

processor via an I/O device. Execution of the computer program results in the central processor retrieving the assay data from the data file, which comprises a binary description of an assay result.

[0123] The target data or record and the computer program can be transferred to secondary memory, which is typically random access memory (*e.g.*, DRAM, SRAM, SGRAM, or SDRAM). Targets are ranked according to the degree of correspondence between a selected assay characteristic (*e.g.*, binding to a selected binding functionality) and the same characteristic of the query target and results are output via an I/O device. For example, a central processor can be a conventional computer (*e.g.*, Intel Pentium, PowerPC, Alpha, PA-8000, SPARC, MIPS 4400, MIPS 10000, VAX, *etc.*); a program can be a commercial or public domain molecular biology software package (*e.g.*, UWGCG Sequence Analysis Software, Darwin); a data file can be an optical or magnetic disk, a data server, a memory device (*e.g.*, DRAM, SRAM, SGRAM, SDRAM, EPROM, bubble memory, flash memory, *etc.*); an I/O device can be a terminal comprising a video display and a keyboard, a modem, an ISDN terminal adapter, an Ethernet port, a punched card reader, a magnetic strip reader, or other suitable I/O device.

[0124] Figure 3 schematically illustrates an exemplary surface enhanced laser desorption/ionization time-of-flight mass spectrometry system **300**. As shown, photon energy produced by laser source **302** impacts biochip **304** at surface feature **306**, which includes one or more selected antigens with captured antibodies. The photon energy causes captured antibodies at surface feature **306** to desorb and ionize. The desorbed ions are then accelerated through flight tube/mass analyzer **308**. Ions are separated according to mass/charge ratios, which as depicted is simply the mass of the ionic species, because each ion is singly charged. As further illustrated, smaller ions travel faster than larger ions, thereby resolving the species according to mass. Ions produce a detectable signal at detector **310** which signal is processed by information appliance or digital device **312** to generate mass spectrum **314**.

[0125] The present invention also provides probes and kits for aiding in the diagnosis and/or prognosis of a disease (*e.g.*, an autoimmune disease, an infectious disease, a prion disease, *etc.*). A probe according to the present invention includes a biochip derivatized with one or more antigens indicative of the disease, which antigens specifically bind antibodies present in a sample. In certain embodiments, the probe

also includes one or more affinity reagents that specifically bind inflammation markers, such as cytokines, leukotrienes, and the like. Additional aspects of the probes of the invention are described above, including in the provided definitions.

[0126] Kits generally include one or more probes comprising biochips
5 derivatized with one or more capture molecules that specifically bind at least one antigen indicative of a disease. The kits of the invention optionally also include affinity reagents, such as antigens indicative of one or more diseases, *e.g.*, either bound to the capture molecules on the probe or packaged separately. Suitable antigens and other affinity reagents are described in greater detail above or are otherwise known in the art.
10 Kits may further include a pre-fractionation spin column (*e.g.*, K-30 size exclusion column) that can be used to prepare samples as described herein.

[0127] In addition, kits also generally include instructions (*e.g.*, in the form of a label or a separate insert) to capture antibodies from a sample with the antigens or other affinity reagents, and to capture the captured antibodies on the probes. The instructions
15 may also include other operational parameters. For example, the kit may have standard instructions informing a consumer how to wash the probe after, *e.g.*, a sample is contacted on the probe. In another example, the kit may have instructions for pre-fractionating a sample to reduce complexity of proteins in the sample. Optionally, the kit may further include a standard or control information for comparison with
20 information derived from test samples.

[0128] While the foregoing invention has been described in some detail for purposes of clarity and understanding, it will be clear to one skilled in the art from a reading of this disclosure that various changes in form and detail can be made without departing from the true scope of the invention. For example, all the techniques and
25 apparatus described above may be used in various combinations. All publications, patents, patent applications, or other documents cited in this application are incorporated by reference in their entirety for all purposes to the same extent as if each individual publication, patent, patent application, or other document were individually indicated to be incorporated by reference for all purposes.

30

WHAT IS CLAIMED IS:

1. A method of aiding in a disease diagnosis, the method comprising:
 - (a) capturing one or more antibodies, if any, present in at least one
5 sample derived from a subject on a probe with one or more antigens indicative of the disease, which antigens specifically bind the antibodies;
 - (b) detecting the captured antibodies, if any, by at least one version of surface enhanced laser desorption/ionization gas phase ion spectrometry to provide antibody capture data; and,
 - 10 (c) correlating the antibody capture data with a probable diagnosis of the disease or a negative diagnosis for the subject.
2. The method of claim 1, wherein the disease comprises an autoimmune disease, an infectious disease, or a prion disease.
3. The method of claim 1, wherein the antibodies comprise
15 autoantibodies.
4. The method of claim 1, wherein the sample comprises a tissue extract or a biological fluid derived from the subject.
5. The method of claim 1, wherein the subject is a mammalian subject.
- 20 6. The method of claim 1, wherein the probe comprises at least two different antigens that are each indicative of the disease.
7. The method of claim 1, wherein the probe comprises a biochip.
8. The method of claim 1, wherein the probe comprises a surface-enhanced neat desorption probe.
- 25 9. The method of claim 1, wherein a matrix material is applied to the probe before laser desorption/ionization.

