Chapter Ⅲ

Plasmon-Enhanced Fluorescence

3.1 Introduction

In this chapter, we focus on early results of enhanced fluorescence observed by metallic nanostructures and the enhanced substrates, including periodical and nonperiodical nanostructures. We will introduce the most important factor of the spacer between molecule, surface and wavelength dependence on PEF. This chapter provides an introduction to fundamentals of PEF, and illustrates current progress in the design of metallic nanostructures for efficient fluorescence signal amplification that utilizes propagating and localized surface plasmons.

3.2 PEF from periodical metallic plasmonic nanostructures

Coupling with incident light, SPR acted as an efficiency interaction platform for the detection of fluorophores with high sensitivity. In general, due to the existence of mismatch in wave vector momentum of the SPPs and the incident light, the excitation of SPPs on a flat continual metal surface is difficult to realize[1]. So it is difficult to obtain the potential plasmonic coupling as well as fluorescence enhancement effect high efficiency based on the metal substrate

with flat morphology[2]. It is shown that the mismatch can be overcome by introduction of an external periodical metal surface such as gratings, nanohole arrays, nanocap arrays, etc.

3.2.1 PEF from nanograting substrate

The first observation of anomalies in the intensity distribution of diffraction spectrum from a metallic grating by Wood in 1902[3], was latterly interpreted based on analyzing the relationship between the incidence photon and a SPR that existed in the grating surface[4]. So fabricating the metal surface with a periodic nanoscale corrugation provided an excellent candidate, that is, the momentum conservation restriction could be relaxed by breaking the translational invariance of the substrate. For the excitation of grating-coupled SPR, the excited condition can be defined as

$$K_{\mathrm{sp}} = K_{\mathrm{px}} + P = \frac{2\pi}{\lambda}\sqrt{\varepsilon_m(\omega)}\sin\theta + \frac{2\pi}{\Lambda}m \tag{3-1}$$

Here, Λ is the grating period, λ is the incident light wavelength, m is the diffraction order, θ is the incidence angle, and $\varepsilon_m(\omega)$ is the frequency-dependent dielectric function of metal given by Drude model.

The mismatch in wave vector between the in-plane momentum $K_x = K\sin\theta$ of impinging photons and can also be overcomed by using diffraction effects at a grating pattern on the substrate. As a result, the moderated diffraction on periodically corrugated metallic surfaces provides an alternative method for simultaneous SPPs-enhanced excitation at λ_{ab} and extraction of SPPs-driven emission of fluorescence light at λ_{em}. By fabricating series of tailored silver plasmonic grating nanostructure, Prof. Tawa systematically investigated the relationship between the grating parameters (depth and duty ratio) and the fluorescence-enhanced effect[2,5], and found that with the condition of depth equal to 20 nm and duty ratio equal to 0.43, the fabricated grating substrate achieved optimal fluorescence enhancement. It was found that the fluorescence enhancement mechanisms from grating arose from both the SPP-

enhanced absorption and SPCE. Prof. Sun also adapted silver sinusoidal nanograting as the enhanced substrate to boost the fluorescence intensity of target molecules as high as 30 fold[6], as shown in Fig. 3-1. With the help of photoluminescence (PL) measurements, the author found that enhanced effect was periodical and angularly dependent. That is, the SPP-enhanced absorption effect turned to be weaker for larger grating pitch, while SPCE became the dominant factor for the enhancement. And the directional emission of high p-polarization of SPCE at different detection angles was observed

