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2017

Lee, M. R., Lee, H. K., Yang, Y., Koh, C. S. L., Lay, C. L., Lee, Y. H., ... Ling, X. Y. (2017). Direct metal writing and precise positioning of gold nanoparticles within microfluidic channels for SERS sensing of gaseous analytes. *ACS Applied Materials & Interfaces*, 9(45), 39584-39593. doi:10.1021/acsami.7b11649

<https://hdl.handle.net/10356/143409>

<https://doi.org/10.1021/acsami.7b11649>

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Direct Metal Writing and Precise Positioning of Gold Nanoparticles within Microfluidic Channels for SERS Sensing of Gaseous Analytes

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Keywords: direct metal writing, two-photon lithography, gold nanoparticles, gas analyte sensing, surface-enhanced Raman scattering

ABSTRACT

We demonstrate a one-step precise direct metal writing of densely packed Au nanoparticles (AuNP) well-defined patterns with tunable physical and optical properties. We achieve this using two-photon lithography from Au precursor comprising poly(vinylpyrrolidone) (PVP) and ethylene glycol (EG), where EG promotes higher reduction rate of Au^{3+} via polyol reduction. Hence, clusters of monodisperse AuNP are generated along raster scanning of the laser, forming high particle density, well-defined structures. By varying PVP concentration, we tune AuNP size from 27.3 – 65.0 nm and density from 172 – 965 particles/ μm^2 , corresponding to surface roughness of 12.9 – 67.1 nm, which is important for surface-based applications such as surface-enhanced Raman scattering (SERS). We find the microstructures exhibit SERS enhancement factor $> 10^5$, and demonstrate remote writing of well-defined Au microstructures within a microfluidic channel for SERS detection of gaseous molecules. We showcase in-situ SERS monitoring of gaseous 4-MBT and real-time detection of multiple small gaseous species with no specific affinity to Au. This one-step, laser-induced fabrication of AuNP microstructures ignites a plethora of possibilities to position desired patterns directly onto or within most surfaces for future creation of multi-functional lab-on-a-chip devices.

INTRODUCTION

Metal nanoparticles exhibit size-dependent optical, catalytic and electronic properties¹⁻³ which are integral to the creation of new materials and technologies such as biosensors, photonic switches and quantum dots.⁴⁻⁶ To fully harvest the potential of these nanoparticles to create functional nano- and micro-devices, they must first be assembled precisely into desired patterns and structures.⁷⁻⁸ Direct metal writing using two-photon lithography emerges as a promising technique to simultaneously fabricate and direct metallic nanostructures easily into predefined designs with high precision due to its high resolution. Consequently, these metallic nanostructures can be selectively imprinted onto designated areas easily to form microreactors, 3D structures,⁹⁻¹⁰ patterned surface-enhanced Raman scattering (SERS) substrates,¹¹ and electrical micropatterns.¹² The most common approach in two-photon laser direct metal writing is the formation of a metal-polymer composite by photoreduction of a metallic precursor within a polymer matrix,¹³⁻¹⁴ where the polymer functions as a physical support to stabilize fabricated nanostructures. However, the presence of an insulating polymer results in decreased metal loading capacity (< 50 % wt)¹⁵ and lowered conductivity,^{9, 13} reducing the efficiency of fabricated nanostructures in both nanoelectronics and photonics.

To resolve this issue, metallic nanostructures without a need for polymeric matrix have also been fabricated using direct metal writing recently.¹⁶ This is achieved by adding a surfactant into the metal precursor solution,¹⁷ to stabilize and facilitate nucleation of reduced metal atoms to form metal NPs. A photoinitiator may also be added to further promote NP synthesis and as a result, individual or interconnected metallic nanostructures can be generated.¹⁸ Unlike the polymer in the metal-polymer composite, the surfactant and photoinitiator on the NPs can be easily removed, making this technique promising for the fabrication of catalytic microreactors¹⁹ and designed SERS substrates for sensing and monitoring of reaction processes.²⁰⁻²¹ However, many of these structures exhibit either sparsely distributed NPs²²⁻²⁴ (from 90 - 360 particles/ μm^2)²⁵ as the surfactant does not contribute to the photoreduction process, or densely packed but polydisperse NPs.¹⁵⁻¹⁶ While the effect of the photoinitiator has been demonstrated to create densely packed NPs, these NPs are generally

inhomogeneous and random¹⁸ owing to the uncontrolled radical photoreduction. Currently, the control over physical properties of fabricated structures, such as nanoparticle size and density is very poor. These properties are especially critical for tuning the plasmonic properties of the structures and maximizing active surface area²⁶⁻²⁸ to pave the way for the future creation of 3D photonic nanocrystals and optical antennas.²⁹⁻³⁰

