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(56) Documents Cited:
US 6248592 B1 **US 5808300 A**
US 20030073145 A1 **US 20030008327 A1**
Int J Mass Spectrom; Vol 24, pp 135-144 (2005). Becker
et al. "Investigation of Cu-, Zn- and Fe-containing
human brain proteins...."
J Anal At Spectrom; Vol 20, pp 210-215 (2005).
Fernandes et al. "Complementary FPLC-IPC-MS and
MALDI-TOF for studying vanadium association to
human serum proteins"

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Other: **WPI, EPODOC, JAPIO, MEDLINE, EMBASE,**
BIOSIS, SCISEARCH, CAPLUS

(54) Abstract Title: **Analysis of biological samples by mass spectrometry**

(57) A method for determining the spatial arrangement of biomolecular and elemental constituents of a tissue sample is disclosed. An area of the tissue sample is exposed to a low energy desorption/ ionisation laser beam to determine its biomolecular constituents, and the same area is subsequently exposed to a high energy ablative laser beam in order to determine the elemental constituents in the sample. The desorption/ ionisation analysis for the biomolecular constituents is preferably performed by MALDI-MS, and the ablative analysis for the elemental constituents is preferably performed by LA-ICP-MS. An apparatus for performing the method is also disclosed. Another embodiment provides a method for preparing a sample for MALDI mass spectrometry.

At least one drawing originally filed was informal and the print reproduced here is taken from a later filed formal copy.

This print takes account of replacement documents submitted after the date of filing to enable the application to comply with the formal requirements of the Patents Rules 1995

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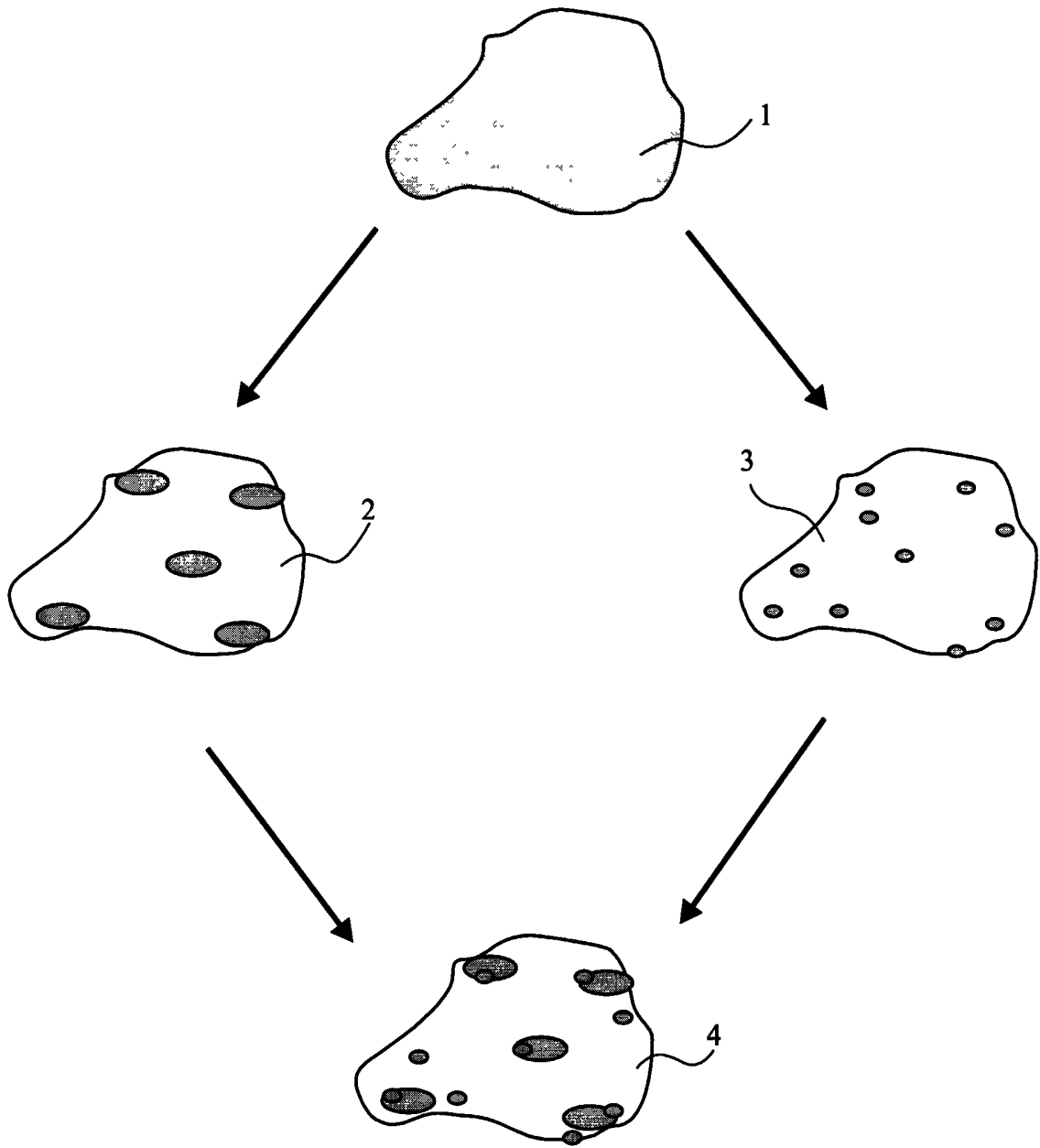


