

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
16 December 2004 (16.12.2004)

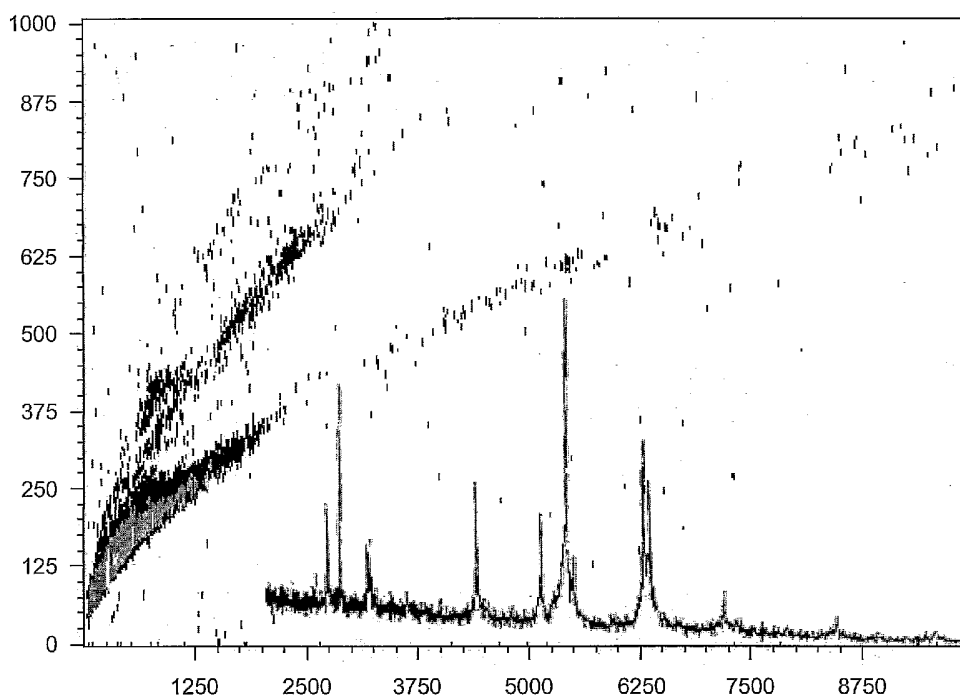
PCT

(10) International Publication Number
WO 2004/109255 A2

- (51) International Patent Classification⁷: **G01N** 4155 South Braeswood, Apartment No. 610-A, Houston, TX 77025 (US).
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- (22) International Filing Date: 4 June 2004 (04.06.2004)
- (25) Filing Language: English (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (26) Publication Language: English
- (30) Priority Data: 60/476,321 6 June 2003 (06.06.2003) US
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[Continued on next page]

(54) Title: RAPID SCREENING OF BIOGENIC PARTICLES IN COLLECTED AEROSOLS



(57) Abstract: The present invention relates to detection of aerosolized biogenic particles using a mass-mobility spectra. More specifically, the biogenic particles are bacteria, viruses, prions or fungi.



SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

Published:

— *without international search report and to be republished upon receipt of that report*

RAPID SCREENING OF BIOGENIC PARTICLES IN COLLECTED AEROSOLS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 60/476,321 filed June 6, 2003, which is incorporated herein by reference in its entirety.

TECHNICAL FIELD

[0002] The present invention relates to detection of aerosolized biogenic particles using a mass-mobility spectra. More specifically, the biogenic particles are bacteria, viruses, prions or fungi.

BACKGROUND OF THE INVENTION

[0003] Typical air samples collected from the environment are complex mixtures and contain bacterial cells and spores, fungal spores and fragments, pollen grains, toxins produced by fungi, viruses, prions and other biogenic compounds. Hence, it is important to develop techniques to rapidly and accurately identify microorganisms to pose health and/or environmental hazards.

[0004] Mass spectrometers have been employed in identifying such microorganisms. Mass spectrometers are commercially available and can include, for example, single or multiple quadrupole, single or multiple magnetic sector, Fourier Transform ion cyclotron resonance (FTICR), ion trap, and combinations thereof (*e.g.*, ion-trap/time-of-flight).

[0005] Generally, mass spectrometry is an analytical technique used for the accurate determination of molecular weights, the identification of chemical structures, the determination of the compositions of mixtures, and qualitative and/or quantitative elemental analysis. In operation, a mass spectrometer generates ions of sample molecules under investigation, separates the ions according to their mass-to-charge ratio (m/z), and measures the relative abundance of each ion. This analysis of the mass distribution of the molecule and its ion fragments can lead to a molecular "fingerprint" for identification.

[0006] One type of mass spectrometer is a time-of-flight (TOF) mass spectrometer which separates ions according to their mass-to-charge ratio by measuring the time it takes

generated ions to travel to a detector. See, *e.g.*, U.S. Pat. Nos. 5,045,694 and 5,160,840, the contents of each are incorporated herein by reference. TOF mass spectrometers are advantageous because they are relatively simple instruments with virtually unlimited mass-to-charge ratio range. TOF mass spectrometers have potentially higher sensitivity than traditional scanning instruments because they can record all the ions generated from each ionization event. TOF mass spectrometers are particularly useful for measuring the mass-to-charge ratio of large organic molecules where conventional magnetic field mass spectrometers lack sensitivity.

[0007] Other ionization techniques used in mass spectrometry are laser desorption (LD) and other "soft" ionization techniques, such as fast atom bombardment (FAB), plasma desorption and electrospray ionization (ESI). These techniques were developed to address the problem of ionizing polar, thermally labile, nonvolatile compounds, such as biogenic molecules, for mass spectrometric analysis. These properties are typical of biogenic molecules (*e.g.*, proteins, nucleic acids, oligosaccharides) and preclude or interfere with the acquisition of their spectra using a "hard" ionization technique such as electron impact.

[0008] The performance of LD is substantially improved by the addition of a small organic matrix molecule to the sample material. This technique, known as matrix-assisted laser desorption/ionization (MALDI), is based on the discovery that the desorption/ionization of large, nonvolatile molecules such as proteins can be effected when a sample of such molecules is irradiated after being codeposited with a large molar excess of an energy-absorbing "matrix" material, even though the molecule does not strongly absorb at the wavelength of the laser radiation. The abrupt energy absorption initiates a phase change in a microvolume of the absorbing sample from a solid to a gas while also inducing ionization of the sample molecules. MALDI is particularly advantageous in biological applications since it facilitates desorption and ionization of large biomolecules in excess of 100,000 Da while keeping them intact. MALDI has been successfully coupled with TOF mass spectrometers to analyze large molecules.

[0009] However, there are major problems using MALDI for biogenic particle detection. For example, MALDI cannot easily discriminate mass spectrum from unwanted aerosol particles such as inorganics without acquiring high resolution spectra from all particles. Another disadvantage of MALDI is that the sensitivity of the MALDI technique needs to be improved over currently attainable attamole levels. Yet further, another disadvantage of MALDI

is that sampling large volumes of air containing only a few hundred microorganisms/liter and then incorporating matrix becomes problematic.

[0010] All these issues make regular MALDI MS incapable when extremely low concentrations need to be detected in a short time period, which is the case of most pathogenic microorganisms, such as anthrax, *etc.* Thus, the present invention is the first to use Matrix Assisted Laser Desorption Ionization—Ion Mobility orthogonal Time-of Flight Mass Spectrometry (MALDI-IM-oTOF MS) technique to rapidly detect and analyze biogenic particles from an atmospheric sample collected on an aerosol collection surface.

