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Improving Correlated SERS Measurements with Scanning Electron Microscopy: An Assessment of the Problem Arising from the Deposition of Amorphous Carbon

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Abstract

For surface-enhanced Raman scattering (SERS) substrates with nonspherical symmetry, it is critical to correlate spectroscopy measurements with imaging by scanning electron microscopy (SEM). However, the deposition of carbon resulting from e-beam exposure during SEM imaging contaminates the surface of nanoparticles, potentially preventing their further functionalization with Raman probe molecules. In addition, the deposited carbon leads to unwanted background SERS signals. In this study, we systematically investigated the deposition of carbon during SEM imaging and examined how it affects the functionalization of nanoparticles with probe molecules and impacts the detection of SERS signals. Significantly, we found that the carbon could be removed or replaced from the surface of Ag nanoparticles through a chemical or physical means, rendering the nanoparticles the capability for correlated SEM/SERS studies.

Keywords

Surface-enhanced Raman scattering; silver nanoparticle; carbon deposition; self-assembled monolayer; plasma etching

1. Introduction

Since its discovery approximately 40 years ago, surface-enhanced Raman scattering (SERS) has emerged as a valuable spectroscopic technique for ultrasensitive detection of molecular species. In comparison with normal Raman scattering, SERS has demonstrated the capability to increase the Raman signals from a given molecule 10^6 to 10^{12} times due to the locally enhanced electric field at the surface of nanoscale metal substrate (typically Ag or Au nanoparticles).¹ It is well known that the strength of this local electric field and thereby the enhancement factor (EF) of Raman signals are both dependent on the physical parameters (e.g., size and shape) of the metal nanoparticle, as well as its orientation relative to the polarization of incident light.^{2–6}

A systematic study of the relationship between the SERS spectrum of a nanoparticle and its physical parameters and orientation is therefore vital for the effort to achieve the highest possible enhancement. To this end, several approaches have been demonstrated to correlate the SERS spectrum of a nanoparticle with its morphology. These include the use of scanning and transmission electron microscopy (SEM and TEM), atomic force microscopy (AFM), and optical imaging via Rayleigh scattering.^{3,7–10} Among them, SEM imaging has proven to

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be a simple yet effective means to obtain details about the morphology of a nanoparticle. In contrast, TEM can only obtain two-dimensional projections of a nanoparticle and it also requires the use of a special substrate; AFM cannot easily resolve the exact profile of a nanoparticle due to tip convolution; and the lateral resolution of Rayleigh scattering imaging is limited by optical diffraction. For assemblies of spherical nanoparticles, techniques based on the polarization effect have been used to determine the orientation of the aggregates.^{11,12} However, for nanoparticles with anisotropic shapes, such as the Ag nanocubes used here, a more definite imaging technique like SEM is necessary.

Currently, a typical correlated SERS/SEM measurement is conducted by first recording the SERS spectra from nanoparticles that have been functionalized with probe molecules and then deposited on a substrate with registration marks. Afterwards, information about the nanoparticles is collected by SEM imaging. This procedure is ineffective since it requires one to collect spectra from a large number of particles, only to find after SEM imaging that the majority of the data are unwanted. Obviously, it will be more effective if we know the morphology and orientation of the nanoparticle first so that collection of SERS spectra can be targeted. However, upon exposure to the e-beam of an SEM machine, the nanoparticles are subjected to contamination due to the deposition of amorphous carbon. The deposited carbon could obstruct further functionalization of the nanoparticles with probe molecules, not to mention introducing the unwanted background Raman signals.

In this study, we used Ag nanocubes as a model system to investigate the impact of carbon deposition on SERS measurements. We used SEM to first identify the Ag nanocubes of a particular orientation prior to recording of its SERS spectrum. We systematically studied the deposition of carbon as a function of e-beam current and exposure time, as well as its impact on the ability to functionalize Ag nanocubes with SERS probe molecules. We also examined methods for replacing or removing the deposited carbon.

