Nanoplasmonics for optical biosensor applications

Optical biosensors are important analytical devices in many sectors of human endeavors, including healthcare, food, and environmental monitoring. However, current optical detection techniques suffer from low sensitivity and resolution, which must be overcome for applications that require low limit-of-detection, such as biomarker quantification for early cancer diagnosis. Nanoplasmonics, the study of nano-scale interaction between light and electrons, may be able to overcome these issues toward an ultrasensitive, high resolution biosensor. This review aims to explore the principles of three nanoplasmonic phenomena: localized surface plasmon resonance (LSPR), surface enhanced raman scattering (SERS), and plasmon resonance energy transfer (PRET), and their implications in biosensing applications.

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Biosensor is a general term for a device used for detection of one or more analytes. A biosensor is important in many aspects of human activity, such as healthcare, food, and environmental monitoring. Especially in medicine, there is an urgent need for ultrasensitive and high resolution biosensors that can detect low levels of biomarkers for disease diagnosis and prognosis. An optical biosensor is one that employs optical techniques, such as absorption, fluorescence, and imaging, to detect and quantify target biomolecules.

While an optical technique is the most commonly used analytical method in a biosensor, it has several limitations that must be overcome in order to achieve the sensitivity and resolution required for diagnostic purposes. For example, a target biomolecule in a sample can be detected and quantified by analyzing the intensity of the fluorescence emitted from a probe binding to that biomolecule. However, the low quantum yields and short fluorescent life-time of many organic fluorophores limits our ability to detect low concentrations of the target biomolecule. Next, optical imaging systems have a diffraction limit, in which the resolution is inversely proportional to the wavelength of the light used in the system. Common light sources, such as ultraviolet, visible, and infrared, have a wavelength in the range between 300 nm to 1000 nm. This means that without even taking into account the aberrations that arise from imperfect lenses, an optical imaging system would never be able to observe or build nanostructures below 300 nm due to the theoretical diffraction limit.

To overcome these limitations, scientists have begun to look into nanoplasmonics. Plasmonics is the study and application arising from the interactions between light and electrons. Thus, nanoplasmonics refers to the study of plasmonics on the nanometer scale, such as the interaction of light near the surface of a gold nanoparticle. The key advantage of nanoplasmonics that allows scientists to overcome optical limits lies in the ability to confine electromagnetic oscillations to a space that is much smaller than the wavelength that would otherwise be generated in free space at that frequency. This confinement of electromagnetic oscillation lead to generation of extremely intense, concentrated electromagnetic fields at optical frequencies. The high intensity can be translated to increased resolution and sensitivity of the biosensor system.

With a recent trend in medicine toward personalized care, more and more diagnoses and treatments require understanding of biological processes at a molecular level. Manipulation, detection, and identification at this scale is now possible with various recently developed nanoplasmonic-based technologies (Fig. 1). In the past few years, there has been a significant increase in the number of studies which utilized these technologies to advance the performances of biosensors.

In this review, I will explore three plasmonic phenomena that occur in both macro- and nano-scale for ultrasensitive and high resolution biosensor applications.

Localized Surface Plasmon Resonance
Surface plasmon is a term for coherent electron oscillation at the planar interface between two materials, usually between a metal and a dielectric. Thus, surface plasmon resonance (SPR) occurs when incident light frequency and the oscillation frequency of electrons match, i.e. when the two frequencies resonate. When resonance occurs, the specific resonant wavelength is absorbed by the two materials, while all other wavelength is reflected. The specific resonant wavelength is related to the dielectric constant of the two materials. The dielectric constant can change when various biomolecules bind to the metal surface, thus inducing a change in the specific resonant wavelength. The change in the specific resonant wavelengths can be easily detected by UV-Vis spectroscopy, which indicates the presence of target analytes.

In the nanoscale, SPR is called localized surface plasmon resonance (LSPR). Unlike SPR where light interacts with a large planar surface, in LSPR light interacts with the surface of nanoparticles, often gold or silver, as shown in Fig. 2a. Both SPR and LSPR are attractive optical methods for biosensors because they do not require additional labels such...
as fluorophores to obtain data. Especially, LSPR is highly advantageous for its spatial resolution of just 1 nanoparticle.

LSPR with Gold Nanoparticles

Gold nanoparticles are spherical particles of diameter 5 nm to 400 nm. At this nano-scale length, gold exhibits different optical and electrical properties compared to gold observed in macro-scale. For example, gold nanoparticles of diameter 30 nm suspended in water has a deep red color, rather than the typical “gold” color observed in macro-scale. As the size of the gold nanoparticles increases, the color shifts from red to blue (Fig. 2b). This phenomena is due to the interaction between light and electrons of the gold nanoparticle.

