Encyclopedia of Nanotechnology
DOI 10.1007/978-94-007-6178-0_100986-1
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Plasmonic Amplification for Fluorescence Bioassays Utilizing Propagating Surface Plasmons

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Synonyms

Plasmon-enhanced fluorescence; Plasmonic amplification of fluorescence; Plasmonic fluorescence biosensor; Surface plasmon-coupled emission

Definition

Plasmonics offers efficient means for enhancing sensitivity of fluorescence bioassays for detection of chemical and biological analytes. In this method, specific capture of target analyte from analyzed liquid sample at metallic sensor surface with attached recognition elements is observed by using fluorophore labels (e.g., organic dyes or quantum dots). These labels are probed by the confined field of surface plasmons that originate from collective oscillations of charge density at a surface of metallic films or metallic nanoparticles. The excitation of surface plasmons is accompanied with strongly increased intensity of electromagnetic field which couples with fluorophores. The combination of surface plasmon-enhanced excitation rate fluorophore absorption wavelength, improved quantum yield, and directional emission at the emission wavelength allows enhancing the detected fluorescence intensity per captured molecule by several orders of magnitude. When implemented to heterogeneous assays, limit of detection can be improved by similar factor.

Overview

The emission of fluorophores placed above a metallic surface was investigated since the 1970s of the last century [1, 2]. These studies revealed that the lifetime of an excited fluorophore is dramatically reduced due to the coupling with so-called surface lossy waves (leading to quenching at distances below Förster radius of ~10 nm) and propagating surface plasmons (that is observed at longer distances up to ~100 nm). The emitted photons that are trapped by propagating surface plasmon (PSP) modes can be partially recovered by reverse Kretschmann configuration [3] or by periodic corrugation of the metal surface [4, 5]. These techniques for out-coupling of PSPs allow extracting the emitted fluorescence photons from the surface to a highly directional beam and deliver them to a detector.

The coupling of fluorophores with PSPs provides efficient means for increasing sensitivity of heterogeneous fluorescence bioassays. In this type of assays, fluorophore emitters are used as labels to report affinity binding of target molecules from analyzed liquid sample at the surface with attached recognition elements such as antibodies or oligonucleotides [6–9]. The interaction of fluorophore labels with PSPs at their absorption λ_{ab} and emission λ_{em} wavelengths can increase their "brightness" and thus offers means to detect smaller number of captured molecules on the surface. In analytical applications, this translates to

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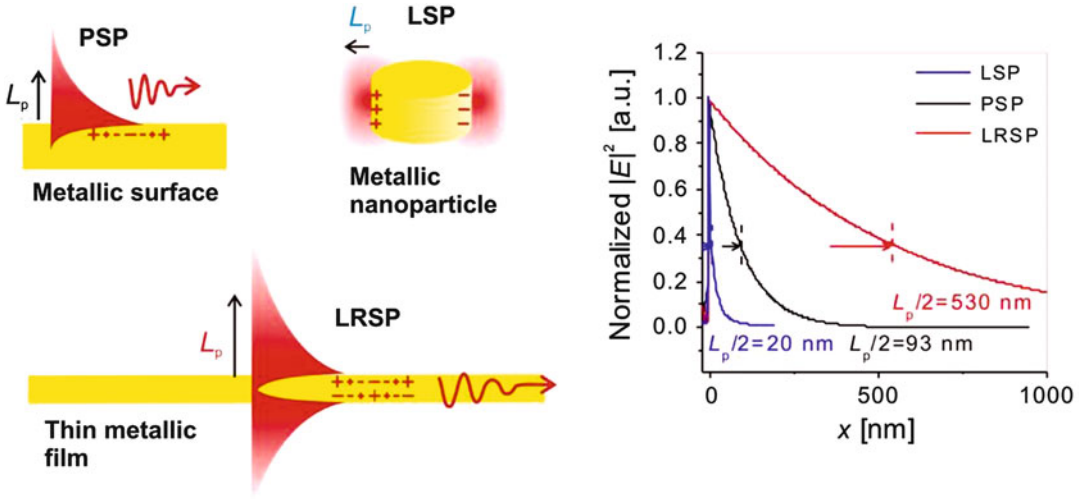

Fig. 1 Examples of surface plasmon modes on metallic films and metallic nanoparticles (*left*). Comparison of probing depth of regular propagating surface plasmons (PSPs) and long-range surface plasmons (LRSPs) on a gold surface in contact with water and of localized surface plasmons supported by a gold disk with diameter of 110 nm and height of 50 nm (*right*). Wavelength of $\lambda = 633$ nm was assumed

the possibility of detecting smaller concentrations of analyte in a sample and improved limit of detection (LOD).