Here, we demonstrate surfactant-based, in-situ two-photon polyol photoreduction of Au³⁺ into monodisperse, densely packed AuNP microstructures with tunable nanoparticle size, density and plasmonic properties. Our strategy aims to increase AuNP formation by addition of a viscous reducing agent, ethylene glycol (EG) to achieve precise fabrication of well-defined, densely packed AuNP microstructures with > 90 % increase in particle density compared to reported works.²⁵ By varying surfactant concentration in the precursor solution, we are able to tune sizes of individual AuNP from (27 – 66) nm, particle density from (172 – 965) particles/ μm^2 and consequently, surface roughness of the microstructure from (13 – 67) nm. Owing to the unique direct metal writing technique to form AuNP microstructures, we are also able to perform metal writing even within enclosed spaces, such as a flat microfluidic channel. The structures are stable and exhibit strong SERS performance where enhancement factor of the best performing platform is $> 10^5$, and can function as indicators to monitor and detect diffusing gaseous analytes within 2 minutes. We further showcase the capability of the microstructures as a recyclable SERS gas detection channel to detect sequentially gaseous ethanol and acetone. Such precise positioning of as-designed AuNP microstructures ignites multiple possibilities to position desired patterns directly onto any transparent surface, even within enclosed spaces for the fabrication of next-generation multi-functional lab-on-a-chip devices.

RESULTS AND DISCUSSION

Direct Metal Writing and Characterization of AuNP Microstructures. We use two-photon lithography to achieve the in-situ, nanoscale polyol photoreduction of Au^{3+} into monodisperse, quasi-spherical Au nanoparticles (AuNP), where densely packed and well-defined AuNP microstructures are precisely formed via the raster scanning of the laser. To begin, we deposit Au precursor solution containing gold(III) chloride (HAuCl_4), poly(vinylpyrrolidone) (PVP, 10 % v/v), and ethylene glycol (EG) on a clean glass substrate functionalized with (3-aminopropyl)triethoxysilane (APTES). Our direct metal writing system is equipped with a femtosecond two-photon laser centered at 780 nm wavelength, which is raster-scanned at an optimized speed (1 $\mu\text{m/s}$) and power (6 mW) (Figure S1) along a predefined x - y plane to reduce Au^{3+} to form precisely-positioned Au microstructures in the form of a word “METAL”, demonstrating the versatility of our lithography technique. We subsequently wash the as-written substrate with water, 2-propanol and 0.5 M potassium iodide (KI) solution to remove excess reactants and PVP.³¹

The dark field and scanning electron microscopy (SEM) images show the written structures to be well-defined, with the structures exhibiting a yellow scattering color under the optical microscope (**Figure 1A, B**). The linewidth of written structures is measured at $(2.1 \pm 0.4) \mu\text{m}$ (Figure 1C). Upon closer examination of SEM images using an image analysis software, each individual letter is revealed to comprise a close-packed cluster of NPs (Figure 1D), with average diameter of $(42.9 \pm 6.7) \text{ nm}$, and particle density of $(600 \pm 102) \text{ particles}/\mu\text{m}^2$ (Figure 1E), giving the microstructures an overall “rough” appearance. We highlight that the amino-terminated glass surface is important to enhance the stability of as-fabricated microstructures to the glass substrate via a weak covalent bond between the amino group and gold.³²⁻³³ In contrast, structures written on non-functionalized glass substrates are usually poorly retained on the substrate surface (Figure S2).

The energy dispersive X-ray (EDX) analysis confirms that the written structures are primarily composed of Au, with the background comprising mainly Si and O which is typical of a glass substrate

(Figure S3). We perform X-ray photoelectron spectroscopy (XPS) analysis to further verify the surface composition in terms of both elements present and the oxidation states of the clustered NP lines. Due to the large scanning area (1 cm²) and high density of AuNP required to perform XPS (and later X-ray diffraction, XRD), we simulate the photoreduction reaction using a solar simulator³⁴ to generate AuNP in bulk for the analyses (Figure S4, S5A). The high-resolution Au 4f XPS spectrum of the AuNP reveals only two sharp peaks at 87.0 and 83.3 eV, where the full-width half-maximum (FWHM) of each of these peaks is approximately 1.0. The difference between the 2 peaks is 3.7 eV, which is characteristic of the Au⁰ 4f doublet spaced at 3.65 eV (Figure 1F, Figure S5B), indicating that the fabricated AuNP composes of Au⁰. Furthermore, the XPS reveals the absence of N on the structures, and the presence of I,³⁵⁻³⁶ indicating that most of the surface N-containing PVP is successfully removed with KI (Figure S5C, D). This is a key advantage of surfactant-based Au microstructure fabrication over polymer-based Au microstructure fabrication, in that while surfactants can be easily removed from the Au structures, the polymer matrix on the other hand, cannot be easily removed without compromising structure integrity. The ability to clean the surface of possibly interfering species is especially important in SERS applications to prevent cross-talking between analyte surface contaminants. The crystallinity of the fabricated AuNP is also determined using (XRD). We observe five sharp diffraction peaks at 38.2 °, 44.4 °, 64.6 °, 77.5 ° and 81.7 ° in the 2 θ range of 20–85 ° (Figure 1G) in the XRD spectrum of the AuNP. They are indexed to the 111, 200, 220, 311 and 222 reflections of fcc phase of metallic gold (JCPDS, card No. 04-0784), attesting the high crystallinity of the AuNP.

