A REVIEW OF PHENOMENA BEHIND OPTICAL BIOSENSORS

Ramesh BabuKodati

Department of Science and Humanities, St. Martin's Engineering College, Secunderabad-500100, Telangana

Abstract: The paper is intended to through light on opticalbiosensors used for various sensing purposes. It provides fundamentals of a sensor and its parameters. It explains the basic phenomena behind optical biosensors qualitatively. It also includes recent advancements in the field of optical biosensors giving emphasis on the plasmon enhanced electric field phenomenon. It creates interest to carry out research in the field of Biosensors.

Key words:Plasmon, florescence, Transducer, sensor chip

1. Introduction:

A sensor is a device which measures a change in one physical parameter in terms of magnitude of a second different parameter which can be measured more conveniently and perhaps more accurately. For example thermometer measures temperature in terms of the length of mercury column. When the temperature of room increases say by two degree centigrade, one hardly feels any change, but this device can tell how much change in temperature occurred by measuring the change in length which can be measured more conveniently and more accurately. Then it translates into temperature.Similarly a conventional glucometer which is commercially available in the market, actually, measures current and it translates the change in current in terms of glucose concentration in blood. Certain other devices like pregnancy kit measures a particular hormone in urine to confirm pregnancy while infectious biosensor kit examines the vapours because of cough to determine whether a person has tuberculosis or not.From the above examples one understands that sensors are of two groups: one group is a sensor which is quantitative, while the other sensor is purely qualitative.

2. Biosensor:A bio sensor [1] is any device that uses a specific biochemical reaction to detect chemical compounds in biological samples. Now a days we are polluting almost everything including air, water or food which in turn causing several diseases. So, it is very important to detect them and it is possible only with biosensors. In global market there is a huge potential and requirement for biosensors. That is why it is very important tostudy the concept of biosensors.

2.1. Components of a Biosensor: In general any type of biosensor will have the following components.

(a.)Sensor surface: It will be a surface which interacts with biological molecule to be examined.

(b).Analytes:Analytes are chemical or biological or environmental elements or molecules that need to be sensed. They can be a natural hazard like pesticides, environmental pollutantssay carbon dioxide, methane; blood or urine analytes say glucose, cholesterol,ions may be bilirubin, vitamins.

(c). Bio-recognition element or bio-receptor (BRE): It leads to specific attachment with particular analyte and does not bind to anything else. This BRE or receptor is something which needs to be fixed on the surface of the sensor so that the analyte comes and interacts with it. It can be enzyme based. Enzymes are molecules which increase or decrease the reaction rates in the body metabolic processes. So, something comes and binds on the enzyme say a ligand. The binding gives a by-product such as change in pH or a refractive index change or maybe change in mass or heat transfer or can change to current . But whatever change can take took place here we want it to be readable.

(d).Transducer:Transducers convert one form of energy to another form of energy. But, in a sensor it basically transforms the binding phenomenon or interaction phenomenon between the analyte and the BRE into a measurable output signal which can be an optical signal or electrical signal or acoustic or maybe change in dimensions or change in length as in thermometer. A transducer can be electrochemical, a piezoelectric, thermo metric or optical. Electrochemical can further be divided in amperometric, conductometric, potentiometric. Measurement of a change in current is called amperometric, measuring a change in conductance is conductometric and measuring the change in potential drop is potentiometric sensing. . In piezoelectric, there is a change in mass which leads to change in the vibration frequencies. In thermometric one measures the change in heat. In the optical transducers, basically one measures the change in optical signals or change in optical properties.

3.Fabrication of a biosensor chip:Various steps involved in the fabrication of the chip are shown in Fig.1. A cross linker is placed over a transducer surface. The job of the cross linker is to attach the bio-recognition element on top. If BRE is notdirectly get attached to the transducer surface then one need to put a cross linker. So a cross linker or a kind of adhesive is required to put this BRE(molecule) on the sensor surface. Now it can catch the analyte, but then there are lots of spaces on the surface which are not filled. So, it is very important to block all these sites so that nothing can go and bind on here. So anti-fouling agent is used.The job of this agent is to block all the nonspecific binding sites. Now the surface has only BREs open for binding, everything else is closed.

