Published in final edited form as:

Small. 2011 February 18; 7(4): . doi:10.1002/sml.201001836.

A New Approach to Solution Phase Gold Seeding for SERS Substrates

Scott M. Tabakman, Zhuo Chen, Hernan Sanchez Casalongue, Hailiang Wang, and Hongjie Dai*

Department of Chemistry, Stanford University. 380 Roth Way, Keck Building Room 125, Stanford, CA, 94305 USA

Abstract

Surface-enhanced Raman scattering (SERS) vastly improves signal-to-noise ratios compared to traditional Raman scattering, making sensitive assays based upon Raman scattering a reality. However, to date, preparation of highly stable SERS-active gold substrates requires complicated and expensive methodologies and instrumentation. Here, we introduce a general and completely solution phase, seed-based approach, capable of producing gold films for surface-enhanced Raman scattering (SERS) applications on a variety of substrates, not requiring surface modification or functionalization. SERS enhancement factors of $\sim 10^7$ were observed. Moreover, solution-phase gold film deposition on highly complex surfaces, such as protein-coated bioassays, is demonstrated for the first time. Protein bioassays coated with such SERS-active gold films were combined with bioconjugated single-walled carbon nanotube Raman labels, affording high sensitivity detection of the cancer biomarker, carcinoembryonic antigen in serum, with a limit of detection of ~ 5 fM (1 pg / mL).

Keywords

Bioassay; Gold Nanoparticles; Nanotechnology; Plasmonics; Surface-enhanced Raman Scattering

1. Introduction

Surface-enhanced Raman scattering (SERS) has proven to be a powerful technique capable of intensifying the emission of weak, inelastic Raman scattering of molecules.^[1, 2] Utilizing the advantages of SERS, glucose, oligonucleotides, explosives and other analytes of interest have been detected at high sensitivity.^[3-6] Recently, our group demonstrated high sensitivity protein detection based upon bioconjugated single-walled carbon nanotube (SWNT) Raman labels and SERS in protein array format.^[7] However, the SERS step required undesirable vacuum deposition of gold films and thermal annealing of the assay substrates at 400 °C.

SERS-active substrates are often made by vacuum evaporation or sputtering,^[7-9] high temperature annealing,^[7] and Langmuir-Blodgett film transfer,^[10] amongst other methods.^[11-16] For many assays, especially those with biological components, it is desirable to produce plasmonic metal nanostructures without exposing the assay components to harsh conditions, such as high temperatures, organic solvents, and high vacuum. Deposition of SERS-active films from the aqueous phase circumvents many of the aforementioned problems, yet provides the opportunity to prepare large area, SERS-active films.

*hdai@stanford.edu.

Supporting Information is available on the WWW under <http://www.small-journal.com> or from the author.

Purely solution phase chemical synthesis of silver substrates has been reported for SERS applications,^[17] but Ag suffers from oxidation and instability problems, especially when reactive species are present, as is the case in bioassays. Gold films are promising as highly stable SERS substrates, and may be prepared from pre-made gold nanoparticle (Au NP) precursor seeds by reduction of chloroauric acid solution by hydroxylamine.^[11, 18] However, deposition of pre-made Au NP seeds onto a substrate requires an amino- or mercaptosilane functionalized substrate, and thus the methodology is not directly amenable to polymeric or other complex surfaces, such as protein microarrays.^[19]

Herein, we present a simple, fast, and completely solution-phase approach to the seeding and growth of SERS active gold films on traditional inorganic substrates, such as glass, quartz and SiO₂, as well as on polymeric, flexible substrates and atop highly complex protein assays. Our strategy for the broadly applicable, solution phase growth of plasmonic Au films begins with rapid, *in situ* “seeding” of gold nanoparticles by deposition/precipitation of Au³⁺ ions onto unmodified surfaces, followed by solution-phase reduction to Au⁰. Subsequently, the gold seeds are “grown” into a film by the hydroxylamine reduction of HAuCl₄,^[18] and the resulting films are referred to as gold-on-gold (Au/Au) films.

