HIBISCUS PUBLISHER

JOURNAL OF BIOCHEMISTRY, MICROBIOLOGY AND BIOTECHNOLOGY

WEBSITE: http://journal.hibiscuspublisher.com

Label-free Photonics Biosensor Transducing Nano-Biological Events

Amir Syahir*

Department of Biochemistry, Faculty of Biotechnology and Biomolecular Science,
Universiti Putra Malaysia, Serdang 43400, Malaysia

*Corresponding Author: amirsyahir@upm.edu.my

HISTORY

Received: 12th of April 2014
Received in revised form: 14th of June 2014
Accepted: 21st of June 2014
Available online: 3rd of July 2014

KEYWORDS

label-free detection methods
nanosensor
protein interactions
nanobio-technology
proteomics

ABSTRACT

Label-free detection method in detecting molecular interactions is one of the most successful innovations in strengthening molecular biological research. The realization of label-free technique has been greatly advanced by the combination of knowledge in material sciences, computational design, and nanofabrication. This rapidly growing new technique is aiming at providing data without the intervention of any label molecule. Here we present a brief overview of photonics label-free techniques in transducing nano-biological events.

Introduction

Nearly two decades has passed since the first draft of the human genome had been published in June 2000 that leaves a remarkable milestone in the history of science [1,2]. Since then, the genome of many other living creatures has been successfully read, and numerous information about the genes can be extracted and extrapolated in order to understand biological processes. One of the most powerful analytical tools to be addressed in these fields is the DNA microarray technology, which can analyze mRNA transcript levels expressed under various conditions [3-6]. However, it is known that the mRNA expression level and the corresponding protein abundances does not always correlate with each other. This is due to the changes in translation rates and protein lifetimes [7,8]. Besides, researchers have no clue in predicting protein activities from the genomic information collected. This is because, the analysis of mRNA expression, as well as DNA sequence, does not provide any information regarding to protein functions, their interactions, activities, three dimensional structures and post-translational modifications such as proteolysis, phosphorylation, glycosylation, methylation, and alkylation. Therefore, post-translational produced proteins need to be analyzed directly in order to obtain such information. Such challenges are now being classified as -omics studies such as transcriptomics, proteomics, and metabolomics.

Studying protein characteristics and their molecular interactions can provide an important route to investigate protein networks in living cell. It can also postulate the functions of newly discovered genes or proteins, thus, holds great value for understanding disease mechanisms [9-11] and provides suitable diagnostic for global-threatening diseases [12,13] (Figure 1). To date, works have vastly being done in these areas (proteomics studies) in order to develop simple and noninvasive test that can indicate disease risk at early stage [14]. One of the keys that plays important role in these research areas is nanobio-sensor technology. This technology provides analytical tools upon knowing the abundance, and also the qualitative characterization of a particular biomolecule. Therefore, the development of biosensor technology is crucial, and is expected to meet the needs of analyzing protein behaviors (interactions, activities, etc.) accurately and effectively.

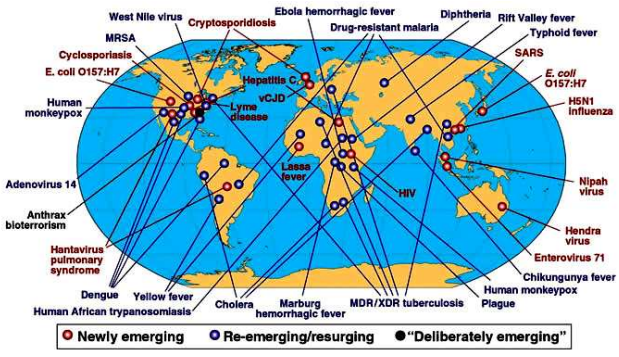

Figure 1. Global examples of emerging and re-emerging infectious diseases. Red represents newly emerging diseases; blue, re-emerging/resurging diseases. Figure was taken from literature 12 and republished with permission.

Nanobio-sensor technology

A biosensor is a device that designed to detect or quantify a nano-bio-chemical molecule such as metabolites, nucleic acids, protein, viruses and nanoparticles. It is a self-contained integrated device [15] that can provide quantitative or qualitative analytical information, in which the biorecognition agent interacts with a target at interfaces thus conveying the information to some physical transduction elements. In biological research involving protein interactions, many biosensors used are solid-supported and affinity-based, in which surface-immobilized capture agents are used to capture targeted analyte selectively and induce some physical changes at localized surface.

This change can be made in a variety of ways because the presence of target protein has a variety of physical significance. For example, nano-electrical biosensors detect electrical charge changes at the localized surface, thus relying on the measurement of current or voltage to detect the bindings or interactions. Others would be subjected to light absorbance / transmittance changes, surface tension changes, and so on.

There are two main purposes of using a nanobio-sensor [16,17],

i) To detect or quantify biomolecules abundance. Jeewon Lee *et al*. reported the use of quantum dots along with virus particles for detecting ultralow levels (unit) of the protein marker relevant to heart-attack patients [18]. This allows the detection of the abundance of a specific biomarker that would lead to further disease diagnosis. Rica and Stevens used gold nano particles that grew on ELISA technique to make cancer marker at the level of 10^{-18} M visible to the naked eye [19]. These are the examples of quantitative analytical information.

ii) To study the characteristics and properties of a particular biomolecule (protein) such as its kinetic behaviour, physical and chemical characteristic, reactions capability, and interactions networking. Igor Sokolov *et al*. [20] has successfully studied the differences of cilia (cell surface brushes) behaviour in normal and cancerous cell using atomic force microscopy (AFM) technique. They found that the cilia of cancerous cell is somewhat differs in length and concentrations in compared to the normal one. This would leads to the characterization of some specific behaviour of a protein on cell surface to differentiate normal cell from the cancerous one. The later one is qualitative analytical information. Thus, depending on these specific purposes, various types of biosensors are being utilized in these two main avenues of nano-biological research.

It is important to realize that the advances in biosensor technologies for *in vitro* diagnostics does have the potential to transform the practice of biological science, medicine and bioterrorism defense. To date, viruses [21-24], disease biomarkers [25-27], cancer related gene [28], bacteria [29,30], dangerous substances/chemicals [31,32], and protein networks [33] have been studied or confirmed thanks to the development or various biosensor technologies. Figure 2 depicts the numbers of scientific literatures and articles published during a period from 1982 to 2013, suggesting that a lot of progress in biosensor field have been made.