Fig. 1



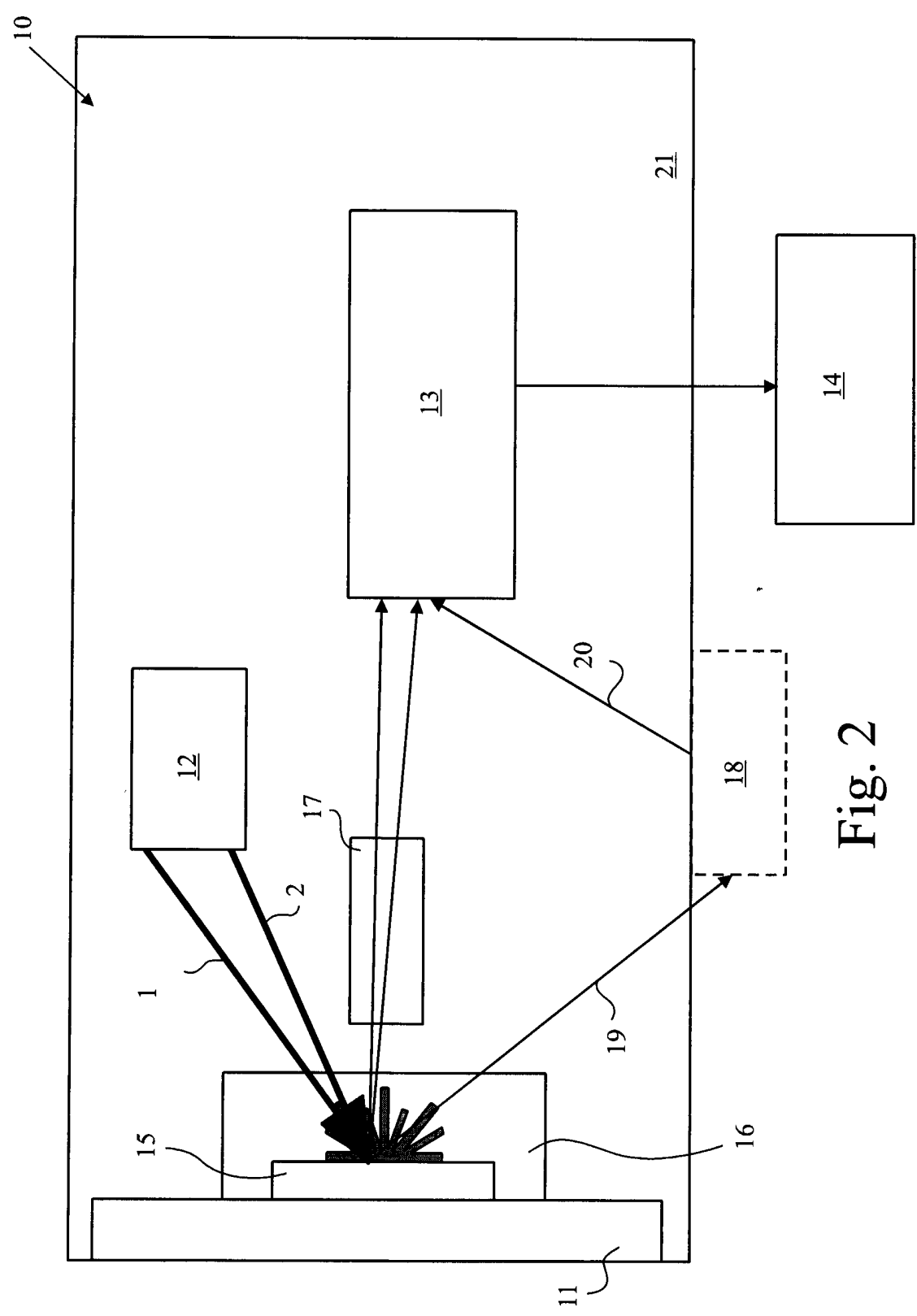


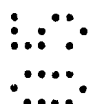
Fig. 2

IMPROVEMENTS IN OR RELATING TO METHODS AND APPARATUS FOR ANALYZING BIOLOGICAL SAMPLES BY MASS SPECTROMETRY

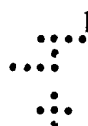
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FIELD OF THE INVENTION

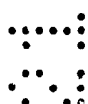
The present invention relates to methods and apparatus for analyzing biological samples by mass spectrometry and more particularly to a method for analysing molecular and
10 elemental components of biopsy tissue. The method and apparatus may find application, for example, in the fields of medical imaging, analysis, monitoring and diagnostics.



BACKGROUND OF THE INVENTION



15 The molecular analysis and imaging of proteins and other biological materials in tissues is an important part of medical imaging, monitoring, analysis, diagnostics and disease classification, and can play a part in the treatment of a variety of disorders. As used herein "biological material" is to be interpreted broadly and can include, for example, nucleic acids, lipids, carbohydrates or any molecule covered in Stryer et al Biochemistry,
20 2002. For example, molecular analysis and imaging may be used as integral building blocks in strategies designed to locate specific proteins that are more highly expressed in tumors relative to normal tissue. Likewise, they may be used to locate specific proteins diminished in expression relative to normal tissue.



25 Laser Desorption Mass Spectrometry (LDMS) is a promising technique for analysis of low molecular weight molecules in tissue, first developed in the 1960's. UV laser spot sizes can be focused to under 5µm, and so the technique is ideally suited to spatially resolved analyses. Laser Desorption Mass Spectrometry (LDMS) to obtain spatial information is often referred to as Laser Microprobe Mass Spectrometry (LMMS). The
30 technique became widely available by the introduction of commercial instruments (Zenobi, 1995). Several reviews (Cotter, 1987) (Hillenkamp book) (L. Van Vaeck et al

1994) (Zenobi 1995) discuss applications, fundamentals and limitations of LD and LMMS.

