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(54) **BIOSENSOR DEVICE AND METHOD FOR MANUFACTURING THEREOF AND METHOD FOR DETECTING BIOLOGICAL MOLECULES**

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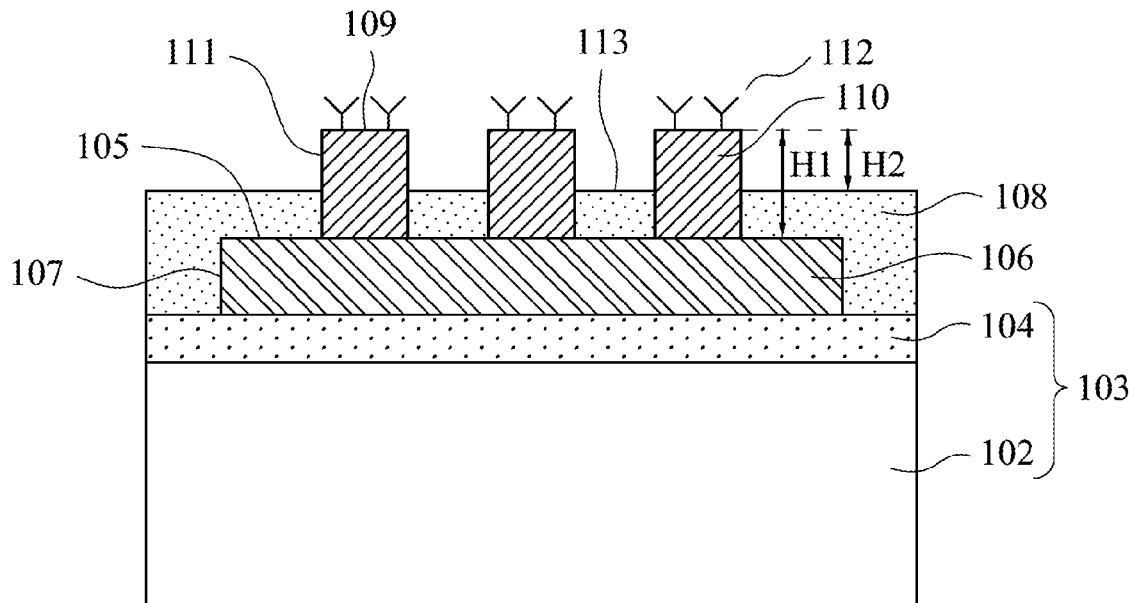
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(57) **ABSTRACT**

A biosensor device includes a substrate plate, a metal conductive layer, a plurality of working electrodes and an insulating layer. The metal conductive layer is disposed over the substrate plate and has an upper surface. The working electrodes are disposed over the upper surface of the metal conductive layer, wherein each of the working electrodes has a top surface and each of the top surfaces is higher than the upper surface of the metal conductive layer. The insulating layer covers the metal conductive layer and surrounds the working electrodes, wherein an upper surface of the insulating layer is located between the top surfaces and the upper surface of the metal conductive layer such that the working electrodes protrude beyond the upper surface of the insulating layer. A method for manufacturing the biosensor device and a method for detecting biological molecules by using the biosensor device are also provided herein.

