

Surface-enhanced fluorescence from metal sculptured thin films with application to biosensing in water

I. Abdulhalim,^{1,a)} Alina Karabchevsky,¹ Christian Patzig,² Bernd Rauschenbach,² Bodo Fuhrmann,³ Evgeni Eltzov,⁴ Robert Marks,⁵ Jian Xu,⁶ Fan Zhang,⁶ and Akhlesh Lakhtakia⁶

¹Department of Electrooptic Engineering, Ben Gurion University of the Negev, Beer Sheva 84105, Israel

²Leibniz-Institut für Oberflächenmodifizierung e.V., Permoserstrasse 15, 04318 Leipzig, Germany

³Interdisziplinäres Zentrum für Materialwissenschaften, Martin-Luther-Universität Halle,

Heinrich-Damerow-Straße 4, 06120 Halle, Germany

⁴Department of Environmental Engineering, Ben Gurion University of the Negev, Beer Sheva 84105, Israel

⁵Department of Biotechnology Engineering, National Institute for Biotechnology in the Negev, Ben Gurion University, Beer Sheva 84105, Israel

⁶Department of Engineering Science and Mechanics, Pennsylvania State University, University Park, Pennsylvania 16802-6812, USA

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Surface-enhanced fluorescence from porous, metallic sculptured thin films (STFs) was demonstrated for sensing of bacteria in water. Enhancement factors larger than 15 were observed using STFs made of silver, aluminum, gold, and copper with respect to their dense film counterparts. The STFs used are assemblies of tilted, shaped, parallel nanowires prepared with several variants of the oblique-angle-deposition technique. Comparison between the different films indicates that the enhancement factor is higher when the tilt is either small ($<30^\circ$) or large ($>80^\circ$); thus, the enhancement is higher when only a single resonance in the nanowires is excited. © 2009 American Institute of Physics. [DOI: 10.1063/1.3081031]

Surface-enhanced fluorescence (SEF) is known for more than a few decades now;¹ however, its potential was rediscovered during the past decade due to the emerging developments in the optics of metallic nanostructures.^{2,3} SEF is a very useful phenomenon with significant applications in biotechnology³ and life sciences, alongside surface-enhanced Raman scattering (SERS). Several physicochemical effects^{4–8} related to the behavior of fluorophores near nanoparticles have been brought into focus by the emerging field of plasmonics. The effect of surfaces near an oscillating dipole on its emission properties was first investigated by Chance *et al.*,¹ which led to the development of the Chance–Prock–Silbey (CPS) theory. Localization of the electromagnetic field near nanotips, corners, holes, needles, etc. has been shown to produce large SEF by factors of up to a few hundreds in what is known as the lightning nanoantenna effect.⁹ Although old¹⁰ and recent reports^{11,12} on polarized SERS from silver thin films with columnar morphology—with application to virus detection¹³—exist, to the best of our knowledge there are no reports on SEF from these films. Here, we present SEF results from sculptured thin films (STFs) (Ref. 14) and highlight the applicability of such kinds of nanobeds for biological sensing in water.

STFs, which are assemblies of shaped parallel nanowires, are prepared using many variants of the basic oblique-angle-deposition (OAD) technique.¹⁵ The growth mechanism is based on self-organized nucleation of nanoparticles and subsequent highly directional growth due to atomic shadowing of the nanoparticle flux reaching the substrate at a (large) oblique angle with respect to the substrate normal. With appropriate substrate rotation during growth,^{14,15} the nanowires

can be varied in shape from tilted rods to helices to chevrons and recently blades.¹⁶

STFs were prepared from different materials (Si, Ag, Au, Al, and Cu) on different substrates and with different morphologies (nanorods and nanohelices). The Si and Cu STFs were grown by ion-beam sputtering OAD,^{17–19} the Au-nanorod STFs were grown with dc sputtering OAD, and the Ag-nanorod STFs by means of electron-beam-evaporation OAD at the IOM. The vapor incidence angle was set to $\sim 85^\circ$ from the substrate normal in all cases. The Al-nanorod STFs were deposited at Penn State by electron-beam evaporation OAD with the vapor incidence angle of 70° to the substrate normal. For reference, dense films of each material were prepared with the vapor incidence parallel to the substrate normal. Some of the films were deposited on substrates templated with monolayers of SiO₂ nanospheres using a colloidal self-assembly method and with Au dots in hexagonal arrangements gained by evaporating Au through the voids of such self-assembled films of hexagonally close-packed nanospheres with subsequent removal of the spheres. Figure 1 contains scanning electron microscope (SEM) micrographs of several STFs.

