Surface enhanced fluorescence by porous alumina with nanohole arrays

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The fluorescence enhancement of Rhodamine 6G (Rh6G) fluorophore in the close vicinity of porous alumina film with ordered nanohole arrays is investigated. Experimental observations show that the nonmetallic substrate with hole arrays enhances the fluorescence intensity. By comparing the fluorescence emissions that are excited with 325 nm and 532 nm, better fluorescence enhancement is obtained with excitation at a shorter wavelength. The study suggests that higher fluorescence excitation efficiency due to the energy transfer from oxygen vacancies to Rh6G fluorophore molecules is responsible for better fluorescence enhancement. The contribution of the scattering of nanohole arrays to the fluorescence enhancement is also proposed based on the intensity increase and reduced lifetime when the energy transfer from oxygen vacancy is absent. The result of the current study is useful for developing non-metal substrates in the study of spectroscopic enhancement, and is expected to advance the applications of porous alumina to microanalysis.

Surface enhanced fluorescence (SEF) has been traditionally termed as an intensity increase of the fluorescence emission from fluorophores sitting in the close vicinity of a metallic surface that has proper nanostructures or configurations [1,2]. Much work on the SEF has been reported in the past decades, which helps us to understand the mechanism of the enhancement phenomena and develop its applications in many fields [3–9]. Up to now, three mechanisms are generally accepted by researchers, which include local electromagnetic (EM) field enhancement [2], surface plasmon coupled emission (SPCE) [7], and fluorescence resonance energy transfer (RET) [8].

The mechanism of local EM field enhancement is based on the enhancement of local electromagnetic field, which is mainly due to the surface plasmon excited by incident light on metallic structures surfaces. For this mechanism, the fluorescence enhancement is basically proportional to the square of the EM field, $|E|^2$. According to what have been reported, experimentally obtained enhancement factor for SEF has reached 10^2 or more [10]. SPCE mechanism deals with the fluorescence enhancement that results from the coupling of the excited fluorophore molecules and the excited surface plasmon. It could be the case that the excited surface plasmon can give the same emission as the fluorophore molecules do, which can enhance the intensity of the fluorescence emission through the coupling with fluorophore molecules. RET mechanism refers to energy transfer

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processes from the substrate to the molecule. Because of the energy transfer, the excitation efficiency of fluorophore molecules becomes higher, which increases the radiative transition probability, and then enhances the fluorescence intensity.

Metallic materials have frequently been used as primary substrates for surface enhanced spectroscopic investigation including SERS (surface enhanced Raman scattering) and SEF. Traditionally, the most effective substrates are Au and Ag nanoparticles or surfaces with nanostructures [10–15]. Other metals such as Cu, Al, have also been used as enhancement substrates [16,17]. It has been firmly proved that the metallic surfaces are the most effective and efficient substrates for SERS and SEF [18], but we also have to admit that most of the metallic substrates are not chemically stable, which may not be a problem for SERS effect, but could induce a significant impact on SEF observation since the electronic transitions are involved in the fluorescence process. Besides, the biological compatibility of metal substrates usually is not good, which limits the applications of fluorescence probes in biological detection. Therefore, developing and exploring enhancement substrates other than metallic ones for spectroscopic enhancement, especially looking for those chemically inert, biocompatible, low cost and easy to obtain, is necessary and worth trying.

Porous alumina film with ordered pore array, which is the anodic aluminum oxide with matrix arrays of pores and also is called AAO film for short, is a honeycomb structure having many attractive properties [19–21]. It is reported that oxygen vacancies (V_o) are formed during the preparation process [19]. Since the energy transfer could occur between V_o and fluorophore molecule, the RET mechanism is possible for the spectroscopic enhancement. In the past years, the RET process has been studied in SEF effect with metal nanostructures, in which the contributions of local EM field and SPCE cannot be ignored. When the non-metallic AAO surface is used as a spectroscopic enhancement substrate, the contribution of RET process to the enhancement effect can be investigated without the influence of other possible mechanisms. This provides us an easy way to separate the RET effect from others, which is certainly helpful for understanding the enhancement mechanism of SEF.

Numerous efforts have been made in finding the applications of AAO film in bioscience and material science including the usage of it as enhancement substrate [22–27]. In the current work, the AAO substrate is employed as an enhancement surface, the fluorescence enhancement effect is obtained and the corresponding enhancement mechanism is explored based on the experimental observations and characteristics of AAO film.