100



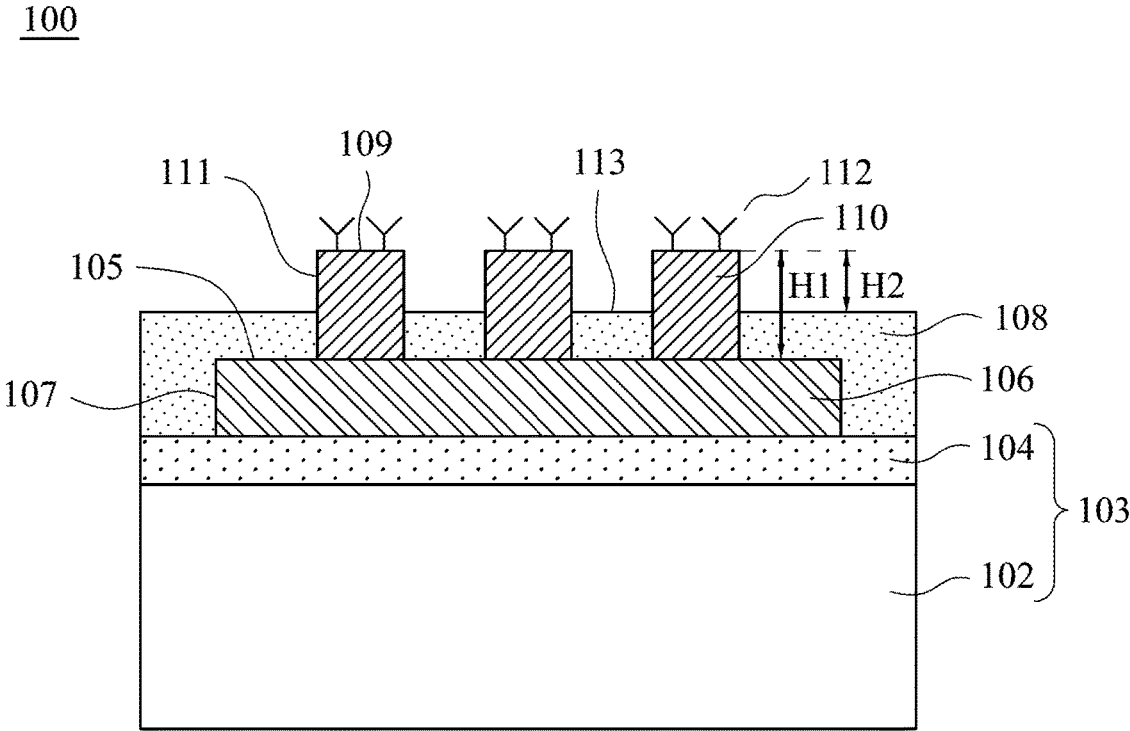


Fig. 1

200

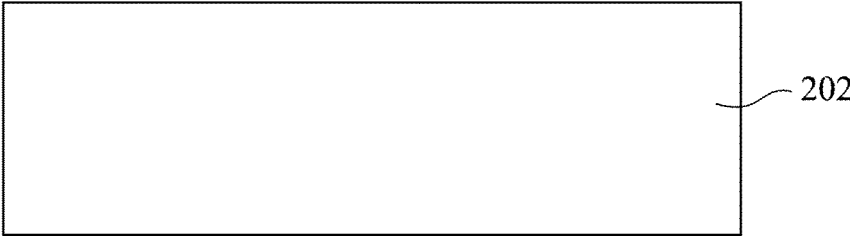


Fig. 2

200

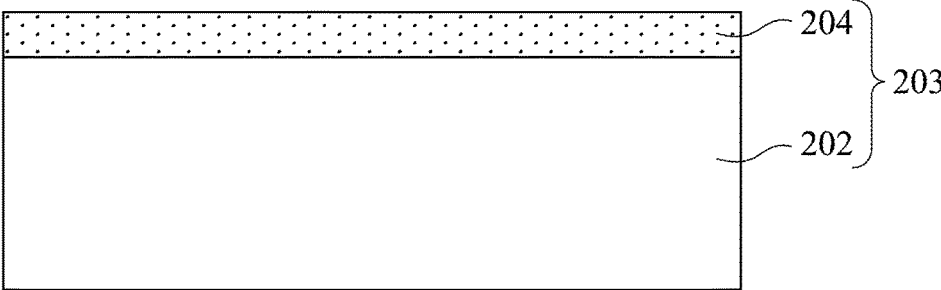


Fig. 3

200

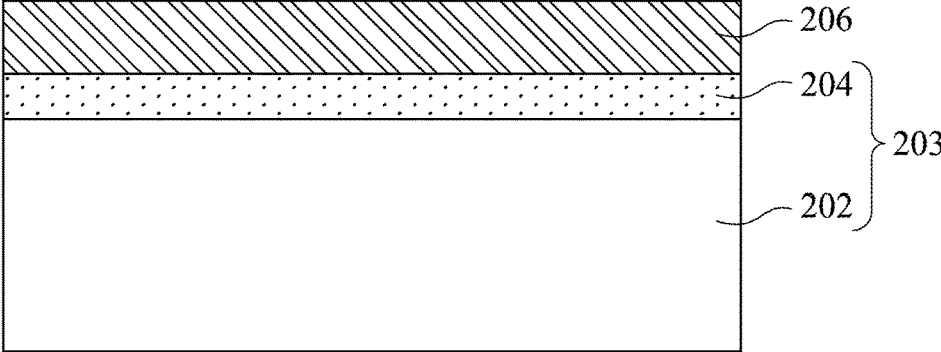


Fig. 4

200

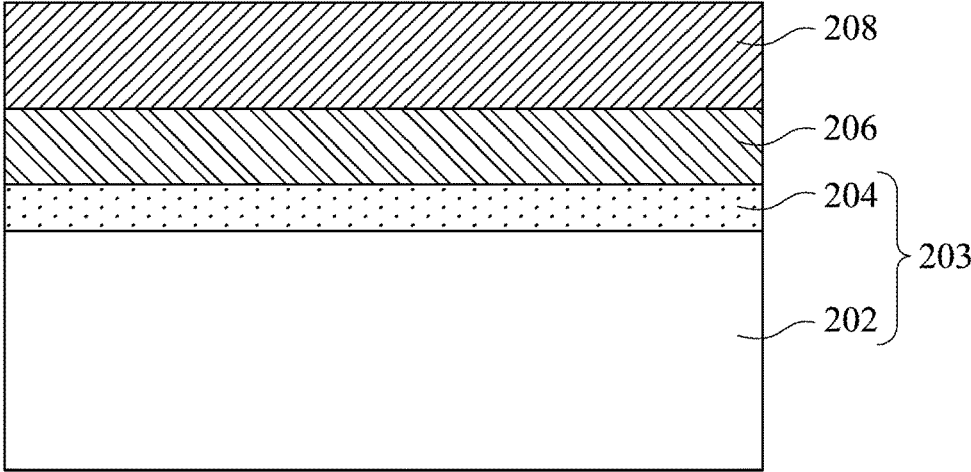


Fig. 5

200

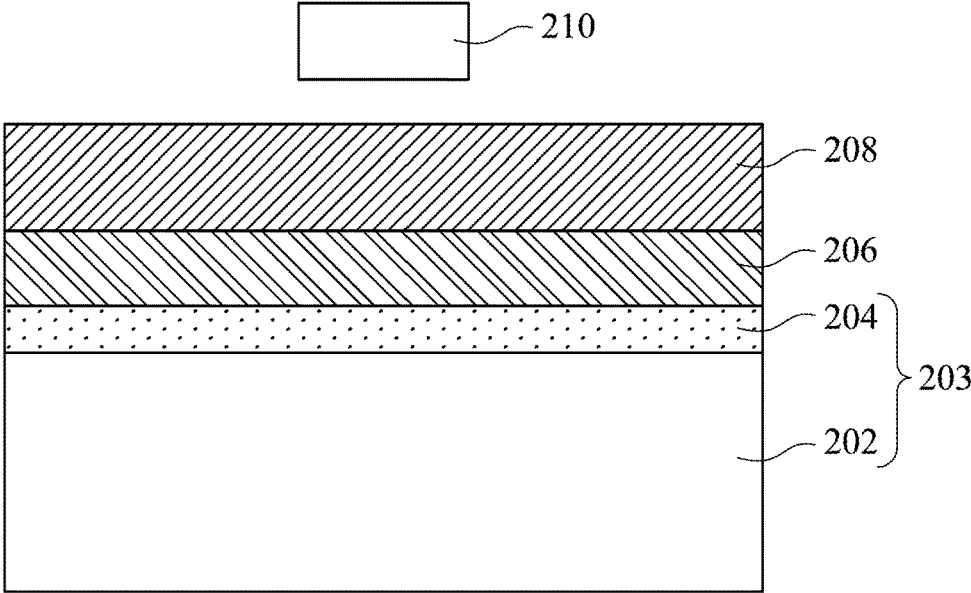


Fig. 6

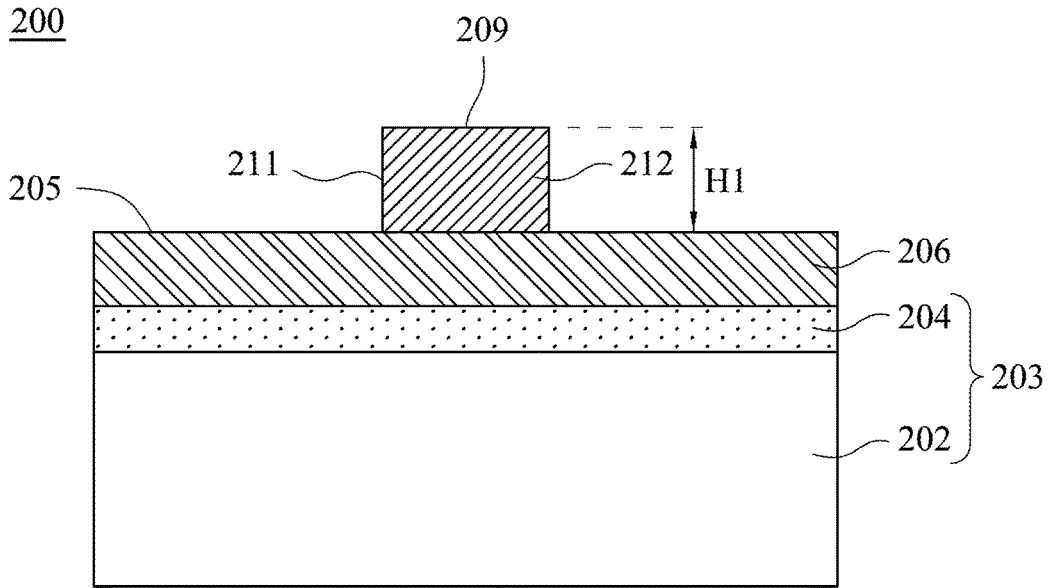


Fig. 7

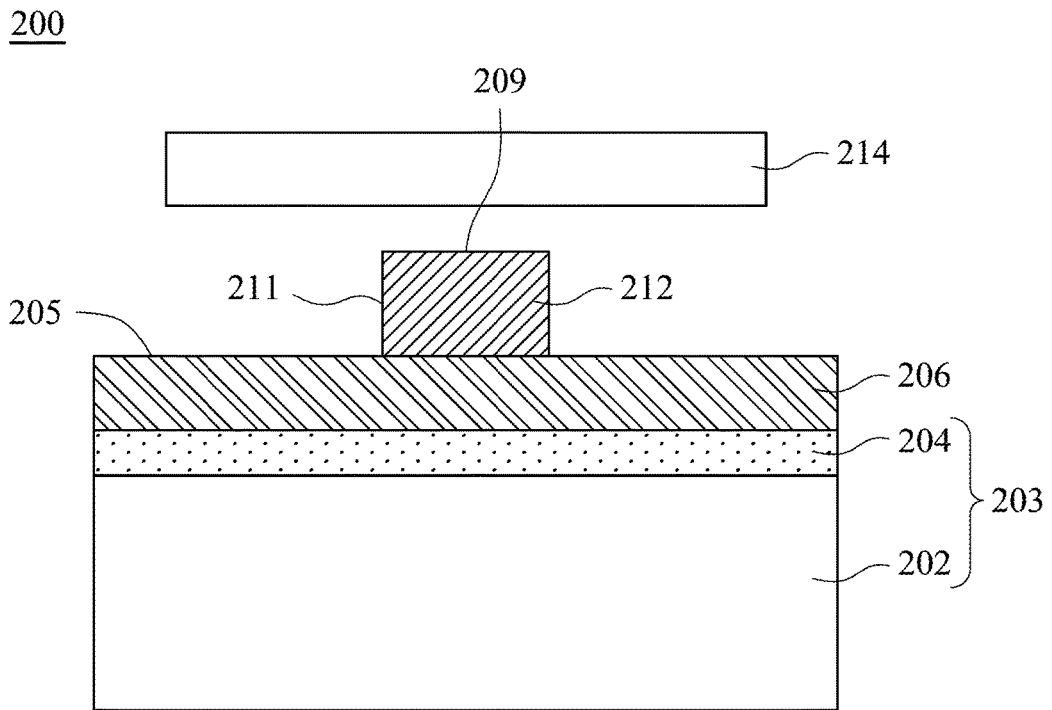


