assays are being developed, including cerebrospinal fluid measurements and brain amyloid plaque imaging through positron emission tomography (PET); however, these tests face limitations as they either require invasive lumbar punctures or are too expensive for wider clinical adoption. As a result, there is intense interest in finding serologic biomarkers of AD to assist in early diagnosis and disease monitoring.

[0006] Accordingly, there is a need to overcome, or at least to alleviate, one or more of the above-mentioned problems.

SUMMARY

[0007] Disclosed herein is a sensor chip and methods for the detection of an analyte via surface plasmon resonance.

[0007a] In one aspect, there is provided a kit comprising: a sensor chip comprising: a conductive layer on a membrane support layer, wherein a plurality of apertures extends through the conductive layer and the membrane support layer and is arranged such that illumination of the membrane support layer produces a surface plasmon resonance; and a first recognition molecule that binds specifically to a first analyte, the first recognition molecule being immobilized onto a surface of the conductive layer; and a second recognition molecule that binds specifically to the first analyte or to a second analyte associated with the first analyte, wherein the second recognition molecule is coupled to a signal amplification moiety, and wherein the signal amplification moiety is capable of inducing formation of an insoluble aggregate of increased optical density relative to that of the first analyte when the first analyte is captured by the first recognition molecule.

[0007b] In one aspect, there is provided an imaging system comprising a light source, a detector, and the sensor chip and second recognition molecule as described herein, wherein the light source is arranged to illuminate the membrane support layer of the sensor chip, and the detector is positioned to detect light transmitted through the sensor chip.

[0007c] In one aspect, there is provided a method of fabricating a sensor chip, the method comprising main steps of: a) providing a top membrane support layer; b) depositing a conductive layer on the top membrane support layer; and c) forming a plurality of apertures that extends through the top membrane support layer, said apertures also extending through the conductive layer, and being arranged such that illumination of the top membrane support layer produces a surface plasmon resonance; and the method further comprising the steps of: coating the top membrane support layer and a bottom membrane support layer on the top and bottom surfaces of a

silicon substrate; providing a layer of photoresist on the top membrane support layer, and defining a plurality of apertures in the photoresist via deep ultraviolet lithography (DUV) and transferring a pattern of the plurality of apertures to the top membrane support layer via reaction ion etching (RIE).

[0007d] In one aspect, provided herein is a method of detecting a first analyte in a sample, the method comprising: a) capturing the first analyte onto the conductive layer of the sensor chip as described herein, the capturing comprising binding the first analyte to the first recognition molecule as described herein; and b) detecting binding of the second recognition molecule as described herein to the first analyte or to a second analyte associated with the first analyte, wherein an increased binding of the second recognition molecule as compared to a control sample indicates a presence of the first analyte in the sample.

[0007e] In one aspect, provided herein is a method of detecting a neurodegenerative disease or amyloidosis in a subject, the method comprising: a) contacting a sample obtained from the subject with the conductive layer of the sensor chip as described herein; b) illuminating the membrane support layer of the sensor chip; and c) detecting binding of the second recognition molecule as described herein to an analyte captured on the conductive layer of the sensor chip, wherein an increased binding of the second recognition molecule as compared to a control sample or control subject indicates that the subject is suffering from the neurodegenerative disease.

[0008] In one aspect, there is provided a sensor chip comprising a conductive layer on a membrane support layer, wherein a plurality of apertures extend through the conductive layer and the membrane support layer and are arranged such that illumination of the conductive layer and/or the membrane support layer produces a surface plasmon resonance.

[0009] In one aspect, there is provided an imaging system comprising a light source, a detector, and a sensor chip as defined herein, wherein the light source is arranged to illuminate the sensor chip and the detector is positioned to detect light transmitted through the sensor chip.

