

CLAIMS

1. Method for analysing an analyte (3) using surface enhanced Raman spectroscopy
5 (SERS), comprising the following steps:
 - a) providing an essentially flat or topologically structured metal surface (1) of a SERS-active metal;
 - b) depositing the analyte (3) or an open pore matrix material (5) on said flat or topologically structured metal surface (1);
 - 10 c) depositing a multitude of nano-droplets (2) of a SERS-active metal on top of the analyte (3) or the open pore matrix material (5), respectively;
 - d) spectroscopically analysing, by scanning laser irradiation and using SERS, the analyte sandwiched between the flat or topologically structured metal surface (1) and the multitude of nano-droplets (2);
 - 15 wherein the number average diameter of the nano-droplets (2) is in the range of 5-70 nm, and wherein the number average distance between adjacent nano-droplets (2) is smaller than their number average diameter, with the proviso that if an open pore matrix material (5) is deposited on the flat or topologically structured metal surface (1) in step b) the analyte (3) is introduced
20 into the pores before step d) characterized in that step c) is carried out by physical vapour deposition (PVD) or by sputtering SERS-active metal.
2. Method according to claim 1, wherein the SERS-active metal of the flat or
25 topologically structured metal surface (1), of the nano-droplets (2), or of both, is selected from the group consisting of a noble metal, including silver, gold, platinum, or copper, sodium, potassium or aluminium, or a mixture thereof or an alloy containing such a metal, wherein preferably the method is carried out in one analytical device, in which the actual generation of the flat or topologically
30 structured metal surface (1) of a SERS-active metal according to step a), the analyte deposition according to step b), the deposition of the nano-droplets according to step c) as well as the spectroscopic analysis according to d) take place within one same analytical device.

3. Method according to any of the preceding claims, wherein step b) includes a step of depositing the analyte (3) as a solution, suspension or emulsion in a carrier liquid, and a step of removing the carrier liquid, wherein the removal is preferably by evaporation, including evaporation by elevated temperature and/or or by reduced pressure, and/or by filtration, wherein in the latter case the flat or topologically structured metal surface (1) can be provided with holes (8) allowing for carrier liquid and non-desired particles to penetrate while keeping analyte particles on the flat or topologically structured metal surface (2).
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4. Method according to any of the preceding claims, wherein step c) is carried out by physical vapour deposition (PVD) or by sputtering SERS-active metal, followed by annealing or accompanied by concomitant annealing, wherein the deposition process is carried out until the layer thickness is not more than 50 nm, preferably not more than 30 nm, nor more than 15 nm or 9 nm
10 and/or wherein in step c) nano-droplets (2) of essentially half spherical shape are generated.
5. Method according to any of the preceding claims, wherein the number average diameter of the nano-droplets (2) is in the range of 10-60 nm, or in the range of 15-50 nm, and/or wherein the number average distance between adjacent nano-droplets (2) is in the range of 1-30 nm, or in the range of 5-50 nm.
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6. Method according to any of the preceding claims, wherein the thickness of the analyte layer and/or of the open pore matrix material (5) sandwiched between the flat or topologically structured metal layer (1) and the nano-droplets (2) is less than 1 μm , or in the range of 1-900 nm, or in the range of 5-100 nm.
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7. Method according to any of the preceding claims, wherein the flat or topologically structured metal surface (1) is a silver and/or gold layer of a thickness in the range of 5-500 nm, or in the range of 10-100 nm, on the substrate material (10), wherein the roughness of the surface of the flat or topologically structured metal surface (1) is below 50%, or below 25%, most or below 10% of its thickness or the roughness
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is less than 100 nm, or less than 50 nm, or less than 20 nm.

8. Method according to any of the preceding claims, wherein the analyte is at least one of the following: inorganic molecule or particle, organic molecule, including small molecules, DNA molecule, protein, peptide, vitamins, food constituent, cell, including bacterial cells, virus, protozoa, human cells, blood cells, cancer cells, circulating tumour cells, wherein in case of cells also morphological information can be determined in the scanning process of step d).
9. Method according to any of the preceding claims, wherein in step d) an irradiation frequency in the range of 600-900 nm, or in the range of 700-800 nm, or in the range of 750-800 nm is used, and wherein a two-dimensional area is scanned for spectroscopic detection and/or wherein in step d) the scanning laser irradiation and using SERS is carried out by way scanning in which the laser and/or the analyte sandwiched between the flat or topologically structured metal surface (1) and the multitude of nano-droplets (2) are moved relative to each other, wherein this scanning can be by way of an X-Y scanning, in which the laser and/or the analyte sandwiched are moved in two, orthogonal, directions, or can be by way of the analyte sandwiched being rotated combined with a translational movement of the laser, or can be by way of an oscillating, including microelectromechanical (MEMS), mirror placed under the Raman laser while translating the sample in single axis direction, and/or wherein for the scanning in step d) first, a scanning is performed using a first magnification objective lens for a fast screening and then, only in the regions providing spectra, scanning is performed using a second magnification objective lens, said second magnification being larger than said first magnification and/or wherein before, during or after the analysis in step d), the analyte is analysed, in another or the same device and most using the same sample space and preparation, using another analytical technique, including XRF, LIBS, or a combination thereof.

