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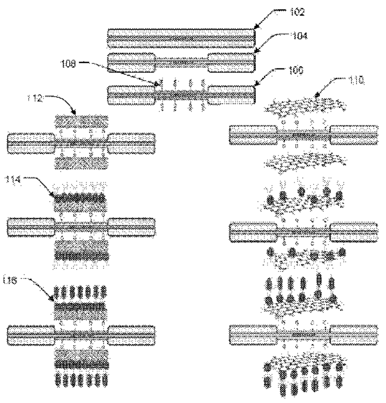

Figure 1(a)

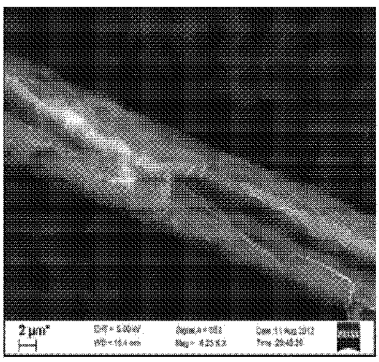

Figure 1(b)

(57) Abstract: The present invention relates to an optical biosensor comprising of an etched fiber Bragg grating (eFBG), having a plurality of coatings comprising of: (a) nano carbon materials and (b) one or more dendrimer molecule(s), wherein the nano carbon materials are attached with one or more dendrimer molecule(s) and coated onto surface of the eFBG. The optical biosensor of the present invention is highly sensitive in detection of antigen-antibody and carbohydrate-protein interactions. A method of fabrication of the optical biosensor is also provided.

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OPTICAL BIOSENSORS HAVING ENHANCED SENSITIVITY

FIELD OF THE INVENTION

[0001] The present invention relates to a highly sensitive optical biosensor for biological and chemical sensing applications. More specifically the present invention relates to dendrimer functionalized nano carbon materials coated on etched fiber Bragg grating (eFBG) and methods of fabrication thereof.

BACKGROUND OF THE INVENTION

[0002] Fiber Bragg grating is a type of distributed Bragg reflector constructed in a short segment of optical fiber that reflects particular wavelength of light and transmits all others. This is achieved by adding periodic variations to refractive index of fiber core, which generates a wavelength specific dielectric mirror.

[0003] Optical sensors based on fiber Bragg gratings (FBGs) have emerged as important sensing elements for measurements of temperature, pressure, chemical and biological agents. These sensors offer attractive characteristics such as small size, light weight, high sensitivity, and low losses, making them very suitable. They are also immune to electro-magnetic interference, are capable of performing remote sensing, and can provide multiplexed sensing and detection within a single device.

[0004] Precise wavelength measurement using FBG sensors has been a challenging problem since the early stages of FBG sensing work. When sensors are employed in the detection of biological materials in particular environment, it is necessary to be able to detect small changes in refractive index. Sensitivity is an important parameter to evaluate sensor performance. This has been brought about in some of current approaches by applying coatings to the surface of the optical sensors. Since FBGs are less sensitive to surrounding medium refractive index, normal FBGs cannot be used in biological sensing application.

[0005] Distributed Bragg reflector constructed in a short segment of optical fiber reflects particular wavelength of light and transmits all others when light is passed through it. This is achieved by grating inscripted in fiber which adds periodic variations to the refractive index of the fiber core, which generates a wavelength specific dielectric mirror. The grating typically has sinusoidal refractive index variation over a defined fiber core length. An interference pattern of maxima and minima is formed causing permanent periodic change to the refractive index of core. This results into a reflection of small amount of wavelength at each modified core segment with reflection of small amount of light at each index variation. The reflected wavelength λ_B is termed as the Bragg wavelength, which is defined by the relationship:

$$\lambda_B = 2n_e\Lambda$$

where n_e is the effective refractive index of the grating in the fiber core and Λ is the grating period. n_e quantifies the velocity of propagating light as compared to its velocity in vacuum. n_e depends not only on the wavelength but also (for multimode waveguides) on the mode in which the light propagates. Therefore, change in Bragg wavelength can be used to study various parameters in biological and chemical fields.

[0006] The existing art in general describes a use of etched FBGs (eFBGs) in DNA hybridization and studying carbohydrate – protein interactions, for example, in Christopher, J. S. et al, 2009. Journal of Sensors. 982658, 1-7, Geunmin R., et al., 2010. Journal of Selected Topics In Quantum Electronics. 16, 647-653, and Chryssis, A. N., et al, 2005. Journal of Selected Topics In Quantum Electronics. 11, 864-872. The use of Single walled carbon nanotubes (SWNTs) and graphene based field effect transistors (FETs) in biological and chemical sensing applications has been reported in the literature for example in Vedala, H. et al, 2011. Nano Lett. 11, 170–175; Vasu, K. S. et al, 2012. Appl. Phys. Lett. 101, 053701; Chen, Y. et al, 2012. ACS Nano. 2012, 6, 760–770; and Yasuhide, O et al, 2010. Biosensors and Bioelectronics. 26, 1727–1730.

[0007] US Patent US 7,010,182 ('182) discloses a coating of dendrimer on the long period gratings (LPGs) surface to detect the binding receptor of the substrate by forming a bond between them causing a change in the refractive index. '182, however, does not disclose dendrimer attached nano carbon materials coated on etched FBG. '182 further does not disclose or teach measuring a shift in Bragg wavelength as an output measurand.

[0008] Stéphane Campidelli et al (2006. Journal of American Chemical Society. 128 (38), 12544–12552 discloses single walled carbon nanotubes functionalized with PAMAM (Polyamidoamine) dendrimer through a tedious process of covalent attachment involving a lot of chemistry. Stephane does not discuss SWNTs – dendrimer complex coated on eFBG and the biosensing application thereof by measuring shift in Bragg wavelenth.

[0009] Lijun Li et al. (2013. Optics Communications. 286, 13-17) discloses coating of carbon nanotubes on the surface of the FBG by drop casting the SWNT solution and drying it on the surface of the FBG. They have shown the increase in the reflectivity in SWNTs coated

FBG over normal FBG caused by the anisotropic property of refractive index of SWNTs. Lijun does not discuss SWNTs – dendrimer complex coated on eFBG and the biosensing application thereof by measuring shift in Bragg wavelength.

[00010] Existing fiber optic biochemical sensors pose complications and inaccuracy to meet requirement in their actual implementation in the fields of biological or chemical materials and are also reported to measure reflectivity change or intensity change as an output measurand or readout, which changes are not highly sensitive for binding receptors.

[00011] There is thus a need to provide new optical biosensors having very high sensitivity and accuracy. The present invention satisfies these needs, as well as others, and efficiently overcomes the deficiencies found in the background art.

OBJECTS OF THE INVENTION

[00012] It is an object of the present invention to provide an optical biosensor that is highly sensitive and accurate for biological and chemical sensing applications.

[00013] It is another object of the present invention to provide an optical biosensor where coating of nano carbon materials attached with dendrimer molecules is disposed on etched fiber Bragg grating (eFBG).