2. Results

2.1. Seeding of gold precursors onto unmodified substrates

Preparation of Au/Au films involves three steps: seeding of gold precursors, reduction into Au⁰ clusters, and selective growth by hydroxylamine reduction of HAuCl₄ (Fig.1). Seeding was accomplished by addition of ammonium hydroxide into a solution of chloroauric acid containing the substrate of choice (see Supporting Methods). Immediately following ammonium hydroxide addition, the transparent yellow, acidic HAuCl₄ solution became cloudy and orange-yellow, with pH ~ 9. The deposition rate of the Au³⁺ species onto the substrate was found to be rapid. Increased exposure times from one minute to twenty minutes did not significantly affect the density or size of gold seeds immobilized on the substrate.

Seeding density of Au seeds was dependent upon the initial concentration of HAuCl₄ into which the substrate was submerged prior to precipitation by ammonium hydroxide. For inorganic substrates such as glass and SiO₂, an increase of HAuCl₄ from 0.5 mM to 5 mM led to significantly increased density and uniformity of Au NP precursor seeds (Fig.2a). Polymeric substrates such as poly(vinyl chloride), PVC, and poly(dimethylsiloxane), PDMS, required slightly Au³⁺ concentrations of 10 mM in order to obtain high density seeding.

2.2. Solution phase reduction of gold precipitate precursors enables gold film growth

Following the deposition of Au seeds by precipitation onto the substrate of choice, the substrate was immersed into a 1 mM solution of sodium borohydride, which led to rapid formation of Au⁰ nanoparticles, evidenced by a faint pink-purple color change of the substrate. Atomic force microscopy revealed formation of Au NPs with diameters of 5-10 nm (Fig. 1b), and UV/Vis absorption spectroscopy revealed a weak surface plasmon resonance at 525 nm, typical of Au NPs in this size range.

2.3. Selective reduction of Au³⁺ onto precursor Au seeds yields plasmonic gold-on-gold (Au/Au) films

Submersion of the seeded substrate into an aqueous solution of chloroauric acid and hydroxylamine initiated selective reduction of Au³⁺ onto the seed layer, and thus the Au precursor seeds were grown into plasmonic nano-islands (Fig.1d). Hydroxylamine-mediated

gold reduction led to a color change of the substrate from pink to blue-purple, and finally a golden color was observed on the substrate as the film thickened (Fig. 1c). SEM imaging revealed that the Au nano-islands formed on the substrate were separated by ~ 10 -100 nm gap spacing, a morphology desirable for local electrical field enhancement and SERS.^[20]

The resulting Au/Au film thickness and structure depended on both the density of gold precursor seeding and the concentration of Au^{3+} ions during hydroxylamine-mediated growth. At a “growth” concentration of 500 μM HAuCl_4 , a substrate seeded at very low density failed to produce a network of interacting plasmonic gold structures, evidenced by SEM imaging, and a surface plasmon resonance at ~ 525 nm, typical of non-interacting Au NPs. Under identical growth conditions, however, a substrate seeded at higher density yielded a network of interacting plasmonic nano-islands, exhibiting a red-shifted plasmon resonance and higher optical density (Fig. 2b & 2c and Supporting Information, Fig S1). As expected, increasing the concentration of HAuCl_4 during growth at a fixed seeding density led to thicker and thicker Au/Au films with higher coverage of gold nano-islands, exhibiting monotonically red-shifted plasmon resonances and increasing optical density (Fig. 3a and Supporting Information, Fig S3).^[18] At very high concentrations of HAuCl_4 during hydroxylamine-mediated growth, the nano-islands coalesced into a continuous, roughened, gold film (Fig. 3b).

Deposition of Au/Au films onto unmodified polymeric substrates such as PVC and PDMS showed similar seeding and growth behavior to that which was observed on glass and SiO_2 . Hydroxylamine-mediated reduction of HAuCl_4 onto Au-seeded PVC and PDMS yielded uniform growth of interacting, plasmonic gold islands, observed by absorbance spectroscopy and SEM imaging (Fig. 4). These substrates retained their flexible character following Au/Au film growth, with no obvious signs of cracking upon repeated bending.

