Metallic nanostructures for plasmonic sensors using surface-enhanced fluorescence and Raman detection

Fei Yan, † Musundi B. Wabuyele, † Guy D. Griffin, Tuan Vo-Dinh*
Advanced Biomedical Science and Technology Group
Oak Ridge National Laboratory, P.O. Box 2008, Oak Ridge, TN 37831-6101

ABSTRACT

We present the fabrication and characterization of silver island films with SiO_2 coatings for application in plasmonic sensors based on surface-enhanced fluorescence and Raman detection. The emission spectral properties of goat anti-mouse immunoglobulin (IgG) $F(ab')_2$ labeled with one or two fluorescein residues were examined on substrates with metallic silver islands. The self-quenching of fluorescein emission was mostly eliminated when this antibody fragment was held 60-90 Å from the surface of metallic silver islands, and our preliminary experiments demonstrated an 8-fold emission intensity increase. Similar surfaces were also examined for surface-enhanced Raman analysis of Rhodamine 123, a potential drug for photodynamic therapy.

Keywords: Metallic nanostructures, silicon oxide coating, surface-enhanced Raman scattering, surface-enhanced fluorescence, fluorescein, rhodamine 123, bioanalysis

1. INTRODUCTION

Surface-enhanced fluorescence (SEF) and surface-enhanced Raman scattering (SERS) are two of the most promising optical techniques for bioanalytical applications yet not fully explored.[1-4] Both processes result from the strong increase of local electromagnetic field in close vicinity to metal (i.e., silver or gold) surfaces that accompanies excitation of surface plasmon resonances in the metals. It was demonstrated lately that close-proximity metallic silver islands, silver colloids or fractal silver structures can alter the radiative decay rate, and/or excitation rate of fluorophores.[5-8] In contrast to SERS, maximal enhancement of fluorescence does not occur from molecules adsorbed directly on the metal surface. Among researchers in this field, there is a general agreement that an optimal distance as large as ~10 nm from the metallic surface provides the maximal fluorescence enhancements. It appears that silver is the most suitable metal for such a phenomenon. However, unprotected silver surfaces are not ideal for many applications due to their great reactivity with surrounding compounds, which leads to their degradation.

In this paper, we report the preparation and application of silver islands surfaces overcoated with well-defined SiO_2 overlayers. Such surfaces allow both SEF and SERS measurements, which are potentially useful in bioanalysis.

2. PREPARATION AND SURFACE MODIFICATION OF SUBSTRATES

The surfaces for enhanced fluorescence experiments were fabricated by depositing well-defined SiO_2 overlayers, by a physical vapor deposition (PVD) method, onto silver island films of 10 nm silver mass thickness. The 10-nm layer of silver was deposited on a cleaned glass surface prepared also by a PVD method. Prior to deposition, glass slides were soaked in a nitric acid: sulfuric acid (1:1) mixture overnight. Then the slides were rinsed with distilled water thoroughly and air-dried. An electron beam evaporation system (CVE 301 EB, Coke Vacuum Products, Norwalk, CT) was used for the PVD process. The deposition rate and PVD chamber pressure were 0.15 Å s^{-1} and 10^{-6} Torr (1 Torr = 133.3 Pa), respectively. The slides were then treated with a 3% solution of 3-aminopropyltriethoxysilane (United Chemical Technologies, Bristol, PA) in anhydrous acetone solution for one minute. (Before treating the slides, the 3% silane solution was stirred for at least 10 minutes to allow for hydrolysis and silanol formation). The

†F.Y. and M.B.W. contributed equally to this paper.
*To whom correspondence should be addressed. E-mail: vodinht@ornl.gov.

slides were then briefly dipped in 100% acetone to remove excess silanol. The adsorbed silane layer was cured at 115 °C for one hour. After cooling to room temperature, the slides were dipped in 100% acetone followed by dipping in water for 1 minute to remove uncoupled reagent. The silanized substrates were immersed in glutaraldehyde (6% in 0.2 M Tris-EDTA buffer, pH 7.7) for 10 minutes, rinsed in deionized water and blown dry. After that, the slides were immersed in FITC-labeled goat IgG $F(ab')_2$ (6 μM solution, diluted 1:50 from stock solution in PBS buffer) for 30 minutes. After thorough rinsing with water, the antibody-immobilized substrates were kept in PBS buffer while fluorescence measurements were taken.