[00014] It is a further object of the present invention to provide an optical biosensor where coating of nano carbon materials attached with multivalent specifically functionalized dendrimer molecules is disposed on eFBG.

[00015] It is another object of the present invention to provide an optical biosensor that is used in detection of carbohydrate-protein interactions as an example of antibody-antigen interactions.

[00016] It is a further object of the present invention to provide an optical biosensor that measures a shift in Bragg wavelength as an output measurand which is very sensitive.

[00017] It is yet another object of the present invention to provide an optical biosensor where multiple gratings of different Bragg wavelengths can be inscribed on a single optical fiber to detect many specific antibody – antigen interactions simultaneously.

[00018] Another object of the present invention is to provide a method of fabrication of an optical biosensor comprising of dendrimer functionalized nano carbon materials coated on eFBG.

[00019] Yet another object of the present invention is to provide a simpler and easier method of coating nano carbon materials on surface of eFBG wherein surfaces of nano carbon materials and eFBG are made hydrophilic.

[00020] Other objects of the present invention will be apparent from the description of the invention herein below.

SUMMARY OF THE INVENTION

[00021] The present invention relates to an optical biosensor comprising of an etched fiber Bragg grating (eFBG) having a plurality of coatings comprising of:

a. nano carbon materials; and

b. one or more dendrimer molecule(s);

wherein the nano carbon materials are attached with one or more dendrimer molecule(s) and coated onto surface of the FBG or eFBG.

[00022] Optical biosensor of the present invention is highly sensitive and accurate for bio-chemical sensing applications, particularly for detecting carbohydrate-protein interactions as an example of antigen-antibody interactions.

[00023] One or more dendrimer molecule(s) of the present invention can be attached with a carbohydrate or antibody to form a carbohydrate or antibody attached dendrimer coated nano carbon materials coated optical biosensor.

[00024] According to one embodiment, nano carbon materials are selected from a group consisting of Single Walled Carbon Nanotubes (SWNTs), Graphene Oxide (GO).

[00025] In another embodiment, the one or more dendrimer molecule(s) are selected from the group consisting of: carboxylic acid terminated poly(propyl ether imine) dendrimers, hydroxyl terminated poly(propyl ether imine) dendrimers, amine terminated poly(propyl ether imine) dendrimers, cyano terminated poly(propyl ether imine) dendrimers, carboxylic acid terminated polyamdioamine dendrimers, amine terminated polyamidoamine dendrimers, amine terminated poly propylene imine dendrimer, and hydroxyl terminated polyamidoamine dendrimers.

[00026] In another embodiment, one or more dendrimer molecule(s) are selected from one or more of carboxylic acid terminated poly (propyl ether imine) dendrimers and amine terminated poly (propyl ether imine) dendrimers attached with various antibodies.

[00027] In one embodiment, optical biosensor of the present disclosure measures a shift in Bragg wavelength ($\Delta\lambda_B$) of eFBG that is coated with dendrimer functionalized nano carbon materials, as an output measurand or readout in detection of carbohydrate-protein or antigen-antibody interactions. In the present invention, it was discovered that the shift in Bragg wavelength is very significant and can measure very small changes in optical properties such as refractive index of the nano carbon materials coated due to antibody-antigen interactions or carbohydrate-protein interactions.

[00028] According to one embodiment, optical biosensor of the present invention can measure about 1-2 pm (10^{-12} meter) shift in Bragg wavelength. Such high sensitivity of 1 2 pm can allow measurement of very small changes that occur in refractive index of nano carbon materials due to antibody – antigen interaction, which makes the optical biosensor of the present invention disclosure highly sensitive and thus accurate. Another embodiment of the present invention allows sensitivity in readout to be increased 5 times in SWNTs coated eFBG and 10 times in GO coated eFBG when compared to the sensitivity without the coating of nano carbon materials. Dendrimer having multiple functionalities further provides a large number of active sites to increase the sensitivity. In one embodiment, dendrimer can be attached to nano carbon materials non – covalently.

[00029] The present invention further allows writing of multiple gratings of different Bragg wavelengths on a single fiber to detect many specific antibody – antigen interactions simultaneously.

[00030] The present disclosure also relates to methods of fabrication of an optical biosensor comprising of a Fiber Bragg grating (FBG), preferably of an etched Fiber Bragg grating (eFBG), with a plurality of coatings comprising of: (a) nano carbon materials and (b) one or more dendrimer molecule(s), wherein dendrimer functionalized nano carbon materials are coated onto eFBG.

[00031] According to another embodiment, surfaces of the nano carbon materials and the eFBG are made hydrophilic to.

[00032] One preferred embodiment of the present invention provides a method of fabrication of an optical biosensor comprising the steps of:

i. optionally decladding the FBG by an etching process to produce eFBG;

ii. treating surface of the eFBG or FBG with a suitable reagent to make the surface of the FBG or eFBG hydrophilic;

iii. coating nano carbon materials onto the surface of the hydrophilic eFBG or FBG made in step (ii); and

iv. attaching dendrimer with the nano carbon materials coated eFBG or FBG made in step (iii).

[00033] In a preferred embodiment, the suitable reagent in step (ii) is a base comprising of NaOH.

[00034] In another preferred embodiment, the nano carbon materials can be selected from a group comprising of Single Walled Carbon Nanotubes (SWNT), Graphene Oxide (GO).

[00035] In one embodiment, the method of fabrication of the present invention further comprises the step of treating surface of the SWNT with a suitable reagent to make the surface of the SWNT hydrophilic.

[00036] In still another embodiment, the SWNT is treated with a suitable reagent to make surface of the SWNT hydrophilic. The suitable reagent can be an acid comprising of H_2SO_4, HNO_3 or mixtures thereof.

[00037] A method of fabrication of the present invention further comprises the step of functionalizing dendrimer with at least a suitable molecule to produce multiple active sites on surface of the dendrimer. The suitable molecule can be a carbohydrate.

[00038] Additional objects and embodiments of the invention will be set forth in part in the description, or may be learned by practice of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[00039] These and other features, aspects and advantages of the invention will become better understood when the following detailed description is read with reference to the accompanying drawings, wherein:

[00040] Figure 1 (a) illustrates a schematic representation of a preferred embodiment of the invention showing coatings of SWNT, GO and dendrimer on etched FBGs and how a binding receptor attaches to the active sites of the dendrimer coating.

[00041] Figure 1 (b) depicts an SEM image of GO coated eFBG.

[00042] Figure 2 illustrates a variation of $\Delta\lambda_B / \lambda^0_B$ with respect to concentration of Con A and PNA.

[00043] Figure 3 illustrates a variation of $\Delta\lambda_B / \lambda^0_B$ of GO – Dendrimer (DM) coated eFBG after dipping it in different concentrations of lectins.

[00044] Figure 4 illustrates a variation of $\Delta\lambda_B / \lambda^0_B$ of the GO – DM coated FBG after dipping it in different concentrations of Con A.

