

CLAIMS

1. ~~A biosensor for the detection of an analyte using surface enhanced Raman spectroscopy (SERS), the biosensor comprising a SERS active substrate and a microfluidic circuit device arranged to be in fluid communication with the SERS active substrate.~~
2. ~~The biosensor according to claim 1, wherein the SERS active substrate is a support comprising at least one SERS active nanostructure disposed on a surface of the support.~~
3. ~~The biosensor according to claim 2, wherein the support comprising at least one SERS active nanostructure disposed on a surface of the support is one of a dielectric support, a semiconductor support, or a paper support, the support comprising at least one nanostructure coated with a layer of a SERS active material.~~
4. ~~The biosensor according to claim 2 or 3, wherein the support comprising at least one SERS active nanostructure disposed on a surface of the support is a dielectric support comprising a plurality of nano islands coated with a layer of a SERS active material disposed on a surface of the support.~~
5. ~~The biosensor according to claim 4, wherein the layer of a SERS active material has a thickness in the range of about 5 nm to about 10 nm.~~
6. ~~The biosensor according to claim 2 or 3, wherein the support comprising at least one SERS active nanostructure disposed on a surface of the support is a semiconductor support comprising a plurality of nanopillars coated with a layer of a SERS active material on a surface of the support.~~
7. ~~The biosensor according to claim 6, wherein the nanopillars has a height in the range of about 250 nm to about 300 nm.~~

8. ~~The biosensor according to claim 6 or 7, wherein spacing between each of the nanopillars is less than 100 nm.~~
9. ~~The biosensor according to any one of claims 6 to 8, wherein the layer of a SERS-active material has a thickness in the range of about 150 nm to about 250 nm.~~
10. ~~The biosensor according to claim 2 or 3, wherein the support comprising at least one SERS-active nanostructure disposed on a surface of the support is a paper support comprising a plurality of nanoparticles of a SERS-active material attached to a surface of the support.~~
11. ~~The biosensor according to any one of claims 1 to 10, wherein the microfluidic circuit device comprises a reaction chamber and the SERS-active substrate forms at least a portion of the reaction chamber of the microfluidic circuit device.~~
12. ~~The biosensor according to any one of claims 2 to 10, wherein the microfluidic circuit device is disposed on the SERS-active substrate so that at least a portion of the microfluidic circuit device is in fluid communication with the surface of the support comprising at least one SERS-active nanostructure.~~
13. ~~The biosensor according to any one of claims 2 to 12, wherein the microfluidic circuit device is removably attached to the SERS-active substrate.~~
14. ~~The biosensor according to claim 1, wherein the SERS-active substrate comprises nanoparticles of a SERS-active material.~~
15. ~~The biosensor according to claim 14, wherein the microfluidic circuit device comprises a reaction chamber and the SERS-active substrate is comprised in the reaction chamber.~~

16. ~~The biosensor according to any one of claims 1 to 15, wherein the microfluidic circuit device comprises a concentration gradient generator having at least two inlets and at least one outlet.~~

17. ~~The biosensor according to claim 16, wherein a microchannel extends from each outlet of the concentration gradient generator.~~

18. ~~The biosensor according to claim 17, wherein the microfluidic circuit device further comprises at least one cross flow line intersecting with and in fluid communication with each microchannel.~~

19. ~~The biosensor according to any one of claims 1 to 18, wherein the SERS-active substrate further comprises at least one analyte binding molecule attached thereto.~~

20. ~~The biosensor according to claim 19, wherein the at least one analyte binding molecule specifically binds the analyte.~~

21.1. ~~A method of manufacturing a biosensor for the detection of an analyte using surface-enhanced Raman spectroscopy (SERS) according to any one of claims 1 to 20, 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 about 10 mTorr to about 15 mTorr for a time period in the range of about 5 minutes to about 10 minutes, and etching the resultant semiconductor support using a combination of SF₆:O₂ gas having a ratio in the range of about 1.1 to about 1.21 at an etch rate in the range of about 2.5 nm/s to about 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, and

- b) arranging a microfluidic circuit device to be in fluid communication with the SERS-active substrate to obtain the biosensor.

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~~22. The method according to claim 21, wherein providing the SERS active substrate comprises providing a support and forming at least one SERS active nanostructure on a surface of the support.~~

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~~23. The method according to claim 22, wherein providing a support and forming at least one SERS active nanostructure on a surface of the support comprises~~

- ~~a) providing a dielectric support;~~
- ~~b) forming at least one nanostructure on a surface of the dielectric support; and~~
- ~~c) forming a layer of a SERS active material on the at least one nanostructure.~~

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~~24. The method according to claim 23, wherein the electron beam evaporation is carried out at a pressure in the range of about 10^{-6} to about 10^{-7} mbar.~~

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~~25. The method according to claim 23 or 24, wherein the layer of a SERS active material is deposited at an evaporation rate in the range of about 0.01 nm/s to about 0.1 nm/s.~~

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~~26. The method according to claim 22, wherein providing a support and forming at least one SERS active nanostructure on a surface of the support comprises~~

- ~~a) providing a semiconductor support;~~
- ~~b) forming at least one nanostructure on a surface of the support by inductively-coupled plasma reactive ion etch; and~~
- ~~c) forming a layer of a SERS active material on the at least one nanostructure.~~

