Besides the above mentioned weak signal, organic fluorescence dyes are also disadvantageous due to the small gap between their excitation wavelength and emission wavelength, in which the gap is just 15 to 30 nm. Since the light intensity of the fluorescence emission is much weaker than the excitation, in order to reduce the large background noise due to the crosstalk of the excitation, either a dark-field condenser or a high quality optical filter must be used, both of which are expensive and inconvenient for building up a point-of-care system.

There is a need to provide a method for detecting a target analyte that overcomes, or at least ameliorates, one or more of the disadvantages described above.

Summary

There is provided a method for detecting the presence or absence of a target analyte in a sample comprising the steps of allowing an inorganic fluorescent particle to conjugate with a detection biomolecule that is bound to said target analyte, if present in said sample, said target analyte additionally being bound by a capture biomolecule immobilized on or within a nanostructure; and detecting the fluorescence emitted by said inorganic fluorescent particle, said nanostructure being capable of generating a surface plasmon resonance effect when excited by a light source to substantially increase the fluorescence emitted by said inorganic fluorescent particle, wherein the detected fluorescence indicates the presence of said target analyte in said sample.

According to a first aspect, there is provided a method for detecting the presence or absence of a target analyte in a sample based on a localized surface plasmon resonance using a kit consisting of:
a substrate having a plurality of nanostructures thereon; a capture biomolecule; a detection biomolecule; an inorganic fluorescent particle, an optional blocking agent; the method comprising the steps of:
allowing said inorganic fluorescent particle to conjugate with said detection biomolecule that is bound specifically to said target analyte, if present in said sample, to form a detection reagent that emits a fluorescence, said target analyte additionally being specifically bound by said capture biomolecule immobilized on or within the plurality of nanostructures; and
detecting the fluorescence emitted by said detection reagent, upon excitation by a light source, said plurality of nanostructures generating a localized surface plasmon resonance effect when the distance between said plurality of nanostructures and said inorganic fluorescent particle is within 5 to 15 nm to thereby enhance the fluorescence emitted by said detection reagent when bound to the target analyte, wherein the detected fluorescence indicates the presence of said target analyte in said sample, wherein a fluorescent emission signal is detected by an optical detector or a camera; and wherein the kit does not use the Kretschmann configuration.

The present inventors have shown for the first time that notwithstanding the larger size of the inorganic fluorescent particles (as compared to the smaller organic fluorescent dyes, which can cause the (localized) surface plasmon resonance field to decay exponentially as the height of the inorganic fluorescent particles increases), inorganic fluorescent particles such as quantum dots can be used in detection methods and kits that utilize (localized) surface plasmon resonance nanostructures. In order to circumvent the larger size of the inorganic fluorescent particles and difficulty in positioning the larger inorganic fluorescent particles within the plasmonic field, the inventors have found that, in one embodiment, by

reducing the size of the biomolecule(s) used in the present method such as by end-on immobilization of the fragmented biomolecule(s) on the surface of the nanostructure, the inorganic fluorescent particles can be drawn closer to areas with a strong (localized) surface plasmon resonance effect. By this additionally, the surface density of the active parts of the capture biomolecule may also be increased, allowing for more interaction and capture of the target analyte. In another embodiment, the detection biomolecule may be fragmented.

It is to be noted that in another embodiment, the capture biomolecule may not be fragmented and may be a complete biomolecule, as long as the inorganic fluorescent particle can be placed appropriately in the plasmonic field and without any substantial decay in the surface plasmon resonance field as the height of the assay (made up of the capture biomolecule/target analyte/ detection biomolecule/inorganic fluorescent particle) increases.

Advantageously, due to the enhancement of the quantum dot fluorescent by the surface plasmon resonance of the nanostructures, the intensity of the quantum dot fluorescence and/or the energy gap between the excitation light and emission signal can be significantly increased by about 10 to about 1000 times (depending on the assay and nanostructure

design) as compared to a assay/biochip where nanostructures are absent. This may aid in the construction of a transmission and imaging based inexpensive point-of-care system.

Due to the surface functionalization of the (localized) surface plasmon resonance chip with the quantum dot sandwich bioassay of the present disclosure, brighter photoluminescent signal, longer life-time, more stable signal, and/or higher sensitivity can be achieved in the present disclosure. The localized surface plasmon resonance-quantum dot bioassay developed in this invention has large energy gap between the quantum dot excitation and emission, which will ease the separation of the relatively weak photoluminescence from the strong excitation and increase the signal-to-noise ratio. Hence, the inability associated with conventional point-of-care systems on detecting low photoluminescence of organic fluorescent dyes may be overcome by the present disclosure with the use of quantum dots on localized surface plasmon resonance chips, the present disclosure being the first to show that quantum dots emissions can be used for analyte detections on a biochip having surface plasmon resonance or localized surface plasmon resonance (or co-excitation therof).

