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APPLICATION NOTE #AN53013

Using Raman microscopy to monitor the surface modifications of disordered array of gold (Au) covered silicon nanowires for SERS biosensing

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Keywords

SERS, plasmonic excitation, Raman microscopy, surface modification, receptor, ligand, biosensor, nanowires, gold

Thermo Fisher Scientific solution

DXR2 Raman Microscope
OMNIC Spectral Software

Thermo Scientific™ DXR2 Raman Microscope

Abstract

The combination of a microscope and a Raman spectrometer provides insights to a multi-stepped surface modification process to fabricate a biosensor for avidin detection using biotin-modified, gold-coated silicon nanowires (Au/SiNWs) deposited on a glass substrate. It is demonstrated that the silicon nanowires morphology enhances light absorption in the overlaying Au layer. In addition, the Raman spectra unequivocally confirmed that the composite structure (SiNWs + Au + Cysteamine + Biotin) can effectively bind the target molecule avidin through specific receptor-ligand interactions.

Introduction

Surface enhanced Raman scattering (SERS) occurring at the surface of rough noble metals is an analytical technique that offers ultrahigh sensitivity and *in situ* recognition of molecules even in a liquid environment. SERS spectroscopy are used in such diverse fields as diagnostics, environmental monitoring, and trace chemical analysis. Recently, we reported the development of a novel, high-performing SERS substrate based on a disordered array of silicon nanowires (SiNWs) covered by an Au film (Au/SiNWs). It is noteworthy that the disordered arrangement is conducive to scalable and low-cost fabrication techniques at relatively low temperatures. This allows the structure to be directly grown onto unconventional substrates such as common microscope slides and facilitates their applications with many commercial Raman spectrometers.

Development and fabrication of SERS substrates is a multi-faceted and multi-disciplined research area that requires a range of research tools, from electron microscopy to vibrational spectroscopy. In this application note, we demonstrate the use of Raman microscopy to monitor sequential surface modifications in fabricating a biotin-modified Au/SiNWs substrate for avidin detection.

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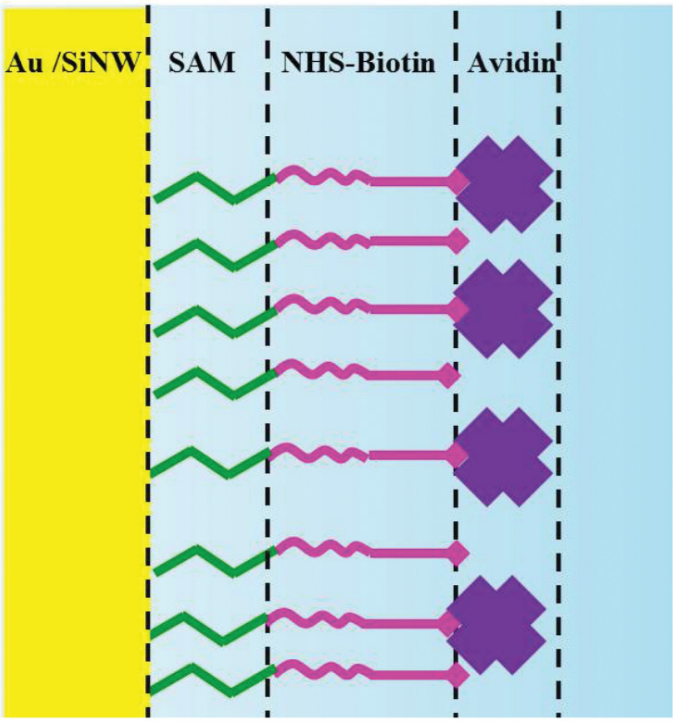


Figure 1: Schematic of the composite structure for avidin detection.

Experimental

Sample preparation

Figure 1 is a schematic showing the composite structure described in this note. First, gold (Au) catalyzed SiNWs were produced by plasma-enhanced chemical vapor deposition (PECVD) on selected areas of the glass slide and coated with an evaporated, thin Au layer of 150 nm thickness[1]. Next, self-assembled monolayers (SAM) of cysteamine were formed after the Au/SiNWs surface was immersed into a 20 mM cysteamine solution and incubated overnight at 70°C. Excess cysteamine was rinsed off with deionized water. The cysteamine modified Au/SiNWs were then immersed into a fresh solution of N-hydroxysuccinimide ester-biotin (NHS-biotin) in phosphate-buffered saline (PBS) at room temperature, and cleaned with PBS. Finally, this biotin-functionalized Au/SiNWs were immersed into 1.5 mL of aqueous 1 µM avidin solution for 30 minutes at room temperature, rinsed with PBS, and dried before being tested.