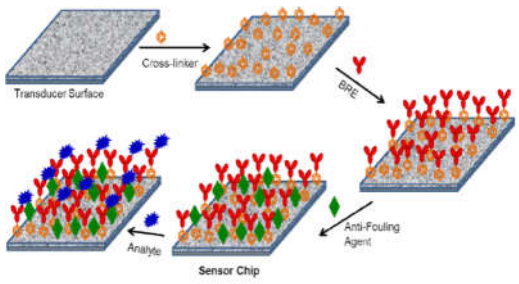

Fig.1 Biosensor chip

And now an analyte goes and binds on these sites. That is how a very good sensor surface is formed[1].Among all types of biosensors optical biosensors are very small in size. They are flexible, they are very fast. They are safe because there is no electrical device to interconnect.

And, they have very good biocompatibility. However the disadvantage is that the optical signal may not be strong enough many times.

3.1. Parameters of a biosensor:

(a) Sensitivity: Sensitivity is the value of the response per analyte concentration. That means it represents the slope of the curve drawn between response and concentration of analytes.
(b) Response time: It is time necessary for having about 95 percent sensor response. That the time to have a confident signal from the sensor.
(c) Resolution: It is the **c**apability of the sensor to monitor the smallest difference in the analyte parameter. Actually the resolution is not the characteristic of the sensing probe but that of the detector.
(d) Repeatability: It is the number of times a sensor can give the similar result in similar conditions of operation.

4.Optical biosensors: Optical biosensors are generally based on measurements of changes in any of theoptical propertiesviz., absorbance in chemical reaction, reflectance and transmittance, refractive index, phase shift, polarization or light energy. So, it can either be a fluorescence based sensor or Raman based sensors.Let us view the phenomena behind optical sensor qualitatively.

4.1. Surfaceplasmonresonance(SPR)[2,3]: Plasmons are basically quanta of longitudinal oscillations of free electrons in metal and we call it plasmon which is synonymous to plasma of charges. That means a metal has solid positive background with free electrons moving randomly and the oscillation is called plasmon oscillation.Thefreeconductionelectronsofametalareinfluenced bya time-dependentforceoppositetothatofthechangingelectromagnetic fieldoftheincidentlight. Theresulting motionoftheelectronswillbeoscillatory,but180°outofphase duetothechargeoftheelectron,andwithdampeningcaused byOhmiclosses.Likealloscillators,theconductionelectrons haveacharacteristicfrequencyknownasthe plasmafrequency.Theplasmafrequencydependsonthedensityofelectrons(n)andtheeffectivemass(m_e),It reflectshow easily the electrons move with respect to incident light. The free electrons in abulk do not oscillate against the restoring force.Ifthelighthasafrequencyabovetheplasma frequencyi.e., intheultraviolet(UV)rangeformetals, the lectronswillnot oscillateandthelightwillsimplybetransmitted or absorbed due to inter band transitions. If the light has a frequencysmallerthantheUVrange,theelectronswilloscillate 180°outofphasewiththeincidentlight,which causesastrong refection.The combination of plasma frequency and interbandtransitionsgivesmetalstheircharacteristiccolour. Theoretically it can be shown that whenthefrequencyoflightisgreater than plasma frequencythedielectricconstantispositiveand the lightistransmitted. Whenthefrequencyoflightislessthantheplasmafrequency,therealpartofthedielectricconstantisnegative,andthemajorityofthelightisreflected. Thus thedielectricconstantdecideswhethermetalelectronscanoscillateor not atthegivenfrequencyoflight. In a bulk metal electron waves have longitudinal nature and are difficult to excite by incident light. Surface plasmons have the transverse nature oscillations and as a result there is a wave which is propagating along the interface and the field associated to this wave is maximum at the interface and it decays exponentially in both the media termed as evanescent wave.

If the bulk metal is reduced into the thin film, oscillation will exist only at the surface and the corresponding propagating charge waves called as surface plasmonpolaritons(SPP) or evanescent waves.The interface between metal surface and surroundings limits frequencies of oscillating electrons. The wave vector or momentum of charge wave will be always greater than that of massless photons.So extra momentum is provided to photons by propagating them through prism or using grating and SPP are excited. At a specific angle i.e. at resonance a dip is observedin the reflection or transmission spectrum.