Our direct metal writing method using EG and PVP is highly robust in achieving quasi-spherical, crystalline AuNP that can be precisely directed into well-defined and densely packed microstructures, which has not been demonstrated previously. The fabrication of highly packed AuNP microstructures arises from a laser-induced polyol reduction of Au³⁺ to form Au⁰. At the laser focal plane, Au³⁺ ions are excited³⁷⁻⁴⁰ and subsequently reduced by EG in a polyol reduction process to form Au⁰ (Figure 1H). The presence of EG as a reducing agent promotes a higher reduction rate of Au³⁺ as compared to

when no reducing agent is added. Furthermore, the high viscosity of EG serves to reduce the diffusion rate of Au atoms, leading to slower growth rates⁴¹⁻⁴² and hence the formation of monodisperse, mainly quasi-spherical AuNP.⁴³ On the other hand, PVP molecules function as a capping agent by competing with Au⁰ atoms for the surface of nucleated AuNP through charge-transfer type interactions⁴⁴ between Au and the carbonyl group in PVP,⁴⁵⁻⁴⁶ to prevent further metallic growth and promote the formation of AuNP. The steric hindrance imposed by the bulky PVP molecule on PVP-capped AuNP further prevents aggregation, allowing dense clusters of stable, monodispersed AuNP to form within written microstructures.

Effect of PVP on Au Structures. We systematically tune the size of AuNP by varying the PVP concentration in the precursor solutions. Keeping the concentrations of Au(III) salt and EG constant, we prepare Au precursor solutions with increasing PVP concentrations of 0 %, 5 %, 10 %, 20 %, and 30 % v/v (termed P0, P05, P10, P20, and P30, respectively) and fabricate horizontal lines using the precursor solutions of varied PVP concentrations (**Figure 2A**). SEM images demonstrate that NP size decreases when PVP concentration increases from 0 % to 30 % (Figure 2B, C, S6). The average NP sizes are measured at (65.0 ± 15.9) nm, (52.3 ± 22.1) nm, (43.0 ± 6.7) nm, (37.4 ± 5.4) nm, and (27.3 ± 4.1) nm for P0, P05, P10, P20 and P30, respectively (Figure 2D). Beyond a PVP concentration of 30 %, the precursor solution is saturated and difficult to dissolve. The poor uniformity of the precursor solution results in the formation of NPs of inhomogeneous sizes (Figure S7), hence, no further increase in PVP concentration to tune nanoparticle size is attempted.

The particle density also increases with increasing PVP concentration, from (172 ± 4) particles/ μm^2 , (600 ± 102) particles/ μm^2 , (795 ± 40) particles/ μm^2 , (892 ± 111) particles/ μm^2 , and (965 ± 65) particles/ μm^2 for P0, P05, P10, P20 and P30, respectively (Figure 2E). The particle density of our AuNP microstructures is higher than previously reported results of 90 - 360 particles/ μm^2 , which we attribute to the presence of EG in our precursor solution that promotes higher reduction rate to Au⁰. In addition, the root-mean-squared surface roughness (Figure S8) of the microstructures measured using

atomic force microscopy (AFM) also increases with increasing PVP concentration, with P0 possessing the “smoothest” surface at (12.5 ± 2.9) nm, followed by P05, P10, P20 and P30 with decreasing surface roughness of (45.2 ± 3.6) nm, (47.9 ± 7.8) nm, (58.0 ± 10.3) nm, and (67.1 ± 16.6) nm, respectively (Figure 2F).

We note that while P05 - P30 microstructures mainly compose of nanoparticles, P0 microstructures are predominantly composed of fused, flat “smooth” Au structure with only the edges composing of sparse AuNP (Figure S9). As a result, P0 microstructures are wider ((6.0 ± 1.3) μ m) and flatter ((83.6 ± 37.9) nm) as compared to P05 - P30 AuNP microstructures, which possess an average width of ~ 2.0 μ m and average height of ~ 286 nm (Figure S10). The fused, “smooth” Au surface is a consequence of the absence of PVP in P0, which results in the aggregation and growth of photoreduced Au atoms with no size control to form large areas of fused, bulk Au structures instead of NP clusters like in P05 - P30.⁴⁷⁻⁴⁸ Such “smooth” surface is undesirable especially for SERS applications, where surface roughness is an important prerequisite for electromagnetic field enhancement.⁴⁹⁻⁵⁰ These results highlight the importance of PVP in controlling the overall morphology of Au microstructures, by preventing particle agglomeration and promoting the formation of AuNP within written lines.⁵¹⁻⁵² The increase in NP density and surface roughness with increasing PVP concentration can be attributed to the higher capping efficiency of the fabricated AuNP, leading to the formation of smaller AuNP,⁵³ hence enabling larger numbers of AuNP clusters to be generated within the same laser volume resulting in higher surface roughness.

Optical Properties of Au Structures. Dark field images of the respective structures demonstrate that under all conditions, written lines are well-defined (Figure S11). We observe that P05 - P30 microstructures appear yellow under the dark field but P0 microstructures appear orange. The extinction spectrum of the P10 microstructures exhibit a peak at 570 nm which corresponds to the localized surface plasmon resonance (LSPR) of AuNP,⁵⁴ indicating that the structures are plasmonically active within the visible-light range of 400 - 700 nm. On the other hand, the extinction

spectrum of P0 microstructures also exhibits a weak peak at 570 nm, however, the spectrum is broadened which is likely due to the fused Au structure, and are hence only weakly plasmonically active (Figure S12).