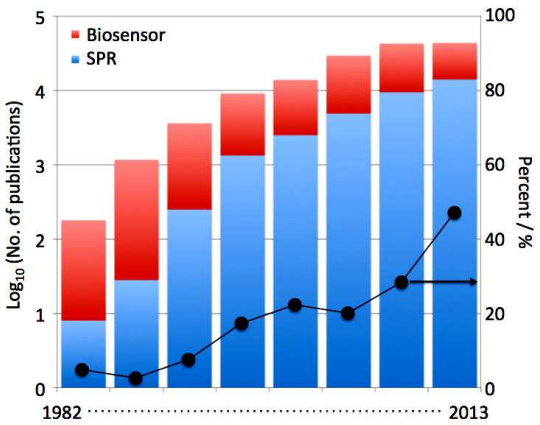

Figure 2. Number of scientific publications for label and label-free biosensor from 1982-2013 (four years period each). The survey was conducted using the *Google Scholar* search engine.

Despite rapid progress in the biosensor field, there is still no general sensing platform that can be ubiquitously applied to clinically detect the constellation of biomolecules in variety type of samples (saliva, urine, serum, or cell lysates) with high sensitivity, large linear dynamic range, rapid, low cost and using less sophisticated devices. Major limitations such as; i) unsatisfied sensitivity, ii) non-native signals interference iii) using too sophisticated devices (unpractical for certain conditions), iv) sensor delicateness (causing errors, high signal-to-noise ratio and doubtful signal), v) small or non-linear dynamic range, and vi) time consuming, forced researchers to keep improving fundamental concepts or exploratory rooms of biosensors by manipulating basic principles or combining the field of studies (e.g. merging of biosensor and electronics [34] or material science [35]).

Label-free detection

Methods in detecting molecular interactions have been studied since 1960's when radioisotope label has been rapidly used to quantitatively determine molecular reactions [36,37]. The system is sensitive, simple, yet bringing about some safety concern due to the usage of radioisotope material. Since then, many label system (other than radioisotope label) that detects molecular interactions have been developed such as Fluorescence/Fourier resonance energy transfer (FRET) [38,39], chemiluminescence [40,41], active enzymes with an easily detectable product, allowing facile target conjugation and convenient detection. These technologies are much safer [42], and being vastly used, producing a lot of sciences in molecular biology [43-45].

Thus, labeled type of detection e.g.with dye molecule or GFP [46] that chemically attached to target molecule can be found in most of the classical examples of biosensors. It can be site-specifically targeted and shows clear signal output that can be easily recognized [47]. During signal readout, the amount of label detected is expected to correspond to the amount of bound targets. However, several steps are required before the molecule can be put in the biosensing system. Target molecule and label molecule coupling reaction, and purification (with broad range of yield) are simply required. In addition, labeling a biomolecule may change its intrinsic properties [48,49].

Changing structure of any proteins caused by label probe or labeling process was not commonly reported in the literatures of protein sciences. However, by labeling a target protein or ligand, its chemical properties (if not physical) would also change. These could lead to different protein behavior during interactions. These are relatively minor problems upon studying DNA interactions, but they are a bigger concern for protein interactions.

Until recent years, labeled detection systems are being mainly developed in searching for more simple and efficient chemistries (in terms of probe size and labeling process), and more sensitive detection. But now, focus has been shifted to the development of so called 'label-free detection system', which requires none of these labels [17,50-55]. Although there have been only a few studies comparing molecular interactions read-out between labeled and label-free detection system, it is generally accepted that molecular interactions with label probes are involving non-native interactions, and could possibly leads to false information [46,57,58].

Label-free detection systems utilize only native physical properties of a target molecule such as weight, refractive index, and molecular charges. Therefore, the detection classes is divided into three; i) nano-mechanical system that are sensitive to weigh of the molecule, ii) optical system that are sensitive to the refractive indices, and iii) electrical system that are sensitive to molecular charges [53]. In a typical biosensing process, ligand is immobilized onto the sensor surface using chemical reaction or physical adsorption to the surface. Then, a protein solution will be allowed to interact with the surface-bound ligand. When interacts to the surface-immobilized ligand, the target protein will transfer its information in a form of mechanical, optical or electrical signals. These will be produced from the physical presence of the target proteins that changed the local environment of the sensor surface, thus, made the protein interactions detectable without any label probe. The main advantage for label-free detection over label detection is that more direct information can be acquired as the method only uses native proteins and ligands. Table 1 summarized the main advantages of the label-free detection system.

Table 1 Main advantages of label-free detection system compare to the detection with label probes. [a]This is a disadvantage of a label-free detection system.

Types of detection	Label-free	Label
Signal readout	Direct information	Indirect information
Interactions	Native	Non-native
Labeling process	-	Low yield labeling process
Sensing process	One step	Multi steps
Distinguishable to non-specific interaction[a]	Non-distinguishable	Distinguishable

Three key technologies

In order to develop nanobio-sensor technology, the following three key technologies must be tied in with each other: i) capturing agents, ii) surface chemistry, and iii) detection systems (Figure 3) [59,60]. Although many of capturing agent like peptides and proteins are commercially available, the other two key technologies (detection system and surface chemistry) are now in a rapid development. To date, many interesting approaches on both technologies to overcome problems and current limitations of the label-free methodology have been reported.

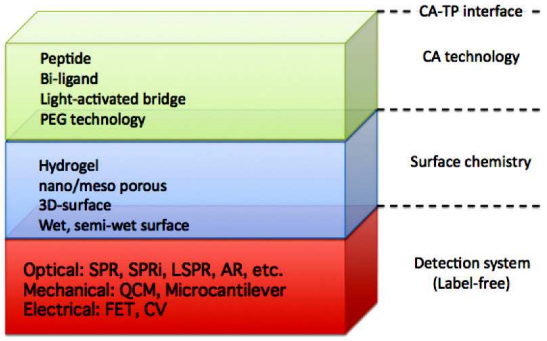

Figure 3. Three key technologies of developing biosensor; i) Capturing agents (CA), ii) surface chemistry, and iii) detection system. CA = capturing agent, CA-TP = capturing agents- target protein.