Fig. 8

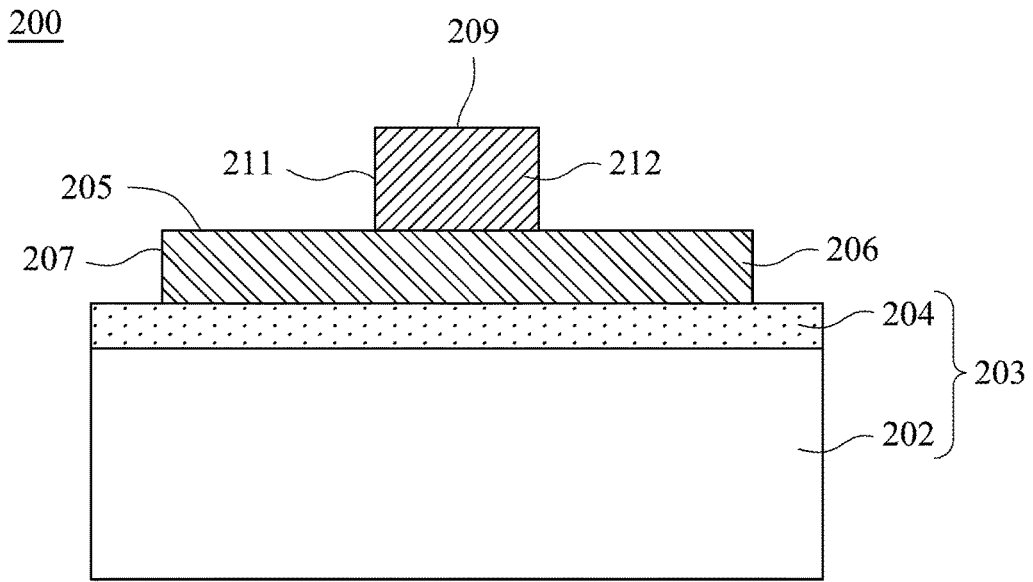


Fig. 9

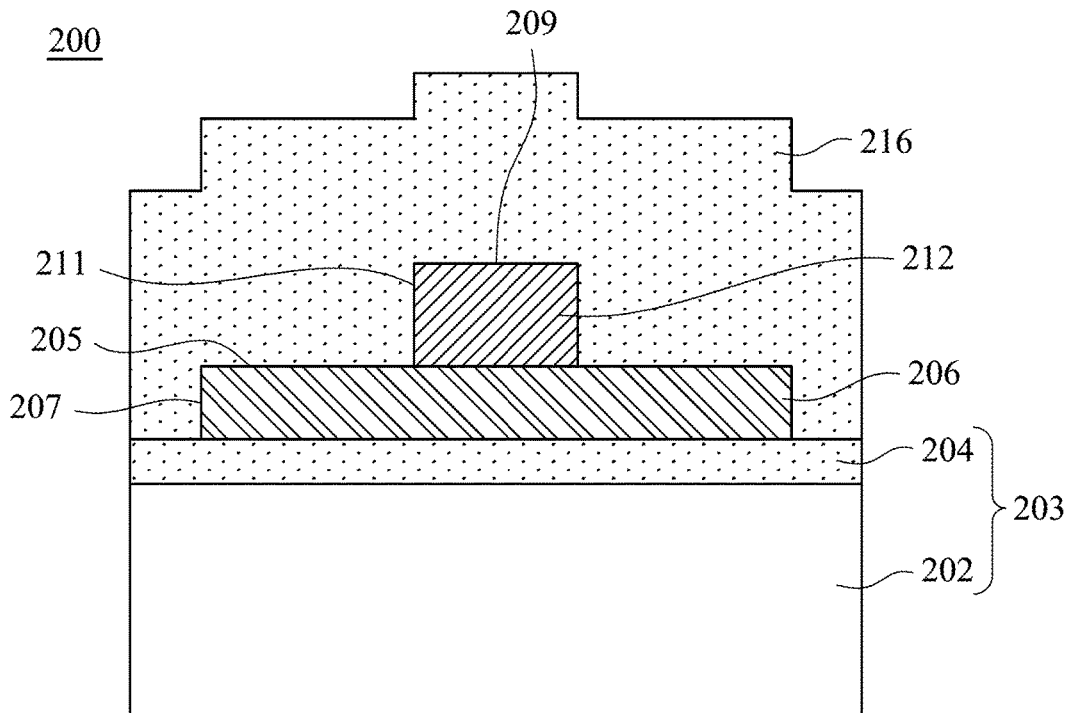


Fig. 10

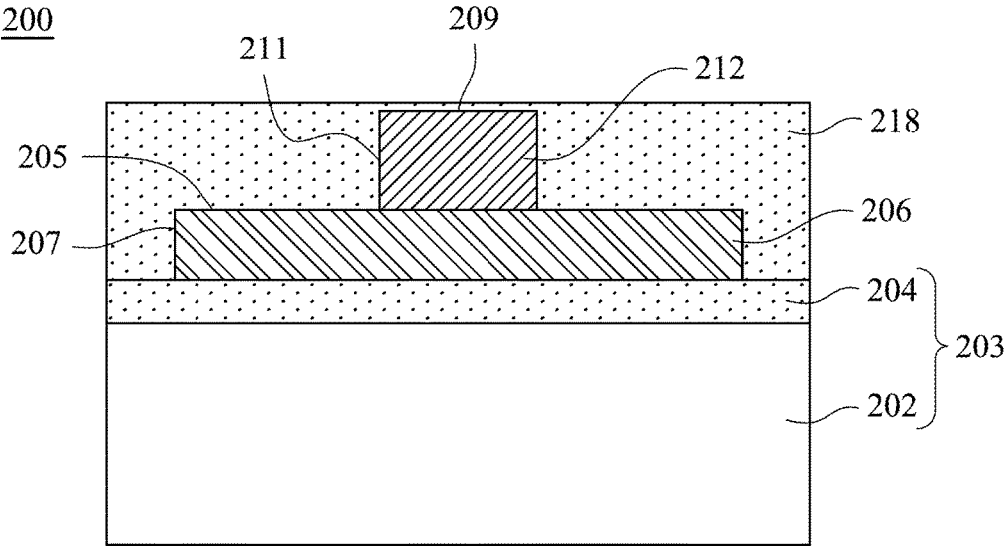


Fig. 11

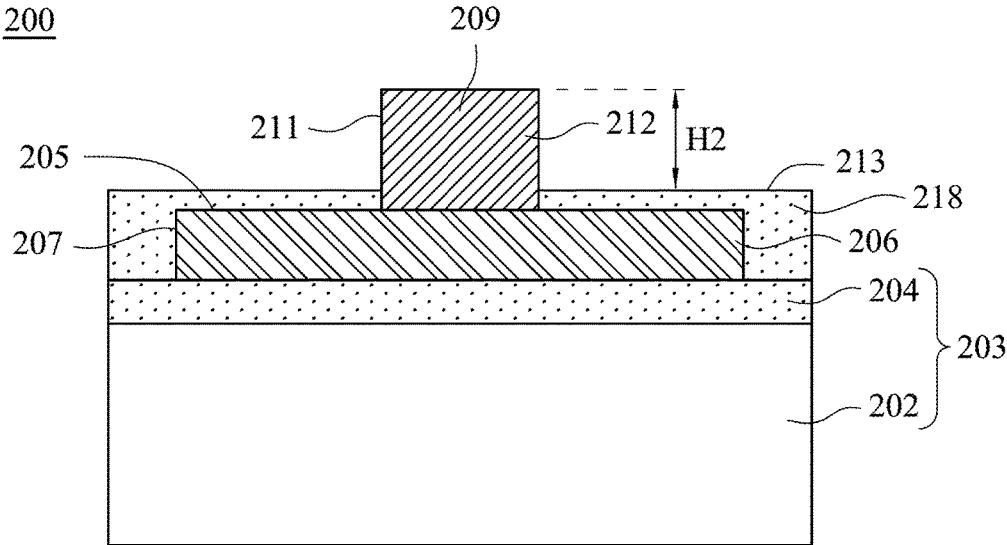


Fig. 12

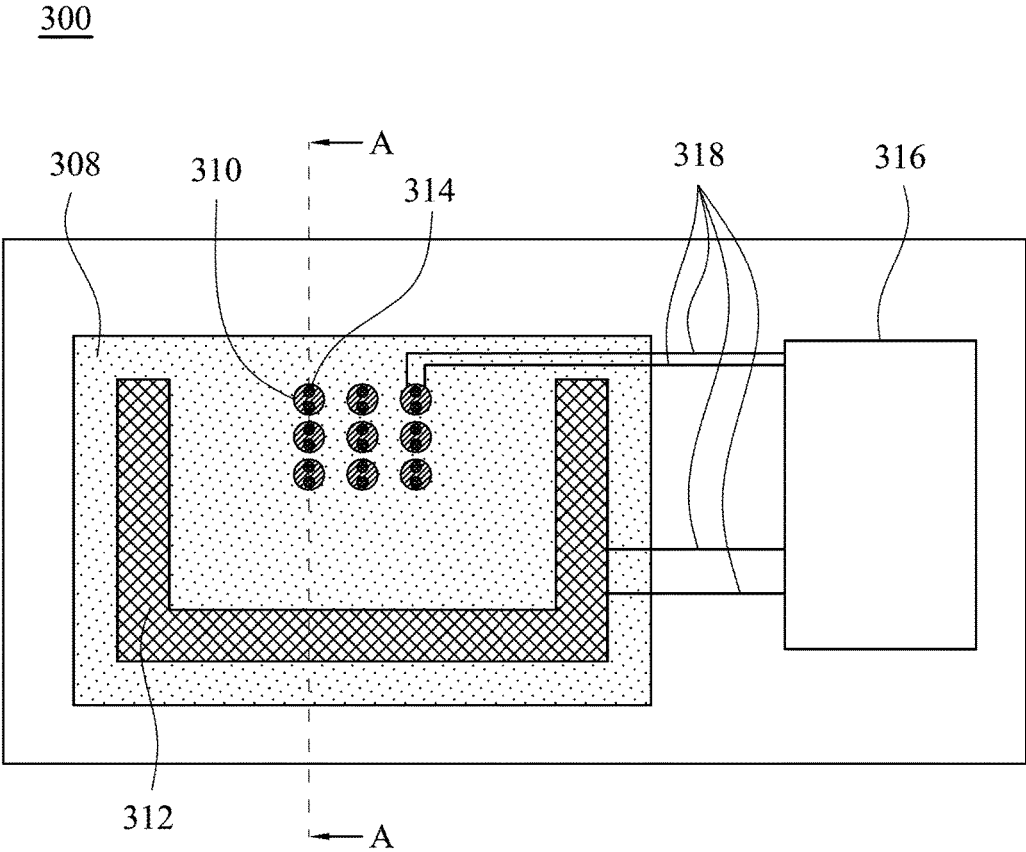


Fig. 13



300

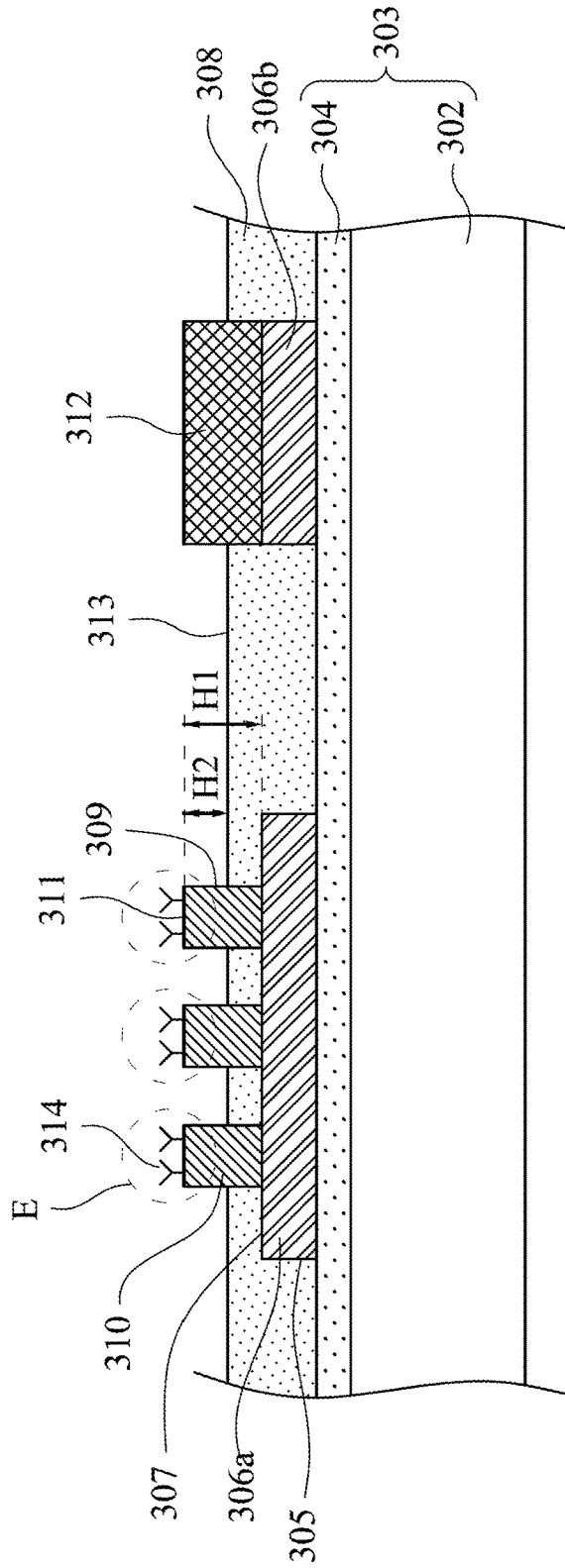


Fig. 14A

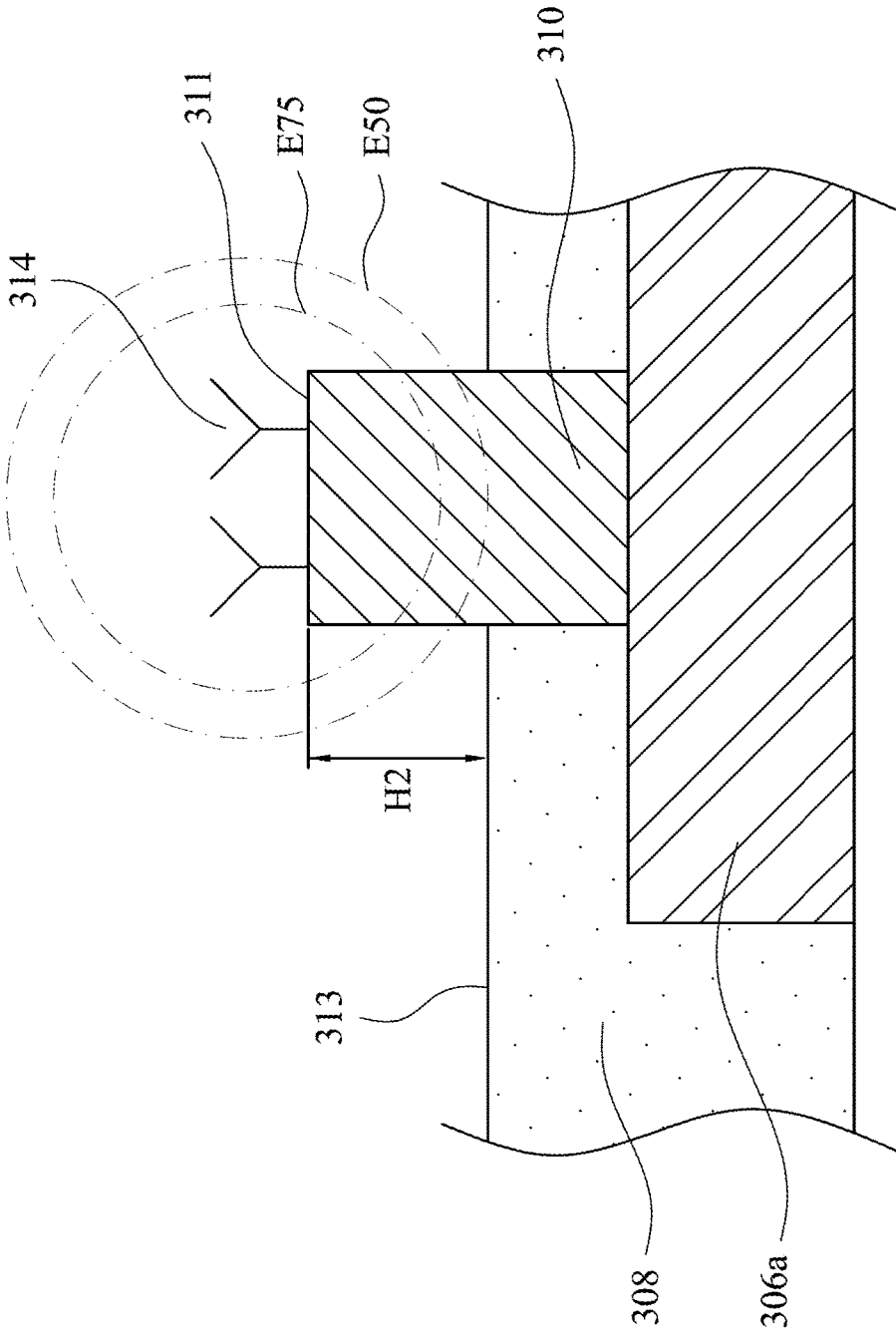


Fig. 14B

**BIOSENSOR DEVICE AND METHOD FOR  
MANUFACTURING THEREOF AND  
METHOD FOR DETECTING BIOLOGICAL  
MOLECULES**

RELATED APPLICATIONS

[0001] This application claims priority to Taiwan Application Serial Number 106121830, filed Jun. 29, 2017, which is herein incorporated by reference.

BACKGROUND

Field of Invention

[0002] The present invention relates to a biosensor device and method for manufacturing thereof, and a method for detecting biological molecules by using the biosensor device.