A major limitation of the technique was the accessible mass range, as ionisation of intact biomolecules over around 1000u was impossible. This was overcome with the introduction of Matrix Assisted Laser Desorption Ionisation MS (MALDI-MS) (Hillenkamp and Tanaka).

Matrix-assisted laser desorption/ionisation ("MALDI") mass spectrometry provides for the spectrometric determination of a mass of poorly ionising or easily fragmented analytes of low volatility by embedding them in a matrix of light-absorbing material. The matrix material, which is present in large excess relative to the analyte, serves to absorb energy from the laser pulse and to transform it into thermal and excitation energy to desorb and ionise the analyte. This technique was introduced in 1988 by Hillenkamp and Karas [Karas, M. and Hillenkamp, F. (1988). Anal. Chem. 60:2299] for use with large biomolecules. Since then, the practice of MALDI mass spectrometry has advanced rapidly and has found applications in the mass determination of molecules ranging from small peptides, oligosaccharides and oligonucleotides to large proteins and synthetic polymers.

More recently, MALDI imaging has been developed as a method for spatially resolving peptides, proteins and drugs in biological tissue and is demonstrating potential as a means of measuring peptides, proteins, and drugs in tissue. Methodologies include both direct tissue examination and indirect analysis of tissue imprinted membranes.

U.S 5,808,300 describes a method and apparatus for imaging biological samples, for example, mammalian tissue, with MALDI MS. Its techniques can be used to generate images of samples in one or more m/z pictures, providing the capability for mapping the concentrations of specific molecules in X, Y coordinates of the original biological sample. Analysis of a biological sample can be carried out directly on a tissue slice or indirectly on an imprint of a tissue sample. The image attained in the analysis can be

displayed in individual m/z values as a selected ion image, as summed ion images, or as a total ion image. The imaging process may also be applied to other separation techniques where a physical track or other X, Y deposition process is utilized, for example, in the CE/MALDI MS combination where a track is deposited on a membrane target.

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US 6756586 describes a further method of analyzing proteins within a sample. A specimen including an energy absorbent matrix is generated. The specimen is interrogated with a laser beam such that a predetermined first laser spot on the specimen releases first sample proteins. The atomic mass of the released first sample proteins is measured over a range of atomic masses. The specimen is moved relative to the laser beam a predetermined linear distance functionally related to a size of the predetermined first laser spot. The specimen is struck again with the laser beam such that a

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predetermined second laser spot on the specimen releases second sample proteins. The atomic mass of the released second sample proteins is measured over a range of atomic masses. An atomic mass window of interest within the range of atomic masses is analysed to determine the specific proteins within the sample. The determined specific proteins can be mapped as a function of the spatial arrangement. The technology is stated to be applicable to MALDI MS and may be used for the analysis of peptides and proteins present on or near the surface of samples such as tissue sections.

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Laser microprobe MS was also established in the 1960s as a powerful microanalytical technique for rapid multi-element analysis of solid samples. The sample is situated in the source compartment that is maintained at reduced pressure. A high energy pulsed laser beam is directed onto the target to effect sample vaporisation/atomisation/excitation/ionisation. The ensuing ions are then transmitted to the MS (usually sector field) for mass analysis and quantitation. The LAMS technique has failed to impact significantly in the field and has been supplanted by combined LA ICP MS systems.

25

30 Laser ablation (LA) inductively coupled plasma mass spectrometry (ICP-MS) has been widely used as a powerful analytical technique for solid micro sampling analyses in

geological, biological, environmental, nuclear, and metallurgical applications. In LA-ICP-MS a high energy laser beam is directed onto the surface of a solid leads to the evaporation of a micro amount of sample. After laser ablation, the micro amount of the laser-ablated material is transported by a carrier gas to a second excitation process (e.g., ICP). In the second process, the ablated material is evaporated, atomized, and excited by the second energy source. An advantage of this two-step operation is the potential of increased efficiencies during the evaporation, atomization, and excitation steps. Recently LA-ICP-MS has been proposed as a technique for mapping of metals distribution in organ tissues (see Kindness et al, Clinical Chemistry 2003; 49: 1916-1923).

Despite the advances that have been made, there is still much that needs to be done to obtain a better understanding of biological function and disease progression. For example, metal transport via proteins is thought to be responsible for metal cytotoxicity in many disease states. However the distribution of elements and molecules in tissue, and in particular the differences between the distribution of elements and molecules in normal and diseased tissue, is poorly understood. Up to the present time there is still no technique that correlates the spatial distribution of both elements and large biomolecules in tissue.

In addition to the problems outlined above, there are also limitations on the current MALDI techniques associated with matrix layer homogeneity, analyte spreading, ion suppression and spatial resolution. Thus there is a clear need to devise improved sample preparation techniques in order to advance the development of this promising approach to tissue analysis.

US 5,372,719 and US 5,453,199 disclose techniques for preparing a chemically active surface so that when a sample is exposed to this surface, a chemical image of the sample is deposited on the surface. The disclosed methods involve the separation of molecules by sorbents.

US 5 770,272 describes a method of forming a continuous matrix-bearing target wherein

a MALDI matrix material is directed at a target surface in a nebulised spray which is enveloped in a sheath of non-reactive gas which confines and entrains the spray and aids in the evaporation of the solvent such that substantial, if not complete solvent evaporation occurs before the matrix material is deposited on the target surface.

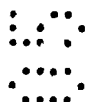
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The entire disclosures of each and every publication listed above are included herein by reference for all purposes.