Detection system

The detection system used is divided into three classes, i) optical-based, ii) mechanical-based, and iii) electrical-based detection. While nano-mechanical-based methods and electrical-based detection methods are being reviewed elsewhere [61,62], herein we concluded several most used photonic-based methods (specifically using plasmonic approaches) in recent years.

Conceivably, the widespread label-free method in the detection of molecular interactions is either optical or photonic-based method. Several methodologies are have been already widely implemented and commercialized. Among these, the most prominent technique is the surface plasmon resonance (SPR) spectroscopy technique. Other specific methods are the localized plasmon using nanoparticle, surface plasmon using nanohole array [63], Raman scattering (especially with surface-enhanced mode), ellipsometry method [64], fabry-perot based technique using porous silicon [65-67], anomalous reflection (AR) of gold method [68], second harmonic generation (SHG) method [69,70], surface-immobilized gold nanospheres (SIGN) [71], and interference-based techniques for example using multilayered silicon surface [72]. The light used therein is in all range from ultra violet (UV), visible light, and into mid-infrared region. Molecular interactions are being monitored either by reflection, transmission or scattering (including dark-field scattering) of the light. The advantages of using optical methods over any other methods (electrical and mechanical) are that it is simple, fast, can be easily miniaturized, and the light does not influence the interactions that are being monitored.

Surface plasmon resonance (SPR) spectroscopy

The surface plasmons (SPs) are light waves that are trapped on the surface of a metal because of their resonance interaction with the free moving electrons oscillate on metallic surface. It is also known as surface plasmon polaritons (SPP), referring to the hybrid nature between light waves and free electron oscillation. It was first predicted by Ritchie in 1957, where plasma energy losses increases with the decreasing of metallic foil thickness [73]. Since then, many scientists such as Raether, Kretschmann and Otto have extensively studied the surface phenomenon.

In the interaction between light wave (irradiated) and free electrons at metal surface, the electrons are responding collectively by oscillating at resonance wavelength. The resonant interaction between the surface charge oscillation and the electromagnetic field of the light constitutes the SP and gives rise to its unique properties. The wave vector (k_{SP}) of SPs is always larger than the wave vector of the incoming photon (k_0). Thus, the irradiated light cannot directly excites the SPs wave [74]. In 1968, Otto introduced the idea of total internal reflection (TIR), where the SPs wave of a metal surface (thinfilm) can be excited with the help of a prism [75]. The metal thinfilm has been put close enough to the interface of the prism surface and air so that the evanescent field produced by the TIR can reach the metal.

The wave vector of the evanescent wave along the interface direction is $k = k_0 n\sin\alpha$, which can be larger than k_0 (when $n \sin\alpha > 1$). Thus, the SPs wave can be excited at some certain point where $k = k(\omega)$ as illustrated (Figure 4). However, this experiment is not easy to repeat since it requires a very precise control of the thickness of the air gap, which is in the order of a couple hundred nm. In 1971, Kretschmann demonstrated a modified Otto geometry [76]. The metal film was put in between the dielectric layer and air, and was kept thin enough (approx. 50 nm) so that the concept of TIR at the interface would still working, while the experimental setup is less requiring.

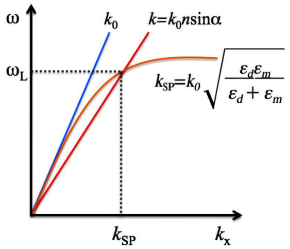

Figure 4 The dispersion curve for a SP mode shows the momentum mismatch problem between illuminated light (blue line) and SP modes (orange line). This problem must be overcome in order to couple light and SP modes together, with the SP mode always lying beyond the light line, that is, it has greater momentum (k_{SP}) than a free space photon (k_0) of the same frequency (dotted line). One way to overcome the problem is by using evanescent wave produced in TIR, which $k = k_0 n \sin\alpha$ (red line).

The SPR technique was first used as a sensor in 1980's, where it was used to detect gas [77,78]. Then, an imaging system of SPR (also known as SPRi or SPR microscopy) was invented in the late 1980's [79-82], and forged the 2-diamensional SPR chips that we see today. In 1995, S. Kawata pioneered the concept of grating-coupled SPR, where there is no longer need for a prism and TIR to excite the SPs [83]. Collective momentum of illuminated light produced by the periodic grating seems to match that of the SPs of metal. This concept allowed direct reflection method of SPR, thus the idea of high-throughput study using imaging system from a CCD camera device could be easily implemented [84-88]. The research progress of SPR is numerous and unbeatable by any other detection method [89-92]. The widespread of Biacore® instrument of SPR devices made the SPR one of the most reliable tools and sources of studying protein interactions especially in thermodynamics and kinetics analyses. Remarkable achievements of SPR analytical tools includes a peny-sized 2-dimensional micro-fluidic chip format detection [93,94], and the detection of small molecules like arsenic and calcium ions by manipulating molecular conformational changes [95,96], reflects its preeminence.

Localized plasmon resonance (LPR) of nanoparticle

The use of nanoparticle goes back to the time of the Romans (4th century AD), when it was used as decorative pigments in the glass of the famous Lycurgus Cup. Analysis of the glass reveals that it contains a small amount of metal crystals

(~70 nm) containing Ag and Au. However, it was not until 1857, when Michael Faraday announced a systematic study of the synthesis and colors of colloidal gold [100], that nanoparticle-based phenomenon began to draw attention. In 2000, Richard Van Duyne invented the concept of a "localized" SPR [101]. Since the pioneering work, thousands of scientific studies have been published that leads to today's understanding of the so-called localized plasmon resonance (LPR) of nanoparticle.

LPR is basically the same plasmon resonance wave as SPR. The only different is that LPR wave is localized around the metallic nanoparticle, rather than propagates along the metal surface like SPR. Advances in nano-fabrication techniques allow researchers to tune the LPR wavelength throughout the visible, near-infrared, and into the Mid infrared region of the EM spectrum, by varying the shape, size, and material of the nanoparticles that support the surface plasmons (Figure 5) [102-106]. This offers additional flexibility when designing LPR biosensing experiments [107]. LPR biosensing is usually observed by the wavelength-shift mode.