BRIEF SUMMARY OF THE INVENTION

[0011] The present invention is directed to identifying biogenic particles, for example bacteria, in an atmospheric sample by analyzing biomarkers that are produced from a mass-mobility spectra of the sample by using MALDI-IM-oTOF MS.

[0012] An embodiment of the present invention is a method of identifying a biogenic particle in a sample of atmosphere comprising the steps of: collecting a sample of atmosphere; analyzing the sampled atmosphere, wherein analyzing comprises obtaining a mass-mobility spectra of the sample; and utilizing a biomarker determined from the mass-mobility spectra to identify the biogenic particle present in the atmosphere sample. The mass-mobility spectra is obtained from a MALDI-IM-oTOF MS.

[0013] In certain embodiments, the biogenic particle can be whole cells or cellular components of a prokaryotic organism, eukaryotic organism or a virus. Eukaryotic organisms can include filamentous fungi, yeasts or molds. Still further, the biogenic particle can be a prion or a component of spores, toxins, or pollen.

[0014] Prokaryotic organisms can include a bacterium, for example a pathogenic bacterium (*e.g.*, gram-negative bacterium and/or a gram-positive bacterium) and/or a non-pathogenic bacterium (*e.g.*, gram-negative bacterium and/or a gram-positive bacterium). Exemplary gram-negative organisms include, but are not limited to, Enterobacteriaceae consisting of *Escherichia*, *Shigella*, *Edwardsiella*, *Salmonella*, *Citrobacter*, *Klebsiella*, *Enterobacter*, *Hafnia*, *Serratia*, *Proteus*, *Morganella*, *Providencia*, *Yersinia*, *Erwinia*, *Buttiauxella*, *Cedecea*, *Ewingella*, *Kluyvera*, *Tatumella* and *Rahnella*. Other exemplary gram-negative organisms not in the family Enterobacteriaceae include, but are not limited to, *Pseudomonas aeruginosa*,

Stenotrophomonas maltophilia, *Burkholderia*, *Cepacia*, *Gardenerella*, *Vaginalis*, and *Acinetobacter* species. In specific embodiments, the pathogenic gram-negative bacterium is a species of *Escherichia*, for example, *Escherichia coli*. Exemplary gram-positive organisms include, but are not limited to, *Staphylococcus aureus*, coagulase-negative staphylococci, streptococci, enterococci, corynebacteria, and *Bacillus* species. More specifically, the pathogenic gram-positive bacterium is a species of *Bacillu*, for example, *Bacillus anthracis* or *Bacillus subtilis*.

[0015] In another specific embodiment, the biomarker can be a protein. The mass of the protein can be any size that identifies the microorganism or the biogenic particle. Yet further, the biomarker can be selected from lipids, oligosaccharides, lipopolysaccharides, glycans or nucleic acids. In specific embodiments, the mass of the biomarker is less than 5000 a.m.u.

[0016] Another embodiment of the present invention is a method of determining the presence of a pathogenic bacterium in a sample of atmosphere comprising the steps of: collecting a sample of atmosphere; analyzing the sampled atmosphere, wherein analyzing comprises obtaining a mass-mobility spectra of the sample; and utilizing a biomarker determined from the mass-mobility spectra to determine the presence of the pathogenic bacterium in the atmosphere sample.

[0017] The foregoing has outlined rather broadly the features and technical advantages of the present invention in order that the detailed description of the invention that follows may be better understood. Additional features and advantages of the invention will be described hereinafter which form the subject of the claims of the invention. It should be appreciated that the conception and specific embodiment disclosed may be readily utilized as a basis for modifying or designing other structures for carrying out the same purposes of the present invention. It should also be realized that such equivalent constructions do not depart from the invention as set forth in the appended claims. The novel features which are believed to be characteristic of the invention, both as to its organization and method of operation, together with further objects and advantages will be better understood from the following description when considered in connection with the accompanying figures. It is to be expressly understood, however, that each of the figures is provided for the purpose of illustration and description only and is not intended as a definition of the limits of the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] For a more complete understanding of the present invention, reference is now made to the following descriptions taken in conjunction with the accompanying drawings.

[0019] FIG. 1 shows the configuration of a MALDI-IM-oTOF MS.

[0020] FIG. 2 is a mobility-mass 2D spectrum of aerosolized gramicidin D impacted onto the layer of DHB crystallites (mass spectrum is shown on top). The gramicidin parent molecular ion shows up at around m/z of 1200.

[0021] FIG. 3 is a mobility-mass 2D spectrum of *E. coli* bacteria (bottom) and its convolution into 1D mass spectrum (top).

[0022] FIG. 4 shows a mobility-mass 2D spectrum of *E. coli* bacteria compared to the mass spectrum of the same sample obtained by high vacuum MALDI (bottom).

[0023] FIG. 5 shows a mobility-mass 2D spectrum of *Bacillus subtilis* compared to the mass spectrum of the same sample obtained by high vacuum MALDI (bottom).

DETAILED DESCRIPTION OF THE INVENTION

[0024] It is readily apparent to one skilled in the art that various embodiments and modifications can be made to the invention disclosed in this Application without departing from the scope and spirit of the invention.

I. Definitions

[0025] As used herein, the use of the word “a” or “an” when used in conjunction with the term “comprising” in the claims and/or the specification may mean “one,” but it is also consistent with the meaning of “one or more,” “at least one,” and “one or more than one.” Still further, the terms “having”, “including”, “containing” and “comprising” are interchangeable and one of skill in the art is cognizant that these terms are open ended terms.

[0026] As used herein the terms “aerosol” or “aerosolized” refers to a suspension of fine solid or liquid particles in gas, such as the atmosphere.

[0027] As used herein the term “aerosolized biogenic particle” refers to biogenic particles that are naturally airborne or biogenic particles that have been modified such that they are airborne.

[0028] As used herein, the terms “aerosolized bacteria” or “aerosolized bacterium” refers to bacteria or bacterium that are naturally airborne bacteria, for example, spore-forming bacteria. Yet further, one of skill in the art also realizes that non-naturally airborne bacteria can be lyophilized and introduced into the atmosphere. Thus, aerosolized bacteria include all naturally occurring airborne bacteria or bacterium that is modified such that it is aerosolized.

[0029] As used herein, the term “atmosphere” refers to the entire gaseous envelope surrounding the earth, including the troposphere, tropopause, and stratosphere.

[0030] As used herein the term “bacteria” or “bacterium” refers to single-cell prokaryotic microorganism.

[0031] As used herein the term “biogenic particle” refers to whole cells or cellular components of prokaryotic organisms (*i.e.*, bacteria), viruses, eukaryotic organisms (*e.g.*, filamentous fungi, yeasts and molds), prions, components of these organisms (*e.g.*, spores and toxins) and pollen that will generate biomarkers when the cells or cellular components are subjected to mass spectrometry.

[0032] As used herein the term “biomarker” refers to an ion or charged molecular fragment produced by mass spectrometry that produces a unique mass-mobility spectrum at the genus, species and strain level. The molecular fragment includes but is not limited to lipids, phospholipids, lipopolysaccharides, oligosaccharides, proteins and nucleic acids. Still further, one of skill in the art realizes that a biomarker can also include trend lines or patterns of lipids, phospholipids, lipopolysaccharides, oligosaccharides, proteins and nucleic acids. Thus, biomarkers include not only an ion or charged molecular fragment, but also trend lines, pattern analysis or cluster analysis of mass-mobility spectra of more than a single ion or charged molecular fragment.