Surface Plasmons

As Fig. 1a illustrates, PSP modes travel along a continuous metallic film and probe the adjacent dielectric medium above the surface with enhanced electric field intensity $|E|^2$. This field exponentially decays as $\exp(-2x/L_p)$, where x states for a distance from the metal surface. The probing depth is typically quantified by a half of penetration depth $L_p/2$ that is equal to about 90 nm for PSPs on a gold surface in contact with aqueous medium at a red wavelength of $\lambda = 633$ nm. The probing depth can be changed by a design of the surface plasmon-supporting metallic structure. For example, the coupling of PSP modes at opposite interfaces of a thin metallic film gives rise to new plasmonic modes with tunable characteristics. This coupling can occur when a thin metallic film is sandwiched between dielectrics with similar refractive index [10]. For probing of aqueous medium that exhibits refractive index close to $n = 1.33$, such geometry can be realized by depositing a thin metal film (with thickness of few tens of nanometers) on the top of a low refractive index fluoropolymer such as Cytop from Asahi Inc. ($n = 1.34$) or Teflon AF from DuPont Inc. ($n = 1.31$) [11, 12]. The near-field coupling of PSPs at opposite interfaces of a metal film gives rise to the occurrence of long-range surface plasmons (LRSP) that exhibit weaker confined field and thus probe deeper away from the metal. An example in Fig. 1 shows that for 20 nm thick gold film on the Teflon AF surface, the aqueous medium is probed by the extended LRSP field with the probing depth of $L_p/2 = 540$ nm at a wavelength of $\lambda = 633$ nm. Let us note that the probing depth can be tuned between that of regular PSPs and (virtually) infinity by changing the thickness metal and by introducing a small perturbation to the refractive index symmetry [13].

Plasmonic Coupling with Fluorescence Emitters

The resonant coupling of light with PSP modes confines its energy to a small volume in vicinity to a metal surface which leads to the strong enhancement of electromagnetic field intensity. The field intensity at metallic surface $|E|^2$ can be normalized with that of the excitation beam $|E_0|^2$ in order to quantify field intensity enhancement. Depending on the confinement and losses of excited PSP modes, the field

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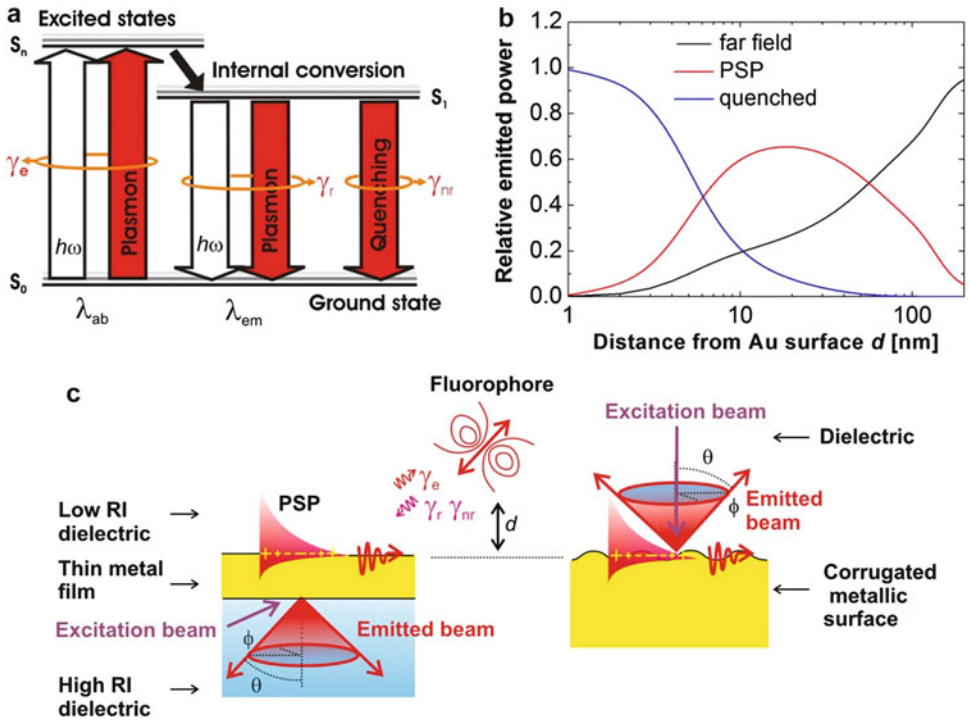

