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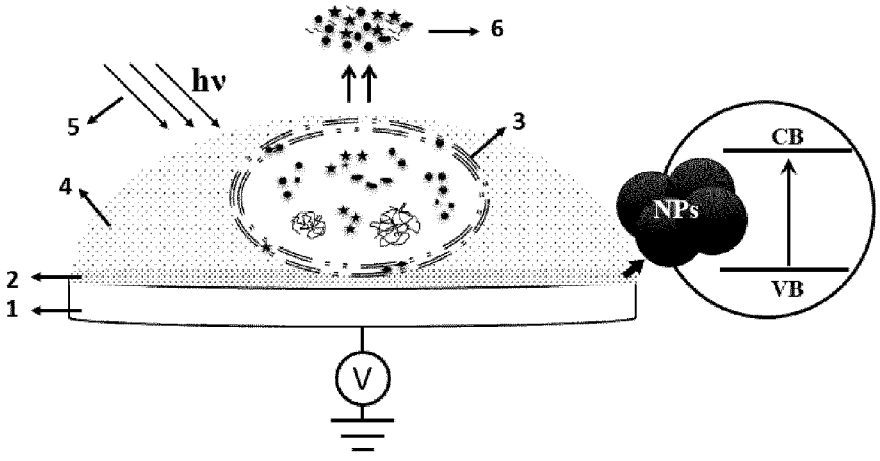

Fig. 1

(57) Abstract: A target plate for enhanced matrix-assisted laser desorption/ionization mass spectrometry (MALDI MS) analysis of intact biological entities. The target plate comprises an electrically conductive substrate (1) covered at least partially with immobilised photo-reactive nanoparticles (2), acting as a photosensitizer and an ionization device, which drives efficient electron-transfer and radical reactions causing in-source disruption of the envelope structure of intact biological entities (3) and helps in desorption/ionization of components such as lipids, peptides and proteins from the intact biological entities to the MS analyser.

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MALDI DEVICE AND METHODS OF PREPARATION AND USE THEREOF

Background to the Invention

The present invention relates to a matrix-assisted laser desorption ionization (MALDI) target plate, comprising a conductive substrate covered with patterns of nanoparticles with a large hydrophilic surface area.

MALDI is a soft ionization technique used in mass spectrometry (MS), allowing analysis of biomolecules (such as DNA, proteins, peptides and sugars)[1] and large organic molecules (such as polymers and dendrimers)[2], which are fragile and easily fragmented when ionized by other ionization methods. MALDI is similar in character to electrospray ionization (ESI) in that both techniques use relatively soft ways of obtaining ions of large molecules in the gas phase, though MALDI produces far fewer multiply charged ions.[3]

In a typical MALDI MS detection, the sample is mixed or overlaid with a solution of a matrix that is commonly a crystallised organic acid, acting as a light absorber and a charge carrier. After drying, the sample co-crystallises with the matrix and retains its integrity. The principle of MALDI ionization lies in the absorption of laser energy by the matrix (typically UV laser *e.g.*, 337 or 355 nm), which transfers the energy to the sample and facilitates its desorption and ionization by protonation or de-protonation.[4] The ions are then accelerated at a fixed potential, and then separated from each other on the basis of their mass-to-charge ratio (m/z). The charged sample is then detected and measured using one or more of mass analysers, for example quadrupole mass analysers, ion trap analysers, time of flight (TOF) analysers, etc.[5]

In recent years, MALDI-TOF MS has emerged as a potential tool for analysis of biological samples such as bacteria, fungi, yeasts, viruses, tissues and cancer

cells.[6-8] Due to its ability to provide information about biomolecules, such as lipids, proteins, peptides, nucleic acids and metabolites, MALDI-TOF MS has been applied for characterization and identification of micro-organisms, epidemiological studies, tissue imaging, detection of biological warfare agents, and biomarker detection, and is a powerful tool for clinical diagnosis and treatment. For example, MALDI-TOF MS has been used for bacterial identification at the genus, species and even strain levels since the pioneer work of Holland *et al.*,[9] Claydon *et al.*[10] and Krishnamurthy *et al.*[11] in 1996. Accordingly, commercial systems, such asMALDI Biotyper ® CA (Bruker Daltonics) and VITEK ® MS (bioMérieux) have been developed for routine identification of bacteria in clinical and microbiology laboratories.

Biological entities can be analysed by MALDI-TOF MS directly in their intact/whole state or after "preparatory" component extraction, depending on their envelope structure. The approach of intact sample analysis is conducted by directly spotting the sample onto a MALDI target plate and overlaying it with matrix solution. Such is the case for bacteria like *Neisseria* spp., *Yersinia* spp., and *Vibrio* spp., where the bacterial cells are directly physically disrupted during MS measurements.[12,13] This intact sample analysis procedure is simple and fast; however, it is not always efficient. For more resistant micro-organisms (*e.g.*, viruses, bacterial spores, yeast cells), it is necessary to first extract the components from the entities and deposit the extractions on a target plate for MS measurements. For example, a combination of formic acid and acetonitrile has often been used to extract proteins from bacterial cells for MS fingerprinting.[14] Compared to intact sample analysis, the "preparatory" component extraction procedure can provide more sample information, but is much more time-consuming. Matsuda *et al.*[15] compared the performance of both procedures for *Staphylococcus* spp. identification. They showed that 60.2% and 80.8% of 273 clinical strains were identified with the intact bacteria profiling method and the "preparatory" protein extraction method, respectively, while the mean process

time required for 48 duplicates was 30 min for the former and 180 min for the latter.

MALDI matrix is often dissolved in a mixture of water, organic solvents (*e.g.*, ethanol, methanol, acetonitrile) and a strong acid (*e.g.*, trifluoroacetic acid).[16] The most frequently used matrices for biological samples are α-cyano-4-hydroxycinnamic acid (CHCA), 2,5-dihydroxy benzoic acid (DHB), and 3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid, SA).[17] The choice of matrix depends on the mass range analysed and the type of information wanted from the samples. Normally, CHCA is more suitable for detection of low molecular weight molecules such as peptides and small proteins (< 10,000 Da); DHB is more often used for phospholipids, glycopeptides and glycoproteins (< 10,000 Da); while SA seems to be the best choice for large proteins (> 15,000 Da).[18]

It has been reported that metal oxides (*e.g.*, TiO_2, ZnO, ZrO_2, SnO_2) and metal oxides doped with different metal ions (*e.g.*, Au, Ag, Co) have microbicidal effects by photocatalysis.[19-22] Their photocatalytic activity is based on their unique electronic structure, which is characterized by a filled valence band and an empty conduction band. Upon absorption of photons from the light source, metal oxide particles generate electron-hole pairs and act as photosensitizers, which drive efficient electron-transfer reactions on their surface and produce reactive oxygen species such as hydroxyl radical ($^{\bullet}OH$), superoxide radical ($^{\bullet}O_2^-$), and singlet oxygen ($^1O_2$). These reactive species can break up the envelope structure of micro-organisms by photo-electrochemical disruption that leads to release of inner components. For example, it has been demonstrated that TiO_2 can kill gram-positive/negative bacteria, viruses, fungi, and even cancer cells in aqueous solutions by photocatalysis.[23]

Metal oxides and noble metals have been proposed to modify the surface of MALDI target plates. Due to their ability of absorbing energy from laser source, transferring energy to samples and assisting desorption/ionization of samples, they can replace often used organic matrices for MS detection and also can be used for in-source tagging reactions, disulfide bridge reductions or ion source decay reactions.[24,25] The modified target plates are usually called surface-assisted laser desorption/ionization (SALDI) plates.