As AuNP clustering in the microstructures potentially leads to increased number of plasmonic hot-spots for SERS enhancement, we demonstrate that SERS properties of the AuNP microstructures can be tuned by modulating the sizes of individual AuNP. We begin by fabricating square and circle structures using P0 - P30 Au precursor solutions. The square and circle structures represent lines and arcs, respectively, which is the basis of all 2D designs. The fabricated ring and square microstructures are well-defined and uniformly written, where the diameter of each ring is 30 μm while the squares are written with lengths of 25 μm (**Figure 3A(i), (ii)**). We functionalize the microstructures with 4-MBT via ligand exchange with PVP, as molecular probe for the SERS measurements. Raman spectra of the written microstructures show typical fingerprint of 4-MBT with clear identification of characteristic peaks at 1079 cm^{-1} and 1563 cm^{-1} . The peak at 1079 cm^{-1} is attributed to a combination of the phenyl ring-breathing mode, C-H in-plane bending, and C-S stretching respectively,⁵⁵ whereas the peak at 1563 cm^{-1} arises from the phenyl stretching motion (Figure S13, Table S1).⁵⁶

The Raman image lights up homogeneously on the ring and square structures when 1079 cm^{-1} is selected, clearly indicating largely consistent SERS intensities across fabricated microstructures (Figure 3B). A cross-sectional SERS profile plot of the P10 square microstructure further demonstrates that the strongest SERS signals are only recorded from the microstructures at (500 ± 128) counts/s, whereas in areas away from the microstructures, SERS intensities average around (6.4 ± 2.6) counts/s (Figure 3C). The average SERS intensity at 1079 cm^{-1} increases from P0 to P30, where P0 exhibits both the lowest and most inhomogeneous SERS intensity of (62 ± 75) counts due to the presence of “smooth” fused areas and sparse AuNP (Figure S14), followed by P05 $((492 \pm 258)$ counts), P10 $((1084 \pm 465)$ counts), P20 $((1150 \pm 537)$ counts), and finally P30, which exhibits the highest SERS intensity at (1453 ± 446) counts (Figure 3D). The enhancement factor (EF) of P0 to P30 substrates

based on the peak at 1079 cm^{-1} are calculated at $\sim 5.6 \times 10^4$, $\sim 9.5 \times 10^4$, $\sim 2.1 \times 10^5$, 2.2×10^5 , and 2.8×10^5 for fabricated microstructures P0, P05, P10, P20, and P30, respectively (Figure 3E), comparable to reported literature values of $10^4 - 10^5$ for similar AuNP sizes using wet chemistry methods.⁵⁷⁻⁵⁹ Generally, the SERS EF increases with PVP concentration. This is due to a greater extent of AuNP clustering as observed from the increasing particle densities and RMS roughness measurements when PVP concentration increases from P0 to P30 (Figure S15), indicating that we are able to achieve tunable SERS performance using our fabrication technique. This result highlights the strong potential of our fabricated clustered AuNP to function as high performing SERS substrates. The tunable, strong SERS enhancement, coupled with the ability to precisely position the AuNP on specific areas of the substrate puts this technique at a strong advantage for the future creation of tailored SERS platforms.

Patterned Microfluidic Channel for In-Situ SERS Detection of Flowing Analytes. The ability to perform one-step, in-situ polyol photoreduction of Au^{3+} into densely packed, well-defined AuNP microstructures ignites a plethora of possibilities, to position desired patterns directly onto or within any transparent surface. We demonstrate the versatility of our fabrication technique via the remote patterning of pre-designed AuNP microstructures *within* a microfluidic channel, to create a SERS detector for gaseous analyte molecules. While gases tend to diffuse and expand indefinitely in air, causing them to become diluted and hence difficult to detect using substrate-based platforms in air, a microfluidic SERS channel is able to confine gaseous molecules within the channel, bringing the molecules close to the SERS platform to be easily detected. Using this channel, we demonstrate that we are able to detect in real-time, the molecular fingerprints of small gaseous molecules both with, and without specific affinity to Au.

To begin, we fabricate a series of P10 Au microstructures within a flat, APTES-functionalized microfluidic channel ($l \times w \times h = 5.0\text{ cm} \times 0.6\text{ cm} \times 0.1\text{ mm}$) (**Figure 4A, B**). Although P30 microstructures exhibit the highest SERS EF, bubbles generated during lithography are observed to linger for longer periods, possibly due to a higher solution viscosity at high PVP concentration. Hence,

we select P10 to allow for easier dissipation of generated bubbles within the microfluidic channel with minimum compromise on SERS EF of the microstructures. We draw the precursor solution into the channel via capillary action and focus the laser on the inner wall to simultaneously reduce and drive the formation of AuNP into pre-defined numbers within the microfluidic channel. Following which, the channel is washed with KI to remove PVP to minimize interference of SERS signals between PVP and analyte (Figure S16). Such precise formation of well-defined metallic Au structures within a microfluidic channel is otherwise not possible using pre-synthesized AuNP in solution.