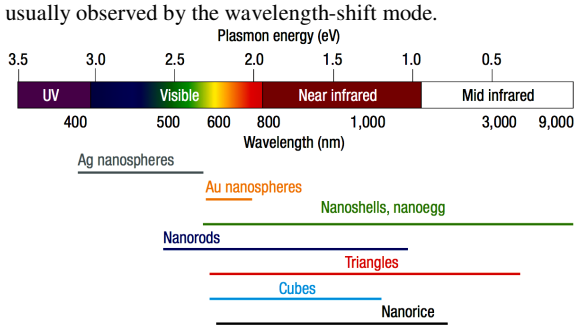

Figure 5. Range of plasmon resonances for a variety of nanoparticle morphologies. Republished with permission of Nature Publishing Group, from Nano-optics from sensing to waveguiding, Surbhi Lal *et. al.*, 1, 2007; permission conveyed through Copyright Clearance Center, Inc.

For sphere nanoparticle geometries, the simplest theoretical approach available for modeling the optical properties is the Mie theory [108]. For other geometries, different approaches have been devised, such as the theoretical calculation derived by Gans [109], which has been used for nanorod geometry [110], and discrete dipole approximation (DDA) method [111], which has been applied to the calculation of nanoprisms [112,113].

Applying the knowledge in fabricating nano-materials produced variety nano-shapes such as gold nano-spherical [114,115], and rod [116,117], pyramid [118-120], disk [121,122], ring [105,123], rice [124], cubes [125], and nano-star shape [126] that revealed their unique features in optical characteristic for biosensor applications. Excellent reviews in the past several years have described the fundamental concepts, preparations, and applications of the LPR biosensor of various nanometals [106,127-136]. It is somewhat difficult to put those nano-particles in a high-throughput biochip format. Nevertheless, Tamiya and colleagues have successfully demonstrated a 300 spots comprised biochip of LPR biosensor in detecting antigen-antibody interactions [137].

The LPR biosensing is a rapidly growing field. The future of this field looks exceptionally bright and promising with awaited wide range of applications from detections to rapid disease diagnostics. The lower fabrication-cost of LPR biosensor compared to SPR biosensor has made it a good candidate for commercialization. However, some limitations have bottlenecked the progress of LPR biosensor, such as i) the difficulties to fabricate a uniform structure of nanoparticle, ii) comparatively shorter linear dynamic range, and iii) immobilization of nanoparticle to a solid surface. More accurate nanomaterial fabrication with desirable design, and the preparation of an LPR-based biochip for a quantitative and rapid screening are the current challenges for this technology.

Conclusion

Plasmonic methods applied in nanobio-sensors are very sensitive to changes at molecular level. Manipulation of the plasmonic principles, combine with other techniques (such as nano-fabrication and bio-techniques) has created many versatile approaches that are able to detect the presence of biomolecule, or characterize the molecule of interest qualitatively. It has been proven that plasmonic methods are able to perform as sensing platform at ultrasensitive ($<10^{-12}$ M) level of detection. Paradigm-shifting thoughts are still needed, however, to made it practical as an on-field technique that can be used as point-of-care diagnostics world widely. These include massive cost reduction and the usage of non-sophisticated machinery [138].

Despite the world-widely usage of SPR and LPR techniques, many other optical-based nanobio-sensors have been invented and progressively being re-invented from time to time. Anomalous reflections (AR) of gold technique [139], surface-immobilized gold nano-sphere (SIGN) [71,140], simple reflection on alloy and composite thin layer surfaces [141], metal-insulator-metal platforms [142,143], gold nano-particle growth ELISA [19] etc., are just a handful examples of the exponentially growing development. It is hoped that the nanobio-sensing technology can be benefited to relief global-threatening diseases, out breaks, and bioterrorism violence to a larger population of the world, and thus contributes to a better social structure of today's modern living.

References

1. The Human Genome. Science. 2001; 291:1145-1434.
2. International Human Genome Sequencing Consortium. Initial sequencing and analysis of the human genome. Nature. 2001; 409:860-921.
3. Lipshutz RJ, Fodor SP, Gingeras TR, Lockhart DJ. High density synthetic oligonucleotide arrays. Nat Genet. 1999; 21: 20-24.
4. Niemeyer CM, Blohm D. DNA microarrays. Angew Chem Int Ed. 1999; 38:2865-2869.
5. Niemeyer CM, Blohm D. DNA-mikroarrays. Angew Chem. 1999; 111:3039-3043.
6. DeRisi JL, Iyer VR, Brown PO. Exploring the metabolic and genetic control of gene expression on a genomic scale. Science. 1997; 278:680-686.
7. Anderson L, Seilhamer J. A comparison of selected mRNA and protein abundances in human liver. Electrophoresis. 1997; 18:533-537.
8. Gygi SP, Rochon Y, Franza BR, Aebersold R. Correlation between protein and mRNA abundance in yeast. Mol Cell Biol. 1999; 19:1720-1730.
9. Stelzl U, Worm U, Lalowski M, Haenig C, Brembeck FH, Goehler H, et al. A human protein-protein interaction network: a resource for annotating the proteome. Cell. 2005; 122:957-968.
10. Rual JF, Venkatesan K, Hao T, Kishikawa TH, Dricot A, Li N, et al. Towards a proteome-scale map of the human protein–protein interaction network. Nature. 2005; 437:1173-1178.
11. Smith EA, Corn RM. Surface plasmon resonance imaging as a tool to monitor biomolecular interactions in an array based format. Appl Spectrosc. 2003; 57:320A-332A.
12. Morens DM, Folkers GK, Fauci AS. Emerging infections: a perpetual challenge. Lancet Infect Dis. 2008; 8:710-719.
13. Fauci AS. Infectious diseases: considerations for the 21st century. Clin Infect Dis. 2001; 32:675-685.