[0033] As used herein, the term “eukaryotic” refers to an organism whose cells have a well-defined nucleus and membrane-bound organelles, such as mitochondria, Golgi bodies, and endoplasmic reticulum.

[0034] As used herein, "gram-negative bacteria" or "gram-negative bacterium" refers to as bacteria which have been classified by the Gram stain as having a red stain. Gram-negative bacteria have thin walled cell membranes consisting of a single layer of peptidoglycan and an outer layer of lipopolysacchacide, lipoprotein, and phospholipid. Exemplary organisms include, but are not limited to, Enterobacteriaceae consisting of *Escherichia*, *Shigella*, *Edwardsiella*, *Salmonella*, *Citrobacter*, *Klebsiella*, *Enterobacter*, *Hafnia*, *Serratia*, *Proteus*, *Morganella*, *Providencia*, *Yersinia*, *Erwinia*, *Buttlauxella*, *Cedecea*, *Ewingella*, *Kluyvera*, *Tatumella* and *Rahnella*. Other exemplary gram-negative organisms not in the family Enterobacteriaceae include, but are not limited to, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, *Burkholderia*, *Cepacia*, *Gardenerella*, *Vaginalis*, and *Acinetobacter* species.

[0035] As used herein, "gram-positive bacteria" or "gram-positive bacterium" refers to bacteria, which have been classified using the Gram stain as having a blue stain. Gram-positive bacteria have a thick cell membrane consisting of multiple layers of peptidoglycan and an outside layer of teichoic acid. Exemplary organisms include, but are not limited to, *Staphylococcus aureus*, coagulase-negative staphylococci, streptococci, enterococci, corynebacteria, and *Bacillus* species.

[0036] As used herein, "IMS" is defined as an ion mobility spectrometer. An ion mobility spectrometer consists of a drift tube in which ions traveling in a gaseous medium in the presence of an electric field are separated according to their ion mobilities. The ion mobilities of specific ion species result from the conditions of drift tube pressure and potential of the ion mobility experiment. The repetitive accelerations in the electric field and collisions at the molecular level result in unique ion mobilities for different ion species.

[0037] As used herein, the term "matrix" refers to a substance mixed with the analyte (typically prior to deposition) and deposited on the sample surface in association with the analyte to absorb at least part of the energy from the energy source (e.g., laser) to facilitate desorption of intact molecules of the analyte. The "matrix" is typically a small organic acid. Typical matrices include, but are not limited to sinapinic acid di-hydroxy-benzoic acid, and cyano-hydroxy-cinnamic acid or other of the "known" matrices in the literature. However, the use of alternative matrices in place of these conventional matrices will become essential. One of the major limitations for the use of MALDI is the inability to quickly and reliably incorporate matrix with the biogenic particle. As a solution to this, copending applications currently filed on

June 4, 2004 having Attorney Registration No. 45,546 and Express Mail No. ER509324104US entitled "Gold Implantation/Deposition of Biological Samples for Laser Desorption Three Dimensional Depth Profiling of Tissues" and Attorney Registration No. 45,546 and Express Mail No. ER509324118US entitled "Fullerene-Based MALDI Matrices for Peptides and Proteins" and claiming priority to U.S. Provisional Application Nos. 60/476,309 and 60/476,718, respectively, each of which is incorporated herein by reference in its entirety, describe the use of derivatized fullerenes and cluster ion implantation. These matrices can be added as a solution onto the MALDI target surface. Alternatively, the MALDI target onto which the aerosols are impacted can be modified by chemically attaching the fullerene or by cluster implantation so that the aerosol capture and matrix incorporation are done in the same process.

[0038] As used herein, "TOF" is defined as a time-of-flight mass spectrometer. A TOF is a type of mass spectrometer in which ions are all accelerated to the same kinetic energy into a field-free region wherein the ions acquire a velocity characteristic of their mass-to-charge ratios. Ions of differing velocities separate and are detected.

[0039] As used herein, "MALDI/IM/o-TOF" is an abbreviation for and defined as a matrix assisted laser desorption ionization/ion mobility/orthogonal time-of-flight mass spectrometer.

[0040] As used herein, "IM/o-TOF" is an abbreviation for and defined as an ion mobility/orthogonal time-of-flight mass spectrometer. Exemplary IM/oTOF apparatuses that may be used in the present invention include, but are not limited to Gillig *et al.* and U.S. Patent Publication Nos. 20010032930, 20010032929 and 20030001087, each of which is incorporated by reference herein in its entirety.

[0041] As used herein, the term "microorganism" refers to a microscopic organism, for example bacteria, viruses, prions, fungi, and protozoa.

[0042] The term "non-pathogenic bacteria" or "non-pathogenic bacterium" includes all known and unknown non-pathogenic bacterium (gram positive or gram negative).

[0043] The term "pathogenic bacteria" or "pathogenic bacterium" includes all known and unknown bacteria (gram positive or gram negative) that cause disease. One specific example of a pathogenic bacterium is *Bacillus anthracis*, which is a spore-forming bacterium that causes the acute infectious disease Anthrax.

[0044] As used herein, the term "prokaryotic" refers to a simple unicellular organism with no nuclear membrane, mitochondria, Golgi bodies, or endoplasmic reticulum.

[0045] As used herein, the term "prion" refer to a small proteinaceous infectious particle. Prions cause spongiform encephalopathies (slow neurodegenerative diseases).

II. Mass spectrometry

[0046] Mass spectrometry is a method of chemical analysis that uses the mass of a substance to identify the substance. To elaborate, associated with every type of molecule is a mass spectrum, a kind of "fingerprint", that is relatively unique to each particular molecule. The chemical analysis of an unknown substance by mass spectrometry involves obtaining a mass spectrum for the substance and comparing the mass spectrum to a library of mass spectra for known substances to identify the chemical components of the unknown substance.

[0047] The MALDI-MS technique is based on the discovery in the late 1980s that desorption/ionization of large, nonvolatile molecules such as proteins can be effected when a sample of such molecules is irradiated after being codeposited with a large molar excess of an energy-absorbing "matrix" material, even though the molecule does not strongly absorb at the wavelength of the laser radiation. The abrupt energy absorption initiates a phase change in a microvolume of the absorbing sample from a solid to a gas while also inducing ionization of the sample molecules.

[0048] Detailed descriptions of the MALDI-TOF-MS technique and its applications may be found in review articles by E. J. Zaluszc et al. (Protein Expression and Purification, Vol. 6, pp. 109-123 (1995)) and D. J. Harvey (Journal of Chromatography A, Vol. 720, pp. 429-4446 (1996)). In brief, the matrix and analyte are mixed to produce a solution with a matrix:analyte molar ratio of approximately 10,000:1. A small volume of this solution, typically 0.5-2 μ l, is applied to a stainless steel probe tip and allowed to dry. During the drying process the matrix codeposits from solution with the analyte.

[0049] Ionization of the analyte is effected by pulsed laser radiation focused onto the probe tip which is located in a short (~5 cm) source region containing an electric field. The ions formed at the probe tip are accelerated by the electric field toward a detector through a flight tube, which is a long (~1 m) field free drift region. Since all ions receive the same amount of energy, the time required for ions to travel the length of the flight tube is dependent on their

mass. Thus, low-mass ions have a shorter time of flight (TOF) than heavier ions. All the ions that reach the detector as the result of a single laser pulse produce a transient TOF signal.