We first demonstrate the real-time, in-situ monitoring of gaseous flow using vapor 4-MBT as an analyte. We warm a solution of 0.5 M of 4-MBT in ethanol in a glass vial and begin the SERS measurement (Figure S17). Within 2 minutes, we observe a faint outline of the microstructures in the Raman image, which becomes gradually more defined to reveal the written micro-pattern of “5 6 7 8 9”, indicating that 4-MBT molecules have diffused to the Au microstructure at 15 minutes (Figure 4C). A plot of SERS intensity against time demonstrates that SERS signal intensity of 4-MBT increases linearly with time (Figure 4D). At the 15 minute mark, the SERS signal intensity appears to be at a maximum, beyond which, we observe no change to the intensity (Figure 4E) or the Raman image, indicating that the microstructures are likely saturated with 4-MBT within 15 minutes.

To showcase the versatility of our microfluidic SERS channel as a universal sensor suitable for the detection of molecules with low Raman cross-sections,⁶⁰⁻⁶¹ we further extend the SERS sensor to the detection of gaseous acetone followed by ethanol, both of which are common, small molecules that have no specific interactions with the AuNP microstructures. Because these molecules are unable to form specific bonds with Au, we heat each solvent for 15 minutes to allow ample time for the gaseous solvent to diffuse to the SERS microfluidic channel and potentially adsorb onto the Au microstructures for detection. We begin with acetone detection, followed by flushing with N₂ gas to remove residual solvent before carrying out the detection of gaseous ethanol (Figure 4F). The SERS spectrum of the written AuNP microstructures prior to gaseous acetone injection shows no peaks, demonstrating that

the washing process to remove most of the PVP is successful. Subsequent SERS spectrum of gaseous acetone exhibits distinct Raman peaks at 1164 cm^{-1} and 1531 cm^{-1} , which correspond to C-C-C bending, CH_3 wagging and scissoring modes of acetone, respectively (Figure S18, Table S2). Raman measurement performed after flushing the channel with N_2 gas for 30 minutes exhibit no Raman peaks, demonstrating that most of the acetone has been successfully removed from the SERS platform. We repeat the detection process with ethanol and observe SERS peaks at 1278 cm^{-1} , 1338 cm^{-1} , 1473 cm^{-1} and 1569 cm^{-1} , which correspond to the OH bending, CH_2 twisting, CH_3 wagging and CH_2 wagging modes of ethanol, respectively (Table S3). By switching between solvents, we demonstrate the versatility of a single microfluidic channel as such to potentially detect different types of small gaseous molecules that have no specific affinity to the AuNP microstructures (Figure 4G).

The SERS microfluidic channel can also potentially be extended to the detection of other specific gaseous molecules or airborne particulates through functionalizing the Au nanoparticle microstructures with other compounds to impart molecular recognition ability to the substrate. This allows the patterns on these substrates to function as indicators to identify the presence of gases through specific binding interactions, such as o-phenylenediamine for the detection of nitric oxide gas,⁶² or APTES for the detection of airborne trinitrotoluene particulates.⁶³

However, we emphasize that the main focus of our manuscript is to showcase a new approach for direct metal writing rather than the creation of a SERS analytical platform for ultratrace detection. The novelty of our new direct metal writing approach lies in an unprecedented bottom-up, in-situ two-photon polyol photoreduction of Au^{3+} into well-defined and tunable AuNP microstructures comprising densely packed, stable AuNP. This strategy effectively eliminates the need for a polymer matrix to assist in metal nanoparticle formation, as well as overcome limitation of sparse distribution and polydisperse AuNP size in previous reports. Hence, while the AuNP microstructures show great promise as tunable SERS platforms, the SERS application is just a simple proof-of-concept as the ability to create precisely-positioned Au patterns can also be potentially extended to other applications

such as microelectrical circuit boards, fabrication and study of tunable, non-linear metamaterials such as split ring resonators which exhibit negative refractive index in the visible light region, creation of localized photothermal microreactor platforms and micro-incubators by selectively imprinting Au microstructures within enclosed spaces for in-vitro live cell studies, where the ability to precisely position AuNP creates the opportunity for region-specific photothermal experiments even within a single cell.^{59, 64-65}

CONCLUSION

In this work, we have demonstrated the ability to create well-defined AuNP microstructures via two-photon laser direct metal writing, with tunable properties such as NP size, particle density, surface roughness and subsequently, plasmonic properties through modulating the PVP concentration in the precursor solution. We find that a precursor solution with 30 % PVP concentration generates AuNP microstructures with the smallest AuNP sizes at (27.3 ± 4.1) nm, highest particle density at (965 ± 65) particles/ μm^2 , highest surface roughness at (67.1 ± 16.6) nm and strongest SERS EF at 2.8×10^5 . The simple fabrication process coupled with the flexibility in design also allowed us to easily create a reusable, patterned SERS microfluidic channel for the detection and in-situ monitoring of simple, gaseous analyte molecules as proof-of-concept. Most importantly, as a one-step laser induced nanoparticle fabrication with precise structure positioning ability, this technique can also potentially be extended to the direct writing of other metals such as Pt, Ag and Cu, and holds great promise for the creation of photothermal microreactor platforms, tunable metamaterials, as well as split-ring resonators for transformation optics studies. Our strategy for direct metal writing could also be coupled with an upright laser system for the writing of 3D metallic structures by minimizing laser penetration issues across initially-formed Au structures when using an inverted laser system.

