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Metal Enhanced Fluorescence on Silicon Wafer Substrates

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Abstract

We report on the fluorescence enhancement induced by silver island film (SIF) deposited on a silicon wafer. The model immunoassay was studied on silvered and unsilvered wafers. The fluorescence brightness of Rhodamine Red X increased about 300% on the SIF, while the lifetime was reduced by several fold and the photostability increased substantially. We discuss potential uses of silicon wafer substrates in multiplex assays in which the fluorescence is enhanced due to the SIF, and the multiplexing is achieved by using micro transponders.

1. Introduction

Fluorescence enhancement on colloidal surfaces was observed a few decades ago [1–3]. Recently however, due to the strong demand for a more sensitive detection of biomarkers, the interest in enhanced fluorescence has been revitalized. A number of research groups have been investigating the emission of fluorescent dyes on surfaces coated with various silver nanostructures. These include Colloids [4,5], Silver Island films (SIFs) [6–8], Roughened Electrodes [9], Structures made by vapor deposition [10], and Electrochemically deposited fractals [11–13].

The dependence of the enhancement on the distance of the fluorophore from the silvered surface was studied for different dyes, and the optimal fluorophore-surface distance was found to be about 100 Å [4,14]. At shorter distances, the quenching by metal dominates the fluorophore-silver interaction. At longer distances, the fluorescence enhancement progressively decreases, although the exact dependence is not yet established. Two phenomena should be taken into account while describing the fluorescence enhancement on silver nanostructures. The first is the enhanced local field which provides stronger excitation. The local field could be extremely high as observed in Surface Enhanced Raman Scattering (SERS) [15–17]. The second is the interaction of the excited molecule with silver nanoparticles which results in a rapid radiation of the excitation energy. This effect increases the quantum yield of the fluorophore and decrease the lifetime [2,18]. The total gain in brightness is the product of these two phenomena.

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Very recently, a new technology was proposed for multiplex assays. This technology is based on micro-transponders (MTP): 100 micron thin, sub-millimeter size silicon chips. The micro-transponder is an integrated circuit composed of photocells, memory, clock and an antenna. It stores information identifying the sequence of an attached oligonucleotide probe in its electronic, 64 bit memory. The memory capacity allows for approximately 10^{17} different IDs. The photocells, when illuminated by light, provide power for electronic circuits on the chip. The purpose of the antenna is to transmit the ID through a variable magnetic field created close to the tag as a result of modulated current in the antenna [19,20]. MTPs can be covered with a polymer coating that optimizes covalent linking of DNA probes. Probe-coated MTPs are stable for over one year at -20°C , making them ideal for use in medical research and commercial assays.

We initiated a project to implement metal-enhanced fluorescence technologies on the surface of the micro-transponder to develop highly sensitive multiplex assays. In this manuscript we report the first observations of fluorescence enhancement on silicon wafer substrates.

2. Experimental

2.1. Materials

For silver deposition on the wafers silver nitrate, D(+)glucose, and sodium hydroxide, obtained from Sigma-Aldrich (MO), and ammonium hydroxide, obtained from Fisher Scientific (PA), were used. Rabbit and goat immunoglobulins (IgGs) (95% pure) were purchased from Sigma. Rhodamine Red-X labeled (Rh Red-X) anti-rabbit IgG, 2mg/ml protein with 3.0:1.0 dye:protein ratio, was purchased from Invitrogen (CA). Buffer components and salts used in the assay (such as bovine serum albumin, poly-L-lysine, sucrose, and sodium phosphate) were purchased from Sigma-Aldrich (MO). Milli-Q[®] purified water was used for all solutions.

2.2. Preparation of the wafers coated with silver island films (SIFs)

The SIF was prepared using the procedure described in the literature [21,22]. First, half of each wafer surface was coated by depositing SIF through chemical reduction of silver nitrate by wet-chemical process using D (+) glucose, while the other half was left uncoated. Next, the MTP wafers were dried in air and a rectangular (14×12 mm) reaction well was prepared on each half (silver coated and uncoated) of wafer surface by covering rest of the area with insulation tape.