The frequency dependence of SPP on thedielectric constant at the interface makes the bulk plasma oscillations into a useful transducer for a sensor. The local electromagnetic field resulting from the charge oscillations of the SPP extends ~100– 200nmintothedielectric. Within this distance, the change of the local environment causes the dielectric constant to differ and the SPP frequency will shift from that in air. The problem with SPP sensors is that they require suitable glass prism or grating arrangement

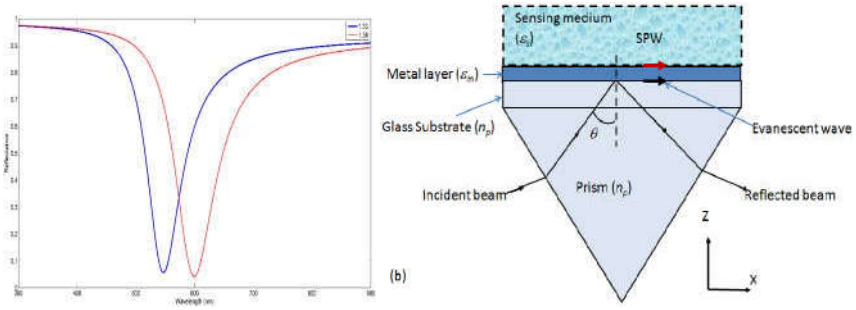

Fig.2(a)Red shift of wavelength and (b) SPR Bioensor

The SPR sensor in kretchmann configuration(where there is very thin metallic film is placed between prism and dielectric or sensing medium) is shown in fig.2(b). When light is incident, there will be dip in reflection spectrum at a particular angle as shown by blue curve in Fig.2(a). If the sensing medium contains analytes the dip shifts as shown by red curve because of change of dielectric constant or refractive index which is known as red shift. This shift is a measure of sensitivity of abiosensor.

4.2. Local surface plasmon Resonance(LSPR)[4-7]:

TherestrictionsofSPPcanbeovercomechangingatwo- dimensional(2D)metalfilmintoazero-dimensional(0D)nanoparticle.Plasmon resonances of metal nanoparticles are basically quanta of charge density oscillations of metal nanoparticles. When metallic nanosphere whose size is smaller than wavelength as shown in the Fig.3 is subjected to a time varying electric field, if the electric field is pointing in upward direction, the electron cloud would slightly shift to the downward direction, leaving behind a dipole here with electrical field in the opposite direction as shown in Fig.3. If applied field is varying at a particular frequency then this dipole will also be oscillating at the same frequency.

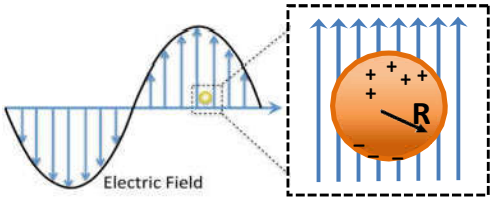

Fig.3. Effect of applied field on nano particle

This kind of oscillations, when the frequency of the incident light becomes equal to the oscillation frequency of conduction electrons is attributed as localized surface plasmon resonance. When there is resonance, light will get absorbed by theseplasmons. This absorbance as well as the resonance frequency is highly dependent on the size, shape and environment of the nanoparticles.As the geometry of the nanoparticle supplies the additional momentum LSPR can be directly excited by the incident field without theneedforadditionalgratingsorprisms. As the change of refractive index of medium surrounding nano particle causes change of resonance frequency, LSPR can be used for sensing. During sensing the light which is scattered from the nanoparticles is captured by the camera. So we can see different colors, which are pertaining to different shapes and sizes of the nanoparticles and here it can seen that they can correspond to different resonances for different dimensions.

4.3 Surface enhanced Raman spectroscopy (SERS)[8-11].If a molecule is shined with a laser, most of the light gets scattered from this is at the same wavelength or frequency of that of the laser. This is called Rayleigh's scattering. But, very small fraction of this light, which is getting scattered from this molecule will have frequencies which are either smaller or larger than the frequency of laser incident and that small shift is very important which is known as Raman shift. It means that in Raman's spectroscopy, a green light have slightly red shifted or slightly blue shifted indicating wavelengths or spectral lines which will be either red shifted or blue shifted in the scattered one. This small change in the frequency is a fingerprint of molecular bonds and crystalline structure of that molecule. Measuring the Raman spectra of different materials, one can say which one is what material. But the problem is that out of 10 million photons only one gets Raman scattered. So in a 10 million-Watt laser, only 1-watt power results from Raman scattering. But if the molecule is brought close to the metallic nanostructure, it will experience enhanced electromagnetic field and can enhance its optical signals - its spectroscopic signals. This is called SERS. It is not just the case for enhancing the Raman signal, it can enhance the fluorescence signal, can enhance absorption - all these things. Suppose if a fluorescent molecule is brought close to the metallic structure, it will experience about 100 times or 1000 times enhanced electromagnetic field and the signal will get enhanced. This is called fluorescence enhanced mechanism FEM. Thus SERS continues to be attractive technique for chemical sensing, biomedical applications and has advantages including unique spectral signatures of analytes ,easy operation without complicated sample preparation.The group of molecules which does not have permanent dipole moment will show Raman signals, while others will show fluorescence or IR signals. Raman spectroscopy can be used to stop fraud. For example comparing Raman spectra for calcium carbonate - natural pearl , faux pearl one can say whether pearl is pure or not[12]. The components of drug tablet can be determined by identifying colors of ramanspectra images. The two constraints of SERS are the molecule under investigation has to be very close to metallic structure and it has to be small. However SERS is also useful to detect bacteria of size of few micron and not very close to nano particle by a method known as non-direct sensing. In this method the bacterium is bonded to BRE which is on the top of cross linker attached