[00045] Figure 5 illustrates a change in a cladding refractive index (surrounding medium) as a function of concentration of Con A solution for both SWNTs (red color open circles) and GO (black color open squares) coated eFBGs.

[00046] Figure 6 illustrates a block diagram of multiple gratings inscribed on eFBG which can be used as optical biosensor for many specific antibody – antigen interactions; (a) GO coated eFBGs with different antibodies (b) SWNTs coated eFBGs with different antibodies.

DETAILED DESCRIPTION OF THE INVENTION

[00047] Abbreviations used:

i. "SWNT" - single- walled nanotube.
ii. "GO" - oxidized graphene sheet/ Graphene Oxide.
iii. "DM" – mannose attached dendrimer/ dendrimers
iv. "FBG" - fiber bragg grating.
v. "FBGs" - fiber bragg gratings.
vi. "eFBG" - etched fiber bragg grating.
vii. "eFBGs" - etched fiber bragg gratings.
viii. "Con A" - concanavalin A.
ix. "PETIM" – poly (propyl ether imine).
x. "FET" - Field effect transistors.
xi. "PNA" - peanut agglutinin

[00048] The embodiments herein and the various features and advantageous details thereof are explained more fully with reference to the non-limiting embodiments that are illustrated in the accompanying figures and detailed in the following description. Descriptions of well-known components and processing techniques are omitted so as to not unnecessarily obscure the embodiments herein. The examples used herein are intended merely to facilitate an understanding of ways in which the embodiments herein may be practiced and to further enable those of skill in the art to practice the embodiments herein. Accordingly, the examples should not be construed as limiting the scope of the embodiments herein.

[00049] The present invention is directed towards optical biosensors which are highly sensitive and accurate for bio-chemical sensing applications particularly for detecting carbohydrate-protein interactions as an example of antigen-antibody interactions.

[00050] The present invention relates to an optical biosensor comprising of etched Fiber Bragg grating (eFBG) having a plurality of coatings comprising of:

a. nano carbon materials;

b. one or more dendrimer molecule(s);

wherein the nano carbon materials are attached with one or more dendrimer molecule(s) and coated onto surface of the eFBG.

[00051] In accordance an embodiment of the present invention, when dendrimer molecules are attached with nano carbon materials coated FBG or eFBG, active sites on the dendrimers are oriented away from the FBG or eFBG, enabling them to complex with specific target molecules having specific binding receptors. Specific target molecules can comprise, for example, carbohydrates and proteins. Specific active sites can exist on commercially available dendrimers on their own or can be created on dendrimers by functionalisation with specific molecules. For example, dendrimer molecules can be reacted with specific carbohydrate molecules to create specific carbohydrate sites on the dendrimers in order to detect specific proteins or antibodies. In one preferred embodiment of the present invention, dendrimer molecule(s) can be attached with a carbohydrate to form a carbohydrate coated dendrimer functionalised nano carbon materials coated optical biosensor, which can bind to a specific protein.

[00052] Protein–carbohydrate interactions play an important role in biological functions such as but not limited to cell adhesion, signal transduction, host-pathogen recognition, and inflammation, among others. Carbohydrates are often specifically recognized by other biomolecules. Antibody binding site of an antigen is constituted by the combination of various amino acids and when the antibody is a kind of carbohydrate, the binding can be regard as antibody-antigen interaction or carbohydrate-protein interaction.

[00053] Dendrimers and dendrimer polymers typically have a central core, an interior dendritic structure, and an exterior dendritic surface, wherein they are highly branched macromolecules with known functional end groups (or terminal groups) and are also sometimes classified as nanomaterials. Different families of dendrimers exist with hundreds of end group modifications, wherein the size of the dendrimer is generally controlled by growth cycle during the synthesis of the material and is referred to as the generation number. For instance, if a dendrimer is made by convergent synthesis and the branching reactions are performed onto the core molecule three times, the resulting dendrimer is considered a fourth generation dendrimer. Each successive generation results in a dendrimer that has roughly twice the molecular weight of the previous generation. Higher generation dendrimers also have more exposed functional groups on the surface.

[00054] In one embodiment of the present invention, one or more dendrimer molecule(s) can be selected from one or more of carboxylic acid terminated poly(propyl ether imine) dendrimers, hydroxyl terminated poly (propyl ether imine) dendrimers, amine terminated poly(propyl ether imine) dendrimers, poly(propyl ether imine) dendrimers, cyano

terminated poly(propyl ether imine) dendrimers, carboxylic acid terminated polyamdioamine dendrimers, amine terminated polyamidoamine dendrimers, amine terminated poly (propyleneimine) dendrimer, and hydroxyl terminated polyamidoamine dendrimers.

[00055] In a preferred embodiment of the present invention, one or more dendrimer molecule(s) can be selected from one or more of poly (propyl ether imine) (PETIM) dendrimers, preferably carboxylic acid terminated PETIM dendrimers.

[00056] In one embodiment, nano carbon materials can be selected from one or more of Single Walled Carbon Nanotubes (SWNT), Graphene Oxide (GO).

[00057] One aspect of the present invention relates to enhancing sensitivity of a biosensor by disposing on an etched Fiber Bragg Grating (eFBG), a coating comprising nano carbon materials attached with dendrimer molecules. According to another aspect of the present invention, sensitivity of biosensor can be greatly enhanced by a coating comprising SWNT or GO attached with multivalent specifically functionalized dendrimer molecules and disposing the coating on an etched fiber bragg grating. The present invention further enables measurement of a shift in Bragg wavelength as an output measurand instead of measuring reflectivity or intensity changes as found in the existing art. The shift in Bragg wavelength $\Delta\lambda_B$ is found to be significant, making the optical sensor of the present invention highly sensitive.

[00058] **Figure 1 (a)** illustrates a schematic representation of a preferred embodiment of the invention. In an embodiment, as illustrated, surface of eFBG **104** or **106** can be coated with a coating comprising SWNT **112** or GO **110**, wherein Fiber Bragg grating (FBG) **102** comprises uniform dimensions all over its surface, which is decladded by an etching process to produce the eFBG **104**.

[00059] Commercially available SWNTs are hydrophobic whereas GO, by preparation, is hydrophilic. One embodiment of the present invention makes surfaces of the SWNTs 112 and the eFBG **104** hydrophilic by virtue of which the two different natured nano carbon materials SWNT and GO can be coated onto the eFBG surface **106**.

[00060] One preferred embodiment of the present invention provides a method of fabrication of an optical biosensor comprising the steps of:

i. optionally decladding the FBG by an etching process to produce eFBG;

ii. treating surface of the eFBG or FBG with a suitable reagent to make the surface of the FBG or eFBG hydrophilic;

iii. coating nano carbon materials onto the surface of the hydrophilic eFBG or FBG made in step (ii); and

iv. attaching dendrimer with the nano carbon materials coated eFBG or FBG made in step (iii).

[00061] In a preferred embodiment, the suitable reagent in step (ii) is a base comprising of NaOH.

[00062] In another preferred embodiment, the nano carbon materials can be selected from a group comprising of Single Walled Carbon Nanotubes (SWNT), Graphene Oxide (GO).

