

Preparation of Plasmonic Platforms of Silver Wires on Gold Mirrors and Their Application to Surface Enhanced Fluorescence

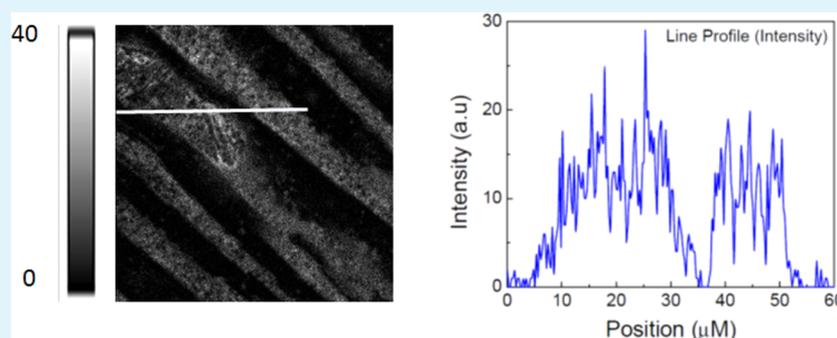
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S Supporting Information



ABSTRACT: In this report we describe a preparation of silver wires (SWs) on gold mirrors and its application to surface enhanced fluorescence (SEF) using a new methodology. Silica protected gold mirrors were drop-coated with a solution of silver triangular nanoprisms. The triangular nanoprisms were slowly air-dried to get silver wires that self-assembled on the gold mirrors. Fluorescence enhancement was studied using methyl azadioxatriangulenium chloride (Me-ADOTA-Cl) dye in PVA spin-coated on a clean glass coverslip. New Plasmonic Platforms (PPs) were assembled by placing a mirror with SWs in contact with a glass coverslip spin-coated with a uniform Me-ADOTA-Cl film. It was shown that surface enhanced fluorescence is a real phenomenon, not just an enhancement of the fluorescence signal due to an accumulation of the fluorophore on rough nanostructure surfaces. The average fluorescence enhancement was found to be about 15-fold. The lifetime of Me-ADOTA-Cl dye was significantly reduced (~ 4 times) in the presence of SWs. Moreover, fluorescence enhancement and lifetime did not show any dependence on the excitation light polarization.

KEYWORDS: plasmonic platform, photonics, metal-enhanced fluorescence, fluorescence lifetime imaging

1. INTRODUCTION

Fabrication and utilization of metal nanostructures has been at the forefront of research for a few decades. Noble metal nanoparticles and nanostructures have been studied intensively due to their unique property: surface plasmon resonance (SPR).^{1–3} The noble metal nanostructures have been employed to enhance both Raman scattering^{4,5} and fluorescence.^{1–3,6,7} Significant effort has been applied recently to application of metallic nanostructures for surface enhanced fluorescence (SEF).^{6–9} Optimal conditions to improve fluorescence sensitivities for practical applications of single molecule detection have been of great interest.^{10,11}

A strong induced electric field at specific locations (“hot spots”),^{12–16} an increase in fluorescence quantum yield, and fluorescence lifetime shortening are the main contributing factors to the SEF.^{1,8,9} However, fluorescence quenching by metallic particles is the main negative factor. The observed

fluorescence enhancement depends strongly on the structure’s size, shape, and environment.^{1,8,9} The enhancement also depends on the distance of the fluorophore from the metallic structure.^{8,9,17–20}

Various silver and gold structures have been applied to the SEF. It has been reported that silver colloids^{12,22} and silver island films (SIFs)^{23,24} on glass substrates enhance fluorescence 5–10-fold and decrease the lifetime a few -fold.⁷ When the excited fluorophore interacts with silver nanostructures, the local field acting on the fluorophore is amplified and the rate of photochemical processes accelerate. As a result, the fluorescence signal is enhanced.