2.4. Optimization of Au/Au films for surface-enhanced Raman scattering

Benzenethiol self-assembled monolayers were used as Raman reporters to study the SERS properties of Au/Au films on glass, PVC and PDMS, as well as on Ag films, prepared on glass. Au/Au films grown from a low density seeding layer yielded only a weak SERS effect at 785 nm excitation, while films seeded at higher densities showed greater enhancement factors (Supporting Information, Fig. S2). SERS enhancement factors of the various Au/Au films were also dependent upon the hydroxylamine-mediated growth conditions. Au/Au films demonstrated increasing SERS enhancements with increasingly red-shifted plasmons and increasing thickness up to a maximum value, followed by a precipitous drop in SERS resulting from complete coalescence of the gold film at very high concentrations of HAuCl_4 during film growth (Fig. 3c & 3d). Optimal Au/Au films produced on glass, seeded at 5 mM HAuCl_4 with selective Au growth of 1 mM of HAuCl_4 and NH_2OH , exhibited a broad surface plasmon resonance at 610 nm, and enhanced the Raman scattering intensity of benzenethiol by a factor of $\sim 10^7$ at 785 nm excitation (Supporting Information, Fig. S3). For comparison, silver films were prepared on glass using previously described methods.^[7, 17] Silver mirror films yielded a similar benzenethiol Raman scattering intensity as the optimal Au/Au film (Fig. 3c), but visibly oxidized over time, while silver films prepared by evaporation afforded benzenethiol Raman scattering intensity 5-fold lower than optimal Au/Au films (Supporting Information, Fig. S7).

SERS was also observed from Au/Au films seeded and grown onto unmodified polymeric substrates. SERS measurements of benzenethiol chemisorbed onto Au/Au films supported by glass, PVC, and PDMS revealed that Raman scattering enhancement for Au/Au films on all three support substrates were of a similar order of magnitude (Fig. 4f). Uniformity and reproducibility of the Au/Au films were excellent, as evidenced by spatially mapping the SERS spectra of benzenethiol-coated films over large areas and on duplicate substrates

(Supporting Information, Fig. S4). It was, however, observed that the Au/Au films SERS properties were reduced when the film was damaged at extremely high laser power densities ($\sim 10 \text{ MW/cm}^2$, Supporting Information, Fig. S5).

2.5. Au/Au films can be deposited from solution onto protein-coated bioassays for SERS-based sensing

Having produced Au/Au films on glass, quartz, SiO_2 , PVC, and PDMS, the versatility of the method was tested by preparing SERS-active Au/Au films atop protein bioassays. Such SWNT-labeled bioassays, described thoroughly elsewhere^[7], are coated by proteins and thus are not suitable substrates for silanization and deposition of pre-made Au NP seeds for film growth^[18]. Moreover, while electron-beam evaporation of silver onto protein bioassays has been shown to yield SERS (Supporting Information, Fig S6) the silver film rapidly oxidizes over the course of a few hours, and the SERS effect is eventually lost.

We first performed sandwich immunoassays on glass substrates, using SWNT Raman labels^[7] (Fig. 5a, and Supporting Methods), which act as high scattering cross-section Raman tags, with a resonance-enhanced Raman G-band at 1590 cm^{-1} . A calibration curve was generated for concentrations from $1 \text{ nM} - 1 \text{ fM}$ of the analyte, the cancer biomarker protein carcinoembryonic antigen (CEA),^[21] spiked into serum. After tagging the protein microarray spots with SWNTs conjugated to the appropriate secondary antibody, Au seeds were deposited by precipitation of 5 mM of chloroauric acid. Subsequent reduction of the Au^{3+} clusters by sodium borohydride yielded a pink-purple color on the substrate, indicating successful seeding of Au NPs onto the protein bioassay substrate. Soaking of the bioassay slide in hydroxylamine and chloroauric acid resulted in growth of a uniform Au/Au film.

Compared to previous methodologies utilized by our group for obtaining SERS of SWNT-labeled protein bioassays, the Au/Au films showed a marked improvement over vacuum deposited metal films (Supporting Information, Figs. S6 & S7) with an enhancement factor up to ~ 250 fold for the SWNT Raman G-band (Fig.5b). The enhanced signal-to-noise ratio afforded a limit of detection of CEA down to $\sim 5 \text{ fM}$ ($\sim 1 \text{ pg/mL}$) (Fig.5c: black squares, and Supporting Information, Fig. S8) with 100 ms Raman scattering integration per pixel. Without Au/Au film enhancement, the detection limit was $> 10 \text{ pM}$ (Fig.5c: red triangles).