2. Experimental

2.1. Preparation of Ag nanocubes for use as SERS substrates

The Ag nanocubes were synthesized using a seed-mediated growth method, and the detailed protocol can be found in our previously reported work.¹³ In brief, single-crystal, Ag spherical seeds of 30 nm in diameter were prepared by reducing silver trifluoroacetate (CF_3COOAg , Aldrich) to elemental silver in ethylene glycol (EG, J. T. Baker). The seeds were then allowed to grow in the presence of silver nitrate (AgNO_3 , Aldrich) and poly(vinyl pyrrolidone) (PVP, $M_w \approx 55,000$, Aldrich) as the Ag precursor and capping agent, respectively. The 100-nm Ag nanocubes were obtained by quenching the reaction with an ice-water bath when the major local surface plasmon resonance (LSPR) peak reached 585 nm. The Ag nanocubes were washed first with acetone, and then three times with DI water to remove excess EG and PVP. The final product was suspended in ethanol at a typical concentration of 10^{10} – 10^{11} particles/mL for further use.

The seeds and 100-nm Ag nanocubes were characterized by a UV-vis spectrometer (Varian, Cary 50), a scanning electron microscope (SEM, FEI, Nova NanoSEM 2300) operated at an accelerating voltage of 15 kV, and a transmission electron microscope (TEM, FEI, G2 Spirit) operated at 120 kV.

2.2. Substrate preparation and imaging

All samples for SERS measurements were prepared by drop-casting a dilute (at a concentration of 10^7 particles/mL) suspension of Ag nanocubes in ethanol onto a small piece of silicon (Si) wafer containing registration marks. Unless otherwise specified, functionalization of the 100-nm Ag nanocubes with 4-methylbenzenethiol (4-MBT, Sigma)

was carried out by submerging the entire Si substrate in a 4-MBT solution in ethanol (1.0 mM) for 60 min either before or after SEM imaging. Functionalization for SERS measurements at different time points was performed by submerging the sample for 1 min in a 1 mM solution of either 4-MBT or toluene (Aldrich), then washing with copious amounts of ethanol and DI water. After taking the SERS spectra, the substrate was returned to the solution of 4-MBT or toluene. This process was repeated such that the substrate experienced cumulative exposure time to the solution of 10 min and 30 min. The SEM was typically operated at an accelerating voltage of 15 kV with a beam current of 0.24 nA. In a current dependent study, we also used e-beam currents of 0.49 nA and 1 nA. Exposure time was typically 1 min, but in some cases varied as noted. Plasma etching was employed as a possible means to remove the carbon coating. Samples were exposed to the plasma for 4 min with the power being set to “high”.

TEM imaging was also used to observe the carbon deposited on the Ag nanocubes during SEM imaging. In this case, a small volume of the Ag nanocubes suspended in ethanol was dropped onto a standard Cu TEM grid and allowed to dry. Small areas of the Cu grid were then imaged by the SEM using the standard settings for exposure times ranging from 1 to 15 min. The same areas of the Cu grid were then imaged using the TEM to confirm the variation in carbon coating thickness.

2.3. Correlated SEM/SERS measurements

The SERS spectra were recorded using a Renishaw inVia confocal Raman spectrometer coupled with a Leica microscope with a 50x objective (NA = 0.09). The excitation wavelength used was 514 nm generated by an Ar laser (5 mW) equipped with a holographic notch filter with a grating of 1200 lines/mm. The backscattered Raman signals were collected on a thermoelectrically cooled (−60 °C) CCD detector. Individual Ag nanocubes that had been targeted by SEM imaging were located on the substrate under dark-field illumination. Spectra were collected at 0.5 mW for 45 sec from each Ag nanocube. All SERS plots represent an average of the spectra acquired from 15–20 individual nanocubes of approximately the same size and orientation.