There may be provided a system comprising a nanostructure with an immobilized capture biomolecule thereon, the capture biomolecule bound to a target analyte that may be present in a sample, the target analyte in turn bound to a detection biomolecule that is conjugated to an inorganic fluorescent particle, the nanostructure being capable of generating a surface plasmon resonance effect when excited by a light source to substantially increase the fluorescence emitted by the inorganic fluorescent particle, wherein the detected fluorescence indicates the presence of the target analyte in the sample. The system may be a bioassay.

The bioassay of the present disclosure can be used to detect antigens having a concentration as low as 100 pg/ml using a simple charge-coupled device (CCD) camera. On the other hand, for conventional bioassays that are based on organic fluorescent dyes, special and expensive detectors such as photomultiplier must be used. Hence, the bioassay of the present disclosure exhibits greater sensitivity improvement compared with bioassays using organic fluorescence dyes.

There is provided a kit for detecting the presence or absence of a target analyte in a sample comprising: a substrate having a nanostructure thereon for immobilizing a capture biomolecule on or within said nanostructure, said capture biomolecule being capable of binding specifically to said target analyte, if present in said sample; a detection biomolecule capable of binding specifically to said target analyte, if present in said sample; and an inorganic fluorescent particle capable of conjugating with said detection biomolecule to form a detection reagent, wherein upon excitation by a light source, said nanostructure is capable of generating a surface plasmon resonance effect to thereby enhance the fluorescence emitted by said detection reagent when bound to said target analyte, if present in said sample.

According to a second aspect, there is provided a kit for detecting the presence or absence of a target analyte in a sample based on a localized surface plasmon resonance, wherein the kit consists of:
a substrate having a plurality of nanostructures thereon; a capture biomolecule; a detection biomolecule; an inorganic fluorescent particle; and an optional blocking agent,
wherein the capture biomolecule is immobilized on or within the plurality of nanostructures; wherein the capture biomolecule binds specifically to the target analyte, if

said target analyte is present in said sample; wherein the detection biomolecule binds specifically to the target analyte, if said target analyte is present in said sample; wherein the inorganic fluorescent particle is conjugated with said detection biomolecule to form a detection reagent that emits a fluorescence, wherein upon excitation of a light source, said plurality of nanostructures generates a localized surface plasmon resonance effect when the distance between said plurality of nanostructures and said inorganic fluorescent particle is within 5 to 15 nm to thereby enhance the fluorescence emitted by said detection reagent when bound to the target analyte, wherein a fluorescent emission signal is detected by an optical detector or a camera, wherein the kit does not use of the Kretschmann configuration.

Definitions

The following words and terms used herein shall have the meaning indicated:

The term "target analyte", as used herein, refers to a substance to be detected that is capable of binding to a capture biomolecule and a detection biomolecule. Hence, the target analyte may have at least two different binding sites, one for binding with the capture biomolecule

Claims

1. A method for detecting the presence or absence of a target analyte in a sample based on a localized surface plasmon resonance using a kit consisting of:

a substrate having a plurality of nanostructures thereon;

a capture biomolecule;

a detection biomolecule;

an inorganic fluorescent particle,

an optional blocking agent; the method comprising the steps of:

allowing said inorganic fluorescent particle to conjugate with said detection biomolecule that is bound specifically to said target analyte, if present in said sample, to form a detection reagent that emits a fluorescence, said target analyte additionally being specifically bound by said capture biomolecule immobilized on or within the plurality of nanostructures; and

detecting the fluorescence emitted by said detection reagent, upon excitation by a light source, said plurality of nanostructures generating a localized surface plasmon resonance effect when the distance between said plurality of nanostructures and said inorganic fluorescent particle is within 5 to 15 nm to thereby enhance the fluorescence emitted by said detection reagent when bound to the target analyte, wherein the detected fluorescence indicates the presence of said target analyte in said sample, wherein a fluorescent emission signal is detected by an optical detector or a camera; and wherein the kit does not use the Kretschmann configuration.

2. The method of claim 1, wherein said plurality of nanostructures includes nanoholes, nanopillars or gratings.

3. The method of claim 1, wherein said plurality of nanostructures comprises a metal selected from the group consisting of aluminum, cobalt, copper, gold, indium, molybdenum, nickel, palladium, platinum, silver, tin, titanium, tungsten, zinc, alloys and combinations thereof.

4. The method of claim 1, wherein said detection biomolecule is an antibody, protein, nucleic acid, antibody fragment, a peptide or an oligonucleotide.