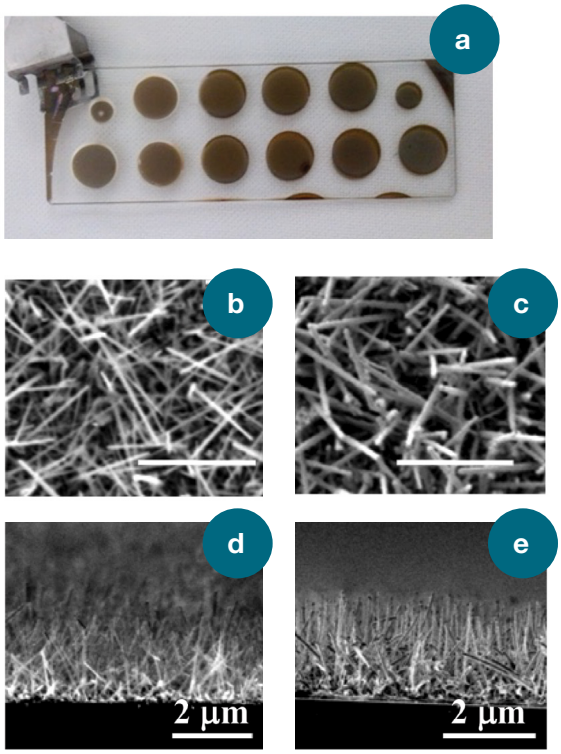
Raman microscopy

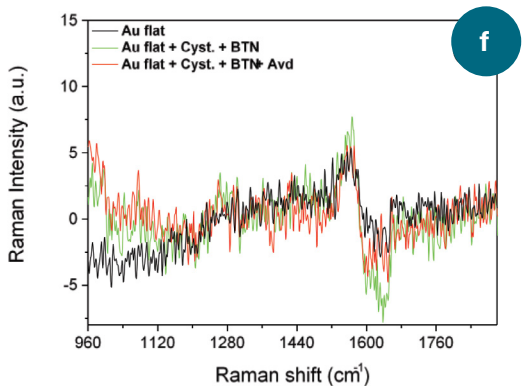
A Thermo Scientific™ DXR2 Raman Microscope was used for all analyses. Visual inspection of the samples after each functionalization step was performed using the microscope with a 50× objective. For Raman spectral acquisition, a 532 nm laser was used. The laser power was set at 5 mW. The measured spectral range was 50–3000 cm^{-1}, and each spectrum resulted from 1s exposure time and 200 accumulations over an area of about 800 nm (laser spot).

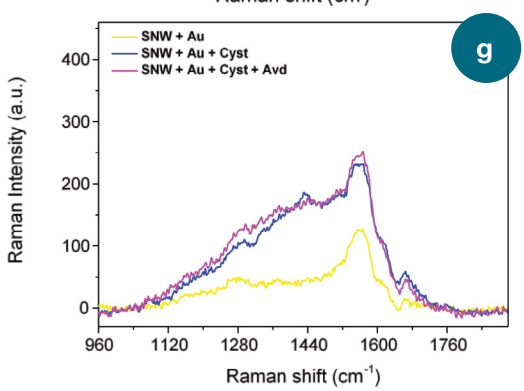
Results and discussion

A picture of the Au/SiNWs on the Corning® glass slide is shown in Figure 2a, where the brown circles are the active areas formed by Au/SiNWs. Representative SEM images of the substrate, both top and side view, are shown in Figure 2b-e. Figures 2f and 2g show the Raman spectra of the planar Au surfaces with and without silicon nanowires after each functionalization step. Comparing the black trace in Figure 2f and the yellow trace in Figure 2g, for example, there is ~20× increase in the Raman signal in the structures with SiNWs. The NWs morphology plays a critical role in enhancing light absorption in the overlaying Au layer by inducing specific plasmonic excitation[1].

Figure 2: (a) Picture of the Au/SiNWs on a Corning® glass slide. SEM images of the SiNWs before (b and d) and after Au coverage (c, e). The images b and c are top view and d and e are side view. (f) Raman spectra of the modified Au film without SiNWs. (g) Raman spectra of the modified Au film with SiNWs.