Description of Related Art

[0003] Recently, a variety of detection methods for biological molecules have been developed to diagnose various diseases, conduct researches associated with physiology, metabolism and monitor environmental factors, etc. The development of Micro-electromechanical Systems (MEMS) have attracted much attention due to its integration of semiconductor processes and precision machinery technologies, which can be used to manufacture a semiconductor microchip for sensing optics, chemicals, biological molecules or others properties. However, as the semiconductor industry has progress into nanometer technology nodes in pursuit of higher device density, higher performance and lower costs, challenges from manufacturing and design issues have resulted in the development in three-dimensional design. Accordingly, there is an urgent need for developing biosensor chips with higher performance and low costs.

SUMMARY

[0004] An aspect of the present disclosure provides a method for manufacturing a biosensor device, including the step of: providing a substrate plate; forming a metal conductive layer over the substrate plate, and the metal conductive layer having an upper surface; forming a plurality of working electrodes over the upper surface of the metal conductive layer, wherein each of the working electrodes has a top surface that is higher than the upper surface of the metal conductive layer; and forming an insulating layer covering the metal conductive layer and surrounding the working electrodes, wherein an upper surface of the insulating layer is between the top surfaces and the upper surface of the metal conductive layer so that the working electrodes protrudes beyond the upper surface of the insulating layer.

[0005] According to some embodiments of the present disclosure, the step of forming the working electrodes includes: depositing an electrically conductive layer over the upper surface of the metal conductive layer; and patterning the electrically conductive layer to form the working electrodes.

[0006] According to some embodiments of the present disclosure, each of the working electrodes has a first height ranged from about 0.05  $\mu\text{m}$  to about 0.6  $\mu\text{m}$ .

[0007] According to some embodiments of the present disclosure, each of the working electrodes has an aspect ratio ranged from about 0.125 to about 7.5.

[0008] According to some embodiments of the present disclosure, the step of forming the insulating layer includes: depositing an insulating material layer over the metal conductive layer and the working electrodes; performing a planarization process on the insulating material layer to form a planarized insulating material layer; and etching the planarized insulating material layer to form the insulating layer.

[0009] According to some embodiments of the present disclosure, each of the working electrodes further includes a sidewall adjoining the top surface, and the insulating layer covers a portion of each of the sidewalls.

[0010] According to some embodiments of the present disclosure, each of the working electrodes protrudes from the upper surface a second height, and the second height is about 0.01  $\mu\text{m}$  to about 0.5  $\mu\text{m}$ .

[0011] According to some embodiments of the present disclosure, the working electrodes are in a shape of a cylinder, a triangular prism, a quadrangular prism, a pentagonal prism, a hexagonal prism or an octagonal prism.

[0012] According to some embodiments of the present disclosure, the method further includes connecting a plurality of biological probes to the working electrodes, wherein the biological probes are nucleic acid, cell, antibody, enzyme, polypeptide or combinations thereof.

[0013] An aspect of the present disclosure provides a biosensor device, including: a substrate plate; a metal conductive layer disposed over the substrate plate and the metal conductive layer having an upper surface; a plurality of working electrodes disposed over the upper surface of the metal conductive layer, wherein each of the working electrodes has a top surface that is higher than the upper surface of the metal conductive layer; and an insulating layer covering the metal conductive layer and surrounding the working electrodes, wherein an upper surface of the insulating layer is between the top surfaces and the upper surface of the metal conductive layer, so that the working electrodes protrudes beyond the upper surface of the insulating layer.

[0014] According to some embodiments of the present disclosure, each of the working electrodes has a first height ranged from about 0.05  $\mu\text{m}$  to about 0.6  $\mu\text{m}$ .

[0015] According to some embodiments of the present disclosure, each of the working electrodes has an aspect ratio ranged from about 0.125 to about 7.5.

[0016] According to some embodiments of the present disclosure, the metal conductive layer further includes a sidewall adjoining the upper surface of the metal conductive layer, and the insulating layer covers the sidewall of the metal conductive layer.

[0017] According to some embodiments of the present disclosure, each of the working electrodes further includes a sidewall adjoining the top surface, and the insulating layer covers a portion of each of the sidewalls.

[0018] According to some embodiments of the present disclosure, each of the working electrodes protrudes from the upper surface a second height, and the second height is about 0.01  $\mu\text{m}$  to about 0.5  $\mu\text{m}$ .

[0019] According to some embodiments of the present disclosure, the working electrodes are in a shape of a cylinder, a triangular prism, a quadrangular prism, a pentagonal prism, a hexagonal prism or an octagonal prism.

[0020] According to some embodiments of the present disclosure, the biosensor device further includes a plurality of biological probes connected to the working electrodes,

wherein the biological probes are nucleic acid, cell, antibody, enzyme, polypeptide or combinations thereof.

**[0021]** Another aspect of the present disclosure provides a method for detecting biological molecules, including: providing a sample comprising a target molecule; providing the biosensor device of claim **10**; connecting a plurality of biological probes to the working electrodes; applying a voltage to the working electrodes such that the working electrodes generate an electric field surrounding the working electrodes; and contacting the sample with the biological probes such that the target molecule in the sample is bound to the biological probes, thereby generating a signal from the working electrodes.

**[0022]** According to some embodiments of the present disclosure, the step of applying the voltage to the working electrodes includes: applying a voltage to the working electrodes such that 75% of the maximal electric field intensity occurs at about 27% to about 40% of the second height from the top surfaces toward the upper surface of the insulating layer.

**[0023]** According to some embodiments of the present disclosure, the step of applying the voltage to the working electrodes includes: applying a voltage to the working electrodes such that 50% of the maximal electric field intensity occurs at about 80% to about 93% of the second height from the top surfaces toward the upper surface of the insulating layer.

#### BRIEF DESCRIPTION OF THE DRAWINGS

**[0024]** The present disclosure can be more fully understood by reading the following detailed description of the embodiment when read with the accompanying figures:

**[0025]** FIG. **1** is a cross-sectional view of a biosensor device according to some embodiments of the present disclosure.

**[0026]** FIGS. **2-12** are cross-sectional views of a biosensor device during various stages of production according to some embodiments of the present disclosure.

**[0027]** FIG. **13** is a top view of a biosensor apparatus according to some embodiments of the present disclosure.

**[0028]** FIG. **14A** is a cross-sectional view along line A-A of the biosensor apparatus according to some embodiments of the present disclosure.

**[0029]** FIG. **14B** is a partial magnified view exemplarily according to the cross-sectional view of FIG. **14A**.

#### DETAILED DESCRIPTION

**[0030]** The following disclosure provides many different embodiments, or examples, for implementing different features of the provided subject matter. Specific examples of components and arrangements are described below to simplify the present disclosure. These are, of course, merely examples and are not intended to be limiting. For example, the formation of a first feature “over” or “on” a second feature in the description that follows may include embodiments in which the first and second features are formed in direct contact, and may also include embodiments in which additional features may be formed between the first and second features, such that the first and second features may not be in direct contact. In addition, the present disclosure may repeat reference numerals and/or letters in the various examples. This repetition is for the purpose of simplicity and

clarity and does not in itself dictate a relationship between the various embodiments and/or configurations discussed.

**[0031]** Further, spatially relative terms, such as “beneath,” “below,” “lower,” “above,” “upper” and the like, may be used herein for ease of description to describe one element or feature’s relationship to another element(s) or feature(s) as illustrated in the figures. The spatially relative terms are intended to encompass different orientations of the device in use or operation in addition to the orientation depicted in the figures. The apparatus may be otherwise oriented (rotated 90 degrees or at other orientations) and the spatially relative descriptors used herein may likewise be interpreted accordingly.

**[0032]** As used herein, the singular forms “a,” “an” and “the” are intended to include the plural forms as well, unless expressly stated otherwise. It will be further understood that the terms “includes,” “comprises,” “including” and/or “comprising,” when used in this specification, specify the presence of stated features, integers, steps, operations, elements, and/or components, but do not preclude the presence or addition of one or more other features, integers, steps, operations, elements, components, and/or groups thereof.

**[0033]** FIG. **1** is a cross-sectional view of a biosensor device in accordance with some embodiments. As shown in FIG. **1**, the biosensor device **100** includes a substrate plate **103**, a metal conductive layer **106**, a second insulating layer **108**, a plurality of working electrodes **110** and a plurality of biological probes **112**. In some embodiments, the substrate plate **103** includes a base substrate **102** and a first insulating layer **104**. The substrate plate **103** may further include, but not limited to, GaN, SiC, SiGe, Ge, or combinations thereof, or other semiconductor materials. The base substrate **102**, for example, may be a silicon substrate. The base substrate **102** may include various doping configurations, depending on design requirements as known in the art. In one embodiment, the base substrate **102** may be a highly-doped and low-resistivity semiconductor substrate. In another embodiment, the substrate plate **103** is a glass substrate without the first insulating layer **104**.