Received: July 7, 2014

Accepted: October 8, 2014

Published: October 8, 2014

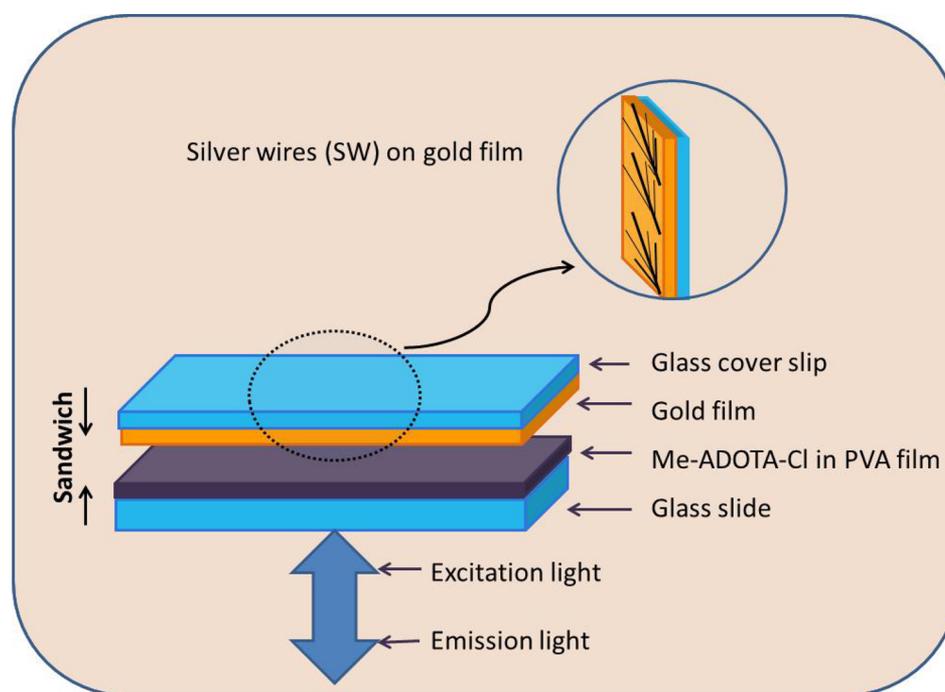


Figure 1. Schematic of plasmonic platform assembly.

Electrochemically deposited silver fractal-like structures on glass and on plastic substrates have been employed for the SEF.^{9,25–28} Dyes have been deposited on silver fractal-like structures for measurements and on fractal free substrates for the control. The lightening nanoantenna effect is produced by localization of the electromagnetic field near needles, corners, holes, and sharp edges.^{29,30} Silver fractals' dense areas with sharp edges create local "hot spots". This results in stronger local fluorescence enhancement of about 100-fold,²⁶ the shortening of the lifetime several folds, and the increase in the fluorophores' photostability.^{9,25–28}

Ion-beam sputtering oblique-angle-deposition (OAD), dc sputtering OAD, and electron-beam-evaporation OAD have been used to prepare sculptured thin silicon, silver, gold, aluminum, and copper films on different substrates.^{30,31} Nanosculptured thin films (STF) take different forms of nanocolumnar structures. Different STFs were studied to find the optimum structure for biosensing based on the SEF.^{30,31} A comparative study was performed with STFs containing the nanocolumnar structures that differ in their shape, height, and tilt angle with respect to the surface, thickness, and arrangement.^{30,31} The greatest enhancement of the fluorescent signal was found for Ag-based STFs on Si(100), giving an enhancement factor of $\times 71$ using fluorescent dye Rhodamine 123.³¹

It has been shown that different morphological patterns are produced when silver nanoparticles are self-assembled by air drying.^{32–34} The patterns depend on size and identity of nanostructures, surface, and thermodynamic state of the solution.^{32–34} Evaporation induced self-assembly of colloidal silver nanoparticles on gold films form small fractal-like structures.^{35–40} The silver self-assembled colloids (Ag-SACs) deposited on silver and gold films strongly enhance fluorescence about 60-fold on average.^{35–40} The "hot" spot fluorescence enhancement on Ag-SACs is about 200-fold.^{35–40} The SEF effect has been observed on silver nanostructures without a gold or silver film. However, the SEF effect is about

5-fold stronger when silver nanostructures are deposited on a 50 nm in thickness noble metal film.³⁶ It has been indicated that the Ag-SACs plasmons interact with the free electrons of the gold film. The interaction assists in the excitation of surface plasmons in the gold film. Both excitations from localized surface plasmons on the silver nanostructures and excited surface plasmons on the gold film induce enhancement of the local field's intensity.³⁸ This results in enhanced fluorescence signal and in decreased lifetime.^{35–40} The ratio of average lifetime of methyl-azadioxatriangulenium chloride (Me-ADOTA-Cl) on glass to the lifetime on Ag-SACs is 64, close to the observed steady state enhancement factor. The reduced lifetime of the excited state of the molecules in the presence of Ag-SACs lowers the probability of bleaching as the excited state reactions leading to photodegradation does not have time to happen.^{35–40}