The optical images depicting each functionalization step are shown in Figure 3. Figures 3a shows the pristine Au/SiNWs. After the treatment with cysteamine, no noticeable change in the image was observed (Figure 3b). After the NHS-biotin functionalization (Figure 3c), however, the surface color changed from green to blue, indicating a uniform adsorption of the biotin molecules onto the Au/SiNWs structure. Upon a 30-minute exposure to 1 µM avidin solution (Figure 3d), the blue color becomes attenuated in a large portion of the surface. In a control experiment (Figure 3e) where cysteamine-modified surface (without NHS-biotin treatment) was immersed in avidin solution and rinsed with PBS, no color change was observed (comparing Figures 3b and 3e). These observations suggest that the avidin was immobilized onto the Au/SiNWs surface through specific avidin-biotin interactions.

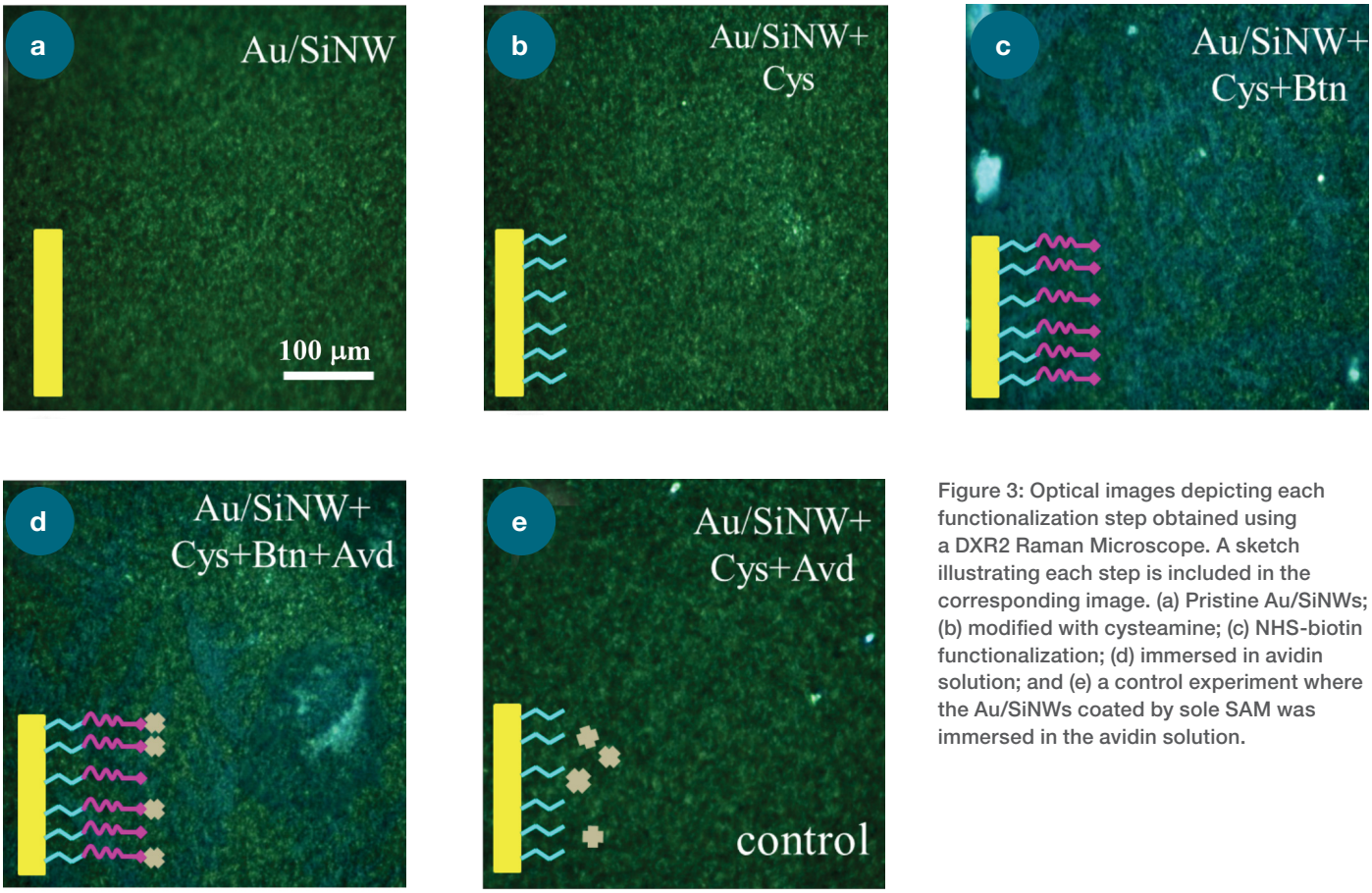


Figure 3: Optical images depicting each functionalization step obtained using a DXR2 Raman Microscope. A sketch illustrating each step is included in the corresponding image. (a) Pristine Au/SiNWs; (b) modified with cysteamine; (c) NHS-biotin functionalization; (d) immersed in avidin solution; and (e) a control experiment where the Au/SiNWs coated by sole SAM was immersed in the avidin solution.

The Raman analyses of the Au/SiNW surfaces after each functionalization step are summarized in Figure 4. Overall, there is a progressive signal increase in the spectral range 1280-1450 cm^{-1} with each modification step. When the cysteamine treated surface (blue trace) was immersed into a 0.1 µM avidin solution without rinse, the corresponding Raman spectrum (brown trace labeled "Avd ctrl" = SNW + Au + Cyst + Avd without rinse) has an additional feature at ~1380 cm^{-1}, originating from the "free" avidin molecules. When the cysteamine treated surface was further functionalized by biotin, the avidin peak at ~1380 cm^{-1} is present even after rinse with PBS to