Data processing for the SERS spectra was performed using OriginPro v. 8.5.1, Student version (OriginLab, Corp., Northampton, MA). All data was baseline-corrected by subtracting the minimum from the data. The spectra were normalized to the peak of Si at 920–1000 cm^{-1} .

3. Results and discussion

3.1 Deposition of amorphous carbon during SEM imaging

Using SEM to image the Ag nanocubes is convenient because a variety of substrates can be used, unlike other nanoscale imaging techniques like TEM. Preparation of SEM samples is also simple and they can be transferred directly into a Raman microscope. Here we used a Si wafer as the substrate but both Au and Ag substrates can also be used to further enhance the SERS signals.¹⁴ The potential drawback of using SEM is that amorphous carbon will be deposited on the sample during exposure to the e-beam in a process known as electron beam-induced deposition (EBID).^{15–20} In a simple demonstration, we deposited Ag nanocubes on a TEM grid and imaged by the SEM at a tilt angle of 45° relative to the incoming e-beam. The faces of the nanocube exposed to the e-beam were coated with a noticeable carbon layer when viewed head-on under TEM (Figure 1A). While EBID is useful for a variety of applications,^{15,17,21} it is a potential problem for SERS applications, which are extremely sensitive to surface contamination.

We used SEM to easily identify individual Ag nanocubes on a marked Si substrate with particular orientations prior to SERS measurements. A previous study from our group indicated that the SERS enhancement factors (EFs) would be maximized when the face diagonal of a Ag nanocube was aligned parallel to the excitation laser polarization, as shown in the inset of Figure 1B, due to the polarization dependence of LSPR excitation.⁷ Therefore, identifying the particles of interest prior to SERS measurements allowed for targeted and more efficient experiments. After SEM imaging, the average SERS spectrum collected from a set of Ag nanocubes indicates that the material that had been deposited was essentially amorphous carbon (Figure 1B). The two major features in this spectrum were the broad peaks centered at 1580 and 1340 cm^{-1} , which can be assigned to the E_{2g} zone center mode of graphite (or G-band) and the disorder-induced band (or D-band), respectively.^{20,22–25}

3.2 Deposition of carbon under certain conditions impairs SERS

As shown in Figure 2, if the Ag nanocubes were first functionalized with 4-MBT, the SERS signals from 4-MBT on those nanocubes were still detectable even after the EBID. The deposition of carbon did not cause any major changes to the SERS peaks of 4-MBT; the intensity of the peak at 1077 cm^{-1} , a combination of the benzene ring breathing and CH in-plane bending modes (7a vibrational mode),^{26–28} remained the same before and after the deposition of amorphous carbon. The other major peak at 1583 cm^{-1} , the benzene ring C-C stretching (8a vibrational mode),^{27–28} did not show any increase in intensity, but rather was superimposed on the background spectrum from carbon. These observations imply that the deposition of amorphous carbon came from vapor phase hydrocarbon contaminants in the vacuum chamber, and it was not caused by degradation of the probe molecules adsorbed on the surface of a Ag nanocube. These results are additionally supported by a body of research, where self-assembled monolayers (SAMs) of aromatic molecules were found to be more stable than aliphatic SAMs under the irradiation of low-energy electrons. Instead of breaking down the aromatic molecules, the electron beam might just crosslink adjacent benzene rings.^{29–30} Note that the beam energy used in our study was even lower than what were used in many of the prior crosslinking studies (15 kV compared to 50 kV), suggesting that the level of crosslinking was low enough to have no effect on the SERS spectra.