14. Hanash SM, Pitteri SJ, Faca VM. Mining the plasma proteome for cancer biomarkers. Nature. 2008; 452:571-579.
15. Ferreira GNM, da-Silva AC, Tome B. Acoustic wave biosensors: physical models and biological applications of quartz crystal microbalance. Trends Biotechnol. 2009; 27:689-697.
16. Tomizaki KY, Usui K, Mihara H. Protein-protein interactions and selection: array-based techniques for screening disease-associated biomarkers in predictive/early diagnosis. FEBS J. 2010; 277:1996-2005.
17. Ray S, Mehta G, Srivastava S. Label-free detection techniques for protein microarrays: Prospects, merits and challenges. Proteomics. 2010; 10:731-748.
18. Park JS, Cho MK, Lee EJ, Alm KY, Lee KE, Jung JH, et al. A highly sensitive and selective diagnostic assay based on virus nanoparticles. Nat Nanotechnol. 2009; 4:259-264.
19. Rica RDL, Stevens MM, Plasmonic ELISA for the ultrasensitive detection of disease biomarkers with the naked eye. Nat Nanotechnol. 2012; 7:821-824.
20. Iyer S, Gaikwad RM, Subba-Rao V, Woodworth CD, Sokolov I. AFM detects differences in the surface brush of normal and cancerous cervical cells. Nat Nanotechnol. 2009; 4:389-393.
21. Yuk JS, Ha KS. Array-based spectral SPR biosensor: analysis of mumps virus infection. Methods Mol Biol. 2009; 503:37-47.
22. Lautner G, Balogh Z, Bardoczy V, Meszaros T, Gyurcsanyi RE. Aptamer-based biochips for label-free detection of plant virus coat proteins by SPR imaging. Analyst, 2010; 135:918-926.
23. Shanmukh S, Jones L, Driskell J, Zhao Y, Dluhy R, Tripp RA. Rapid and sensitive detection of respiratory virus molecular signatures using a silver nanorod array sers substrate. Nano Lett. 2006; 6:2630-2636.
24. Patolsky F, Zheng G, Hayden O, Lakadamyali M, Zhuang X, Lieber CM. Electrical detection of single viruses. Proc Natl Acad Sci. 2004; 101:14017-14022.
25. Li Y, Lee HJ, Corn RM. Detection of protein biomarkers using rna aptamer microarrays and enzymatically amplified surface plasmon resonance imaging. Anal Chem. 2007; 79:1082-1088.
26. Haes AJ, Chang L, Klein WL, Van Duyne, RP. Detection of a biomarker for alzheimer's disease from synthetic and clinical samples using a nanoscale optical biosensor. J Am Chem Soc. 2005; 127:2264–2271.
27. Chua JH, Chee RE, Agarwal A, Wong SM, Zhang GJ. Label-free electrical detection of cardiac biomarker with complementary metal-oxide semiconductor-compatible silicon nanowire sensor arrays. Anal Chem. 2009; 81:6266–6271.
28. Huh YS, Lowe AJ, Strickland AD, Batt CA, Erickson D. Surface-enhanced Raman scattering based ligase detection reaction. J Am Chem Soc. 2009; 131:2208–2213.
29. Sanvicens N, Pastells C, Pascual N, Marco MP. Nanoparticle-based biosensors for detection of pathogenic bacteria. TrAC-trend Anal Chem. 2009; 28:1243-1252.
30. Dudak FC, Boyac IH. Rapid and label-free bacteria detection by surface plasmon resonance (SPR) biosensors. Biotechnol J. 2009; 4:1003-1011.
31. Primera-Pedrozo OM, Jerez-Rozo JI, De La Cruz-Montoya E, Luna-Pineda T, Pacheco-Londono LC, Hernandez-Rivera SP. Nanotechnology-based detection of explosives and biological agents stimulants. IEEE Sens J. 2008; 8:963–973.
32. Alava T, Berthet-Duroure N, Ayela C, Trévisiol E, Pugnière M, Morel Y, et al. Parallel acoustic detection of biological warfare agents surrogates by means of piezoelectric immunochips. Sens Actuat B-Chem. 2009; 138:532-538.
33. Jones RB, Gordus A, Krall JA, MacBeath G. Nature. 2006; 439:168-174.
34. Neher, E. Molecular biology meets microelectronics. Nat Biotechnol. 2001; 19:114.
35. Sun Y, Xia Y. Shape-controlled synthesis of gold and silver nanoparticles. Science. 2002; 298:2176-2179.
36. Potts JT Jr., Sherwood LM, O'Riordan JL, Aurbach GD. Radioimmunoassay of polypeptide hormones. Adv Intern Med. 1967; 13:183-240.
37. Odell WD, Wilber JF, Paul WE. Metabolism. 1965; 14:465-467.
38. Karlström A, Nygren PÅ. Dual labeling of a binding protein allows for specific fluorescence detection of native protein. Anal Biochem. 2001; 295:22-30.