2.3. Immunoassay procedure

Model immunoassays were performed on the MTP wafer surface in the wells as described earlier [3]. First, the surface of the well was coated with poly-lysine for better protein adsorption. Approximately 0.5 ml of freshly prepared poly-L-lysine solution (0.01% poly-L-lysine in 5mM Na-Phosphate buffer, pH 7.2) was added to each slide, incubated for 30 min at room temperature and finally then rinsed with water. Next, rabbit IgG was non-covalently immobilized on the "sample" slide, or goat IgG on the "control" slide, by physical adsorption (overnight incubation of the rabbit or goat IgG solution of 40 $\mu\text{g}/\text{mL}$ in 50 mM Na-phosphate buffer, pH 7.2, 200 μL per well, at room temperature). Then, all remaining protein binding sites were blocked by adding 200 μL of blocking buffer (1% bovine serum albumin, 1% sucrose, 0.05% NaN_3 , 0.05% Tween-20 in 50 mM Na-phosphate buffer, pH 7.3 per well and incubating it for 2 hrs at room temperature. After washing, 150 μL per well labeled reporter conjugate, anti-rabbit antibody (at 5 $\mu\text{g}/\text{mL}$ in blocking buffer) was added, followed by incubation of 1 hr at room temperature. The labeled antibody supernatants were removed, and the surfaces were rinsed, covered with 50 mM Na-phosphate buffer, pH 7.3, and stored at $+4^{\circ}\text{C}$ until fluorescence measurements (spectrum, lifetime or photobleaching) were done.

Since the physical properties of the surfaces are different and we immobilized the antigen by physical adsorption, it is possible that binding percentage may be different for the uncoated and SIF-coated wafer surfaces. Therefore, the binding effectiveness was estimated by measuring the labeled antibody concentrations before and after incubation on each surface (by collecting the emission spectra of the supernatants removed from the reaction wells). We found that the binding efficiency on the SIF-coated wafers was the same as on bare wafers, about 50% of the labeled antibodies from the supernatant.

2.4. Fluorescence measurements

Fluorescence measurements were carried on Fluo200 (PicoQuant, Inc.) fluorometer equipped with a monochromator and a R3809U-50 (Head-on, 45 mm) microchannel plate (Hamamatsu, Inc.) on detection side. In addition, we used a 495 nm long wave pass cut-off filter. The excitation was from pulsed 475 nm laser diode with a pulse with 68 ps. For the photostability measurements we used 568 nm illumination from a argon/krypton small frame laser. The intensity decays were analyzed in term of multi-exponential model using fitting program FluoFit4 (PicoQuant, Inc.). All fluorescence measurements were done in a 'front-face' configuration.

2.5. Atomic force microscopy measurements

Atomic force microscopy (AFM) images were collected by scanning dry sample wafers with an Atomic Force Microscope (TMX 2100 Explorer SPM, Veeco), equipped with AFM dry scanner, over 100 μm . The AFM scanner was calibrated using a standard calibration grid as well as 100 nm diameter gold nanoparticles, from Ted Pella. Images were analyzed using SPMLab software.

3. Results and Discussion

On visual detection reliable SIF deposition was observed. The wafers are rather fragile and non-transparent. Photographs on Figure 1, top show the half-coated silicon wafer slide. The silvered area is clearly visible, and the color depends on the angle of observation (Fig. 1A and B). These observations suggests of surfaces that are active in MEF study. The AFM image (Figure 1, bottom) shows that silver nanoparticles are partially clustered on the surface, which we found to be beneficial for fluorescence enhancements [5]. As silicon MTP wafers are not transparent, direct absorption measurements cannot be done. To circumvent this problem, we produced SIF surfaces on similar size glass substrates simultaneously during the preparation. These silvered glass slides showed absorption characteristic for SIF with optical density of about 0.4. We assume that similar SIFs were deposited on silicon wafer substrates.

3.1. Fluorescence spectra and brightness

Fluorescence spectra were measured the same excitation/observation conditions for both, silvered and unsilvered areas. Figure 2 shows both spectra using 475 nm excitation. Although this is not a preferable excitation for Rh Red-X dye, we did not notice any problem with spectral data collection. It should be noted that the background from silicon wafer slides was minimal, well below 1%. The control measurement with non-specific antigen shows only about 5% signal proving that non-specific binding is not significant.