**[0034]** The first insulating layer **104** is disposed over the base substrate **102**. In one embodiment, the first insulating layer **104** may include, but not limited to, an oxide, a nitride, an oxynitride or combinations thereof, such as silicon oxide, silicon nitride, and silicon oxynitride. The first insulating layer **104** is formed of low-k dielectric material such that the biosensor device **100** has excellent insulating properties. In some embodiments, the first insulating layer **104** may have a thickness in a range from about 0.02  $\mu\text{m}$  to about 0.25  $\mu\text{m}$ , for example, about 0.10  $\mu\text{m}$ , about 0.15  $\mu\text{m}$  or about 0.20  $\mu\text{m}$ .

**[0035]** The metal conductive layer **106** is disposed over the substrate plate **103**, and has a sidewall **107** and upper surface **105**. The sidewall **107** adjoins the upper surface **105** and the second insulating layer **108** covers the sidewall **107**. In one embodiment, the metal conductive layer **106** may include, but not limited to, Ti, Ni, Ag, Al, Al/Cu alloy, Al/Si/Cu alloy or combinations thereof. In one embodiment, the metal conductive layer **106** may have a thickness in a range from about 0.02  $\mu\text{m}$  to about 0.7  $\mu\text{m}$ , for example, about 0.1  $\mu\text{m}$ , about 0.2  $\mu\text{m}$ , about 0.3  $\mu\text{m}$ , about 0.4  $\mu\text{m}$ , about 0.5  $\mu\text{m}$  or about 0.6  $\mu\text{m}$ .

**[0036]** Each working electrode **110** is disposed over the upper surface **105** of the metal conductive layer **106**, and has a top surface **109** and a sidewall **111**. Each top surface **109**

is higher than the upper surface **105** of the metal conductive layer **106**, each sidewall **111** adjoins each top surface **109**, and the second insulating layer **108** merely covers a portion of each sidewall **111**. Each working electrode **110** has a first height **H1** protruding beyond the metal conductive layer **106**. In one embodiment, each working electrode **110** may have the first height **H1** in a range from about 0.05  $\mu\text{m}$  to about 0.6  $\mu\text{m}$ , for example, about 0.05  $\mu\text{m}$ , about 0.1  $\mu\text{m}$ , about 0.2  $\mu\text{m}$ , about 0.3  $\mu\text{m}$  or about 0.4  $\mu\text{m}$ . In some embodiments, each working electrode **110** may have a width of about 0.08  $\mu\text{m}$  to about 0.4  $\mu\text{m}$ , for example, about 0.08  $\mu\text{m}$ , about 0.1  $\mu\text{m}$ , about 0.2  $\mu\text{m}$  or about 0.3  $\mu\text{m}$ . In one embodiment, each working electrode **110** may have an aspect ratio in a range from about 0.125 to about 7.5, for example, about 0.2 or about 0.3.

[0037] In some embodiments of the present disclosure, the working electrodes **110** may be in a shape of a cylinder, a triangular prism, a quadrangular prism, a pentagonal prism, a hexagonal prism or an octagonal prism. In some embodiments, the working electrodes **110** may include, but not limited to, Ta, TaN, Cu, Ti, TiN, W, Ti, Ni, Ag, Al, Al/Cu alloy, Al/Si/Cu alloy or combinations thereof. In some embodiments, the material of the working electrodes **110** may be TiN, preferably.

[0038] The biological probes **112** may be modified and connected to the working electrodes **110** using various methods known in the art. In accordance with some embodiments of the present disclosure, the biological probes **112** may include, but not limited to, nucleic acid, cell, antibody, enzyme or combinations thereof. It is noted that the biological probes **112** may detect various biological molecules. For example, when using antibody as the biological probe **112**, a target molecule (i.e., antigen) in a sample may be bound or reacted with the biological probe **112**, thereby detecting the presence of the target molecule using various techniques known in the art.

[0039] The second insulating layer **108** covers the metal conductive layer **106** and surrounds the working electrodes **110**. The upper surface **113** of the second insulating layer **108** is positioned between the top surfaces **109** of the working electrodes **110** and the upper surfaces **105** of the metal conductive layer **106** such that the working electrodes **110** protrudes beyond the upper surface **113** of the second insulating layer **108**. The protruding portion has a second height **H2**, which is the vertical distance from each top surface **109** to the upper surface **113** of the second insulating layer **108**. In some embodiments, the second height **H2** may be in a range from about 0.01  $\mu\text{m}$  to about 0.5  $\mu\text{m}$ , for example, about 0.05  $\mu\text{m}$ , about 0.15  $\mu\text{m}$ , about 0.3  $\mu\text{m}$  or about 0.45  $\mu\text{m}$ . Therefore, when a voltage is applied to the working electrodes **110**, the working electrodes **110** generate an electric field surrounding the protruding working electrodes **110**. The coverage of the electric field is not limited to the top surfaces **109** of the working electrodes **110** and further extends to the sidewalls **111** of the working electrodes **110** so that the electrochemical reaction is greatly enhanced, thereby increasing the strength of signal. At the same applied voltage, the working electrode **110** having three-dimensional structure provides superior sensitivity over the planar working electrode of the prior art.

[0040] In some embodiments, the second insulating layer **108** may include, but not limited to, an oxide, a nitride, an oxynitride or combinations thereof, such as silicon oxide, silicon nitride, and silicon oxynitride. In some embodiments,

the material of the first insulating layer **104** is the same as the material of the second insulating layer **108**. In yet some embodiments, the material of the first insulating layer **104** is different from the material of the second insulating layer **108**.

[0041] In addition, when a voltage is applied to the working electrodes **110**, background signal is also generated, and that interferes the detection result. The generation of the background signal is related to the cross-sectional area of electrode. When the cross-sectional area (CA) is larger, the background signal is higher. In accordance with some embodiments of the present disclosure, when the voltage is applied to the working electrodes **110**, the working electrodes **110** generate the electric field having a coverage range that is larger than the planar working electrodes of the prior art. The coverage range of the electric field is not limited to the top surfaces **109** of the working electrodes **110** and further extends to the sidewalls **111** of the working electrodes **110**. Accordingly, the widths of the working electrodes **110** can be adjusted to be smaller than that of the planar working electrodes in the prior art while sustaining the same effective coverage of the electric field. Therefore, in accordance with embodiments of the present disclosure, the widths of the working electrodes **110** may be smaller than the widths of the planar working electrodes in the prior art, and have smaller cross-sectional area than the planar working electrodes of the prior art, thereby reducing the generation of the background signal.

[0042] As described above, in some embodiments, the first height **H1** of each working electrode **110** is ranged from about 0.05  $\mu\text{m}$  to about 0.6  $\mu\text{m}$ . When the first height **H1** of each working electrode **110** is less than 0.05  $\mu\text{m}$ , the second height **H2** of each protruding portion of working electrode **110** may be less than 0.01  $\mu\text{m}$ . In this situation, when the voltage is applied to the working electrodes **110**, the extension of the coverage of the effective electric field is limited and thus the enhancement of the electrochemical reaction of the biological probe **112** is unobvious. Accordingly, it can be seen that the higher the protruding portion of the working electrodes **110**, the wider the effective electric field coverage is and the better the electrochemical reaction is. It is noted that each working electrode **110** has an aspect ratio ranged from about 0.125 to about 7.5. When each working electrode **110** has the aspect ratio greater than 7.5, the working electrodes are easily formed with defects in structure, thereby reducing the reliability of entire device.

[0043] FIGS. 2-12 are cross-sectional views of a biosensor device during various stages of production in accordance with some embodiments of the present disclosure. As shown in FIG. 2 and FIG. 3, a substrate plate **203** is provided. In some embodiments, the substrate plate **203** includes a base substrate **202** and a first insulating layer **204** formed over the base substrate **202**. The first insulating layer **204** may be formed using atomic layer deposition (ALD), physical vapor deposition (PVD), chemical vapor deposition (CVD), chemical oxidation, heat oxidation and/or other suitable process. In one embodiment, the first insulating layer **204** may include, but not limited to, an oxide, a nitride, an oxynitride or combinations thereof, such as silicon oxide, silicon nitride, and silicon oxynitride. In some embodiments, the first insulating layer **204** is formed with a thickness ranged from about 0.02  $\mu\text{m}$  to about 0.25  $\mu\text{m}$ , for example, about 0.10  $\mu\text{m}$ , about 0.15  $\mu\text{m}$  or about 0.20  $\mu\text{m}$ .

