

Evolutionary Optimized, Monocrystalline Gold Double Wire Gratings as a Novel SERS Sensing Platform

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Achieving reliable and quantifiable performance in large-area surface-enhanced Raman spectroscopy (SERS) substrates poses a formidable challenge, demanding signal enhancement while ensuring response uniformity and reproducibility. Conventional SERS substrates often made of inhomogeneous materials with random resonator geometries, resulting in multiple or broadened plasmonic resonances, undesired absorptive losses, and uneven field enhancement. These limitations hamper reproducibility, making it difficult to conduct comparative studies with high sensitivity. This study introduces an innovative approach that addresses these challenges by utilizing monocrystalline gold flakes to fabricate well-defined plasmonic double-wire resonators through focused ion-beam lithography. Inspired by biological strategy, the double-wire grating substrate (DWGS) geometry is evolutionarily optimized to maximize the SERS signal by enhancing both excitation and emission processes. The use of monocrystalline material minimizes absorption losses and ensures shape fidelity during nanofabrication. DWGS demonstrates notable reproducibility (RSD = 6.6%), repeatability (RSD = 5.6%), and large-area homogeneity $> 10^4 \mu\text{m}^2$. It provides a SERS enhancement for sub-monolayer coverage detection of 4-Aminothiophenol analyte. Furthermore, DWGS demonstrates reusability, long-term stability on the shelf, and sustained analyte signal stability over time. Validation with diverse analytes, across different states of matter, including biological macromolecules, confirms the sensitive and reproducible nature of DWGSs, thereby establishing them as a promising platform for future sensing applications.

1. Introduction

Spectroscopy with scattered light is invaluable in nearly every aspect of modern life. Raman scattering (RS), in particular,^[1] has emerged as a tool for detecting chemicals and identifying them by their fingerprint-like spectral features, harnessing the very specific molecular vibrations of the studied molecule. However, unaided conventional Raman spectroscopy suffers from low efficiency ($\eta \approx 10^{-6}$),^[2] restricting its application to concentrations greater than 0.01 M.

Plasmonic resonances of metallic nanostructures have been extensively studied and optimized since the discovery of surface-enhanced Raman scattering (SERS).^[3,4] SERS takes advantage of the sub-diffraction light focusing properties of plasmonic resonances at the surface of metallic nanostructures, together with the non-linear nature of RS, leading to a signal enhancement up to 10^8 or even higher.^[5] Nowadays, chemical detection,^[6] medical^[7] and agricultural^[8] diagnostics as well as public safety^[9] are just a few examples of the ubiquitous fields of application where SERS is already established.

SERS substrates rely on three essential factors to achieve enhanced signals together

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with the reliable quantitative and repeatable application: i) Small gaps within resonant plasmonic structures to attain the highest possible electromagnetic field enhancement; ii) Large areas covered with dense and regular arrays of resonators to use every incident photon and capture every molecule in low concentrations; iii) Nanometer-level fabrication precision on these large areas is crucial for accurately analyzing the signal, assessing the amount of analyte, and ensuring reproducibility. Consequently, grating or other long-range resonances can thus be effectively harnessed.

Various approaches have been explored, including bottom-up synthesis of agglomerated resonators (e.g., Refs. [10–12] and many mores in Ref. [4]), top-down fabrication from evaporated gold films,^[13–16] or a combination of both approaches.^[17–19] While these approaches have resulted in effective SERS substrates, they have not fully met all three crucial aspects (i)–(iii). In addition, we want to address a fourth point that is often overlooked: iv) The substrate should exhibit plasmonic near-field enhancement at both the excitation wavelength and the emission wavelength of the analyte,^[20–22] which can be several tens of nanometers separated from each other.^[23,24]

In this work, we have realized SERS substrates fulfilling requirements (i)–(iv) by applying a novel combination of three main ideas. First, we start with wet-chemically grown monocrystalline gold flakes,^[25] a material without lateral grain boundaries, even across large spans of hundreds of micrometers. This enables the highest geometrical control using top-down focused ion-beam (FIB) fabrication and minimizes ohmic losses of the plasmon resonance currents. Second, we employ gratings consisting of two wire plasmonic resonators, which we call double wire grating substrate (DWGS). While large-area grating resonances gather light from expansive regions and provide a first resonance for in-plane scattering,^[26] the gaps of the double wires generate sub-diffraction-limit electrical near-field hotspots at a second resonance, which strongly enhances excitation and re-emission of the Raman-active probe molecules. Finally, we numerically optimize the geometry of the DWGS using particle swarm optimization (PSO). The optimization process happens using the parameters of the planned experiment and is based on a fitness function that ensures maximal SERS sensitivity for two predefined wavelengths.

We numerically and experimentally evaluated not only DWGS but also, for comparison, a single wire grating substrate (SWGS) as well as planar non-structured gold surfaces. All three substrate types were realized using both monocrystalline and polycrystalline materials. Our experimental results show that the monocrystalline DWGS outperforms all other tested SERS substrates in performance, consistently exhibits reproducible large-area enhancement factors up to several 10^6 even with a relatively large gap of 15 nm. This advancement overcomes several technological challenges associated with large-scale applications, including reproducibility, repeatability, large-area homogeneity, and reusability, among others. Finally, we successfully tested the DWGS on eight different use cases, spanning from chemisorbed, physisorbed and even gaseous species to proteins and DNA strains. These findings establish SERS based on DWGSs as an auspicious Raman spectroscopy methodology. The scalability of this approach can be achieved using large electrochemically grown monocrystalline gold layers,^[27] and it can be further extended through fabrication with nano-imprint

lithography.^[28] Additionally, the DWGS can be utilized in new applications, such as serving as electrodes^[29] in electrochemical environments and sensing technology.

2. Results and Discussion

2.1. Theory and Optimization

Stokes Raman scattering (RS) involves the excitation of vibrational modes in an analyte by a photon with higher energy than the vibrational levels. In this process, the photon can be re-emitted inelastically with slightly lower energy, as sketched in **Figure 1A**, after exciting an electron to a virtual energy level. RS is highly inefficient,^[2] occurring alongside the elastic Rayleigh scattering. However, RS scales non-linearly as both excitation and emission rates are proportional to the electromagnetic field intensity at the analyte position, defined as the local density of states (LDOS).^[30] Increasing the LDOS allows for strongly enhances RS. Plasmonic resonances provided by metallic nanostructures do this by concentrating light into sub-diffraction-limited volumes close to their surface. The resulting electromagnetic near-field intensities can surpass diffraction-limited focal fields by several orders of magnitude. This fundamental working principle forms the basis of SERS.

Most SERS substrates utilizing metal plasmons typically display one or more broad resonances that span both the excitation wavelength λ_i and the Raman scattered wavelength λ_s as both involved wavelengths only differ in the small energy amount needed to excite a molecular vibration. This allows to express the SERS efficiency in a first-order approximation to be proportional to $E^4(\lambda_i)$, where E represents the electrical field strength at the location of the molecule to be detected.^[4] On the contrary, a well-designed single-particle plasmonic resonance can exhibit a typical Q-factor of $Q = \frac{\omega_r}{\Delta\omega} = 15$, where ω_r is the resonance frequency and $\Delta\omega$ is the resonance FWHM. For example, when exciting a 4-Aminothiophenol (4-ATP) molecule^[31] with an excitation wavelength $\lambda_i = 633$ nm, one important Raman peak occurs at 1570 cm^{-1} , corresponding to an emission wavelength of $\lambda_s \approx 704$ nm (see **Figure 1A** inset). A plasmonic resonator optimized for excitation with $Q = 15$ exhibits half its intensity at a wavelength of 654 nm. Consequently, the emission enhancement at λ_s of a high Q plasmonic resonator will be only weak. This is why in this study, we introduce a second resonance in the SERS substrate design to enhance both emission and excitation by incorporating a grating of plasmonic resonators.^[26,32] While double gratings for SERS sensing have been previously realized using plasmonic materials,^[33] these designs did not leverage fundamental antenna modes as resonances inspired by the working principle of optical nanoantennas.^[34] Optical nanoantennas possess the ability to efficiently transduce light between nanoscale gaps and far-field radiation via a dipolar mode. Additionally, we established a second resonance in the SERS substrate geometry, differing from the approach of the Crozier group, which introduced the second resonance either through a second particle type^[20] or by coupling to a second, thin metal layer.^[21] We introduce the double wire grating substrate (DWGS) with the cross-section of its elementary cell sketched in **Figure 1B** which has several practical benefits: i) Fabrication via focused ion beam