10. The method of claim 1, wherein the antigens are selected from the group consisting of: organic molecules, inorganic molecules, allergens, biomolecules, nucleic acids, proteins, peptides, peptide nucleic acids, prions, haptens, hapten-carrier conjugates, carbohydrates, and lipids.
- 5 11. The method of claim 1, wherein the antigens are covalently attached to the probe.
12. The method of claim 1, wherein the antigens are non-covalently attached to the probe.
- 10 13. The method of claim 1, wherein the version of surface enhanced laser desorption/ionization gas phase ion spectrometry comprises surface enhanced laser desorption/ionization mass spectrometry.
14. The method of claim 1, wherein the probe further comprises one or more affinity reagents that specifically bind one or more inflammation markers and the method further comprises capturing and detecting the inflammation markers, if any,
15 present in the sample to provide inflammation marker capture data, and correlating the inflammation marker capture data with the probable diagnosis of the disease or the negative diagnosis for the subject.
15. The method of claim 14, wherein the inflammation markers comprise cytokines and/or leukotrienes.
- 20 16. The method of claim 1, wherein detecting further comprises quantifying the captured antibodies such that the antibody capture data comprises quantified antibody data.
17. The method of claim 16, wherein the captured antibodies are quantified by a quantitative surface scanning technique.
- 25 18. The method of claim 16, wherein correlating comprises comparing the quantified antibody data to at least one control.

19. The method of claim 18, wherein an amount of antibody above the control correlates with a positive diagnosis of the disease.

20. The method of claim 1, wherein the probe is derivatized with one or more capture molecules that capture the antigens, which antigens are captured by the capture molecules before or after capturing the antibodies in the sample.

21. The method of claim 20, wherein the capture molecules comprise linker molecules, receptors, linker antibodies, Protein A, Protein G, or mercaptoheterocyclic ligands.

22. A method of aiding in a disease prognosis, the method comprising:
10 (a) profiling at least a first sample derived from a subject diagnosed with a disease, wherein profiling comprises detecting antibodies in the first sample using the method according to claim 1;

(b) profiling at least a second sample derived from the subject diagnosed with the disease, wherein profiling comprises detecting the antibodies in the second sample using the method according to claim 1; and,

15 (c) comparing the relative amounts of the antibodies in the first and second samples detected by profiling, thereby aiding in the prognosis of the disease for the subject.

23. A probe comprising a biochip derivatized with one or more antigens indicative of a disease, which antigens specifically bind antibodies present in a sample.

24. A kit comprising:
(a) a probe comprising a biochip derivatized with one or more capture molecules that specifically bind at least one antigen indicative of a disease; and,
25 (b) instructions to capture antibodies from a sample with the antigen, which antigen specifically binds the antibodies, and to capture the captured antibodies on the probe.