Fig. 2 (**a**) Jablonski diagram showing surface plasmon-mediated transitions between the fluorophore ground state and higher excited states. (**b**) Comparison of the emitted power from randomly oriented dipole that is quenched, emitted via PSPs, and radiated directly to the far field depending on the distance from a flat gold surface *d*. (**c**) Schematic out-coupling of PSP modes via reverse Kretschmann geometry of ATR method (*left*) and by using diffraction on periodically corrugated metallic surface (*right*)

enhancement $|E/E_0|^2$ can reach values up to $\sim 10^2$ (it should be noted that much stronger enhancement was observed upon the excitation of localized surface plasmons on sharp metallic nanoparticles that feature more confined field [14]). As Jablonski diagram in Fig. 2a illustrates, PSPs couple with a fluorophore at both emission and excitation wavelengths λ_{ab} and λ_{em}, respectively. This interaction strongly depends on the distance d between the fluorophore and metallic surface. Figure 2b shows the relative power emitted by a fluorophore at a red wavelength $\lambda_{em} = 670$ nm above a gold surface. At distances d below $\sim$10 nm, the fluorescence intensity is mostly quenched due to the Förster energy transfer. At the distance of about $\sim$20 nm, the majority of energy is emitted via PSPs. When moving farther away from the surface, the fluorophore emits preferably to the far field.

Figure 2c shows two common schemes that allow converting the fluorescence light that is trapped by PSPs into waves propagating into far field. PSPs that are generated by fluorescence at λ_{em} can leak through a thin metallic film when the reverse Kretschmann geometry of attenuated total reflection (ATR) method is used. For randomly oriented emitters (fluorophores), the fluorescence light is reemitted to a cone beam that propagates below the thin metal at a polar angle θ at which surface plasmon resonance occurs. Alternatively, diffraction on periodically corrugated metallic surface allows for out-coupling of PSPs into the far field.

For the plasmonic amplification of fluorescence, the emitter (which is typically a fluorophore label anchored to a biomolecule) is placed on a metallic surface and exposed to the intense surface plasmon field. The fluorescence intensity that is delivered to a detector can be increased by three effects. Firstly, the coupling of an excitation beam with PSPs increases the excitation rate of emitter that is (far from saturation) proportional to the field enhancement $|E/E_0|^2$ at the absorption wavelength λ_{ab}. Secondly, the emitter radiates fluorescence light at the emission wavelength λ_{em} via PSPs that can be afterward