Fig. 1 demonstrates the immobilization chemistry we utilized in this study for surface-enhanced fluorescence experiments. Silanes are a group of molecules where a silicon atom is bound to four variable groups. These molecules can be used to alter the characteristics of a silicon or glass surface. The oxygen of hydroxyl groups at a silicon oxide surface may substitute one or several of the four groups in the silane. Chlorosilanes, mercapto-silanes and aminosilanes are examples of different silanes used for surface modification. In the case of silver island-coated glass slides, 2-mercaptoethylamine (cysteamine) is used instead of aminosilanes. The reaction between silanes and the surface hydroxyl groups seems rather simple but is nevertheless rather complex. In organic solvents miscible with water, the amine group catalyzes the rapid hydrolysis of the alkoxy groups. These hydrolized silanes do not only couple very easily with the surface hydroxyl groups, but also condense with each other to form siloxane oligomers. [9] Silanization of silicon oxide substrates with an aminosilane can either be done by incubating the substrate in water:ethanol aminosilane solution or by depositing aminosilanes on the substrate in a vacuum chamber. Biological molecules such as an antibody can be bound covalently to a silanized silicon oxide surface by the use of a linker molecule, e.g. glutaraldehyde. The dialdehyde will link proteins to the aminosilane through reactions with the amino group on the silane and with amino groups on the protein. The resulting molecule is a so-called protonated Schiff base.

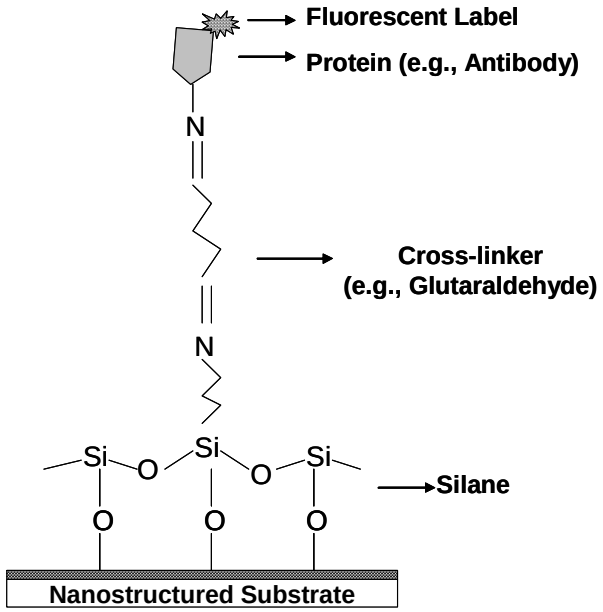

Figure 1. Schematic of the immobilization of fluorescence-labeled proteins on nanostructured substrates.

3. CHARACTERIZATION OF SUBSTRATES

An explorer SPM model AFM (ThermoMicroscopes, Sunnyvale, CA) was used for imaging of the surfaces. The AFM was operated in the non-contact mode, and measurements were performed under ambient conditions. Silicon tips with a resonance frequency of 240-420 kHz and a force constant of 40 N/m were used.