Nanostructures have also been used to fabricate MALDI target plates. The term "nanostructures" refers to structures with nanoscale size (1 - 1000 nm) in at least one dimension. For example, Niu *et al.*[26] have proposed to produce a nanostructured thin film (such as an alumina or aluminium thin film) on a supporting plate for MALDI MS analysis of proteins, peptides, small molecules, etc. The nanostructured thin film was used to generate enhanced surface area and the appropriate structural dimensions.

Summary of the Invention

The present invention provides a target plate according to claim 1, for enhanced MALDI MS analysis of intact biological entities such as bacteria, fungi, yeasts, virus, tissues and cancer cells. The target plate comprises an electrically conductive substrate partially covered with sintered photo-reactive nanoparticles, which provide a hydrophilic surface area for homogenous sample distribution. During MALDI MS detection, the nanoparticles absorb energy from the laser to generate electron-hole pairs and act as photosensitizers. They drive electron-transfer and radical reactions and cause in-source photo-electrochemical disruption of the biological entities' envelope structure, as well as assisting in desorption and ionization of components from the entities. Compared to the usage of classic bare metallic MALDI target plates, more sample components, especially large proteins (> 10,000 Da) or low-abundant biomolecules, can be

detected using the target plate of this invention. It is an efficient tool for rapid profiling of biological entities without the need for "preparatory" component extraction. The invention can be applied for characterization and identification of micro-organisms, analysis of micro-organisms' resistance to antibiotics, detection of biomarkers, imaging of tissues, etc., and serve for quick clinical diagnosis and treatment.

The invention also provides a method of preparing a plate, according to claim 9, and a method of use of a plate, according to claim 13. Optional features of the invention are set out in the dependent claims.

Brief Description of the Drawings

Embodiments of the invention will now be described in more detail, by way of example only, with reference to the accompanying drawings, in which:

Fig. 1 schematically shows a photo-reactive MALDI plate for enhanced MS analysis of intact biological entities according to the invention;

Fig. 2 shows the mass spectra of intact gram-negative bacteria *Escherichia coli (E.coli)* obtained using a classic bare metallic MALDI plate and a MALDI plate presented in **Fig. 1**;

Fig. 3 shows the mass spectra of intact gram-positive bacteria *Bacillus subtilis* (*B.subtilis*) obtained using a classic bare metallic MALDI plate and a MALDI plate presented in **Fig. 1**;

Fig. 4 shows the mass spectra of intact human melanoma cancer line *wm115* obtained using a classic bare metallic MALDI plate and a MALDI plate presented in **Fig. 1**;

Fig. 5 shows the mass spectra obtained from ampicillin-resistant *E.coli* XL1 and ampicillin-susceptible *E.coli* XL1 by using a classic bare metallic MALDI plate and a MALDI plate presented in **Fig. 1**;

Fig. 6 shows the mass spectra obtained from kanamycin-resistant *E.coli* BL21 and kanamycin-susceptible *E.coli* BL21 by using a classic bare metallic MALDI plate and a MALDI plate presented in **Fig. 1**;

Fig. 7 shows the mass spectra obtained from melanoma cell line *wm115* with or without the expression of green fluorescent protein by using a classic bare metallic MALDI plate and a MALDI plate presented in **Fig. 1**.

Detailed Description of Particular Embodiments

Fig. 1 shows a MALDI plate comprising an electrically conductive substrate **1,** partially covered with immobilized photo-reactive nanoparticles **2**. Nanoparticles **2** act as sensitive photo-reactive materials and an ionization device when covered by an organic matrix. A whole detection process is conducted as following: *i*) a sample of biological entities **3** (*e.g.*, bacteria, fungi, yeasts, viruses, cancer cells, tissues) in their intact state is placed on the plate in contact with the nanoparticles **2**, which provide a hydrophilic surface area for the homogenous sample distribution; *ii*) a light-sensitive organic matrix **4** is added after the sample **3** is dried at room temperature; *iii*) upon irradiation by laser **5**, photo-reactive nanoparticles **2** absorb the laser energy, generate electron-hole pairs and trigger redox and radical reactions. The envelope structure of the intact biological sample **3** is disrupted by these reactions leading to exposure of sample's components **6**; *iv*) components **6** are desorbed from sample **3** and ionized with the assistance of photo-reactive nanoparticles 2 and organic matrix **4**, released into the gas phase and driven by an electric field to the analyser of a mass spectrometer.

Substrate **1** in **Fig. 1**:

The substrate can be a commercially available MALDI plate or a homemade target plate made of any conducting material. Typically, the target plate is made of aluminium, nickel, stainless steel or a conductive polymer. It can present a flat, unmodified surface, or a surface with patterned spots, dots or annular groves to assist in locating samples. Alternatively, the substrate can be made of a non-conductive material coated with a thin layer of conductive material such as one or more evaporated metals, or a semi-conducting material. Also, the conducting substrate can be a metallic foil placed in contact with a commercially available MALDI plate. A foil with a thickness below 250 μm is suitable, such as commercially available aluminium foil used for cooking or packaging food. When carrying out MALDI detection, a high voltage is applied to the substrate with respect to the mass spectrometer. The electric field thereby generated drives the ions released upon energy absorption to the analyser of the mass spectrometer.

Photo-reactive nanoparticles **2** in **Fig. 1**:

Drops of a nanoparticle suspension are deposited on substrate **1** to form a specific area, such as a set of stripes or an array of spots. Usually, the array of spots allows a high throughput analysis of biological samples and is convenient for accurate positioning of samples. Alternatively, inkjet printing, screen-printing, rotogravure printing or other deposition techniques can be used to prepare the specific area of nanoparticles. Several layers of a nanoparticle suspension can be deposited on the substrate to form areas of different thicknesses, ranging from 50 nanometers to 50 micrometres. After the solvent evaporation, the particles can be sintered to ensure their mutual adhesion and adhesion to the substrate. Sintering can be performed either by thermally heating the nanoparticles below their melting point or by low-thermal techniques such as photonic flash sintering. The sintered nanoparticles provide a mesoporous structure with an extremely high surface-to-volume ratio and a high hydrophilic surface for homogeneous distribution of samples. The

nanoparticles can be metal oxides (*e.g.*, TiO_2, ZnO, ZrO_2, SnO_2), metal oxides doped with different metal ions (*e.g.*, Au, Ag, Co) and other materials with photo-catalytic activity. It is important that the band gap of the nanoparticles matches the laser wavelength in the MALDI mass spectrometer.

Biological sample **3** in **Fig. 1**:

The sample can include whole or intact micro-organisms (*e.g.*, bacteria, fungi, yeasts, virus), cells (*e.g.*, cancer cells), tissues, or any other biological entities. If the sample is suspended in a liquid solution it can be deposited dropwise on the plate. If the sample is in a solid state it can be directly placed on the plate.

Light sensitive organic matrix **4** in **Fig. 1**:

The light sensitive organic matrix **4** can be a classic MALDI matrix containing usually a crystalline acid, such as α-cyano-4-hydroxycinnamic acid (CHCA), sinapic acid (SA), 2,5-dihydroxybenzoic acid (DHB) or 2-(4-hydroxyphenylazo)-benzoic acid (HABA). The acid plays the role of a light absorber and a charge carrier, assisting desorption/ionization of components from sample **3**.

Photoionization process:

The gist of the present invention is to use photo-reactive nanoparticles for in-source photo-electrochemical disruption of the envelope of intact biological entities and for desorption/ionization of the entities' components in the presence of an organic matrix. Samples are homogeneously distributed and adsorbed on the hydrophilic surface of nanoparticles. Under the irradiation of a pulsed laser light, the nanoparticles absorb energy from the laser, generate electron-hole pairs, and induce electron-transfer and radical reactions. These reactions cause break-up of the analysed entities, facilitating desorption and ionization of inner components together with the assistance of the matrix.