to surface. Now the signal from cross linker is assessed which changes due to binding of bacteria with BRE.

5. Various sensors and their mechanisms: Based on the above phenomena various sensors can be fabricated.

5.1 Localized surface Plasmon enhanced electric field(LSPR) biosensor: LSPR is like a dipole sitting at the center of the nanoparticle and this was oscillating. The oscillating dipole there causes the enhancement of the electric field in the vicinity of the nanoparticle on light incidence.The enhanced electromagnetic fields can be used for enhancing optical signals. It is possible to develop a variety of nanostructures using silver and gold, and they have different optical properties. So, basically making different structures one can tune the optical properties at desired way.During sensing the light which is scattered from the nanoparticles is captured by the camera. So we can see different colors, which are pertaining to different shapes and sizes of the nanoparticles and here it can seen that they can correspond to different resonances for different dimensions

We can have nanoshells, where one have a core and then on top of it one can have a shell. For different thicknesses of the core and shell one can have different resonance peaks and also the colors. We can have single silver particle biosensors. Fig.4 shows one single nanoparticle (i)before and (ii) after exposure of 10 Nanomolar streptavidin that a shift of about 13 nanometers occurs by adding streptavidin. These measurements were collected in a nitrogen environment. So, that is how nanoparticles are used for localized surface plasmon resonance space sensing[13].

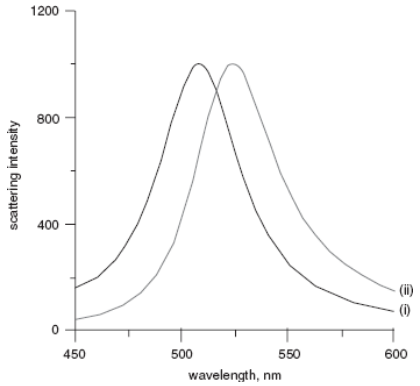

Fig.4. Single Ag nanoparticle biosensor

5.2 Surface enhanced fluorescence(SEF) biosensor: 'Fluorescence' was named by George Gabriel Stokes when he saw mineral fluorite which lights up when illuminated with ultraviolet and that is how he named it fluorescence. Actually if a molecule is shined light of certain wave length, it gets excited to higher state and after certain time, it comes back to the ground state emitting light. That is called fluorescence. It is also possible that it may go from, say, a singlet state to triplet state and then to ground state which something is called phosphorescence. The fluorescence time is much smaller than phosphorescence time. Common Flourophores are Flouroscences, BOZ 7, RHODAMINE 6G.

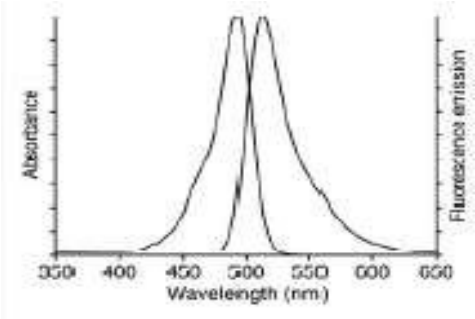

Fig.5. Fluorescein molecule red shift

For example if Fluorescein molecule is shined with certain light, peak absorption takes place at about 575 nm and gets adsorbed there. After this kind of vibrational relaxation, it comes down emitting light of wavelength 525 nm which is larger than the absorption wavelength as depicted in Fig.5. This is called fluorescence. Molecules of Various proteins and compounds, say for example ATGC in DNA, tryptophan present in human body also have certain fluorescence. In case of a fluorescence biosensor we attach this fluorophore to the receptor via some linker. The linker provides the means for triggering the change in the fluorescence of the attached fluorophore. What happens actually is when an analyte is brought near this receptor, it leads to change in the fluorescence. If we bring this kind of fluorophore near a metallic nano structured surface - plasmonic surface, then its fluorescence can be enhanced and that is why it is called surface enhanced fluorescence[14,15]. In a pH biosensor, the change in pH on introducing analyte can be estimated by measuring the change in intensity of fluorescence as shown in Fig.6.