For SEF measurements, the STFs were spin coated with the fluorescent dye Rhodamine 123 diluted in methanol at 0.6 wt %. The thickness of the dye layer was estimated by atomic-force-microscope measurements to be ~ 50 nm. Care was taken in selecting the spinning conditions to obtain uniform dye layers and similar thicknesses both on the STFs and dense reference films. To demonstrate biosensing, the films were inserted in an aqueous solution of *E. coli* (TV1061) with concentration 3.6×10^8 cfu/ml to observe the fluorescence from the bacteria. Fluorescence measurements were performed using an Olympus fluorescence microscope with an Hg arc lamp as excitation light. The green

^{a)}Electronic mail: abdulhm@bgu.ac.il.

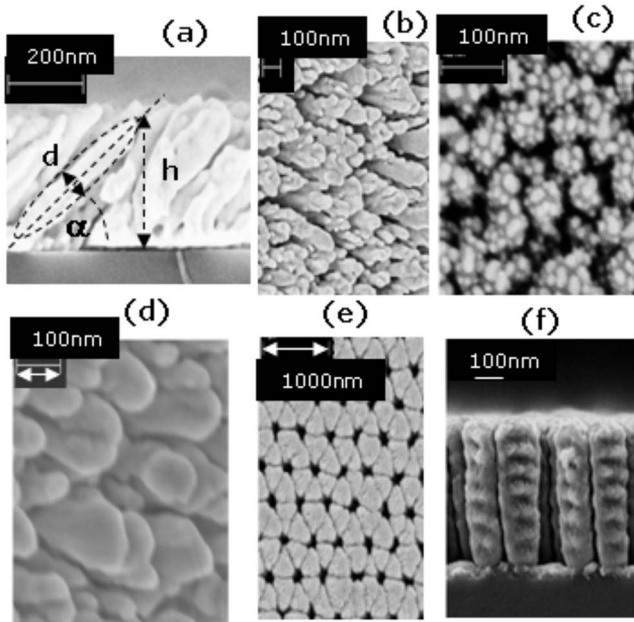


FIG. 1. SEM micrographs of typical STFs prepared for this study: (a) side view of Au-nanorod STF, (b) top view of (a), (c) top view of 5 nm Ag layer deposited on top of Si nanorods, (d) top view of Cu STFs, (e) top view of Si nanohelices deposited on Au dots, and (f) side view of (e). These films were prepared at the Institut für Oberflächenmodifizierung (IOM).

Hg line at 546 nm was used for excitation and the emission was detected using a red filter at 590 nm. The detection was done using a high-sensitivity cooled charge coupled device camera with a controlled exposure time. The grabbed images were then analyzed using MATLAB and the average intensity was compared between a STF and its dense reference film made of the same material on a similar substrate. For more quantitative measurements, a fiber-optic-based spectrometer was connected to the fluorescence microscope with a specially designed mechanical jig that allows adjustment and focusing so that the fiber entrance plane is conjugate to the plane of the camera.

In Figs. 2(a) and 2(b), fluorescence images of the Ag-nanorod STF and the corresponding dense Ag reference film inserted in an aqueous solution of *E. coli* are compared. Undoubtedly, the nanorod STF exhibits SEF to a higher degree than the reference film. In Fig. 2(c), fluorescence spectra are presented for the Ag and Al STFs together with that for their reference samples. The ratio between the peaks at 590 nm gives enhancement factors of 15 for Ag and 11 for Al. The enhancement factors for the nanorod STFs are with respect to dense films, which are effectively metal mirrors that can be expected to exhibit some enhancement with respect to fused silica substrates. Hence while other researchers reported^{2,3} in the past enhancement factors of 16 or larger from metal nanoisland films or from metal-coated nanoparticles with respect to glass substrates, when we compare our results to the same glass substrates the enhancement factors are 20 or larger. Furthermore, if we subtract the background from the fluorescence peak values and then calculate the ratio we get an enhancement factor of 19 instead of 15.

Table I summarizes the STFs evaluated, their morphological description, and the enhancement factors obtained. Several conclusions can be drawn from this table. (1) STFs of metals exhibit the highest enhancement factors. (2) Nanorod STFs with high (>80 deg) or low (<30 deg) values of