[0050] The mass of an unknown analyte is determined by comparing its experimentally determined TOF to TOF signals obtained with ions of known mass. The MALDI-TOF-MS technique is capable of determining the mass of proteins of between 1 and 40 kDa with a typical accuracy of $\pm 0.1\%$, and a somewhat lower accuracy for proteins of molecular mass above 40 kDa.

[0051] Yet further, it may be necessary to separate the bulk of ions in time prior to supplying them to the ion acceleration region of the MS instrument. Mass spectrometry is then performed sequentially on "packets" of separated ion samples, rather than simultaneously on the bulk of the generated ions. In this manner, mass spectral information provided by the MS instrument is spread out in another dimension to thereby reduce the localized congestion of mass information associated with the bulk ion analysis.

[0052] One known ion separation technique which is used to pre-separate the bulk of the ions in time prior to MS analysis is ion mobility spectrometry (IMS). As is known in the art, IMS instruments typically include a pressurized static buffer gas contained in a drift tube which defines a constant electric field from one end of the tube to the other. Gaseous ions entering the constant electric field area are accelerated thereby and experience repeated collisions with the buffer gas molecules as they travel through the drift tube. As a result of the repeated accelerations and collisions, each of the gaseous ions achieves a constant velocity through the drift tube. The ratio of ion velocity to the magnitude of the electric field defines an ion mobility, wherein the mobility of any given ion through a high pressure buffer gas is a function of the collision cross-section of the ion with the buffer gas and the charge of the ion. Generally, compact conformers, *i.e.*, those having smaller collision cross-sectional areas, have higher mobilities, and hence higher velocities through the buffer gas, than diffuse conformers of the same mass, *i.e.*, those having larger collision cross-sectional areas. Thus, ions having larger collision cross-sections move more slowly through the drift tube of an IMS instrument than those having smaller collision cross-sections, even though the ions having smaller collision cross-sections may have the same or even greater mass than those having higher collision cross-sections.

III. Identification of Biogenic Particles

[0053] The present invention is directed to identifying biogenic particles in an atmospheric sample by analyzing biomarkers that are produced from a mass-mobility spectra of the sample by using MALDI-IM-oTOF MS. This technique allows for fast or rapid sampling rates.

[0054] In specific embodiments, it is envisioned that the present invention is used to determine the presence of a pathogenic bacterium in an atmospheric sample. The presence of pathogenic bacterium is determined by analyzing biomarkers that are produced from a mass-mobility spectra of an atmospheric sample by using MALDI-IM-oTOF MS

[0055] Biomarkers that are used in the present invention are protein molecules which are specific to the genetic code. The average mass of the protein molecule can be any size that identifies the microorganism of interest. Other biomarkers include in a mass range of less than 5,000 a.m.u., but are not limited to lipids, oligosaccharides, lipopolysaccharides, glycans, or nucleic acid molecules (RNA or DNA). Still further, one of skill in the art realizes that the biomarker of the present invention may also include trend lines of spectra, patterns of spectra and cluster analysis of spectra. Trend lines, pattern analysis and cluster analysis are standard statistical techniques that are known and used in the art.

[0056] The biogenic particle is whole cells or cellular components of prokaryotic organisms (*i.e.*, bacteria), viruses, prions, eukaryotic organisms (*e.g.*, filamentous fungi, yeasts and molds), components of these organisms (*e.g.*, spores and toxins) and pollen that will generate biomarkers when the cells or cellular components are subjected to mass spectrometry.

[0057] It is envisioned that the present invention can identify non-pathogenic bacterium or pathogenic bacterium. The bacterium (pathogenic or non-pathogenic) can be a gram-negative bacterium or gram-positive. Exemplary organisms include, but are not limited to *Escherichia*, *Shigella*, *Edwardsiella*, *Salmonella*, *Citrobacter*, *Klebsiella*, *Enterobacter*, *Hafnia*, *Serratia*, *Proteus*, *Morganella*, *Providencia*, *Yersinia*, *Erwinia*, *Buttiauxella*, *Cedecea*, *Ewingella*, *Kluyvera*, *Tatumella*, *Rahnella*, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, *Burkholderia*, *Cepacia*, *Gardenerella*, *Vaginalis*, *Acinetobacter* species, *Staphylococcus aureus*, coagulase-negative staphylococci, streptococci, enterococci, corynebacteria, and *Bacillus* species.

[0058] More specifically, the gram-negative bacterium is a species of *Escherichia*, more particularly, *Escherichia coli*. An example of the gram-positive bacterium is a species of *Bacillus*, more particularly, *Bacillus anthracis* or *Bacillus subtilis*.

[0059] Another embodiment of the present invention is to identify the presence of viruses in the atmosphere. Viruses are propagated by infecting host animal cells with a virus. The virus within a host cell uses the resources and environment of the host cell to reproduce. At some point, the viruses produced within a cell rupture the cell wall and move on to infect other cells and repeat the process. Exemplary viruses include, but are not limited to adenovirus, varicella-zoster virus (chicken pox), poxviruses (*e.g.*, smallpox or molluscum contagiosum), parvoviruses, picornaviruses (*e.g.*, rhinoviruses, poliovirus), paramyxoviruses (*e.g.*, measles virus, parainfluenza viruses, respiratory syncytial virus, mumps virus) orthomyxoviruses (*e.g.*, influenza viruses A and B) and reoviruses.

[0060] Still further, another biogenic particle includes prions, which are also known as slow virus agents. Prions are modified host protein that transmit disease.

[0061] It is envisioned that the present invention can be used to identify filamentous fungi. Filamentous fungi for analyzing herein are eukaryotic organisms with multicellular structures bounded by a rigid cell wall containing, for example, chitin. Fungi ordinarily grow as multicellular colonies, *e.g.*, mushrooms or molds, and form a mycellum through a mass of branching, interlocking filaments, or hyphae. Although these branches are interrupted by cross-walls, the passage of cytoplasm between compartments is possible. Both sexual and asexual reproduction can occur in fungi. In asexual reproduction, spores, known as "conidia", are borne externally at the tips of budding projections formed at various locations along the filaments. Most fungal spores range in size, *e.g.*, from about 2 to about 50 μm . Spores and hyphal fragments of fungi are ubiquitous in air where they are sometimes the major pollutant and sources of infection or allergic reactions.

[0062] The families represented by the filamentous fungi herein include, but are not limited to, Phycomycetes, Ascomycetes, *e.g.*, *Neurospora*, *Aspergillus* and *Penicillium*, *Basidiomycetes* and *Deuteromycetes*.

[0063] Yeasts and molds are known to those skilled in the art to be members of the fungal kingdom. Yeast for use herein are ordinarily a simple form of fungi that generally consist

of single cells rather than hyphae. They typically reproduce asexually by budding (*e.g.*, a small outgrowth on the cell's surface which increases in size until a wall forms to separate the new individual from the parent) and fission. Some yeast, such as, for example, *Saccharomyces cerevisiae*, (also referred to as brewer's yeast or baker's yeast) can exhibit, under certain conditions, a filamentous mold-like form. These filamentous cells, often referred to as pseudohyphal cells, have an elongated morphology.

