

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, wherein the microfluidic circuit device comprises a concentration gradient generator having at least two inlets and at least one outlet, wherein a microchannel extends from each of the at least one outlet of the concentration gradient generator, and wherein the microfluidic circuit device further comprises at least one cross-flow line intersecting with and in fluid communication with each of the at least one microchannel, wherein each of the at least one cross-flow line has an inlet for introducing an analyte binding molecule into the microfluidic circuit device.
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 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.
4. The biosensor according to claim 2, 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.
5. The biosensor according to claim 2, 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.

6. The biosensor according to any one of claims 1 to 5, 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.
- 5 7. The biosensor according to any one of claims 2 to 5, 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.
- 10 8. The biosensor according to any one of claims 2 to 7, wherein the microfluidic circuit device is removably attached to the SERS-active substrate.
9. The biosensor according to claim 1, wherein the SERS-active substrate comprises nanoparticles of a SERS-active material.
- 15 10. The biosensor according to claim 9, wherein the microfluidic circuit device comprises a reaction chamber and the SERS-active substrate is comprised in the reaction chamber.
- 20 11. The biosensor according to any one of claims 1 to 10, wherein the SERS-active substrate further comprises at least one analyte binding molecule attached thereto.
12. 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 11, the method comprising
25 a) providing a SERS-active substrate, and
b) arranging a microfluidic circuit device to be in fluid communication with the SERS-active substrate, wherein the microfluidic circuit device comprises a concentration gradient generator having at least two inlets and at least one
30 outlet, wherein a microchannel extends from each of the at least one outlet of the concentration gradient generator, and wherein the microfluidic circuit device further comprises at least one cross-flow line intersecting with and in

fluid communication with each of the at least one microchannel, wherein each of the at least one cross-flow line has an inlet for introducing an analyte binding molecule into the microfluidic circuit device, to obtain the biosensor.

- 5 13. 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 11;
 - 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;
 - 10 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;
 - d) detecting a surface enhanced Raman signal from the SERS-active substrate; and
 - 15 e) checking the obtained signal against a reference to correlate the obtained signal with the amount of the one or more analytes.
14. The method according to claim 13, 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.
- 20 15. The method according to claim 13 or 14, further comprising introducing a further SERS-active substrate to the microfluidic circuit device after step c).
- 25 16. The method according to claim 15, 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.
- 30 17. The method according to claim 16, wherein the further analyte binding molecule is the same as or different from the analyte binding molecule.

18. 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.
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19. The method according to any one of claims 13 to 18, 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.
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20. A method for detecting haptoglobin (Hp) using surface enhanced Raman spectroscopy (SERS), the method comprising
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- a) providing a biosensor according to any one of claims 1 to 11;
 - 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
 - 20 f) detecting a surface enhanced Raman signal from the SERS-active substrate.