

**CLAIMS**

1. A method of manufacturing a biosensor for the detection of an analyte using surface-enhanced Raman spectroscopy (SERS), the method comprising
  - a) providing a SERS-active substrate, wherein providing the SERS-active substrate comprises (i) providing a semiconductor support, forming at least one nanostructure on a surface of the support by inductively-coupled plasma reactive ion etch, wherein the inductively-coupled plasma reactive ion etch comprises subjecting the semiconductor support to an oxygen plasma treatment carried out at a pressure in the range of 10 mTorr to 15 mTorr for a time period in the range of 5 minutes to 10 minutes, and etching the resultant semiconductor support using a combination of SF<sub>6</sub>:O<sub>2</sub> gas having a ratio in the range of 1.1 to 1.21 at an etch rate in the range of 2.5 nm/s to 2.8 nm/s, and forming a layer of a SERS-active material on the at least one nanostructure, or (ii) providing a paper support, and attaching a plurality of silver and/or gold nanoparticles to a surface of the paper support, wherein the SERS-active substrate is operable to directly attach at least one analyte binding molecule thereon without any linker agent, and
  - b) arranging a microfluidic circuit device over the SERS-active substrate and to be in fluid communication with the SERS-active substrate, wherein the microfluidic circuit device comprises a reaction chamber and wherein an interior portion of the reaction chamber is defined by the microfluidic circuit device and the SERS-active substrate, to obtain the biosensor.
2. The method according to claim 1, wherein forming a layer of a SERS-active material on the at least one nanostructure is carried out by at least one of e-beam deposition or sputtering.
3. The method according to claim 1, wherein attaching the plurality of silver and/or gold nanoparticles to a surface of the paper support is carried out by at least one of e-beam vapor deposition, sputtering, or syringe filtration.

4. The method according to any one of claims 1 to 3, further comprising subjecting the SERS-active substrate and the microfluidic circuit device to an oxygen plasma treatment prior to arranging the microfluidic circuit device to be in fluid communication with the SERS-active substrate.  
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5. The method according to any one of claims 1 to 4, further comprising heat treating the SERS-active substrate and the microfluidic circuit device at a temperature in the range of 80 °C to 150 °C for a time period in the range of 30 minutes to 90 minutes after arranging the microfluidic circuit device to be in fluid communication with the SERS-active substrate.  
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6. A method for detecting at least one analyte using surface enhanced Raman spectroscopy (SERS), the method comprising  
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  - a) providing a biosensor comprising a SERS-active substrate and a microfluidic circuit device arranged over the SERS-active substrate and in fluid communication with the SERS-active substrate, wherein the microfluidic circuit device comprises a reaction chamber and wherein an interior portion of the reaction chamber is defined by the microfluidic circuit device and the SERS-active substrate;  
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  - b) introducing at least one analyte binding molecule to the microfluidic circuit device to attach the at least one analyte binding molecule directly on the SERS-active substrate without any linker agent;
  - c) introducing one or more analytes to the microfluidic circuit device to contact the at least one analyte binding molecule with the one or more analytes, and introducing a further SERS-active substrate to the microfluidic circuit device thereafter, wherein the further SERS-active substrate comprises a further analyte binding molecule, a Raman-active marker compound and a metallic nanoparticle, wherein the further analyte binding molecule and the Raman-active marker compound are independently covalently attached to the metallic nanoparticle;  
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- d) detecting a surface enhanced Raman signal from the SERS-active substrate and the further SERS-active substrate; and
- e) checking the obtained signal against a reference to correlate the obtained signal with the amount of the one or more analytes.

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- 7. The method according to claim 6, wherein the one or more analytes are selected from the group consisting of proteins, peptides, nucleic acids, carbohydrates, lipids, cells, viruses, small molecules, and combinations thereof.

- 10 8. The method according to claim 6 or 7, wherein the at least one analyte binding molecule specifically binds the one or more analytes.

- 9. The method according to any one of claims 6 to 8, wherein the at least one analyte binding molecule is selected from the group consisting of an antibody, antibody fragment or antibody like molecules.

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- 10. The method according to any one of claims 6 to 9, wherein the Raman-active marker compound is selected from the group consisting of 4-aminothiophenol (4-ATP), 2-naphthalenethiol (2-NT), 4-methylbenzenethiol, rhodamine B isothiocyanate, methylene blue, coumarin, melamine, and combinations thereof.

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- 11. The method according to any one of claims 6 to 10, wherein the metallic nanoparticle is coated with or consists of a metal selected from the group consisting of a noble metal, copper, aluminum, and alloys thereof.

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- 12. The method according to any one of claims 6 to 11, wherein the further analyte binding molecule is the same as or different from the analyte binding molecule.

- 13. The method according to any one of claims 6 to 12, wherein the microfluidic circuit device comprises a concentration gradient generator and/or a single mixing structure having at least two inlets and at least one outlet.

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14. The method according to claim 13, wherein the reference is generated using the microfluidic circuit device and expresses intensity of SERS signal as a function of analyte concentration.
- 5 15. The method according to any one of claims 6 to 14, wherein the method is a multiplex method for detecting more than one analyte, wherein more than one type of analyte binding molecules are introduced to the microfluidic circuit device.