Since the 4-MBT monolayer was not disturbed by the deposition of amorphous carbon during SEM imaging, SERS probe molecules with strong peaks outside the range of the carbon background can be intentionally chosen for correlated SEM/SERS analysis. It should be pointed out that there are many cases where functionalization of nanoparticles should be performed after SEM imaging. In order to determine the impact of the amorphous carbon on the ability to functionalize the Ag nanocubes post SEM imaging, we investigated the dependence of carbon deposition on e-beam current and exposure time, and then evaluated its impact on the SERS signals coming from the probe molecules.^{15–17}

We first investigated the effect of e-beam exposure time on the deposition of carbon. It was found that more carbon was deposited onto the surface of the Ag nanocubes when the e-beam exposure time was increased, as shown by the TEM images in Figure 3A. As expected, the SERS signals from carbon also increased dramatically with the exposure time (Figure 3B). Since a thicker layer of carbon made it more difficult for 4-MBT molecules to access the Ag surface during modification, the SERS signals from 4-MBT became very weak even just after 5 min of exposure to the e-beam of the SEM (Figure 3B).

Besides exposure time, the e-beam current was found to have an impact on the deposition of carbon. Three substrates with Ag nanocubes deposited were first imaged in the SEM for 1 min at currents of 0.24, 0.49, or 1 nA prior to functionalization with 4-MBT (Figure 4). While the SERS intensities of the amorphous carbon peaks did not vary significantly between the three samples, the intensities from 4-MBT decreased as current increased,

suggesting that fewer 4-MBT molecules were able to access the Ag surface. Increasing beam current generally resulted in a higher rate of carbon deposition until a point of saturation was reached.¹⁸ The currents used in this study were higher than the saturation point, which was reported to be around 0.1 nA.^{18,19} Since there were no observable changes to the carbon SERS intensity or carbon layer thickness, the decreased accessibility of 4-MBT to the Ag surface is likely related to a scenario in which nanocubes imaged at a higher beam current might have a denser film of carbon deposited on the surface. If deposition of carbon is not the goal, the impact of EBID on the SERS measurements can be reduced by minimizing the current used and shortening the time exposed to the e-beam when the sample is imaged under SEM.

3.3 Removal of amorphous carbon

In addition to manipulation of the conditions under which the SEM images are taken, we would ideally remove the deposited carbon to greatly reduce its SERS background and simultaneously improve the ability of SERS probes to access the Ag surface. It is obvious from Figures 3 and 4 that simply immersing the sample for a short period of time in a dilute solution of thiol-based analyte was not enough to remove the carbon layer. However, it is highly possible to replace the deposited amorphous carbon with a thiol molecule that can form a SAM on Ag surface.²² Incorporating a series of washing steps into the functionalization process can potentially lead to complete removal of the carbon, while forming a SAM of the analyte.

To replace carbon with thiol molecules, Ag nanocubes with only the appropriate orientations were located using the SEM operating at the standard settings (15 kV, 1 min, 0.24 nA), and then the entire substrate was immersed in a 1 mM solution of 4-MBT in ethanol. After 1 min, the substrate was removed from the 4-MBT solution, rinsed with ethanol and DI water, and SERS spectra were collected from the imaged Ag nanocubes. The substrate was returned to the 4-MBT solution for another 9 min, or for a total immersion time of 10 min. The process was repeated as schematically illustrated in Figure 5A, and the SERS spectra indicate that after only 10 min, the signals from the amorphous carbon were substantially reduced, and essentially disappeared after a total of 30 min (Figure 5B). This observation demonstrated that the carbon layer was not bound strongly to the Ag surface. Interestingly, we found that the carbon layer could also be washed away by toluene using a procedure similar to what was used for the 4-MBT solution (Figure 5C).

When 4-MBT was used, the strength of the Ag-S bond was able to overcome the Ag-C interaction. The surface of a Ag nanocube was simultaneously cleaned and functionalized for SERS measurements. The toluene most likely acted as a good solvent for the amorphous carbon. Since the expected SERS signals were not detected from the Ag nanocubes after toluene treatment,³¹ the toluene molecules did not adsorb onto the surface, but washed off the carbon, and presumably left behind an exposed Ag surface available for the adsorption of other SERS probes.