Similar fluorescence enhancements have been reported on silver nanoprisms⁴¹ deposited on gold films. In that study, drop-coated silver nanoprism solutions were relatively quickly air-dried. While there was not complete coverage on the slide, the self-assembled triangular structures had a uniform distribution on the gold film. The most significant increase in fluorescence, measured on "hot" spots, was over 50-fold.⁴¹

In this paper, we prove that the SEF is a real phenomenon, not an enhancement of the fluorescence signal due to a simple accumulation of the fluorophore on rough nanostructures' surfaces. In all previous reports, the fluorophore has been directly deposited on the formed nanostructures. The nanostructures have very different roughnesses than the substrates. Many concerns have been raised that nonuniform coverage of the fluorophore takes place. If the fluorophore's coverage is higher on the rough nanostructures surfaces than the substrate, higher fluorescence can result on the nanostructure. In this report, we present a new method of preparation of silver wires on silica protected gold films. The silver wires are used to assemble a new type of the plasmonic platform (PP). The new PP consists of two parts that are put in contact: a

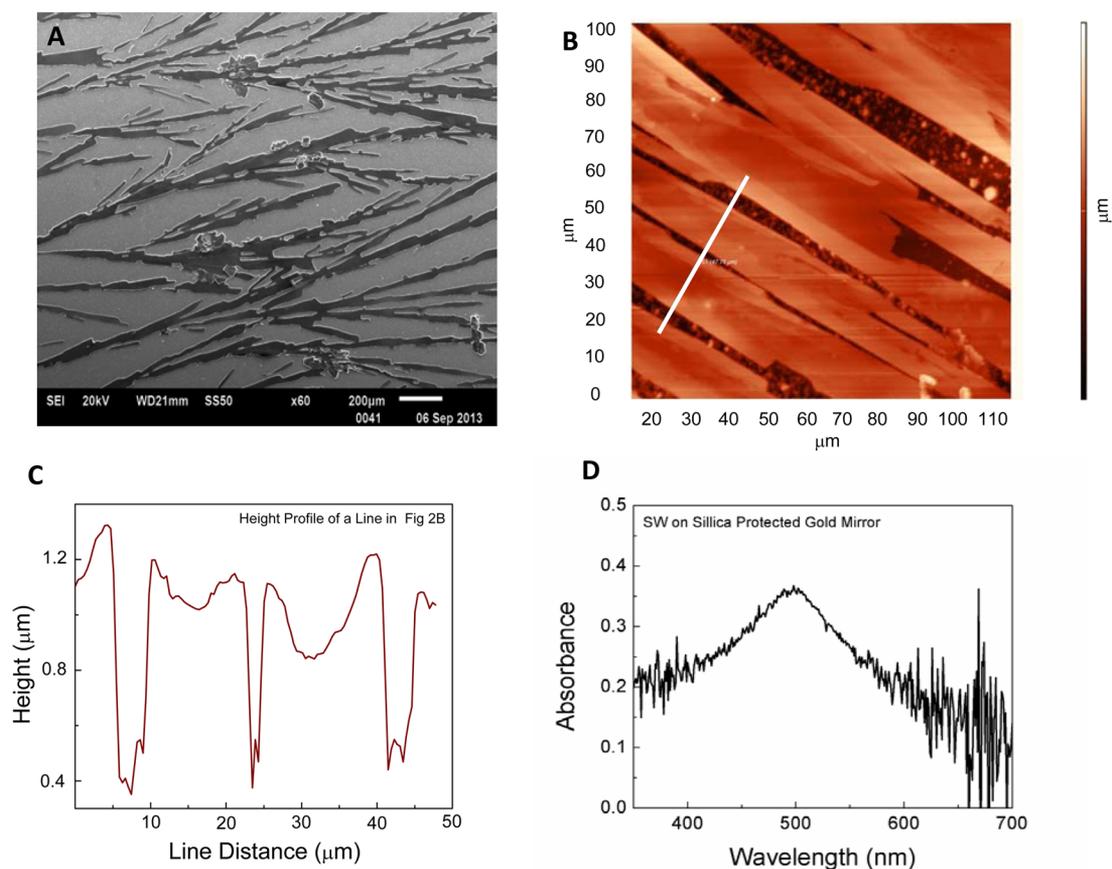


Figure 2. (A) SEM photomicrograph of silver wires on silica protected gold mirror. (B) AFM image ($100\ \mu\text{m} \times 110\ \mu\text{m}$) of silver wires on silica protected gold mirror. (C) Height profile of SWs on silica protected gold mirror for the line drawn in panel B. (D) Absorption spectrum of SW on silica protected gold mirror.

uniform film of the fluorophore on a glass coverslip and silver wires on silica protected gold mirror on a glass slide. The new PP confirms that the fluorescence enhancement is not due to accumulation of the fluorophore on silver structures but results from the SEF phenomenon.