3. Discussion

Herein we have demonstrated the seeding and growth of uniform, SERS-active gold-on-gold (Au/Au) films purely from the solution phase onto a variety of traditional and non-traditional substrates, without requiring modification of the surface. The *in situ* Au seeding step is critical to the entire Au/Au film synthesis process. We hypothesize that the addition of ammonium hydroxide to chloroauric acid in basic pH leads to ligand exchange of chloride for ammine (or amino) ligands around the Au^{3+} center, with a general form $\text{Au}(\text{NH}_3)_2(\text{H}_2\text{O})_{2-x}(\text{OH})_x^{(3-x)+}$.^[22] The resulting ammine-gold complexes do not rapidly hydrolyze in basic solutions^[23] and aggregate into clusters. In moderately basic pH, the low-solubility, cationic clusters are then deposited onto negatively charged substrate surfaces. The deposited gold precipitates are subsequently reduced into Au^0 nanoparticles by sodium borohydride in aqueous solution. The Au NP seeding methodology presented here appears to be very broadly applicable to a variety of substrates. Unlike previous reports of solution-phase gold film growth, our method is not restricted to surfaces bearing amino- or mercapto-functionality^[18, 19].

Gold cluster seeding density was optimized to yield uniform and dense seed distribution. Seeding density increased with increasing Au^{3+} concentration (Fig.2a), thus allowing one to control the film density and morphology. Seeding of cationic Au clusters was also found to

be dependent on pH, resulting in a uniform seed layer only at pH 8-10. Replacement of ammonium hydroxide with sodium hydroxide in the seeding step led to colorless cationic Au solutions and failed to produce Au NP seeding, thus exemplifying the role of nitrogen-containing basic ligands in the precipitation and deposition process.

Reduction of the deposited small clusters of gold cations by NaBH_4 was necessary prior to the “growth” of the final Au/Au film, without which, no reduction of Au^{3+} by hydroxylamine was observed. Following reduction of the seeds, AFM confirmed the presence of nanoscopic spheres with heights 5-10 nm (Fig. 1b), and absorbance spectroscopy revealed a plasmon resonance at 525 nm confirming conversion of the cationic clusters to nanoscale Au^0 particles.

Growth of the Au film occurred by hydroxylamine reduction of additional chloroauric acid onto the “seeds.” The rate of HAuCl_4 reduction by hydroxylamine is much greater for surface-bound Au^{3+} ions than those in solution, and thus Au^0 formation is specific to the seed layer.^[18] Optimal synthesis conditions for SERS-active Au/Au films were identified by independently varying the Au^{3+} concentrations during both seeding and growth (Fig. 3 and Supporting Fig. 2). The Au^{3+} concentration during growth had a profound effect on the resulting plasmon peak, film thickness, and particle morphology (Fig. 3a & 3b). Morphology of the Au/Au films imaged by SEM shows individualized Au nanoparticles at low concentrations, growing into islands at higher concentrations, and finally forming a continuous rough gold film at very high Au^{3+} concentrations.

Enhancement factors ranging from 10^5 through $\sim 10^8$ have been reported for gold, silver, and silver-on-gold films produced by a variety of methods.^[10, 17, 24, 25] In agreement with literature, Au/Au film SERS enhancement factors of $10^6 - 10^7$ were observed for benzenethiol (at 785 nm excitation) chemisorbed onto Au/Au films grown on unfunctionalized glass and polymeric substrates (Fig. 3 and Supporting Information, Fig. S2). SERS enhancement factors increased with increasingly red-shifted plasmons and increasing film thickness up to a point, followed by a rapid drop in enhancement factor, resulting from complete coalescence of the gold film under very high concentration Au^{3+} growth condition. This behavior is consistent with previous reports that have suggested that maximal SERS may be obtained within the gaps between separated plasmonic particles, owing to vastly increased electric fields resulting from coupled localized surface plasmons.^[26, 27] Thus, for Au/Au films grown onto glass substrates, the optimal HAuCl_4 concentration for seeding was determined to be 5 mM, while the optimal HAuCl_4 concentration during the growth step was 1 mM (Supporting Information, Fig. S3).