39. Rizzo MA, Springer GH, Granada B, Piston DW. An improved cyan fluorescent protein variant useful for FRET. Nat Biotechnol. 2004; 22:445-449.
40. Moody MD, Van Arsdell SW, Murphy KP, Orencole SF, Burns C. Array-based ELISAs for high- throughput analysis of human cytokines. Biotechniques. 2001; 31:186-190.
41. Spencer KA, Osorio FA, Hiscox JA. Recombinant viral proteins for use in diagnostic ELISAs to detect virus infection. Vaccine. 2007; 25:5653-5659.
42. Forster AC, McInnes JL, Skingle DC, Symons RH. Non-radioactive hybridization probes prepared by the chemical labelling of DNA and RNA with a novel reagent, photobiotin. Nucleic Acids Res. 1985; 3:745-761.
43. Zhu H, Bilgin M, Bangham R, Hall D, Casamayor A, Bertone P, et al. Global analysis of protein activities using proteome chips. Science. 2001; 293:2101-2105.
44. Ivanov SS, Chung AS, Yuan ZL, Guan YJ, Sachs KV, Reichner JS, et al. Antibodies immobilized as arrays to profile protein post-translational modifications in mammalian cells. Mol Cell Proteomics. 2004; 3:788-795.
45. Zhu J, Gopinath K, Murali A, Yi G, Hayward SD, Zhu H. et al. RNA-binding proteins that inhibit RNA virus infection. Proc Natl Acad Sci. 2007; 104:3129-3134.
46. Lippincott-Schwartz J, Patterson GH. Development and use of fluorescent protein markers in living cells. Science. 2003; 300:87–91.
47. Popp MW, Antos JM, Grotenbreg GM, Spooner E, Ploegh HL. Sortagging: a versatile method for protein labeling. Nat Chem Biol. 2007; 3:707-708.
48. Haab BB. Methods and applications of antibody microarrays in cancer research. Proteomics. 2003; 3:2116-2122.
49. Li Z, Adams RM, Chourey K, Hurst GB, Hettich RL, Pan C. Systematic comparison of label-free, metabolic labeling, and isobaric chemical labeling for quantitative proteomics on ltq orbitrap velos. J Proteome Res. 2012; 11:1582–1590.
50. Ramachandran N, Larson DN, Stark PRH, Hainsworth E, LaBaer J. Emerging tools for real-time label-free detection of interactions on functional protein microarrays. FEBS J. 2005; 272:5412-5425.
51. Yu X, Xu D, Cheng Q. Proteomics. Label-free detection methods for protein microarrays. 2006; 6:5493-5503.
52. Daniels JS, Pourmand N. Label-free impedance biosensors: opportunities and challenges. Electroanalysis. 2007; 19:1239-1257.
53. Erickson D, Mandal S, Yang AHJ, Cordovez B. Nanobiosensors: optofluidic, electrical and mechanical approaches to biomolecular detection at the nanoscale. Microfluid Nanofluid. 2008; 4:33-52.
54. Szmacinski H, Ray K, Lakowicz JR. Metal-enhanced fluorescence of tryptophan residues in proteins: Application toward label-free bioassays. Anal Biochem. 2009; 385:358-364.
55. Kambhampati D. ed. Protein Microarray Technology. Weinheim Germany: Wiley-VCH; 2003.
56. Davis F, Higson SP. Label-free immunochemistry approach to detect and identity antibiotics in milk. Pediatr Res. 2010; 67:476-480.
57. Chen AK, Behlke MA, Tsourkas A. Avoiding false-positive signals with nuclease-vulnerable molecular beacons in single living cells. Nucleic Acids Res. 2007; 35:e105(1-12).
58. Benesch J, Askendal A, Tengvall P. Quantification of adsorbed human serum albumin at solid interfaces: a comparison between radioimmunoassay (RIA) and simple null ellipsometry. Colloids Surf B. 2000; 18:71-81.
59. Mitchell P. A perspective on protein microarrays. Nat Biotechnol. 2002; 20:225-229.
60. Tomizaki KY, Usui K, Mihara H. Protein-detecting microarrays: current accomplishments and requirements. ChemBioChem. 2005; 6:782-799.
61. Calleja M, Kosaka PM, San Paulo Á, Tamayo J. Challenges for nanomechanical sensors in biological detection. Nanoscale. 2012; 4:4925-4938.
62. Luo X, Davis JJ. Electrical biosensors and the label free detection of protein disease biomarkers. Chem Soc Rev. 2013; 42:5944-5962.
63. Leebeeck AD, Swaroop Kumar LK, de Lange V, Sinton D, Gordon R, Brolo AG. On-chip surface-based detection with nanohole arrays. Anal Chem. 2007; 79:4094-4100.