Results

Fig. 2 shows the comparison of mass spectra obtained from intact gram-negative bacteria *E.coli* by using a classic stainless steel MALDI plate and a photo-reactive MALDI plate from **Fig. 1** where the immobilized nanoparticles **2** are TiO_2. Sinapinic acid was used as the organic matrix. **Fig. 2a** is in the mass range *m/z* 6,000 - 15,000 and **Fig. 2b** is for *m/z* 15,000 - 34,000. The obtained results show that the photo-reactive MALDI plate brings more relevant information from intact gram-negative bacteria.

Fig. 3 shows the comparison of mass spectra obtained from intact gram-positive bacteria *B.subtilis* by using a classic stainless steel MALDI plate and a photo-reactive MALDI plate from **Fig. 1** where the immobilized nanoparticles **2** are TiO_2. Sinapinic acid was used as the organic matrix. **Fig. 3a** is in the mass range *m/z* 6,000 - 12,000 and **Fig. 3b** is for *m/z* 12,000 - 34,000. The obtained results show that photo-reactive MALDI plate brings more relevant information from intact gram-positive bacteria. **Figs. 2** and **3** imply that identification of bacteria on the basis of protein fingerprinting can be significantly improved by using the present invention, especially at high mass range (*m/z* > 10,000).

Fig. 4 shows the comparison of mass spectra obtained from the intact human melanoma cell line *wm115* by using a classic stainless steel MALDI plate and a photo-reactive MALDI plate from **Fig. 1** where the immobilised nanoparticles **2** are TiO_2. Sinapinic acid was used as the organic matrix. **Fig. 4a** is in the mass range *m/z* 5,000 - 15,000 and **Fig. 4b** is for *m/z* 15,000 - 50,000. The obtained results show that more peaks can be obtained with the photo-reactive MALDI plate, implying that more components, especially with high molecular weight (> 10,000 Da) can be detected from intact cancer cells with the present invention.

Fig. 5 compares the performance of a classic stainless steel MALDI plate (**Fig. 5a**) and a plate from **Fig. 1** where the nanoparticles **2** are TiO_2 (**Fig. 5b**) for detection of

bacterial resistance to ampicillin. In both **Fig. 5a** and **Fig. 5b**, the mass spectrum obtained from ampicillin-resistant *E.coli* XL1 was compared with that from ampicillin-susceptible *E.coli* XL1 at the mass range of *m/z* 27,800 - 29,800. Sinapinic acid was used as the organic matrix. The ampicillin-resistance gene, expressed as a protein of 28,970 Da, was detected from the resistant strain using the invented plate (**Fig. 5b)**, whereas this gene is undetectable using a classic bare MALDI plate (**Fig. 5a**).

Fig. 6 compares the performance of a classic stainless steel MALDI plate (**Fig. 6a**) and a plate from **Fig. 1** where the immobilised nanoparticles **2** are TiO_2 (**Fig. 6b**) for detection of bacterial resistance to kanamycin. In both **Fig. 6a** and **Fig. 6b**, the mass spectrum obtained from kanamycin-resistant *E.coli* BL21 was compared with that from kanamycin-susceptible *E.coli* BL21 at the mass range of *m/z* 19,000 - 20,000. Sinapinic acid was used as organic matrix. The kanamycin-resistance gene, expressed as a protein of 29,050 Da, was detected from the resistant strain with the invented plate (**Fig. 6b)**, whereas the product of this gene is undetectable with a classic bare MALDI plate (**Fig. 6a**). **Fig. 5** and **Fig. 6** imply potential applications of the present invention for fast detection of bacterial resistance to antibiotics.

Fig. 7 compares the performance of a classic stainless steel MALDI plate (**Fig. 7a**) and a plate from **Fig. 1** where the immobilized nanoparticles **2** are TiO_2 (**Fig. 7b**), for detection of a specific marker from cancer cells. In both **Fig. 7a** and **Fig. 7b**, the mass spectra obtained from intact human melanoma cell *wm115* with and without the GFP marker (green fluorescent protein, $M_w \approx$ 26,900 Da) were compared at the mass range of *m/z* 25,000 - 29,000. Sinapinic acid was used as the organic matrix. In **Fig. 7b,** a peak around m/z 26,900 is clearly detected from GFP-expressed *wm115* with high intensity and resolution using the invented plate, while this marker is hardly detected using the classic bare MALDI plate (**Fig. 7a**). This finding implies that the present invention is advantageous in detecting biomarkers from cancer cells.

Advantages of the present work

To study biological samples such as micro-organisms, cells and tissues by mass spectrometry, two procedures are usually adopted. One is to directly place intact entities on a classic bare MALDI plate for MS detection; the other one includes "preparatory" component extraction prior to MS detection. The former is simple and fast, but may not be always sufficient for biological analysis. The latter can provide more sample information, but is time-consuming; also, the extracted components vary greatly with the extraction protocol. Herein, using the present invention, biological entities on the target plate undergo an in-source photo-induced electrochemical disruption process during MALDI detection, with their envelope structures being broken up by redox and radical reactions. This invented device also enhances the desorption/ionization process of the entities' components. Accordingly, more components (especially large molecular weight proteins or low-abundant molecules) can be detected directly from intact entities. Moreover, different redox proton donor/acceptor probes (such as salicylic acid or dopamine) could be added to the matrix to promote proton transfer and redox reactions. Additionally, with fixed MALDI detection parameters, the invented device can produce highly reproducible results, as the disruption process of the entities depends only on the photo-reactive nanomaterial. Generally, this invention provides a quick way of precise profiling of biological entities with high reproducibility. It therefore helps to broaden the applications of MALDI MS for biochemical research and clinical medicine.

Examples

Example 1 - Detection of Bacteria Resistance to Antibiotics Using TiO_2-modified MALDI Target Plates

Fabrication of TiO_2-modified Target Plate

An aluminium plate or a stainless steel plate is used as a substrate. A TiO_2 suspension is prepared by dissolving a commercially available TiO_2 paste (Solaronix Ti-Nanoxide D20/SP, Switzerland, 20 - 25 nm anatase nanoparticles; suspension $V_{isopropanol}/V_{water}/V_{Triton\ X\text{-}100}$ 78/19.5/2.5) to reach a final concentration of 0.2 % (m/m). The obtained suspension is inkjet-printed using a disposable cartridge DMC-11610 (Dimatix Fujifilm, USA) containing 16 individually addressable nozzles with 10 pL nominal droplet volume, forming an array of spots (3 mm diameter) on the substrate. An optimum substrate temperature of 60 ^{0}C is used during the printing, ensuring rapid drying of the ink on the substrate and good printing resolution. Afterwards, the printed plate is heated at 400 ^{0}C for one hour to sinter the nanoparticles and then cooled down to room temperature. The TiO_2 suspension can also be screen-printed, rotogravure-printed, or directly deposited with a micropipette (1-10 µL) onto the metal substrate.

Preparation of Bacteria Samples

Bacteria (*e.g.*, *E.coli*, *B.sutilis*) resistant or susceptible to different antibiotics, (*e.g.*, ampicillin, carbenicillin, kanamycin, chloramphenicol, gentamicin, spectinomycin, erythromycin, hygromycin, tetracycline, etc.) are separated from the growing media by centrifugation (13,000 rpm × 3 min), and washed three times with deionized water. Finally, the pellet is resuspended in deionized water with the concentration around 10^8 cells·mL^{-1}. The solution (1 - 2 µL) is deposited with a micropipette onto a spot on the TiO_2-modified target plate, and dried at room temperature (~ 5 min). All practical activities with bacteria and antibiotics are conducted in a biosafety level 1 or 2 (P1 or P2) laboratory. All wastes are autoclaved and disposed properly according to the safety guidelines. Instruments, facilities and benches are wiped with ethanol 70%/water 30% when activities are finished.