As previously reported, the plasmonic enhancement is much higher for molecules with low intrinsic quantum yield.^{42,43} We use ADOTA-Cl dye with the original quantum yield of 0.42 in ethanol and an extinction coefficient of $9770\ \text{M}^{-1}\ \text{cm}^{-1}$ at 539 nm. The thin film of Me-ADOTA-Cl in PVA is uniformly deposited on a different clean glass slide. A slide with silver wires and a slide with the thin fluorophore film are placed in contact and used as a PP for surface enhanced fluorescence studies.

2. EXPERIMENTAL METHODS

2.1. Chemicals. All chemicals and materials were used as received. The water used for all solutions and washings was Millipore grade with $>18.2\ \text{M}\Omega$ resistivity.

2.2. Synthesis of Silver Triangular Nanoprisms. Silver nanoprisms were prepared by a chemical reduction method as previously described.^{41,44} Solutions were added to a 25 mL Erlenmeyer flask in the following order: 2.0 mL of 12.4 mM sodium citrate tribasic (Sigma-Aldrich 99.0%), 5.0 mL of 0.375 mM silver nitrate (Sigma-Aldrich 99.9999%), and 5.0 mL of 50 mM hydrogen peroxide (Fisher Scientific). Then, 25 μL of 1 mM potassium bromide (Fisher Scientific) was added using a micropipette. Lastly, 2.5 mL of fresh 5 mM sodium borohydride (J.T. Baker 98%) was added. The flask was covered and shaken to mix the reactants. After formation of the

nanoprisms, the solution of nanoprisms was blue in color and stable for several weeks.

2.3. Preparation of Silver Wires on Gold Slides. The solution of silver nanoprisms was drop-coated on the silica (5 nm thick) protected gold mirror (48 nm thick) manufactured by EMF, Inc., Ithaca, NY. The gold mirrors were placed in a near-isolation chamber and allowed to evaporate very slowly for at least a week at room temperature during which time self-assembled SWs were formed on gold films.

2.4. Synthesis of Methyl-Azadioxatriangulenium Chloride (Me-ADOTA-Cl). Methyl-azadioxatriangulenium chloride (Me-ADOTA-Cl) was prepared as previously described.³⁸ Azadioxatriangulenium chloride (ADOTA) was prepared from a solution of 100 mg of ADOTA-PF₆ and 1 g of an Amberlite IRA-400 ion-exchange resin which was mixed and stirred for 1 h. This resin was filtered off and replaced fresh three times.

2.5. Film Preparation and Plasmonic Platform (PP) Assembly. The sample was prepared by spin coating a $10\ \mu\text{M}$ or $100\ \mu\text{M}$ aqueous solution of the dye Me-ADOTA-Cl in 0.2% PVA onto a clean glass coverslip. As shown in Figure 1, the PP was assembled, by putting in contact two parts: a Me-ADOTA-Cl covered glass coverslip and a gold-coated slide with silver wires. The glass coverslip was brought in contact with the SW platform by adding a drop of mineral oil on it which ensured they remain together for the duration of the measurement. The enhancement factor is a distance dependent.^{17–20} However, in this report the distance between the dye and silver wires was not controlled.

2.6. Structural and Morphological Characterization. The structural and morphological characterization of the SWs was performed by scanning electron microscope (JEOL JSM-6510LV) and AFM. Scanning atomic force microscopy utilized the NTEGRA Prima scanning probe microscope manufactured by NT-MDT