64. Qi C, Zhu W, Niu Y, Zhang HG, Zhu GY, Meng YH, et al. Detection of hepatitis B virus markers using a biosensor based on imaging ellipsometry. J Viral Hepatitis. 2009; 16:822-832.
65. Wang ZH, Jin G. A label-free multisensing immunosensor based on imaging ellipsometry. Anal Chem. 2003; 75:6119-6123.
66. Lin VSY, Motesharei K, Dancil KPS, Sailor MJ, Ghadiri MR. A porous silicon-based optical interferometric biosensor. Science. 1997; 278:840-843.
67. Minas G, Wolffenbuttel RF, Correia JH. A lab-on-a-chip for spectrophotometric analysis of biological fluids. Lab Chip. 2005; 5:1303-1309.
68. Schwartz MP, Alvarez SD, Sailor MJ. Porous SiO_2 interferometric biosensor for quantitative determination of protein interactions: binding of protein a to immunoglobulins derived from different species. Anal Chem. 2007; 79:327-334.
69. Simon HJ, Benner RE, Rako JG. Optical second harmonic generation with surface plasmons in piezoelectric crystals. Opt Commun. 1977; 23:245-248.
70. Corn RM, Romagnoli M, Levenson MD, Philpott MR. The potential dependence of surface plasmon-enhanced second-harmonic generation at thin film silver electrodes. Chem Phys Lett. 1984; 106:30-35.
71. Tsuboi K, Fukuba S, Naraoka R, Fujita K, Kajikawa K. Multichannel biosensing platform of surface-immobilized gold nanospheres for linear and nonlinear optical imaging. Appl Opt. 2007; 46:4486-4490.
72. Schmitt K, Schirmer B, Hoffmann C, Brandenburg A, Meyrueis P. Interferometric biosensor based on planar optical waveguide sensor chips for label-free detection of surface bound bioreactions. Biosens Bioelectron. 2007; 22:2591-2597.
73. Ritchie RH. Plasma Losses by Fast Electrons in Thin Films. Phys Rev. 1957; 106:874-881.
74. Barnes WL, Dereux A, Ebbesen TW. Surface plasmon subwavelength optics. Nature. 2003; 424:824-830.
75. Otto A. Excitation of nonradiative surface plasma waves in silver by the method of frustrated total reflection. Z. Phys. 1968; 216:398-410.
76. Kretschmann E, Raether H. Radiative decay of nonradiative surface plasmons excited by light. Z. Naturforsch A. 1968; 23:2135-2136.
77. Nylander C, Liedberg B, Lind T. Gas detection by means of surface plasmon resonance. Sensor Actuator. 1982/83; 3:79-88.
78. Liedberg B, Nylander C, Lundstorm I. Surface plasmon resonance for gas detection and biosensing. Sensor Actuator. 1983; 4:299-304.
79. Yeatman E, Ash EA. Surface plasmon microscopy. Electron Lett. 1987; 23:1091-1092.
80. Yeatman E, Ash EA. Surface Plasmon Scanning Microscopy. SPIE Scanning Microsc Technol Appl. 1988; 897:100-107.
81. Rothenhausler B, Knoll W. Surface–plasmon microscopy. Nature. 1988; 332:615-617.
82. Hickel W, Kamp D, Knoll W. Surface-plasmon microscopy. Nature. 1989; 339:186-188.
83. Kano H, Kawata S. Grating-coupled surface plasmon for measuring the refractive index of a liquid sample. Jpn J Appl Phys. 1995; 34:331-335.
84. Brockman JM, Fernandez SM. Grating-coupled surface plasmon resonance for rapid, label-free, array-based sensing. Am Lab. 2001; 33:37-40.
85. Dostalek J, Homola J, Miler M. Rich information format surface plasmon resonance biosensor based on array of diffraction gratings. Sens Actuat B-Chem. 2005; 107:154-161.
86. Singh BK, Hillier AC. Surface plasmon resonance imaging of biomolecular interactions on a grating-based sensor array. Anal Chem. 2006; 78:2009-2018.
87. Singh BK, Hillier AC. Multicolor surface plasmon resonance imaging of ink jet-printed protein microarrays. Anal Chem. 2007; 79:5124-5132.
88. Dostalek J, Homola J. Surface plasmon resonance sensor based on an array of diffraction gratings for highly parallelized observation of biomolecular interactions. Sens Actuat B-Chem. 2008; 129:303-310.
89. Homola J. Surface plasmon resonance sensors for detection of chemical and biological species. Chem Rev. 2008; 108:462-493.
90. Hoa XD, Kirk AG, Tabrizian M. Towards integrated and sensitive surface plasmon resonance biosensors: A review of recent progress. Biosens Bioelectron. 2007; 23:151-160.
91. Boozer C, Kim G, Cong S, Guan HW, Londergan T. Looking towards label-free biomolecular interaction analysis in a high-throughput format: a review of new surface plasmon resonance technologies. Curr Opin Biotechnol. 2006; 17:400-405.
92. Homola J, Yee SS, Gauglitz G. Surface plasmon resonance sensors: review. Sens Actuat B-Chem. 1999; 54:3-15.
93. Luo Y, Yu F, Zare RN. Microfluidic device for immunoassays based on surface plasmon resonance imaging. Lab Chip. 2008; 8:694-700.
94. Safsten P, Klakamp SL, Drake AW, Karlsson R, Myszka DG. Screening antibody–antigen interactions in parallel using Biacore A100. Anal Biochem. 2006; 353:181-190.
95. Forzani ES, Foley K, Weaterhoff P, Tao N. Detection of arsenic in groundwater using a surface plasmon resonance sensor. Sens Actuat B-Chem. 2007; 123:82-88.
96. Gestwicki JE, Hsieh HV, Pitner JB. Using receptor conformational change to detect low molecular weight analytes by surface plasmon resonance. Anal Chem. 2001; 73:5732-5737.
97. Nakatani K, Hagihara S, Goto Y, Kobori A, Hagihara M, Hayashi G, et al. Small-molecule ligand induces nucleotide flipping in (CAG)n trinucleotide repeats. Nat Chem Biol. 2005; 1:39-43.
98. Knoll W. Interfaces and thin films as seen by bound electromagnetic waves. Annu Rev Phys Chem. 1998; 49:569-638.
99. Brockman JM, Nelson BP, Corn RM. Surface plasmon resonance imaging measurements of ultrathin organic films. Annu Rev Phys Chem. 2000; 51:41-63.
100. Faraday M. The Bakerian Lecture: Experimental Relations of Gold (and Other Metals) to Light. Philos Trans Royal Soc London. 1857; 147:145-181.
101. Jensen TR Malinsky MD, Haynes CL, Van Duyne RP. Nanosphere Lithography: Tunable Localized Surface Plasmon Resonance Spectra of Silver Nanoparticles. J Phys Chem B. 2000; 104:10549-10556.
102. Liz-Marzan, LM. Tailoring surface plasmons through the morphology and assembly of metal nanoparticles. Langmuir. 2006; 22:32-41.
103. Mock JJ, Barbie M, Smith DR, Schultz DA, Schultz S. Shape effects in plasmon resonance of individual colloidal silver nanoparticles. J Chem Phys. 2002; 116:6755-6759.
104. Ringe E, Zhang J, Langille MR, Sohn K, Cobley C, Au L, et al. Effect of size, shape, composition, and support film on localized surface plasmon resonance frequency: a single particle approach applied to silver bipyramids and gold and silver nanocubes. Mater Res Soc Symp Proc. 2010; 1208 © Materials Research Society 1208-010-02.
105. Aizpurua J, Hanarp P, Sutherland DS, Kali M, Bryant GW, Garcia de Abajo FJ. Optical properties of gold nanorings. Phys Rev Lett. 2003; 90:057401-(1-4).
106. Lal S, Link S, Halas NJ. Nano-optics from sensing to waveguiding. Nat Photonics. 2007; 1:641-648.
107. Yonzon CR, Jeoung E, Zou S, Schatz GC, Mrksich M, Van Duyne RP. A comparative analysis of localized and propagating surface plasmon resonance sensors: the binding of concanavalin a to a monosaccharide functionalized self-assembled monolayer. J Am Chem Soc. 2004; 126:12669-12676.
108. Hergert W, Wriedt T. ed. Mie Theory 1908-2008, Present Development and Interdisciplinary Aspect of Light Scattering. 2008 September 15–17; Martin Luther University Halle - Wittenberg Halle, Germany; Universität Bremen, Bremen 2008.
109. Gans, R. Form of ultramicroscopic particles of silver. Ann. Phys. 1915; 47:270-284.
110. Link S, El-Sayed MA. Spectral properties and relaxation dynamics of surface plasmon electronic oscillations in gold and silver nanodots and nanorods. J Phys Chem B. 1999; 103:8410-8426.
111. Kelly KL, Coronado E, Zhao L, Schatz GC. The optical properties of metal nanoparticles: the influence of size, shape, and dielectric environment. J Phys Chem B. 2003; 107:668-677.
112. Haes AJ, Zou S, Schatz GC, Van Duyne RP. Nanoscale optical biosensor: short range distance dependence of the localized surface plasmon resonance of noble metal nanoparticles. J Phys Chem B. 2004; 108:6961-6968.