MALDI Matrix Deposition and MS Measurements

Matrix solution (1 - 2 uL, sinapinic acid, 15 mg/mL in $V_{acetonitrile}/V_{water}/V_{trifluoroacetic\ acid}$ 50/49.5/0.1) is added to cover the bacteria sample spot and dried at room temperature (~ 5 min). MALDI-TOF MS detection was performed on a Bruker microflex ® LRF under linear positive mode. The laser source used in this instrument is a nitrogen laser (337.1 nm). One mass spectrum is obtained from each sample spot by accumulation of 500 laser shots. Each test is repeated for 3 times and the average mass spectrum is presented in all figures.

Antibiotic Resistance Results

Non-pathogenic gram-negative *E.coli* and gram-positive *B.subtilis* are used as models to investigate the performance of the invented TiO_2-MALDI plate for intact bacteria profiling and compare it with a classic bare MALDI plate. Results show that more peaks are obtained from the invented TiO_2-MALDI plate, especially at the mass range > 10,000 Da, as shown in **Fig. 2** and **Fig. 3.** The bacterial envelope is mainly composed of lipid bilayers and peptidoglycan, which can be broken up by TiO_2-induced photo-chemical redox reactions. The TiO_2 nanoparticles also facilitate desorption and ionization of inner components together with the presence of organic matrix. Accordingly, more information about the internal components can be produced by the TiO_2-MALDI plate directly from intact bacteria.

Antibiotic-resistant bacteria often contain special resistance genes in their genome or on plasmids, which they have acquired from other bacteria and which they can spread to make non-resistant bacteria resistant to specific antibiotic compounds. Such antibiotic resistance genes encode proteins that perform enzymatic reactions to degrade or modify antibiotic molecules making them non-functional. These proteins conferring resistance to antibiotics normally appear in the mass range higher than 10,000 Da. Ampicillin and kanamycin resistance of *E.coli* are firstly analysed as models. As shown in **Fig. 5b**, compared to the ampicillin-susceptible

E.coli XL1-blue strain, an additional peak at *m/z* 28,970 is detected from the ampicillin-resistant strain, by using the invented TiO_2-MALDI plate. This peak comes from a protein called ß-lactamase that is responsible for resistance to ß-lactam antibiotics. Similarly, the product of the gene *aph* (29,050 Da) is detected specially from a kanamycin-resistant strain by using the TiO_2-MALDI plate, as shown in **Fig. 6b**. Neither of these two types of resistance is detectable by using a classic bare MALDI plate, as shown in **Fig. 5a** and **Fig. 6a**.

In addition to ampicillin and kanamycin, the resistance of *E.coli* strains to other antibiotics (*e.g.* carbenicillin, chloramphenicol, gentamicin, spectinomycin, erythromycin, hygromycin, tetracycline, etc.) can also be detected by the invented plate from intact bacteria.

Example 2 – Detection of Specific Marker from Cancer Cells Using TiO_2-modified MALDI Target Plates

Fabrication of a TiO_2-modified MALDI target plate is similar to that of *Example 1.*

The human melanoma cell line *wm115* is employed as model. A cell pellet is collected from culture media by centrifugation (2,000 rpm × 4 min), and resuspended in the same volume of deionized water. The solution (1 µL) is deposited onto a TiO_2 spot on the target plate and dried at room temperature. The cells are covered by 1 µL of matrix solution (sinapinic acid, 15 mg/mL in $V_{acetonitrile}/V_{water}/V_{trifluoroacetic\ acid}$ 50/49.5/0.1) and dried at room temperature prior MALDI MS detection. MS measurements are similar to those of *Example 1.*

In-source photo-electrochemical disruption of intact *wm115* happens during MS detection when using the TiO_2-modified MALDI plate. Thereafter, more cellular components are detected, especially at the mass range higher than 15,000 Da compared to the result from a classic bare MALDI plate (**Fig. 4)**. The obtained

results imply that the invented TiO_2-MALDI plate is more efficient for profiling of intact cancer cells and shows advantage in cellular biomarker detection and differentiation of cancer cells in different growth phases.

The green fluorescent protein (GFP, 26,900 Da) is used as a model marker in *wm115* cell line. MS fingerprints from the intact *wm115* cell line with and without the GFP marker by using a classic bare plate (**Fig. 7a**) and a TiO_2-modified plate (**Fig. 7b**) are compared. It is obvious that the peak from GFP at m/z 26,900 is much more clearly detected with TiO_2-modified plate, confirming the potential ability of the invented device for the fast detection of cancer biomarkers.

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Claims

1. A plate for matrix-assisted laser desorption ionization (MALDI) mass spectrometry comprising: an electrically conductive substrate partially covered with immobilized photo-reactive nanoparticles, a sample comprising intact biological entities, having envelopes, distributed over the photo-reactive nanoparticles, and a matrix that comprises a light absorber and a charge carrier acid, the plate facilitating in-source photo-electrochemical disruption of the envelopes and desorption and ionization of components of the entities.

2. A plate according to claim 1 wherein the sample comprises at least one of micro-organisms (e.g., bacteria, fungi, yeasts, virus), cells (e.g., cancer cells) or tissues.

3. A plate according to claim 1 or 2 wherein the nanoparticles comprise metal oxides (e.g., TiO_2, ZnO, ZrO_2, SnO_2), or metal oxides doped with different metal species (e.g., Au, Ag, Co).

4. A plate according to claim 1, 2 or 3 wherein the nanoparticles are made of metals that are oxidized on their surface to form a thin layer of metallic oxides.

5. A plate according to any preceding claim wherein the nanoparticles are spherical or cylindrical with a mean radius between 1.5 and 50 nanometers.

6. A plate according to any preceding claim wherein the nanoparticles are deposited on the substrate in a pattern selected from a specific area, a set of stripes, and an array of individual spots.

7. A plate according to any preceding claims wherein the nanoparticles are immobilized in a layer of thickness ranging from 50 nanometres to 50 micrometres.

8. A plate according to any preceding claims wherein the electrically conductive substrate comprises stainless steel, aluminium, nickel, zinc, copper, silicon, tin-indium oxide on glass or a conductive/semi-conductive polymer.

9. A method of preparing the plate according to any preceding claim comprising the steps of: (a) preparing a suspension of nanoparticles according to one of claims 3, 4

and 5, and (b) applying this suspension to the conductive substrate, according to claim 6 or 7.

10. A method according to claims 9, wherein the suspension of nanoparticles is deposited on the substrate by inkjet-printing, screen-printing, rotogravure printing, drop casting or another deposition technique.

11. A method according to claim 9 or 10, comprising the step of thermal or photonic flash curing in order to obtain sintering of the nanoparticles to ensure their mutual adhesion and their adhesion to the substrate.

13. A method of use of a plate according to any one of claims 1 to 8, wherein components are released from the intact biological entities for the mass spectrometry analysis by photo-electrochemical disruption of the intact biological entities' envelope structure.

14. A method according to claim 12, wherein the components released from the intact biological entities for mass spectrometry analysis are biomolecules, such as lipids, proteins, peptides, nucleic acids and metabolites.

15. A method according to claim 13, wherein the components released for mass spectrometry analysis are proteins, which are specific markers for detection of micro-organisms antibiotic resistance, when the intact biological entities include intact bacteria, fungi, viruses, yeasts, etc.