Beyond unmodified inorganic and polymeric substrates, the broad applicability of Au NP seeding by precipitation was tested by depositing Au precursor seeds onto a protein bioassay in order to obtain a SERS effect of underlying SWNT Raman labels.^[7, 28-30] Precipitation and deposition of cationic gold clusters onto the protein-coated assay slide was successful, as evidenced by a uniform color change upon reduction of the gold seeds to Au^0 . It is plausible that proteins on the bioassay surface contained negatively charged domains or functional groups that promote seeding of Au^{3+} clusters. Growth of the Au seeds by hydroxylamine-mediated Au^{3+} reduction led to uniform Au/Au film growth and enhanced the Raman scattering intensity of the SWNT labels by 250-fold. Compared to silver films evaporated onto protein bioassays, the Au/Au film deposition technique led to a 7-fold increase in SERS enhancements of the SWNT Raman modes, as well as improved stability, owing to the inert nature of Au^0 (Supporting Information, Fig. S6). The improved signal-to-noise ratio of surface-enhanced SWNT Raman-label scattering afforded detection of the protein biomarker carcinoembryonic antigen (CEA) in serum at concentrations as low as 5 fM (1 pg/mL), a nearly three order of magnitude improvement over traditional CEA

measurement techniques (e.g. ELISA). To our knowledge, this is the first time that SERS substrates were formed on protein microarrays by solution phase chemistry.

4. Conclusions

In conclusion, precipitation of cationic Au by ammonium hydroxide appears to be a powerful and general method to generate uniform Au NP seeds onto a wide variety of unmodified or complex substrates. Coupled with selective reduction of Au^{3+} by hydroxylamine, Au/Au films affording surface-enhanced Raman scattering have been generated on unfunctionalized glass, quartz, and SiO_2 , as well as on polymeric, flexible substrates such as poly(vinyl chloride) and poly(dimethylsiloxane). Both seeding and growth conditions of Au/Au films were optimized to yield the greatest SERS effect for the Raman reporter molecule benzenethiol, with enhancement factors of 10^7 observed uniformly and reproducibly. For the first time, SERS active Au films were deposited from solution onto protein bioassays to yield high sensitivity Raman detection of a protein analyte in sandwich assay format. Deposition of Au seeds onto such a broad range of substrates by the methodology presented herein is expected to be widely applicable for the preparation of uniform plasmonic films, as well as other applications requiring uniform gold film growth from the solution phase.

5. Experimental Section

Briefly, gold-on-gold (Au/Au) substrates were produced in the following general manner (see Supporting Information for detailed materials and methods): The substrate of choice was submersed in a solution of chloroauric acid, to which ammonium hydroxide was added at 20 μL / mL (0.6% ammonia), under vigorous agitation for one minute. Following incubation in the seeding solution, the substrate was washed by sequential immersion into two water baths. Immediately following the wash steps, the Au^{3+} seeded substrate was submersed into a solution of 1 mM sodium borohydride at room temp on an orbital shaker. Reduction was allowed to proceed for 5 minutes, followed by two submersions of the substrate in water baths. Au nanoparticle-seeded substrates were moved directly from wash water baths to a 1:1 (by concentration) solution of HAuCl_4 and NH_2OH under vigorous agitation to initiate growth. Growth proceeded at room temperature on an orbital shaker at 100 RPM until obvious development of the film ceased, 15-20 minutes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported in part by the National Institutes of Health (NIH)-National Cancer Institution (NCI) grant R01 CA135109-01 Center for Cancer Nanotechnology Excellence Focused on Therapeutic Response at Stanford, Stanford Bio-X Initiative, and a grant from Ensysce Biosciences.