[0064] Additionally, certain fungi, including molds, produce toxins such as T-2 mycotoxins and Aflatoxin B₁. In some instances, these toxins can be airborne.

[0065] Still further, it is envisioned that pollen can be identified using the present invention. Pollen herein are ordinarily in the form of grains which are microspores of seed plants containing, for example, a male gametophyte. Some pollen grains are dispersed by the wind and may be allergenic to pollen-sensitive individuals. Common pollen grains include those produced by grasses, trees, shrubs and weeds. Pollen can also include, for example, proteins, polypeptides, polysaccharides, glycoproteins, and lipoproteins in both the pollen outer wall and cytoplasm.

[0066] MALDI-TOF-MS technique is combined with IM (as shown in Figure 1) to generate the biomarkers of the biogenic particle, such as bacteria. This technique obtains 2D mass-mobility spectra of biological samples and adds a new dimension to the regular MALDI MS, which produces only the mass information. In MALDI-IM-oTOF MS, the ions are separated by their shape in a gas filled drift tube before they enter the mass spectrometer. Most of biomolecular components of the cell, such as proteins lipids, oligonucleotides and others can be easily distinguished by their mobility from other bio- and non-biological components of the MALDI sample.

[0067] Due to the mobility separation, it can be determined when the laser has located a biogenic particle on an aerosol collector surface such as found in an Andersen N-6 single-stage bioaerosol sampler. Using MALDI-IM-oTOF MS it is possible to simultaneously see ions from the major components of the biogenic particle, for example a microorganism cell - peptides/proteins on one trend line and lipopolysaccharides on another. This particular simultaneous combination of ions is characteristic of a particle of biological origin. Since very few counts are needed to establish the existence of trend lines, and since these counts are in

regions of the 2D mobility plot which are unique to their chemical class, one can - within one or two laser shots - quickly determine which spatial region contains biogenic particles.

[0068] Thus, a complete mass spectrum does not have to be collected to decide that the laser is focused onto a microorganism rich surface area on the aerosol collector surface. As soon as the characteristic microorganism molecules are present, the laser can remain long enough at this position to determine the exact mass of the genetically determined proteins and thus determine the microorganism. The laser spot can remain in this location until the peptide and polysaccharide signals associated with the microorganism are lost (*i.e.* until all the microorganism in the region has been laser ablated). At this point the laser is moved to another spot and the question is again asked within a few laser shots—are there characteristic microorganism trend lines? If not, then the decision can be made with equal certainty to move the laser to the next spot.

[0069] Thus, the present invention works by seeking the strongly correlated MALDI ion signals from (initially) intact microorganism which, after laser irradiation appear in the mobility spectra as well resolved lipopolysaccharides and peptide/protein ions in both the low and high mass region. A combination of a high repetition rate laser and an X-Y stage for moving the sample would allow quick interrogation of different surface regions of the collector using the ion mobility correlations to determine which region contains bacteria and which does not. If biogenic material is found, an entire spectra is acquired at this spot, otherwise the laser is moved to the next position in the X-Y grid.

[0070] Using this method one can rapidly interrogate an aerosol collector surface area of approximately 6 cm² in a reasonable time of one minute if the following assumptions are made. For example, a laser with a repetition rate of 2 kHz is used (thus allowing a mobility drift time of 500 microseconds). The laser beam is focused into a laser spot diameter of 0.2 mm (hence covering an area of 0.03 mm²). This allows for the coverage of a reasonably sized surface area rather quickly. Thus, probably two laser shots are needed to decide if a spot contains biogenic aerosol or not. If not, then the sample is moved by 0.1mm (overlapping the new location area with half of the already interrogated area). By overlapping the new location area with the interrogated area, the laser covers an area of .015 mm² at each spatial location by overlapping adjacent laser positions so that no area of the samples collector surface is missed. In one second, an area on the aerosol collector surface equal to 0.015mm² x 1000 (15

mm²/second) is covered. So, in 45 seconds, a 675 mm² area is interrogated for the presence or absence of bacteria. This leaves 15 seconds (30,000 laser shots) for detailed mass measurement of the particular regions containing biogenic material to determine the type of microorganism present. This technique allows complete interrogation of a 1 inch diameter collection area in a minute. This may help to increase the speed of sampling large gas volumes which is at present limited by having to pass the gas through small collector areas. It is necessary to sample several liters of gas which may in the end only contain a few hundred bacteria.

[0071] Alternatively, the laser is focused to 20 microns or less which is the size of many microorganisms or spores. The collector area which is scanned in one minute would then be 100 times smaller (a few mm in diameter). However, this approach may be advantageous because the laser beam would then either be entirely on or entirely off of a single bacterium. Thus, only spectra from the microorganism on the collector surface is acquired. Any spectra lacking the requisite mobility patterns are saved separately or discarded. This technique can be combined with an optical microscopic location of particles above 1 micron if a particular particle size is known before-hand. Such combined techniques for positioning the laser to specific collected particles eliminates or reduces the need to laser scan the entire collector area or other biogenic particles.

IV. Examples

[0072] The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Example 1

MALDI-IM-oTOF instrument

[0073] MALDI-IM-oTOF instrument was used for analysis of aerosol particles. A matrix layer was prepared by depositing 200 µL of matrix solution deposited on the impactor target. Then the target was placed into an Andersen N-6 single-stage bioaerosol sampler, and the

analyte solution (1 mg/mL gramicidin in MeOH) was placed into a Collison nebulizer, where the particles are generated at an airflow rate of 5 L/M. Particles are drawn into the impactor and deposited on top of pre-coated matrix target (collection time – 2 minutes).

[0074] Figure 2 shows a typical spectrum of gramicidin D aerosolized and impacted onto the target. The success of the analysis depended on the type of the collection matrix. For example, an additional deposition of CHCA solution on top of the impacted layer was necessary to obtain high intensity of the gramicidin signal. On the other hand, when gentisic acid (DHB) was used as a matrix, no additional deposition was required. This is, most likely, the result of different crystalline structure of the two matrices, as well as different mechanisms of the matrix-analyte integration.

Example 2

Analysis of *E. coli*

[0075] Mobility-mass 2D spectra of several samples of bacteria prepared by the modified dried droplet method were obtained using α -cyano-4-hydroxycinnamic acid (CHCA) as a matrix. *E. coli* (lyophilized) b strain, American Type Culture Collection (ATCC) # 11303, was used in concentration of 20 mg/mL in 70:30 acetonitrile: water in .1% TFA.

[0076] The matrix was prepared as a saturated solution of CHCA in 70:30 acetonitrile: water in .1% TFA. Then 10 μ L of bacteria solution was deposited on target area (5 spots) and allowed to air dry. Each spot contained 10^7 bacteria. Next, 10 μ L of matrix solution deposited on top of bacteria and allowed to air dry.

[0077] Figure 3 shows the 2D mobility/mass plot for the *E. coli* (strain b) along with the 1D mass spectrum (plotted on top). The 1D spectrum was derived by summing all ions irrespective of their mobility at each value of m/z . High repetition rate (up to 500Hz) 3rd harmonics of the Nd:YLF laser was used in this data acquisition. For comparison to high vacuum MALDI results, the mobility/mass data are re-plotted in figure 4 and the high vacuum MALDI spectrum obtained from the same sample is shown as an insert along the m/z axis in figure 4.