16. A method according to claim 13, wherein the components released for mass spectrometry analysis are biomolecules that are specific cancer markers, when the intact biological entities include cancer cells or cancer tissues.

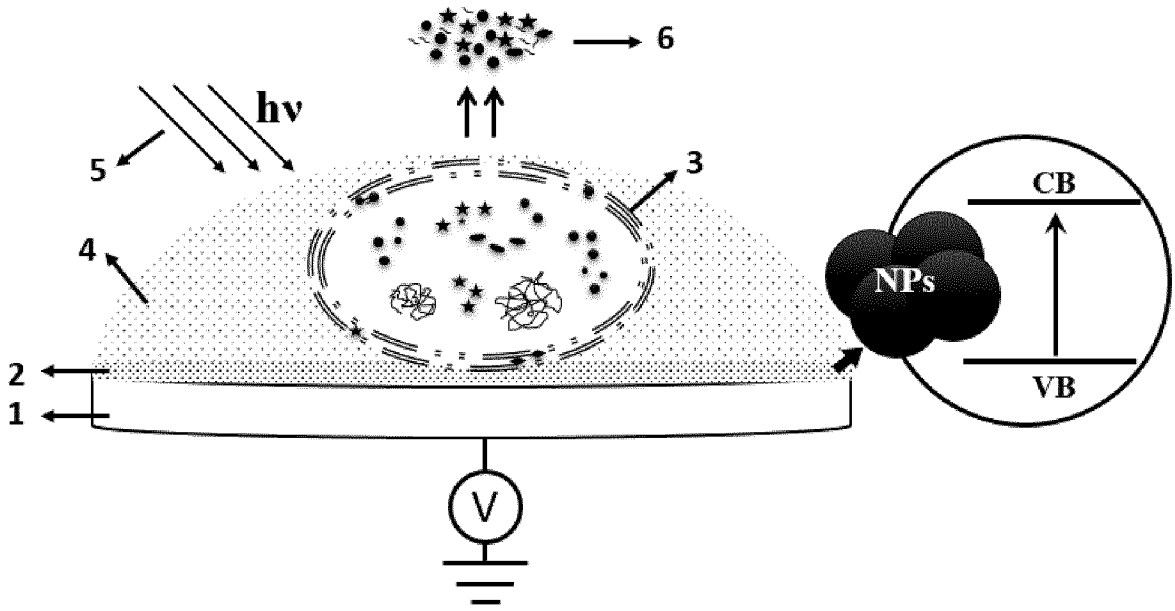

Fig. 1

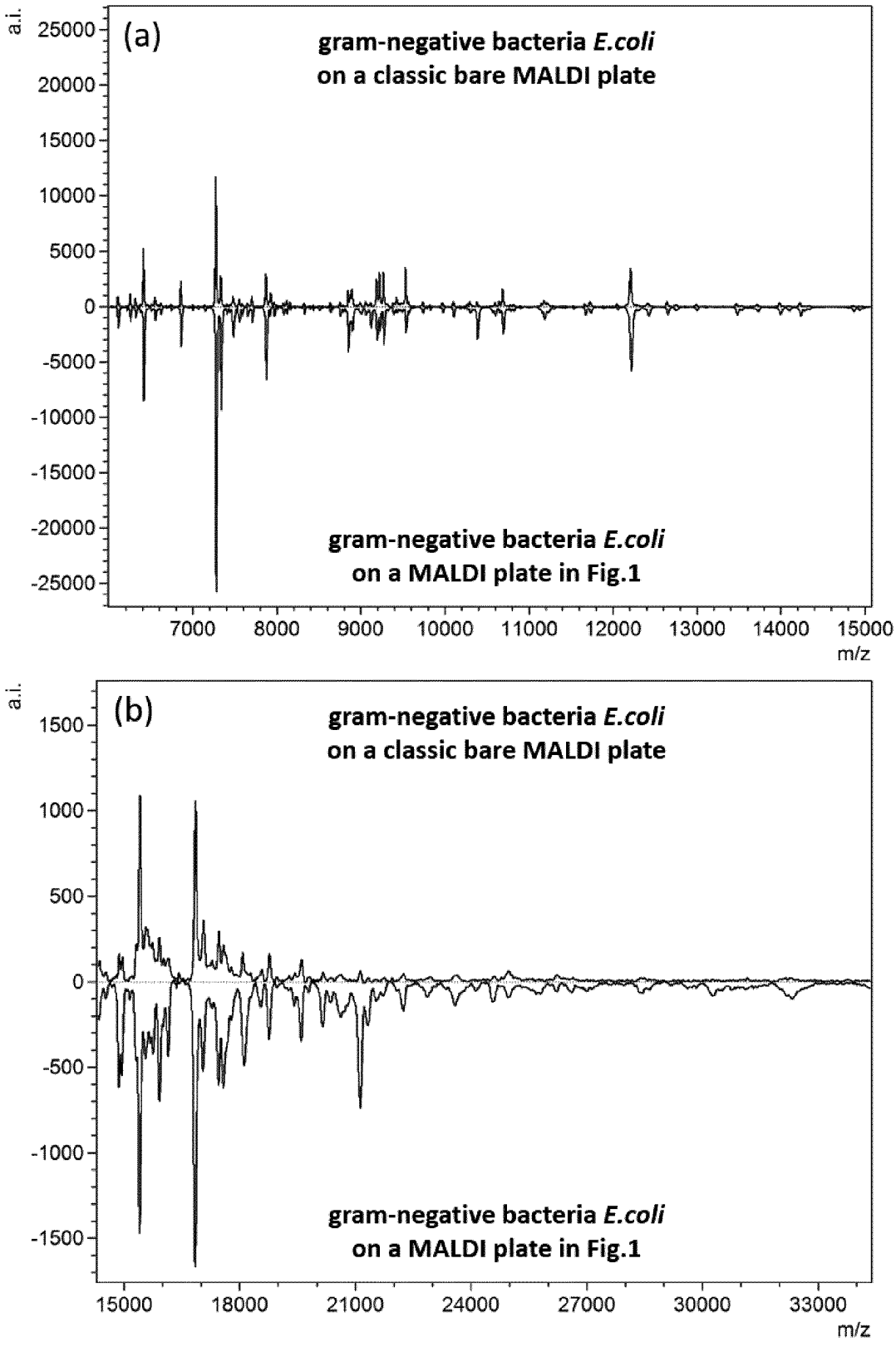
(a)
gram-negative bacteria E.coli
on a classic bare MALDI plate
gram-negative bacteria E.coli
on a MALDI plate in Fig.1
a.i.
m/z
(b)
gram-negative bacteria E.coli
on a classic bare MALDI plate
gram-negative bacteria E.coli
on a MALDI plate in Fig.1
a.i.
m/z