[0010] In one aspect, there is provided a method of fabricating a sensor chip, the method comprising steps of:

a) providing a top membrane support layer;

b) depositing a conductive layer on the top membrane support layer;

c) forming a plurality of apertures that extend through the membrane support layer, said apertures also extending through the conductive layer, and being arranged such that

illumination of the conductive layer and/or the top membrane support layer produces a surface plasmon resonance.

[0011] In one aspect, there is provided a method of detecting an analyte in a sample, the method comprising:

a) capturing an analyte onto a surface of a sensor chip as defined herein;

b) detecting binding of a second recognition molecule to the captured analyte on the surface of the sensor chip, wherein the second recognition molecule is specific to the analyte, wherein an

WHAT IS CLAIMED IS:

1. A kit comprising:

a sensor chip comprising:

a conductive layer on a membrane support layer, wherein a plurality of apertures ~~extend~~ extends through the conductive layer and the membrane support layer and ~~are~~ is arranged such that illumination of the ~~conductive layer and/or the~~ membrane support layer produces a surface plasmon resonance; and

a first recognition molecule that binds specifically to a first analyte, the first recognition molecule being immobilized onto a surface of the conductive layer; and

a second recognition molecule that binds specifically to the first analyte or to a second analyte associated with the first analyte, wherein the second recognition molecule is coupled to a signal amplification moiety, and wherein the signal amplification moiety is capable of inducing formation of an insoluble aggregate of increased optical density relative to that of the first analyte when the first analyte is captured by the first recognition molecule.

2. The kit of claim 1, wherein the first analyte comprises a protein, a nucleic acid, or a combination, association, or organizational state thereof.

3. The kit of claim 1, wherein the first analyte is an amyloidogenic protein, and wherein the second recognition molecule binds specifically to an exosome-associated biomarker.

4. The kit of claim 3, wherein the amyloidogenic protein is amyloid β (Aβ), amyloid precursor protein (APP), α-synuclein (α-Syn), insulin receptor substrate 1 (IRS-1), tau protein, lipoprotein (APOE), SOD1, TDP-43, bassoon, or fibronectin.

5. The kit of claim 3, wherein the amyloidogenic protein is Aβ, APP, IRS-1, NCAM or tau.

6. The kit of claim 3, wherein the amyloidogenic protein is Aβ42, Aβ40, Aβ39 or Aβ38.

7. The kit of any one of claims 3-6, wherein the exosome-associated biomarker is CD9, CD63, CD81, ALIX, TSG101, Flotillin-1, LAMP-1, HSP70, or HSP90

8. The kit of any one of claims 3-6, wherein the exosome-associated biomarker is CD63, CD9, or CD81.

~~2~~9. The ~~sensor chip~~ kit of ~~claim~~ any one of claims 1-8, wherein the conductive layer is gold, silver, aluminum, sodium, indium or titanium.

~~3~~10. The ~~sensor chip~~ kit of ~~claim 1 or 2~~ any one of claims 1-9, wherein the membrane support layer is silicon nitride or sodium dioxide.

~~4~~11. The ~~sensor chip~~ kit of ~~claim 1~~ any one of claims 1-10, wherein the apertures have a diameter of ~~about~~ 150 nm to ~~about~~ 450 nm.

~~5~~12. The ~~sensor chip~~ kit of any one of claims ~~1-4~~ 1-7, wherein the apertures are arranged periodically.

~~6~~13. The ~~sensor chip~~ kit of claim ~~5~~ 12, wherein the apertures have a periodicity of ~~about~~ 250 nm to ~~about~~ 650 nm.

~~7. The sensor chip of any one of claims 1-6, comprising a first recognition molecule immobilized onto the surface of the conductive layer.~~

~~8~~14. The ~~sensor chip~~ kit of ~~claim 7~~ any one of claims 1-13, wherein the apertures are arranged such that the surface plasmon resonance produced on illumination has a decay length approximately equal to a diameter of ~~a target of the first recognition molecule~~ the first analyte.