BRIEF SUMMARY OF THE DISCLOSURE

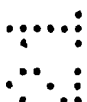
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Throughout the description and claims of this specification, the words “comprise” and “contain” and variations of the words, for example, “comprising” and “comprises”, means “including but not limited to”, and is not intended to (and does not) exclude other moieties, additives, components, integers or steps.



15

Throughout the description and claims of this specification, the singular encompasses the plural unless the context otherwise requires. In particular, where the indefinite article is used, the specification is to be understood as contemplating plurality as well as singularity, unless the context requires otherwise.



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Features, integers, characteristics, compounds, chemical moieties or groups described in conjunction with a particular aspect, embodiment or example of the invention are to be understood to be applicable to any other aspect, embodiment or example described herein unless incompatible therewith.

25

The present invention relates to a method and apparatus for establishing the spatial arrangement of biomolecular and elemental constituents of a tissue sample and an improved sample preparation method.

30

In a first aspect, the invention provides a method for determining the spatial arrangement of biomolecular and elemental constituents of a tissue sample, which comprises:

(a) forming a first test specimen comprising a thin first layer of the tissue sample coated with an energy absorbing matrix;

5 (b) interrogating the test specimen with a first low energy laser beam such that a predetermined first laser spot on the test specimen desorbs and ionises first sample molecules;

(c) measuring the molecular mass of the ionised first sample molecules over a range of molecular masses;

10 (d) moving the first test specimen relative to the first laser beam a predetermined linear distance;

(e) thereafter interrogating the first test specimen with the first laser beam such that a predetermined second laser spot on the test specimen desorbs and ionises second sample molecules;

15 (f) measuring the molecular mass of the ionised second sample molecules over the range of molecular masses

(g) repeating steps (d), (e) and (f) for a plurality of laser spots on the test specimen arranged within a first X,Y plot;

20 (h) selecting a molecular mass window of interest within the range of molecular masses and graphically depicting the molecular mass of molecules within the molecular mass window of interest as a function of the plurality of laser spots on the test specimen arranged within the first X,Y plot;

(i) optionally forming a second test specimen comprising a second layer of the tissue sample, said second layer being proximate to said first layer in said tissue sample;

25 (j) interrogating either the first test specimen or the second test specimen with a second high energy laser beam, such that third sample molecules are ablated from a predetermined third laser spot on the test specimen.

(k) measuring the elemental atomic mass of said ablated third sample molecules over a range of elemental atomic masses;

30 (l) moving the first test specimen or the second test specimen relative to the second laser beam a predetermined linear distance;

(m) thereafter interrogating the first test specimen or the second test specimen with the

second laser beam such that fourth sample molecules are ablated from a predetermined fourth laser spot on the test specimen;

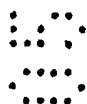
(n) measuring the elemental atomic mass of said ablated fourth sample molecules over a range of elemental atomic masses;

5 (o) repeating steps (l), (m) and (n) for a plurality of laser spots on the test specimen arranged within a second X,Y plot;

(p) selecting an elemental atomic mass window of interest within the range of elemental atomic masses to determine the spatial arrangement of specific elements within the sample and graphically depicting the atomic mass of elements within the atomic mass

10 window of interest as a function of the plurality of laser spots on the test specimen arranged within the second X,Y plot ; and

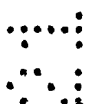
(q) combining the first X,Y plot and the second X,Y plot to obtain a graphical depiction of the spatial arrangement of molecules and elements of interest in the tissue sample.



15 In a second aspect the invention provides an apparatus for analyzing a test sample containing molecules of interest, comprising:



(i) a first test specimen comprising sample molecules of interest coated with an energy-absorbing matrix;



(ii) a first laser source for generating a low energy laser beam for sequentially

20 interrogating the first test specimen with a laser beam at a plurality of laser spots on the test specimen, thereby sequentially desorbing/ionising sample molecules from each laser spot;

(iii) a first mass analyser for measuring the molecular mass of the ionised sample molecules over a range of molecular masses;

25 (iv) a first moving mechanism for sequentially moving the first test specimen relative to the laser beam a predetermined linear distance between the spots;

(v) optionally a second test specimen comprising sample molecules of interest;

(vi) a second laser source, for generating a high energy laser beam, which may be the same or different from the first laser source, for sequentially interrogating the first test

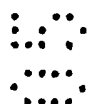
30 specimen or the second test specimen with a laser beam at a plurality of laser spots on the first or second test specimen, thereby sequentially ablating sample molecules from each

laser spot;

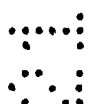
(vii) a second mass analyser, which may be the same or different from the first mass analyser, for measuring the elemental atomic mass of the released sample atoms over a range of elemental atomic masses;

5 (viii) a second moving mechanism, which may be the same or different from the first moving mechanism, for sequentially moving the first test specimen or the second test specimen relative to the second laser beam a predetermined linear distance between the spots;

10 (ix) a work station for receiving, combining, and graphically presenting the elemental and molecular mass spectrometric information from the first and second mass analysers, as arranged within an X,Y plot.



15 In a third aspect the invention provides a method of preparing a tissue specimen for matrix-assisted laser desorption/ionisation ("MALDI") mass spectrometry, which comprises directing at the tissue surface a cone-shaped gas-atomized spray of a solution of a matrix-assisted laser desorption/ionisation crystalline matrix material dissolved in a suitable solvent to form a relatively homogeneous matrix crystal layer in terms of crystal morphology and crystal distribution of said matrix material on said tissue sample.



20 In still further aspects, the invention also includes methods of monitoring and diagnosing disease using any one of the first, second and third aspects of the invention.

25 Test specimens in step (a) in accordance with the invention may be generated from any suitable tissues, although in general the invention finds particular application in the analysis of mammalian tissues. The test specimen may be animal or human tissue and may be healthy or diseased. Examples of diseased tissues include cancerous tissues, for example, testicular, prostate, liver, breast, colon, kidney, lymph, and brain cancerous tissues.

