Plasmon-Enhanced Fluorescence of FITC Labeled Human IgG

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***Abstract* — Plasmonic enhancement of fluorescence up to 7 times was demonstrated for human IgG labeled by FITC near silver nanoparticles. Nanostructured Ag films were formed by chemical route without expensive processes of nanolithography, molecular beam epitaxy, vacuum deposition. For excitation with linear polarized light the enhancement factor was shown to increase for p-polarization and to decrease for s-polarization.**

***Index Terms* — IgG-FITC, nanoplasmonics, polyelectrolyte layer, silver nanoparticles, surface enhanced fluorescence..**

I. INTRODUCTION

Plasmonic enhancement of fluorescence is being extensively investigated during last 20 years in the context of practical applications in analytical spectroscopy, display and light-emitting devices. It has being realized for organic molecules, quantum dots, rare-earth ions [1-4]. Experiments performed by a number of groups did show luminescence enhancement from 2…3 то 30 times when the object is placed near Ag or Au nanoparticles [5-7]. The maximal enhancement occurs for spatially organized nanostructures developed by means of submicrometer nanolithography, vacuum deposition and etching [8, 9].

A huge number of experimental and theoretical investigations are devoted to luminescence of organic dyes. The reason is that organic fluorophores are nowadays the most convenient and popular labels used for marking of various biomolecules (i. e. albumins, antibodies, immunoglobulins). Plasmonic enhancement of its fluorescence has being reported elsewhere [10, 11]. However, many authors have considered weak luminescent dyes, such as Cy 5 and so on.

Fluorescence enhancement factor F (i.e. the ratio of luminescence intensities near metal nanobody and without it) depends on the size of metal nanoparticles, their patterning, and also on distance between metal and a fluorophore. So, the theoretical calculations given in Ref. [12] for Ag nanoparticles and fluorescein isothiocyanate (FITC) show that the enhancement factor reaches its maximum (F~50) at the distance between FITC and Ag nanoparticles approximately 6 nm and the size of the latter ones ~ 50 nm.

It is important to control the distance between metal and fluorophore because the fluorescence enhancement factor is a combination of the enhancing and quenching effects changing with change of the distance. A simple chemical approach has been proposed by our group [13] for fabrication metal-fluorophore spacer based on electrostatic deposition of counter-charged polyelectrolyte molecules. We reported 7-9 fold enhancement of fluorescence of bovine serum albumin labeled with FITC near Ag plasmonic film with the spacer thickness 3-6 nm [3]. In this paper, we present the results of fluorescence enhancement of FITC labeled human IgG near silver nanoparticles. The highlight of this work is the using of an organic label with high quantum yield. Both the plasmonic Ag film and polyelectrolyte spacer were formed without expensive processes of nanolithography, molecular beam epitaxy, vacuum deposition and can be used in mass measurements.

II. EXPERIMENT

Polydiallyldimethylammonium chloride (PDADMAC, M = 200000 g/M, Aldrich) and sodium polystyrene sulfonate (PSS, M = 70000 g/M, Aldrich) were used as positively and negatively charged polyelectrolytes (PE). Other chemicals were $AgNO_3$, $Na_3C_6H_5O_7 \cdot 5{,}5H_2O$ (Aldrich), fluorescein isothiocyanate (isomer I, Sigma), purified human IgG in lyophilized form (Edobulin trademark, Immuno AG), Tris-EDTA buffer solution (pH 7.6, Fisher BioReagents).

Silver sol was synthesized by $AgNO_3$ citrate reduction technique [14]. The synthesis of IgG – FITC conjugates was made according to standard procedure

[15], the concentration of product is being 5.2 mg/ml with IgG:FITC molar ratio 1:7.

Glass substrates of 1×2 cm^2 size were cleaned in piranha ($H_2SO_4 + H_2O_2$), washed and kept in a mixture of H_2O-H_2O_2-NH_3 (1:1:1) at 70^{o}C for 15 minutes. After washing in water substrates were covered with a polycation layer (PDADMAC, 1 g/L in 0.5 M NaCl) during 20 minutes to develop positive charge on a glass surface. To deposit Ag nanoparticles on the substrates, the half of a substrate surface was immersed into a silver sol for 24 hrs. The silver-free remaining portion of the substrate served as a reference sample.

To develop a dielectric spacer, we used a method described in [13]. Labeled IgG was diluted by Tris-EDTA buffer solution in a volume proportion 1:100 directly before using. Two aliquots of IgG-FITC solution were put on Ag and glass parts of the substrate and remained for 1 hour at room temperature. At last, the samples were washed by buffer solution and dried on air.