As expected, no difference in the emission spectra were recorded, and estimated enhancement in fluorescence brightness is above 300%, which is close to enhancement observed on a glass cover slip for Rhodamine-phalloidin (4 fold) [23].

The difference in the brightness is demonstrated on the photographs in Figure 3. These photographs were taken using, 532 nm laser (2mW) with an expanded beam for the excitation and a 570 nm long wave pass filter on observation.

3.2. Fluorescence lifetimes

The interaction of excited molecules with silver nanoparticles modifies (increases) the radiative rate of fluorophore deactivation. The consequence is a higher quantum yield and shorter lifetime. Usually, the deactivation processes modify (increases) the nonradiative rate, which decreases both, quantum yield and lifetime. The intensity decays measured on silvered and unsilvered areas of the silicon wafer slide are presented in Figure 4. The decays were normalized to compare the changes in the lifetimes. The decay parameters are summarized in Table 1.

The lifetime of Rh Red-X is significantly shorter in the presence of SIFs. The intensity decay becomes also more heterogeneous. This is expected because fluorophores on the surface are distributed in a slightly different plasmonic environment, i.e., at a different distance from each other and having a different orientation to nearby nanoparticles such that “hot” micro-regions exhibiting very strong interactions and enhancement are interspersed among areas where the enhancement is only modest.

3.3. Photostability

We previously observed increased photostability of fluorophores deposited on silver nanoparticles [23–27]. Both, decrease in the fluorescence lifetime and increase in the quantum yield lead to a significant reduction of photodegradation. First, decrease in lifetime decreases the bleaching; as shorter lifetime decreases the opportunity of free oxygen radicals to damage the fluorophore. Second, the increase in quantum yield diminishes bleaching because it allows a decrease of the excitation light intensity needed to maintain the same signal intensity. A comparison of photostabilities observed on SIFs and on uncoated silicon wafer is shown in Figure 5. It is clear that the presence of silver nanoparticles allows extraction of larger number of emitted photons from the studied system.

4. Conclusions

We demonstrated that silver nanostructures such as SIFs can be deposited on silicon wafer substrates and observed a three-fold fluorescence enhancement due to the presence of the SIF in a model immunoassay. A silicon surface is very attractive solid support in immunoassay because of its very low fluorescence background in addition to its suitability for fluorescence enhancement approaches. In addition, the increase of the photostability of fluorophores on silver nanostructures should be beneficial in future advanced silicon-based sensing devices for multiplex assays for genomics and drug screening.

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Fig 1.

Top: The silicon wafers half coated with silver island films (SIFs). The color observed at the SIF region depends on the observation angle as seen in photographs A and B. Bottom: The AFM image of SIF coated silicon wafer with IgG.

Fig 2.
Emission spectra of immobilized Rh-Red-X labeled IgG on silvered and unsilvered silicon wafers

Fig. 3. Photographs of fluorescent spots on silver coated area (left) and on bare Silicon wafer. Entire slide (silvered and unsilvered) was coated with Rh-Red-X labeled IgG. The excitation was from a low power 532 laser. The photographs were taken through a long wave pass 570 nm filter.

Fig. 4. normalized fluorescent intensity decays of Rh-Red-X labeled IgG deposited on silvered and unsilvered silicon wafer slide.

Fig. 5.
Dependence of fluorescence intensity on the time of exposure to 568 nm Argon/Krypton laser illumination.

Table 1

Multi exponential analysis of fluorescence intensity decays of Rh-Red-X labeled IgG in absence and presence of SIFs

conditions	τ_1 (ns)	a_1	τ_2 (ns)	a_2	T_3 (ns)	a_3	$\bar{\tau}$ (ns)	$\langle \tau \rangle$ (ns)	χ^2
Silicon wafer only	0.14	0.387	1.07	0.478	3.60	0.135	2.19 ^a	1.05 ^b	1.02
Silicon Wafer -SIF	0.03	0.734	0.33	0.222	1.38	0.044	0.69	0.16	1.06

$$^a \bar{\tau} = \frac{\sum_i f_i \tau_i}{\sum_i f_i}$$
$$^b \langle \tau \rangle = \sum_i a_i \tau_i$$