The study by Haes et al. demonstrates the use of LSPR to detect amyloid-derived diffusible ligands (ADDLs) as a biomarker for Alzheimer’s disease. The researchers first fabricated a monodisperse, surface-confined gold nanoparticles in a triangular shape. Then, anti-ADDL antibodies were covalently linked onto the nanoparticle surface. The researchers performed a “sandwich” assay by exposing their functionalized nanoparticle surface with synthetic ADDLs and second anti-ADDL antibody to detect as low as 100 fM of ADDL. The binding event between synthetic ADDLs and the second anti-ADDL antibody caused a change in the refractive index of the system, shown by a shift in the surface plasmon resonance wavelength at maximum extinction. Finally, the researchers also obtained cerebrospinal fluid from patients with and without Alzheimer’s disease in order to test the validity of their LSPR-based nanobiosensor. Indeed, the SPR wavelength shift was much greater for patients with Alzheimer’s than that of those without Alzheimer’s, suggesting that the biosensor is capable of correctly diagnosing patients with the disease.

The sensitivity at which LSPR detected the biomarker for Alzheimer’s disease shown above is quite remarkable and clinically relevant. However, developing the platform to perform LSPR on a larger scale is currently not feasible due to the difficult fabrication process called nanoparticle lithography.

LSPR with Quantum Dots

Quantum dots (QDs) are semiconductor nanocrystals that are used as fluorescent probes. Just like other fluorescent molecules, QDs are able to absorb photons of light, then re-emit the photons at a lower energy, or longer wavelength. The most basic structure of a QD is made up of a semiconductor core and a shell. The core is often composed of cadmium mixed with selenium (denoted CdSe) or with tellurium (CdTe), and the shell is often composed of zinc sulfide (denoted ZnS). Unlike traditional fluorophores involving excitation of electrons, QDs form excitons, which have a long lifetime and high intrinsic brightness. QDs offer many
advantages over the conventionally used fluorescent markers, such as:

1. Size-tunable emission spectra
2. Broad absorption spectra
3. Strong luminescence
4. Robust photostability

The size-tunable emission and broad absorption spectra lend the QDs the ability to multiplex and parallel process. Simply, many differently sized QDs conjugated to different biomolecules can be all excited at once due to their broad absorption spectra, and each biomolecule can be distinguished as differently-sized QDs will emit at different wavelengths, as seen in Figure 2. Strong luminescence and robust photostability lead to high signal-to-noise ratio of the output, thus allowing for ultrasensitivity.

At first glance, QDs are unusual source of material for LSPR as QDs are semiconductors and therefore do not possess free electrons of a metal. However, scientists have developed ways to dope semiconducting QDs with charge carriers, thus inducing optical resonances similar to that of metal nanoparticles. With the ability to control the doping level of the semiconductor, scientists can create a range of electron densities around the QD, allowing for tunability for resonant wavelengths.

In a letter by Luther et al., researchers successfully demonstrated near infrared LSPR in QDs of the semiconductor cuprous (copper I) sulfide. Cuprous sulfide was an attractive choice as it is a self-doped semiconductor. The researchers controlled the doping level of cuprous sulfide by adjusting the stoichiometry of the copper and sulfur. Fully stoichiometric Cu$_2$S showed no LSPR feature, while deficient stoichiometry such as Cu$_{1.94}$S$_{0.06}$ resulted in LSPR. With smaller ratios of copper ions to sulfur, the LSPR progressively blue shifted. While this field is still nascent, dynamically tunable LSPRs using QDs may open to a new field of active plasmonics. The development of tunable LSPR will likely yield to multiplexing capabilities (i.e. ability to detect multiple analytes at the same time), which is a highly desirable characteristic for biosensing applications.

Surface Enhanced Raman Scattering

The phenomenon of Raman scattering is the inelastic scattering of a photon. When light hits an atom or molecule, most are elastically scattered, i.e. light does not lose energy after interacting with the target biomolecule, and is called Rayleigh scattering. However, a small fraction of light is scattered inelastically, i.e. incident light has different energy from emitted light (Fig. 3). Raman scattering is similar to fluorescence in that both result in an emission of photon that has a different frequency from the incident photon. However, while fluorescence involves absorption and emission at specific frequencies depending on the fluorophore, Raman scattering can occur at any frequency, but maintain a constant separation.