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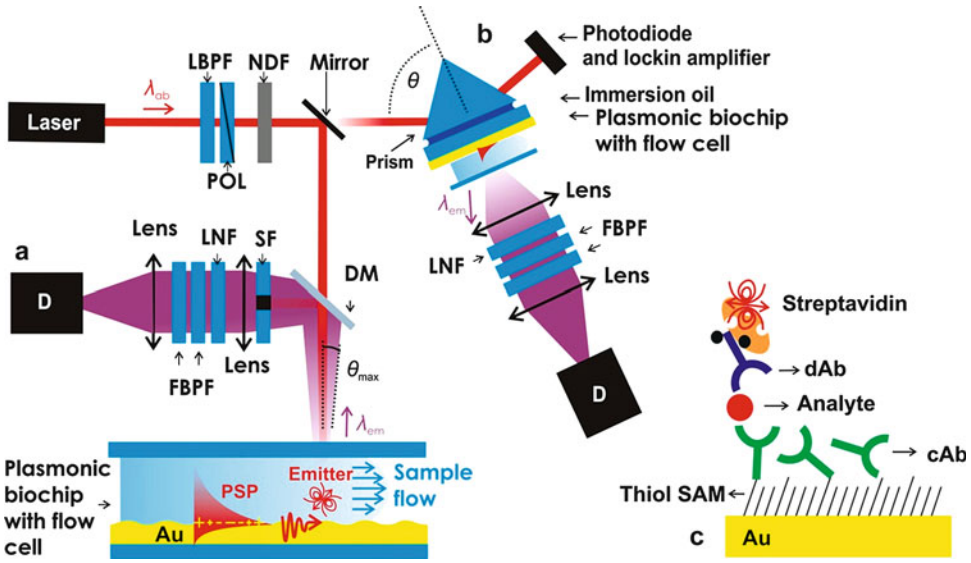

Fig. 3 Schematics of (**a**) total internal reflection fluorescence (TIRF) and (**b**) epifluorescence geometry with the surface plasmon-based amplification of fluorescence light intensity. (**c**) An example of sandwich immunoassay with the fluorescence detection that utilizes capture antibody (cAb) attached to a surface and biotinylated detection antibodies (dAb) that are decorated with fluorophore-labeled streptavidin

reemitted to a specific direction toward a detector. This feature improves the fraction of emitted photons that are delivered to the detector from above the plasmonic structure f_d compared to that without it f_{d0}. Thirdly, near-field coupling of the emitter with PSPs can improve its quantum yield η (particularly for emitters with low intrinsic quantum yield η_0). Let us note that these three effects can be combined and the total enhancement factor EF (defined as fluorescence intensity emitted by a fluorophore at a metallic structure F normalized with that without the structure F_0) can be expressed as

$$EF = F/F_0 \propto |E/E_0|^2 \times f_d/f_{d0} \times \eta/\eta_0. \quad (1)$$

It should be noted that the EF strongly depends on the emitter orientation which is in most experiments not controlled. For ensembles of fluorophores, Eq. 1 typically needs to be averaged over all possible orientations and locations of emitter on plasmonic structure (mode details on such models can be found in literature [15, 16]).

Implementations of Plasmon-Enhanced Fluorescence Biosensors

The employing of PSPs for the amplification of fluorescence signal mostly utilizes epifluorescence or total internal reflection fluorescence (TIRF) geometries that are depicted in Fig. 3a, b, respectively. In the epifluorescence-based sensors, an excitation beam at a wavelength that coincides with the absorption band of used fluorophore label at λ_{ab} is made incident on a sensor chip a with plasmonic structure. The surface plasmon-enhanced field intensity at this wavelength probes the assay on the surface (see Fig. 3c) and excites fluorophore labels that report the target analyte binding events. The fluorophore emits light at the emission wavelength λ_{em} directly to the far field or via PSPs that are subsequently out-coupled toward a detector.

The TIRF-like geometry allows for combined surface plasmon resonance (SPR) and fluorescence measurements based on ATR method with Kretschmann geometry (see Fig. 3b). In this implementation, the resonantly excited surface plasmon waves on a thin metal film can probe the binding events by (i) changes in refractive index due to the capture of target analyte leading to a shift of the SPR angle and