METHODS

Materials. Poly(vinyl pyrrolidone) (PVP, $M_w = 10\,000$ g/mol), ethylene glycol (EG, $\geq 99.8\%$), gold(III) chloride trihydrate (HAuCl_4 , $\geq 99.9\%$), , 4-methylbenzenethiol (4-MBT, 98%) and (3-

aminopropyl)triethoxysilane (APTES, $\geq 99.9\%$), were purchased from Sigma-Aldrich. Potassium iodide and ethanol (ACS, ISO, Reag.) were purchased from EMSURE. Hydrochloric acid (HCl, 37 %) was purchased from VWR Chemicals. Acetone (AR grade) was purchased from Tedia Chemicals. 2-propanol (Certified ACS) was purchased from Fisher Scientific. All chemicals were used without further purification. Milli-Q water ($> 18.0 \text{ M}\Omega \text{ cm}$) was purified with a Sartorius arium611 UV ultrapure water system.

Preparation of Au precursor solutions. To a solution of 100 μL of EG and 20 μL of 600 mM HAuCl_4 , 0 μL , 10 μL , 20 μL , 40 μL , 60 μL and 80 μL of PVP solution (1 g in 50 mL H_2O) are added, and the mixtures are topped up with ultrapure water to 200 μL to make the precursor solutions of 0 %, 5 %, 10 %, 20 %, 30 % and 40 % (v/v) PVP respectively. The mixtures are then vortexed and sonicated at room temperature until they are fully dissolved to form a clear yellow solution. It is observed that at a PVP concentration of 40 %, the precursor solution turns cloudy, possibly from oversaturation of PVP, and needs a longer time (~ 1 hour) to dissolve completely. Post lithography, the as-written substrate is subsequently washed with water, 2-propanol and 0.5 M potassium iodide (KI) solution (dissolved in a binary solvent mixture of 1:1 water to ethanol) to remove excess reactants and PVP. The surface N-containing PVP is removed through ligand exchange with I^- ions, where I^- binds to Au through charge-transfer interactions.³⁵

Functionalization of glass surfaces for lithography. Glass cover slips (Deckglasser cover glass, 22 mm $\times$ 22 mm, with thickness of 0.13 – 0.16 mm), and flat microfluidic tubes ($l \times w \times h = 5.0 \text{ cm} \times 0.6 \text{ cm} \times 0.1 \text{ mm}$, VitroTubes^{TM}, VitroCom, with thickness of 0.035 mm) are functionalized for 5 minutes in 2 % v/v APTES in ethanol and then rinsed with a 50 % water/ethanol mixture. The substrates are then blown dry with nitrogen gas and left to dry overnight in a 65 °C oven.

Lithography of Au microstructures. Lithography of the Au microstructures (P0 - P40) were carried out by the Nanoscribe® Photonic Professional within a liquid precursor. 100 μL of the respective Au precursor solutions (P0 - P40) are deposited on the functionalised glass substrates. The system is equipped with an erbium doped, femtosecond laser source, with a center wavelength of 780 nm, repetition rate of 80 MHz and pulse length of 150 fs. Hence, the power per pulse, termed the “peak

power” of the two-photon laser operating at 780 nm wavelength, with 150 fs pulse width and at a repetition rate of 80 MHz is 500 W, and the corresponding energy per pulse is 8×10^{-11} J. The focus plane is automatically located by the system through detecting a change in refractive index between glass cover slip and the precursor solution. The stage is systematically moved upwards in a stepwise manner towards the glass surface until a difference in refractive index is detected. Consequently, this process is highly tolerant with an error of ≤ 100 nm. Structures for fabrication were designed using a CAD software, 3ds Max®. Parameters of the structures were defined by the DeScribe software. All structures were written with 6 mW laser power and scan speed of 1 $\mu\text{m/s}$ to achieve densely packed AuNP microstructures (Figure S1). Objective lens used is a 100 $\times$ oil immersion objective, N.A. 1.4, with working distance of 0.17 mm, parfocal length is 45.06 mm, and field of view of 25 mm. The focal depth of the laser spot is 340 μm , and the focal volume is 3.77×10^7 nm^3 .

During the lithography, small bubbles are observed as a result of absorption by fabricated Au nanoparticles. However, it is observed that the bubbles dissipate quickly upon generation, and hence generally do not compromise the stability and appearance of the written microstructures. Currently, we are unable to write vertically in the z-direction because our two-photon lithography system employs an inverted laser configuration which requires a laser-transparent medium to create 3D structures. In our work, the fabricated Au microstructures are opaque at the two-photon laser operating wavelength of 780 nm. Consequently, we observe that the two-photon laser is unable to penetrate the initially-formed Au structures to write 3D structures of varying thickness.