30 The first test specimen comprises a thin first layer of the tissue sample, preferably having a thickness of from 5 to 50 microns, and also comprises an energy absorbing matrix. The

energy absorbing matrix is a material that will absorb UV, or light energy at other chosen wavelengths. The energy absorbing matrix can comprise, for example, an organic or inorganic compound having a relatively high extinction coefficient for absorption of light energy and is preferably applied as a thin layer over the layer of the tissue sample or may
5 be otherwise incorporated into the layer of the tissue sample. Where the energy absorbing matrix is applied as a layer over the first layer of the tissue sample, the volumes required may be between 500uL and 10 mL per tissue section.

Suitable energy absorbing matrices can include, for example, organic acids such as 2,5 –
10 dihydroxybenzoic acid, α – cyano – 4 - hydroxycinnamic acid (CHCA), sinapinic acid (SPA), 3 – hydroxypicolinic acid, 5 – (trifloro – methyl) uracil, caffeic acid, succinic acid, anthranilic acid, 3 – aminopyrazine – 2 – carboxylic acid and ferulic acid.

Preferably either CHCA or SPA are used.

15 The second test specimen may comprise a layer of tissue sample with or without an additional energy absorbing matrix. Where the energy absorbing matrix is absent in the second test specimen the energy is absorbed only by the sample and is not first incident on an exogenous energy absorbing material.

20 In step (b) of the method of the invention the test specimen is interrogated with a laser configured so that it strikes a predetermined first spot on the specimen. The laser is preferably one that can be operated in either low energy (for MALDI) or high energy (for LA) regimes. The action of the laser in step (b) is to desorb molecules of the sample from the first spot forming an analyte plume of sample and matrix molecules. The power
25 of the laser is configured to be relatively low such that molecules are desorbed, permitting analysis of high molecular masses that can include, for example, proteins, peptides, nucleotides and other biomolecules.

30 In step (c) the molecular mass of the desorbed sample molecules is measured over a range of molecular masses. MALDI TOF (Time-of-Flight) MS or MALDI-Q-TOF instrumentation can be, and are preferably, used for this purpose.

In step (d) the test specimen is moved relative to the laser beam by a predetermined distance. This distance may be functionally related to the size of the laser spot in order to achieve an effective scan pattern. By varying the amount of the translation, it is possible to affect the resolution of the scan. The mechanism used to translate the specimen is preferably computer-controlled and can be of any suitable type. The translation may be one, two, or three-dimensional, depending upon the application.

In steps (e) to (g) the operation is repeated until the specimen has been effectively scanned.

In step (h) a window of interest is defined from among the range of molecular masses encompassed by the molecules released from the specimen by the laser beam. This window of interest may include the entire range of molecular masses of the released molecules or any portion thereof. Within the window of interest, masses of the desorbed/ionised molecules may be selected so that the spatial arrangement of these molecules may be displayed, as a function of original location within the sample. Any suitable mapping/graphical techniques may be applied to enable this data manipulation.

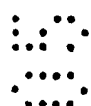
In step (i) a second test specimen comprising a second layer of the tissue sample proximate to the first layer is optionally generated. In some instances, however, it will be preferable to carry out the elemental atomic mass analysis on the first test specimen in order to ensure that the superimposed spatial arrangements of molecular and elemental maps are strictly comparable. Where it is necessary to use two test specimens they are preferably prepared from microtomed adjacent sections of tissue and care should be taken to ensure that there are no significant observable differences between the adjacent microtomed tissue sections.

In step (j) the specimen is interrogated with a second laser beam having high energy sufficient to ablate sample molecules from the surface of the specimen. The ablated material, if sufficiently ionised, may be directly measured by mass spectrometry,

preferably employing a quadrupole mass analyser. Alternatively, ablated material may be transferred to a high temperature ICP source for atomization/ionisation prior to mass spectrometric measurement.

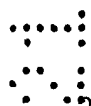
- 5 The preferred laser for use in steps (b) and (j) is an Nd:YAG laser, operating at 266nm, in either low or high energy modes for MALDI or LA respectively. Whilst a dual mode laser is preferred for many applications it is possible within the invention to use two or more lasers, as appropriate.

- 10 In step (k) the elemental atomic mass of the ions produced in step (j) is measured using a suitable mass analyser, preferably a quadrupole or mass sector field analyser.



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In steps (l) to (n) the procedure is repeated with translation of the specimen so that elemental information is acquired across the entire sample area, permitting selected ions of interest to be extracted from the data acquired from each location and represented graphically as a display of the spatial distribution of selected elements within the tissue section, as in step (o).



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In step (p) a window of interest is defined from among the range of atomic masses encompassed by the elements ablated from the specimen by the laser beam. This window of interest may include the entire range of atomic masses of the ablated elements or any portion thereof. Within the window of interest, masses of the ablated/ionised elements may be selected so that the spatial arrangement of these elements may be displayed, as a function of original location within the sample. Any suitable mapping/graphical techniques may be applied to enable this data manipulation.

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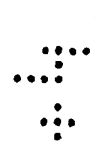
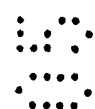
Finally, in step (q) the molecular map (the first X,Y plot) and the elemental map (the second X,Y plot) are combined to obtain a graphical depiction of the spatial arrangement of molecules and elements of interest in the tissue sample.

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The overlaid spatial data can relate, for example, to the distribution of a single protein

and metal, or to groups of proteins and metals as appropriate. Other biomolecules and elements can of course be chosen as desired.