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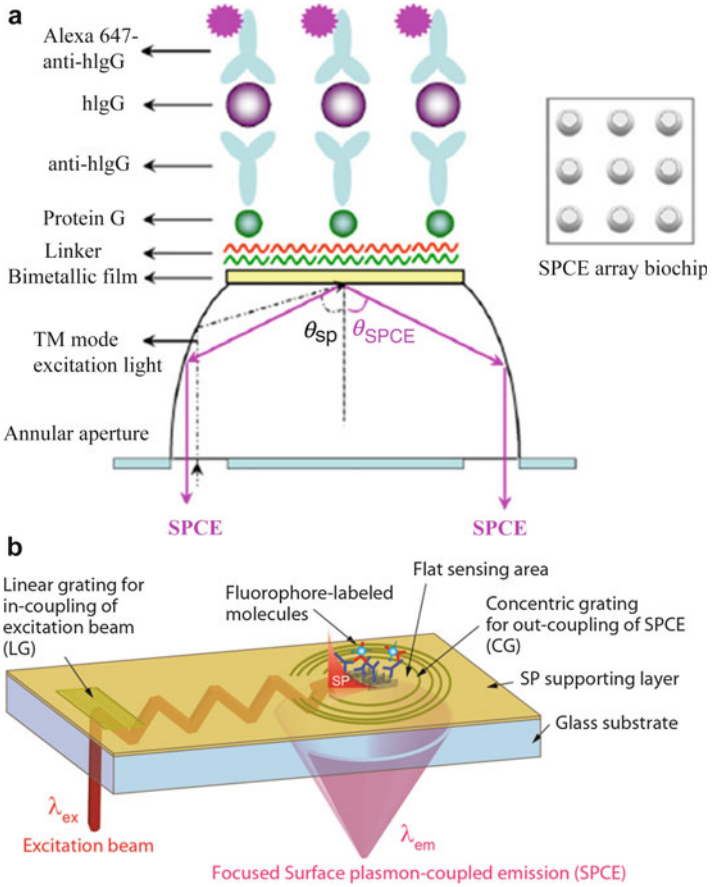

Fig. 4 Optical elements for the extraction of SPCE utilizing reverse Kretschmann configuration and (**a**) paraboloid elements and (**b**) concentric diffractive lens (Reproduced with permission from Yuk et al. 18 and Toma et al. 9)

(ii) fluorescence light that is excited by the enhanced surface plasmon field intensity when fluorophore labels are used [7]. The fluorescence light that is directly emitted perpendicular to the surface through the sample can be detected. Optical filters are used in order to decrease background signal (see Fig. 3). The laser band-pass filter (LBPF) blocks light propagation at wavelengths different from the excitation laser wavelength that is close to the absorption wavelength of used fluorophore λ_{ab}. Laser notch filter (LNF) is employed to block the laser light intensity that is typically much stronger than that of emitted fluorescence light at λ_{em}. In addition, fluorescence band-pass filters (FBPF) are used to select only the light at wavelengths around λ_{em}. In general, the fluorescence intensity is weak, and thus low light intensity detectors (D) such as photomultiplier, avalanche photodiode, or cooled CCD are used.

In the TIRF-like geometry depicted in Fig. 3b, the majority of fluorescence light intensity is leaking through the thin metallic film and propagates in the form of a characteristic surface plasmon-coupled emission (SPCE) cone. In order to retrieve this fluorescence light and deliver it to a detector, glass hemispherical prisms were used [17]. Later on, this approach was implemented by using miniaturized parabolic elements that can be embossed to polymer chips [18]. As Fig. 4a shows, such element allows directly imaging SPCE intensity to a detector and offers means for measurements with array detection format. Alternatively, concentric diffraction grating was proposed for out-coupling of SPCE signal and its imaging to a detector [19]; see Fig. 4b. This approach can be implemented by mass production-compatible technologies such as nanoimprint lithography and holds potential for simplification of the more traditionally used optical schemes.