In addition to the aforementioned two methods, plasma etching was also found to be capable of removing much of the amorphous carbon layer (see the Experimental Section for detailed procedure). After imaging two samples of Ag nanocubes on a Si wafer with the standard SEM protocol, one of them was directly functionalized with 4-MBT, while the other was plasma etched for 4 min and then functionalized. The short exposure time was effective in removing the carbon while minimizing the oxidation of Ag surface.¹⁰ Figure 6 shows that without plasma etching, amorphous carbon provided a strong background which made the SERS signal from 4-MBT difficult to detect. After plasma etching, this background was greatly reduced, indicating the removal of most of the carbon layer. The similarity of the 4-MBT spectrum on the plasma cleaned sample to the negative control sample, not exposed to

SEM or plasma, confirms that the Ag nanocubes were not adversely affected by the plasma treatment. It should be pointed out that the conditions for functionalizing particles and collecting SERS spectra were the same for both Figure 2 and Figure 6, and the nanocubes were from the same original batch of synthesis. The difference in intensity for the SERS peaks (the top trace in Figure 2 vs. the bottom trace in Figure 6) can be attributed to the fact that the nanocubes used for Figure 6 had been stored as an aqueous suspension for several months. It is well documented that this aging process contributes to rounding of the corners of the cubes.^{4,32} As a result, the SERS spectra tend to be less intense due to the diminishing enhancement for the corner-based hot spots.⁴

4. Conclusions

This work demonstrates a simple approach to increase the efficiency and reliability of correlated SEM/SERS measurements. To overcome problems caused by the background signals from amorphous carbon deposited during exposure to the e-beam of an SEM, we found that optimizing the operating conditions could alleviate the effect of carbon deposition. In particular, reducing the time and current had the greatest impact. However, other parameters such as magnification, beam energy, and operating mode may also help reduce the amount of carbon deposited as well as reduce the time necessary to image the particles. In addition, three strategies, including replacement with thiol molecules, washing with a good solvent, and plasma cleaning, were all demonstrated to be effective in removing the amorphous carbon. Correlated studies between individual nanocrystals or aggregates and SERS can be streamlined and tailored to each custom application by using a combination of techniques for imaging and carbon removal explored here. We believe that this knowledge will greatly facilitate a deeper understanding of the complex relationship between the physical parameters of a metal nanocrystal and its SERS capabilities.

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References

1. Le Ru EC, Blackie E, Meyer M, Etchegoin PG. *J Phys Chem C*. 2007; 111:13794–13803.
2. Willets KA, Van Duyne RP. *Annu Rev Phys Chem*. 2007; 58:267–297. [PubMed: 17067281]
3. Henry AI, Bingham JM, Ringe E, Marks LD, Schatz GC, Van Duyne RP. *J Phys Chem C*. 2011; 115:9291–9305.
4. Cogley CM, Skrabalak SE, Campbell DJ, Xia Y. *Plasmonics*. 2009; 4:171–179.
5. McLellan JM, Li ZY, Siekkinen AR, Xia Y. *Nano Lett*. 2007; 7:1013–1017. [PubMed: 17375965]
6. Xu H, Käll M. *Chem Phys Chem*. 2003; 4:1001–1005. [PubMed: 14562448]
7. Michaels AM, Nirmal M, Brus LE. *J Am Chem Soc*. 1999; 121:9932–9939.
8. Weber ML, Willets KA. *J Phys Chem Lett*. 2011; 2:1766–1770.
9. Camden JP, Dieringer JA, Wang Y, Masiello DJ, Marks LD, Schatz GC, Van Duyne RP. *J Am Chem Soc*. 2008; 130:12616–12617. [PubMed: 18761451]
10. Camargo PHC, Rycenga M, Au L, Xia Y. *Angew Chem Int Ed*. 2009; 48:2180–2184.
11. Shegai T, Brian B, Miljkovi VD, Käll M. *ACS Nano*. 2011; 5:2036–2041. [PubMed: 21323329]
12. Stranahan SM, Titus EJ, Willets KA. *J Phys Chem Lett*. 2011; 2:2711–2715.
13. Zhang Q, Li W, Moran C, Zeng J, Chen J, Wen LP, Xia Y. *J Am Chem Soc*. 2010; 132:11372–11378. [PubMed: 20698704]
14. Rycenga M, Xia X, Moran CH, Zhou F, Qin D, Li ZY, Xia Y. *Angew Chem Int Ed*. 2011; 50:5473–5477.