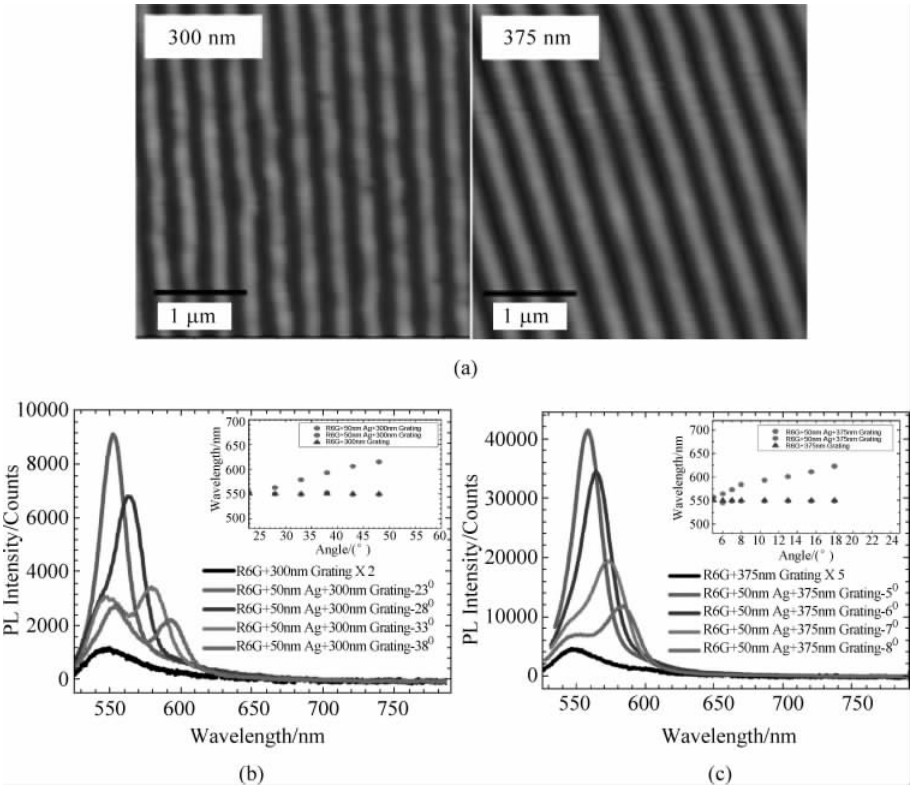

Figure 3-1 (a) AFM images of gratings with different periods: (left) 300 nm; (right) 375 nm, and fluorescence spectra of Rh6G deposited on silver grating films with various detected angles θ_F and with fixed excitation angles θ_E. Grating periods: (b) 300 nm and (c) 375 nm[6]

experimentally[7]. With the increase of the metal film thickness in the grating-based SPCE system, the enhanced spectra were demonstrated significantly affected by both depth and film thickness. Furthermore, for the short wavelength range, the higher angular sensitivity would be obtained[8].

3.2.2 PEF from nanohole arrays substrate

Since the enhanced extraordinary optical transmission (EOT) effect which exhibits greater transmittance of light energy than that occupied by the nanoholes[10]. The noble metallic opticall films decorated with subwavelength nanohole arrays are introduced to act like an excellent antenna to couple the incident EM wave into surface plasmons at a given frequency, and both the position and the width of the transmission peak can be modulated by adjusting the parameters of symmetry and period. As a result, the position of transmission tips λ_{tip}, under normal incidence, can be given by the following dispersion relation[11]:

$$\lambda_{\text{tip}} \sqrt{i^2 + j^2} \approx a_0 \sqrt{\frac{\varepsilon_m \varepsilon_d}{\varepsilon_m + \varepsilon_d}} \tag{3-2}$$

where a_0 is the period pitch of hole arrays, and i and j are the scattering orders of the nanohole array respectively[12].

On a free-standing nanohole array film, the incident light coupled with the SPs can enhance the fields associated with the evanescent waves, then increase the transmittance. As a result, continuous metallic film perforated with arrays of nanoholes can both supports the excitation of SPPs and LSP[13]. The enhanced EM field which will help to increase fluorescence efficiency of the fluorophore locate in vicinity of substrate. In order to collect the mostly enhanced fluorescence signals, the peak of the LSPR should be around 40-120 meV, which is higher in energy than the emission peak of dye, and the shape of LSPR spectra should be the same to the excitation spectrum of dye[14]. The conditions of plasmon coupling, such as incidence angle, laser frequency,

metal composition, and excitation and emission properties of fluorophores, play an important role in PEF on the metal nanohole arrays. By using various geometric parameters of the arrays in the Au film, Prof. Brolo reported a significantly, et al. enhanced fluorescence effect compared with the unpatterned identical films on glass. They found that the Au films decorated with nanohole arrays at resonance condition could improve the fluorescence sensitivity, and the detected fluorescence was strongly dependent on the nanohole radius and periodical distance, as shown in Fig. 3-2[15]. In order to avoid the fluorescence quench effect, silicon oxidation film was also introduced as spacer layer to separate the fluorescent molecule from substrate[16]. Recently, a periodical nanostructure with nanohole arrays formed in continued silver films was fabricated with template-stripping technique first, and then with an atomic layer deposition grown oxide layer on it, such as silica shell or alumina, to prevent fluorophore quenching[17,18].