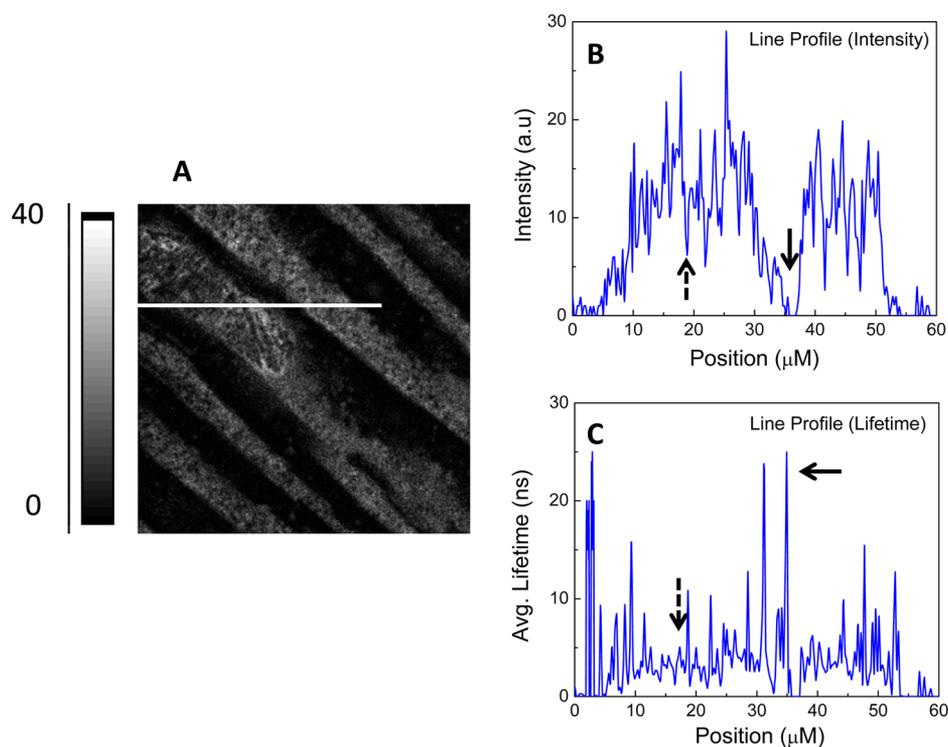


Figure 3. (A) Confocal fluorescence microscopy intensity image of Me-ADOTA-Cl on SWs on silica protected gold mirror. (B) Confocal fluorescence microscopy intensity profile [a.u.] of Me-ADOTA-Cl over 60 μm of the line drawn in panel A. (C) Lifetime profile (ns) of the line drawn in panel A. This is the same line used for the fluorescence intensity profile. Higher intensity corresponds to shorter lifetime associated with the presence of SW (dashed black arrow) and lower intensity corresponds to longer lifetime correlating to the absence of SW (solid black arrow).

(Moscow, Russia) with the closed-loop feedback semicontact mode at rate 0.5 Hz. Scanning was controlled and images were analyzed with NOVA software by NT-MDT instrument manufacturer.

2.7. Fluorescence and Lifetime Microscopy Measurements.

A confocal MicroTime 200 (Picoquant GmbH, Germany) system coupled with an Olympus IX71 microscope was used to obtain time-resolved images. Fluorescence photons were gathered from different places on the sample using a 60 \times water immersed objective (N.A 1.2, Olympus). A 500 nm long-pass filter with additional two 488 R band-pass filters (Shemrock) were applied to remove scattered light. A pulsed laser (470 nm-LDH-P-C470B) with repetition rate of 20 MHz was used as a light source. Fluorescence photons were collected using a photon counting module (SPCM = AQR-14, PerkinElmer) with processing accomplished by the PicoHarp300 time correlated single photon counting (TCSPC) module. Data analysis was performed using a SymPhoTime (5.2.4) software package. An average lifetime was calculated using FastLT method in Symphotime software. FastLT is a method for estimating the average lifetime as a function of the pseudo-pixels based on calculating the barycenter of the pseudo-pixel's decay. The time span from the barycenter of the IRF to the barycenter of the decay equals the average lifetime. This estimate does not require any nonlinear data fitting and is therefore very fast and does not suffer as much from low statistics. If an IRF is not available, the "time zero" has to be estimated differently, for example, by using the rising flank of the decay. However, this may introduce a systematic shift in the estimated average lifetimes.

3. RESULTS AND DISCUSSION

3.1. Structural and Morphological Characterization.

Freshly synthesized silver nanoprisms were prepared with a lateral dimension of 40 ± 5 nm and a thickness of 8.5 ± 1.4 nm.⁴⁴ Supporting Information shows the AFM image of triangular nanoprisms. The solution of silver triangular nanoprisms was drop coated onto the slide. It was discovered that silver nanoprisms self-assembled differently on dissimilar