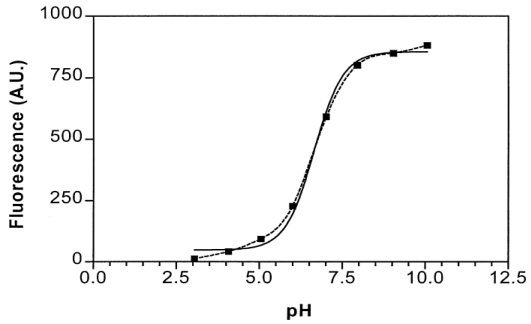

Fig. 6. pHbiosensor graph

5.3 ESP-LSP coupling based ultra high enhancementbiosensor: If there are surface plasmons then enhancement of the electromagnetic field on the surface will be up to order of 10^2. If there are LSP, the enhancement of field will beabout 10^3. The ESP-LSP coupling can enhance field by 10^5. Supposing this kind of structure, these are extended plasmons coupled to localized plasmons and slightly displaced, say about 1 or 2 nanometres, we find a high enhanced electromagnetic field here which is called plasmonic hotspot. And if we bring a molecule here, basically you can enhance its electromagnetic signal. So, the molecule placed experiences an electromagnetic field of about 10^5 in the hotspot.

If a fluorescent molecule is placed at the hot spot, it experiences enhanced fluorescence intensity and do not see any enhancement when it go off ESP with fluorescence intensity going down[16,17]. Thus there is better control over fluorescence by controlling the ESP

resonance angle and hence better sensitivity. The diagram of ESP-LSP coupled biosensor is shown in Fig.7.

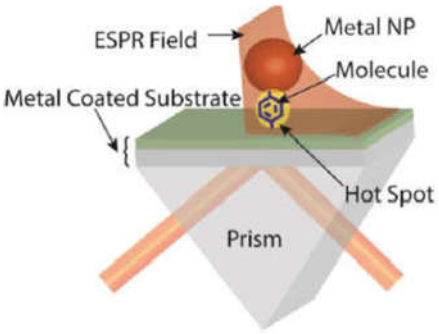

Fig.7. ESP-LSP coupled biosensor

5.4 Colorimetric biosensors; In this kind of biosensors, it is the change of colour when the interaction occurs. So, it is a kind of sensor where just by seeing - using eye, one can say that this is the particular analyte that is making the change here. For example, the pH strip is dip into the solution, it changes the colour and then we know how much pH it occurred. So, mostly it is qualitative[l8].

5.5Fibre opticFabry-Perot Interferometer(FPI) based biosensors:Fabry-Pérot interferometer basically is a thin film which has two mirrors facing each other.When a ray of light enters to it, it gets partially reflected from one boundary and then transmitted through the medium and then move back and forth between mirrors.Because of multiple reflections, an interference pattern of more contrast and visibility than that of Michelson Interferometer, is formed as it has a greater number of reflections.In case of fiber optic Fabry-Pérotbiosensor two small pieces of this opticalfiber are seperated a gap called Fabry-Perot cavity.The ends of fibre pieces have metallic coatings which act as mirrors.

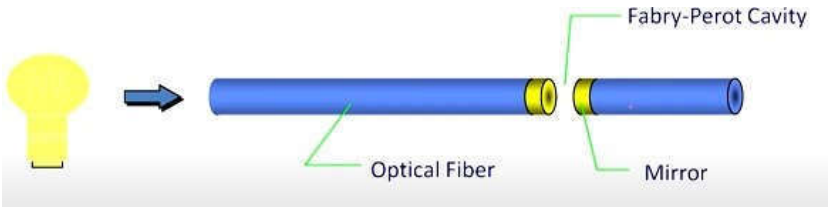

Fig.8 Fibre optic FPI biosensor

This can be used for detection of ammonia. When the tip of optical fiber which was sending collimated light passes through a polymer layer of Polydimethylsilane(PDMS) exposed to various concentrations of ammonia, say from 0 to a 500 ppm for 30 seconds, the interference fringe showed a shift in wavelength. For the exposure of 60 seconds the shift is found to be larger[19]. Fig.9 illustrates shift of fringe pattern on detection of Ammonia.