15. Chansin GAT, Hong J, Dusting J, deMello AJ, Albrecht T, Edel JB. *Small*. 2011; 7:2736–2741. [PubMed: 21953785]
16. Egerton RF, Li P, Malac M. *Micron*. 2004; 35:399–409. [PubMed: 15120123]
17. Li J, Toth M, Tileli V, Dunn KA, Lobo CJ, Thil BL. *Appl Phys Lett*. 2008; 93:023130.
18. Roediger P, Wanzenboeck HD, Hochleitner G, Bertagnolli E. *J Vac Sci Technol B*. 2009; 27:2711–2717.
19. Rykaczewski K, Henry MR, Kim SK, Federov AG, Kulkarni D, Singamaneni S, Tsukruk VV. *Nanotechnology*. 2010; 21:035202. [PubMed: 19966395]
20. Kulkarni DD, Rykaczewski K, Singamaneni S, Kim S, Fedorov AG, Tsukruk VV. *ACS Appl Mater Interfaces*. 2011; 3:710–720. [PubMed: 21319745]
21. Ding W, Dikin A, Chen X, Piner RD, Ruoff RS, Zussman E, Wang X, Li X. *J Appl Phys*. 2005; 98:014905.
22. Norrod KL, Rowlen KL. *Anal Chem*. 1998; 70:4218–4221. [PubMed: 21651259]
23. Itoh K, Kudryashov I, Yamagata J, Nishizawa T, Fujii M, Osaka N. *J Phys Chem B*. 2005; 109:271–276. [PubMed: 16851013]
24. Ishida H, Fukuda H, Katagiri G, Ishitani A. *Appl Spectros*. 1986; 40:322–330.
25. Kudelski A, Pettinger B. *Chem Phys Lett*. 2000; 321:356–362.
26. Sauer G, Brehm G, Schneider S. *J Raman Spectrosc*. 2004; 35:568–576.
27. Rycenga M, Kim MH, Camargo PHC, Cobley C, Li ZY, Xia Y. *J Phys Chem A*. 2009; 113:3932–3939. [PubMed: 19175302]
28. Merlin JC, Cornard JP. *J Chem Educ*. 2006; 9:1393–1398.
29. Waske PA, Meyerbröcker N, Eck W, Zharnikov M. *J Phys Chem C*. 2012; 116:13559–13568.
30. Geyer W, Stadler V, Eck W, Zharnikov M, Götzhäuser A, Grunze M. *Appl Phys Lett*. 1999; 75:2401–2403.
31. Chang ASP, Maiti A, Ileri N, Bora M, Larson CC, Britten JA, Bond TC. *Proc SPIE*. 2012; 8366:83660S.
32. Zhang Q, Li W, Wen LP, Chen J, Xia Y. *Chem Eur J*. 2010; 16:10234–10239. [PubMed: 20593441]

Figure 1.

Deposition of carbon on Ag nanocubes during SEM imaging was confirmed by both TEM imaging and SERS measurements. (A) After SEM imaging at a 45° tilt angle (left), the Ag nanocube was imaged head-on in the TEM (right). Carbon was only deposited on the exposed faces, as indicated by the arrow in the inset. (B) SERS spectra taken from a single 100-nm Ag nanocube before (bottom trace) and after SEM imaging (top trace). The broad SERS peaks correspond to the bands of amorphous carbon: 1340 cm^{-1} for the disorder-induced band, and 1580 cm^{-1} for the in-plane E_{2g} center mode of graphite. The broad peak in both spectra at 930–1000 cm^{-1} was from the Si substrate, and was used to normalize the spectra. Each SERS spectrum represents the average from 15–20 similar Ag nanocubes, and one of them is shown in the inset (scale bar: 100 nm). The polarization of the excitation laser was parallel to the face diagonal of the Ag nanocube.