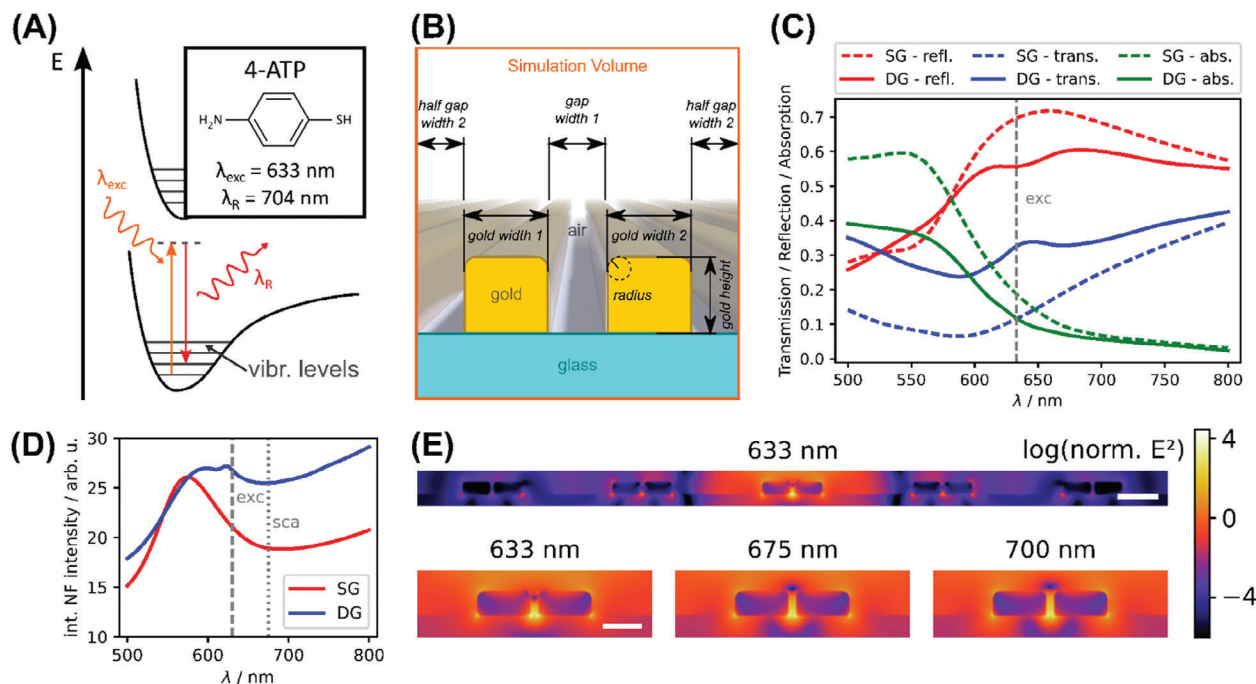


Figure 1. Numerical optimization. A) Simplified energy level scheme of the Stokes–Raman-scattering process. The inset shows the 4-Aminothiophenol (4-ATP) molecule structure, and two possible wavelengths involved in measuring the 1570 cm^{-1} Raman peak. B) The elementary cell cross-section illustrates the potential possible free parameters for the optimization of DWGS. C) Spectra of transmission (blue), reflection (red), and absorption (green) for optimized SWGS (dashed lines) and DWGS (solid lines). D) Integrated near-field intensity enhancement for the optimized SWGS and DWGS. E) The optimized DWGS SERS substrates near-fields are showcased in a vertical cross-section, illuminated from above, at three different wavelengths. It is clearly visible that two different resonances constructively interfere, resulting in high surface field intensities, particularly between and on top of the Au features. Conversely, destructive interference leads to low fields in the central top area of the narrow gap. Scalebar E): top – 200 nm; bottom – 100 nm.

milling or other methods is straightforward^[28] for large-scale high-throughput substrate preparation; ii) The lineal geometry (Figure 1B) makes use of small gaps in one dimension to enhance the optical near-fields but still is extended to the second dimension, which makes the substrate polarization sensitive and also will allow the application of an external electrical potential^[29] for future electrically modulated SERS (E-SERS) experiments; iii) Since we only need to simulate in two dimensions, fast evolutionary optimization is possible. The optimization was performed using particle swarm optimization (PSO), a variation of evolutionary optimization implemented in Lumerical/Ansys FDTD Solutions. The fitness function F – needed to compare different DWGS geometries during the evolution – is designed to capture the physics of SERS: enhance the plasmonic near-fields for both excitation $E^2(\lambda_i)$ and emission $E^2(\lambda_s)$ in a volume V_s . This volume was set to a layer only a few nanometers thick above the gold surface, as the target analyte is typically situated near the substrate surface through physical or chemical absorption. This leads to:

$$F = \int_{V_s} E^2(\lambda_i) \cdot E^2(\lambda_s) d^3r \quad (1)$$

Further details regarding the simulation and optimization are briefly commented on in the experimental section and Supporting Information (Figure S1A,B, Supporting Information). The specific results we chose to investigate further are characterized

by “gold width 1” = 139 nm, “gold width 2” = 136 nm, a small “gap width 1” = 15 nm, and “gap width 2” = 441 nm. Furthermore, optimization was also conducted on a grating with a SWGS in the elementary cell, resulting in a gold width of 80 nm and a gap width of 20 nm. Figure 1C–E presents the simulated DWGS and SWGS properties under broad-band optical excitation. Figure 1C shows far-field spectra, where reflection is proportional to the received signal when detection occurs in the excitation direction due to reciprocity.^[35] The DWGS exhibits a transmission up to $\approx 40\%$ due to its large gaps, which are necessary to hit the grating resonance for the given numerical aperture. This additional resonance is visible by an additional feature in both reflection and transmission at the excitation wavelength when compared to the SWGS. This is even more pronounced in the near-field enhancement depicted in Figure 1D. The near-field spectra are obtained from the region surrounding the central five two-wire building blocks, as depicted in the top field map of Figure 1E, and are normalized to the lateral dimension. The DWGS outperforms the SWGS in terms of both excitation and emission wavelength (denoted by the grey dashed lines in Figure 1D). Since the excitation near-field peak corresponds to a dip in transmission and reflection, the additional mode is dark and not effectively coupling to the far-field. Instead, it is only coupled via the bright plasmonic double wire mode, scattering into the grating plane. This dark mode has reduced radiative losses and is comparably narrow. The close-up views of the near field in Figure 1E demonstrate that this additional resonance causes the near fields to cancel out in

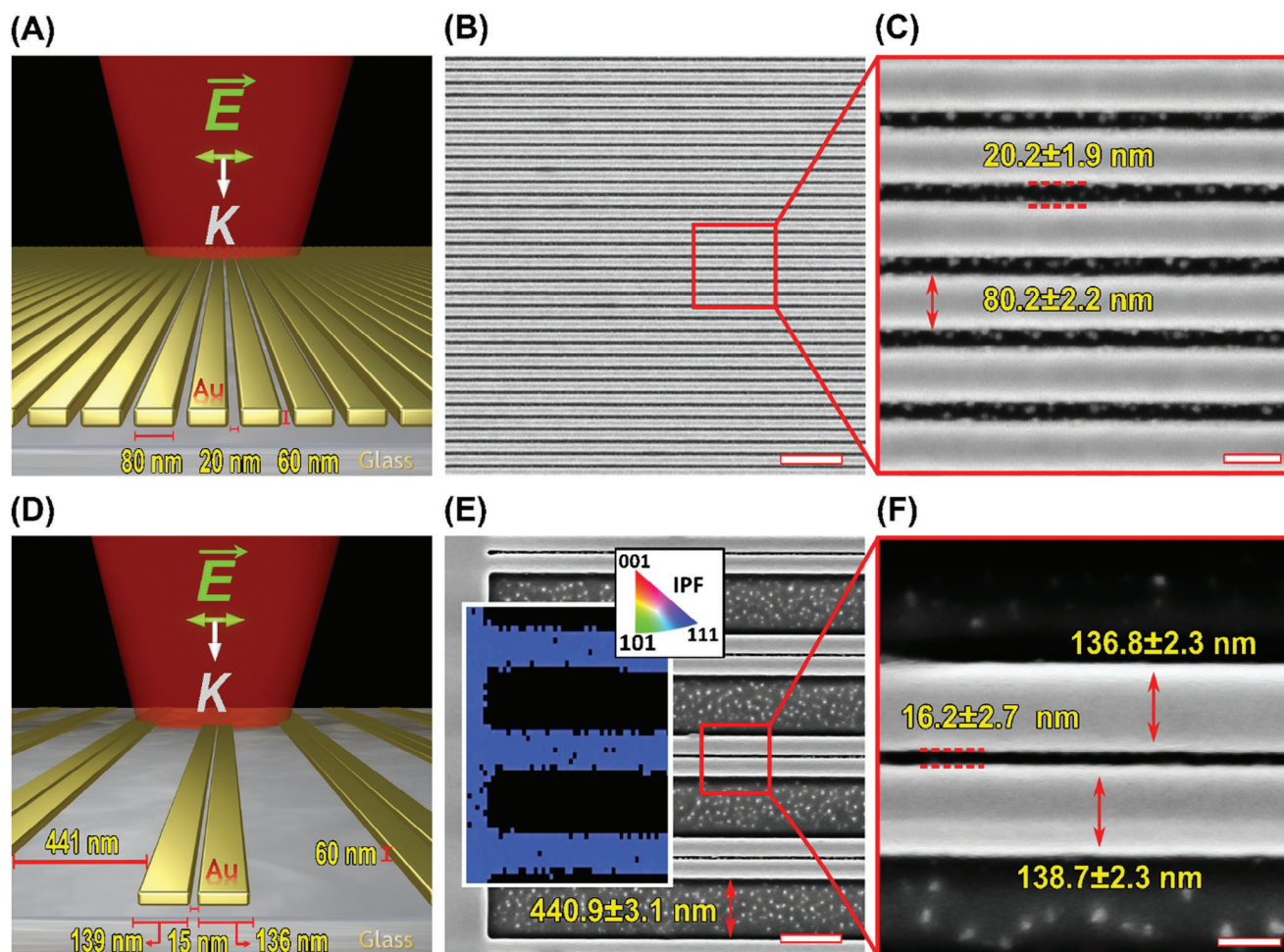


Figure 2. Geometry of the SERS platforms. A) and D), 3D schematics of the SWGS and DWGS SERS sensing platforms. The laser polarization and k-vector are represented by green and white arrows, respectively. SEM images of the monocrystalline gold gratings are displayed in (B), (C), (E), and (F) along with the measured dimensions. (B) depicts the SWGS structure, while a higher magnification view is provided in (C). Similarly, (E) shows the DWGS structure, and a higher magnification view is presented in (F). To prove the mono-crystallinity of the final gratings, the inverse pole figure (IPF) map obtained from electron beam scattering (EBSD) measurements are superimposed on the corresponding SEM image in (E). The stereographic triangle of the IPF color map is located on the upper side of (E). Scale bars: B), E) –500 nm, C) and F) –100 nm.

specific regions within the gap but results in a substantial enhancement closely above the gold surface, as intended by the specific choice of the fitness function F . The presence of the initial broad peak in the near fields around 580 nm can be attributed to the hidden constraint of the illumination numerical aperture, as briefly discussed in the Supporting Information (Figure S1C, Supporting Information). A closer inspection of the resonances with parameter sweeps and eigenmode analysis will be part of a future publication; however, some aspects can already be found in the (Figure S1D,E, Supporting Information). In this work, our focus lies on the experimental aspects of DWGSs from here on.

2.2. Synthesis, Fabrication, and Characterization

Both DWGS and SWGS platforms were fabricated via Ga-FIB milling using monocrystalline gold flakes^[25] as well as evaporated poly-crystalline gold. Figure 2A,D illustrates the resulting

SWGS and DWGS, along with their respective geometric parameters and illumination conditions. Both grating designs are situated on a glass substrate and are illuminated from the top with a linearly polarized Gaussian beam (spot size $\approx 1.5 \mu\text{m}$). Figure 2B,C,E,F shows scanning electron micrographs (SEM) of the experimentally realized geometries after FIB milling, showcasing high-precision fabrication and high fidelity achieved by using monocrystalline gold^[25] compared to evaporated polycrystalline milling (Figure S2, Supporting Information). Due to the resolution of the Ga ion beam ($\approx 5 \text{ nm}$) and sample drift during the large area patterning, the intended geometries could only be fabricated with an accuracy of $\pm 5 \text{ nm}$. Since the plasmonic resonances are comparably broad, this limitation does not hamper the DWGS performance. The bright contrast spots within the gap regions indicate the presence of residual gold particles. Such residues are caused by intermixing during the local physical sputtering in FIB milling or redeposition. Since such particles exhibit an absorption-dominated and polarization-independent

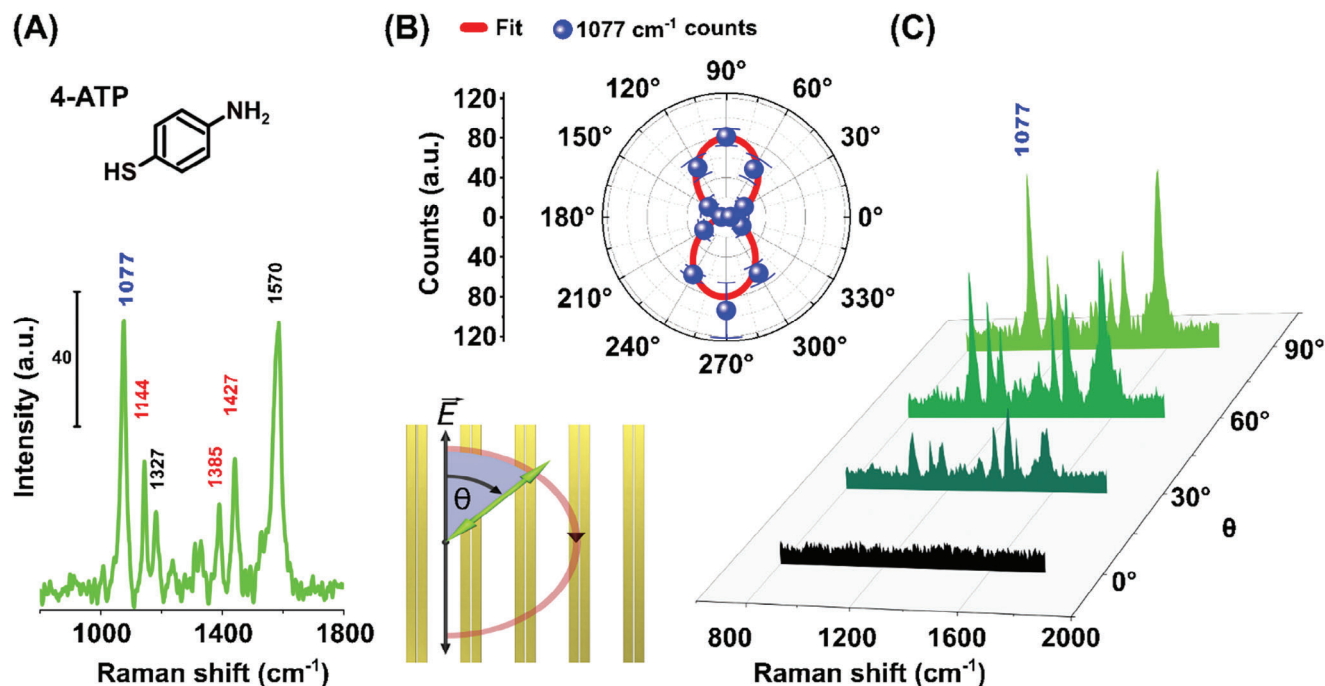


Figure 3. Physical properties of DWGS. In (A), the 4-ATP SERS signature is displayed. In (B), angle-resolved polarization spectroscopy showing Raman spectra of 4-ATP on DWGS presented as a polar plot, demonstrating the sinusoidal behavior of 1077 cm^{-1} Raman peak intensity with respect to the sample rotation angle θ . The blue dots represent the experimental data, and the red lines depict the theoretical fitting curve. The lower inset in (B) visually represents the changing angle between the electric field and the DWGS gold wires. The angle is obtained by rotating the sample from 0° to 360° while maintaining the laser polarization fixed in y-axis. Panel (C) provides a plot illustrating the SERS spectrum signature of 4-ATP at different representative angles. Each spectrum represents an average of 3–6 measurements.

resonance slightly above a wavelength of 500 nm, they are not excited by a 633 nm laser as used in the experiments.^[36] As a result, their influence can be safely neglected. The inset in Figure 2E also depicts an electron beam backscatter diffraction (EBSD) map supporting that monocrystallinity is maintained after FIB processing.^[37] Since the spatial resolution of EBSD under normal conditions is 20–50 nm, the small 15 nm gap is not visible in the map. FIB nanofabrication results and EBSD of polycrystalline gold were included in the, Figure S2C–F (Supporting Information).

2.3. Spectroscopic Properties

To investigate the SERS-enhancing capabilities of DWGSs, we used several model molecules: 4-Aminothiophenol (4-ATP),^[31] Methylene blue (M-blue),^[38] Rhodamine 6G (R6G),^[39] 3-Mercaptopropionic acid (3-MPA),^[40] Methyl benzoate (M-Benzate),^[41] Benzenethiol (BT),^[42] as well as biological molecules, including DNA^[43] and Bovine Serum albumin protein (BSA).^[44]

First, we investigated the SERS signature of 4-ATP self-assembled monolayers (SAM) on DWGS. Our observations in Figure 3A align consistently with the literature.^[45] The two most prominent peaks at 1077 and 1570 cm^{-1} correspond to the stretching modes of C–S and C=C ring, respectively.^[46] These peaks were selected for multiple experiments carried out during the course of this study. To assess the enhancement factor

(EF),^[5] a reference Raman measurement of 4-ATP was conducted (SI). The resulting SERS enhancement factor for the DWGS falls within the range of $\approx 10^6$, as determined by our protocols and sample preparation methods (experimental section and Supporting Information).

We investigated the polarization dependency of the SERS signal from a 4-ATP-DWGS sample (Experimental Section). Figure 3B,C illustrates the results dependent on θ , the angle between the wires and the excitation polarization. As we excite the gap plasmons in the grating via a dipolar resonance perpendicular to the wires, we expect the Raman signal strength to follow a $\sin^2\theta$ behavior, which is confirmed by the experimental data. Figure 3B,C shows the angle-dependent intensity of the 1077 cm^{-1} peak. The intensity of the peak increased significantly when the polarization angle varied from 0° to 90° . This confirms the strong enhancement of the Raman signal by the DWGS, which behaves similarly to a flat gold film when the plasmonic gap mode is not excited (Figures S8 and S9, Supporting Information). To rule out the polarization sensitivity of the experiment setup, angle-dependent conventional RS was conducted on bulk 4-ATP analyte deposited on flat gold substrate. The results, along with a polar plot, are concisely discussed in the (Figure S3, Supporting Information). As a result, a polarization angle of $\theta = 90^\circ$ was selected for all subsequent experiments. Finally, switching the near-field enhancement by controlling the polarization is advantageous as it allows for confirming the DWGS plasmonic signal enhancement. When the plasmonic gap mode is not excited, the DWGS behaves similarly to a non-plasmonic surface.

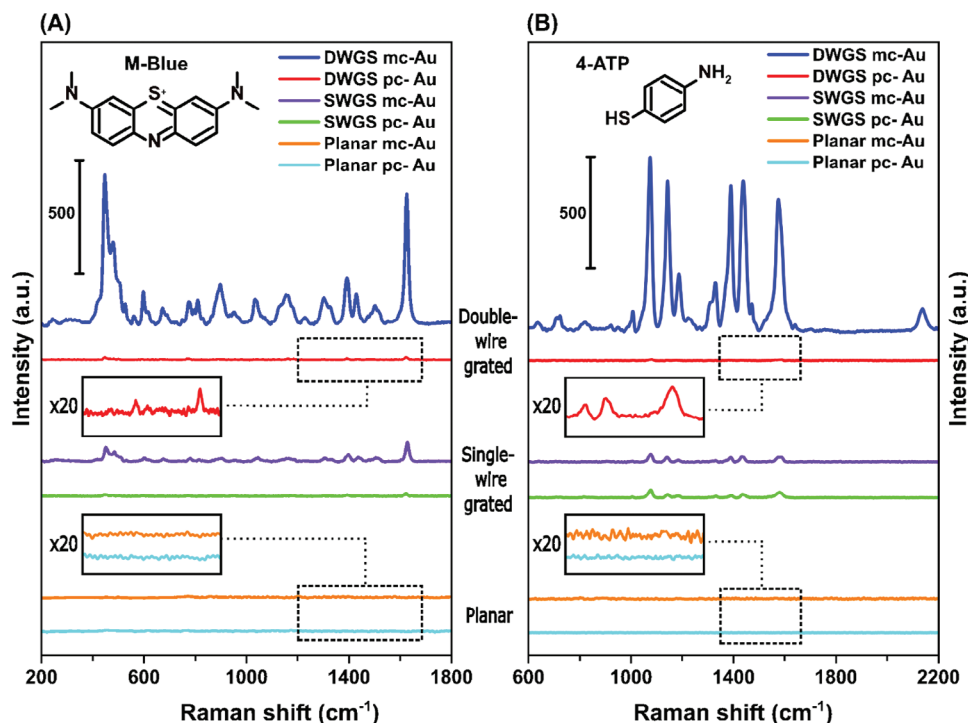


Figure 4. Substrate and crystallinity comparison. Panel A) displays SERS measurements of M-Blue (1 μM) on dip-casted planar Au (non-grated), single-wire grating substrate (SWGS), and double-wire grating substrate (DWGS). Both monocrystalline (mc-Au) and polycrystalline (pc-Au) gold materials were utilized for each substrate type. A zoomed-in view of the interval between 1200–1700 cm⁻¹ is presented to highlight the relevant peaks. B) Depicts SERS signals of 4-ATP SAM on the same substrates as in panel (A). Here, a zoomed-in view of the intervals between 1350–1650 cm⁻¹ is provided.

The polarization measurement delivered an additional result: During rotation, an ongoing increase in the peaks at 1144, 1385 and 1427 cm⁻¹ (highlighted in red in Figure 3A) is observed, which can be seen in Figure 3C. This increase is attributed to the formation of 4,4'-dimercaptoazobenzene (DMAB) dimers, which are expected to occur due to continuous irradiation and prolonged exposure to oxygen. These dimers are believed to result from oxidation of the 4-ATP molecules induced by the Raman laser. Consequently, this process introduces additional vibrational modes.^[45] For the present work, we do not investigate this effect further.

To investigate the relationship between morphology, plasmonic properties, and the spatial distribution of gold nanostructures in terms of hot-spots, we performed a scanning confocal 2D Raman mapping of 4-ATP SAM on DWGS sample (SI). The mapping reveals the expected hot-spots along the gold wires, and the expected behavior of the plasmon at the edges. The results are depicted in (Figures S4 and S5, Supporting Information).

Next, both M-Blue and 4-ATP molecules were investigated using chemical or physical immobilization methods respectively, as described in the experimental methods section. These molecules were deposited on six different substrates: planar non-grated gold substrates, SWGS, and DWGS, each fabricated using both monocrystalline (Figure 2) as well as polycrystalline gold materials (Figure S2, Supporting Information). The outcomes of these experiments are summarized in Figure 4.

The DWGS made from monocrystalline gold exhibits the highest SERS signal enhancements for both M-blue and 4-ATP, as shown in Figure 4A,B. These SERS spectral signatures align with

previous studies on these molecules.^[45,47] In contrast, the signals obtained from monocrystalline SWGS substrates are approximately two orders of magnitude lower for both probe molecules. Furthermore, the DWGS monocrystalline substrates demonstrate the ability to detect concentrations as low as $\approx 10^{-12}$ M, as evidenced by a 4-ATP measurement (Figure S6, Supporting Information). No SERS signatures were observed from planar monocrystalline or planar polycrystalline gold substrates. Both monocrystalline DWGS and SWGS exhibit higher peak signals when compared to their polycrystalline counterparts. This indicates that the additional surface roughness present on polycrystalline substrates does not sufficiently improve the Raman signal enough to counteract the increased losses caused by the amplified scattering of plasmonic mode currents at grain boundaries.

2.4. Technological Properties

A high-quality SERS substrate should possess several important characteristics to meet the demand for SERS sensing-based technology, as outlined in Refs.[48–50]. These characteristics include exceptional sensitivity, homogeneity over a large area, reproducibility in fabrication and signal, long-term stability, cleanliness, and ideally, the ability to be reused. Furthermore, it should be cost-effective to produce. In Figure 5, we show successful tests of the DWGS for these characteristics, except for the criterion of cost-effectiveness, which remains a challenge for the nano-engineers.^[28] For addressing the technological aspects, all substrate samples were prepared using the same protocol and

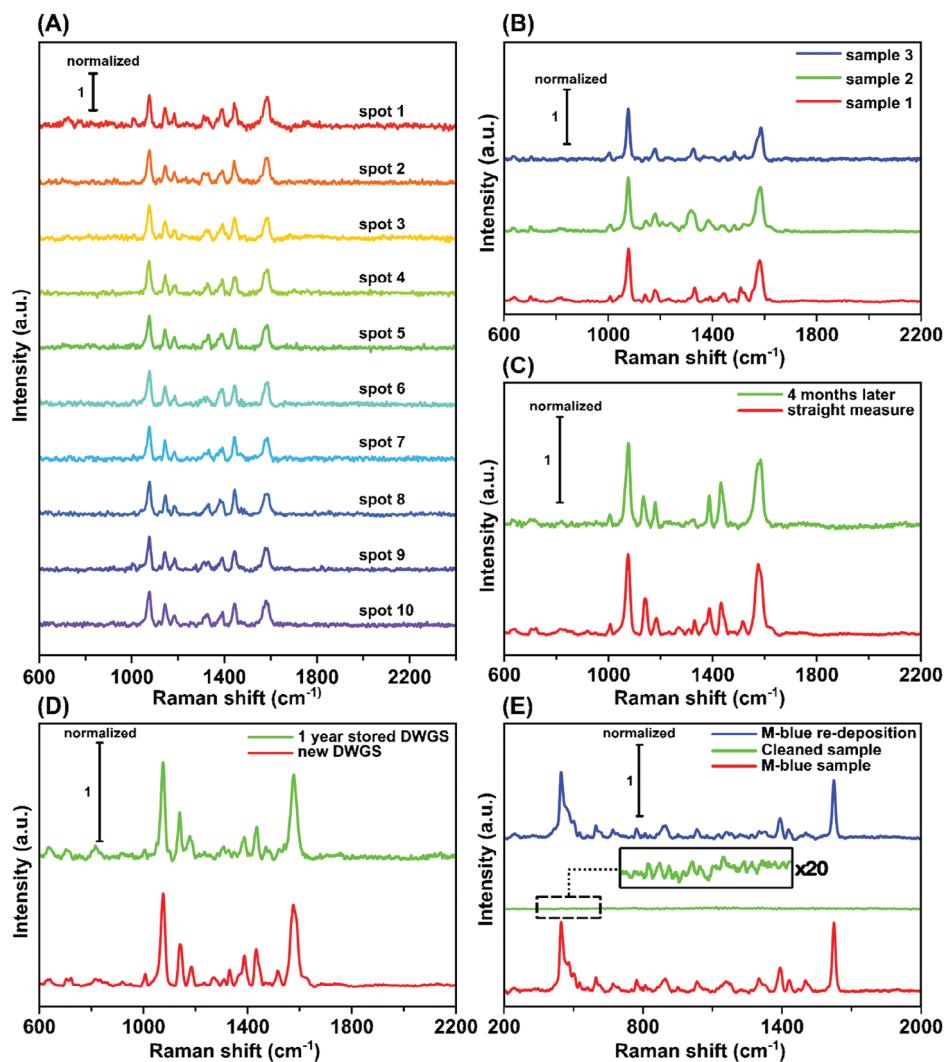


Figure 5. DWGS monocrystalline gold platform robustness and essential properties for sensing technology. A) SERS signal repeatability on the same SERS platform, showing consistent Raman signals of the 4-ATP SAM for ten sequential measurements on the same sample at different positions. B) SERS signature reproducibility among three different fabricated DWGS gold substrate samples, all with the 4-ATP SAM. C) Long-term signal stability of 4-ATP molecule after being left undisturbed at room temperature for 4 months without any special handling. D) Long shelf time demonstrated with sustained Raman signal generation of the 4-ATP SAM deposited on a one-year-old substrate. E) DWGS reusability and signal regeneration after a simple cleaning procedure and re-deposition of the M-blue probe molecule. A $\times 20$ zoom-in within the $300\text{--}600\text{ cm}^{-1}$ interval highlights relevant peaks. Intensities were normalized to improve visual clarity of each experiment.

experimental settings. Figure 5A provides evidence of the consistency and repeatability of multiple measurements conducted using the same SERS platform sample under identical conditions. The platform exhibits high repeatability between measurements ($\text{RSD} = 5.6\%$) when analyzing the intensities of the 4-ATP molecules at the 1077 cm^{-1} peak using 10 different measurement spots covering about 15% of the single substrate area in the center of DWGS. Furthermore, Figure 5B attests to high reproducibility achieved using three different substrates ($\text{RSD} = 6.6\%$), for the same peak (1077 cm^{-1}). This is better than most available commercial substrates (Table S2, Supporting Information) based on semi-random pointy nano-particle distributions, which typically exhibit RSD of at least $> 20\%$,^[51] except for the one formerly fabricated by Hamamatsu. The Hamamatsu substrate shows an average $\text{RSD} < 10\%$,^[51] but is no longer available for sale. An-

other group^[52] reported an average $\text{RSD} < 100\%$ for RAM-SERS-SP commercial substrate, around 20% for Hamamatsu, between 5–10% for Q-SERS and $\approx 10\%$ of Klarite 309 substrate,^[53] which is also discontinued. Figure 5C supports the claim of long-term signal stability by demonstrating the signal's consistency both after deposition and four months later. Remarkably, the sample was kept at ambient room temperature without any special handling or sealing. The stability of the signal signature can be attributed to the physical and chemical stability of the structural rigid substrate of crystalline gold and the thiol-gold chemical bond. Additionally, Figure 5D confirms that unused DWGS SERS substrates do not deteriorate over time, demonstrating a long shelf life of at least one year. Samples prepared with these one-year-old unused substrates display a nearly identical Raman signature of the 4-ATP molecules compared to samples prepared with new

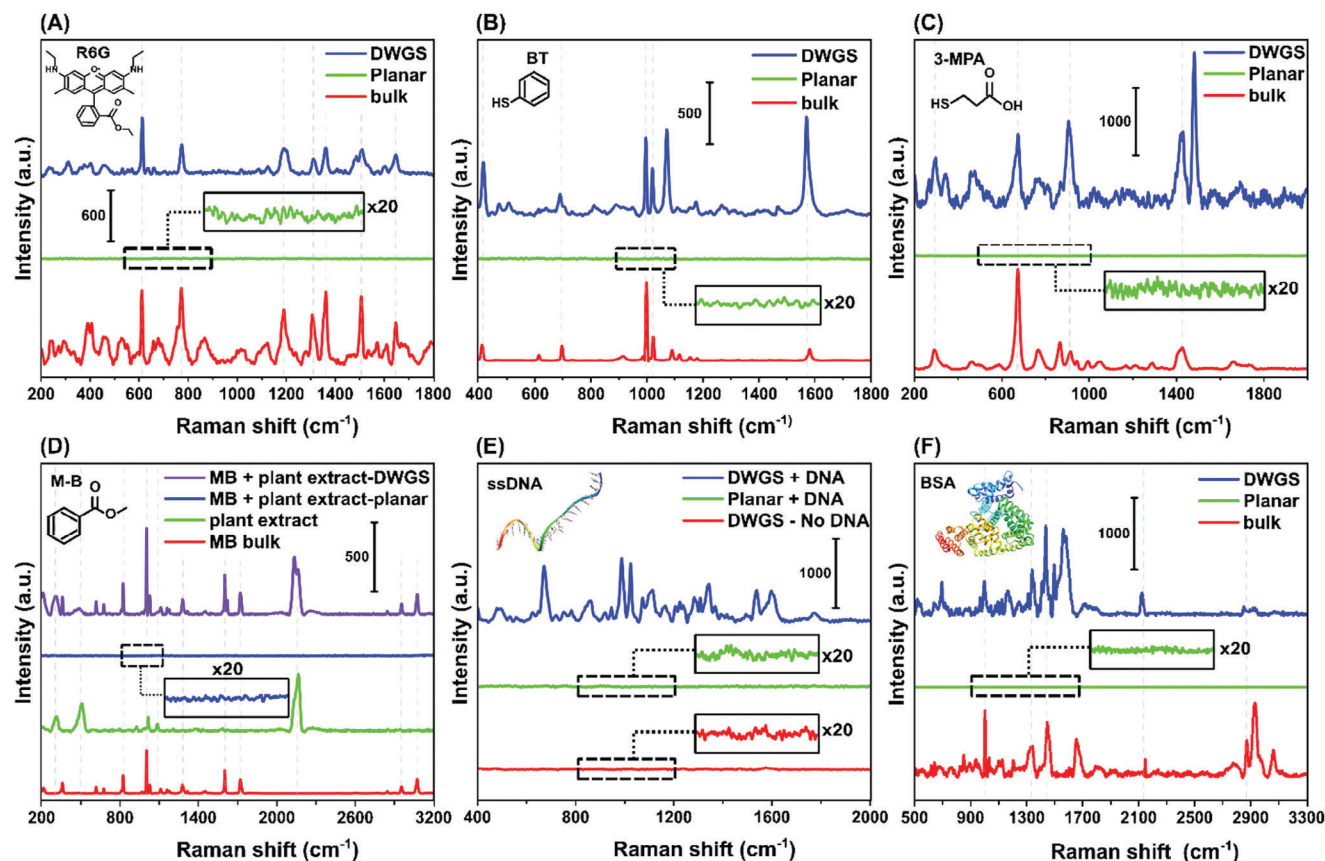


Figure 6. Showcases the potential future applications of DWGS platform. The substrates are: Planar Au flake (non-grated), and double wire grating substrate (DWGS). A) illustrates the platform's ability to detect R6G from the solution. (B) shows the direct detection of the BT probe molecule from the gas phase. C) presents the detection of volatile 3-MPA analytes that were solubilized in a liquid medium containing the substrate, followed by detection after substrate drying. D) serves as a proof of concept by detecting the Methyl benzoate volatile molecule in plant extract sample. (E) and (F) depict the detection of SERS signatures of biological macromolecules in solution, specifically ssDNA and BSA protein, respectively. Zoomed-in views with $\times 20$ magnification are provided to emphasize the relevant peaks. The model of ssDNA is based on the coordination of DNA polymerase-clamp-exonuclease determined by Cryo-EM,^[67] while the BSA model is based on Crystal Structure of Bovine Serum Albumin.^[68] Molecular graphics and analyses of biomolecules were performed with UCSF Chimera software.^[69]

unused substrates. The substrates were stored at room temperature under ambient conditions without any special treatment or sealing except being boxed against dust contamination. Finally, the reusability of each substrate is made possible due to the inert nature and chemical stability of gold, as well as the intrinsic rigidity of the substrate. Cleaning procedures using various chemical solvents and physical methods can be utilized for the cleaning process of the double grating substrate,^[54] enabling its reuse. Mild cleaning procedures are suitable for achieving reusability of pre-used substrates (refer to the experimental methods section). Proof of concept of cleaned reused substrate's reusability is shown in Figure 5E.

2.5. Sensing in Challenging Environments and Future Possible Applications

Finally, we explore the potential applications of the SERS DWGS in diverse challenging environments and discuss their prospects. These applications encompass the detection of analytes across different states of matter,^[55,56] target molecules in

extracts or biological media,^[57] and challenging environments such as chemical probes in water,^[58] metabolites in solutions,^[57] biomedical markers in physiological samples,^[59] and volatile gas molecules.^[55] We showcase the direct identification of analytes from liquid solution (Figure 6A), detection of gas molecules through gas flow or solubilization procedures of volatile gaseous molecules (Figure 6B,C). Furthermore, we investigated the detection of M-Benzoate, a plant metabolic analyte, in plant extracts (Figure 6D), as well as different biological macromolecules such as DNA (Figure 6E) and BSA proteins (Figure 6F).

These various analytes show the versatility of our substrate optimization approach. The fact that we are not working in the resonant Raman regime. Our light is therefore scattered inelastically at a virtual energy level, without exciting the molecules. This scattering is independent of the molecule under observation, and thus, a SERS substrate only needs to consider the wavelength of the excitation laser. The emission process, on the other hand, is highly dependent on the vibrational energy levels of the molecule. Here, we utilize the existence of the so-called fingerprint region, which refers to the highly analyte-specific Raman shifts occurring in the range between 400 and 1800 cm^{-1} . This energy difference

is also dependent on the excitation wavelength. For our 633 nm excitation, this falls between about 640 and 710 nm. This is why we optimize for two wavelengths at 635 and 675 nm (the center of the fingerprint region). As the plasmon resonance at the emission wavelength is quite broad, we enhance a large interval of the fingerprint region, allowing us to detect a variety of different organic molecules (Figure 6).

To detect target molecules in liquids, R6G was then applied to the DWGS (experimental methods section). The presence of molecules not close to the substrate did not interfere with the measurements, as the SERS effect is surface-sensitive within a short range of a few nanometers.^[60] The characteristic peaks of R6G were clearly observed, particularly at 612, 774, 1310 and 1361 cm^{-1} , which were compared and found to be consistent with the bulk R6G powder material. Furthermore, these peaks were found to be in good agreement with literature.^[61]

The detection of volatile gas molecules was demonstrated in two experiments (experimental section and, Figure S7, Supporting Information). The results are presented in Figure 6B for BT detection and Figure 6C for 3-MPA detection. Both 3-MPA and BT exhibited successfully detected peaks that are consistent with the literature.^[42,62] Moreover, the SERS peaks maintained similarity to the Raman peaks of the neat bulk analyte. The SERS signature of the BT analyte showed five prominent peaks at 417, 998, 1022, 1073 and 1572 cm^{-1} , most of which were similar to the conventional Raman spectrum of the bulk BT. Regarding 3-MPA, significant peaks were observed at 295, 672, 904 and 1428 cm^{-1} , with the distinctive peaks at 672 and 904 cm^{-1} that appear both in SERS and neat solution, could be assigned to CS stretching and C-COO stretching respectively.^[62]

The detection of the M-Benzoate molecule in plant extract, as described in the experimental methods section, is summarized in Figure 6D. The Raman signature of the M-Benzoate molecule was clearly observed in the sample mixture, appearing as a superposition of the pure analyte and the prominent peaks from the plant extract.

The detection of two biological macromolecules exhibited consistency with the literature.^[63–66] For the DNA SERS signature, the spectrum depends on various parameters such as DNA sequence, orientation, and sample preparation.^[66] However, certain peaks, including the one at 670 cm^{-1} , are commonly observed due to similar functional groups of DNA building blocks.^[65] The DNA SERS signature obtained using DWGS provided several distinct peaks, as shown in Figure 6E, including peaks at 670, 988, 1029, 1340 and 1538 cm^{-1} . The major peaks at 670, 988, 1029 cm^{-1} could be attributed to guanine base, sugar backbone, and cytosine, respectively.^[63,65] The Raman signals of Bovine Serum Albumin (BSA) were easily observed using SERS, as depicted in Figure 6F, compared to the bulk signal of BSA powder and previously reported literature.^[64] Particularly, distinct peaks at 1000, 1560 and 1580 cm^{-1} were assigned to phenylalanine and tryptophane amino acid.

3. Conclusion

In summary, we have introduced the double wire grating substrate (DWGS), an innovative SERS platform based on a grating of two-wire waveguides – double resonant nanostructures fabricated from monocrystalline gold using focused ion beam lithog-

raphy. Through careful design of the fitness function for evolutionary optimization, these DWGSs achieve large local field enhancement for both excitation and emission processes involved in Raman scattering. The monocrystalline material optimizes the optical properties and enables precision and fidelity in the nanofabrication process over a large area, with enhancement factors reaching several 10^6 and the ability to detect concentrations as low as $\approx 10^{-12}$ M in solution. The dipolar double wire resonances allow to “switch” the surface enhancement via the excitation polarization angle. The DWGS platform presents numerous technological advantages, namely high sensitivity, and noteworthy homogeneity over large areas ($>10^4 \mu\text{m}^2$), notable repeatability (RSD = 5.6%), and reproducibility (RSD = 6.6%). Moreover, it is long-term stable up to at least one year under ambient conditions, requiring no special handling or sealing, and can be reused multiple times without a noticeable loss in performance. To highlight its practical usability, we successfully applied the DWGS platform with both chemical and physical deposition methods, enabling the detection and identification of trace amounts of analytes across different states of matter. This includes gaseous analytes, plant extracts, and biological macromolecules. The investigation of DWGS in this study reveals its potential as an advanced SERS platform, capable of enhancing analytical capabilities in various fields. For even greater SERS enhancement, the use of Helium FIB will allow for gaps down to a few nanometers, which is expected to further localize and enhance the fields. In large-scale applications, initial experiments on large electrochemically grown gold layers,^[27] patterned with nano-imprint lithography (as shown, for example, in ^[28]), promise the possibility of achieving higher substrate output without compromising the beneficial properties.

4. Experimental Section

Simulation: Simulations were performed with Ansys Lumerical FDTD Solutions (Release 2021 R2.5) in a purely 2D simulation area. Several parameters were fixed to factor in fabrication constraints. Gaps were not allowed to be smaller than 15 nm, which represented the smallest reliably realizable gap by means of Ga-FIB milling. The upper grating edge also had a radius of 15 nm due to the point spread function of the Ga-beam (referred to as “radius” in Figure 1B). Additionally, the height of the DWGS was fixed at 60 nm, a commonly observed height in wet-chemically grown gold flakes,^[70] which facilitated quick experimental realization after optimization. Excitation was performed via a broadband pulse with a 500–800 nm wavelength interval and a thin lens approximation of an NA = 0.6 objective to mimic the experimental conditions. The materials employed were glass as user-defined dielectric with the fixed index at $n = 1.518$ representing borosilicate glass, and gold as a fit of the Etchegoin material model in its corrected version.^[71] The grating contained 21 double-grating elementary cells, which ensures convergence against a hypothetical infinite grating. Only the five central elementary cells were evaluated for the fitness function used in the particle swarm optimization (100 generations containing 20 individuals) of the grating parameters. A mesh refinement area with fixed 2 nm Yee cell size was placed around all gold within the simulation up to 20 nm distance. Distance from the resonator to the boundary PML (standard settings) was set to be 500 nm to ensure a distance larger than half of the maximal wavelength.

Gold Synthesis: Gold flakes were synthesized via a modified wet-chemical procedure based on Ref. [70] Briefly, in a typical procedure, Hydrogen tetrachloroaurate trihydrate (Aldrich, SKU: 520918) was dissolved in 1 mL DDW (1 M final concentration) and mixed by stirring in 20 mL ethylene glycol (Aldrich, SKU: 102466), serving as the solvent in 50 mL

polypropylene centrifugation tube. A microscopy coverslip was used as a substrate for the gold flake growth. Prior to immersion in the gold solution, the substrate was thoroughly cleaned in ethanol and acetone, followed by rinsing with DDW and drying with a stream of Nitrogen (N_2). Two substrates were immersed into the homogeneous gold solution back-to-back. Subsequently, the solution was heated in an oven and kept at 90 °C for 18 h. Then, the substrates were removed from the solution, rinsed with ethanol and water, and dried with a N_2 -stream. Typically, the resulting flake thickness ranges between 40 and 80 nm, which can be measured by fitting a broadband transmission spectrum.^[70] Gold flakes with a thickness of 60 nm were identified and transferred onto partly evaporated gold / glass substrates (marker structures & ground contact) with a PMMA-mediated method^[72] to ensure ground contact for subsequent focused ion-beam milling. The evaporated gold substrate was produced by thermally depositing a gold film on silicon or glass substrates under a high vacuum. A 5 nm Titanium/Chromium adhesive layer is deposited on the substrate, followed by a 60 nm gold layer.

FIB Fabrication: Focused ion beam milling is conducted using the Gallium beam of a Zeiss ORION NanoFab system, operating at an acceleration voltage of 30 kV and a beam current of 10 pA. For the larger gaps, the beam is scanned with 35 passes, a 1 μ s dwell time, and a serpentine direction, applying an ion dose of 4.7 mC cm⁻². The small gaps were fabricated using a single line pass and a dwell time of 300 μ s.

SERS Model Molecule Sample Preparations: This 4-ATP (Aldrich, SKU: 422967) was chemically deposited using the standard gold thiolation method in solution.^[73] The substrate was immersed in a glass vial containing 5 mL of 1.5 mM ethanol solution of 4-ATP at room temperature for 24 h. This incubation led to the formation of a self-assembled monolayer of 4-ATP on the gold substrates. Similarly, to calculate the enhancing factor, the DWGS substrate was prepared according to the standard gold thiolation procedure. The reference for EF calculation was obtained by drop-casting 0.5 μ L of 15 mM 4-ATP (in Abs. Ethanol) on a planar gold surface, followed by solvent drying in a hood for 10–30 min. The 0.5 μ L ethanol solvent covers ≈ 1 mm² of the gold surface. Both measurements were done under identical experimental conditions. The enhancing factor was then calculated by averaging the 1077 cm⁻¹ integrated peaks from 3 spectra.

M-Blue (Aldrich, SKU: 66720) molecules were physically deposited by dip-casting the sample into corresponding concentrations of the analyte in DDW (Ranging from μ M to mM concentration) for 2 h. Subsequently, the sample was snap-rinsed 10 times in clean Ethanol and 10 times in DDW, then dried with a stream of Nitrogen (N_2).

For sample reuse, the surface was cleaned by mild basic piranha treating for 20 min at 60 °C using a 4:1:1 mixture of DDW:NH₄OH:H₂O₂. Adjusting the treating time or mixture ratio is recommended for the desired cleaning without damaging the sample.

Liquid-phase SERS direct measuring of R6G (Aldrich, SKU: R4127) was obtained by preparing a 1 μ M solution in DDW, applying 1 μ L on the substrate surface, covering it with a microscopy glass coverslip. Measurements were performed directly in solution by focusing the laser spot through the solution onto the substrate.

The setup for sensing gaseous volatiles of BT (Benzenethiol; Aldrich, SKU: T32808) molecule is shown schematically in the Figure S7A (Supporting Information). The gas was actively flown through a tube to the SERS substrate. A 0.5 mL sample of BT ($\approx 17\%$ (v/v) in ethanol) was applied into a warmed container (30 °C). An active flow of Argon (≈ 100 mL min⁻¹) was maintained for 30 min, followed by removing the substrate and performing measurements.

For sensing solubilized gaseous molecules, 3-MPA (Aldrich, SKU: M5801) was drop-casted (50 μ L) and pumped from a warmed container (30 °C) into an adjacent chemical beaker containing the SERS substrate immersed in pure DDW as shown in the Figure S7B (Supporting Information). The inert pumping flow was carried out using active argon flow (≈ 100 mL min⁻¹) for 1 h, followed by drying the sample for 10 min in a hood and measuring the 3-MPA attached to the SERS substrate.

Both biological molecules were prepared in a similar way. Both the single-strand DNA (Sequence: 5'CGATCCACCTCCG-

GAACCTCCACCTTTTTCGAATTGTGGATGACTCCAAGCGGAGCCGC-CTTTACCGGGGACAGGGAGAG -3') and the bovine serum albumin (BSA) were prepared by applying of 1 μ L analyte solution (1 μ M in DDW) to the DWGS sample, covering it with a microscopy glass coverslip and measuring directly.

The plant sample extract was prepared following methods described in Ref. [74]. Briefly, Tomato plants (*Solanum lycopersicum*) of the cultivar named Ikram (Syngenta) were grown for four weeks under controlled growth conditions (16 h/8 h light/dark, 24 $\pm$ 2 °C). Fresh leaves are then harvested, frozen, and homogenized. Metabolites were extracted using an organic phase composed of Methanol, Chloroform, and MiliQ H₂O ratio of 2.5:1:1 (v/v) respectively, to remove chlorophyll and avoid fluorescence interference. The extract was sonicated for 10 min at room temperature, centrifuged, and the upper supernatant was collected. Next, it was mixed with 1 mM M-Benzoate (Aldrich, SKU: 8223301000) solution (In Ethanol) in a 1:50 v/v M-Benzoate solution to plant extract. The mixture was applied on the SERS substrate, covered with a microscope coverslip, and directly measured.

Raman Measurements and Data Analysis: Raman spectra were collected using a confocal Horiba LabRam HR Evolution with a 633 nm excitation laser and a power at the sample 0.09 mW (except of 4-ATP 10⁻¹² M experiment shown in: Figure S6 (Supporting Information), the power at the sample was 0.5 mW). The measurements were performed using 50 $\times$ LWD objective (Olympus LMPlanFL-N, NA = 0.5, spot size ≈ 1.5 μ m). The measurements were taken with a 600 gr mm⁻¹ grating and a 100 μ m confocal hole. The instrument was equipped with a Sincerity multichannel 1024 $\times$ 256-pixels CCD detector cooled to a temperature of -60 °C. The approximate spectral resolution was 1.3 cm⁻¹. Each spectrum was acquired for 20 s with 3 accumulations. System calibration was performed by assessing the first-order phonon band of Silicon (111) substrate at 520 cm⁻¹. Most measurements were conducted using the same experimental settings. In cases where different exposure times or laser intensities were used, the data were normalized to obtain counts per 1 sec and 1 mW, allowing for comparison under adjusted uniform setting. LabSpec software (version 6.5.2.11) was used to operate the spectrometer. To assess reproducibility and repeatability, at least three consecutive accumulation spectra were acquired back-to-back at the same location, and a second set of at least three repeat spectra was collected immediately thereafter at a slightly offset location (1–3 μ m). The average SERS intensities and relative standard deviations of the whole spectrum were calculated based on 3–25 SERS spectra, with the relative standard deviation (RSD) calculated for specific peaks. Raman data processing, including spectra smoothing, baseline correction, and normalization was performed using OriginPro 2023. The smoothing algorithm was Savitzky-Golay with the second polynomial order. Baseline subtraction was done using a spline interpolating method with a second derivative. After performing a basic processing for each spectrum, such as despiking. Spectra obtained from the same experiment (at least 3) were averaged and plotted as one spectrum. To improve visual clarity, the SERS intensities were normalized using a min-max spectral normalization approach.

To measure the polarization dependency of the 4-ATP-DWGS SERS signal, a continuous 360° manual micrometer-based rotation stage was used. The polarization direction of the excitation laser along the y-axis of the sample was maintained throughout the experiment. The DWGS substrate was rotated in steps of 30°. Data were fitted by $\gamma(\theta) = \gamma_0 + A \sin^2(\pi(\frac{\theta - \theta_0}{\omega}))$, where θ_0 was fixed to 0°.

SEM and EBSD Characterization: SEM imaging was conducted using a Thermo Fisher Verios 460L field-emission scanning electron microscope, equipped with an electron backscatter diffraction (EBSD) detector (OXFORD instruments). Images were acquired in the secondary electron mode, with an accelerating voltage of 3 kV, a beam current of 25 pA, and a working distance of 5.9 mm. For EBSD analysis, the sample was tilted by 70°, using an electron beam with an acceleration voltage of 20 kV and a beam current of 0.8 nA. The backscattered electrons, which undergo partial diffraction, enabled the visualization of characteristic Kikuchi patterns. The OXFORD Channel 5 software was employed to analyze these patterns and determine the relevant 3D orientation information for each pixel.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

The manuscript was written through the contributions of all authors. All authors have given approval to the final version of the manuscript. A.S. and M.Y.B. planned and carried out the SERS experiments, performed methodology validations, and software analysis including models. A.S. wrote the first draft and took the lead in writing the manuscript. In addition, M.Y.B. conceived the benchmarking SERS experiments using chemical/physical bonding of liquid or gaseous (bio)-chemical compounds. M.J.P. assisted in analyzing the results and revising the manuscript. E.S., K.H., and H.M. managed the top-down fabrication, contributed to result analysis, and participated in discussions. V.T. and A.T. performed the plant extract preparation. P.M. revised the manuscript and participated in discussions. T.F. conceived the presented idea, including the initial substrate geometry, performed numerical design optimization, and numerically analyzed the plasmonic properties of the final DWGS. M.Y.B. and T.F. co-supervised the project and acquired the funding.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords

double-resonance, focused ion-beam lithography, grating nanostructures, monocrystalline gold flakes, plasmonic resonator, SERS substrate, surface-enhanced Raman spectroscopy

- [1] C. V. Raman, K. S. Krishnan, *Nature* **1928**, 121, 501.
- [2] D. A. Long, *The Raman Effect: A Unified Treatment of the Theory of Raman Scattering by Molecules*, John Wiley & Sons, New Jersey, United States **2002**.
- [3] M. Fleischmann, P. J. Hendra, A. J. McQuillan, *Chem. Phys. Lett.* **1974**, 26, 163.
- [4] J. Langer, D. J. de Aberasturi, J. Aizpurua, R. A. Alvarez-Puebla, B. Auguie, J. J. Baumberg, G. C. Bazan, S. E. J. Bell, A. Boisen, A. G. Brolo, J. Choo, D. Cialla-May, V. Deckert, L. Fabris, K. Faulds, F. J. G. de Abajo, R. Goodacre, D. Graham, A. J. Haes, C. L. Haynes, C. Huck, T. Itoh, M. Ka, J. Kneipp, N. A. Kotov, H. Kuang, E. C. Le Ru, H. K. Lee, J. F. Li, X. Y. Ling, et al., *ACS Nano* **2020**, 14, 28.
- [5] C. E. Le Ru, E. Blackie, M. Meyer, P. G. Etchegoin, *J. Phys. Chem. C* **2007**, 111, 13794.
- [6] P. K. Kannan, P. Shankar, C. Blackman, C. H. Chung, *Adv. Mater.* **2019**, 31, 1803432.
- [7] Z. C. Huang, A. M. Zhang, Q. Zhang, D. X. Cui, *J. Mater. Chem. B* **2019**, 7, 3755.
- [8] M. Petersen, Z. L. Yu, X. N. Lu, *Biosensors-Basel* **2021**, 11, 187.
- [9] R. Gillibert, J. Q. Huang, Y. Zhang, W. L. Fu, M. L. de la Chapelle, *TrAC, Trends Anal. Chem.* **2018**, 105, 166.
- [10] K. Kneipp, Y. Wang, H. Kneipp, L. T. Perelman, I. Itzkan, R. Dasari, M. S. Feld, *Phys. Rev. Lett.* **1997**, 78, 1667.
- [11] L. Scarabelli, M. Coronado-Puchau, J. J. Giner-Casares, J. Langer, L. M. Liz-Marzan, *ACS Nano* **2014**, 8, 5833.
- [12] J. F. Li, Y. J. Zhang, S. Y. Ding, R. Panneerselvam, Z. Q. Tian, *Chem. Rev.* **2017**, 117, 5002.
- [13] X. Yao, S. Jiang, S. S. Luo, B. W. Liu, T. X. Huang, S. Hu, J. F. Zhu, X. Wang, B. Ren, *ACS Appl. Mater. Interfaces* **2020**, 12, 36505.
- [14] S. S. Acimovic, H. Sipova, G. Emilsson, A. B. Dahlin, T. J. Antosiewicz, M. Kall, *Light Sci Appl* **2017**, 6, e17042.
- [15] N. A. Hatab, C. H. Hsueh, A. L. Gaddis, S. T. Retterer, J. H. Li, G. Eres, Z. Y. Zhang, B. H. Gu, *Nano Lett.* **2010**, 10, 4952.
- [16] H. G. Duan, H. L. Hu, K. Kumar, Z. X. Shen, J. K. W. Yang, *ACS Nano* **2011**, 5, 7593.
- [17] K. N. Kanipe, P. P. F. Chidester, G. D. Stucky, M. Moskovits, *ACS Nano* **2016**, 10, 7566.
- [18] C. Zhang, C. H. Lia, J. Yu, S. Z. Jiang, S. C. Xu, C. Yang, Y. J. Liu, X. G. Gao, A. H. Liu, B. Y. Man, *Sens Actuators B Chem* **2018**, 258, 163.
- [19] K. Jung, J. Hahn, S. In, Y. Bae, H. Lee, P. V. Pikhitsa, K. Ahn, K. Ha, J. K. Lee, N. Park, M. Choi, *Adv. Mater.* **2014**, 26, 5924.
- [20] M. G. Banaee, K. B. Crozier, *ACS Nano* **2011**, 5, 307.
- [21] Y. Z. Chu, M. G. Banaee, K. B. Crozier, *ACS Nano* **2010**, 4, 2804.
- [22] N. A. Cinel, S. Butun, G. Ertas, E. Ozbay, *Small* **2013**, 9, 531.
- [23] C. E. Le Ru, P. G. Etchegoin, *Chem. Phys. Lett.* **2006**, 423, 63.
- [24] C. E. Le Ru, J. Grand, N. Felidj, J. Aubard, G. Levi, A. Hohenau, J. R. Krenn, E. Blackie, P. G. Etchegoin, *J. Phys. Chem. C* **2008**, 112, 8117.
- [25] J. S. Huang, V. Callegari, P. Geisler, C. Bruning, J. Kern, J. C. Prangasma, X. F. Wu, T. Feichtner, J. Ziegler, P. Weinmann, M. Kamp, A. Forchel, P. Biagioni, U. Sennhauser, B. Hecht, *Nat. Commun.* **2010**, 1, 150.
- [26] V. G. Kravets, A. V. Kabashin, W. L. Barnes, A. N. Grigorenko, *Chem. Rev.* **2018**, 118, 5912.
- [27] S. V. Grayli, X. Zhang, F. C. MacNab, S. Kamal, D. Star, G. W. Leach, *ACS Nano* **2020**, 14, 7581.
- [28] Y. Q. Chen, Z. W. Shu, S. Zhang, P. Zeng, H. K. Liang, M. J. Zheng, H. G. Duan, *Int J Extrem Manuf* **2021**, 3, 032002.
- [29] M. Ochs, L. Jucker, M. Rödel, M. Emmerling, R. Kullock, J. Pflaum, M. Mayor, B. Hecht, *Nanoscale* **2023**, 15, 5249.

- [30] J. Michon, M. Benzaouia, W. J. Yao, O. D. Miller, S. G. Johnson, *Opt. Express* **2019**, 27, 35189.
- [31] W. Hill, B. Wehling, *J. Phys. Chem.* **1993**, 97, 9451.
- [32] P. F. Liao, J. G. Bergman, D. S. Chemla, A. Wokaun, J. Melngailis, A. M. Hawryluk, N. P. Economou, *Chem. Phys. Lett.* **1981**, 82, 355.
- [33] S. J. Bauman, Z. T. Brawley, A. A. Darweesh, J. B. Herzog, *Sensors* **2017**, 17, 1530.
- [34] P. Mühlischlegel, H. J. Eisler, O. J. F. Martin, B. Hecht, D. W. Pohl, *Science* **2005**, 308, 1607.
- [35] R. J. Potton, *Rep Prog Phys* **2004**, 67, 717.
- [36] U. Kreibig, M. Vollmer, *Optical Properties of Metal Clusters*, Springer, Berlin **1995**.
- [37] B. Hoffmann, M. Y. Bashouti, T. Feichtner, M. Mackovic, C. Dieker, A. M. Salaheldin, P. Richter, O. D. Gordan, D. R. T. Zahn, E. Spiecker, S. Christiansen, *Nanoscale* **2016**, 8, 4529.
- [38] B. J. Barner, R. M. Corn, *Langmuir* **1990**, 6, 1023.
- [39] P. Hildebrandt, M. Stockburger, *J. Phys. Chem.* **1984**, 88, 5935.
- [40] P. Smejkal, B. Vlckova, M. Prochazka, P. Mojzes, J. Pflieger, *Vib. Spectrosc.* **1999**, 19, 243.
- [41] G. S. Shin, J. J. Kim, *Surf. Sci.* **1985**, 158, 286.
- [42] T. H. Joo, M. S. Kim, K. Kim, *J. Raman Spectrosc.* **1987**, 18, 57.
- [43] J. M. Sequaris, E. Koglin, P. Valenta, H. W. Nurnberg, *Ber. Bunsen-Ges. Phys. Chem. Chem. Phys.* **1981**, 85, 512.
- [44] E. Koglin, J. M. Sequaris, *Top Curr Chem* **1986**, 134, 1.
- [45] Y. F. Huang, H. P. Zhu, G. K. Liu, D. Y. Wu, B. Ren, Z. Q. Tian, *J. Am. Chem. Soc.* **2010**, 132, 9244.
- [46] K. Uetsuki, P. Verma, T. Yano, Y. Saito, T. Ichimura, S. Kawata, *J. Phys. Chem. C* **2010**, 114, 7515.
- [47] N. Leopold, B. Lendl, *J. Phys. Chem. B* **2003**, 107, 5723.
- [48] P. A. Mosier-Boss, *Nanomaterials* **2017**, 7, 142.
- [49] B. Sharma, R. R. Frontiera, A. I. Henry, E. Ringe, R. P. Van Duyne, *Mater. Today* **2012**, 15, 16.
- [50] R. J. C. Brown, M. J. T. Milton, *J. Raman Spectrosc.* **2008**, 39, 1313.
- [51] Y. Liu, Y. Zhang, M. Tardivel, M. Lequeux, X. P. Chen, W. Liu, J. Q. Huang, H. Y. Tian, Q. Q. Liu, G. R. Huang, R. Gillibert, M. L. de la Chapelle, W. L. Fu, *Plasmonics* **2020**, 15, 743.
- [52] A. Azziz, W. Safar, Y. Xiang, M. Edely, M. L. de la Chapelle, *J. Mol. Struct.* **2022**, 1248, 131519.
- [53] S. Almagiva, S. Botti, L. Cantarini, R. Fantoni, S. Lecci, A. Palucci, A. Puiu, A. Rufoloni, *J. Raman Spectrosc.* **2014**, 45, 41.
- [54] L. M. Fischer, M. Tenje, A. R. Heiskanen, N. Masuda, J. Castillo, A. Bentien, J. Emneus, M. H. Jakobsen, A. Boisen, *Microelectron. Eng.* **2009**, 86, 1282.
- [55] T. Seesaard, N. Goel, M. Kumar, C. Wongchoosuk, *Comput Electron Agric* **2022**, 193, 106673.
- [56] L. T. Qian, S. Durairaj, S. Prins, A. C. Chen, *Biosens. Bioelectron.* **2021**, 175, 112836.
- [57] J. Kim, A. S. Campbell, B. E. F. de Avila, J. Wang, *Nat. Biotechnol.* **2019**, 37, 389.
- [58] H. B. Zhou, D. T. Yang, N. P. Ivleva, N. E. Mircescu, R. Niessner, C. Haisch, *Anal. Chem.* **2014**, 86, 1525.
- [59] H. F. Liu, E. H. Dai, R. Xiao, Z. H. Zhou, M. L. Zhang, Z. K. Bai, Y. Shao, K. Z. Qi, J. Tu, C. W. Wang, S. Q. Wang, *Sens Actuators B Chem* **2021**, 329, 129196.
- [60] S. Lal, N. K. Grady, G. P. Goodrich, N. J. Halas, *Nano Lett.* **2006**, 6, 2338.
- [61] A. M. Michaels, J. Jiang, L. Brus, *J. Phys. Chem. B* **2000**, 104, 11965.
- [62] J. L. Castro, M. R. Lopez-Ramirez, J. F. Arenas, J. C. Otero, *J. Raman Spectrosc.* **2004**, 35, 997.
- [63] E. Pyrak, J. Krajczewski, A. Kowalik, A. Kudelski, A. Jaworska, *Molecules* **2019**, 24, 4423.
- [64] B. Fazio, C. D'Andrea, A. Foti, E. Messina, A. Irrera, M. G. Donato, V. Villari, N. Micali, O. M. Marago, P. G. Gucciardi, *Sci. Rep.* **2016**, 6, 26952.
- [65] W. L. Peticolas, *Biophys. Biochem. Aspects Fluoresc. Spectrosc.* **1995**, 246, 389.
- [66] S. Rubin, P. H. L. Nguyen, Y. Fainman, *Nanophotonics* **2021**, 10, 1581.
- [67] R. Fernandez-Leiro, J. Conrad, J. C. Yang, S. M. V. Freund, S. H. W. Scheres, M. H. Lamers, *Nat. Struct. Mol. Biol.* **2017**, 24, 140.
- [68] A. Bujacz, *Acta Crystallogr D Biol Crystallogr* **2012**, 68, 1278.
- [69] E. F. Pettersen, T. D. Goddard, C. C. Huang, G. S. Couch, D. M. Greenblatt, E. C. Meng, T. E. Ferrin, *J. Comput. Chem.* **2004**, 25, 1605.
- [70] E. Krauss, R. Kullock, X. F. Wu, P. Geisler, N. Lundt, M. Kamp, B. Hecht, *Cryst. Growth Des.* **2018**, 18, 1297.
- [71] P. G. Etchegoin, E. C. Le Ru, M. Meyer, *J. Chem. Phys.* **2006**, 125, 164705.
- [72] E. Krauss, G. Razinskis, D. Kock, S. Grossmann, B. Hecht, *Nano Lett.* **2019**, 19, 3364.
- [73] K. Kim, D. Shin, J. Y. Choi, K. L. Kim, K. S. Shin, *J. Phys. Chem. C* **2011**, 115, 24960.
- [74] Z. S. Batyrshina, B. Yaakov, R. Shavit, A. Singh, V. Tzin, *BMC Plant Biol* **2020**, 20, 20.