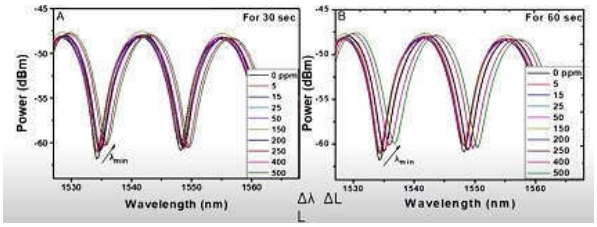

Fig. 9 FPI sensor detection of Ammonia

6. **Recent Trends in the area of biosensors:**The recent advances are in COVID-19, including the insights in the virus, the responses of the host cells, the cytokine release syndrome, and the therapeutic approaches to inhibit the virus and alleviate the cytokine storm[20]. Detection of biomarkers has raised much interest recently due to the need for disease diagnosis and personalized medicine in future point-of-care systems. Among various biomarkers, antibodies are an important type of detection target due to their potential for indicating disease progression stage and the efficiency of therapeutic antibody drug treatment[21].

7. **Conclusion**: Colorimetricbiosensors are simple and can be constructed easily but they have low sensitivity. The ESP-LSP coupling can enhance electric field but not that much as high enhancement as in case of SERS. On the other hand Fluorescence enhanced biosensors and SERS are widely used in various fields because of their high sensitivity, low cost and abundant availability. Among available biosensors, SERS biosensors are relatively new and have outstanding capability of performing and imaging at larger penetration depths when compared other optical biosensors. But SERS instruments are large in size and expensive so they have limited applications. Nowadays with the availability of commercial benchtop or palm sized Raman readers, plasmon enhanced SERS technology is developing towards compact SERS biosensors. Recent research [19,21] focus is on SERS substrates because of their flexibility, conformability and easy uptake of analytes. In the future, plasomonics are developed for not only enhancing the sensing signal but also to develop new sensing schemes based on fluorescence and SERS devices.

References:

[1] Sachin Kumar Srivastava,Onlne NPTEL course on" Optical sensors",2019
[2] N. W. Ashcroftand D. N. Mermin, Solid Stat. Physics,
Cengage Learning, 1976.
[3] T. W. H. Oates, H. Wormeester and H. Arwin, Prog. Surf.Sci., 2011, 86, 328–376.
[4] J. R. Lackowicz, Plasmonics, 2006, 1, 5–33.
[5]Hutter&Fendler, Adv. Mater. 16, 1685-1706 (2004)
[6]Pelton et.al., LaserandPhotonicsReview3, 136-159(2008)
[7] Maier, Plasmonics: Fundamentals and Applictions, Springer, 2007
[8] E. C. Le Ru, E. Blackie, M. Meyer and P. G. Etchegoin, J.
Phys. Chem. C, 2007, 111, 13794–13803.
[9] 8 S. Y. Lee, L. Hung, G. S. Lang, J. E. Cornett, I. D. Mayergoyz
and O. Rabin, ACS Nano, 2010, 4, 5763–5772.
[10] F. S. Ameer, W. Hu, S. M. Ansa, K. Siriwardana,
W. E. Collier, S. Zou and D. Zhang, J. Phys. Chem. C, 2013,
117, 3483–3488.
[11] M. Li, S. K. Cushing, H. Liang, S. Suri, D. Ma and N. Wu,
Anal. Chem., 2013, 85, 2072–2078

[12] Lewis, et.al.,Handbook of Raman Spectroscopy: From the Research Laboratory to the Process Line, Marcel Dekker, New York: 2001
[13] Srivastavaet al., Analyst, 140, 3201 (2015)
2010, 10, 813–820.
[14] R.P.V. Duyne, J. Fluorescence, 14, 2004 flourescene
[15]Srivastavaet al., Optics Express, **25**, 4761 (2017)
[16]Li, et al. J. Phys. Chem. C 119, 19382 (2015)
[17]Li, et al. Nanoscale 8, 15658 (2016)
[18]Mondal et al., Front. Microbiol.9179 (2018)
[19]Kanawade et al, JOSA B, 36, 684(2019)
[20]X. Li, W. Zeng, X. Li, H. Chen, L. Shi, X. Li, H. Xiang, Y. Cao, H. Chen, C. Liu, J. Wang, J. Transl. Med. (2020)
[21]Wei Xu,et al. Int J Mol Sci.,v.21(1); 2020 Jdoi: 10.3390/ijms2101013