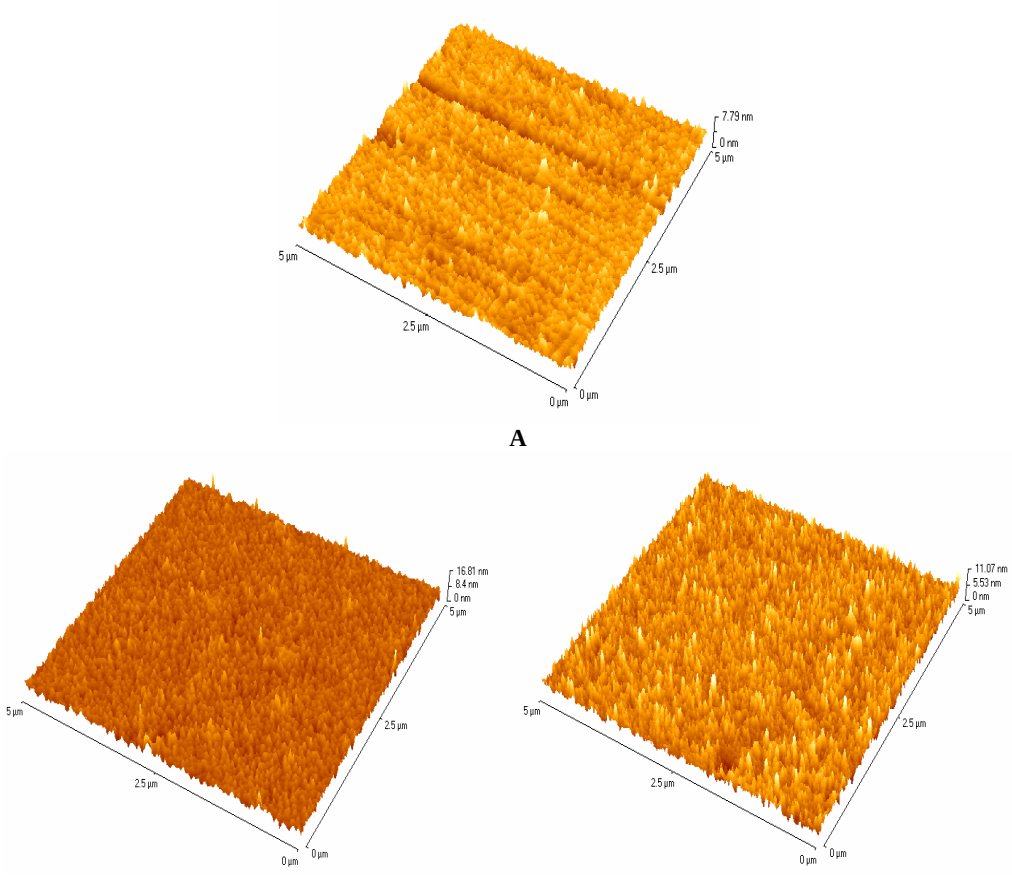

Figure 2. AFM images of A) plain glass, B) 10-nm silver coated glass, and C) 7-nm SiO_2 overlayer on top of 10-nm silver coated glass.

As demonstrated in Fig. 2, the average surface height of the silver islands was decreased from 16.81 nm to 11.07 nm with a 7-nm SiO_2 coating, which seemed to result in a much rougher surface.

4. SURFACE-ENHANCED FLUORESCENCE STUDIES

Fig. 3 shows the schematic diagram of the instrumental setup for SEF experiments. 488 nm radiation from an Argon laser is used with an excitation power of ~30 mW, which was collimated and defocused using a 60× microscopic objective. The actual spot size was not directly measured but is assumed to have been near the diffraction limit of the objective lens. The objective lens was used to both excite and collect the SEF signal in front-face geometry.

To determine the enhancement of fluorescence, silver island-coated glass slides with and without SiO_2 coating, as well as reference slides (plain glass) were pretreated with 2-mercaptoethylamine and aminosilanes accordingly, before further modification (shown in Fig. 1). All slides were washed in 0.1 M PBS buffer, pH 7.5, and fluorescence measurements of surface-immobilized FITC-antibody were performed in PBS solution. The experimental design sandwiched the PBS solution as a thin aqueous layer between two slides, the bottom slide having the metal coating.