References

1. Schatz GC, Young MA, Duyne RPV. *Physics*. 2006; 46:19.
2. Nie SM, Emery SR. *Science*. 1997; 275:1102. [PubMed: 9027306]
3. Yonzon CR, Haynes CL, Zhang XY, Walsh JT, Van Duyne RP. *Analytical Chemistry*. 2004; 76:78. [PubMed: 14697035]
4. Cao YWC, Jin RC, Mirkin CA. *Science*. 2002; 297:1536. [PubMed: 12202825]
5. Sylvia JM, Janni JA, Klein JD, Spencer KM. *Analytical chemistry*. 2000; 72:5834. [PubMed: 11128944]

6. Zhou Q, Yang Y, Ni JE, Li ZC, Zhang ZJ. Nano Research. 2010; 3:423.
7. Chen Z, Tabakman SM, Goodwin AP, Kattah MG, Daranciang D, Wang XR, Zhang GY, Li XL, Liu Z, Utz PJ, Jiang KL, Fan SS, Dai HJ. Nature Biotechnology. 2008; 26:1285.
8. Hulteen JC, Duyne VRP. Vacuum Sci Tech A. 1995; 13:1553.
9. Constantino CJL, Lemma T, Antunes PA, Aroca R. Analytical Chemistry. 2001; 73:3674. [PubMed: 11510833]
10. Tao A, Kim F, Hess C, Goldberger J, He RR, Sun YG, Xia YN, Yang PD. Nano Lett. 2003; 3pp: 1229.
11. Freeman RG, Grabar KC, Allison KJ, Bright RM, Davis JA, Guthrie AP, Hommer MB, Jackson MA, Smith PC, Walter DG, Natan MJ. Science. 1995; 267:1629. [PubMed: 17808180]
12. Chumanov G, Sokolov K, Gregory BW, Cotton TM. Journal of Physical Chemistry. 1995; 99:9466.
13. Chaney SB, Shanmukh S, Dluhy RA, Zhao YP. Applied Physics Letters. 2005; 87:031908.
14. Neddersen J, Chumanov G, Cotton TM. Appl Spectrosc. 1993; 47:1959.
15. Jackson JB, Halas NJ. Proceedings of the National Academy of Sciences of the United States of America. 2004; 101:17930. [PubMed: 15608058]
16. Au L, Chen YC, Zhou F, Camargo PHC, Lim B, Li ZY, Ginger DS, Xia YA. Nano Research. 2008; 1:441. [PubMed: 20200595]
17. Ni F, Cotton TM. Analytical Chemistry. 1986; 58:3159. [PubMed: 3813029]
18. Brown KR, Lyon LA, Fox AP, Reiss BD, Natan MJ. Chemistry of Materials. 2000; 12:314.
19. Supriya L, Claus RO. Langmuir. 2004; 20:8870. [PubMed: 15379520]
20. Schuck P, Fromm D, Sundaramurthy a, Kino G, Moerner W. Physical Review Letters. 2005; 94:14.
21. Chau I, Allen MJ, Cunningham D, Norman AR, Brown G, Ford HER, Tebbutt N, Tait D, Hill M, Ross PJ, Oates J. Journal of Clinical Oncology. 2004; 22:1420. [PubMed: 15007086]
22. Somodi F, Borbath I, Hegedus M, Tompos A, Sajó IE, Szegedi A, Rojas S, Fierro JLG, Margitfalvi JL. Applied Catalysis a-General. 2008; 347:216.
23. Bronnum B, Johansen HS, Skibsted LH. Inorganic Chemistry. 1988; 27:1859.
24. Baker BE, Kline NJ, Treado PJ, Natan MJ. Journal of the American Chemical Society. 1996; 118:8721.
25. Felidj N, Aubard J, Levi G, Krenn JR, Hohenau A, Schider G, Leitner A, Aussenegg FR. Applied Physics Letters. 2003; 82:3095.
26. Camden JP, Dieringer JA, Wang YM, Masiello DJ, Marks LD, Schatz GC, Van Duyne RP. Journal of the American Chemical Society. 2008; 130:12616. [PubMed: 18761451]
27. Garcia-Vidal FJ, Pendry JB. Physical Review Letters. 1996; 77:1163. [PubMed: 10063006]
28. Liu Z, Tabakman S, Sherlock S, Li XL, Chen Z, Jiang KL, Fan SS, Dai HJ. Nano Research. 2010; 3:222. [PubMed: 21442006]
29. Liu Z, Tabakman S, Welsher K, Dai HJ. Nano Research. 2009; 2:85. [PubMed: 20174481]
30. Liu ZA, Li XL, Tabakman SM, Jiang KL, Fan SS, Dai HJ. Journal of the American Chemical Society. 2008; 130:13540. [PubMed: 18803379]

Figure 1.