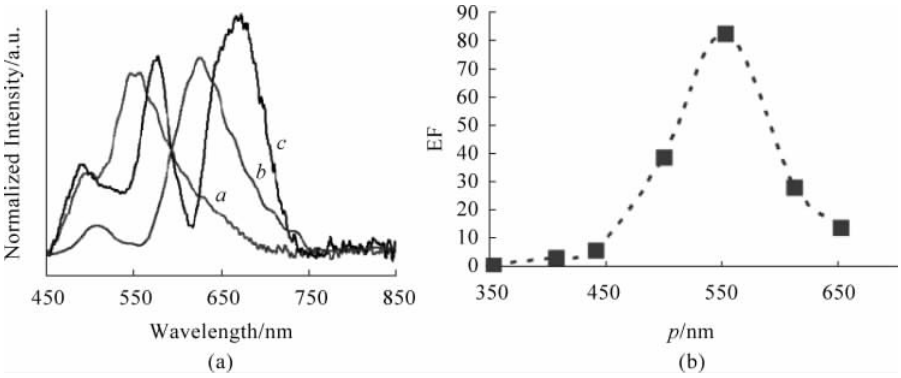

Figure 3-2 (a) The transmission spectra of white light across nanohole arrays. The uncoated nanohole array film with pitch $p=440$ nm (curve *a*), $p=550$ nm (curve *b*), and coated with a dye film of oxazine 720 with $p=440$ nm (curved *c*); (b) The calculated factor of enhancement from nanohole arrays with different pitch[15]

Selecting Rh6G-Quasar670™ FRET pair as acceptor molecule, through tuning the plasmon wavelength with a maximal overlap of the acceptor

excitation and donor's emission, the mostly efficient enhancement effect can be obtained by using the nanohole arrays[19]. In comparison with the backside illumination, the frontside one stimulates a stronger coupling between the incident EM waves and the substrates[20]. Furthermore, Bai's group systematically studied the plasmonic responses of four plasmonic metals (Au, Ag, Cu, Al) and three composite plasmonic metals (Ag/Au, Cu/Au, Al/Au) nanohole arrays in the plasmon-enhanced fluorescence spectroscopy biosensing setup[21]. In addition, the large-area golden nanohole arrays integrated with conductive glass (indium tin oxide: ITO) were also used to demonstrate the dielectrophoresis (DEP)-enhanced SPR sensing. Governed molecules diffuse to the sensor, are significantly accelerated by using applied alternating current (AC) electric field forces on the BSA molecules, as shown in Fig. 3-3[22]. It is

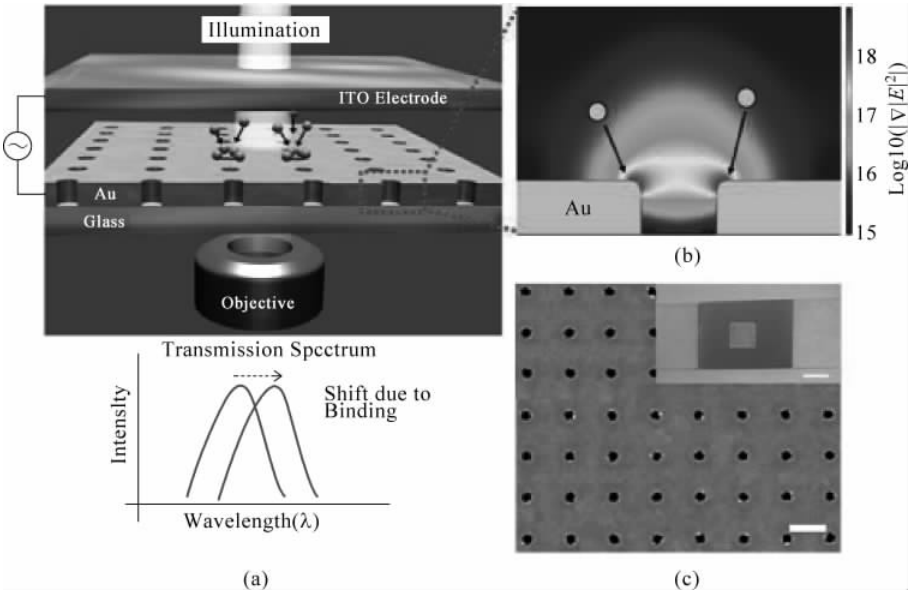

Figure 3-3 (a) Schematic of the experimental setup; (b) Dielectrophoretic forces attract target molecules to the edge of each hole as the electric field intensity gradient is the strongest (red color) along the rim of the holes; (c) The SEM image of the nanohole array (periodicity pitch $p = 600$ nm and nanohole diameter $d = 140$ nm), the scale bar here is 500 nm[22]