113. Haes AJ, Zou S, Zhao J, Schatz GC, Van Duyne RP. Localized surface plasmon resonance spectroscopy near molecular resonances. J Am Chem Soc. 2006; 128:10905-10914.
114. Mock JJ, Smith DR, Schultz S. Local refractive index dependence of plasmon resonance spectra from individual nanoparticles. Nano Lett. 2003; 3:485-491.
115. Sönnichsen C, Reinhard BM, Liphardt J, Alivisatos AP. A molecular ruler based on plasmon coupling of single gold and silver nanoparticles. Nat Biotechnol. 2005; 23:741-745.
116. Sönnichsen C, Franzl T, Wilk T, von Plessen G, Feldmann J. Drastic reduction of plasmon damping in gold nanorods. Phys Rev Lett. 2002; 88:077402(1-4).
117. Payne EK, Shuford KL, Park S, Schatz GC, Mirkin, CA. Multipole plasmon resonances in gold nanorods. J Phys Chem B. 2006; 110:2150-2154.
118. Haes AJ, Van Duyne RP. A nanoscale optical biosensor: sensitivity and selectivity of an approach based on the localized surface plasmon resonance spectroscopy of triangular silver nanoparticles. J Am Chem Soc. 2002; 124:10596-10604.
119. Murray WA, Suckling JR, Barnes WL. Overlayers on silver nanotriangles: field confinement and spectral position of localized surface plasmon resonances. Nano Lett. 2006; 6:1772-1777.
120. Whitney AV, Elam JW, Zou S, Zinovev AV, Stair PC, Schatz GC, et al. Localized surface plasmon resonance nanosensor: a high-resolution distance-dependence study using atomic layer deposition. J Phys Chem B. 2005; 109:20522-20528.
121. Qin L, Banholzer MJ, Millstone JE, Mirkin CA. Nanodisk codes Nano Lett. 2007; 7:3849-3853.
122. Hanarp P, Kall M, Sutherland DS. Optical properties of short range ordered arrays of nanometer gold disks prepared by colloidal lithography. J Phys Chem B. 2003; 107:5768-5772.
123. Larsson EM, Alegret J, Kall M, Sutherland DS. Sensing characteristics of NIR localized surface plasmon resonances in gold nanorings for application as ultrasensitive biosensors. Nano Lett. 2007; 7:1256-1263.
124. Wang H, Brandl DW, Le F, Nordlander P, Halas NJ. Nanorice: a hybrid plasmonic nanostructure. Nano Lett. 2006; 6:827-832.
125. Sherry LJ, Chang SH, Schatz GC, Van Duyne RP, Wiley BJ, Xia, Y. Localized surface plasmon resonance spectroscopy of single silver nanocubes. Nano Lett. 2005; 5:2034-2038.
126. Nehl CL, Liao H, Hafner JH. Optical properties of star-shaped gold nanoparticles. Nano Lett. 2006; 6:683-688.
127. Liz-Marzan LM. Nanometals: formation and color. Mater Today. 2004; 7:26-31.
128. Willets KA, Van Duyne RP. Localized surface plasmon resonance spectroscopy and sensing. Annu Rev Phys Chem. 2007; 58:267-297.
129. Hutter E, Fendler JH. Recent advances in the exploitation of localized surface plasmon resonance. Adv Mater. 2004; 16:1685-1706.
130. Jain PK, Huang X, El-Sayed IH, El-Sayed MA. Noble metals on the nanoscale: optical and photothermal properties and some applications in imaging, sensing, biology, and medicine. Acc Chem Res. 2008; 41:1578-1586.
131. Stuart DA, Haes AJ, Yonzon CR, Hicks EM, Van Duyne RP. Biological applications of localised surface plasmonic phenomenae. IEE Proc Nanobiotechnol. 2005; 152:12-32.
132. Zhao J, Zhang X, Yonzon CR, Haes AJ, Van Duyne RP. Localized surface plasmon resonance biosensors. Nanomedicine. 2006; 1:219-228.
133. Sepulveda B, Angelome PC, Lechuga LM, Liz-Marzan LM. LSPR-based nanobiosensors. Nano Today. 2009; 4:244-251.
134. Haes AJ, Van Duyne RP. A unified view of propagating and localized surface plasmon resonance biosensors. Anal Bioanal Chem. 2004; 379:920-930.
135. Anker JN, Hall WP, Lyandres O, Shah NC, Zhao J, Van Duyne RP. Biosensing with plasmonic nanosensors. Nat Mater. 2008; 7:442-453.
136. Heber J. Plasmonics: Surfing the wave. Nature. 2009; 461:720-722.
137. Endo T, Kerman K, Nagatani N, Hiepa HM, Kim DK, Yonezawa Y, et al. Multiple label-free detection of antigen-antibody reaction using localized surface plasmon resonance-based core-shell structured nanoparticle layer nanochip. Anal Chem. 2006; 78:6465-6475.
138. Ayas S, Cupallari A, Ekiz OO, Kaya Y, Dana A. Counting molecules with a mobile phone camera using plasmonic enhancement. ACS Photonics. 2014; 1:17-26
139. Syahir A, Tomizaki KY, Kajikawa K, Mihara H. Poly(amidoamine)-dendrimer-modified gold surfaces for anomalous reflection of gold to detect biomolecular interactions. Langmuir. 2009; 25:3667-3674.
140. Fukuba S, Tsuboi K, Abe S, Kajikawa K. Nonlinear optical detection of proteins based on localized surface plasmons in surface immobilized gold nanospheres. Langmuir. 2008; 24:8367-8372.
141. Syahir A, Kajikawa K, Mihara H. Enhanced refractive index sensitivity for anomalous reflection of gold to improve performance of bio-molecular detection. Sens Actuat B. 2014; 190:357-362.
142. Syahir A, Mihara H, Kajikawa K. A new optical label-free biosensing platform based on a metal-insulator-metal structure. Langmuir. 2010; 26:6053-6057.
143. Syahir A, Kajikawa K, Mihara H. Sensitive detection of small molecule-protein interactions on a metal-insulator-metal label-free biosensing platform. Chem Asian J. 2012; 7:1867-1874.