[00063] In one embodiment, the method of fabrication of the present invention further comprises the step of treating surface of the SWNT with a suitable reagent to make the surface of the SWNT hydrophilic.

[00064] In still another embodiment, the SWNT is treated with a suitable reagent to make surface of the SWNT hydrophilic. The suitable reagent can be an acid comprising of H_2SO_4, HNO_3 or mixtures thereof.

[00065] A method of fabrication of the present invention further comprises the step of functionalizing dendrimer with at least a suitable molecule to produce multiple active sites on surface of the dendrimer. The suitable molecule can be a carbohydrate.

[00066] In an embodiment of the present invention, eFBG **104** can be treated with NaOH and the like to produce hydroxyl functions **108** resulting onto eFBG **106**. The treatment of the surface eFBG with NaOH and the like produces surface roughness and few hydroxyl functions making surface of the eFBG hydrophilic. In an exemplary embodiment, surface of eFBG **104** surface is treated with 0.2 to 0.4 N NaOH solution at 30 °C - 50 °C, more preferably at 40 °C for a few hours, preferably 3-4 hours, more preferably 3.5 hours and subsequently kept in the NaOH solution for about 30 minutes at room temperature. The treated eFBG 106 is rinsed with deionised water for about 10 minutes. The surface treatment can lead to a decrease in the Bragg wavelength (λ_B) by 15-25 pm.

[00067] According to one embodiment, SWNTs **112** have an average diameter in range of 0.7 nm -1nm, more preferably around 0.8nm, with chirality (6, 5), purchased from M/s South-West Nanotechnologies. Surface of SWNTs can be made hydrophilic by following a procedure known in the literature for example in Hussain, S., Jha, P. *et al*, 2011. Journal of Modern Physics, 2, 538-543, by treating with 3:1 conc. H_2SO_4:conc. HNO_3 for 3 hrs at 40 ^oC.

The mixture is centrifuged and the residue is washed with water several times and dried at 100 °C.

[00068] According to one embodiment, cladding of the FBG **102** can be removed by an etching process. The etching can be carried out by dipping only the grating region of the FBG **102** in a range of 200-300μL of 40% Hydro Fluoric acid (HF) solution placed in a teflon block for few hours, more preferably approximately 2 hours. This process can render the diameter of the etched FBG **106** to 6μm -12 μm more preferably 8μm – 10μm with a few nm downshift more preferably 1nm downshift in λ_B.

[00069] In one embodiment of the present invention, surface roughness and hydrophilicity of eFBG **106** leads to attachment of the acid treated SWNTs and λ_B is found to increase by about 10-14 pm after the attachment of SWNTs (with respect to the λ_B after NaOH treatment). This increase in λ_B is due to the increase in the effective refractive index due to the SWNTs coating.

[00070] According to an exemplary embodiment of the present invention, GO **110** suspensions (0.25mg/ml) can be prepared according to a method known in the literature (Vasu, K. S., Biswanath *et al*, 2010. *Solid State Communications*. 150, 1295-1298; Stankovich, S. *et al*, 2007. *Carbon*. 45, 1558-1565; Hummers *et al*, 1958. *J. Am. Chem. Soc.* 80, 1339). **Figure 1 (b)** illustrates a scanning electron microscopic image of GO coated eFBG captured using ULTRA 55, Field Emission Scanning Electron Microscope (M/s Karl Zeiss).

[00071] In an embodiment of the present invention, one or more dendrimer molecule(s) can be reacted with specific molecules to create specific active sites on the dendrimer in order to detect specific proteins or antigens. Preferably, the one or more dendrimer molecule(s) can be attached with a carbohydrate to form a carbohydrate coated dendrimer functionalized nano carbon materials coated optical biosensor, which can then bind to a specific protein. In a more preferred embodiment, the carbohydrate is mannose.

[00072] In one exemplary embodiment, carbohydrate–protein interactions can be detected using mannose attached PETIM dendrimer as a multivalent carbohydrate ligand. Mannose attached PETIM dendrimer (generation 4) solutions in water with a concentration of 0.5mM can be prepared by a method as described in Vasu, K. S. *et al*, 2012. *Appl. Phys. Lett.* 101, 053701. The nano carbon materials **110** or **112** coated eFBG **106** can be dipped in the Dendrimer solution to create carbohydrate (mannose) sites. As the SWNTs are p – type, DM molecules **114** form SWNT – DM complexes, through charge transfer interactions. The formation of SWNT **112** – DM **114** complexes can result in the decrease of λ_B by about 8-12pm more preferably 10 pm with respect to the λ_B of eFBG **106** coated with SWNTs **112**. The Bragg wavelength (λ_B^0) of the eFBG **106**, after the formation of the SWNT – DM complex is 1539.09 nm.

[00073] Carbohydrate coated dendrimer functionalized nano carbon materials coated eFBG of the present invention can then bind to a specific protein such as Lectin for detecting carbohydrate-protein interactions or antigen-antibody interactions. Lectins are a group of proteins that bind specific carbohydrate structures including but not limited to for example mannose binding lectins such as Concanavalin A (ConA), Galactose binding lectins such as Peanut agglutinin, Jacalin and Wheat Germ Agglutinin, among others.

[00074] Concanavalin A (ConA) is a lectin originally extracted from the jack-bean, Canavalia ensiformis that interacts with diverse receptors containing mannose carbohydrates, notably rhodopsin, blood group markers, insulin-receptor the Immunoglobulins and the carcino-embryonary antigen (CEA). It also interacts with lipoproteins. Peanut agglutinin (PNA) is plant lectin protein derived from the fruits of Arachis hypogaea. Peanut agglutinin binds preferentially to T-antigen, a galactosyl (β-1, 3) N-acetylgalactosamine structure.

[00075] In an exemplary embodiment of the present invention, solutions of Con A specific to mannose and non-specific PNA can be prepared in water (pH ~ 6.5 to 6.7) in a concentration range of 100 pM to 5μM. The Bragg wavelength values can be monitored after treating the SWNT **112** – DM **114** coated eFBG **106** at different concentrations of aqueous solutions of Con A from 1nM to 5μM. It was found that with increase of Con A concentration, the difference $\Delta\lambda_B$ ($\lambda_B - \lambda_B^0$) significantly increases. Millipore water of 18MΩ resistance and pH varying from 6.5 to 6.7 can be used for making dendrimer mannose, Con A and PNA solutions, SWNTs and GO suspensions.

[00076] **Figure 2** shows variation in the ratio $\Delta\lambda_B/ \lambda^0_B$ with respect to concentration of Con A and PNA. As shown in **Figure 2**, even for 1nM of Con A, the shift $\Delta\lambda_B$ is ~ 2pm and after an addition of 5μM Con A, the shift $\Delta\lambda_B$ increases to ~75pm.