found that the fabricated nanostructure enabled label-free and real-time detection of target molecules in the concentrations as low as 1 pM in very short time. Compared with traditional diffusion-based binding method, the current protocol exhibited very high detection efficiency.

3.2.3 PEF from nanoparticle arrays substrate

It is reported that the plasmonics properties of nanoparticles (NPs) critically depend on its characteristics, such as the dielectric constant, interparticle distance, shape, and dimension, etc. As a result, many researches have performed to study the fluorescence enhancement on such a NP substrate, particularly the relationship between the orientation and the distance of fluorescent molecules, as well as the environment effect, with respect to the substrate[23]. Due to the coupling effect between the emission of fluorophore and the SPR band of SNP arrays nanostructure, the intensity of PEF, moderated by adjusting the distributed characteristics of nanopartice arrays, was also observed experimentally[24-26]. Though the deposition of metal nanoparticles onto the substrate often produces some local "hot spots" randomly, which will be helpful to obtain a high fluorescence signal, the pursuing efficiency fluorescence-enhanced substrate with large area, the uniform-and high-enhancement factor is still difficult to realize[27]. With the thermally deposited method, Cu periodical nanostructures were fabricated. For a longer wavelength region, LSPR of the fabricated substrate can be precisely controlled. It is found that the quenched fluorescence effects from fluorophore near the plasmonic pure Cu nanostructures are observed. With tuning the position of SPR properly, the highest fluorescence-enhanced factor (EF) of target molecules by 89.2-fold was obtained as shown in Fig. 3-4, compared with reference[28] sample.

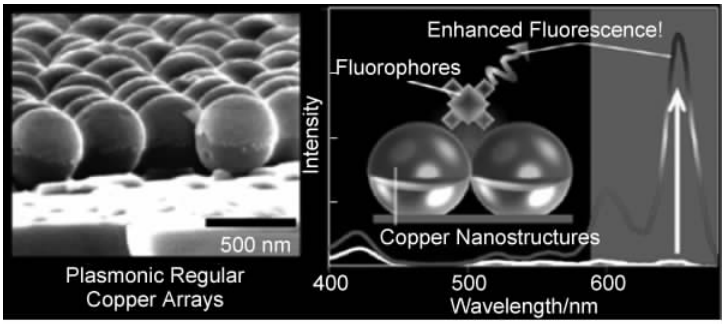

Figure 3-4 (a) SEM image acquired from the copper arrays; (b) Schematic of the interaction between the copper nanostructure and fluorophores[28]

3.2.4 PEF from nanorod arrays substrate

The properties of LSPR critically depend on the morphology of metallic nanostructure. Compared with local enhanced EM field generated in randomly patterned metallic nanostructures, the SP near-field coupling between particle dimers can lead to frequency shift of LSPR and unusual huge EM fields for nanostructure with order arrays. As a result, the precise tuning of LSPR, such as adjusting the shape and arrangement of metal nanopartices, plays an important role in pursuing the high-efficiency coupling effect of the fluorophore and the local enhanced EM field[29, 30]. It is reported that considerable fluorescence enhancement factor of 100-fold, from the silver nanorod arrays nanostructure with highly regular distributed, was obtained as compared to the controlled sample[31]. Recently, Abdulhalim, et al. has investigated the effect of silver nanorod arrays morphology on the PEF with the Glancing angle deposition (GLAD) method. It is found that the nanorod length has a significant effect on the PEF enhancement factor. Based on the lighting-rod and SPR effects, the variation of PEF efficiency from silver nanorod arrays substrate is qualitatively analyzed, see Fig.3-5[32].