Fig. 2

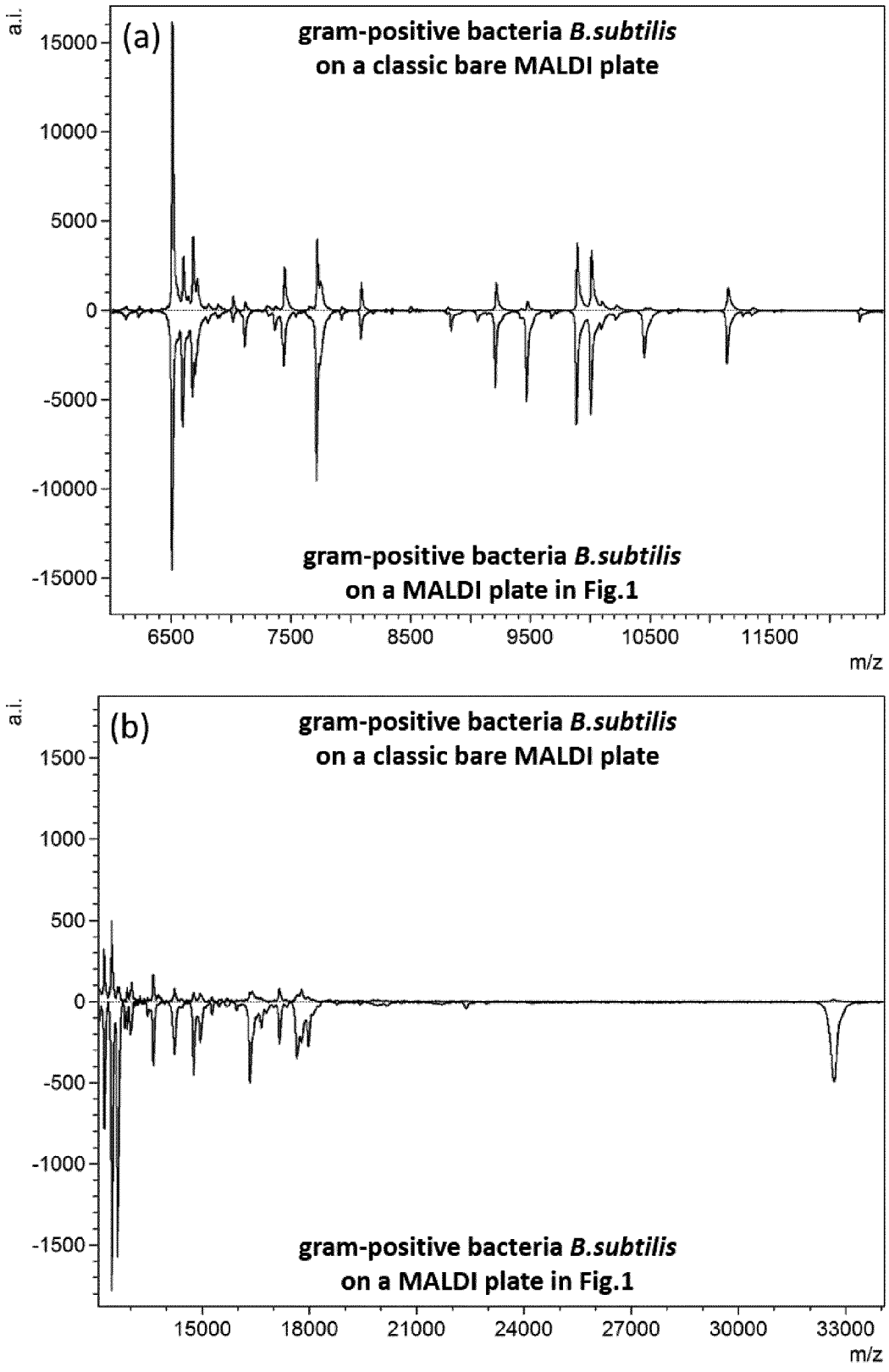
(a)
gram-positive bacteria B.subtilis
on a classic bare MALDI plate
gram-positive bacteria B.subtilis
on a MALDI plate in Fig.1
a.i.
15000
10000
5000
0
-5000
-10000
-15000
6500
7500
8500
9500
10500
11500
m/z
(b)
gram-positive bacteria B.subtilis
on a classic bare MALDI plate
gram-positive bacteria B.subtilis
on a MALDI plate in Fig.1
a.i.
1500
1000
500
0
-500
-1000
-1500
15000
18000
21000
24000
27000
30000
33000
m/z

Fig. 3

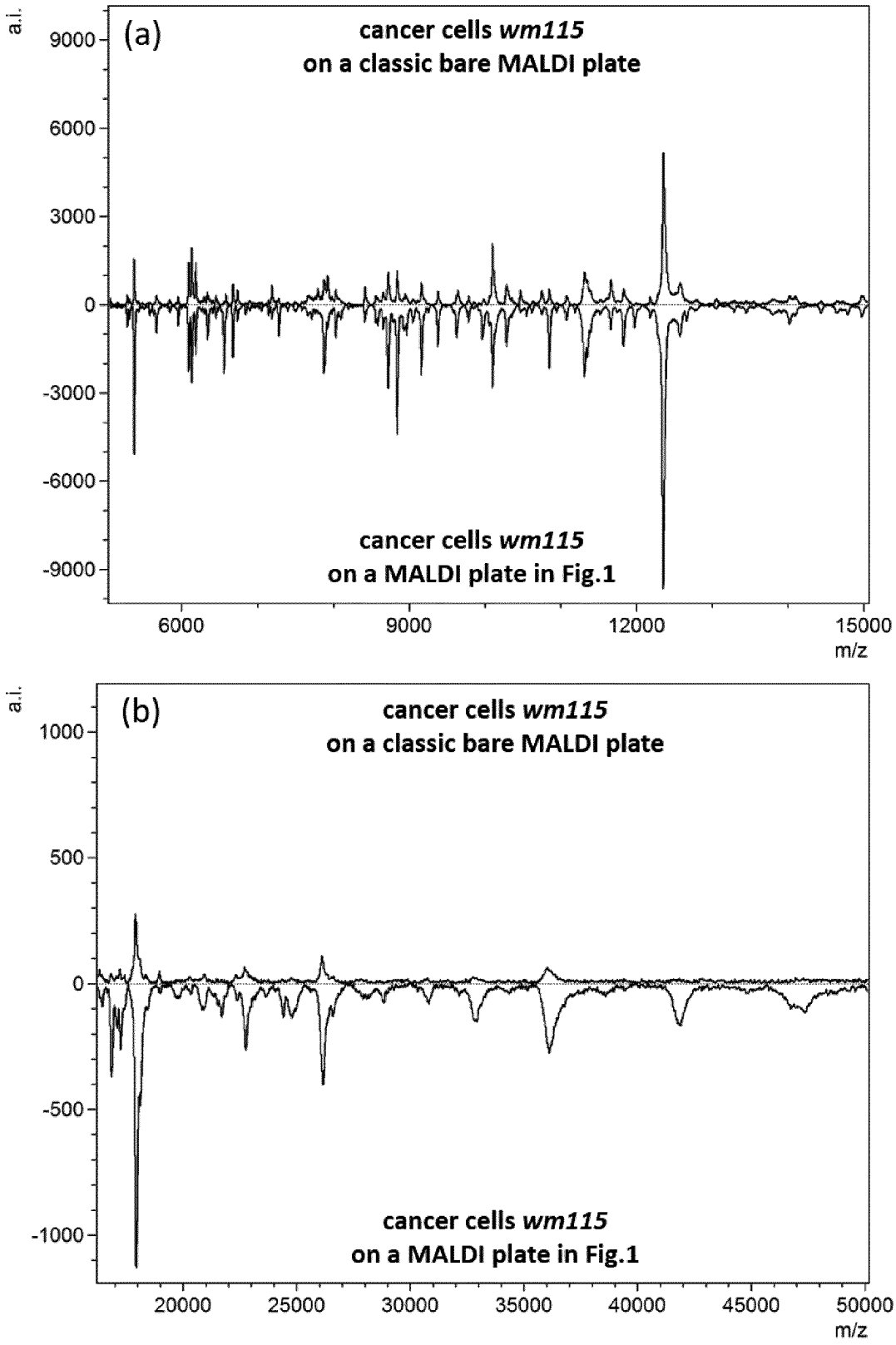
(a)
cancer cells wm115
on a classic bare MALDI plate
cancer cells wm115
on a MALDI plate in Fig.1
a.i.
9000
6000
3000
0
-3000
-6000
-9000
6000
9000
12000
15000
m/z
(b)
cancer cells wm115
on a classic bare MALDI plate
cancer cells wm115
on a MALDI plate in Fig.1
a.i.
1000
500
0
-500
-1000
20000
25000
30000
35000
40000
45000
50000
m/z