In Surface-Enhanced Raman Scattering (SERS), the effects of Raman scattering is enhanced by nearly a factor of 10. The effect is enhanced when the target biomolecule is absorbed on metal surfaces, typically gold or silver. The exact mechanism of the enhancement effects of SERS is not known, however, many scientists theorize that the increased signal intensity of the Raman signal is due to the enhancement of electrical field provided by the metal surface, similar to the mechanism of LSPR.

One may graph the spectrum of Raman-scattered light, similar to a nuclear magnetic resonance graph, as shown in Fig. 3. The spectrum depends on the molecular constituents and their state. Thus, Raman spectroscopy is useful in identifying various materials or analytes.

In the study by Zhang et al., researchers explored two characteristics to optimize SERS in terms of its limit of detection, namely the relationship between surface nanostructure and excitation wavelength and also analyze-surface binding chemistry. The researchers concluded that SERS is optimized when the energy of LSPR is within the range of the energy of excitation wavelength and vibration band of interest.

While more research needs to be done to exactly pinpoint the origin of SERS, scientists are beginning to combine LSPR and SERS for biosensing applications, as the two phenomena may be related to each other. A combined LSPR-SERS system is attractive as each technique serves different but complementary functions: LSPR for quantification of analyte, and SERS for identification of analyte. It would be interesting to see in the future a biosensor utilizing the two nanoplasmonic phenomena.
To understand plasmon resonance energy transfer (PRET), one must understand fluorescent resonance energy transfer (FRET). FRET involves a special pair of fluorescent molecules, in which one fluorophore’s emission spectrum overlaps with the other fluorophore’s excitation spectrum. A well-known example of molecules that undergo FRET are cyan fluorescent protein (CFP) and yellow fluorescent protein (YFP). Both are derivatives of the biomolecule green fluorescent protein (GFP), except with modified excitation and emission spectra. For CFP, the excitation frequency is around the high-energy violet light and emission frequency around cyan light. For YFP, the excitation frequency matches the cyan light, and emission frequency in yellow light.

Suppose both CFP and YFP are mixed in a solution and excited with violet light. This will excite the CFP and release cyan light. Similarly, the solution can be excited with cyan light, causing YFP to emit yellow light. An interesting phenomenon occurs when the two molecules are in a biologically close range, around 2 nm – 8 nm, an energy transfer may occur. That is, one may first excite CFP by violet light, which will subsequently cause CFP to emit cyan light. This emitted cyan light is immediately used to excite YFP, and the final result is the emission of yellow light, contrary to the expected cyan light (Fig. 4a). Thus, FRET is a highly sensitive tool to measure the interaction between two proteins.

PRET was first observed in a study by Liu et al., when the Rayleigh scattering spectrum showed quenching dips at certain resonant frequencies, shown in Fig. 4b. These frequencies were found out to be PRET between a single nanoplasmonic particle and a metalloprotein called cytochrome c. While PRET is still a relatively new phenomenon, I believe it has the potential to monitor protein-protein interaction at a single molecule level.

Conclusions

In this review, I have covered the fundamentals of three nanoplasmonic phenomena and their corresponding macroscale phenomena. Nanoplasmonics offer solutions to the current limitations of optical detection and optical imaging, namely low sensitivity and low resolution. Nanoplasmonics overcome these by confining electromagnetic waves onto a nature structure, which results in a high intensity electromagnetic field locally. While nanoplasmonics may offer superior sensitivity and resolution to optical techniques, the field faces several challenges of its own. First, various nanostructures such as gold nanoparticles and doped quantum dots are difficult to fabricate with good reproducibility, leading to higher costs than their optical counterparts. Thus, there needs to be significant research done in nanofabrication, which is currently an active area of study. Ideally in the near future, there would be standard nanofabrication processes that are high-throughput and yield uniform nanostructures for scientists in all disciplines to use. Second, these nanoplasmonic-based methods need to be integrated into a larger system of microfluidic devices. While nanoplasmonic-based techniques provide the ability to perform ultrasensitive detection and high resolution imaging, it does not provide a solution for other aspects of biosensors such as sample processing and readout. A microfluidic device is capable of handling such aspects and may also enhance the inherent advantages of nanoplasmonics. More research done toward a complete, integrated device that may purify a sample, detect analytes, and output a user-friendly report would be ideal in order to translate the technology to clinical uses. Such improvements would lead to a true “marriage” between nanoplasmonics and microfluidics, with nanoplasmonics offering high sensitivity and high resolution detection while microfluidics offering low cost, high throughput, and multiplexing capabilities.
References