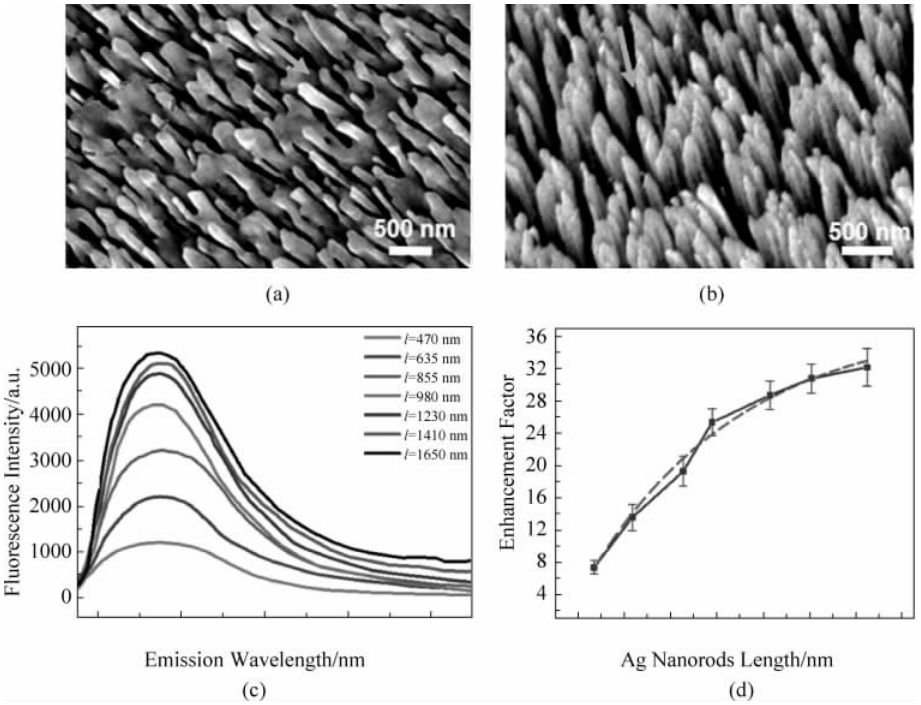

Figure 3-5 The SEM image of the silver nanorods fabricated at (a) room temperature (320 K) and (b) low temperature (140 K); (c) PEF spectra of R6G on silver nanorod arrays substrate with various nanorod lengths; (d) The relationship between EF and the length of nanorods[32]

3.3 PEF from nonperiodical metallic plasmonic nanostructure

Spatial correlations and distribution of EM field have been extensively investigated in pursuing the nature of wave transport in nonlinear disordered systems[33]. Due to the LSPs and the lightning-rod effect which governed by structural inhomogeneities, the correlations of local EM enhanced effect existed on disordered metallic nanostructure have been widely studied, as reviewed by Prof. Shalaev[34]. The resonant dipolar excitations localized in disorder nanostructures with subwavelength size exhibiting sensitive frequency

and polarization dependence, which are critically determined by the local topography of nanostructure[35]. For the giant local EM field excited in the nonperiodic structure, the spectrum properties of molecules located in the vicinity of "hot spots" existed on the plasmonic nanostructure can be moderated. As a result, the investigation into enhanced fluorescence effect of many typed nonperodic nanostructures, such as silver island film (SiF), fractal-like, etc. on fluorescence have been widely performed.

3.3.1 PEF from metallic silver island substrate

Fluorescence enhancement of substrates with metallic islands film has been the subject to research since the 1980s[36]. It offers the advantage of a relatively simple preparation method and provides moderate enhancement factors through the combined effect of PEF excitation rate and increased quantum yield. With the thermal vapor deposition method, copper films with various thicknesses deposited onto glass substrate have been used to study the enhanced fluorescence effect. The experimental observations show that fluorescence intensities of fluorophores located near the Cu films are critical dependence on the film thickness, and reach maximum EF at certain thickness[37]. It is reported that the PEF derived from coupling effect of fluorescent molecules with excited states with the SP in the metallic nanostructure firstly, then local EM enhancement effect stimulated the enhanced absorption of the fluorophores[39], see Fig.3-6.

3.3.2 PEF from metallic fractal-like substrate

Due to the strong optical response fluctuations of the local EM fields, which could be moderated by adjusting the fraction dimension and morphology of nanostructure, the nonlinear optical characteristics of disorder metal nanostructure have been attracted much attention recently[40-42]. It has been reported that fluorescence enhancement effects fluorophore emission was