Scanning electron microscopy (SEM) has been performed with Hitachi S-806 (Japan) microscope. Luminescence spectra were measured using a home-made spectrometer based on S-3801 spectrograph (Solar TII, Belarus) with a 1200 lines/mm grating and a liquid nitrogen cooled silicon CCD camera LN-CCD-1152-E PROD FG (Princeton Instruments, USA). A light emitting diode (LED) ARPL-3W Blue (Arlight, Russia) was used as the excitation light source with maximum at 460 nm and spectral half-width ~25 nm.

III. RESULTS

EM image of Ag film formed on a PDADMAC-modified substrate shows that silver nanoparticles cover the surface nearly continuously with some of them forming aggregates (Fig. 1). The particles size is 30 – 120 nm, average diameter is near 55 nm.

Compared to the reference sample, FITC fluorescence enhancement near Ag nanoparticles is observed even on a single PE layer. We consider that rather big size of immunoglobulin molecules allows spacing a label from a silver surface. On such distance, the increase of the probability of spontaneous photon emission becomes dominant and prevails over the processes of nonradiative energy transfer on metal. The biggest value of fluorescence enhancement factor $F = 6.7$ is being reached when the distance from FITC to Ag becomes equal to 3 PE layers.

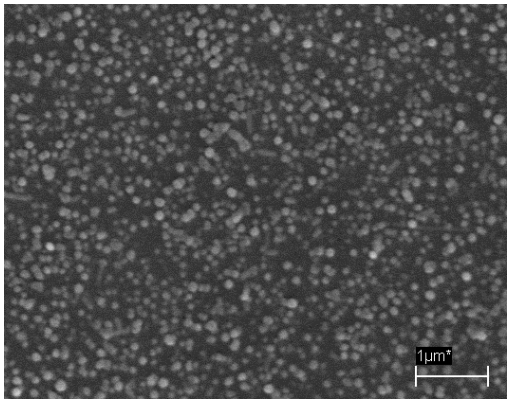

Fig. 1. SEM image of silver nanoparticles in Ag film.

Further measurements were performed in two standard geometries: *p*-polarization when incident light polarizations lies within the plane of incidence, and *s*-polarization when incident light polarization is normal to the plane of incidence. In all experimental observations for excitation with linear polarized light the enhancement factor increases noticeably for *p*-polarization and decreases for *s*-polarization. For instance, the fluorescence enhancement factor for *p*-polarization rose up to 7.4 when a PE thickness equals 3 layers (Fig. 2, 3) and down to 6 for *s*-polarization. These results are in remarkable coincidence with the behavior of BSA-FITC molecules studied earlier [3].

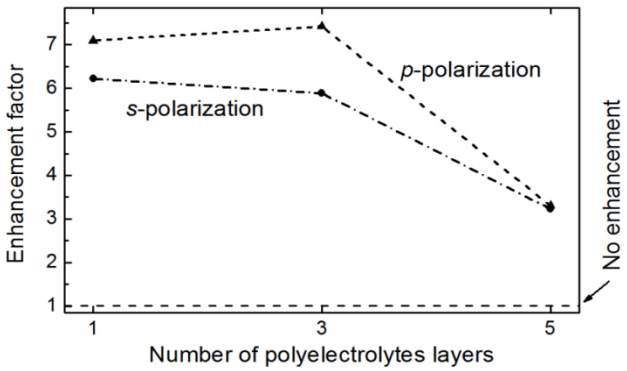

Fig. 2. Dependence of the fluorescence enhancement factor on the number of polyelectrolyte layers (*p*- and *s*-polarization of incident light).

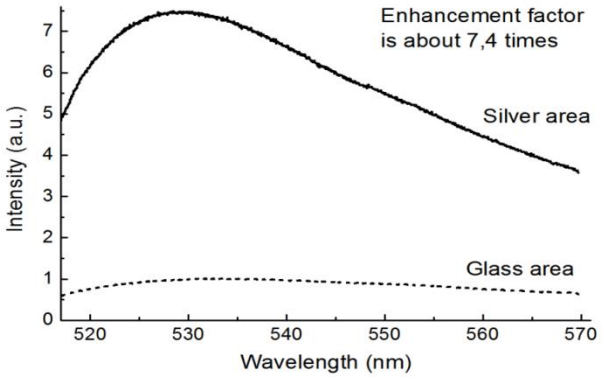

Fig. 3. A part of IgG-FITC photoluminescence spectra around its maximum for silver and glass surfaces covered by 3 polyelectrolyte layers (*p*-polarization of incident light).

IV. CONCLUSION

In conclusion, plasmonic enhancement of fluorescence up to 7 times was demonstrated for IgG-FITC conjugates near silver nanoparticles. Nanostructured Ag films were formed by chemical route; the technology can be easily reproducible and applied for any other biomolecules labeled with FITC.

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