Fig. 4

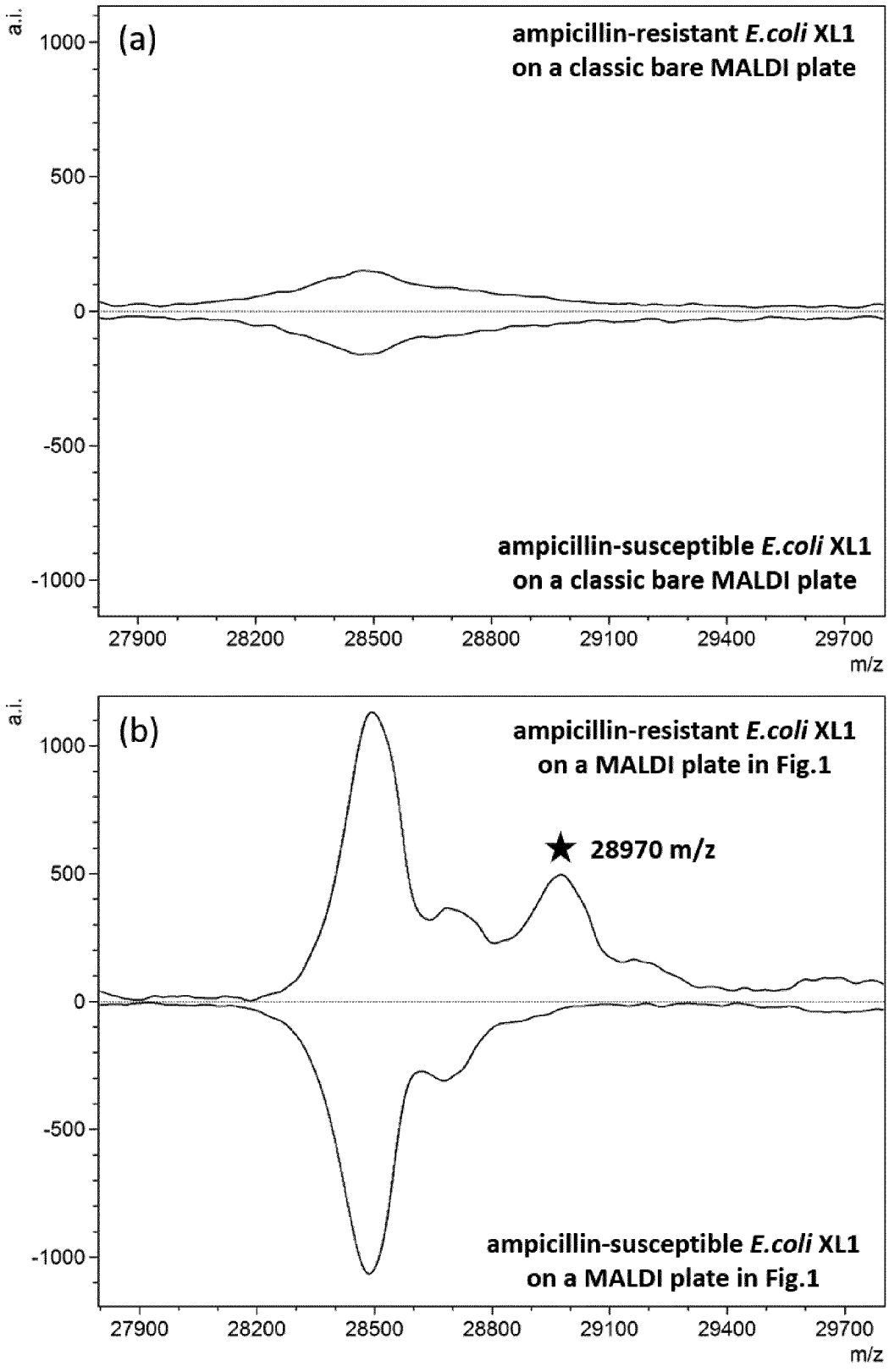
a.i.
(a)
ampicillin-resistant E.coli XL1
on a classic bare MALDI plate
1000
500
0
-500
-1000
ampicillin-susceptible E.coli XL1
on a classic bare MALDI plate
27900
28200
28500
28800
29100
29400
29700
m/z
a.i.
(b)
ampicillin-resistant E.coli XL1
on a MALDI plate in Fig.1
28970 m/z
1000
500
0
-500
-1000
ampicillin-susceptible E.coli XL1
on a MALDI plate in Fig.1
27900
28200
28500
28800
29100
29400
29700
m/z

Fig. 5

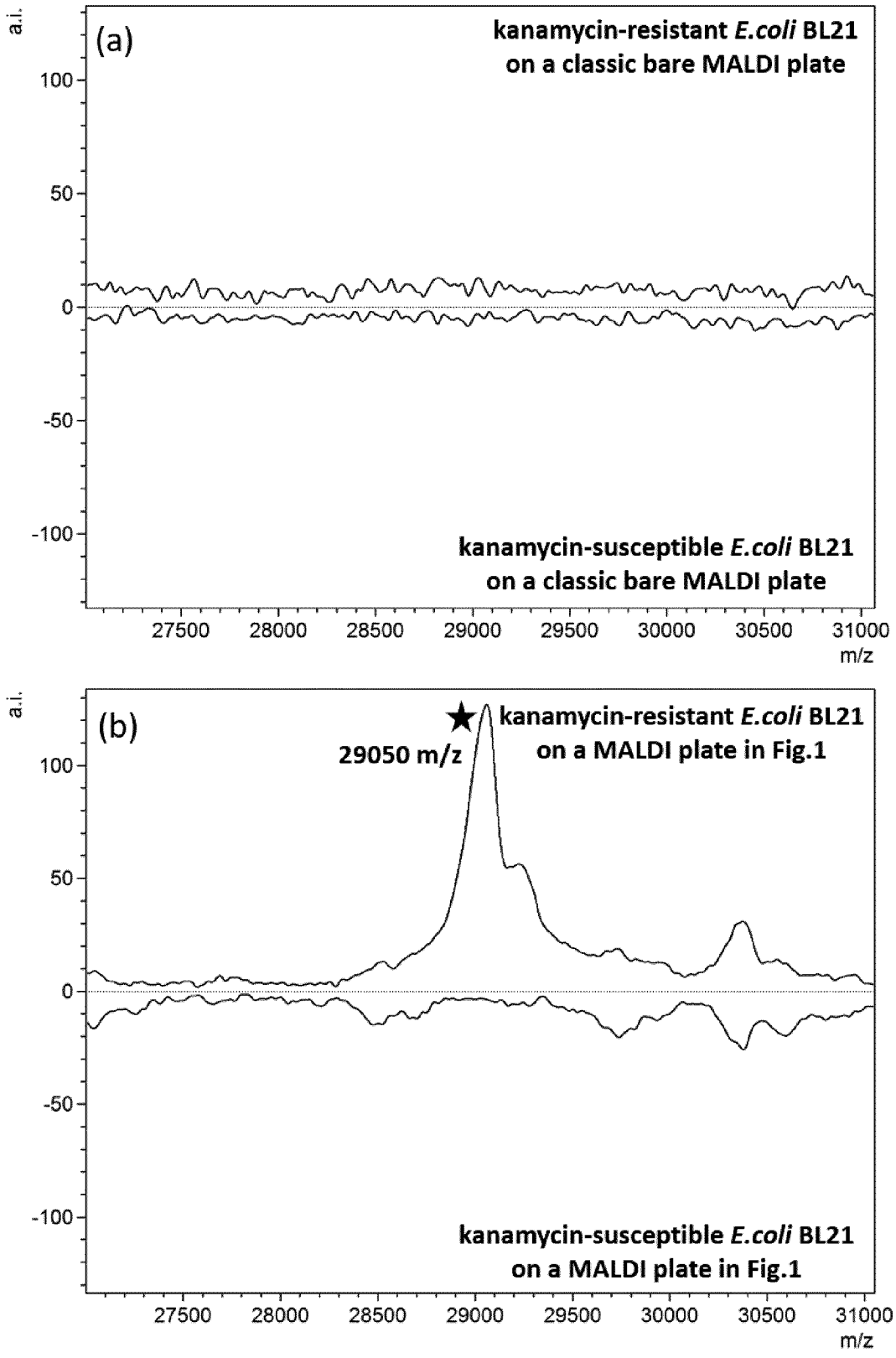
(a)
kanamycin-resistant E.coli BL21
on a classic bare MALDI plate
kanamycin-susceptible E.coli BL21
on a classic bare MALDI plate
a.i.
100
50
0
-50
-100
27500
28000
28500
29000
29500
30000
30500
31000
m/z
(b)
29050 m/z
kanamycin-resistant E.coli BL21
on a MALDI plate in Fig.1
kanamycin-susceptible E.coli BL21
on a MALDI plate in Fig.1
a.i.
100
50
0
-50
-100
27500
28000
28500
29000
29500
30000
30500
31000
m/z