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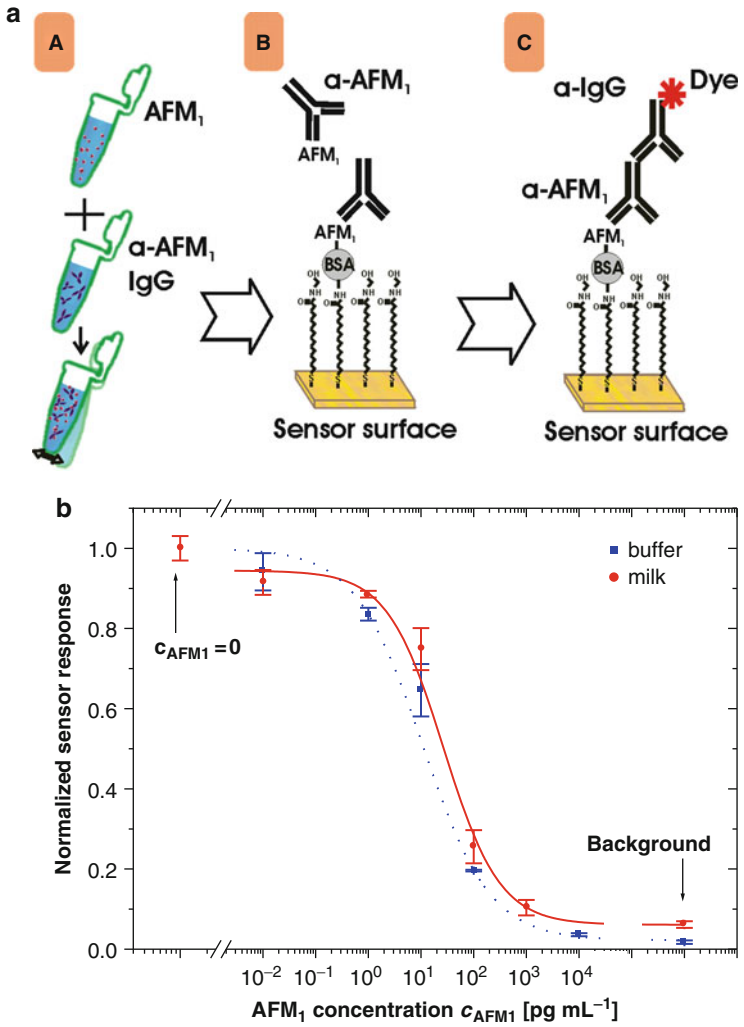

Fig. 5 (**a**) Schematic of inhibition competitive immunoassay for detection of aflatoxin M1 and (**b**) a calibration curve for the aflatoxin M1 surface plasmon-enhanced fluorescence biosensor (Reproduced with permission from Wang et al. 22)

Examples of Applications

Since the end of the last century, numerous fluorescence biosensors that utilize plasmonic amplification of fluorescence signal were developed for detection of nucleic acids [20], proteins [6], as well as whole cells [21]. These efforts were mainly motivated by the increasing needs for assays with enhanced sensitivity in important areas of medical diagnostics (analysis of biomarkers), food control, and security (detection of harmful compounds such as toxins or pathogens). This section illustrates the plethora of sensor architectures and achieved performance characteristics by several examples. It should be noted that it does not cover the whole area and does not present a critical review in this field.

Plasmon-enhanced fluorescence biosensors were utilized for detection of low molecular weight analytes by using an inhibition competitive immunoassay. For instance, Wang et al. detected aflatoxin M_1 (AFM_1) with the molecular weight of 328 Da by using an optical arrangement similar to that depicted in Fig. 3b [22]. This harmful analyte is a metabolite of mycotoxin aflatoxin B_1 produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus* pathogens. The gold sensor surface was functionalized by using a thiol self-assembled monolayer with a conjugate of AFM_1 and bovine serum albumin for the inhibition competitive immunoassay; see Fig. 5a. Monoclonal rat antibody against AFM_1 was incubated with a sample containing AFM_1, and the unreacted antibody was flowed over the sensor surface and