5 In the apparatus of the invention, the MALDI and LA-ICP-MS analyses are preferably carried out on a single test specimen using a dual function laser. Preferably the specimen is mounted on a single translation stage movable within a sample inlet source. In a preferred embodiment the apparatus is provided with ion extraction to a Q-TOF mass analyser for molecular (MALDI) and atomic (LA-MS and LA-ICP-MS) mass spectrometry measurement. MALDI-MS and LA-MS or LA-ICP-MS measurements can
10 be carried out on the test specimen sequentially and processed to produce the desired overlaid spatial data.



15 In the third aspect of the invention a tissue specimen for MALDI and/or LA – (ICP)–MS is prepared by directing at the tissue surface a cone-shaped gas-atomized spray of a solution of a MALDI matrix material in a volatile solvent to form a continuous matrix layer on the tissue surface. Preferably the spray is dispensed using a nebuliser. In preferred embodiments of the method, it has been found that improved more homogeneous crystal coating (in terms of crystal morphology and uniform distribution of crystals upon the sample tissue) of matrix material is obtained when the nebuliser and the
20 sample are in a fixed spatial arrangement, that is to say, the matrix material is deposited without moving either the nebuliser or the sample.

BRIEF DESCRIPTION OF THE DRAWINGS

25 The invention will now be more particularly described, by way of example only, with reference to the accompanying Drawings in which:

Figure 1 shows a schematic diagram of the method steps leading to a combined MALDI and LA-ICP-MS derived molecular and elemental map;

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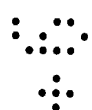
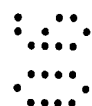
Figure 2 shows a diagrammatic representation of an embodiment of a combined MALDI

and LA-ICP-MS mass spectrometer

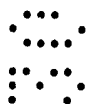
DETAILED DESCRIPTION

5 Referring firstly to Figure 1, there is shown a tissue section 1, a molecular image 2 produced by MALDI analysis, an elemental image 3 produced by LA-ICP-MS analysis and a combined molecular and elemental image 4 produced by overlaying the molecular image 2 and the elemental image 3. The method of the invention enables the overlaying of any selected molecular mass image and element image to obtain a plot of the spatial
10 distribution of the molecule(s) and element(s) of interest.

Turning now to Figure 2, there is shown a mass spectrometer illustrated generally at 10, which comprises a computer controlled XYZ translation stage 11, a dual mode laser 12, a mass analyser 13 and a workstation 14 for data analysis. A matrix-coated tissue
15 specimen 15 is mounted on the translation stage 11 in a sample inlet source 16. The specimen 15 is connected to the mass analyser 13 by an ion extraction means 17. An optional inductively coupled plasma (ICP) device 18, illustrated by broken lines, is connected by carrier gas line 19 to the vacuum environment of the sample inlet source 16 and by ion extraction means 20 to the mass analyser 13. All components of the described
20 instrumentation except for the ICP source and the workstation are enclosed within a vacuum chamber 21.

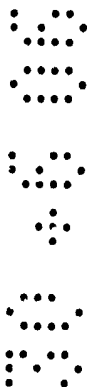


The dual mode laser is preferably a Nd:YAG laser, operating at 266nm, and the mass analyser is selected from quadrupole, sector field, MALDI-TOF and MALDI-Q-TOF
25 mass analysers. The laser has a low energy mode for desorption and a high energy mode for laser ablation.



In operation, the tissue specimen 15 such as, for example, a matrix coated tissue is mounted on the translation stage 11 and interrogated with the laser 12, operating at low
30 power. Desorbed molecules from the specimen are transferred by the ion extraction means 17 to the mass analyser 13 for measurement of molecular mass via quadrupole,

quadruple-time of flight or sector field.. Between each reading the specimen 15 is moved to a new position by the translation stage 11. In this way data can be collected providing information about the spatial distribution of a molecule of interest across the surface of



the specimen.

Next, the specimen 15, or a similar specimen prepared from a tissue section taken from a proximate region of the tissue sample, is interrogated with the laser 12, operating at high power. Ablated molecules, if sufficiently ionised, are collected in the ion extraction means 17 and transferred to the mass analyser 13 for measurement of atomic mass. In a further, preferred embodiment, the ablated molecules are collected by the carrier gas line 19 and transferred to the ICP device 18 for ionisation/atomization. Ions from the ICP device 18 are then transferred to the mass analyser 13 by the ion extraction means 20 for measurement of atomic mass. Between each reading the specimen 15 is moved to a new position by the translation stage 11. In this way data can be collected providing information about the spatial distribution of an element of interest across the surface of the specimen.

Finally the data from the mass analyser 13 is collected by the workstation 14 and displayed as overlaid spatial depictions of the populations of the molecule and element of interest.

Example 1

Biopsy samples from rat kidney and liver and human diseased and normal lymph tissue were collected at the Manchester Royal Infirmary. Samples were spiked with 1uL of a metallodrug or metalloprotein.

For MALDI imaging tissue sections were microtomed to approximately 20um and mounted directly onto a glass cover slip. The samples were subsequently coated in a-CHCA or SPA matrix material by micro-concentric pneumatic or ultrasonic nebulisation and adhered to a MALDI target. Samples were analysed in an Applied Biosystems Qstar, a hybrid quadrupole time-of-flight mass spectrometer, fitted with an orthogonal MALDI ion source and ion imaging software.

For elemental mapping biopsies were frozen and/or embedded in carboxymethyl cellulose and microtomed (50 μm). Tissues were subjected to analysis and quantitation via LA-ICP-MS.

- 5 Images obtained from the MALDI and LA – ICP – MS mapping were overlaid by processing in a Hewlett Packard computer with a Pentium 4 processor and sent to a display screen and printer.