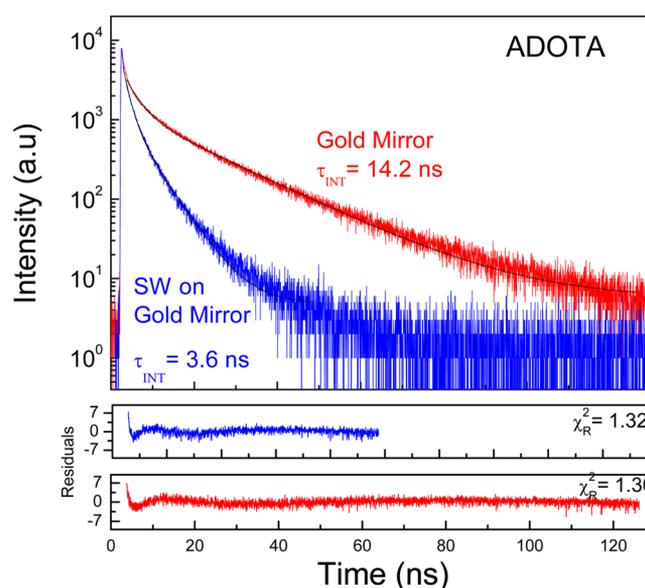


Figure 4. Fluorescence intensity decays of Me-ADOTA-Cl on a bare gold film and on SWs on the silica protected gold film. Me-ADOTA-Cl is more strongly quenched on silver wires than on the silica protected gold mirror alone, leading to a short fluorescence lifetime of 3.6 ns for the SW compared to 14.2 ns for the silica protected gold mirror alone.

surfaces. The self-assembly also depends on the drying conditions and the drying time. Allowing the solution of silver nanoprisms to air-dry on a bare gold mirror resulted in individual nanoprisms deposited on the gold mirror.⁴¹ While there was not complete coverage, the self-assembled structures had a uniform distribution on the slide.⁴¹ Here, a silver colloid

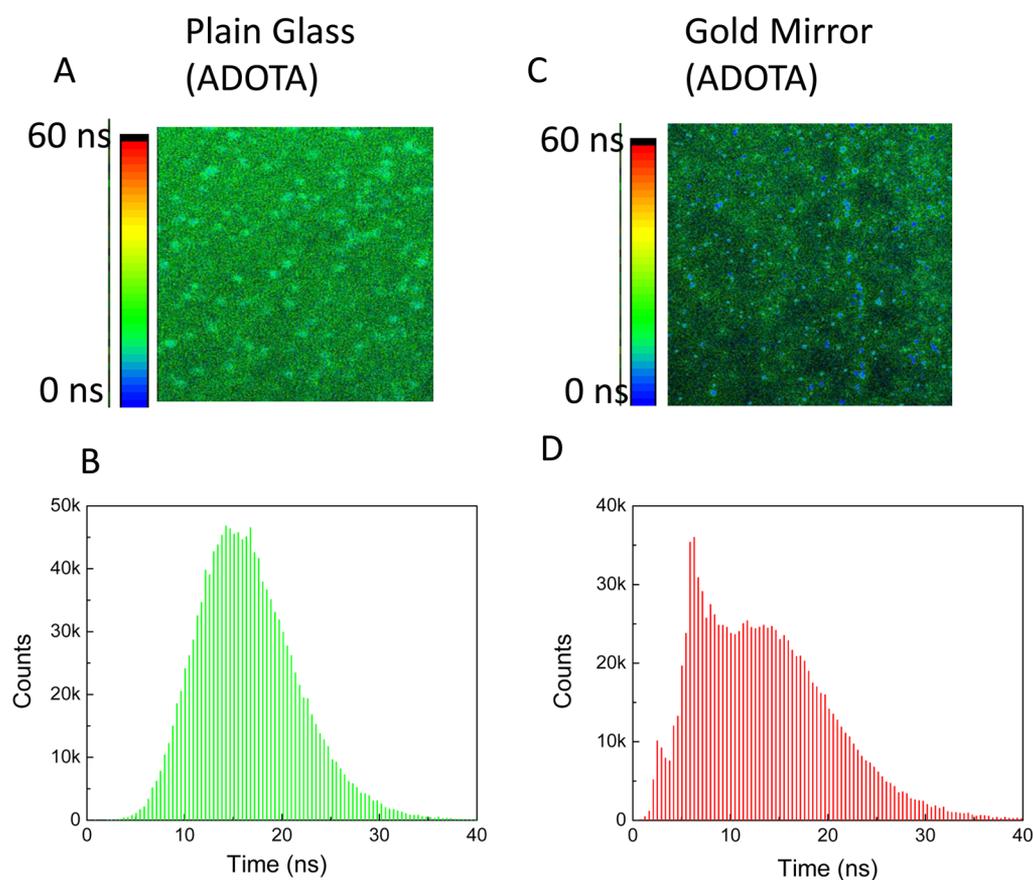


Figure 5. Panels A and B show the lifetime image and lifetime histogram, respectively, of Me-ADOTA-Cl between plain glass slides. C and D show the lifetime image and the lifetime histogram, respectively, of Me-ADOTA-Cl between plain glass and the silica protected gold mirror.