[0078] Figure 4 shows that the main peaks in the mass range of 5000-10000 a.m.u. in the high vacuum MALDI spectrum corresponded to known protein markers and were aligned along a trend line which was known from previous work to be a peptide/protein “trend line”. In contrast, the ions in the mass region below 5,000 comprise both peptide/protein ions as well as

ions having the same masses, but significantly slower mobility. These ions appear along a trend line which was approximately 60% slower than the peptide line. This trend line most likely comprised lipopolysaccharides or glycans which were known to be components of bacterial cell wall surfaces. There was also a “third trend line” of ions with even slower mobility; the nature of those ions was unclear.

Example 2

Analysis of *B. subtilis*

[0079] Mobility-mass 2D spectra of several samples of bacteria prepared by the modified dried droplet method were obtained using α -cyano-4-hydroxycinnamic acid (CHCA) as a matrix. *Bacillus subtilis* (lyophilized, ATCC # 6633), was used in concentration of 20 mg/mL in 70:30 acetonitrile: water in .1% TFA.

[0080] The matrix was prepared as a saturated solution of CHCA in 70:30 acetonitrile: water in .1% TFA. Then 10 μ L of bacteria solution was deposited on target area (5 spots) and allowed to air dry. Each spot contained 10^7 bacteria. Next, 10 μ L of matrix solution deposited on top of bacteria and allowed to air dry.

[0081] Figure 5 shows mobility and mass spectra obtained for *B. subtilis* using high vacuum MALDI. While the same trend lines were observed as in figure 4, the characteristic masses of the peptide and protein peaks along the trend line were very different between the two bacteria types. Also the low mobility components from *Bacillus subtilis* are very different compared to those in the *E.coli* spectrum. The separation of the peptide and glycolipid constituents by the ion mobility allowed the glycolipid ions to be considered as additionally useful marker molecules for bacteria which may further supplement the identification of bacteria type instead of relying solely on identifying large proteins. Furthermore, for the first time, peptides and proteins below mass 5000 became useful bacteria biomarkers because the ion mobility provided separation from the glycolipids which allowed their unambiguous mass and chemical type assignment. Until these experiments the lower mass region was not used for marker ions because of the spectral congestion and known glycolipid contamination in the 1D mass spectrum. Use of the low mass region is highly desirable if possible, because it is so much easier to detect the smaller ions.

REFERENCES

[0082] All patents and publications mentioned in the specifications are indicative of the levels of those skilled in the art to which the invention pertains. All patents and publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

U.S. Patent Publication No. 20010032930.

U.S. Patent Publication No. 20010032929.

U.S. Patent Publication No. 20030001087.

Gillig *et al.*, Anal. Chem. pp. 72, 3965 (2000).

Harvey, Journal of Chromatography A, Vol. 720, pp. 429-4446 (1996).

Zaluzec *et al.*, Protein Expression and Purification, Vol. 6, pp. 109-123 (1995)

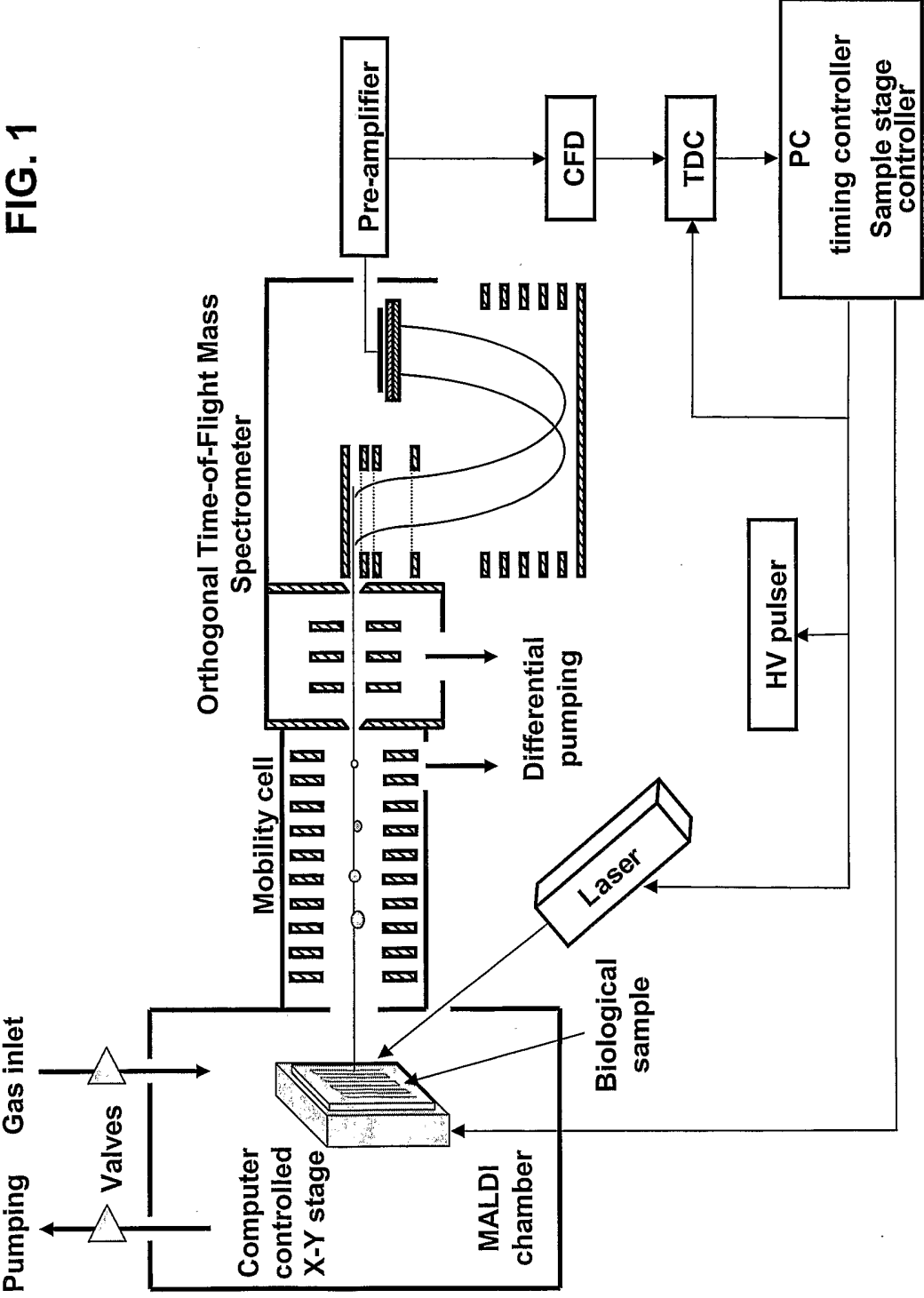
[0083] Although the present invention and its advantages have been described in detail, it should be understood that various changes, substitutions and alterations can be made herein without departing from the spirit and scope of the invention as defined by the appended claims. Moreover, the scope of the present application is not intended to be limited to the particular embodiments of the process, machine, manufacture, composition of matter, means, methods and steps described in the specification. As one of ordinary skill in the art will readily appreciate from the disclosure of the present invention, processes, machines, manufacture, compositions of matter, means, methods, or steps, presently existing or later to be developed that perform substantially the same function or achieve substantially the same result as the corresponding embodiments described herein may be utilized according to the present invention. Accordingly, the appended claims are intended to include within their scope such processes, machines, manufacture, compositions of matter, means, methods, or steps.