[00077] Another exemplary embodiment of the present invention is configured to measure variation in the ratio $\Delta\lambda_B/ \lambda^0_B$ with respect to concentration of a non-specific lectin, PNA. It is observed that $\Delta\lambda_B$ value after 5μM PNA treatment is only ~ 5pm. The $\Delta\lambda_B$ for 5μM of PNA treatment is lesser than the $\Delta\lambda_B$ for 2nM of Con A treatment, showing a very high specificity of the optical biosensor for the mannose - Con A interactions. Further, in Figure 2 the variation of $\Delta\lambda_B/ \lambda^0_B$ as a function of concentration of the Con A without coating of

SWNTs is shown, which indicates that the eFBG coated only with DM is much less sensitive when compared to the SWNT – DM coated eFBG.

[00078] **Figure 3** depicts another embodiment of the present invention wherein a change in $\Delta\lambda_B/\lambda^0_B$ of the GO – DM coated eFBG is shown after dipping in different concentrations of lectins.

[00079] In an exemplary embodiment of the present invention, a change in $\Delta\lambda_B/\lambda^0_B$ is measured for the GO-DM coated eFBG as a function of lectin concentration. The Bragg wavelength (λ^0_B) of the eFBG coated with the GO – DM complex is found to be approximately 1546.55nm. The change in λ_B after addition of 1µM Con A $\Delta\lambda_B$ is found to be ~ 150pm as compared to a $\Delta\lambda_B$ of ~ 20pm for 1µM PNA. The $\Delta\lambda_B$ value for 1µM PNA detection is same as the $\Delta\lambda_B$ value for 500pM of Con A. It was found that for 1µM concentration of Con A treatment, value of $\Delta\lambda_B$ for the GO coated eFBG is twice as compared to the value of $\Delta\lambda_B$ for SWNTs coated eFBG. The enhanced sensitivity of GO coated eFBG over SWNTs coated eFBG is due to the fact that the surface area of GO is more than SWNTs and also GO covers more area on the surface of eFBG, resulting in larger number of DM molecules to get attached to the GO coated eFBG. Furthermore, the lowest Con A concentration sensed using GO coated eFBG is about 0.5nM ($\Delta\lambda_B$ ~ 9pm) as compared to Con A concentration sensed using SWNTs coated eFBG to be about 1nM ($\Delta\lambda_B$ = 2pm).

[00080] In another exemplary embodiment of the present invention, sensor readout or output measurand, a relative change in the Bragg wavelength $\Delta\lambda_B/\lambda^0_B$ as a function of lectin concentration is shown to obey the Langmuir type isotherm, giving the affinity constant value of 4.2 x 10^7 M^{-1} for SWNTs coated eFBG and 3.4 x 10^8 M^{-1} for GO coated eFBG.

[00081] The Langmuir type adsorption isotherm is given by formula:

$$\frac{\Delta\lambda_B}{\lambda^0_B} = S\ [\log\ C\ +\ \log(7.389\ K_A)] \qquad (1)$$

wherein parameter S is called structure factor, C is the concentration of lectin solution and K_A is the affinity constant.

[00082] **In Figure 2** and **Figure 3**, Equation (1) can be applied to extract K_A values of Con A –mannose interaction yielding 4.2 x 10^7 M^{-1} for SWNTs coated eFBG and 3.4 x 10^8 M^{-1} for GO coated eFBG. The observed K_A value in SWNT coated eFBG optical sensor is 40 times more than the K_A value in the SWNT FET sensor as measured in Vasu, K. S., Naresh, K., Bagul, R. S., Jayaraman, N., Sood, A. K., 2012. Appl. Phys. Lett. 101, 053701.

[00083] **Figure 4** depicts variation in $\Delta\lambda_B / \lambda^0_B$ of the GO coated directly on cladding of the FBG with respect to different concentrations of Con A wherein sensitivity of carbohydrate - protein interactions are found to be negligible when the GO is directly coated on cladding of the FBG. Thus showing that etching of FBG enhances the sensitivity.

[00084] **Figure 5** depicts change in cladding refractive index (surrounding medium) as a function of concentration of Con A solution for both SWNTs (red color open circles) and GO (black color open squares) coated eFBGs. The change in the refractive index of the surrounding medium which acts as cladding to the etched FBG, causes the change in the effective refractive index which in turn causes a change in λ_B. The change in the cladding refractive index (surrounding medium) was calculated using the equation given by Matthew, P. D., Zheng, Z., Mira, S., Saeed, P., Christopher, C. D., James, S. S., William,E. B., 2000. Anal. Chem. 72, 2895–2900.

$$n_{eff} = n_{clad}\left[\left(\frac{n_{eff}^2 - n_{clad}^2}{n_{cor}^2 - n_{eff}^2}\right)\left(\frac{n_{cor} - n_{clad}}{n_{clad}}\right) + 1\right] \qquad (2)$$

where n_{clad}, n_{cor} are refractive indices of cladding and core of a FBG. In an exemplary embodiment, core refractive index is taken as 1.471 and not accounted for periodic change in it to calculate the change in the cladding refractive index. Also, by calculating the cladding refractive index after SWNT or GO coating on eFBG, it is found that the effective area of coating by SWNTs and GO is 50%-60% more preferably 55% and 60%-70% more preferably 65% respectively.

[00085] The change $\Delta\lambda_B$ in Bragg wavelength for different concentrations of Con A are much higher than that for non-specific lectin PNA showing the specificity of the sensor.

[00086] **Figure 6** illustrates another embodiment of the present invention showing a block diagram of multiple gratings inscribed on eFBG **106** which can be used as an optical biosensor for many specific antibody – antigen interactions. **Figure 6(a)** illustrates eFBG coated with graphene oxide (GO) **110** attached with different antibodies such as Antibody 1 **602**, Antibody 2 **604**, Antibody 3 **606** and so on upto Antibody n **608**. Similarly **Figure 6(b)** illustrates eFBG **106** coated with SWNTs **112** attached with different antibodies such as Antibody 1 **602**, Antibody 2 **604**, Antibody 3 **606** and so on upto Antibody n **608**. The antibodies can bind with specific antigens such as Antigen 1 **601**, Antigen 2 **603**, Antigen 3

605 and so on upto Antigen n **609**. Thus the biosensor of the present invention can be implied to detect a large number of specific antibody- antigen interactions.

[00087] The biosensor of the present invention can be used in different biological and chemical sensing applications by attaching specific ligands to the nano carbon materials coated on etched Fiber Bragg Grating.

[00088] In a further embodiment, the present invention provides to write multiple gratings of different bragg wavelengths on a single optical fiber to detect many specific antibody – antigen interactions simultaneously.

[00089] It is understood that the specific order or hierarchy of steps in the methodology disclosed is an illustration of exemplary approaches. Based upon design preferences, it is understood that the specific order or hierarchy of steps in the processes may be rearranged. Some of the steps may be performed simultaneously. The accompanying method claims present elements of various steps in a sample order, and are not meant to be limited to the specific order or hierarchy presented.

[00090] There may be many other ways to implement the invention. Various functions and elements described herein may be portioned differently from those shown without departing from the spirit and scope of the invention. Various modifications of these embodiments will readily apparent to those skilled in the art in view of present disclosure, and generic method defined herein may be applied to other embodiments.