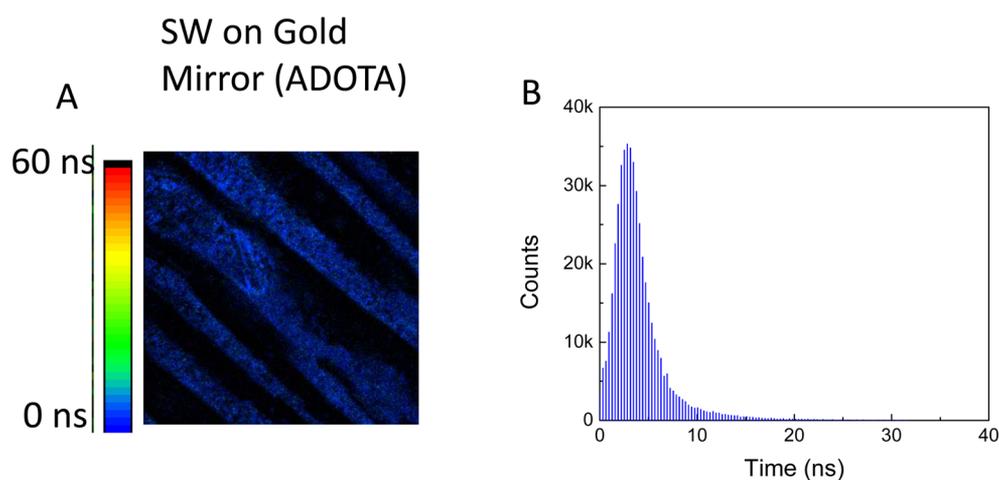


Figure 6. A and B show the lifetime image and lifetime histogram, respectively, of Me-ADOTA-Cl between plain glass and SW on the silica protected gold mirror.

solution was cast on a 48 nm thick gold mirror surface protected with 5 nm silica and slow dried in a near isolation chamber at room temperature. In this case, silver nanowires assembled rather than individual nanoprisms as previously seen. The SEM and AFM images of typical self-assembled SWs on silica protected gold mirrors are shown in Figures 2A,B, respectively. While there is not complete coverage on the slide, the self-assembled SWs have a uniform distribution. The assembled wires are from 10 to 100 μm in width and a few centimeters long. The images were zoomed to see the edges of

the protected gold film substrate. The z -dimension of SWs is less than 1 μm high from the surface of the slide, as shown in Figure 2C. The coverage is also very reproducible. Moreover, it is important to understand that these structures sustain plasmonic absorption despite the large dimensions. Figure 2D shows the typical plasmonic absorption of SW on the silica protected gold film on a glass slide with the absorption maximum at about 500 nm. SW on a plain glass slide shows absorption maximum around 450 nm (data not shown). The

red-shifted absorption on the silica protected gold film could be due to the plasmonic interaction of SW with gold mirror.

3.2. Fluorescence and Lifetime Microscopy Measurements. The majority of the metal enhanced fluorescence studies in the literature are done by coating a homogeneous layer of fluorophore solutions directly onto metallic surfaces.^{25–32,35–41} In this study of fluorescence enhancement measurements, the samples were prepared in a different configuration, as shown in Figure 1. SW structures collapsed and washed away while spin coating the fluorophore solution on the SW coated gold surfaces. That led us to develop this alternative assembly, where the fluorophore layer on the plain glass coverslip was brought in contact with the SW coated gold surface. The fluorophore was spin coated to get a uniform layer on the plain glass coverslip prior to the PP assembly. The glass coverslip was brought in contact with the SW platform by adding a drop of mineral oil on it. The distance between the dye and silver wires was not controlled.

Figure 3A shows the intensity image of one such a sample which was prepared using Me-ADOTA-Cl, a long-lived (~18 ns fluorescence lifetime) organic fluorophore. SWs are visible as enhanced fluorescence intensity areas. The area between the SWs here acts as an experimental control as the dye solution was uniformly distributed on the plain glass, and we do not see increased intensity counts in those areas compared to the ones on the SW. Figure 3B shows the intensity profile of the line drawn on Figure 3A. It is clearly visible that the SW areas show more than 15 times fluorescence intensity enhancement compared to areas void of SW (both ends of the line). Different concentrations (10 μM and 100 μM) of the dye did not have any effect on the average fluorescence enhancement. It is well-known that plasmon coupled fluorescence enhancement is associated with decreased fluorescence lifetime.^{1,8,9} Panel C in Figure 3 shows the fluorescence lifetime profile of the same line as in panel B. One can see that areas with enhanced fluorescence intensity have shorter lifetime (dashed black arrow) than areas with no SWs (solid black arrow).