1   Experimental details

The AAO film is fabricated by using a process of two-step anodic oxidation of aluminum, in which a highly ordered hexagonal array is formed by uniform nanoholes. During the preparation process, pure aluminum foils (99.99%) were firstly degreased in acetone for an hour and annealed at 350°C for about 20 min. The electro-polishing process was accomplished to reduce the surface roughness in a mixture of $HClO_4$ (70%, Panreac) and C_2H_5OH (1:4, *v/v*) at ambient temperature. Then the polished samples were anodically oxidized in a 0.3 mol/L aqueous oxalic acid solution at a constant voltage (40 V). Repeating the anodization process twice, and rinsing the samples in H_3PO_4 and H_2CrO_4 (0.5 mol/L and 0.2 mol/L, respectively) aqueous solution, one improved the formation of the porous array. The unreacted aluminum was removed from the film with a mixed solution of $CuCl_2$ (0.1 mol/L) and chromic acids to release the AAO membrane. The barrier layer at the bottom of the pores was removed in the H_3PO_4 solution, making the pores opening at both ends and expending the pores as well.

The obtained AAO film has a pore diameter of 40 nm, and the average periodicity (center-to-center distance) is about 130 nm. Figure 1 shows the scanning electron micro-
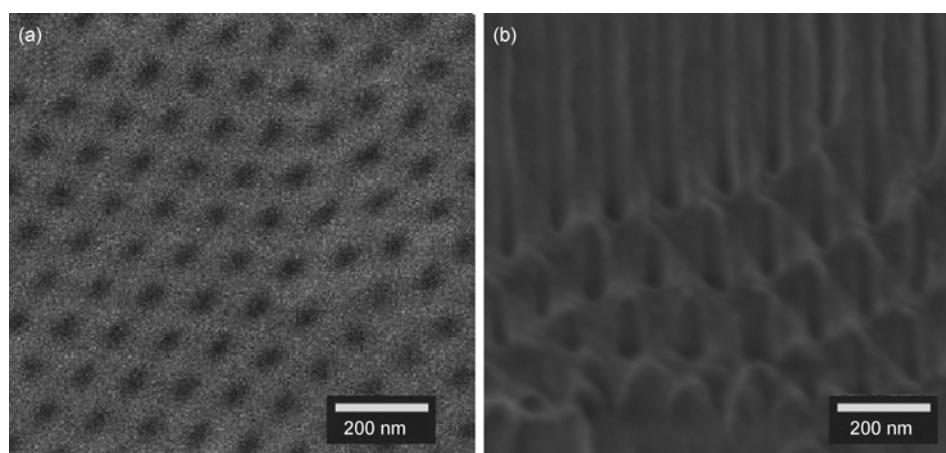

Figure 1   The SEM images of (a) the top surface and (b) the cross section of AAO film.

scope (SEM) images of the top surface and the cross section of AAO film with typical pore arrays obtained through the above process. The percentage element contents of oxygen to aluminum in AAO substrate is measured with energy dispersive spectrum (EDS). The obtained image and element ratio is presented in Figure 2.

A frequently used fluorescence probe, Rhodamine 6G (Rh6G), is employed to study the SEF on the non-metallic AAO substrate. Figure 3 displays the absorption spectrum of Rh6G and the emission spectrum of bare AAO substrate. During the fluorescence spectroscopic measurement, the Rh6G water solution (5×10^{-6} mol/L) is sandwiched between the glass and the AAO film. The samples are excited with laser lines of 325 nm and 532 nm, respectively. A SP2750i monochromator (Acton Research Corporation, USA) equipped with a PIXIS100 CCD (Charge Coupled Device) system is used for signal collection and detection. Fluorescence lifetime measurements are performed with an Edinburg FLS 920 fluorescence spectrometer through time-correlated
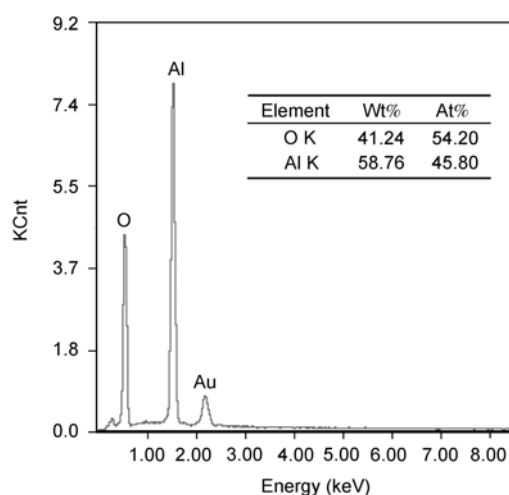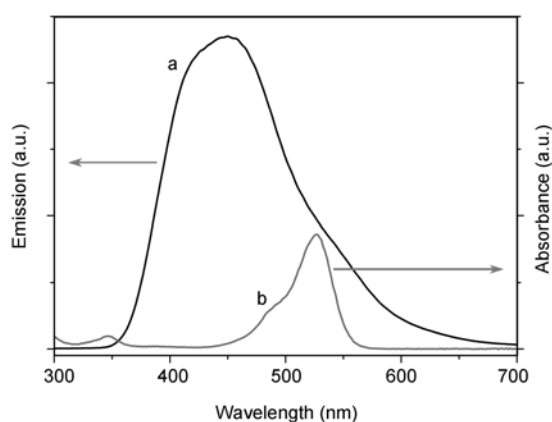
single photon counting. Properly selected notch and edge filters are placed in front of the entrance of the spectrometer to block the scattering light. All of the measurements are conducted at room temperature.