Figure 2.

The SERS spectrum of 4-MBT adsorbed on the 100-nm Ag nanocubes before SEM imaging (a, bottom trace) was retained after SEM imaging (b, top trace). However, the background from deposition of carbon was detected under the 4-MBT peaks after SEM imaging (b, red). The SERS peaks from 4-MBT were at 1077 cm^{-1} , a combination of the benzene ring breathing and CH in-plane bending modes, and at 1583 cm^{-1} , the benzene ring C-C stretching. The broad peak from 930–1000 cm^{-1} was from the Si substrate, and was used to normalize all the spectra. Each SERS spectrum represents the average from 15–20 similar Ag nanocubes with similar orientation relative to laser polarization. The SEM was operated at 15 kV, with a current of 0.24 nA.

Figure 3.

(A) TEM images of Ag nanocubes (a) that had not been exposed to the e-beam, and after exposure to the e-beam at 15 kV and 0.24 nA for (b) 1 min, (c) 5 min, and (d) 15 min. All scale bars are 50 nm. (B) The duration of exposure of Ag nanocubes to the e-beam affects not only the amount of carbon deposited, but also the capability of the Ag nanocubes for functionalization. The individual 100-nm Ag nanocubes on a Si substrate were exposed to the e-beam for 1, 5, and 10 min, at a voltage of 15 kV and a current of 0.24 nA. The samples were then immersed in 1 mM 4-MBT for functionalization for 1 h prior to recording of the SERS spectra (from bottom to top, 1 min, 5 min, and 10 min).

Figure 4.

Three different samples of Ag nanocubes were imaged in the SEM at three different currents of 0.24, 0.49, and 1 nA (in the order from top to bottom). After imaging, the samples were functionalized with 4-MBT (1 mM in ethanol) for 1 h. The SERS intensity of 4-MBT on Ag nanocubes imaged at higher currents was much lower than those imaged at a lower current, but all showed broad peaks from amorphous carbon with the same intensities between 1340 and 1580 cm^{-1} . The peak at 1077 cm^{-1} represents a combination of the benzene ring breathing and CH bending modes from 4-MBT. The scale bar is 100 counts.

Figure 5.

The amorphous carbon could be removed by repeatedly immersing the samples in a thiol or toluene solution in ethanol and then washing with ethanol and DI water. Prior to SERS measurements, the Ag nanocubes were imaged using the SEM on a Si substrate (1 min, 15 kV, 0.24 nA). (A) A schematic of the experimental process. The substrate was immersed in either a 4-MBT or toluene solution (each 1 mM in ethanol). At three time points, the substrate was removed and washed thoroughly with ethanol and DI water, and SERS spectra were collected from the imaged Ag nanocubes to monitor the carbon removal. The substrate was then returned to the solution. The SERS spectra collected over time from the SEM-imaged Ag nanocubes after immersion in (B) a 4-MBT solution and (C) a toluene solution showed a marked decrease in signal from carbon after just 10 min immersion and essential disappearance after 30 min. The scale bars in both (B) and (C) are 100 counts.

Figure 6.

Much of the amorphous carbon could be removed through plasma etching. After SEM imaging, the Ag nanocubes were derivatized with 4-MBT directly (top trace) or subjected to plasma etching for 4 min prior to functionalization with 4-MBT (middle trace). As a control, Ag nanocubes with no exposure to the electron beam of an SEM were functionalized and included for comparison (bottom trace).