

5 CLAIMS

What is claimed is:

1. A surface-enhanced Raman scattering (SERS)-active photonic crystal fiber (PCF) probe
10 for integrated sample collection and SERS sensing of one or more analytes comprised in the
sample, the probe comprising a biopsy needle and a SERS-active PCF integrated into or in
communication with the biopsy needle,

wherein the PCF has a longitudinal axis and comprises a solid core extending along the length
15 of the longitudinal axis and a cladding region surrounding the solid core, wherein at least the
cladding region comprises one or more air holes extending along the longitudinal axis of the
PCF,

wherein the inner surface of the one or more air holes is functionalized by metallic nanoparticles
20 immobilized thereon that enhance Raman scattering, and the one or more air holes are adapted
for SERS sensing of the one or more analytes.
2. The SERS-active PCF probe of claim 1, wherein the core and the one or more air holes
in the cladding region are arranged in a pattern as illustrated in any one of Figures 2-6 of the
25 drawings.
3. The SERS-active PCF probe of claim 1 or 2, wherein the inner surface of the one or
more air holes comprises, consists essentially of or is made of silica glass.
- 30 4. The SERS-active PCF probe of any one of claims 1-3, wherein the metallic
nanoparticles comprise or consist of a noble metal or copper.
5. The SERS-active PCF probe of claim 4, wherein the noble metal is gold or silver.
- 35 6. The SERS-active PCF probe of any one of claims 1-5, wherein the metallic
nanoparticles have a mean diameter in the range from 5 nm to 250 nm, preferably in the range
from 40 nm to 100 nm.
7. The SERS-active PCF probe of any one of claims 1-6, wherein the metallic
40 nanoparticles comprise or consist of gold nanoparticles preferably having a mean diameter of
60 nm.

5 8. The SERS-active PCF probe of any one of claims 1-7, wherein the metallic nanoparticles are immobilized onto the inner surface via a coupling agent, preferably a silane coupling agent, more preferably a molecule comprising a di- or trialkoxysilane group and a functional group capable of binding to the metallic nanoparticles, such as an amino or mercapto group, the coupling agent being preferably selected from (3-mercaptopropyl)trimethoxysilane
10 (MPTMS) and 3-Aminopropyltriethoxysilane (APTES).

9. The SERS-active PCF probe of any one of claims 1-8, wherein the metallic nanoparticles are further functionalized by a first analyte-binding molecule immobilized thereon.

15 10. The SERS-active PCF probe of claim 9, wherein the first analyte-binding molecule is an antibody, antibody fragment, or antibody-like molecule.

11. The SERS-active PCF probe of claim 10, wherein the first analyte-binding molecule is a monoclonal or polyclonal antibody.

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12. A method for detecting one or more analytes using surface-enhanced Raman scattering (SERS), the method comprising the steps of:

- (i) loading a sample suspected of containing the one or more analytes into a SERS-active PCF probe of any one of claims 1-11 via the biopsy needle comprised therein;
- 25 (ii) immobilizing the one or more analytes to the metallic nanoparticles coated on the one or more air holes of the PCF probe;
- (iii) loading a second analyte-binding molecule comprising a Raman reporter moiety into the SERS-active PCF probe; and
- (iv) detecting the one or more analytes by measuring a SERS signal from the SERS-active
30 PCF probe.

13. The method of claim 12, wherein the method comprises an additional blocking step immediately prior to step (ii).

35 14. The method of claim 11 or 12, wherein the method comprises an additional washing step immediately prior to step (iii) and/or (iv) to remove the unbound analytes and/or analyte-binding molecules, respectively.

40 15. The method of any one of claims 12-14, wherein the sample is a body fluid or biological fluid.

- 5 16. The method of any one of claims 12-15, wherein the sample is selected from the group consisting of whole blood, serum, plasma, cerebrospinal fluid, sputum, bronchial washing, bronchial aspirates, urine, semen, lymph fluids, wound exudates, tears, saliva, milk, and cell culture supernatants.
- 10 17. The method of any one of claims 12-16, wherein the one or more analytes are selected from the group consisting of proteins, peptides, nucleic acids, carbohydrates, lipids, small molecules, haptens, cells, and viruses.
- 15 18. The method of any one of claims 12-17, wherein the second analyte-binding molecule is an antibody, antibody fragment, or antibody-like molecule.
19. The method of claim 18, wherein the second analyte-binding molecule is a monoclonal or polyclonal antibody.
- 20 20. The method of any one of claims 12-19, wherein the Raman reporter moiety comprised in the second analyte-binding molecule is selected from the group consisting of malachite green isothiocyanate (MGITC), tetramethylrhodamine-5-isothiocyanate (TRITC), X-rhodamine-5-(and-6)-isothiocyanate (XRITC), and 3, 3'-diethylthiadicarbocyanine iodide (DTDC).
- 25 21. The method of any one of claims 12-20, wherein the method is a multiplex method for detecting more than one analyte, wherein in the contacting step more than one analyte-binding molecule is used.
22. The method of any one of claims 12-21, wherein the detection is *in vivo* or *in vitro*.
- 30 23. Use of the SERS-active PCF probe of any one of claims 1-11 for the detection of one or more analytes.
24. The use of claim 23, wherein the detection is *in vivo* or *in vitro*.