~~9~~15. The ~~sensor chip~~ kit of any one of claims ~~1-8~~ 1-14, wherein the conductive layer and the membrane support layer are disposed on a substrate, the substrate ~~having a void formed therein in a region adjacent to the plurality of apertures to enable~~ enabling illumination of the ~~conductive layer and/or the~~ membrane support layer ~~in either direction~~ to produce the surface plasmon resonance.

16. The kit of any one of claims 1-15, wherein the second recognition molecule is fused to the signal amplification moiety.

17. The kit of any one of claims 1-16, wherein the signal amplification moiety is an enzyme.

18. The kit of claim 17, wherein the enzyme is horse radish peroxidase.

19. The kit of claim 17 or 18, wherein the kit further comprises a substrate of the enzyme.

20. The kit of any one of claims 1-19, wherein the signal amplification moiety is a secondary antibody capable of binding to the second recognition molecule.

~~10~~21. An imaging system comprising a light source, a detector, and ~~a~~ the sensor chip and second recognition molecule of any one of claims ~~1-9~~ 1-20, wherein the light source is arranged to illuminate the membrane support layer of the sensor chip, and the detector is positioned to detect light transmitted through the sensor chip.

~~11~~22. A method of fabricating a sensor chip, the method comprising main steps of:

a) providing a top membrane support layer;

b) depositing a conductive layer on the top membrane support layer; and

c) forming a plurality of apertures that ~~extend~~ extends through the top membrane support layer, said apertures also extending through the conductive layer, and being arranged such that illumination of the ~~conductive layer and/or the~~ top membrane support layer produces a surface plasmon resonance~~.~~

~~12. The method according to claim 11,~~; and the method further comprising the steps of:

coating the top membrane support layer and a bottom membrane support layer on the top and bottom surfaces of a silicon substrate;

providing a layer of photoresist on the top membrane support layer, and

defining a plurality of apertures in the photoresist via deep ultraviolet lithography (DUV) and transferring ~~the~~ a pattern of the plurality of apertures to the top membrane support layer via reaction ion etching (RIE).

~~13~~23. The method according to claim ~~12~~ 22, further comprising the steps of:

removing the photoresist on the top membrane support layer and coating a silicon dioxide protective layer on ~~the~~ a surface of the top membrane support layer;

coating a layer of photoresist on the bottom membrane support layer;

defining a sensing area in the photoresist via photolithography;

and transferring the pattern of the sensing area to the bottom membrane support layer via reaction ion etching (RIE);

transferring the pattern of the sensing area to the silicon substrate;

removing the protective layer on the surface of the top membrane support layer with diluted hydrogen fluoride;

and depositing the conductive layer on the top membrane support layer.

~~14~~24. A method of detecting ~~an~~ a first analyte in a sample, the method comprising:

a) capturing ~~an~~ the first analyte onto ~~a surface of a~~ the conductive layer of the sensor chip of ~~anyone~~ any one of claims ~~1-9~~ 1-20, the capturing comprising binding the first analyte to the first recognition molecule of any one of claims 1-20; and

b) detecting binding of ~~a~~ the second recognition molecule of any one of claims 1-20 to the ~~captured analyte on the surface of the sensor chip~~ first analyte or to a second analyte associated with the first analyte, ~~wherein the second recognition molecule is specific to the analyte,~~ wherein an increased binding of the second recognition molecule as compared to a control sample indicates ~~the~~ a presence of the first analyte in the sample.

~~15. The method of claim 14, wherein the second recognition molecule is specific to the analyte, wherein the second recognition molecule is coupled to a signal amplification moiety, and wherein the signal amplification moiety is capable of inducing the formation of an insoluble aggregate of increased optical density relative to that of the captured analyte.~~

~~16. The method of claim 14 or 15, wherein the second recognition molecule is fused to the signal amplification moiety.~~

~~17. The method of claim 15 or 16, wherein the signal amplification moiety is an enzyme.~~

~~18. The method of claim 17, wherein the enzyme is horse radish peroxidase.~~

~~19~~25. The method of claim ~~17 or 18~~ 24, wherein:

the signal amplification moiety of the second recognition molecule is an enzyme; and

~~-~~the method further comprises contacting the enzyme with ~~an enzyme~~ a substrate of the enzyme.