2   Experimental results and discussions

To investigate the SEF effect with non-metallic pore array substrate, we have done spectroscopic measurement of the fluorescence of Rh6G molecules in the close vicinity of AAO film and reference glass surface with sandwiched configuration containing a very thin layer of Rh6G water solution. Curves (b) and (c) show the structured fluorescence emission spectra observed on different substrates with excitation at 325 nm (Figures 4). The fluorescence emissions obtained at AAO substrates are clearly made of two peaks at 550 nm and 420 nm. It can be seen that the fluorescence signals at 550 nm, which is from Rh6G fluorophores, is clearly enhanced. The fluorescence intensity is much stronger than that on the reference glass substrate. If we define the enhancement factor as the ratio of fluorescence intensity of Rh6G at 550 nm on the AAO substrate to that on the glass, then the enhancement factor is found to be about 5 corresponding to the excitation of the system at 325 nm. Since the emission peak at 420 nm is only observed in the system with AAO substrate, it must be closely related to AAO and the excitation of it. Based on the experimental observation and previous study [28], we suggest that the observed emission at 420 nm (Curve a in Figure 4), which is only presented in the SEF measurements with AAO substrates, is related to the oxygen vacancies (V_o) formed during the preparation of AAO.

According to the theoretical mechanism of AAO preparation, oxygen in alumina comes from OH^- in the electrolyte solution [19]. During the growth of alumina, OH^- group gradually migrates to the oxide/metal interface, moving into

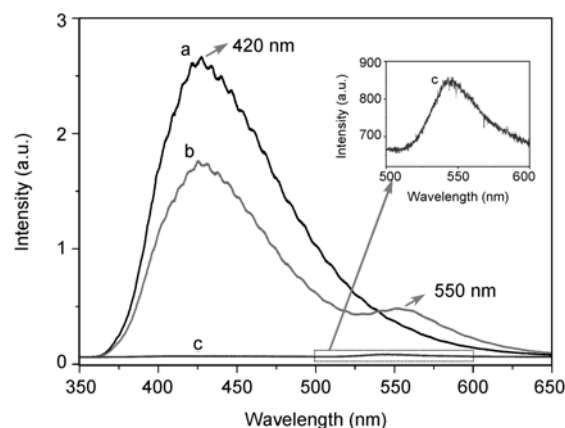**Figure 2**   EDS image of the AAO substrate. Inset: the proportion of oxygen and aluminum elements.

Figure 3   The emission spectrum of AAO substrate (Curve a) and the absorption spectrum of Rh6G molecules (Curve b).


Figure 4   Fluorescence spectra of AAO without Rh6G (Curve a) and with Rh6G (Curve b), and Rh6G on the reference glass substrate (Curve c) with UV excitation at 325 nm. Inset: the amplified Curve c.

O⁻ and then forming new alumina layers. In the sample that is anodized in oxalic acid, the electric current increase results in more consumption of OH⁻ group, which then reduces the OH⁻ concentration in the solution. The amount of V_o formed in AAO can be estimated by the element ratio of oxygen to aluminum in the sample. More oxygen vacancy results in a lower ratio of O to Al. The EDS result presented by Figure 2 shows that the actual atomic ratio of O to Al is 54.2/45.8=1.18, which is much smaller than the standard ratio of 1.50. This directly proves the existence of oxygen vacancies in AAO substrate.

Fluorescence resonance energy transfer is a distance-dependent excited state interaction in which the emission of one center (acceptor) is coupled to the excitation of another (donor). The excitation energy can be transferred by a radiationless process to a neighboring fluorophore center when the energy level difference matches. If the acceptor center is fluorescent, the transferred energy can be emitted as fluorescence that results in an enhancement of fluorescence emission. According to RET theory [9,29], the condition for obtaining energy transfer is that the distance between the donor and acceptor is in the range of about 1 nm to 10 nm, and the fluorescent emission spectrum of the donor center must overlap with the absorption spectrum of the acceptor center. As it is shown in Figure 3, the emission of AAO overlaps with the absorption of Rh6G. Through comparing Curves (a) and (b) in Figure 4, the emission intensity of V_o decreases with the increase of the peak at 550 nm, which directly proves the RET between the AAO substrate and the fluorophore molecules. Thus the experimental observation of SEF is in good agreement with the RET mechanism for the system with nonmetallic AAO substrate.