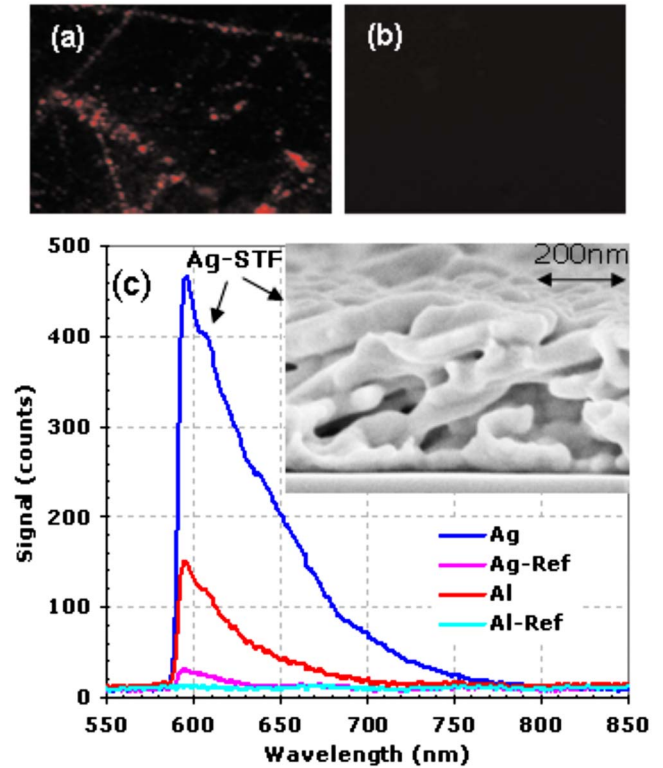


FIG. 2. (Color online) Fluorescence images from (a) Ag-nanorod STF and (b) a dense Ag film immersed in an aqueous solution of luminescent *E. coli*. (c) Typical SEF spectra from a Ag-nanorod STF and an Al-nanorod STF and from the corresponding reference films. Inset: SEM micrograph of a Ag-nanorod STF showing the highest enhancement factor.

the inclination angle α [see Fig. 1(a)] exhibit the highest enhancement factors because the SEF signal is stronger when only one resonance is excited, which is the case with normal-incidence excitation when α is either very low or very high. In these two cases the dipole excited is perpendicular to the incidence direction and therefore its emission is maximum in the backward direction. (3) Au-nanorod STFs show no enhancement which is somehow surprising. It could be related to the fact that these films were sputtered with broader flux distribution and because the films are not thick enough, thereby causing broader distribution of nanorod

TABLE I. STF samples used, their geometric attributes, and the measured enhancement factors EF. FS: fused silica, Sub.: substrate. Ag-Si 1/2: 5–15 nm Ag on Si rods. Si spirals 1: Si spirals on 30 nm Au dots. Si spirals 2: Si spirals on 350 nm SiO₂ spheres.

Samples	EF	h (nm)	d (nm)	α (deg)	Sub.
Ag rods	15	400	75	23	FS
Al rods	11	1000	30	20	FS
Ag-Si-1	8	50		90	Si
Cu rods	8	220		90	Si
Ag islands	7	5, 15			Si, FS
Ag-Si 2	5	50, 100		90	FS
Si spirals 1	4	740			Si
Si rods	3	50–100		90	Si, FS
Cu rods	2	45–100	30–60	20	FS
Cu rods	0.5–1	1100	40–150	30–42	FS
Si spirals 2	0.5–1	1090			Si
Au rods	0.5–1	285	40	35	FS

sizes and orientations, thus averaging out the lightning nanoantenna effect. For the STF of Si nanohelices on Au dots, an enhancement factor of 4 was observed. This enhancement must be due to the underlying Au dots (~ 150 nm diameter and ~ 30 nm height) because Si nanohelices on SiO₂ nanospheres did not show enhancement.

Several mechanisms can contribute to the SEF from metallic STFs because such a STF can act as (a) a reflective interface in the context of the CPS theory, (b) a porous material with high surface-to-volume ratio, and (c) a matrix of metal nanorods that enhances the local electromagnetic field and acts as nanoantennas or through the dipole-dipole^{20,21} coupling between neighboring nanorods to contribute to SEF. Porosity is believed not to play a role here because the thin layer of dye used by us covers mainly the top surface of the film. The same is true for the case of fluorescence from bacteria since the bacteria are much larger than the voids in the STFs. Local field plasmon enhancement effects might be the main contributors but that could not be proved directly as absorption resonances corresponding to localized surface plasmon resonance excitations were not observed.

Besides the well-known lightning nanoantenna mechanism, in STFs the nanorods are separated by few nanometers to a few tens of nanometers and therefore dipole-dipole interaction can play a major role in the enhancement mechanism.^{20,21} The dipole-dipole interaction occurs when light incident on a nanorod induces across it an electric field E that depends both on the shape of the nanorod and on the contributions from the neighboring nanorods. Near-field effects ($E \propto 1/r^3$) play a role for internanorod distance much less than the wavelength λ while far-field effects ($E \propto 1/r$) can play a role for nanorods of larger size. The far-field effects perhaps enhance SEF from the luminescent bacteria which have a size of about $1 \mu\text{m}$. For more quantitative physical explanations we believe that rigorous electromagnetic simulations of such disordered system of nanorods are necessary which we plan to perform.

To conclude, enhancement of SEF from porous, metallic STFs was observed and applied to biosensing in water. The main SEF mechanisms are believed to be the lightning nanoantenna effect and the dipole-dipole interaction. This

observation makes STFs potential candidates as SEF nano-beds for biosensing and bioimaging.

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