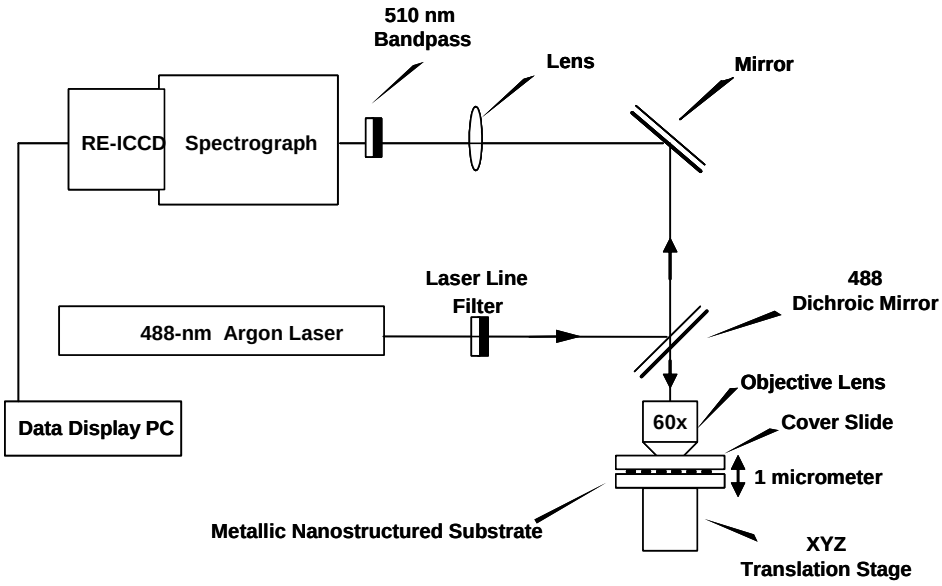

Figure 3. Schematic of the instrument setup used for SEF detection of the FITC-labeled antibody

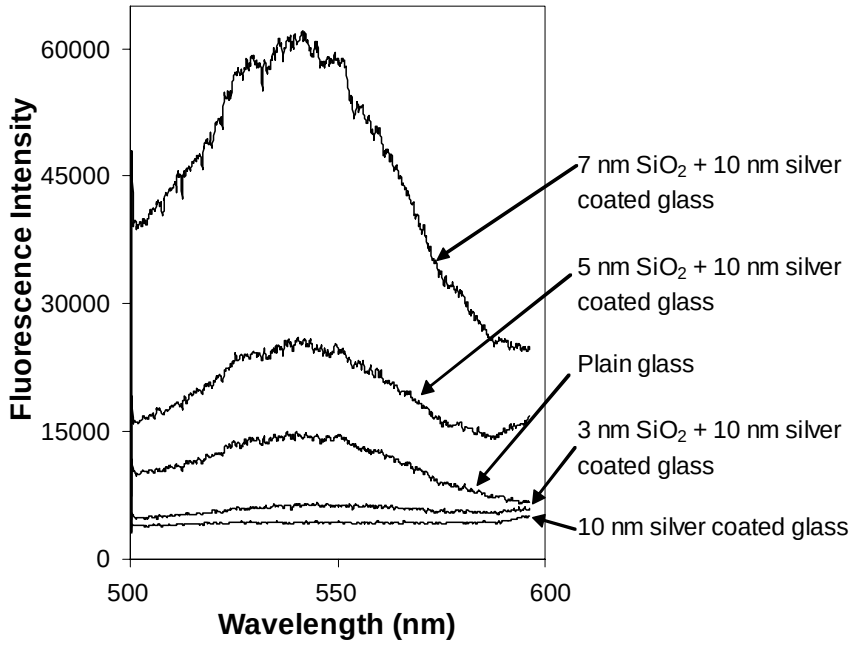

Figure 4. Fluorescence emission spectra of FITC-labeled goat anti-mouse immunoglobulin (IgG) $F(ab')_2$ tethered to silanized substrates; 488 nm laser, 30 mW.

As illustrated in Fig. 4, direction immobilization of FITC-labeled anti-mouse immunoglobulin (IgG) $F(ab')_2$ on a 10 nm silver coated glass resulted in complete quenching of FITC fluorescence, and partial quenching was observed when only a 3-nm SiO_2 coating was added. The introduction of a thicker SiO_2 spacer, along with layering of other linking compounds, gave rise to an enhancement factor between 2 and 8 in this preliminary study. It was estimated that a total thickness of ~30-40 Å could result from the silanization and antibody cross-linkage processes. [10] Therefore, the optimum distance between the fluorescent label and the substrate for SEF, ~10 nm in our case, agrees very well with many previous reports. [5-8]