5. The method of claim 1, further comprising, before said allowing step, the steps of providing a sample suspected of containing said target analyte to said immobilized capture biomolecule and allowing said target analyte to bind to said immobilized capture biomolecule.

6. The method of claim 5, further comprising, before said providing and allowing steps, the step of immobilizing said capture biomolecule on or within said plurality of nanostructures.

7. The method of claim 1, wherein said inorganic fluorescent particle is a semiconductor nanocrystal.

8. The method of claim 7, wherein said semiconductor nanocrystal is a quantum dot selected from the group consisting of CdO, CdS, CdSe, CdTe, CdSeTe, CdHgTe, ZnS, ZnSe, ZnTe, ZnO, MgTe, MgS, MgSe, MgO, GaAs, GaP, GaSb, GaN, HgO, HgS, HgSe, HgTe, CaS, CaSe, CaTe, CaO, SrS, SrSe, SrTe, SrO, BaS, BaSe, BaTe, BaO, InAs, InP, InSb, InN, AlAs, AlN, AlP, AlSb, AlS, PbO, PbS, PbSe, PdTe, Ge, Si, ZnO, ZnS, ZnSe, ZnTe and combinations thereof.

9. The method of claim 1, wherein said capture biomolecule is an antibody, protein, nucleic acid, antibody fragment, a peptide or an oligonucleotide.

10. The method of claim 9, wherein said antibody fragment is selected from the group consisting of a $F(ab')_2$, Fab, Fab', Fd, Fv dAb, camelid antibody fragments, nanobody, isolated complementarity determining region, sFv, scFv and r IgG fragment.

11. The method of claim 9, wherein said antibody fragment, peptide or oligonucleotide is functionalized with a chemical moiety for binding to said nanostructure.

12. A kit for detecting the presence or absence of a target analyte in a sample based on a localized surface plasmon resonance, wherein the kit consists of:

a substrate having a plurality of nanostructures thereon;

a capture biomolecule;

a detection biomolecule;

an inorganic fluorescent particle; and

an optional blocking agent,

wherein the capture biomolecule is immobilized on or within the plurality of nanostructures;

wherein the capture biomolecule binds specifically to the target analyte, if said target analyte is present in said sample;

wherein the detection biomolecule binds specifically to the target analyte, if said target analyte is present in said sample;

wherein the inorganic fluorescent particle is conjugated with said detection biomolecule to form a detection reagent that emits a fluorescence,

wherein upon excitation of a light source, said plurality of nanostructures generates a localized surface plasmon resonance effect when the distance between said plurality of nanostructures and said inorganic fluorescent particle is within 5 to 15 nm to thereby enhance the fluorescence emitted by said detection reagent when bound to the target analyte,

wherein a fluorescent emission signal is detected by an optical detector or a camera,

wherein the kit does not use the Kretschmann configuration.

13. The kit of claim 12, wherein said plurality of nanostructures includes nanoholes, nanopillars or gratings.

14. The kit of claim 12, wherein said plurality of nanostructures comprises a metal selected from the group consisting of aluminum, cobalt, copper, gold, indium, molybdenum, nickel, palladium, platinum, silver, tin, titanium, tungsten, zinc, alloys and combinations thereof.

15. The kit of claim 12, wherein said detection biomolecule is an antibody, protein, nucleic acid, functional antibody fragment, a peptide or an oligonucleotide.

16. The kit of claim 12, wherein said inorganic fluorescent particle is a semiconductor nanocrystal.

17. The kit of claim 16, wherein said semiconductor nanocrystal is a quantum dot selected from the group consisting of CdO, CdS, CdSe, CdTe, CdSeTe, CdHgTe, ZnS, ZnSe, ZnTe, ZnO, MgTe, MgS, MgSe, MgO, GaAs, GaP, GaSb, GaN, HgO, HgS, HgSe, HgTe, CaS, CaSe, CaTe, CaO, SrS, SrSe, SrTe, SrO, BaS, BaSe, BaTe, BaO, InAs, InP, InSb, InN, AlAs, AlN, AlP, AlSb, AlS, PbO, PbS, PbSe, PdTe, Ge, Si, ZnO, ZnS, ZnSe, ZnTe and combinations thereof.

18. The kit of claim 12, wherein said capture biomolecule is an antibody, protein, nucleic acid, functional antibody fragment, a peptide or an oligonucleotide.

19. The kit of claim 18, wherein said antibody fragment is selected from the group consisting of a $F(ab')_2$, Fab, Fab', Fd, Fv dAb, camelid antibody fragments, nanobody, isolated complementarity determining region, sFv, scFv and r IgG fragment.

20. The kit of claim 18, wherein said antibody fragment, peptide or oligonucleotide is functionalized with a chemical moiety for binding to said nanostructure.