The above discussion indicates that the energy transfer from the excited oxygen vacancy contributes to the SEF at AAO substrate, but it may not be the only mechanism for the observation. If we change the excitation wavelength so that the oxygen vacancy is not excited, and hence the energy transfer does not occur, then the observation on the fluorescence enhancement can give us the answer. Based on this idea, the SEF measurement is also carried out by exciting the sample with 532 nm laser. Obvious fluorescence enhancement is obtained too. Figure 5 shows fluorescence spectra detected from Rh6G in the close vicinity of AAO substrate and on the reference glass surface. Since the excitation photon energy of 532 nm is too low to excite the oxygen vacancies of AAO substrate, there is no peak associated with oxygen vacancies appearing in the spectrum. According to the previous discussion on the RET mechanism, no contribution from the V_o through the energy transfer to the fluorescence enhancement is expected with excitation at 532 nm. In order to understand the experimental observation of Figure 5, we briefly review the Mie theory and discuss the scattering properties of pore arrays in AAO film. According to the scattering theory, the scattering property, C_S, of pore arrays depends on the size and period
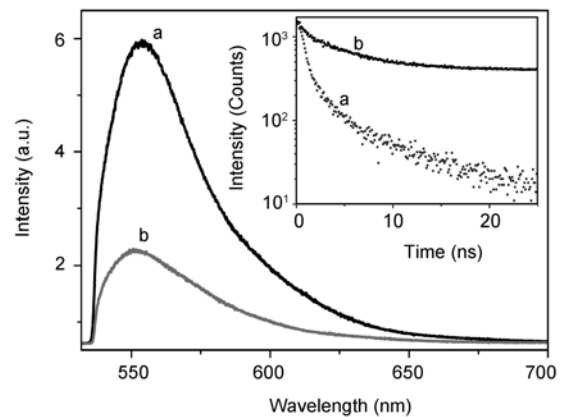
Figure 5   Fluorescence spectra of Rh6G on AAO (a) and reference glass substrate (Curve b) with 532 nm excitation. Inset: the intensity decay of fluorescence emission on AAO (Curve a) and of reference glass substrate (Curve b).

of pores, and the excitation wavelength [30]. Fluorescence enhancement could be obtained on the AAO substrate due to the high scattering cross section of periodic nanohole structure.

In addition, we have studied the fluorescence lifetime of Rh6G on the AAO substrate and on the reference glass substrate. The decay curves are shown in the inset of Figure 5, and the corresponding lifetime from the data fitting is listed in Table 1. It can be seen that the fluorescence intensity decay of Rh6G on the AAO substrate is faster than that on the glass substrate. The average fluorescence lifetime of Rh6G decreases from 4.6 ns on the glass to 2.2 ns on the AAO substrate. It is known that fluorescence emission quantum yield (Q) can be increased by decreasing the lifetime (τ). Fluorescence intensity is proportional to the quantum yield that is determined by radiative decay rate (Γ) and non-radiative decay rate (K_{nr}) through the following equation [31]:

$$Q = \frac{\Gamma}{\Gamma + K_{nr}},$$
$$\tau = \frac{1}{\Gamma + K_{nr}}. \tag{1}$$

In fact, a highly efficient structured emission, where the radiative decay rate is increased, would be expected to have a reduced fluorophore lifetime and fluorescence enhancement. Thus, the RET and scattering mechanisms are suggested for understanding SEF observation with non-metallic AAO substrate.

Table 1   Fluorescence lifetime obtained from the inset of Figure 5 by fitting the experimental data (A_n, amplitudes; τ, mean lifetime)

Substrate	τ_1 (ns)	A_1 (%)	τ_2 (ns)	A_2 (%)	T (ns)
Glass	0.60	7.32	4.91	92.68	4.60
AAO	0.76	69.83	5.40	30.17	2.16

3   Summary

In summary, the fluorescence enhancement on non-metallic AAO substrate is investigated in the current study. Up to five folds enhancement is observed compared to a reference glass substrate with excitation at 325 nm and about three times enhancement with 532 nm excitation. The resonance energy transfer from the excited oxygen vacancy to fluorophore molecules is one contribution to the fluorescence enhancement. Another contribution is due to the efficient scattering of pore arrays in AAO film, which is proved by the fluorescence enhancement accompanied with a decrease in lifetime. Therefore we suggest that the SEF effect on AAO substrate results from the RET of oxygen vacancy and the efficient scattering of nanohole arrays. The result helps studying the basic properties of AAO and non-metal enhancement mechanism, and expanding the application of AAO to microanalysis.

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