[0044] Referring to FIG. 4, in this step, a metal conductive layer 206 is formed over the first insulating layer 204. In some embodiments, the metal conductive layer 206 may be formed using PVD, CVD, electron beam evaporation, sputtering, electroplating and/or other suitable process. In one embodiment, the metal conductive layer 206 may include, but not limited to, Ti, Ni, Ag, Al, Al/Cu alloy, Al/Si/Cu alloy or combinations thereof. In one embodiment, the metal conductive layer 206 is formed with a thickness ranged from about 0.3  $\mu\text{m}$  to about 0.5  $\mu\text{m}$ , for example, about 0.3  $\mu\text{m}$ , about 0.4  $\mu\text{m}$  or about 0.5  $\mu\text{m}$ .

[0045] Referring to FIG. 5, in this step, an electrically conductive layer 208 is deposited over the metal conductive layer 206. In some embodiments, the electrically conductive layer 208 may be formed using PVD, CVD, electron beam evaporation, sputtering, electroplating and/or other suitable process. In some embodiments, the electrically conductive layer 208 may include, but not limited to, Ta, TaN, Cu, Ti, TiN, W or combinations thereof. In some embodiments, the electrically conductive layer 208 may have a thickness ranged from about 0.05  $\mu\text{m}$  to about 0.6  $\mu\text{m}$ , for example, about 0.05  $\mu\text{m}$ , about 0.1  $\mu\text{m}$ , about 0.2  $\mu\text{m}$ , about 0.3  $\mu\text{m}$  or about 0.4  $\mu\text{m}$ .

[0046] Referring to FIG. 6 and FIG. 7, a patterning process is performed on the electrically conductive layer 208, thereby forming a plurality of working electrodes 212 (only a single working electrode is shown exemplarily). As shown in FIG. 6, a patterned photoresist layer (not shown) is formed on the electrically conductive layer 208 by using a photomask 210 in a photolithographic process, and the photoresist layer may be positive photoresist or negative photoresist. Next, in FIG. 7, an etching process is performed on the electrically conductive layer 208 upon which the patterned photoresist layer is utilized, thereby forming a plurality of working electrode 212 and exposing an upper surface 205 of the metal conductive layer 206. Each working electrode 212 has a first height H1. Each working electrode 212 has a top surface 209 and a sidewall 211. Each top surface 209 is higher than the upper surface 205 of the metal conductive layer 206. Each sidewall 211 adjoins each top surface 209.

[0047] Referring to FIG. 8 and FIG. 9, a patterning process is performed on the metal conductive layer 206. As shown in FIG. 8, a patterned photoresist layer (not shown) is formed on the working electrodes 212 and the metal conductive layer 206 by using a photomask 214 in a photolithographic process, and the photoresist layer may include a positive photoresist or a negative photoresist. Next in FIG. 9, an etching process is performed on the metal conductive layer 206 upon which the patterned photoresist layer is utilized, thereby exposing a portion of an upper surface of the first insulating layer 204 below. Therefore, the metal conductive layer 206 has a sidewall 207 adjoins the upper surface 205 and the exposed portion of the upper surface of the first insulating layer 204.

[0048] Referring to FIG. 10, an insulating material layer 216 is deposited over the first insulating layer 204, the metal conductive layer 206 and the working electrodes 212. In this step, the insulating material layer 216 may conformally cover the first insulating layer 204, the metal conductive layer 206 and the working electrodes 212. In some embodiments, the insulating material layer 216 may have multiple sub-layers and the material of each sub-layer is different from one another. In yet some embodiments, the insulating

material layer 216 may have multiple sub-layers and the material of each sub-layer is the same as one another. In some embodiments, the insulating material layer 216 may be formed using PVD, CVD, plasma enhanced CVD (PECVD) and/or other suitable process.

[0049] In one embodiment, the insulating material layer 216 may include, but not limited to, an oxide, a nitride, an oxynitride or combinations thereof, such as silicon oxide, silicon nitride, and silicon oxynitride. In one embodiment, the insulating material layer 216 is made of tetraethoxysilane (TEOS).

[0050] Referring to FIG. 11, a planarization process is performed on the insulating material layer 216 to form the second insulating layer 218. In this step, the planarization process is performed on the insulating material layer 216 such that the second insulating layer 218 has a substantial flat upper surface. In one embodiment, the planarization process may include chemical mechanical planarization (CMP) and/or other suitable process. In some embodiments, the insulating material layer 216 may have multiple sub-layers and the material of each sub-layer is different from one another such that the planarization process is more efficient.

[0051] Referring to FIG. 12, a portion of the second insulating layer 218 is removed by using a suitable etching process such that an upper surface 213 of the second insulating layer 218 is positioned between the upper surface 205 of the metal conductive layer 206 and the top surfaces 209 of the working electrodes 212. Therefore, the working electrodes 212 protrude beyond the upper surface 213 of the second insulating layer 218, and the protruding portion has a second height H2 which is the vertical distance from the top surface 209 to the upper surface 213 of second insulating layer 218. In some embodiments, the second height H2 may be in a range from about 0.01  $\mu\text{m}$  to about 0.5  $\mu\text{m}$ , for example, about 0.05  $\mu\text{m}$ , about 0.15  $\mu\text{m}$ , about 0.3  $\mu\text{m}$  or about 0.45  $\mu\text{m}$ . In some embodiments, a plurality of biological probes may further be modified onto the working electrodes 212 such that the biological probes are connected to the top surfaces 209 of the working electrodes 212.

[0052] The biosensor device manufactured in accordance with various embodiments of the present disclosure is compatible with various biosensor apparatuses. FIG. 13 is a top view of a biosensor apparatus in accordance with some embodiments of the present disclosure. FIG. 14A is a cross-sectional view taken along line A-A in FIG. 13. As shown in FIG. 13 and FIG. 14A, a biosensor apparatus 300 includes a substrate plate 303, a metal conductive layer 306a, a metal conductive layer 306b, a second insulating layer 308, a plurality of working electrode 310, a counter electrode 312, a plurality of biological probes 314, a signal measurement unit 316 and wires 318.

[0053] Each of the working electrodes 310 and the counter electrode 312 may be electrically connected to the signal measurement unit 316 via one or more wires 318. Therefore, as shown in FIG. 14A, when a voltage is applied to the working electrodes 310, each of the working electrodes 310 generates an electric field E surrounding the corresponding working electrode 310. In the meantime, a test sample is provided and in contact with the biological probes 314. If a target molecule in the test sample is bound to the biological probe 314, the working electrodes 310 generate a signal such

that the generated signal is transmitted to the signal measurement unit 316, thereby detecting the presence of the target molecules.

[0054] Referring to FIG. 14A, the metal conductive layer 306b is disposed over the first insulating layer 304. The counter electrode 312 is disposed over the metal conductive layer 306b. In some embodiments, the material of the metal conductive layer 306b is the same as the material of the metal conductive layer 306a. In some embodiments, the material of the metal conductive layer 306b is different from the material of the metal conductive layer 306a. The substrate plate 303, the metal conductive layer 306a, the second insulating layer 308, the working electrodes 310 and the biological probes 314 may be respectively the same as the substrate plate 103, metal conductive layer 106, the second insulating layer 108, the working electrodes 110 and the biological probes 112 described hereinbefore, and the descriptions thereof are omitted to avoid repetition.

[0055] Referring to FIG. 14B, FIG. 14B is a partial magnified view exemplarily showing the cross-sectional view of FIG. 14A. When the voltage is applied to the working electrodes 310, each of the working electrodes 310 generates a corresponding electric field. Exemplarily, the dotted line E75 represents an iso-electric-field-intensity curve where the electric field intensity is 75% of the maximal electric field intensity in space. In other words, the electric field intensity of each point “inside” the dotted line E75 is greater than 75% of the maximal electric field intensity. Exemplarily, the dotted line E50 represents an iso-electric-field-intensity curve where the electric field intensity is 50% of the maximal electric field intensity in space. In other words, the electric field intensity of each point “inside” the dotted line E50 is greater than 55% of the maximal electric field intensity. In some embodiments, when a voltage is applied to the working electrodes 310, 75% of the maximum electric field intensity (i.e., the maximal magnitude of the electric field multiplied by 0.75) occurs at about 27-40% of the second height H2 from the top surfaces 311 downwardly (i.e., the intersection of the working electrodes 310 and the dotted line E75); in other words, 75% of the maximal electric field intensity occurs at about 60-73% of the second height H2 from the upper surface 313 upwardly. In some embodiments, when a voltage is applied to the working electrodes 310, 50% of the maximal electric field intensity (i.e., the maximal magnitude of the electric field multiplied by 0.5) occurs at about 80-93% of the second height H2 from the top surfaces 311 downwardly (i.e., the intersection of the working electrodes 310 and the dotted line E50); in other words, 50% of the maximal electric field intensity occurs at about 7-20% of the second height H2 from the upper surface 313 upwardly.