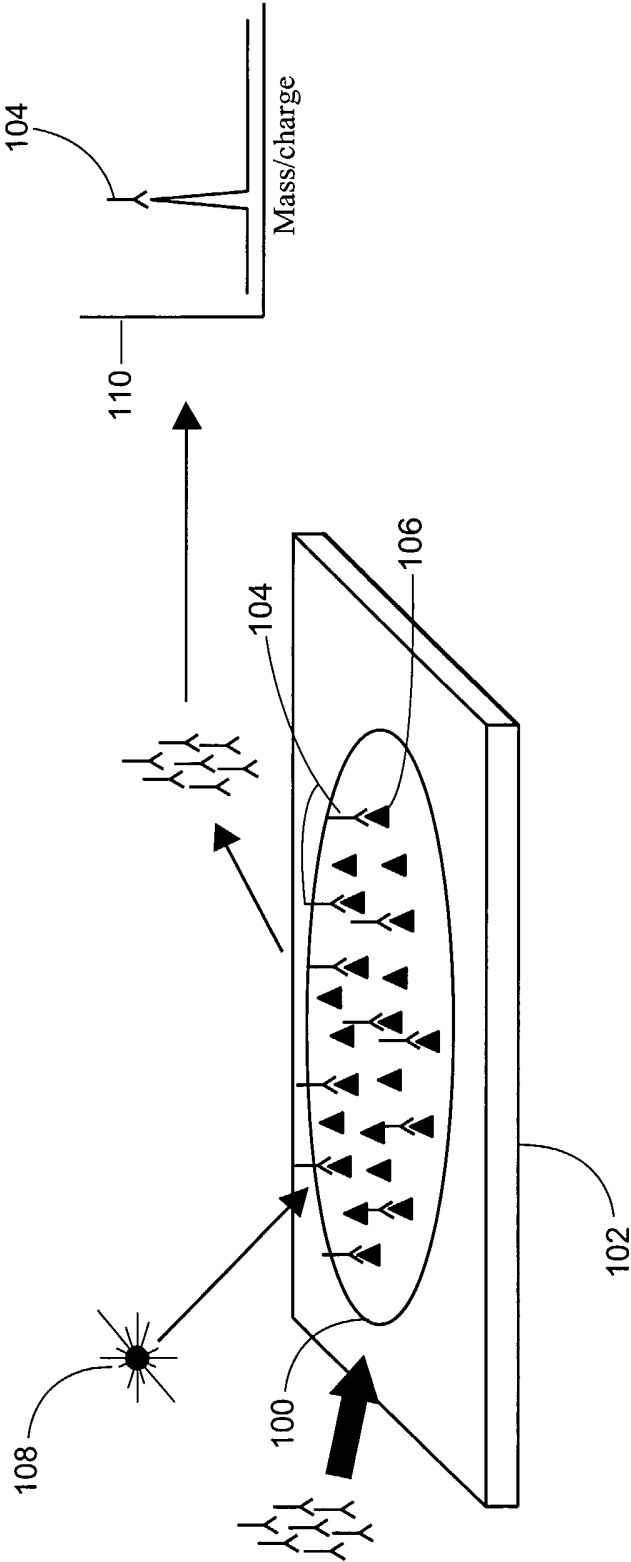


Fig. 1

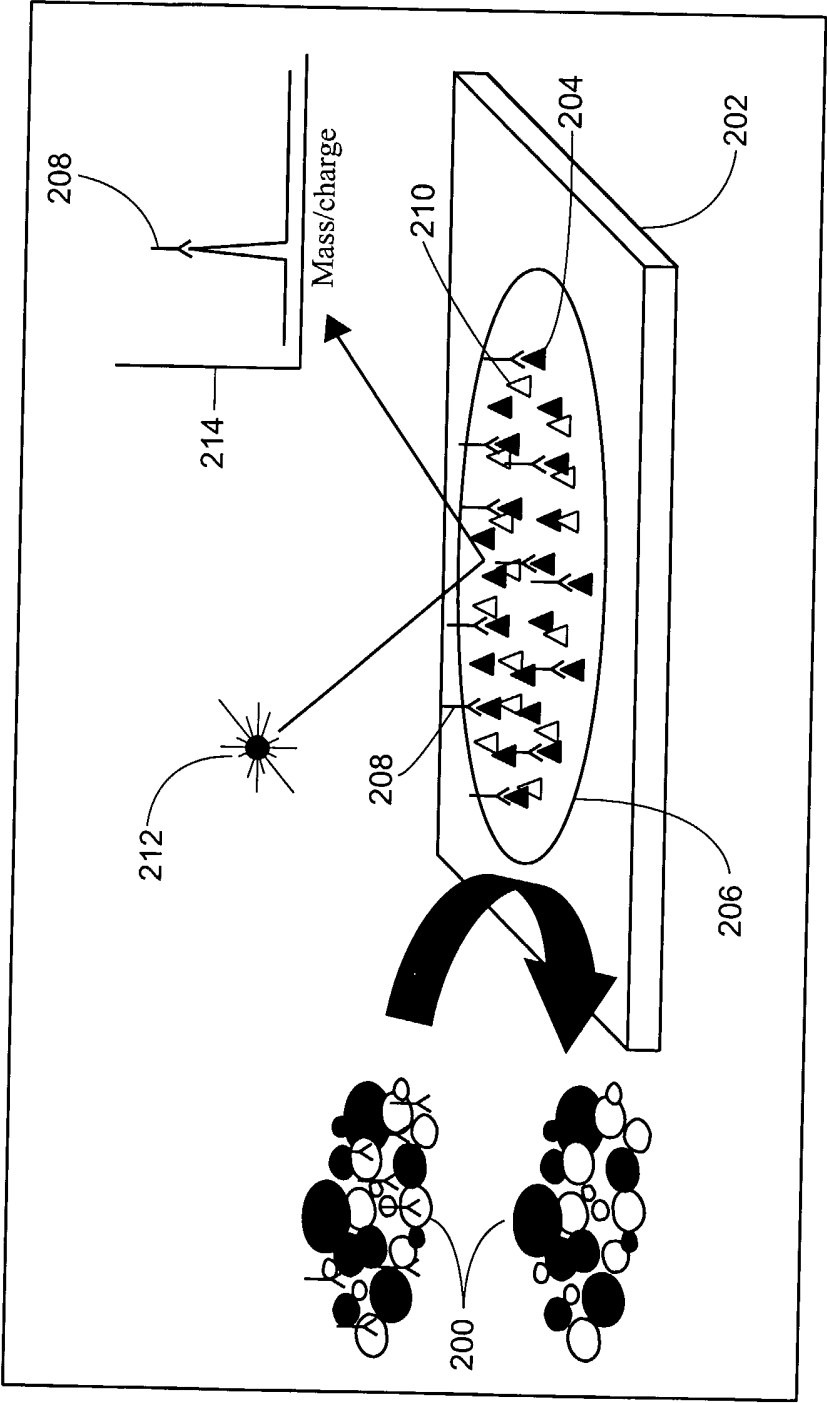