[00091] All structural and functional equivalents to the elements of the various embodiments of the invention described throughout the disclosure that are known or later come to be known to those ordinary skills in the art are expressly incorporated herein by reference and intended to be encompassed by the invention.

[00092] The above description and drawings are only illustrative of preferred embodiments which achieve the objects, features and advantages of the present invention, and it is not intended that present invention be limited thereto. Any modification of the present invention which comes within the spirit and scope of following claims is considered part of the present invention. Furthermore, to extent that the term “include”, “have” or like is used in the description or the claims, such term is intended to be inclusive in manner similar to the term “comprise” is interpreted when employed as a transitional word in claim.

ADVANTAGES OF THE INVENTION

[00093] The present invention provides an optical biosensor which is highly sensitive and accurate for biological and chemical sensing applications wherein a coating of nano carbon materials attached with dendrimer molecules is disposed on etched fiber Bragg grating (eFBG).

[00094] The present invention further provides an optical biosensor which is used in detection of carbohydrate-protein interactions as an example of antibody-antigen interactions.

[00095] The present invention further provides an optical biosensor which measures a shift in Bragg wavelength as an output measurand wherein the shift in wavelength is very sensitive to small changes in optical properties such as refractive index of the nano carbon materials coated eFBG due to antibody-antigen interactions or carbohydrate-protein interactions.

[00096] The present invention further provides an optical biosensor where multiple gratings of different Bragg wavelengths can be inscribed on a single optical fiber to detect many specific antibody – antigen interactions simultaneously.

[00097] The present invention further provides an optical biosensor wherein a multivalent Dendrimer having multiple functionalities further provides a large number of active sites to increase the sensitivity in detection of antibody-antigen interactions or carbohydrate-protein interactions.

[00098] The present invention further provides a method of fabrication of an optical biosensor comprising of dendrimer functionalized nano carbon materials coated on eFBG.

[00099] The present invention further provides a simpler and easier method of coating of nano carbon materials on surface of eFBG wherein surfaces of nano carbon materials and eFBG are made hydrophilic.

[000100] Other Advantages of the present invention will be apparent from the description of the invention hereinabove.

CLAIMS

We claim:

1. An optical biosensor comprising of an etched Fiber Bragg grating (eFBG) having a plurality of coatings comprising of:
   a. nano carbon materials; and
   b. one or more dendrimer molecule(s);

   wherein the nano carbon materials are attached with the one or more dendrimer molecule(s) and coated onto surface of the eFBG.

2. The optical biosensor of claim 1, wherein the nano carbon materials are selected from a group comprising of Single Walled Carbon Nanotubes (SWNT), Graphene Oxide (GO).

3. The optical biosensor of claim 1, wherein the one or more dendrimer molecule(s) are selected from the group consisting of: carboxylic acid terminated poly(propyl ether imine) dendrimers, hydroxyl terminated poly(propyl ether imine) dendrimers, amine terminated poly(propyl ether imine) dendrimers, cyano terminated poly(propyl ether imine) dendrimers, carboxylic acid terminated polyamdioamine dendrimers, amine terminated polyamidoamine dendrimers, amine terminated poly (propylene imine) dendrimer, and hydroxyl terminated polyamidoamine dendrimers.

4. The optical biosensor of claim 1, wherein said biosensor measures a shift in Bragg wavelength ($\Delta\lambda_B$) as an output measurand or readout in detection of carbohydrate-protein or antigen-antibody interactions.

5. The optical biosensor of claim 1, wherein a shift in Bragg wavelength ($\Delta\lambda_B$) ranges from about 1 to 2 pm.

6. The optical biosensor of claim 1, wherein the dendrimer is a multivalent functionalised dendrimer having multiple specific active sites suitable to bind to specific binding receptors.

7. The optical biosensor of claim 1, wherein carbohydrate attached dendrimer binds to a specific protein in detection of protein-carbohydrate interactions.
8. The optical biosensor of claim 1, wherein multiple gratings of different Bragg wavelengths are written on a single fiber to detect many specific antibody – antigen interactions simultaneously.

9. A method of fabrication of optical biosensor comprising the steps of:

i. optionally decladding the FBG by an etching process to produce eFBG;

ii. treating surface of the eFBG or FBG with a suitable reagent to make the surface of the eFBG hydrophilic;

iii. coating nano carbon materials onto the surface of the hydrophilic eFBG made in step (ii); and

iv. attaching dendrimer with the nano carbon materials coated eFBG made in step (iii).

10. The method of claim 9, wherein the suitable reagent in step (ii) is a base comprising of NaOH.

11. The method of claim 9, wherein the nano carbon materials are selected from a group comprising of Single Walled Carbon Nanotubes (SWNT), Graphene Oxide (GO).

12. The method of claim 11, further comprising the step of treating surface of the SWNT with a suitable reagent to make the surface of the SWNT hydrophilic.

13. The method of claim 13, wherein the suitable reagent in step is an acid comprising of H_2SO_4, HNO_3 or mixtures thereof.

14. The method of claim 10, further comprising the step of functionalising dendrimer with at least a suitable molecule to produce multiple active sites on surface of the dendrimer.