26. The method of claim 24 or 25, wherein the detecting of the binding of the second recognition molecule comprises analyzing localized measurements of one or both of the first analyte and the second analyte.

~~20. The method of claim 15 or 16, wherein the signal amplification moiety is a secondary antibody capable of binding to the second recognition molecule.~~

~~21. The method of any one of claims 15-20, wherein a first recognition molecule is immobilized on the surface of the surface plasmon resonance sensor chip, wherein the first recognition molecule is capable of capturing the analyte on the surface of the sensor chip.~~

~~22. The method of claim 21, wherein the analyte is an exosome-bound biomarker.~~

~~23~~27. The method of ~~claim 14~~ any one of claims 24-26, wherein the method comprises detecting two or more analytes that are co-localized in the sample.

~~24. A kit comprising a sensor chip according to any one of claims 1-9.~~

~~25. The kit according to claim 24, wherein the kit comprises a second recognition molecule for detecting a captured analyte on the surface of the sensor chip.~~

~~26. The kit according to claim 25, wherein the second recognition molecule is coupled to a signal amplification moiety, and wherein the signal amplification moiety is capable of inducing the formation of an insoluble aggregate of increased optical density relative to that of the captured analyte.~~

~~27~~28. A method of detecting a neurodegenerative disease or amyloidosis in a subject, the method comprising:

a) contacting a sample obtained from the subject with the ~~surface~~ conductive layer of ~~a~~ the sensor chip of any one of claims ~~1-9~~ 1-20;

b) illuminating the membrane support layer of the sensor chip; and

~~b~~c) detecting binding of ~~a~~ the second recognition molecule of any one of claims 1-20 to an analyte captured on the ~~surface~~ conductive layer of the sensor chip, ~~wherein the second recognition molecule is specific to the analyte,~~ wherein an increased binding of the second recognition molecule as compared to a control sample or control subject indicates that the subject is suffering from ~~a~~ the neurodegenerative disease.

~~28. The method according to claim 27, wherein the second recognition molecule is coupled to a signal amplification moiety, and wherein the signal amplification moiety is capable of inducing the formation of an insoluble aggregate of increased optical density relative to that of the captured analyte.~~

~~29. The method according to claim 27 or 28, wherein the analyte is an exosome-associated biomarker.~~

~~30. The method according to claim 27 or 28, wherein the analyte is an exosome-associated biomarker or biomarker contained within exosome.~~

~~31. The method of any one of claims 27 to 30, wherein the biomarker is selected from the group consisting of Aβ, APP, α-Syn, CD9, CD63, CD81, ALIX, TSG101, Flotilin-1, Flotilin-2, LAMP-1, HSP70, HSP90, CHL1, IRS-1, L1CAM, NCAM, Tau, APOE, SOD1, TDP-43, bassoon, fibronectin, DNA, and RNA or combinations and associated complexes thereof.~~

~~32. The method of claim 31, wherein the Aβ is Aβ42, Aβ40, Aβ39 or Aβ38.~~

29. The method of claim 28, wherein the detecting of the binding of the second recognition molecule to the analyte comprises measuring a change of a transmission light wavelength or intensity.

~~33~~30. The method of ~~any one of claims 27 to 31~~ claim 28 or 29, wherein the neurodegenerative disease is selected from the group consisting of Alzheimer~~'~~'s disease, mild cognitive impairment, vascular dementia, dementia, Parkinson's disease, Amyotrophic lateral sclerosis, Multiple sclerosis, Progressive supranuclear palsy, Taupathies, and vascular mild cognitive impairment.

~~34~~31. The method of ~~claims 28-32~~ any one of claims 28-30, wherein the method further comprises that the subject found to be suffering from a neurodegenerative disease is to be treated.