Fabrication of Au nanoparticles in bulk for XPS and XRD analyses. 100 μL of P10 Au precursor solution was deposited onto a glass substrate, and exposed to UV-light from a 300 W Xenon lamp Solar Simulator (Newport Corporation, Model: 69911, $\lambda = 200 - 420$ nm) for 15 minutes at ambient conditions. The solution is observed to decolorize and turn dark purple. The dark purple solution is washed to remove excess resist and redispersed in ethanol for characterization.

Functionalization of Au microstructures for SERS measurements. We perform a direct ligand exchange with 4-methylbenzenethiol (4-MBT). The structures are immersed in 15 mM of 4-MBT solution for 24 hours to ensure ample time for functionalization. We select 4-MBT as a Raman probe

due to the strong coordination bond between the thiol group and metal, which favors the formation of a 4-MBT self-assembled monolayer (SAM) on the gold surface. This in turn allows for a more accurate calculation of the number of molecules on the surface of assembled Au nanoparticles contributing to the SERS response.

Fabrication of Au microstructures inside microfluidic tube. The P10 Au precursor solution is drawn up the microfluidic tube via capillary action for lithography, and the excess is removed by draining the liquid out and rinsing the tube with sufficient water and ethanol. Following which, we rinse the microfluidic tubes with 0.5 M KI solution and 1M HCl solution 3 times for 10 minutes each before immersing overnight in 0.5 M KI solution for ligand exchange with PVP on the surface of the written structures.

We select P10 as the precursor solution even though it does not exhibit the strongest SERS performance because we observe that bubbles generated during lithography will linger for longer periods of time in solutions with higher PVP concentration, which is possibly due to a higher solution viscosity. Hence, in order to allow for easier dissipation of generated bubbles in the microfluidic tube but at the same time maximise the SERS capability of the microstructures, we select P10 as the precursor solution.

Microfluidic flow cell setup. 15 mL of acetone (AR grade, 13.6 mol/dm^3) or 12 mL ethanol (AR grade, 17.3 mol/dm^3) or 0.5 M 4-MBT in ethanol is placed in a 20 mL glass vial and the vial is sealed with parafilm and connected through a flexible teflon tubing to the flat microfluidic tube (microfluidic channel). The vial is placed on a heat plate and heated gently to facilitate the evaporation and diffusion of the respective solvents into the SERS microfluidic channel. We begin the experiment with the detection of gaseous acetone. The acetone is gently warmed at 50°C for 15 minutes before performing the SERS measurement. After which, the microfluidic channel is flushed with N_2 gas at a flow rate of 50 sccm for 30 minutes to remove the acetone from the substrate. The SERS detection of ethanol is carried out in a similar fashion by heating the ethanol at 70°C for 15 minutes before taking the SERS measurement. In performing the in-situ monitoring of 4-MBT, the 4-MBT/ethanol mixture is heated

at 60 °C to produce gaseous, 4-MBT vapor molecules. SERS measurements are taken every 2 minutes until no further change to SERS signal intensity or Raman image is observed.

SERS measurements of fabricated structures. Raman x-y imaging was obtained by the Ramantouch microspectrometer (Nanophoton Inc, Osaka, Japan). The wavelength and power of excitation laser was set at 785 nm and 0.39 mW, respectively. The laser power of 0.39 mW refers to the effective power of a single spot when the incident laser beam is split into 400 individual spots during a line-scan SERS measurement. Collectively, the total laser power applied is therefore 156 mW. The excitation laser light was focused into a line on the sample through a cylindrical lens and a Nikon LU Plan objective (50× -, NA 0.55). For each line, the samples were measured with an exposure time of 1 s.

Density Functional Theory (DFT) simulations. The calculation of acetone Raman peaks on Au cluster (10 atom pyramid) was carried out using the B3LYP (default) exchange-correlation functional, as implemented in the Gaussian 09 computational chemistry package, for which the LANL2DZ basis set was employed.

Materials characterization. Scanning electron microscopy (SEM) was done with a JEOL-JSM-7600F microscope, and dark field spectroscopy was done with an Olympus BX51BD microscope. The extinction spectra were obtained using a UV-Vis spectrometer, Cary 60 UV-Vis from Agilent Technologies. Energy dispersive X-ray (EDX) analysis was performed with AZtecEnergy Oxford Instruments. Au microstructure width, AuNP sizes and AuNP density for all samples, P0, P05, P10, P20, P30 were measured using ImageJ. In the case of P0, as the main body of the Au structure is fused while the edges of these structures are composed of distinct nanoparticles, we obtain the average diameter of AuNP in P0 by measuring the size of distinct AuNP formed at the edge. The physical size of AuNP at the edge is representative of those in the main body when we consider that the main body of the Au structures is composed of multiple Au nano-islands fused together. The diameter of the nano-islands is similar to the AuNP formed at the edges (Figure S9). For AuNP size, the histograms were plotted by measuring 100 AuNP to give a representative average diameter and associated standard deviation for accurate comparisons. To determine the particle density (particles/ μm^2), we