5. SURFACE-ENHANCED RAMAN SCATTERING STUDIES

Fig. 5 shows the schematic diagram of the instrumental setup for SERS experiments. All SERS measurements were performed with a SPEX 1403 double-grating spectrometer with 2-cm^{-1} band-pass. In this system, 632.8 nm radiation from a Helium–Neon laser is used with an excitation power of ~5 mW. Signal collection is performed at 0° with respect to the incident laser beam. This coaxial excitation/collection geometry was achieved with a small prism, which was used to direct the excitation beam to the sample while allowing most of the collected signal to pass on to the collection fiber. A Raman holographic filter is used to reject the Rayleigh scattered radiation from the collected Raman signal. The Raman signal is focused onto the entrance slit of a spectrograph (ISA, HR-320), which is equipped with a red-enhanced intensified charge-coupled device (CCD) detector (Princeton Instruments, RE-ICCD-576S), with a total accumulation time of 10 s per spectrum.

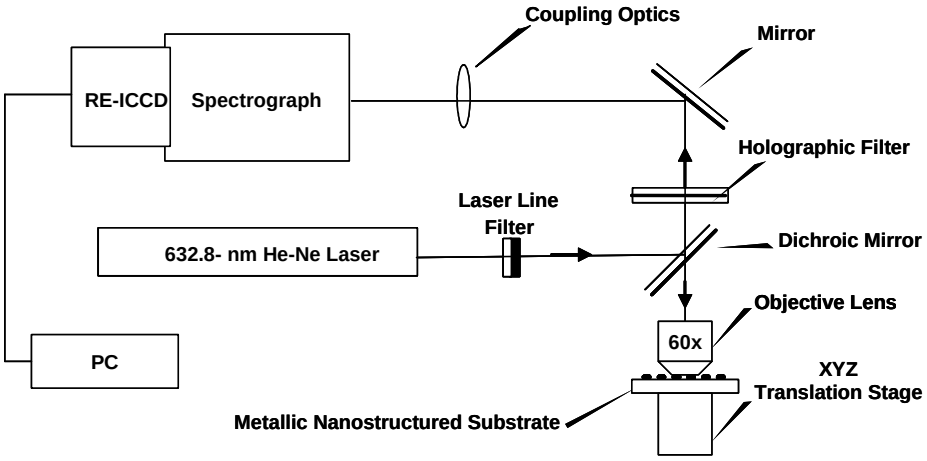

Figure 5. Schematic of the instrument setup used for SERS detection of Rhodamine 123 dye.

Rhodamine 123 has been termed a mitochondria-specific dye, because this cationic cyanine dye is accumulated in electrically negative compartments such as mitochondria in healthy cells. Uncoupling agents and inhibitors reduce mitochondrial fluorescence. [11] The large membrane surface area in the mitochondrial matrix may contribute to the staining by binding large amounts of accumulated probe. Rhodamine 123 has an absorption maximum at about 485 nm and an emission maximum at 530 nm.

As shown in Fig. 6, SERS signals from Rhodamine 123, although intensity decreased almost by half, retained characteristic peak signatures when the silver islands were coated by a 7-nm SiO_2 overlayer. . By properly adjusting the thickness of the SiO_2 overlayers, it may be possible to retain the SER signals to a greater extent, while achieving a maximal SEF enhancement. This property could be very useful in applications which require better Raman fingerprints than only fluorescence dyes. [12]