Fig. 6

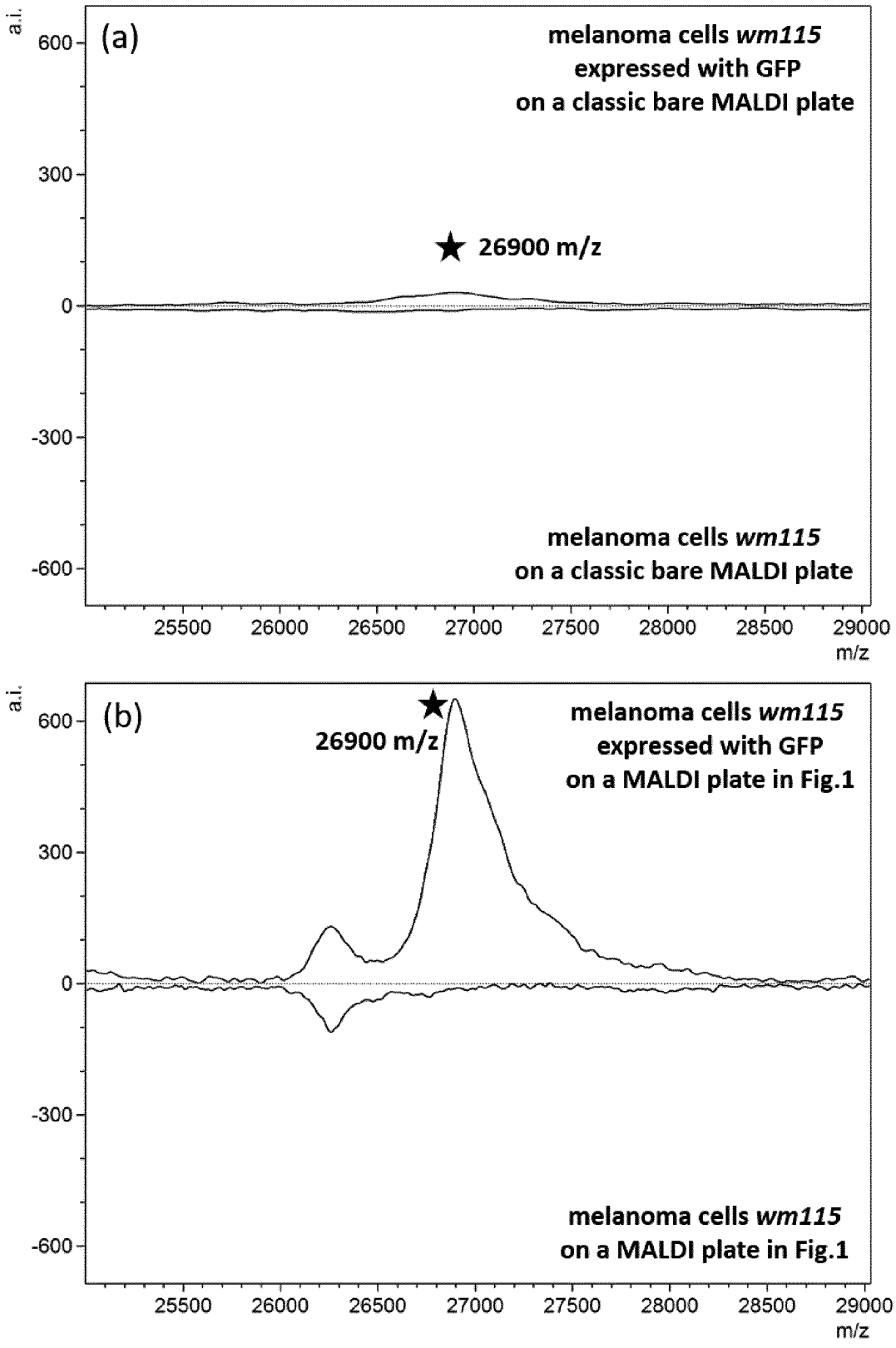
a.i.
600
300
0
-300
-600
(a)
melanoma cells wm115
expressed with GFP
on a classic bare MALDI plate
26900 m/z
melanoma cells wm115
on a classic bare MALDI plate
25500
26000
26500
27000
27500
28000
28500
29000
m/z
a.i.
600
300
0
-300
-600
(b)
26900 m/z
melanoma cells wm115
expressed with GFP
on a MALDI plate in Fig.1
melanoma cells wm115
on a MALDI plate in Fig.1
25500
26000
26500
27000
27500
28000
28500
29000
m/z

Fig. 7

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2018/051080

A. CLASSIFICATION OF SUBJECT MATTER
INV. H01J49/04
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
H01J

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

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

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2009/003673 A2 (ECOLE POLYTECH [CH]; LIU BAOHONG [CN]; LIANG QIAO [CN]; LION NIELS [CH) 8 January 2009 (2009-01-08) paragraphs [0071], [0080] claims 1,4,11,15,17,18	1-11, 14-16
X	WO 2006/118595 A2 (NANOSYS INC [US]; NIU CHUNMING [US]; DANIELS ROBERT HUGH [US]; DUBROW) 9 November 2006 (2006-11-09) claims 1, 2, 5, 16, 23 paragraphs [0003], [0018]	1-3,7-9, 13,14
X	US 2006/145068 A1 (CHEN YU-CHIE [TW] ET AL) 6 July 2006 (2006-07-06) claims 1,2,8	1

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 March 2018	03/04/2018

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Peters, Volker

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2018/051080

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2009003673 A2	08-01-2009	EP 2168139 A2	31-03-2010
		US 2010248388 A1	30-09-2010
		WO 2009003673 A2	08-01-2009
WO 2006118595 A2	09-11-2006	AU 2005331265 A1	09-11-2006
		CA 2578359 A1	09-11-2006
		EP 1804958 A2	11-07-2007
		JP 2008513781 A	01-05-2008
		US 2008073505 A1	27-03-2008
		US 2009124034 A1	14-05-2009
		WO 2006118595 A2	09-11-2006
US 2006145068 A1	06-07-2006	TW I274874 B	01-03-2007
		US 2006145068 A1	06-07-2006