15. The method of claim 15, wherein the suitable molecule is a carbohydrate.

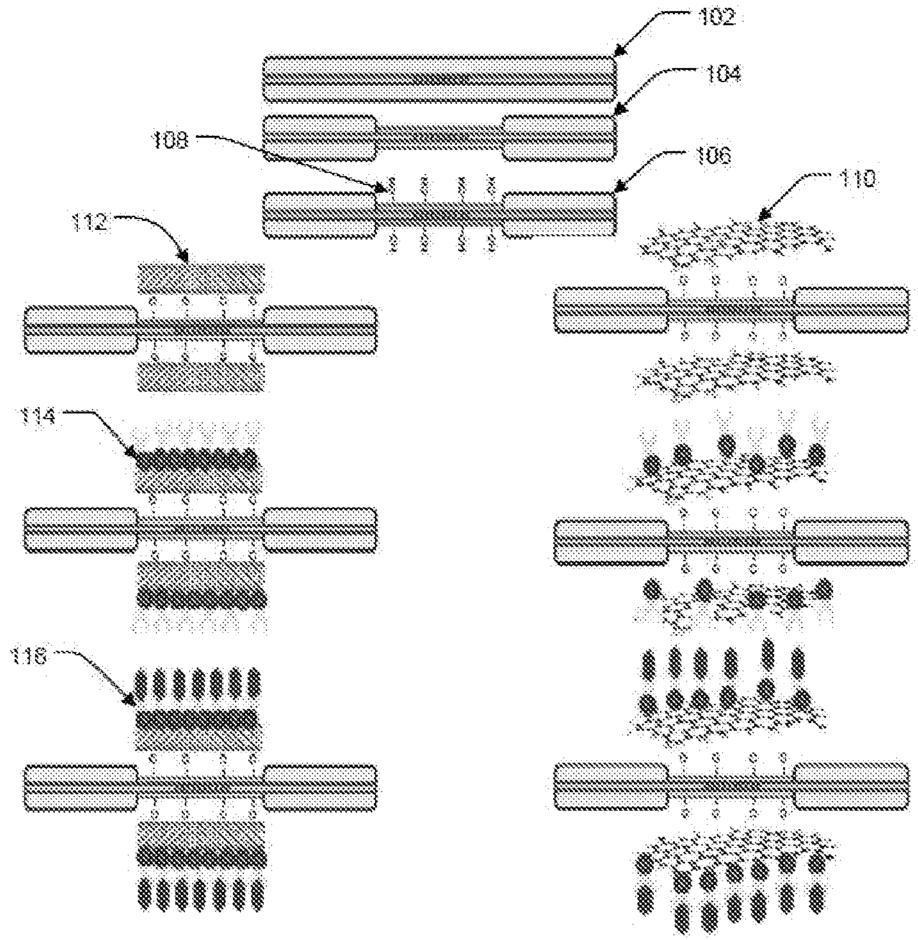

Figure 1(a)

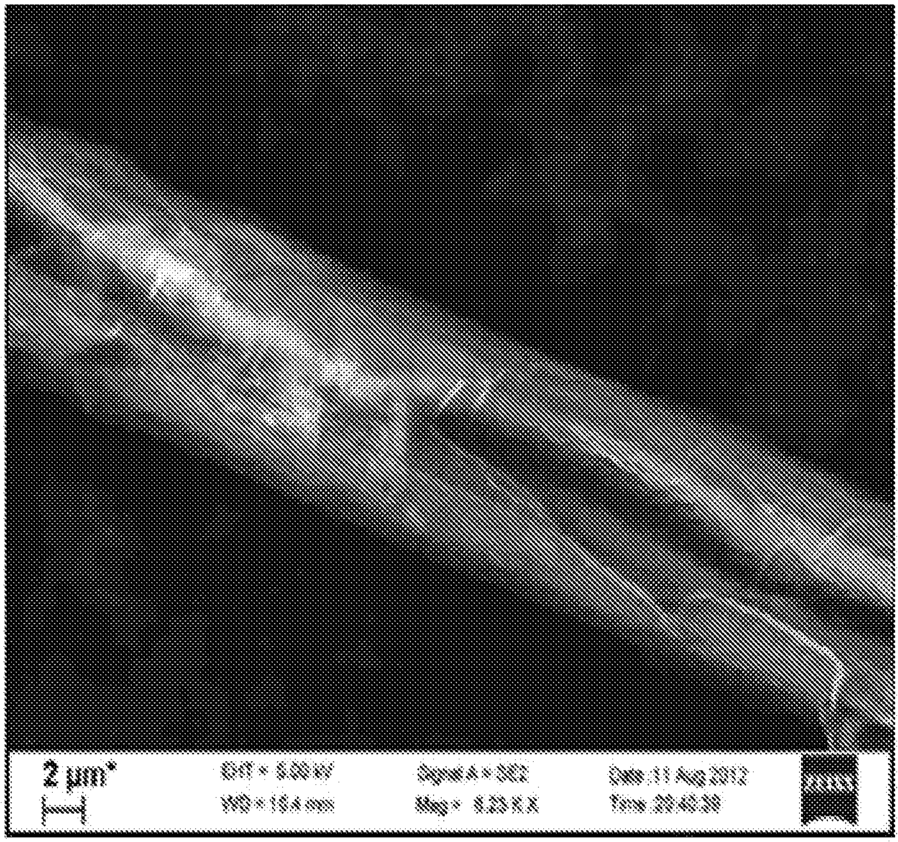

Figure 1(b)

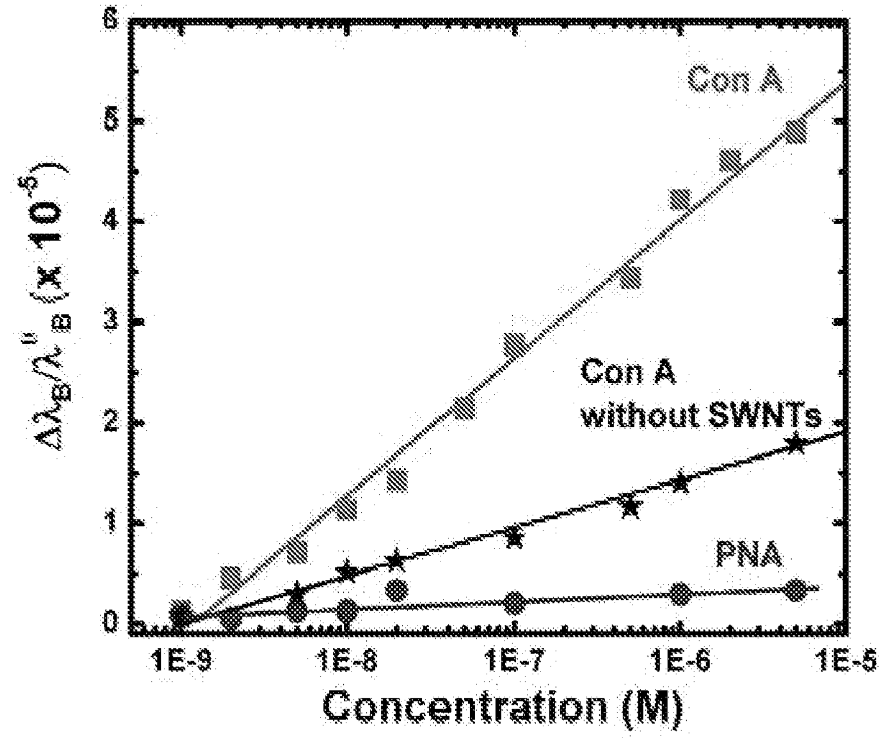
Con A
Con A
without SWNTs
PNA
0
1
2
3
4
5
6
1E-9
1E-8
1E-7
1E-6
1E-5
Concentration (M)
Δλ_B/λ^0_B (x 10^-5)