10. Method according to any of the preceding claims, wherein the flat or topologically structured metal surface (1) comprises a multitude of holes (8), having a diameter smaller than the analyte particles to be measured, wherein the holes (8) can have a diameter in the range of 20-200 nm, or in the range of 50-100 nm.
- 5 11. Method according to any of the preceding claims, wherein step b) includes a step of fixing the analyte on the flat or topologically structured metal surface (1), preferably by adding a further fixing layer, adding a fixing substance, or by a cross-linking carrier material either deposited together with the analyte (3) on the flat or
10 topologically structured metal surface (1) or before or after deposition of the analyte on the flat or topologically structured metal surface (1)
and/or wherein, if an open pore matrix material (5) is deposited on the flat or topologically structured surface (1) in step b) the analyte (3) is introduced into the pores before step d), or after step c), wherein the analyte can be introduced into the
15 pores from the gas phase by diffusion or in the liquid phase or as a solution by immersion.
12. Use of a method for analysing an analyte (3) according to any of the preceding claims for solvent, in particular water quality testing, preferably for high purity
20 water testing, in particular in the chip manufacturing field, food and beverage quality testing, pharmaceutical drug discovery, medical diagnostics preferably using one analytical device in which all steps a)-d) are carried out.
13. Analytical device having at least one module for a) generating an essentially flat or
25 topologically structured metal surface (1) of a SERS-active metal;
at least one module for b) depositing the analyte (3) or an open pore matrix material (5) on said flat or topologically structured metal surface (1);
at least one module for c) depositing a multitude of nano-droplets (2) of a SERS-active metal on top of the analyte (3) or the open pore matrix material (5),
30 respectively;
at least one module for d) spectroscopically analysing, by scanning laser irradiation and using SERS, the analyte sandwiched between the flat or topologically structured metal surface (1) and the multitude of nano-droplets (2);

wherein the function of the above-mentioned modules can be carried out by individual units or within joint units, and wherein the module for a) and the module for c) can be one same unit, which can be an inline device for a supply and which can be fully-automated

characterized in that said module for c) depositing a multitude of nano-droplets (2) of a SERS-active metal on top of the analyte (3) or the open pore matrix material (5), respectively, is a module for depositing a multitude of nano-droplets (2) of a SERS-active metal by physical vapour deposition (PVD) or by sputtering SERS-active metal.

14. Multilayer structure for analysing an analyte (3) using surface enhanced Raman spectroscopy (SERS), comprising the following elements:

a) an essentially flat or topologically structured metal surface (1) of a SERS-active metal;

b) the analyte (3) or an open pore matrix material (5) on said flat or topologically structured metal surface (1);

c) a multitude of nano-droplets (2) of a SERS-active metal deposited on top of the analyte (3) or the open pore matrix material (5), respectively

adapted to be spectroscopically analysed, by scanning laser irradiation and using SERS, the analyte sandwiched between the flat or topologically structured metal surface (1) and the multitude of nano-droplets (2);

wherein the number average diameter of the nano-droplets (2) is in the range of 5-70 nm, and wherein the number average distance between adjacent nano-droplets (2) is smaller than their number average diameter,

with the proviso that if an open pore matrix material (5) is deposited on the flat or topologically structured metal surface (1) in step b) the analyte (3) is introduced into the pores before step d)

characterized in that said multitude of nano-droplets (2) of a SERS-active metal are deposited by physical vapour deposition (PVD) or by sputtering SERS-active metal.

15. Multilayer structure according to claim 14, wherein the number average diameter of the nano-droplets (2) is in the range of 10-60 nm, or in the range of 15-15 nm,

and/or wherein the number average distance between adjacent nano-droplets (2) is in the range of 1-30 nm, or in the range of 5-50 nm

and/or wherein the flat or topologically structured metal surface (1) is a silver and/or gold layer of a thickness in the range of 5-500 nm, or in the range of 10-100 nm, on

5 the substrate material (10), wherein the roughness of the surface of the flat or topologically structured metal surface (1) can be below 50%, or below 25%, or below 10% of its thickness or the roughness can be less than 100 nm, or less than 50 nm, or less than 20 nm.