Moreover, fluorescence intensity decays were measured independently at several areas to confirm the results. Figure 4 shows representative fluorescence intensity decays of Me-ADOTA-Cl measured on SWs (blue line) and on empty areas (red line). The recovered average lifetimes after multi-exponential fittings are 14.2 ns on the gold mirror (void of SWs) and 3.6 ns on the SW area. We saw about a fourfold decrease in the fluorescence lifetime compared to the lifetime on the area void of SWs.

Furthermore, in order to validate these results, measurements were performed with all the appropriate control samples. First, Me-ADOTA-Cl on plain glass was tested, followed by Me-ADOTA-Cl on the silica protected gold mirror. Figure 5 shows the fluorescence lifetime images (FLIM) and fluorescence lifetime distribution histograms (intensity weighted averaging) of respective samples of Me-ADOTA-Cl on plain glass and on the silica protected gold film. As expected, plain glass gives the monoexponential decay with a lifetime of 16 ns (panels A and B). Me-ADOTA-Cl on the silica protected gold mirror showed heterogeneous (three exponential fit: τ_1 , 19.8 ± 0.21 ns; amplitude, 0.61; τ_2 , 6.13 ± 0.17 ns; amplitude, 0.30; and τ_3 , 1.13 ± 0.07 ns; amplitude, 0.09) fluorescence decay due to gold induced quenching with average lifetime of 14.2 ns (panels C and D). Only part of the population was quenched, possibly due to the 5 nm silica layer on the gold film. Figure 6 shows the FLIM images of Me-ADOTA-Cl on SWs and of the control

experiment. Figure 6A shows the FLIM image of the Me-ADOTA-Cl sample put in contact between plain glass and SWs on the silica protected gold film on a glass slide (test sample). Figure 6B shows the lifetime histogram of the image. The blue color in the image is associated with the shorter lifetime on the lifetime scale, and as shown in histogram, the average lifetime is 3.6 ns. Moreover, we wanted to determine if there is any effect of orientation of excitation light polarization on the intensity and the lifetime. We found that changing the excitation light polarization did not affect the total intensity counts or fluorescence lifetime of ADOTA in SW deposited on silica protected gold mirror (see Supporting Information). This is due to the random orientation of dye molecules on glass coverslip which leads to depolarized emission.

4. CONCLUSIONS

A new method of preparation of silver wires on silica protected gold films was presented. Silver wires self-assembled on silica protected gold films when the solution of silver nanoprisms was allowed to evaporate slowly in a near-isolation chamber at room temperature. The silver wires were used to assemble a new type of plasmonic platform. This new PP consisted of two parts: a uniform film of Me-ADOTA-Cl in PVA on a glass coverslip and silver wires on silica protected gold mirror on a glass slide. These parts were put in contact using a drop of mineral oil, which ensured they remained together for the duration of the experiment. The plasmonic platform with self-assembled silver wires was considered a good candidate for the SEF. It was demonstrated that the SEF was a real phenomenon, not an enhancement of the fluorescence signal due to a simple accumulation of the fluorophore on rough nanostructure surfaces. The measurements of SEF on the PP show that fluorescence emission of Me-ADOTA-Cl was greatly enhanced, and the lifetime of the dye decreased when silver wires were present. The average increase of fluorescence emission was over 15-fold. The new PP may be utilized for improving bioassays and cell imaging.

■ ASSOCIATED CONTENT

Supporting Information

An AFM image of silver triangular nanoprisms is shown in Figure S1. Figure S2 displays dye doped PVA layer on the glass coverslip using AFM. Figure S3 represents the height profile across the scratch made on the dye layer from Figure S2. Figure S4 shows the effect of orientation of light excitation polarization on fluorescence intensity of Me-ADOTA-Cl on SW on silica protected gold film. The intensity image of Me-ADOTA-Cl on SW on a silica protected gold mirror where orientation of SW is parallel to excitation light polarization is presented on the left. The intensity image of Me-ADOTA-Cl on SW on a silica protected gold mirror with SW orientation perpendicular to excitation light polarization is presented on the right. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by Welch Foundation (BP-0037), NIH Grant R01EB12003, and NSF Grant CBET-126408. We appreciate Thomas Sorensen for his help with Me-ADOTA-Cl synthesis.

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