We claim:

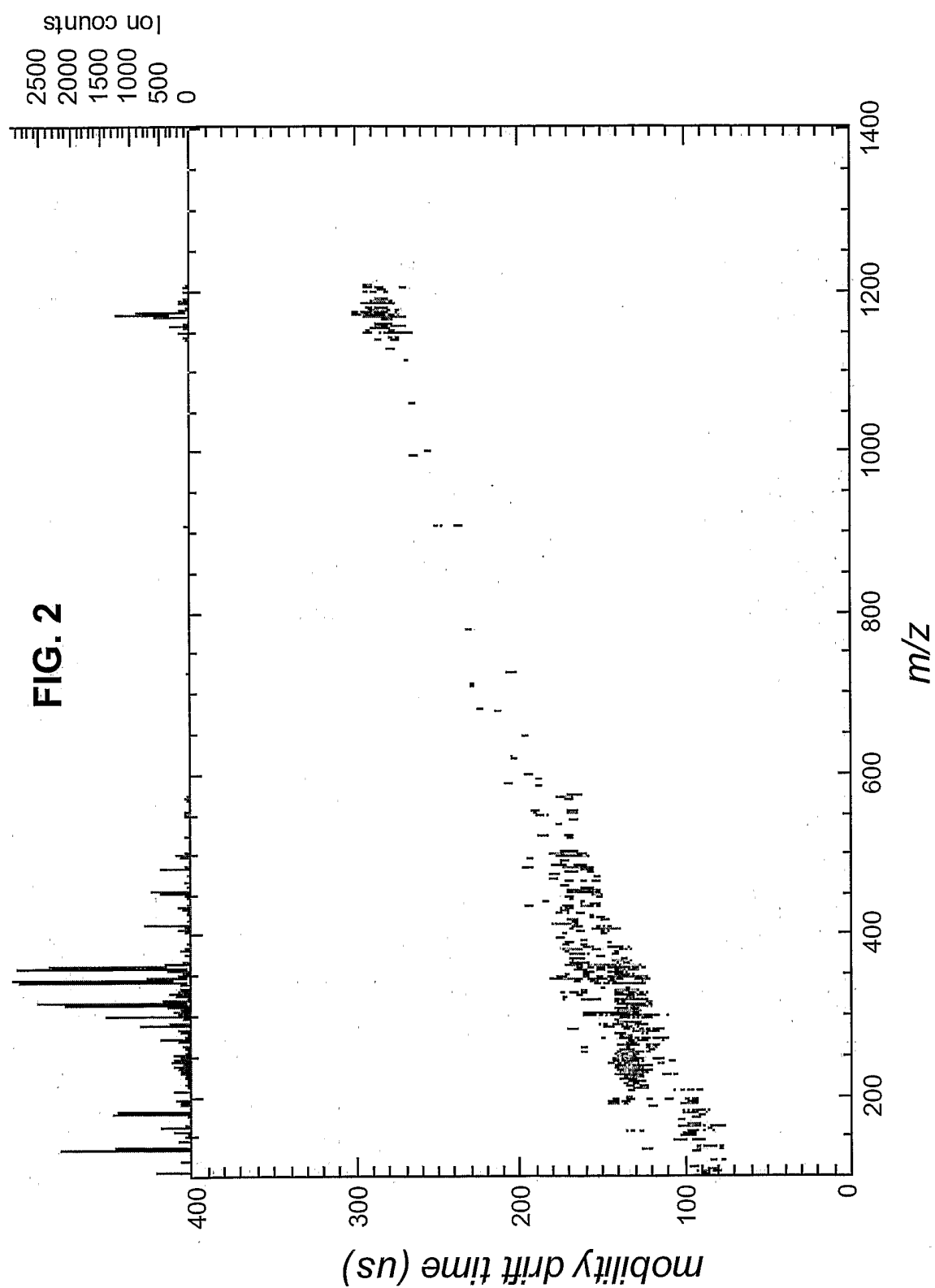
1. A method of identifying a biogenic particle in a sample of atmosphere comprising the steps of:
 - collecting a sample of atmosphere;
 - analyzing the sample of atmosphere, wherein analyzing comprises obtaining a mass-mobility spectra of the sample; and
 - utilizing a biomarker determined from the mass-mobility spectra to identify the biogenic particle present in the sample of atmosphere.
2. The method of claim 1, wherein the biogenic particle is a prokaryotic or eukaryotic organism.
3. The method of claim 1, wherein the biogenic particle is a virus or a prion.
4. The method of claim 2, wherein prokaryotic organism is a bacterium.
5. The method of claim 4, wherein the bacterium is a non-pathogenic bacterium.
6. The method of claim 4, wherein the bacterium is a pathogenic bacterium.
7. The method of claim 6, wherein the pathogenic bacterium is a gram-negative bacterium.
8. The method of claim 7, wherein the gram-negative bacterium is a species of *Escherichia*.
9. The method of claim 8, wherein the species of *Escherichia* is *Escherichia coli*.
10. The method of claim 6, wherein the pathogenic bacterium is a gram-positive bacterium.
11. The method of claim 10, wherein the gram-positive bacterium is a species of *Bacillus*.
12. The method of claim 11, wherein the species of *Bacillus* is *Bacillus anthracis* or *Bacillus subtilis*.
13. The method of claim 2, wherein the eukaryotic organism is filamentous fungi, yeasts or molds.

14. The method of claim 1, wherein the biogenic particle is a component of spores, toxins, or pollen.
15. The method of claim 1, wherein the biomarker is a protein.
16. The method of claim 1, wherein the biomarker is selected from the group consisting of lipids, oligosaccharides, lipopolysaccharides, glycans and nucleic acids.
17. The method of claim 16, wherein the mass of the biomarker is less than 5000 a.m.u.
18. The method of claim 1, wherein the mass-mobility spectra is obtained from a MALDI-IM-oTOF MS.
19. A method of determining the presence of a pathogenic bacterium in a sample of atmosphere comprising the steps of:
 - collecting a sample of atmosphere;
 - analyzing the sample of atmosphere, wherein analyzing comprises obtaining a mass-mobility spectra of the sample; and
 - utilizing a biomarker determined from the mass-mobility spectra to determine the presence of the pathogenic bacterium in the sample of atmosphere.
20. The method of claim 19, wherein the biomarker is a protein.
21. The method of claim 19, wherein the biomarker is selected from the group consisting of lipids, oligosaccharides, lipopolysaccharides, glycans and nucleic acids.
22. The method of claim 21, wherein the mass of the biomarker is less than 5000 a.m.u.
23. The method of claim 19, wherein the pathogenic bacterium is a gram-negative bacterium.
24. The method of claim 23, wherein the gram-negative bacterium is *Escherichia coli*.
25. The method of claim 19, wherein the pathogenic bacterium is a gram-positive bacterium.
26. The method of claim 25, wherein the gram-positive bacterium is *Bacillus anthracis* or *Bacillus subtilis*.