Diagram of gold-on-gold film (Au/Au) formation on (a) glass. Deposition-precipitation of chloroauric acid with ammonium hydroxide, followed by reduction rapidly yields densely packed Au⁰ nanoparticles on a variety of substrates, as observed by (b) tapping mode AFM. Subsequent reduction of additional chloroauric acid by hydroxylamine “grows” the gold nanoparticles into (c) a uniform gold-on-gold film with nanoscale structure observed by (d) SEM imaging revealed gold nano-islands separated by small gaps. The entire process is complete in less than 30 minutes on a variety of unmodified substrates.

Figure 2.

Gold seeding density and uniformity is dependent on chloroauric acid concentration. **a)** Atomic force microscopy topography images of Au⁰ seeds deposited on SiO₂ demonstrating the dependence on seeding density with HAuCl₄ concentration. **b)** Digital photographs of Au/Au films grown on glass at the indicated HAuCl₄ seeding concentration, following reduction and growth at a fixed HAuCl₄ and NH₂OH concentration of 500 μM. **c)** SEM images of the films in **(b)**.

Figure 3.

Au/Au film growth conditions were varied to optimize the resulting SERS properties. **a)** Absorbance spectra of Au/Au films seeded at a fixed concentration of 5 mM HAuCl_4 , and grown at the indicated concentrations of HAuCl_4 and NH_2OH . The Au/Au film plasmon resonance red-shifts and its optical density increases with increasing growth concentration. **b)** SEM images of Au/Au films in **(a)**, seeded at a fixed concentration (5 mM HAuCl_4) and grown at HAuCl_4 and NH_2OH concentrations of (i) 100 μM , (ii) 250 μM , (iii) 500 μM , (iv) 1000 μM , and (v) 2000 μM . Note that the gold islands coalesce into a continuous film at a growth concentration of 2000 μM . **c)** Benzenethiol Raman scattering spectra of self-assembled monolayers formed on the various Au/Au films in **(a)**, shown with a benzenethiol spectrum obtained from a silver mirror (Tollen's) substrate.^[17] **d)** Estimated enhancement factors (See Supporting Methods for details) for benzenethiol monolayers on the various Au/Au films shown in **(a)**. A decrease in SERS intensity is observed when the gold islands coalesce into a continuous film at a growth concentration of 2000 μM .

Figure 4.

Formation of Au/Au films on unmodified polymeric substrates. **a)** UV/Vis/NIR absorbance spectrum of an Au/Au film grown on an unmodified poly(vinyl chloride) (PVC) coverslip. **b)** Digital photograph of an Au/Au film grown on a PVC coverslip, which retains its flexibility even after Au/Au coating (inset). **c)** SEM image of Au/Au film from **(b)**. **d)** Digital photograph of Au/Au film grown on poly(dimethylsiloxane) (PDMS). **e)** SEM image of Au/Au film from **(d)**. **f)** Surface-enhanced Raman scattering spectra of benzenethiol chemisorbed onto Au/Au films grown on PDMS, PVC, and glass, showing SERS enhancements of approximately the same order of magnitude for each substrate.

Figure 5.

Au/Au film deposition onto a SERS-based bioassay yields high sensitivity detection of analyte protein. **(a)** Schematic of a microarray immunoassay performed on glass, with Au/Au film deposited atop the bioassay substrate, from the solution phase, to provide SERS of SWNT Raman labels. **(b)** Raman scattering spectrum of SWNT G-band with and without Au/Au film deposition demonstrating the SERS effect. Deposition of the Au/Au film from solution onto the SWNT-labeled protein assay yields a G-band enhancement of ~ 200-fold. Inset shows a digital photograph of protein assay slides before (left) and after (right) Au/Au film growth. **(c)** Observed G-band Raman intensity for 1 nM – 1 fM CEA spiked into dilute serum with (black) and without (red) Au/Au SERS, along with blank control ("0M"). Error bars represent the standard deviation of the means of 9 duplicate microarray spots for each CEA concentration (see Supporting Information for details).