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~~27. The method according to claim 26, wherein the inductively-coupled plasma reactive ion etch comprises~~

a) ~~subjecting the semiconductor support to an oxygen plasma treatment carried out at a pressure in the range of about 10 mTorr to about 15 mTorr for a time period in the range of about 5 minutes to about 10 minutes; and~~

b) ~~etching the resultant semiconductor support using a combination of SF₆:O₂ gas having a ratio in the range of about 1.1 to about 1.21 at an etch rate in the range of about 2.5 nm/s to about 2.8 nm/s.~~

28.2. The method according to claim ~~26~~ or ~~27~~ 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.

~~29.~~ The method according to claim 22, wherein providing a support and forming at least one SERS active nanostructure on a surface of the support comprises

a) ~~providing a paper support, such as a chromatography paper support, and~~

b) ~~attaching a plurality of silver and/or gold nanoparticles to a surface of the paper support.~~

30.3. The method according to claim ~~29~~ 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.

31.4. The method according to any one of claims ~~21~~ 1 to ~~30~~ 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.

32.5. The method according to any one of claims ~~21~~ 1 to ~~31~~ 4, further comprising heat treating the SERS-active substrate and the microfluidic circuit device at a temperature in the range of about 80 °C to about 150 °C for a time period in the range of about 30 minutes to about 90 minutes after arranging the microfluidic circuit device to be in fluid communication with the SERS-active substrate.

~~33.6.~~ A method for detecting at least one analyte using surface enhanced Raman spectroscopy (SERS), the method comprising

- a) providing a biosensor ~~according to any one of claims 1 to 20~~ comprising a SERS-active substrate and a microfluidic circuit device arranged to be in fluid communication with the SERS-active substrate;
- b) introducing at least one analyte binding molecule to the microfluidic circuit device to attach the at least one analyte binding molecule on the SERS-active substrate;
- 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;
- 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.

~~34.7.~~ The method according to claim ~~33.6~~, 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.

~~35.8.~~ The method according to claim ~~33.6~~ or ~~34.7~~, wherein the at least one analyte binding molecule specifically binds the one or more analytes.

~~36.9.~~ The method according to any one of claims ~~33 to 35.6~~ 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.

~~37. The method according to any one of claims 33 to 36, further comprising introducing a further SERS active substrate to the microfluidic circuit device after step c).~~

~~38. The method according to claim 37, 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.~~

~~39.10. The method according to any one of claims 386 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.~~

~~40.11. The method according to any one of claims 38 or 39 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.~~

~~41.12. The method according to any one of claims ~~38 to 40~~ 6 to 11, wherein the further analyte binding molecule is the same as or different from the analyte binding molecule.~~

~~42.13. The method according to any one of claims ~~33 to 41~~ 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.~~

~~43.14. The method according to claim ~~42~~ 13, wherein the reference is generated using the microfluidic circuit device and expresses intensity of SERS signal as a function of analyte concentration.~~

~~44.15.~~ The method according to any one of claims ~~33 to 43~~ 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.

~~45.~~ A method for detecting haptoglobin (Hp) using surface enhanced Raman spectroscopy (SERS), the method comprising

- ~~a) providing a biosensor according to any one of claims 1 to 20;~~
- ~~b) introducing a first mixture comprising haemoglobin and a sample suspected to comprise haptoglobin to the microfluidic circuit device;~~
- ~~c) introducing a peroxidase substrate and a peroxide source to the microfluidic circuit device and contacting with the first mixture to form a second mixture;~~
- ~~d) introducing a quenching agent to the microfluidic circuit device and contacting with the second mixture to form a third mixture;~~
- ~~e) contacting the third mixture with the SERS active substrate; and~~
- ~~f) detecting a surface enhanced Raman signal from the SERS active substrate.~~

~~46.~~ The method according to claim 45, wherein the first mixture, the peroxidase substrate, the peroxide source, and the quenching agent are comprised in a cartridge with each being spaced apart by an air gap.

~~47.~~ The method according to claim 45 or 46, wherein the first mixture, the peroxidase substrate, the peroxide source, and the quenching agent are comprised in a cartridge and arranged in order based on their addition sequence to the microfluidic circuit device.

~~48.16.~~ A method for detecting haptoglobin (Hp) using surface enhanced Raman spectroscopy (SERS), the method comprising

- a) providing a microfluidic circuit device;
- b) introducing a first mixture comprising haemoglobin and a sample suspected to comprise haptoglobin to the microfluidic circuit device;
- c) introducing a peroxidase substrate and a peroxide source to the microfluidic circuit device and contacting with the first mixture to form a second mixture;

- d) introducing a quenching agent to the microfluidic circuit device and contacting with the second mixture to form a third mixture;
- e) introducing a SERS-active substrate to the microfluidic circuit device and contacting the third mixture with the SERS-active substrate; and
- f) detecting a surface enhanced Raman signal from the SERS-active substrate.

49.17. The method according to claim 4816, wherein the first mixture, the peroxidase substrate, the peroxide source, the quenching agent, and the SERS-active substrate are comprised in a cartridge with each being spaced apart by an air gap.

50.18. The method according to claim 4816 or 4917, wherein the first mixture, the peroxidase substrate, the peroxide source, the quenching agent, and the SERS-active substrate are comprised in a cartridge and arranged in order based on their addition sequence to the microfluidic circuit device.