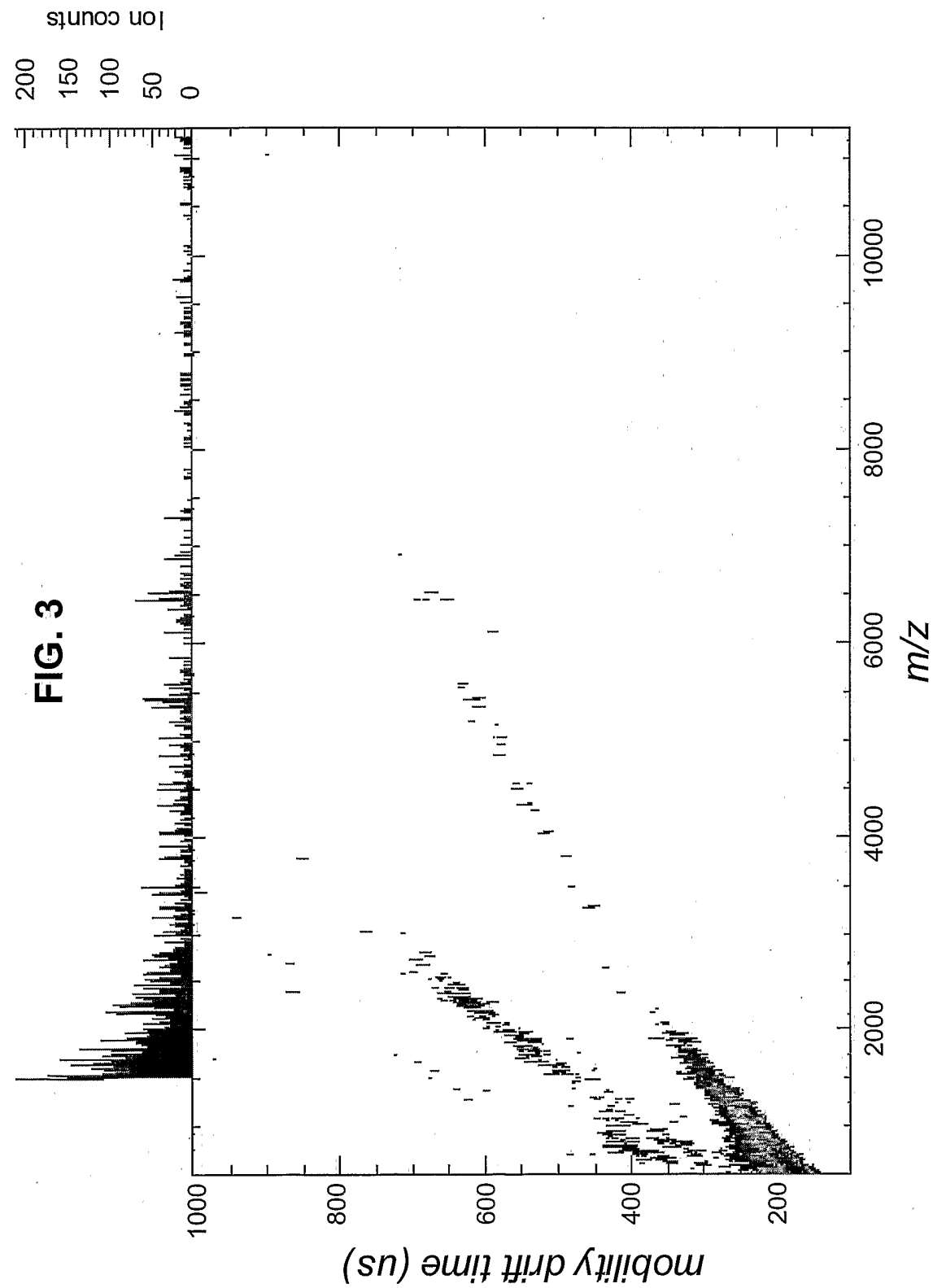
FIG. 1



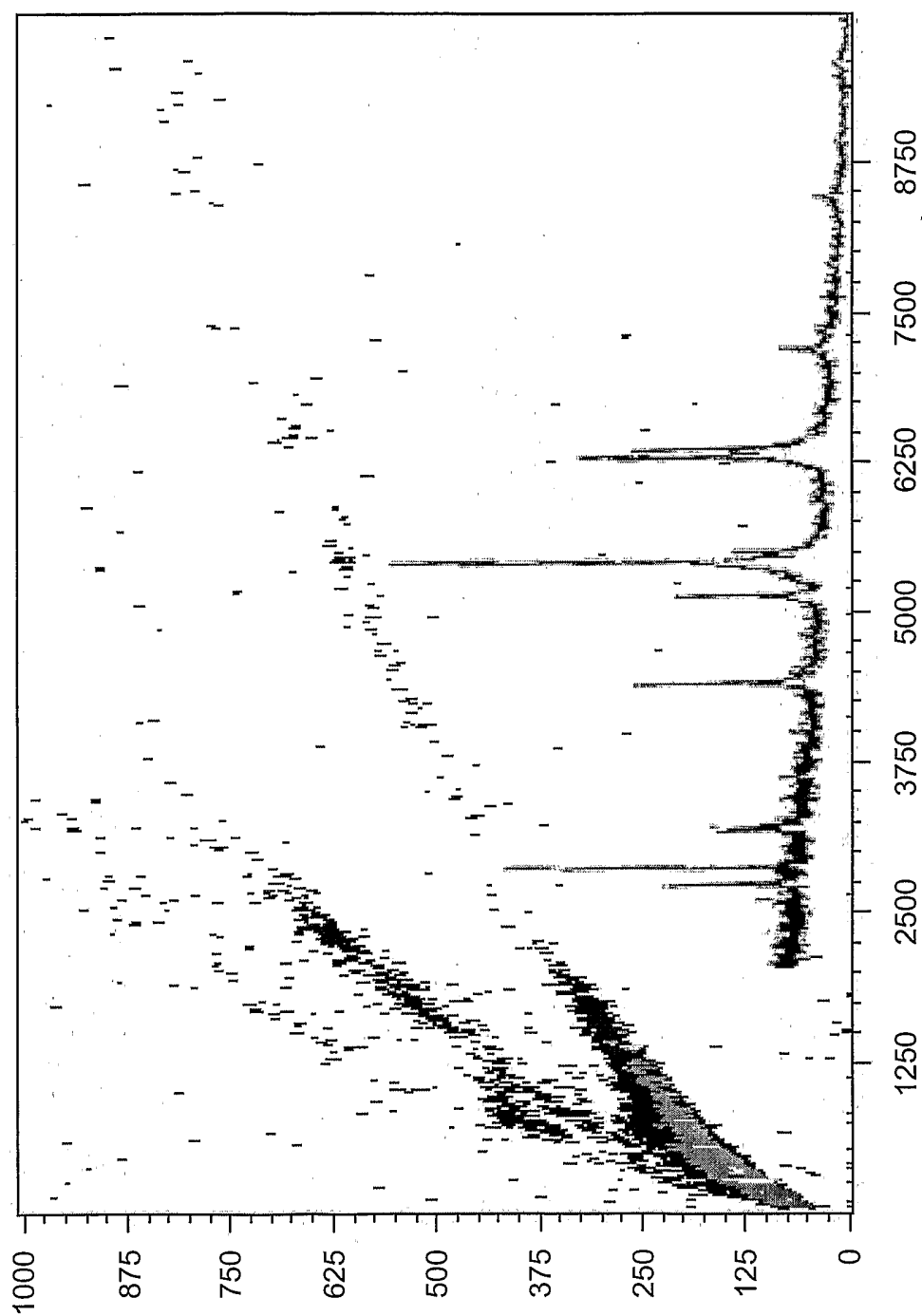
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FIG. 4

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FIG. 5