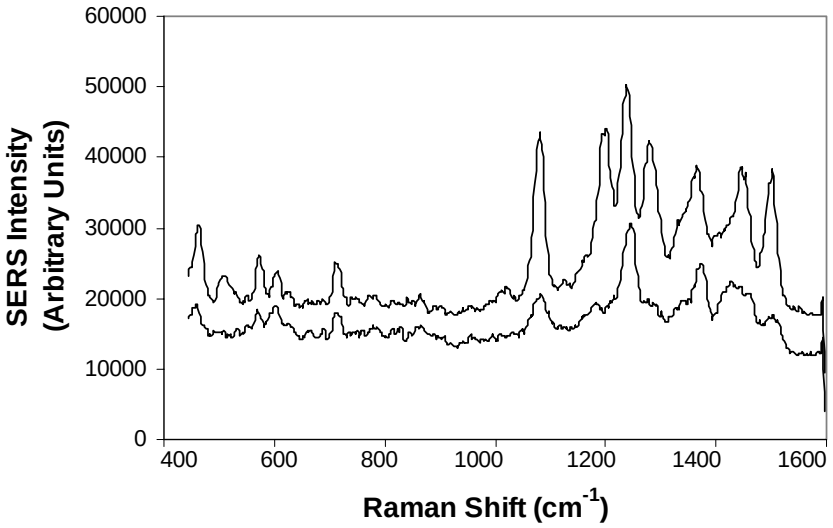

Figure 6. SERS spectra of Rhodamine 123 on a 10-nm silver coated glass (top) and on a 7-nm SiO_2 + 10-nm silver coated glass (bottom); 632.8 nm laser, 8 mW.

6. CONCLUSIONS

The preparation and characterization of silver islands films with SiO_2 coating for surface-enhanced fluorescence and Raman bioanalysis have been illustrated. We have demonstrated the requirement for optimal spacing in order to obtain maximal fluorescence enhancement. Our preliminary study suggested that the surface density of the underlying silver islands, and the immobilization procedures for dye-labeled proteins, as well as the experimental configurations etc., could be the main factors for both better SEF and SERS enhancement.

ACKNOWLEDGEMENTS

This research was sponsored by the U.S Department of Energy (DOE) Office of Chemical and Biological National Security and the DOE Office of Biological and Environmental Research, under contract DEAC05-000OR22725 with UT-Battelle. Fei Yan and Musundi B. Wabuyele are also supported by an appointment to the Oak Ridge National Laboratory Postdoctoral Research Associates Program, administered jointly by the Oak Ridge National Laboratory and Oak Ridge Institute for Science and Education.

REFERENCES

1. K. Sokolov, G. Chumanov, T. M. Cotton, Enhancement of molecular fluorescence near the surface of colloidal metal films, *Anal. Chem*. 70, 3898-3905, 1998.
2. A. Wokaun, Surface-enhanced electromagnetic processes, *Solid State Phys.* 38, 223-294, 1984.
3. T. Vo-Dinh, F. Yan, D. L. Stokes, Metallic nanostructures for surface-enhanced Raman scattering bioanalysis, in *Protein Nanotechnology* (T. Vo-Dinh ed.), Humana Press, Totowa, NJ, in press. 2004.

4. T. Vo-Dinh, L. R. Allain, D. L. Stokes, Cancer gene detection using surface-enhanced Raman scattering (SERS), *J. Raman Spectrosc*. 33, 511-516, 2002.
5. C. D. Geddes, J. R. Lakowicz, Metal-enhanced fluorescence, *J. Fluoresc.* 12, 131-139, 2002.
6. C. D. Geddes, A. Parfenov, J. R. Lakowicz, Photodeposition of silver can result in metal-enhanced fluorescence, *Appl. Spectrosc*. 57, 526-531, 2003.
7. A. Parfenov, I. Gryczynski, J. Malicka, C. D. Geddes, J. R. Lakowicz, Enhanced fluorescence from fluorophores on fractal silver surfaces, *J. Phys. Chem. B* 107, 8829-8833, 2003.
8. C. D. Geddes, A. Pardenov, D. Roll, J. Fang, J. R. Lakowicz, *Langmuir* 19, 6236-6241, 2003.
9. F. J. Boerio, L. Armogan, S. Y. Cheng, Structure of gamma-aminopropyltriethoxysilane films on iron mirrors, *J. Colloid Interface Sci.,* 1980, 73, 416.
10. P. M. St. John, R. Davis, N. Cady, J. Czajka, C. A. Batt, H. Craighead, Diffraction-based cell detection using a microcontact printed antibody grating, *Anal. Chem*. 70, 1108-1111, 1998.
11. Uchida N, Combs J, Chen S, Zanjani E, Hoffman R, Tsukamoto A, Primitive human hematopoietic cells displaying differential efflux of the rhodamine 123 dye have distinct biological activities, *Blood* 88, 1297-1305, 1996.
12. Y. C. Cao, R. Jin, J. Nam, C. S. Thaxton, C.A. Mirkin, Raman dye-labeled nanoparticle probes for proteins, *J. Am. Chem. Soc*. 125, 14676 -14677, 2003.