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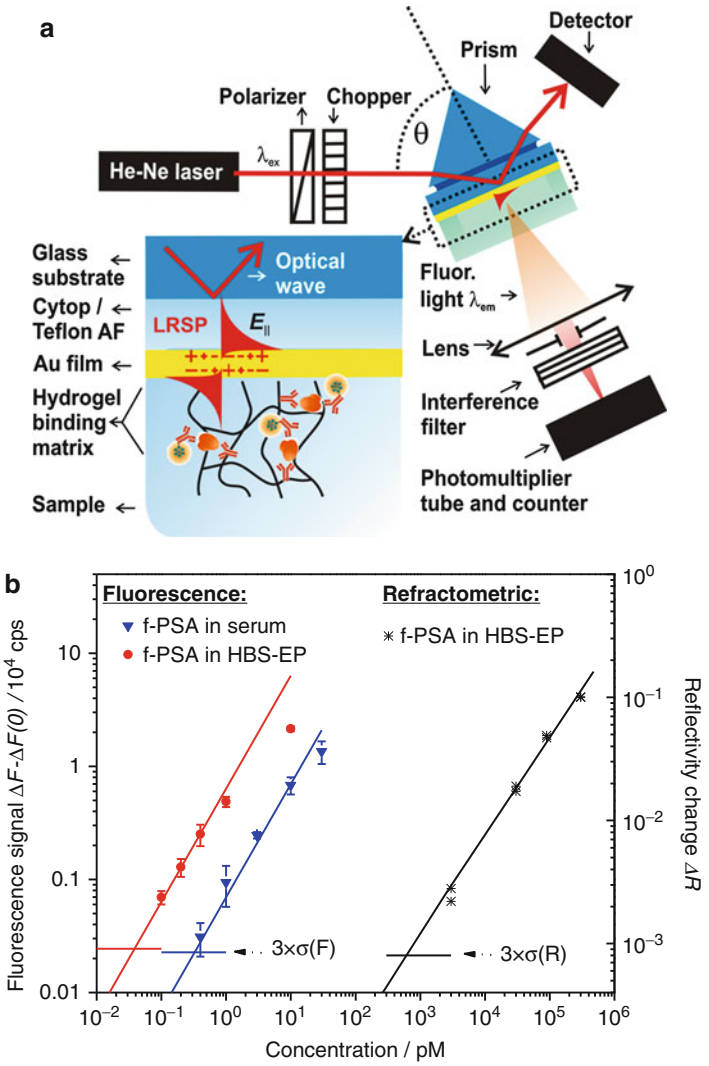

Fig. 6 (**a**) Surface plasmon-enhanced fluorescence biosensor with a biointerface architecture based on three-dimensional hydrogel matrix that is probed by LRSPs. (**b**) Comparison of calibration curves of free prostate-specific antigen (f-PSA) assay with direct SPR readout (*black curve*) and with plasmonically amplified fluorescence readout for serum (*blue curve*) and buffer (*red curve*) samples (Reproduced with permission from Wang et al. 23)

detected by using a secondary anti-rat antibody that was labeled with a Cy5 dye. As the calibration curve in Fig. 5b shows, the limit of detection of AFM_1 present in milk was determined to be 0.6 pg/ml (1.8 pM) and the whole analysis required 53 min.

Prostate-specific antigen (PSA) is a biomarker with a molecular weight of 33–34 kDa that is present in the blood at around nM concentrations and used for a diagnosis of prostate cancer. Improving the sensitivity of PSA assays for the analysis at lower concentrations has been pursued for point-of-care diagnosis (POC) of female breast cancer [108], for early identification of prostate cancer relapse [109], and in forensic applications [110]. A biosensor for detection of free prostate-specific antigen (f-PSA) using long-range surface plasmon-enhanced fluorescence spectroscopy (LRSP-FS) and photo-cross-linked carboxymethylated dextran hydrogel matrix (depicted in Fig. 6a) was reported by Wang et al. [23]. In this approach, there was used a three-dimensional hydrogel binding matrix with a thickness of around 1 μm that offers the advantage of large binding capacity which can translate to higher efficiency in capturing of target analyte from a sample. In order to probe the whole volume of the binding matrix,