#### [0056] Simulation of Electric Field

[0057] In this experiment, COMSOL Multiphysics 4.4 simulation software was used to simulate the electric field intensity. As shown in Table 1 below, the used working electrode is in a shape of a cylinder and the cylindrical working electrode in Embodiment 1 has a radius of 0.05  $\mu\text{m}$ , the maximal electric field intensity is  $2.86 \times 10^6$  (v/m); the cylindrical working electrode in Embodiment 2 has a radius of 0.1  $\mu\text{m}$ , and the maximal electric field intensity is  $1.85 \times 10^6$  (v/m); the cylindrical working electrode in Embodiment 3 has a radius of 0.2  $\mu\text{m}$ , and the maximal electric field intensity is  $7.75 \times 10^5$  (v/m).

TABLE 1

Working electrode	Electrode radius ( $\mu\text{m}$ )	Maximum magnitude of electric field (v/m)
Embodiment 1	0.05	$2.86 \times 10^6$
Embodiment 2	0.1	$1.85 \times 10^6$
Embodiment 3	0.2	$7.75 \times 10^5$

[0058] As described above, the maximum magnitude of the electric field increases as electrode radius decreases. Next, as shown in Table 2 below, the coverage and the intensity of the electric field generated by the protruding part of the working electrode were simulated. When the working electrode is a conventional planar working electrode, it has a height of 0  $\mu\text{m}$  (i.e., without any sidewall), and all the 100%, 75% and 50% of maximum magnitudes of the electric field occur at the level of the surface of working electrode. Then also referring to Table 2 below, calculated from the top surface of working electrode toward the insulating layer below, when the working electrode has a second height H2 of 0.15  $\mu\text{m}$ , 75% of the maximal electric field intensity occurs at 0.05  $\mu\text{m}$  from the top surface of working electrode; and 50% of the maximal electric field intensity occurs at 0.13  $\mu\text{m}$  from the top surface of working electrode.

TABLE 2

Height of working electrode ( $\mu\text{m}$ )	Height of 100% maximum magnitude of electric field	Height of 75% maximum magnitude of electric field	Height of 50% maximum magnitude of electric field
0	0	0	0
0.15	0	0.05	0.13

[0059] To sum up, the biosensor device has the working electrode protruding beyond the insulating layer in accordance with various embodiments of the present disclosure. In electrochemical reactions, the greater the electric field, the faster the motion of the charged object is. As a result, the current density is higher.

[0060] A known equation of electrochemical reaction (Electrochemical Methods: Fundamentals and Applications. Allen J. Bard, Larry R. Faulkner, Wiley. ISBN: 0471043729) is shown as follows:

$$J_A(x, t) = -\left(\frac{F}{RT}\right) z_A D_A C_A(x, t) \mathcal{E}(x)$$

[0061]  $J_A(x, t)$  represents a current density of a charged object A located at a site “x” in a time “t”.  $Z_A$  represents a valence number of the charged object A.  $D_A$  represents a diffusion coefficient of the charged object A.  $C_A(x, t)$  represents a concentration of the charged object A located at the site x in the time t.  $\mathcal{E}(x)$  represents an electric field of the charged object A located at the site x. The protruding working electrode results in the wider coverage of the electric field that affects the motion of the charge object, which is conducive to enhancing the efficiency of electrochemical reaction, thereby increasing the strength of signal. Therefore, the working electrode manufactured in accordance with the embodiment of the present disclosure may have a smaller width than the width of planar electrode known in the art, thereby enhancing the sensitivity.

**[0062]** Although the present disclosure has been described in considerable detail with reference to certain embodiments thereof, other embodiments are possible. Therefore, the spirit and scope of the appended claims should not be limited to the description of the embodiments contained herein.

**[0063]** It will be apparent to those skilled in the art that various modifications and variations can be made to the structure of the present invention without departing from the scope or spirit of the invention. In view of the foregoing, it is intended that the present invention cover modifications and variations of this invention provided they fall within the scope of the following claims.

What is claimed is:

1. A method for manufacturing a biosensor device, comprising the step of:

providing a substrate plate;  
forming a metal conductive layer over the substrate plate, and the metal conductive layer having an upper surface;  
forming a plurality of working electrodes over the upper surface of the metal conductive layer, wherein each of the working electrodes has a top surface that is higher than the upper surface of the metal conductive layer; and

forming an insulating layer covering the metal conductive layer and surrounding the working electrodes, wherein an upper surface of the insulating layer is between the top surfaces and the upper surface of the metal conductive layer so that the working electrodes protrudes beyond the upper surface of the insulating layer.

2. The method of claim 1, wherein the step of forming the working electrodes comprises:

depositing an electrically conductive layer over the upper surface of the metal conductive layer; and  
patterning the electrically conductive layer to form the working electrodes.

3. The method of claim 2, wherein each of the working electrodes has a first height ranged from about 0.05  $\mu\text{m}$  to about 0.6  $\mu\text{m}$ .

4. The method of claim 2, wherein each of the working electrodes has an aspect ratio ranged from about 0.125 to about 7.5.

5. The method of claim 1, wherein the step of forming the insulating layer comprises:

depositing an insulating material layer over the metal conductive layer and the working electrodes;  
performing a planarization process on the insulating material layer to form a planarized insulating material layer; and  
etching the planarized insulating material layer to form the insulating layer.

6. The method of claim 1, wherein each of the working electrodes further comprises a sidewall adjoining the top surface, and the insulating layer covers a portion of each of the sidewalls.

7. The method of claim 1, wherein each of the working electrodes protrudes from the upper surface a second height, and the second height is about 0.01  $\mu\text{m}$  to about 0.5  $\mu\text{m}$ .

8. The method of claim 1, wherein the working electrodes are in a shape of a cylinder, a triangular prism, a quadrangular prism, a pentagonal prism, a hexagonal prism or an octagonal prism.

9. The method of claim 1, further comprising connecting a plurality of biological probes to the working electrodes,

wherein the biological probes are nucleic acid, cell, antibody, enzyme, polypeptide or combinations thereof.

10. A biosensor device, comprising:

a substrate plate;  
a metal conductive layer disposed over the substrate plate and the metal conductive layer having an upper surface;  
a plurality of working electrodes disposed over the upper surface of the metal conductive layer, wherein each of the working electrodes has a top surface that is higher than the upper surface of the metal conductive layer; and

an insulating layer covering the metal conductive layer and surrounding the working electrodes, wherein an upper surface of the insulating layer is between the top surfaces and the upper surface of the metal conductive layer, so that the working electrodes protrudes beyond the upper surface of the insulating layer.

11. The biosensor device of claim 10, wherein each of the working electrodes has a first height ranged from about 0.05  $\mu\text{m}$  to about 0.6  $\mu\text{m}$ .

12. The biosensor device of claim 10, wherein each of the working electrodes has an aspect ratio ranged from about 0.125 to about 7.5.

13. The biosensor device of claim 10, wherein the metal conductive layer further comprises a sidewall adjoining the upper surface of the metal conductive layer, and the insulating layer covers the sidewall of the metal conductive layer.

14. The biosensor device of claim 10, wherein each of the working electrodes further comprises a sidewall adjoining the top surface, and the insulating layer covers a portion of each of the sidewalls.

15. The biosensor device of claim 10, wherein each of the working electrodes protrudes from the upper surface a second height, and the second height is about 0.01  $\mu\text{m}$  to about 0.5  $\mu\text{m}$ .

16. The biosensor device of claim 10, wherein the working electrodes are in a shape of a cylinder, a triangular prism, a quadrangular prism, a pentagonal prism, a hexagonal prism or an octagonal prism.

17. The biosensor device of claim 10, further comprising a plurality of biological probes connected to the working electrodes, wherein the biological probes are nucleic acid, cell, antibody, enzyme, polypeptide or combinations thereof.

18. A method for detecting biological molecules, comprising:

providing a sample comprising a target molecule;  
providing the biosensor device of claim 10;  
connecting a plurality of biological probes to the working electrodes;  
applying a voltage to the working electrodes such that the working electrodes generate an electric field surrounding the working electrodes; and  
contacting the sample with the biological probes such that the target molecule in the sample is bound to the biological probes, thereby generating a signal from the working electrodes.

19. The method of claim 18, wherein the step of applying the voltage to the working electrodes comprises: applying a voltage to the working electrodes such that 75% of the maximal electric field intensity occurs at about 27% to about 40% of the second height from the top surfaces toward the upper surface of the insulating layer.



20. The method of claim 18, wherein the step of applying the voltage to the working electrodes comprises: applying a voltage to the working electrodes such that 50% of the maximal electric field intensity occurs at about 80% to about 93% of the second height from the top surfaces toward the upper surface of the insulating layer.

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