Fig. 2

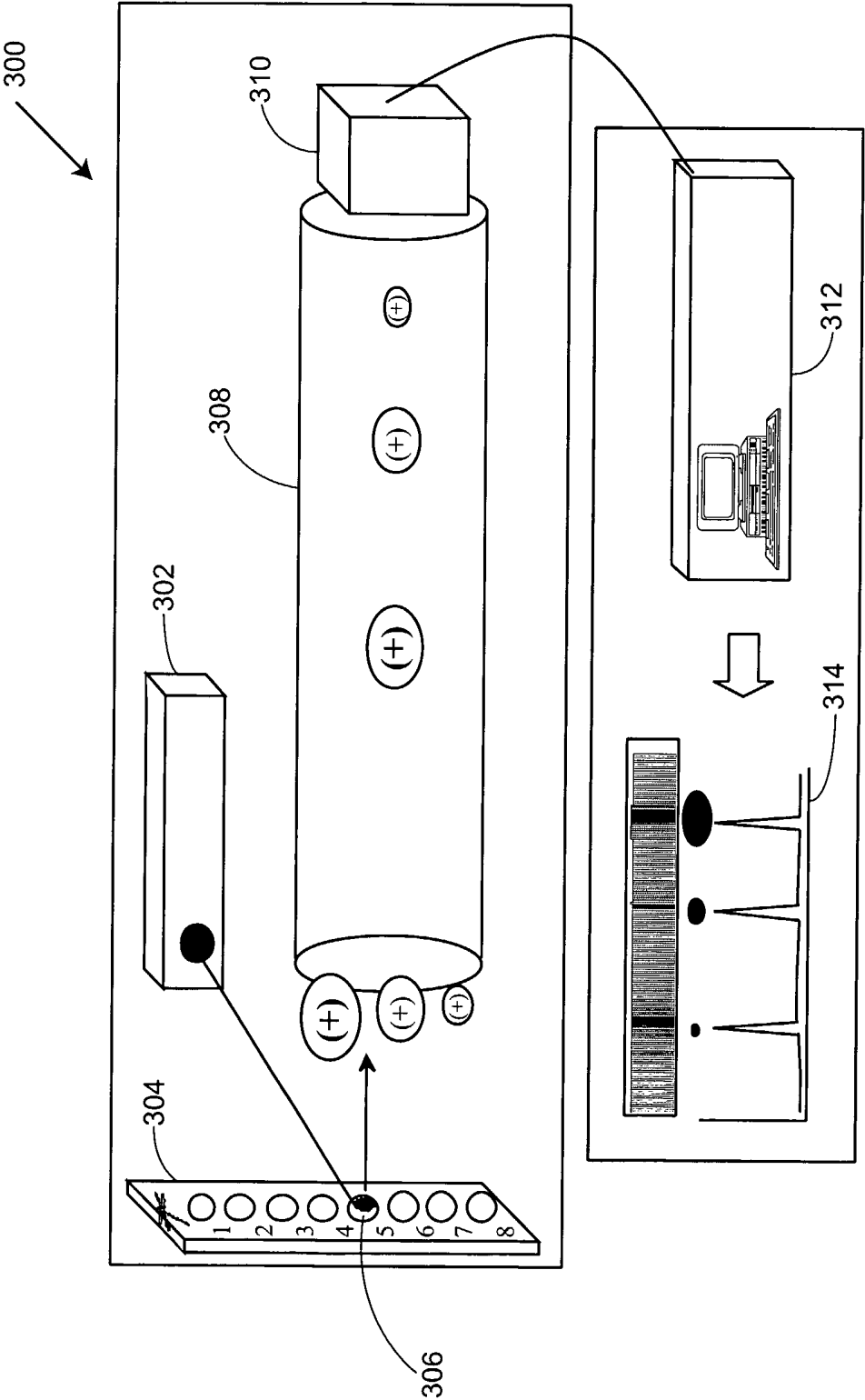


Fig. 3