remove "free" avidin (the red trace in Figure 4), suggesting that avidin was immobilized onto the substrate via receptor-ligand interactions. These observations unequivocally confirm that the composite structure (SiNWs + Au + Cysteamine + Biotin) can serve as a biosensor to effectively bind the target molecule avidin through specific receptor-ligand interactions.

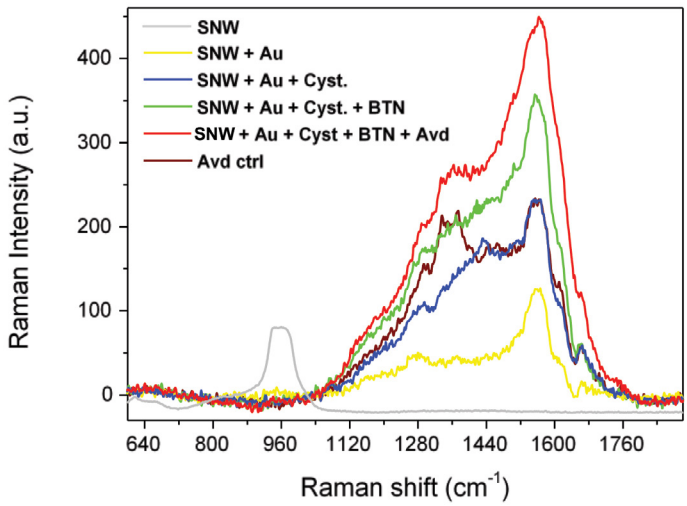


Figure 4. Raman spectra corresponding to each step involved in the SERS biosensing experiment.

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Conclusions

Developing effective SERS active materials that are amenable to easy fabrication and analysis with commercial Raman spectrometers is a multi-faceted research subject. To that end, the DXR2 Raman Microscope has proven a valuable tool for assessing biosensor construction and binding of the target molecule to the biosensor. In this application note, we demonstrated that the combination of microscope and Raman spectrometer can provide a holistic understanding of the multi-stepped surface modification process towards a biotin-modified Au/SiNWs SERS substrate for avidin detection. While microscope provides direct visualization of the morphology of the modified surfaces, the Raman spectra offers insight into the underlying chemistry of each modification step. Specifically, it is demonstrated that the nanowires morphology enhances light absorption in the overlaying Au layer due to plasmonic excitation. Furthermore, Raman spectra for different surface modifications unequivocally confirm that the composite structure (SiNWs + Au +Cysteamine + Biotin) can serve as a biosensor to effectively bind the target molecule avidin through specific receptor-ligand interactions.

Reference

1. Convertino, V. Mussi & L. Maiolo, “Disordered array of Au covered Silicon nanowires for SERS biosensing combined with electrochemical detection”, Scientific Reports **6**:25099 (2016).

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