Example 2

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Samples for MALDI examination were prepared by microtoming tissue sections to approximately 20 μm and mounting directly onto a glass cover slip. The samples were subsequently coated in a-CHCA or SPA matrix material by micro-concentric pneumatic or ultrasonic nebulisation and adhered to a MALDI target. During the coating process the membrane and the nebuliser were maintained in a fixed relationship with no relative movement. The nebuliser delivered a cone-shaped spray that formed an even, homogeneous coating on the tissue section.

Discussion

The invention provides a powerful new tool for the potential diagnosis and monitoring of disease states. It is likely that changes in the elemental content of a tissue have an enormous effect on the molecular and cellular activity. By combining spatially resolved images of specific elements, improved understanding of basic biochemical and biological function may be realized. For example, the ability to measure elements (atomic MS) and proteins (molecular MS) within the same tissue section is likely to be a powerful method of identifying not only the carrier protein molecules, but also the range of transported metals, providing an insight into the mechanism of metal cytotoxicity and drug action.

Similarly a merging of images of metallodrugs (via either MALDI imaging or LA-ICP-MS or LA-MS) with maps of other elements (by LA-ICP-MS or LA-MS) may be useful in terms of assessing a reduction in cytotoxicity, along with changes in target proteins (as measured by MALDI imaging). The invention therefore provides a wholly new strategy
5 for studying the fate and metabolic activity of metallodrugs in organ and tissue. This will have high significance in drug targeting and drug metabolite profiling.

In addition, combining the data sets acquired by two different techniques in accordance with the invention may provide a means of quantification by MALDI imaging. MALDI is
10 non-quantitative without inclusion of an internal standard, which is problematic in imaging. However, LA-ICP-MS provides quantitative information. It may therefore be possible to normalise and thus quantify MALDI data via a direct comparison with elemental maps.



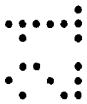
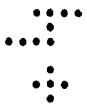
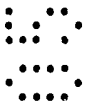
15 Whilst the invention has been described herein in relation to the analysis of biological materials, it will be apparent to those skilled in the art that many other applications are possible. Thus, for example, the method and apparatus of the invention can be applied to the analysis of samples of a wide range of polymeric materials, for example, polyolefins and other industrially important polymers, for the determination of the spatial distribution
20 of additives and high and low molecular weight species within the polymer structure.

The reader's attention is directed to all papers and documents which are filed concurrently with or previous to this specification in connection with this application and which are open to public inspection with this specification, and the contents of all such papers and
25 documents are incorporated herein by reference.

All of the features disclosed in this specification (including any accompanying claims, abstract and drawings), and/or all of the steps of any method or process so disclosed, may be combined in any combination, except combinations where at least some of such
30 features and/or steps are mutually exclusive.

Each feature disclosed in this specification (including any accompanying claims, abstract and drawings), may be replaced by alternative features serving the same, equivalent, or similar purpose, unless expressly stated otherwise. Thus, unless expressly stated otherwise, each feature disclosed is one example only of a generic series of equivalent or similar features.

The invention is not restricted to the details of any foregoing embodiments. The invention extends to any novel one, or any novel combination, of the features disclosed in this specification (including any accompanying claims, abstract and drawings), or to any novel one, or any novel combination, of the steps of any method or process so disclosed.



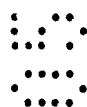
CLAIMS

5 1. A method for determining the spatial arrangement of biomolecular and elemental constituents of a tissue sample, which comprises:

(a) forming a first test specimen comprising a thin first layer of the tissue sample coated with an energy absorbing matrix;

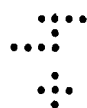
10 (b) interrogating the test specimen with a first low energy laser beam such that a predetermined first laser spot on the test specimen desorbs and ionises first sample molecules;

(c) measuring the molecular mass of the ionised first sample molecules over a range of molecular masses;



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(d) moving the first test specimen relative to the first laser beam a predetermined linear distance;



(e) thereafter interrogating the first test specimen with the first laser beam such that a predetermined second laser spot on the test specimen desorbs and ionises second sample molecules;



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(f) measuring the molecular mass of the ionised second sample molecules over the range of molecular masses

(g) repeating steps (d), (e) and (f) for a plurality of laser spots on the test specimen arranged within a first X,Y plot;

25 (h) selecting a molecular mass window of interest within the range of molecular masses and graphically depicting the molecular mass of molecules within the molecular mass window of interest as a function of the plurality of laser spots on the test specimen arranged within the first X,Y plot;

30 (i) optionally forming a second test specimen comprising a second layer of the tissue sample, said second layer being proximate to said first layer in said tissue sample;

(j) interrogating either the first test specimen or the second test specimen with a second high energy laser beam, such that third sample molecules are ablated from a predetermined third laser spot on the test specimen.

(k) measuring the elemental atomic mass of said ablated third sample molecules over a range of elemental atomic masses;

(l) moving the first test specimen or the second test specimen relative to the second laser beam a predetermined linear distance;

5 (m) thereafter interrogating the first test specimen or the second test specimen with the second laser beam such that fourth sample molecules are ablated from a predetermined fourth laser spot on the test specimen;

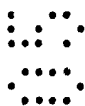
(n) measuring the elemental atomic mass of said ablated fourth sample molecules over a range of elemental atomic masses;

10 (o) repeating steps (l), (m) and (n) for a plurality of laser spots on the test specimen arranged within a second X,Y plot;

(p) selecting an elemental atomic mass window of interest within the range of elemental atomic masses to determine the spatial arrangement of specific elements within the sample and graphically depicting the atomic mass of elements within the atomic mass

15 window of interest as a function of the plurality of laser spots on the test specimen arranged within the second X,Y plot ; and

(q) combining the first X,Y plot and the second X,Y plot to obtain a graphical depiction of the spatial arrangement of molecules and elements of interest in the tissue sample.