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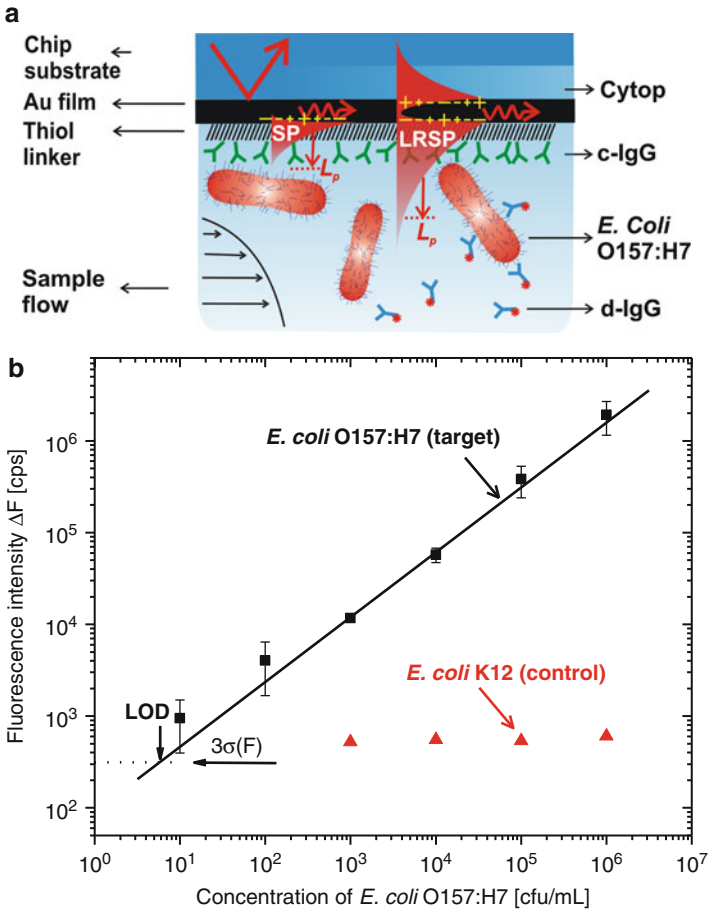

Fig. 7 Plasmonically amplified fluorescence biosensor for detection of bacterial pathogens by using sandwich immunoassay. (**a**) Schematic of the interface architecture and (**b**) calibration curve for the *E. coli* O157:H7 (Reproduced with permission from Huang et al. [21, 24] respectively)

long-range surface plasmons with increased probing depth were used. By using the sandwich immunoassays, the sensor allowed for detection of f-PSA in buffer and human serum with the limit of detection (LOD) of 34 f. and 0.33 pM, respectively, and the analysis time was of 35 min. This LOD was about four orders of magnitude better than that for SPR-based detection as can be seen from calibration curves presented in Fig. 6b.

Similar optics that allows probing to about micrometer distance from the metallic sensor surface was implemented for detection of large bacterial pathogens with around micrometer size by Huang et al. [21]. As depicted in Fig. 7a, sandwich immunoassay was used, and labeled detection antibodies were bound to the analyte that was affinity captured on the sensor surface. By using the PSP modes with increased probing depth, fluorophore labels on the whole area of the analyte were excited and contributed to the sensor signal. This fluorescence readout principle was carried out for *E. coli* O157:H7 and provided the limit of detection of six colony-forming units in one milliliter (cfu/mL). As can be seen in the calibration curve presented in Fig. 7b, the assay was highly specific and required 20 min.

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Conclusion and Perspectives

Plasmonics provides highly efficient amplification in important analytical tools based on surface-enhanced Raman spectroscopy (SERS), surface-enhanced infrared absorption spectroscopy (SEIRA), and plasmon-enhanced fluorescence spectroscopy (PEF). In the fluorescence spectroscopy, plasmonic structures supporting propagating surface plasmons can provide enhancement of fluorescence signal by a factor EF $> 10^2$, and in conjunction with immunoassays, it offers the limit of detection at low fM concentrations and the analysis time of several ten minutes. By tuning the probing depth of intense field of surface plasmons, a range of analytes can be detected and various biointerface architectures can be used. In order to translate this method to viable commercial technology, plasmonic sensor chips that are compatible with established fluorescence scanners and microscopes are pursued [25, 26]. For these applications, plasmonic structures that can be prepared by using mass production – compatible methods such as nanoimprint lithography – are of high interest [27]. In parallel, dedicated devices for research applications where fluorescence detection is combined with surface plasmon resonance were described [7]. In addition, the plasmonic amplification of fluorescence signal is subject of implementation to miniaturized devices for sensitive analysis in field that does not rely on using specialized laboratory environment [28].

Cross-References

- ▶ Fluorescence
- ▶ Fluorophore
- ▶ Localized Surface Plasmon
- ▶ Plasmonic Nanostructures
- ▶ Plasmonics
- ▶ Quantum Dot
- ▶ Surface Plasmon Polaritons
- ▶ Surface Plasmon Resonance

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