count the number of nanoparticles in a $0.5\ \mu\text{m} \times 0.5\ \mu\text{m}$ square region of a SEM image ($120\ 000\times$ magnification) depicting the fabricated Au microstructure lines. We use ImageJ (an image analysis software) to assist in the tagging and counting of nanoparticles to ensure that no nanoparticle is counted twice. To further ensure the accuracy of our data, the average particle density is subsequently calculated from three individual measurement using different SEM images. X-ray photoelectron spectroscopy (XPS) spectra were measured using a Phoibos 100 spectrometer with a monochromatic Mg X-ray radiation source. All XPS spectra were fitted using XPS Peak 4.1 (freeware accessible at <http://www.phy.cuhk.edu.hk/~surface>). Powder XRD patterns were recorded on a Bruker GADDS XRD diffractometer with Cu K_α radiation. Atomic force microscopy (AFM) RMS roughness measurements were carried out using the Bruker Dimension ICON with NanoScope V controller from Bruker. Tapping mode (non-contact mode) image was acquired using silicon probes (Tap300Al-G with 30-nm aluminium reflex coating) from BudgetSensor. Data analysis was carried out using WSxM Scanning Probe Microscopy Software, a free program from Nanotec Electrónica S.L36. To characterize the surface roughness of Au microstructures, we use AFM which is a technique capable of providing the 3D topography images through the tapping of its cantilever on the sample.⁶⁶ Using AFM nanoscope analysis software, the root-mean-square (RMS) surface roughness (Figure S8) is subsequently extracted from these AFM images and denotes the standard deviation of Z-values (height) in a selected area on the microstructure.⁶⁷ For all analysis, we measure the RMS roughness in a $1\ \mu\text{m} \times 1\ \mu\text{m}$ square on fabricated Au microstructure lines, which equate to 99.4 pixels and hence ~ 99 height measurements. We perform such measurement at least 10 times for all samples of Au microstructures to ensure a representative result.

ASSOCIATED CONTENT

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The manuscript was written through contributions of all authors. M.R.L performed the experiments and wrote the manuscript. Y.J.Y., C.S.L.K, C.L.L. contributed to the experimental results. H.K.L., Y.H.L., I.Y.P and X.Y.L. carried out editing of the manuscript. All authors have given approval to the final version of the manuscript.

ACKNOWLEDGEMENTS

X.Y.L. thanks the support from National Research Foundation, Singapore (NRF-NRFF2012-04), Singapore Ministry of Education, Tier 1 (RG21/16) and Tier 2 (MOE2016-T2-1-043), and Nanyang Technological University. M.R.L and C.S.L.K thank the support from Nanyang President's Graduate Scholarship. H.K.L and C.L.L. thank the support from A*STAR graduate scholarship. The authors will also like to thank Lim Poh Chong from IMRE, A*STAR for his help in XRD measurements.

SUPPORTING INFORMATION

Supporting Information Available: miscellaneous information, supplementary figures and calculations. This material is available free of charge via the internet at <http://pubs.acs.org>.

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FIGURES

Figure 1. Fabrication and characterization of Au structures. (A) Dark field image and (B) SEM images of direct laser written Au patterns. (C) A magnified SEM image demonstrates that the written structures are predominantly comprised of nanoparticles, (D, E) with average size distribution of the nanoparticles being $(42.9 \pm 6.7) \text{ nm}$. (F) XPS and (G) XRD spectra of the nanoparticles demonstrate that the structures are made of crystalline Au^0 FCC lattice. (H) The mechanism of direct gold writing in the presence of ethylene glycol and PVP via two-photon lithography to form Au nanoparticles which can be precisely positioned to form desired micropatterns.

Figure 2. (A) SEM image of written Au microstructures. SEM images of written structures using (B) 0 % and (C) 30 % of PVP during the two-photon lithography. (D) Size distribution of nanoparticles, (E) particle density and (F) RMS roughness of fabricated AuNP microstructures with respect to different amount of PVP.

Figure 3. SERS performance of written Au structures P0 - P30. (A) (i - ii) SEM images and (B) the corresponding Raman image showing an example of the P10 Au microstructure written for SERS detection (C) Cross-sectional SERS intensity profile along the red dotted line in the SEM image (inset). (D) The SERS intensity and (E) corresponding SERS enhancement factor of Au structures P0 - P30, which increases with decreasing nanoparticle size, increasing particle density as well as increasing surface roughness.

Figure 4. Writing AuNP microstructures within microfluidic channel for SERS sensing of gaseous analytes. (A) A camera image and (B) dark field image of the microfluidic channel written with Au metal structures; inset: a magnified optical image of the structures written within the tube. The highly versatile lithography technique can be used to easily arrange AuNP structures into desired patterns. (C) In-situ monitoring of the diffusion of gaseous 4-MBT molecules through the microfluidic channel. Time-resolved Raman images demonstrate the gradual saturation of the SERS substrate with time to eventually reveal the design of the substrate. (D) SERS intensity is observed to increase with time and (E) A plot of SERS intensity over time demonstrates the increase in SERS intensity with plateau after 15 min. (F) Schematic illustrating the SERS sensing of various gaseous analytes, including acetone and ethanol, passing through the microfluidic channel. (G) SERS spectra demonstrating the detection of gaseous acetone followed by flushing with nitrogen gas, and then the detection of ethanol using the microfluidic device.

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