Figure 2

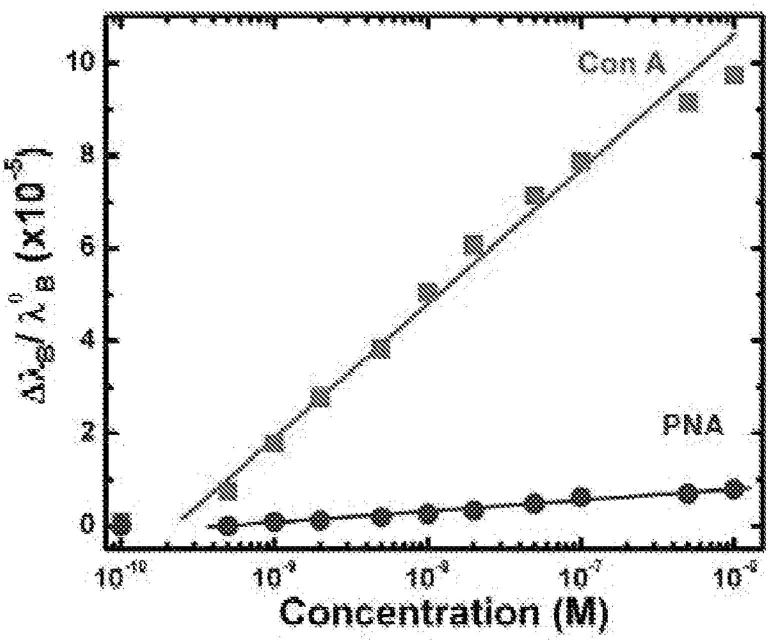

Figure 3

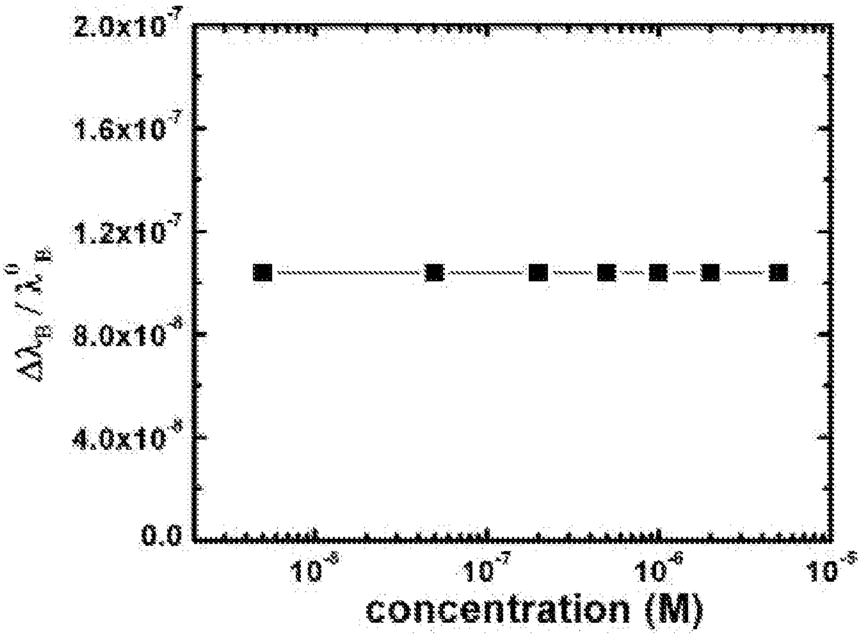
2.0x10-7
1.6x10-7
1.2x10-7
8.0x10-8
4.0x10-8
0.0
Δλ_B / λ^0_B
10-8
10-7
10-6
10-5
concentration (M)

Figure 4

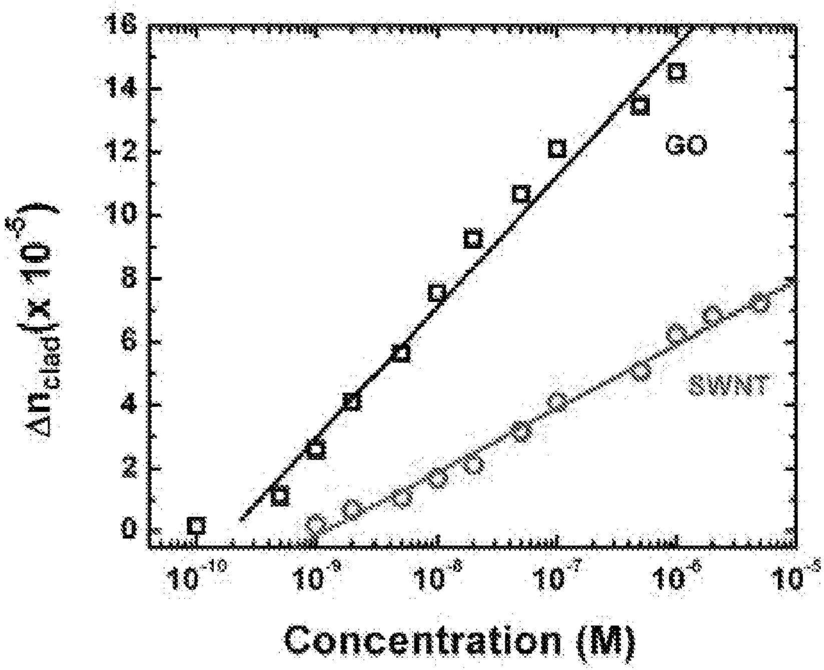
16
14
12
10
8
6
4
2
0
Δn_clad(x 10^-5)
GO
SWNT
10^-10
10^-9
10^-8
10^-7
10^-6
10^-5
Concentration (M)

Figure 5

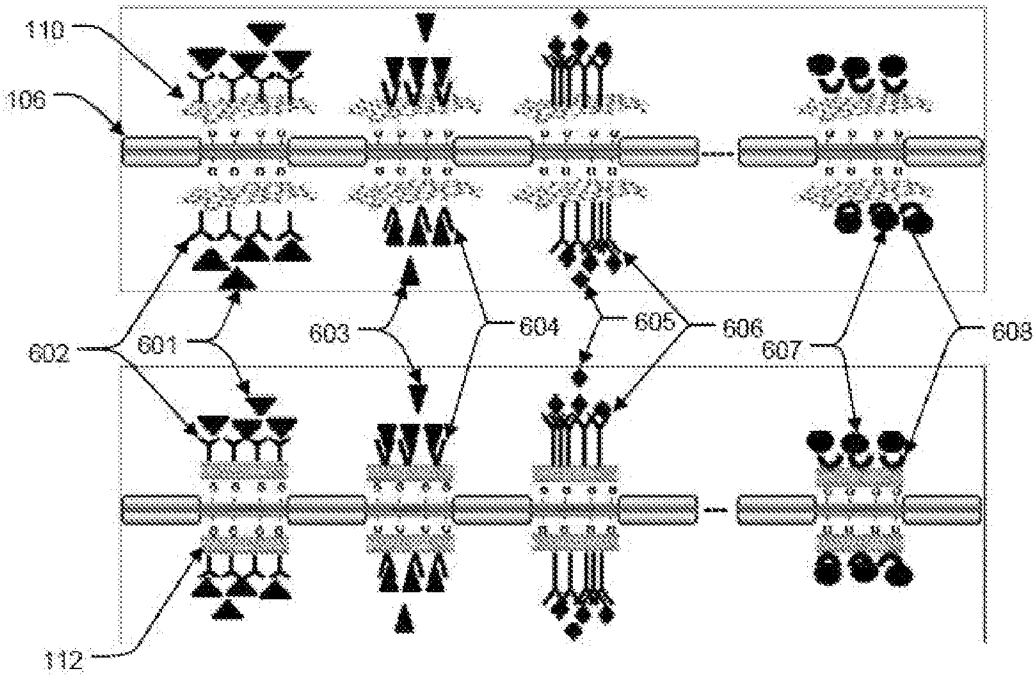

Figure 6