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2. A method according to claim 1, wherein the tissue is mammalian tissue.

3. A method according to claim 2, wherein the tissue is human tissue.

4. A method according to claim 3, wherein the human tissue is diseased.

25

5. A method according to any one of the preceding claims, wherein steps (b) and (c) are carried out using a MALDI TOF MS mass analyser.

6. A method according to any one of the preceding claims, wherein steps (j), (k), (l) and (m) are carried out using an LA-ICP- MS mass analyser.

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7. A method according to claim 1 substantially as described in the Examples.

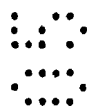
8. A method for determining the spatial arrangement of biomolecular and elemental constituents of a tissue sample substantially as hereinbefore described

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9. An apparatus for analyzing a test sample containing molecules of interest, comprising:

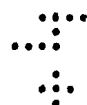
(i) a first test specimen comprising sample molecules of interest coated with an energy-absorbing matrix;

10 (ii) a first laser source for generating a low energy laser beam for sequentially interrogating the first test specimen with a laser beam at a plurality of laser spots on the test specimen, thereby sequentially desorbing/ionising sample molecules from each laser spot;



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(iii) a first mass analyser for measuring the molecular mass of the ionised sample molecules over a range of molecular masses;



(iv) a first moving mechanism for sequentially moving the first test specimen relative to the laser beam a predetermined linear distance between the spots;



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(v) optionally a second test specimen comprising sample molecules of interest;

(vi) a second laser source, for generating a high energy laser beam, which may be the same or different from the first laser source, for sequentially interrogating the first test specimen or the second test specimen with a laser beam at a plurality of laser spots on the first or second test specimen, thereby sequentially ablating sample molecules from each laser spot;

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(vii) a second mass analyser, which may be the same or different from the first mass analyser, for measuring the elemental atomic mass of the released sample atoms over a range of elemental atomic masses;

(viii) a second moving mechanism, which may be the same or different from the first moving mechanism, for sequentially moving the first test specimen or the second test specimen relative to the second laser beam a predetermined linear distance between the spots;

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(ix) a work station for receiving, combining, and graphically presenting the elemental and

molecular mass spectrometric information from the first and second mass analysers, as arranged within an X,Y plot.

10. An apparatus according to claim 9, wherein the first mass analyser is a TOF or
5 QTOF mass analyser.

11. An apparatus according to claim 9 or 10, wherein the second mass analyser is a quadrupole, time-of-flight, QTOF or sector field mass analyser.

10 12. An apparatus according to any one of claims 9 to 11, wherein the molecular and elemental analyses are carried out on a single test specimen.

13. An apparatus according to any one of claims 9 to 12, wherein there is used a
single laser source, capable of operating at low and high energy to enable respectively
15 either desorption or ablation of molecules from the specimen.

14. An apparatus according to claim 9, which is provided with both ion extraction
means for direct MS measurement, and a carrier gas line to transport ablated material to a
high energy ICP source for atomization/ionisation prior to MS measurement.

15. An apparatus for analyzing a test sample substantially as hereinbefore described.

16. A method of preparing a tissue specimen for matrix-assisted laser
desorption/ionisation ("MALDI") mass spectrometry, which comprises directing at the
25 tissue surface a cone-shaped gas-atomized spray of a solution of a matrix-assisted laser
desorption/ionisation matrix material dissolved in a solvent to form a continuous matrix
layer of said matrix material on said tissue sample.

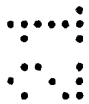
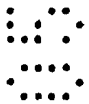
17. A method according to claim 16, wherein the spray is dispensed using a nebuliser.

18. A method according to claim 16 or claim 17, wherein the nebuliser and the

sample are in a fixed spatial arrangement.

19. A method according to claim 16, substantially as described in the Examples.

5 20. A method of monitoring and/or diagnosing disease using the method of claim 1 or the apparatus of claim 9.





INVESTOR IN PEOPLE

Application No: GB0507457.0

Examiner: Dr Rowena Dinham

Claims searched: 1-15 & 20

Date of search: 29 August 2005

Patents Act 1977: Search Report under Section 17**Documents considered to be relevant:**

Category	Relevant to claims	Identity of document and passage or figure of particular relevance
A	-	Int J Mass Spectrom; Vol 24, pp 135-144 (2005). Becker et al. "Investigation of Cu-, Zn- and Fe-containing human brain proteins...." See entire document, especially Results and Discussion, sections 3.1-3.3
A	-	J Anal At Spectrom; Vol 20, pp 210-215 (2005). Fernandes et al. "Complementary FPLC-IPC-MS and MALDI-TOF for studying vanadium association to human serum proteins" See entire document, especially Results and Discussion
A	-	US 2003/0008327 A1 (ORNATSKAIA) See entire document, especially para [0006], [0015], [0018], [0019] and [0167]
A	-	US 5808300 A (CAPRIOLI) See entire document, especially column 4 line 1-65 and Examples
A	-	US 2003/0073145 A1 (CAPRIOLI) See entire document, especially para [0017]-[0019] and Examples
A	-	US 6248592 B1 (NOGAR) See entire document, especially column 2 line 48-58

Categories:

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application

Field of Search:Search of GB, EP, WO & US patent documents classified in the following areas of the UKC^X :
Worldwide search of patent documents classified in the following areas of the IPC⁰⁷

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The following online and other databases have been used in the preparation of this search report

WPI, EPODOC, JAPIO, MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS