



Nanofabrication Method of Self-Organized Au-Pd Bimetallic Nanostructures Through Thermal Dewetting for LSPR-Biosensing

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The development of efficient plasmonic biosensing platforms relies heavily on precisely engineering noble metallic nanostructures. This study presents a novel approach for the nanofabrication of Au-Pd bimetallic nano-islands on a quartz glass substrate using a self-organization method by thermal dewetting technique. Firstly, the deposition of Au thin films using the sputter coating process followed by controlled thermal annealing to induce the formation of well-defined gold nano-islands on a substrate. Secondly, the Pd thin film is deposited as the second layer on the gold nano-islands film. Thermal annealing was utilized to aggregate the Au-Pd bimetallic nano-islands on a substrate. The influence of annealing parameters such as temperature and duration on the morphology of the Au-Pd bimetallic nano-islands was systematically investigated. Scanning electron microscopy (SEM) and energy dispersive X-ray spectrometer (EDS) were employed to characterize the fabricated nano-island's size, shape, and elemental composition on a substrate.

Furthermore, the plasmonic properties of the Au-Pd bimetallic nano-islands are evaluated using UV-visible spectroscopy. The potential of the fabricated Au-Pd nano-islands as plasmonic biosensing was demonstrated through the Localized Surface Plasmon Resonance property (LSPR). Our results highlight the promising application of Au-Pd bimetallic nano-islands as efficient and sensitive platforms with enhanced plasmonic properties. This study provides insights into the nanofabrication process of bimetallic nanostructures and opens avenues for

developing advanced plasmonic biosensing devices with improved performance.

Keywords: Au-Pd bimetallic nanostructures; Localized Surface Plasmon Resonance (LSPR); Thermal dewetting method, Plasmonic biosensing

1. Introduction

Metallic nanostructures have garnered significant attention in recent years for their remarkable optical properties and potential biosensing applications [1-3]. Optical plasmonic biosensing has emerged as a promising field where metallic nanostructures play a central role [4, 5]. These nanostructures, typically made of noble metals such as gold, silver, palladium, platinum, or aluminum, exhibit localized surface plasmon resonance (LSPR) [6-10]. The LSPR phenomenon arises from the collective oscillation of free electrons in response to incident light [11-13]. The substantial enhancement of the electromagnetic field at the surface of metallic nanostructures enables the susceptible detection of analyte molecules and makes them ideal candidates for bio-sensing applications [14-16]. Furthermore, metallic nanostructures in biosensing platforms offer numerous advantages, including label-free detection, real-time monitoring, high sensitivity, and the potential for multiplexed detection [17-19]. The various types of metallic nanostructures utilized in optical plasmonic bio-sensing and their applications in medical diagnostics [20-22], environmental monitoring [23], food safety [24], agricultural safety [25-27], and beyond.

Conventional nanofabrication processes, while pivotal in creating novel metallic nanostructures on substrates, are not without their challenges. These techniques, which include methods like electron beam lithography (EBL), focused ion beam (FIB) milling, ultraviolet lithography (UVL), imprinting lithography, and physical vapor deposition, have been extensively employed to design and fabricate novel metallic nanostructures with precise control over size, shape, and arrangement. However, their utility is often hampered by several drawbacks. Among the key challenges are high fabrication costs, limited scalability, intricate process steps, and restricted compatibility with diverse substrates. Moreover, achieving uniformity and reproducibility across large areas remains a significant challenge, hindering the practical implementation of metal nanostructures in numerous fields, including electronics, photonics, and sensing. Thus, the need to address these drawbacks and explore alternative nanofabrication approaches becomes evident, underscoring the importance of advancing the development and deployment of metal nanostructures for next-generation technologies.

Traditionally, plasmonic nanostructures have been fabricated using single-metal layers deposited onto substrates, exhibiting distinct LSPR characteristics determined by the metal's properties and the geometry of the nanostructures [41-43]. However, recent research has shown that the optical properties of plasmonic nanostructures can be further optimized by engineering complex architectures, such as bimetallic composed of two types of metal nano-islands deposited on a substrate [44]. Integrating bimetallic nanostructures offers a versatile platform for tailoring the optical response of plasmonic nanostructures [45]. By carefully selecting the materials composing each layer and controlling their geometrical parameters, researchers can tune the LSPR properties to achieve desired functionalities with unprecedented precision. Moreover, the interaction between the two layers enables the manipulation of

plasmonic coupling effects, enhancing the overall performance of the nanostructures, which underscores the potential of bimetallic nanostructures in advancing the field of plasmonic biosensing.

The emergence of nanofabrication techniques has revolutionized various fields, particularly in the realm of optical plasmonic biosensing, where precise control over nanostructures is crucial for enhancing sensitivity and selectivity. Among these methods, the thermal dewetting process stands out as a promising approach for the self-organization of Au-Pd bimetallic nano-islands on substrates. This method harnesses the thermodynamic instabilities at the nanoscale, enabling the spontaneous formation of nanoscale features through surface diffusion and aggregation of thin metal films upon heating. Integrating gold (Au) and palladium (Pd) in a bimetallic configuration offers distinct advantages, including tunable plasmonic properties, enhanced stability, and improved biocompatibility compared to pure Au or Pd nanostructures. Moreover, the self-organization nature of this process facilitates large-scale fabrication with high reproducibility and uniformity, overcoming the limitations associated with conventional nanofabrication techniques. These attributes make self-organized Au-Pd bimetallic nano-islands highly promising for optical plasmonic biosensing applications, where their unique optical properties can be leveraged to achieve ultrasensitive detection of biomolecules.

2. Materials And Methods

2.1 Self-organized Au-Pd bimetallic nanostructures on the substrate by the thermal dewetting process.

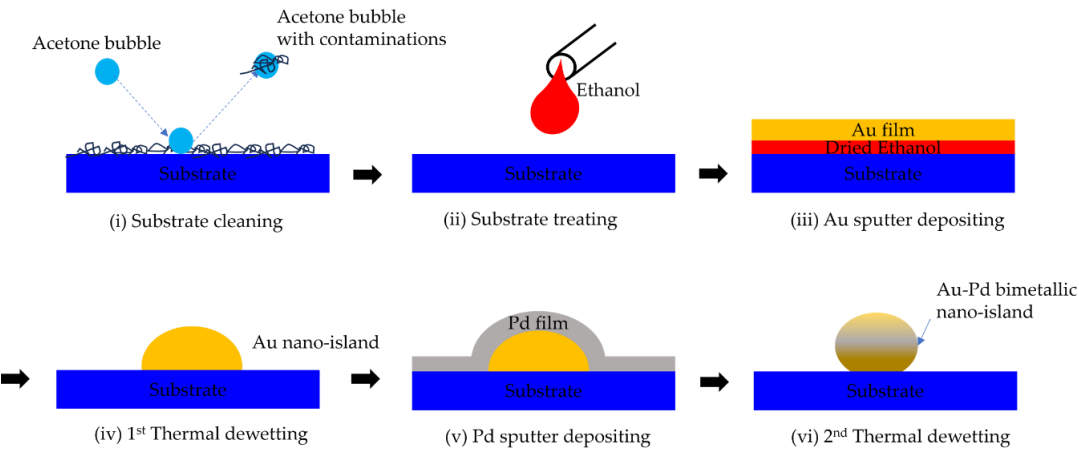


Figure 1. Schematic diagram of self-organized Au-Pd bimetallic nanostructures on the substrate.

Figure 1 illustrates the schematic diagram of the self-organized metallic nanofabrication process through the thermal dewetting process to fabricate Au-Pd bimetallic nanostructures on a quartz glass substrate. Firstly (i), a 1 mm. thickness quartz glass substrate was cut into a square of 1 cm². The substrate surface was thoroughly cleaned in the acetone bath with an ultrasonic cleaning machine (GT Sonic, VGT-1613T) for 15 minutes to remove contaminants. Secondly (ii), a drop of ethanol was treated on the cleaned surface of the substrate to ensure

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good adhesion of the metallic film. Thirdly (iii), a thin film of the gold was deposited onto the prepared substrate surface by sputter coating technique (DC-MCM-200 Ion Sputter Coater, target materials: Au, diameter = 50 mm, target to sample distance: 35 mm). The thickness of the gold film was 30 nm by controlling a sputtering time. Then, (iv) the substrate with the deposited gold thin film was subjected to thermal annealing through an electric furnace (Nabertherm, N 31/H). During annealing, the substrate was heated at a temperature of 700°C for 30 minutes, below the quartz glass substrate's deformation temperature (long-time working temperature: 1050°C) [51]. This critical temperature allowed the gold film to undergo dewetting while preventing damage to the substrate. The gold film was heated with surface diffusion behavior, and capillary forces became dominant; this led to the formation of nano-islands on the substrate surface. The thin gold film was retracted and broken up due to surface energy minimization. The gold droplets coalesced and rearranged into gold nano-islands film spontaneously. As a result, the first layer of gold nano-islands was fabricated onto the substrate surface [52].

However, this experiment wanted to prove that nano-islands made from other materials in the second stage, such as Palladium (Pd), may affect the LSPR optical properties. Then, (v) Palladium is deposited onto the first layer of Au nano-islands film on a quartz glass substrate using a sputtering process (DC-Sputter coater machine, Quorum Tech SC7620), as shown in Figure 2. In this process, Palladium atoms are ejected from a palladium target material and travel through the chamber's high-vacuum environment. These atoms condense onto the substrate, forming a thin film over the gold nano-islands. These techniques allow for the controlled deposition of Pd nanoparticles into the Au nano-islands. The thickness of Pd was 30 nm by adjusting the sputtering time. Finally, (vi) After deposition, gold nano-islands film on a substrate with the deposited Pd was subjected to a thermal annealing process. During thermal annealing, the substrate was heated to 700°C for 30 minutes, the same as the first stage of thermal annealing, in an electric furnace. Annealing temperature and duration can also influence the morphology of the Au-Pd bimetallic nanostructures on a substrate. By controlling these parameters, it is possible to achieve the desired shapes and sizes of Au-Pd nanoparticles as spherical on a substrate, which can have implications for various sensing applications.



Figure 2. Sputter coater machine for Pd depositing process, Quorum Tech SC7620.

2.2 Characterization of Au-Pd bimetallic nanostructures on substrate.

The fabricated Au-Pd bimetallic nanostructures were characterized using scanning electron microscopy and energy dispersive X-ray spectrometer (SEM-EDS, High Vacuum, Quanta 450 FE with Oxford INCA Energy E2H I). SEM-EDS is a powerful tool for analyzing the elemental composition of Au-Pd nano-islands on a substrate, providing valuable information for understanding their properties and potential sensing applications. SEM characterization helps evaluate the fabricated Au-Pd bimetallic nanostructures' dimensions, shape, and distribution. ImageJ was employed to quantify the size distribution of nano-islands on a substrate accurately.

Furthermore, EDS was used in conjunction with SEM to comprehensively analyze the elemental composition of Au-Pd bimetallic nano-islands on a substrate at room temperature using a beam voltage of 15 kV. The SEM was used to locate and focus on the Au-Pd nano-islands. Once the nano-islands were in focus, the electron beam was directed onto the nano-islands to induce X-ray emission. As the electron beam interacts with the Au-Pd nano-islands, X-rays are emitted from the sample. The EDS detector collects these X-rays and measures their energies. Since gold and palladium have distinct X-ray emission spectra, the EDS system can differentiate between them based on the energies of the detected X-rays. EDS can provide both qualitative and quantitative information about the elemental composition of the Au-Pd bimetallic nano-islands. Qualitatively, EDS can confirm the presence of Au and Pd in the nano-islands. Quantitatively, EDS can determine the relative concentrations of Au and Pd in the bimetallic nano-islands film on a quartz glass substrate by measuring the intensity of their characteristic X-ray peaks. In addition to point analysis, SEM-EDS can also be used for elemental mapping of the sample. Elemental maps provide spatial information about the distribution of Au and Pd within the nano-islands and substrate.

2.3 Localized surface plasmon resonance (LSPR) analysis.

In this study, the LSPR analysis using a UV-Vis spectrometer (Aventes, AvaSpec-ULS2048CL-EVO-UA-50) was utilized as a technique specifically designed for studying the optical properties of Au-Pd nano-islands on a substrate. Figure 3 illustrates how the UV-Vis spectrometer was used to measure the optical absorbance spectrum of the Au-Pd nano-islands over a range of wavelengths, typically in the UV to visible range (200-800 nm). The spectrometer, with its UV-Vis light source, monochromator or filter to select specific wavelengths, and a detector to measure the intensity of transmitted light, is ideal for this purpose. The characteristic peaks corresponding to LSPR were observed, typically appearing as sharp absorption bands in the spectrum. The position, shape, and intensity of the LSPR peaks provide valuable information about the size, shape, and dielectric environment of the Au-Pd bimetallic nano-islands.

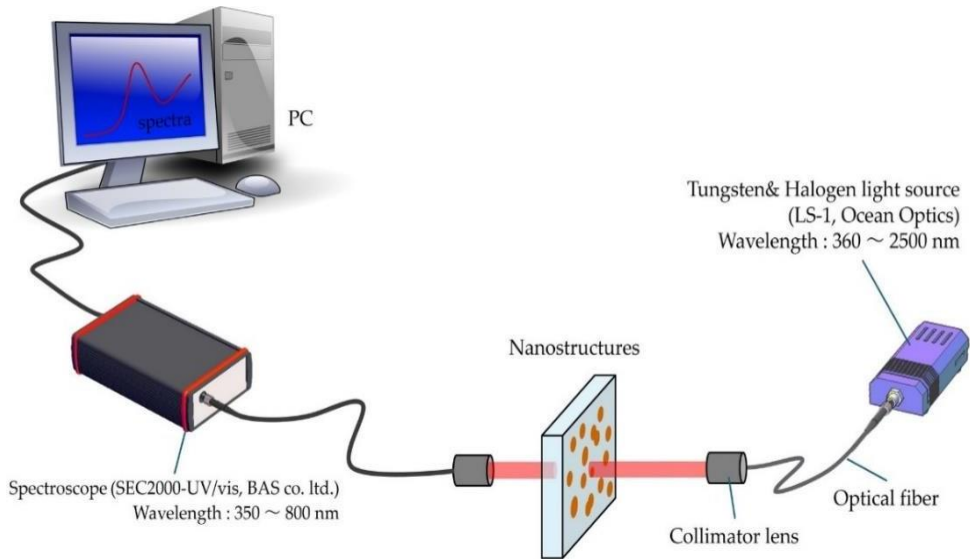


Figure 3. Schematic diagram of the UV-Vis spectrometer to analyze the absorbance spectrum of Au-Pd bimetallic nano-islands on the substrate.

3. Results

3.1 The thermal dewetting process of Au-Pd bimetallic nanostructures on a substrate.

Figure 4 illustrates that the gold nano-islands were aggregated on a quartz glass substrate as the first layer of nanostructures. The gold thin film, with a thickness of 30 nm, was subjected to a thermal annealing process on a quartz glass substrate at 700°C for 30 minutes. Overall, it was found that gold nano-lands had a spherical shape and were distributed throughout the area of the glass substrate. As can be seen, the grain structure of the gold nano-island film was well visible. The average diameter of Au nano-islands was about 20 nm by ImageJ. The experimental parameters of this first layer will be applied to the process of the second layer of palladium using thermal dewetting for Au-Pd bimetallic nanostructures.

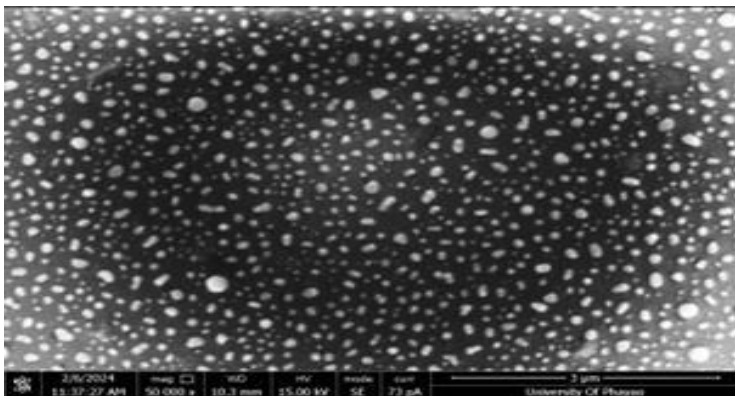


Figure 4. Gold nanostructures on a quartz glass substrate by thermal dewetting process.

Figure 5 illustrates the Au-Pd bimetallic nano-islands agglomerated onto the surface of the quartz glass substrate by the thermal dewetting process. ImageJ found that the average diameter of the Au-Pd nano-islands was about 32 nm. This measurement was significantly larger than the gold nano-island in Figure 4, indicating a clear size difference. However, the Au-Pd bimetallic nano-islands were widely dispersed on a quartz glass substrate. The thermal dewetting temperature for the Pd layer was about 700°C for 30 minutes in an electric furnace, the same as the first stage of thermal dewetting of gold. The results showed that this thermal annealing temperature causes palladium and gold to melt together into an Au-Pd alloy. The Au-Pd bimetallic nano-islands were agglomerated onto the substrate surface as the bimetallic plasmonic nanostructures.

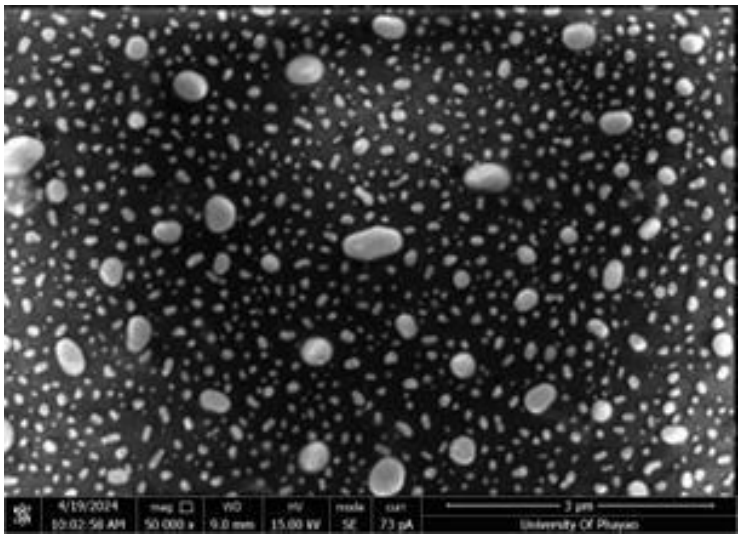


Figure 5. The agglomeration of Au-Pd bimetallic nanostructures on a substrate.

3.2 Energy dispersive X-ray spectrometer analysis of Au-Pd bimetallic nanostructures on a substrate.

The sample under consideration involves a quartz glass substrate hosting a coating containing bimetallic nanostructures composed of gold (Au) and palladium (Pd). Figure 6 (a) displays the outcomes of energy dispersive X-ray spectrometer (EDS) analysis conducted on Au-Pd bimetallic nanostructures deposited on the substrate. This analysis provided crucial insights into the elemental composition and distribution within the substrate. Additionally, leveraging the spatial mapping capabilities of EDS, elemental distribution across the substrate surface was visualized, offering valuable information on the uniformity and morphology of the nanostructures.

The examination confirmed the successful deposition of the bimetallic material onto the substrate, as both gold (Au) and palladium (Pd) elements were found in the nanostructures. Figure 6(b) shows the spatial mapping of gold, indicating that approximately 88.82% of the coating on the quartz glass substrate comprises gold atoms. Similarly, Figure 6(c) illustrates the spatial mapping of palladium (Pd), revealing that palladium constitutes approximately 11.18% of the nanostructures. Further detailed in Figure 7 and Table 1, these findings provide

valuable insight into the relative atomic percentages of gold and palladium within the nanostructures on the quartz glass substrate.

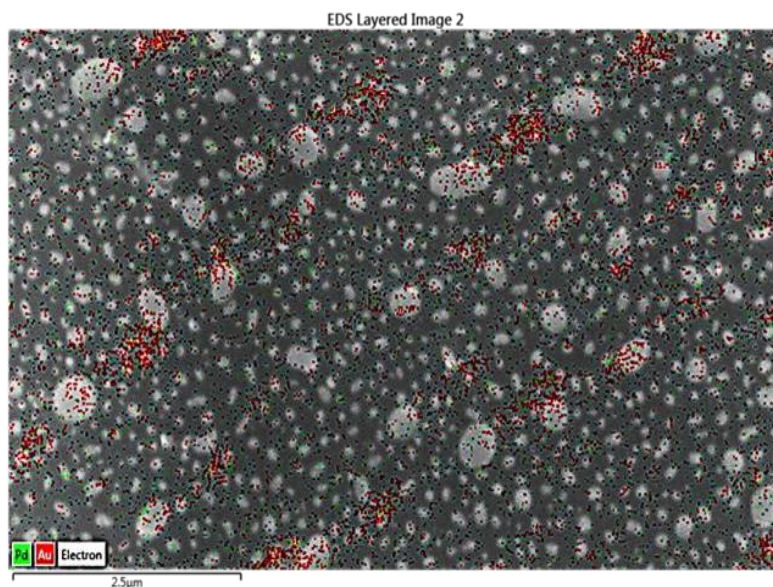


Figure 6 (a). Energy dispersive X-ray spectrometer (EDS) analysis of Au-Pd bimetallic nanostructures on a substrate.

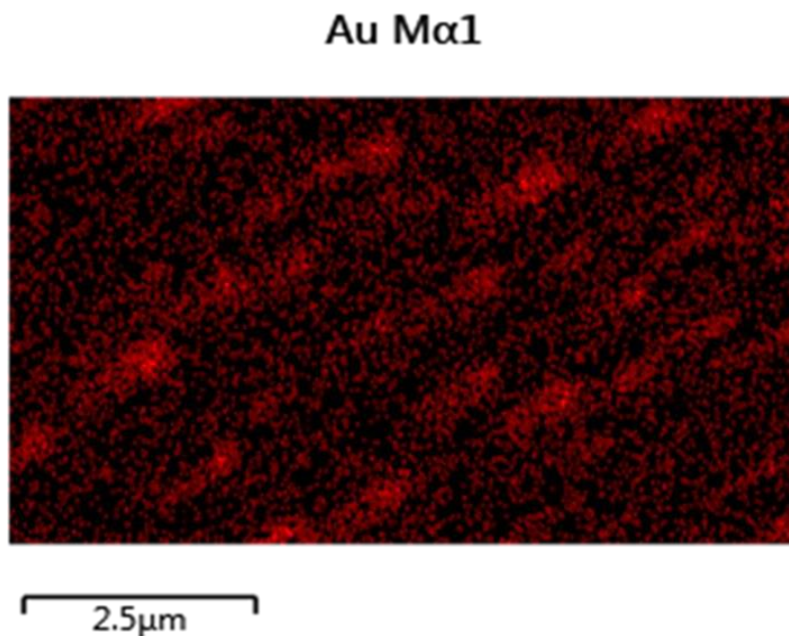


Figure 6 (b). EDS mapping for Au element: the region of mapping corresponds to (a)., acquisition time 80 s.

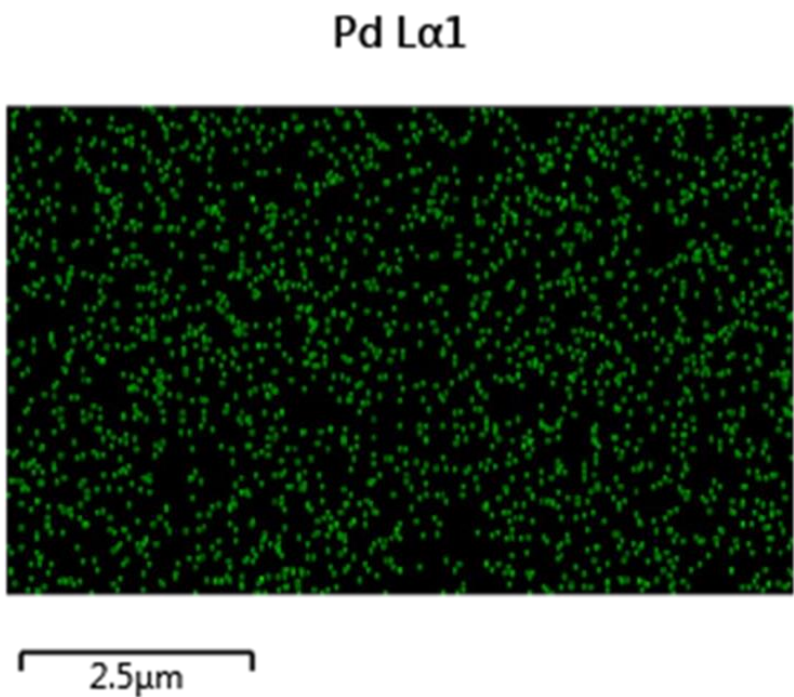


Figure 6 (c). EDS mapping for Pd element: the region of mapping corresponds to (a), acquisition time 80 s.

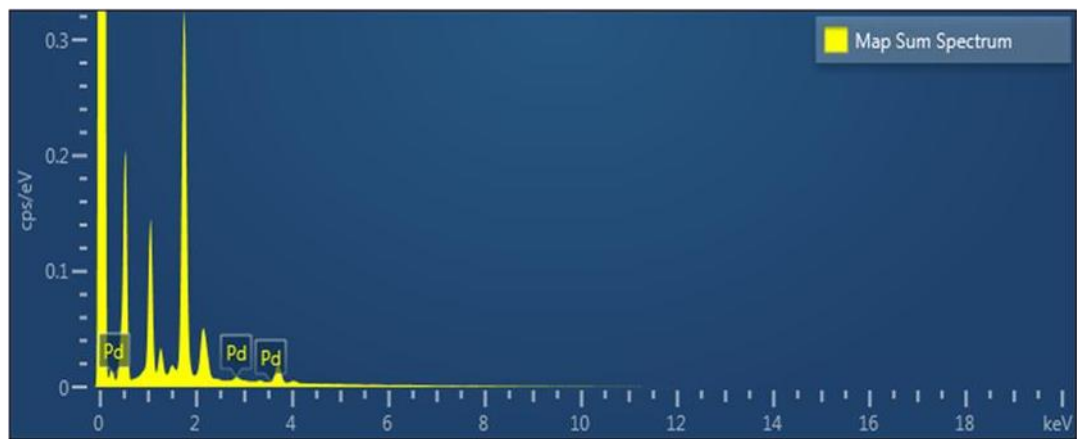


Figure 7. EDS spectrum of Au-Pd bimetallic nanostructures on a quartz glass substrate.

Table 1. Element table of Au-Pd bimetallic nanostructures on a quartz glass substrate.

Element	Weight %	Weight % sigma	Atomic %
Au	88.82	1.53	81.11
Pd	11.18	1.53	18.89
Total	100		100

3.3 Comparison of extinction spectrum of metal nanostructures on substrates.

As shown in Figure 8, the absorbance spectrum of gold nano-islands on a quart glass substrate exhibiting a peak of 0.38 arbitrary units (a.u.) at a wavelength of 550 nm suggests significant absorption of light within the visible spectrum, indicative of the plasmonic properties of gold nanoparticles. This peak likely corresponds to the excitation of surface plasmons in the gold nano-islands, leading to enhanced electromagnetic fields near the surface. In contrast, the absorbance spectrum of Au-Pd bimetallic nano-islands on the same substrate with a peak of 0.6 a.u. at 560 nm wavelength implies a broader absorption profile and potentially different plasmonic behavior due to the presence of palladium alongside gold. The higher absorbance and slightly red-shifted peak wavelength in the Au-Pd bimetallic nano-islands could be attributed to the alloying or surface modification effects induced by the incorporation of palladium. This comparison suggests that the addition of palladium alters the optical properties of the nano-islands, potentially offering tunability and enhanced functionality for various applications such as sensing, catalysis, or photonics.

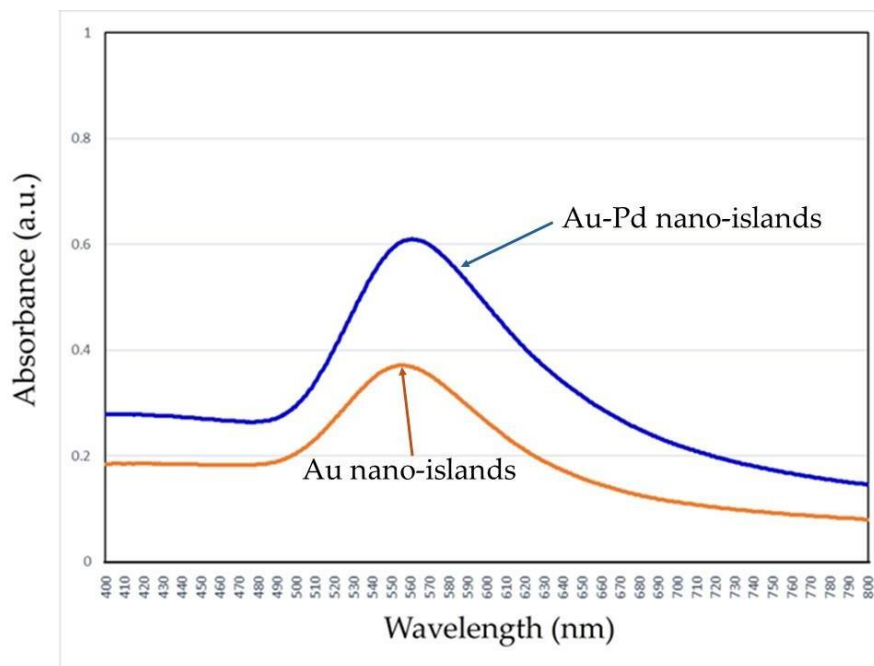


Figure 8. The comparison of absorbance spectrum for Au nano-island with Au-Pd bimetallic nano-islands on a quartz glass substrate.

4. Discussion

The deposition of palladium (Pd) nano-islands on gold (Au) nano-islands on a substrate can yield fascinating properties and applications in nanotechnology. This hierarchical structure combines the unique properties of both Au and Pd, offering synergistic effects that can be leveraged for various purposes, such as catalysis, sensing, and nanoelectronics. Precisely controlling these dimensions of the nano-islands, shapes, and arrangement allows for tailored functionalities and enhanced performance in diverse fields. Moreover, the substrate provides a solid foundation for anchoring the nano-islands, ensuring stability, and facilitating further functionalization into devices.

The self-organization of the thermal dewetting process was employed in this study to create randomized bimetallic nanostructures on a quartz glass substrate. Gold and palladium can be subjected to thermal annealing to enhance their agglomeration behavior. Gold exhibits relative stability and high oxidation resistance, even at elevated temperatures. Typically, the suitable thermal annealing temperature for gold ranges from 400°C to 700°C depending on the thickness of gold thin film [49]. Annealing gold within this temperature range can enhance its agglomeration without significant oxidation.

Similarly, palladium demonstrates stability and oxidation resistance at high temperatures. The suitable thermal annealing temperature for palladium ranges from approximately 300°C to 700°C [50]. However, if gold is alloyed with other metals, the annealing temperature may vary depending on the alloy composition and desired properties [51]. Therefore, this study's selection of thermal dewetting temperatures at 700°C aimed to prevent the oxidation of gold and palladium in the thermal dewetting process.

The deposition of a first layer of gold nano-islands on a glass substrate plays a crucial role in influencing the agglomeration behavior of the subsequent layer of palladium nano-islands in Au-Pd bimetallic nano-islands on the substrate. The gold nano-islands serve as anchors for the attachment of palladium atoms during the deposition process, facilitating the growth of the Au-Pd nano-islands. Additionally, the presence of the gold layer alters the surface energy and diffusion kinetics of palladium atoms, affecting their aggregation behavior on the substrate, which can result in controlled palladium nano-island growth, preventing excessive agglomeration during deposition. Moreover, the intimate contact and interdiffusion between the gold and palladium atoms at the interface contribute to forming alloyed regions, further stabilizing the bimetallic nanostructures and hindering agglomeration. Overall, the presence of the first layer of gold nano-islands on the glass substrate plays a pivotal role in regulating the agglomeration behavior of the second layer of palladium nano-islands, thereby influencing the morphology, stability, and optical properties of the resulting Au-Pd bimetallic nanostructures on the substrate. Further experimental investigations can provide valuable guidance for the rational design and fabrication of functional nanomaterials.

The enhanced LSPR absorbance spectrum of AU-Pd alloy nano-islands on a quartz glass substrate compared to gold nano-islands can be attributed to several factors. Incorporating palladium into the gold matrix alters states' electronic structure and density, enhancing plasmonic properties [53]. Additionally, the presence of palladium promotes the formation of alloy nanostructures with improved stability and increased density of active sites for plasmonic resonance [54]. These alloy nano-islands exhibit a more pronounced absorbance spectrum due

to the synergistic effects of alloy composition and morphology, resulting in a higher and steeper absorbance profile than pure gold nano-islands [55]. This phenomenon highlights the potential of Au-Pd alloy nanostructures for applications in sensing, catalysis, and optical devices where enhanced plasmonic properties are desirable. The presented nanofabrication method offers several advantages for synthesizing Au-Pd bimetallic nanostructures with enhanced plasmonic properties, particularly for biosensing applications. The self-organization approach through thermal dewetting simplifies the process, allowing for the spontaneous formation of well-defined nano-islands without complex lithographic techniques. This results in a more straightforward and more cost-effective fabrication process. Overall, the demonstrated capability of the fabricated Au-Pd nano-islands to exhibit Localized Surface Plasmon Resonance (LSPR) highlights their suitability as efficient platforms for label-free biosensing with improved performance and paves the way for the development of advanced plasmonic biosensing devices with broader applications in medical diagnostics.

The nanofabrication method of self-organized Au-Pd bimetallic nanostructures through thermal dewetting offers several advantages over conventional techniques. Thermal dewetting leverages the natural tendency of thin metal films to dewet when heated, forming nanostructures straightforwardly and cost-effectively. By heating a thin film of Au-Pd alloy on a substrate, the film breaks into isolated nanoparticles due to the depreciation of surface energy. This scalable process can be applied to large-area substrates, enabling high-throughput production of nanostructures. In contrast, conventional methods like electron-beam lithography (EBL) and focused ion beam (FIB) lithography are expensive, complex, and limited in scalability and throughput, requiring sophisticated equipment and cleanroom facilities. These limitations hinder the precise fabrication of nanostructures needed for high-performance plasmonic biosensors. Thermal dewetting provides fine control over nanostructures by adjusting parameters such as film thickness, alloy composition, and annealing temperature. This process is an attractive alternative for cost-effective and precise plasmonic biosensor fabrication.

The reproducibility of the nanofabrication method for self-organized Au-Pd bimetallic nanostructures via thermal dewetting is significantly influenced by the initial conditions and precision in controlling the process parameters. Uniform deposition of the Au-Pd film is crucial as even slight variations in film thickness can lead to discrepancies in the resulting nanostructure size and distribution. Pre-patterned substrates can enhance reproducibility by providing consistent nucleation sites, leading to uniform dewetting behavior across different samples. Ensuring high-quality deposition techniques can mitigate variability and improve reproducibility [56]. However, several challenges and limitations exist in this nanofabrication method. The interaction between the metal film and the substrate can introduce inconsistencies, especially if the surface is not uniform. The annealing process also requires precise control; deviations in temperature or duration can significantly alter the diffusion dynamics, leading to either insufficient dewetting or excessive coalescence of the nanostructures. Environmental factors, such as ambient temperature, humidity, and contamination levels, can further impact the dewetting process, necessitating controlled environments to ensure consistent results. Overcoming these challenges requires careful optimization of deposition techniques, thermal control systems, and the use of high-quality substrates to achieve stable and reproducible nanostructures.

Plasmonic biosensing platforms are highly valued for their exceptional sensitivity and low detection limits, making them suitable for various biomedical and environmental applications. These platforms leverage the unique optical properties of metallic nanoparticles, such as gold, silver, palladium, platinum, or aluminum, which exhibit localized surface plasmon resonances (LSPR). When these nanoparticles interact with biomolecules, changes in the local refractive index near the nanoparticle surface lead to measurable shifts in the LSPR wavelength, enabling highly sensitive detection. Sensitivity in plasmonic biosensors is influenced by factors such as nanoparticle material and shape, with gold preferred for stability and biocompatibility [57]. Surface functionalization with specific ligands or antibodies increases selectivity and reduces background noise. Precision in measuring LSPR peak shifts using advanced spectroscopic techniques enhances sensitivity. Maintaining controlled conditions like temperature, pH, and ionic strength is essential for reproducible sensitivity. Low detection limits are achieved through high surface-to-volume ratios of nanoparticles, signal amplification techniques, optimized sensing architectures, and innovative data analysis methods [58]. Compared to traditional methods like ELISAs or fluorescence-based assays, plasmonic biosensors offer label-free detection, real-time monitoring, and the potential for miniaturization and portable integration.

Au-Pd bimetallic nano-islands significantly enhance biosensor sensitivity and selectivity by combining gold's biocompatibility and stability with palladium's catalytic properties, leading to pronounced shifts in the LSPR effect upon analyte binding. Palladium's catalytic activity amplifies signals in biosensing applications through detectable changes, such as colorimetric or electrochemical signals, which is helpful in enzyme-mimicking activities. Incorporating palladium improves the sensors' structural stability and durability, protecting the gold surface from degradation and ensuring long-term reliability. Additionally, Au-Pd nano-islands support multiplex detection by functionalizing their surface with different recognition elements, enabling simultaneous detection of multiple analytes. Their enhanced properties make them ideal for point-of-care testing (POCT), offering rapid and accurate diagnostics, and for environmental monitoring, detecting trace pollutants, toxins, or pathogens. In the food industry, this platform ensures safety by detecting contaminants, and in biomedical research, it also facilitates early disease diagnosis and personalized medicine [59]. Integrating Au-Pd bimetallic nano-islands into practical biosensing devices involves synthesizing and characterizing these nano-islands to ensure optimal size and distribution, followed by functionalizing their surfaces with specific ligands, antibodies, or aptamers for selective target biomolecule binding. These nano-islands, known for their plasmonic and catalytic properties, enhance signal amplification. They are integrated with transducers, such as optical or electrochemical interfaces, to convert biological interactions into measurable signals. Incorporating these nano-islands into microfluidic devices allows precise sample flow control, improving sensitivity and efficiency.

Future developments in plasmonic biosensing aim to further improve sensitivity and detection limits through material innovations that explore new plasmonic materials and composites with enhanced optical properties, advancements in surface chemistry for more robust and specific functionalization techniques, integration with microfluidic systems to handle small sample volumes and to improve analyte transport, and leveraging AI for better signal interpretation and reduction of false positives/negatives. Ongoing research and technological advancements

promise to push these limits further, expanding the applicability of plasmonic biosensors in various fields.

5. Conclusions

The efficient nanofabrication process described in the manuscript involves the formation of gold (Au) nano-islands as the first layer on a quartz glass substrate, followed by the deposition of a second layer of palladium (Pd) nano-islands to create Au-Pd bimetallic nanostructures. The thermal dewetting process applied to the 30 nm thick gold film at 700°C for 30 minutes resulted in the aggregation of spherical gold nano-islands with an average diameter of approximately 20 nm, well-distributed across the glass substrate. These experimental parameters were then applied to the deposition of the Pd layer, which also underwent thermal dewetting at the same temperature and duration. The resulting Au-Pd bimetallic nano-islands exhibited a larger average diameter of about 32 nm than the gold nano-islands. However, they were widely dispersed on the substrate surface. Energy dispersive X-ray spectrometer (EDS) analysis confirmed the presence of both Au and Pd elements in the nanostructures, with gold constituting approximately 88.82% and palladium approximately 11.18%. The absorbance spectra of the Au and Au-Pd nano-islands revealed distinct optical properties, with the Au-Pd nano-islands exhibiting a higher absorbance peak at 560 nm compared to the Au nano-islands at 550 nm. This difference suggests altered plasmonic behavior in the bimetallic nanostructures due to the incorporation of palladium, potentially enhancing their functionality for sensing applications.

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References

1. Kumar, S., & Seo, S. (2023). Plasmonic sensors: A new frontier in nanotechnology. *Biosensors*, 13(385).
2. Hammond, J. L., Bhalla, N., Rafiee, S. D., & Estrela, P. (2014). Localized surface plasmon resonance as a biosensing platform for developing countries. *Biosensors*, 4(172-188).
3. Côco, A. S., Campos, F. V., Díaz, C. A. R., Guimarães, M. C. C., Prado, A. R., & de Oliveira, J. P. (2023). Localized surface plasmon resonance-based nanosensor for rapid detection of glyphosate in food samples. *Biosensors*, 13(512).
4. Hamza, M. E., Othman, M. A., & Swillam, M. A. (2022). Plasmonic biosensors: Review. *Biology*, 11(621).
5. Acunzo, A., Scardapane, E., De Luca, M., Marra, D., Velotta, R., & Minopoli, A. (2022). Plasmonic nanomaterials for colorimetric biosensing: A review. *Chemosensors*, 10(136).
6. Morsin, M., Mat Salleh, M., Ali Umar, A., & Sahdan, M. Z. (2017). Gold nanoplates for a

- localized surface plasmon resonance-based boric acid sensor. *Sensors*, 17(947).
7. Phetsahai, A., Eiamchai, P., Thamaphat, K., & Limsuwan, P. (2023). The morphological evolution of self-assembled silver nanoparticles under photoirradiation and their SERS performance. *Processes*, 11(2207).
8. Sousanis, A., & Biskos, G. (2021). Thin film and nanostructured Pd-based materials for optical H₂ sensors: A review. *Nanomaterials*, 11(3100).
9. Demishkevich, E., Zyubin, A., Seteikin, A., Samusev, I., Park, I., Hwangbo, C. K., Choi, E. H., & Lee, G. J. (2023). Synthesis methods and optical sensing applications of plasmonic metal nanoparticles made from rhodium, platinum, gold, or silver. *Materials*, 16(3342).
10. Ushanov, V. I., Ereemeev, S. V., Silkin, V. M., & Chaldyshev, V. V. (2024). Plasmon resonance in a system of Bi nanoparticles embedded into (Al,Ga)As matrix. *Nanomaterials*, 14(109).
11. Unser, S., Bruzas, I., He, J., & Sagle, L. (2015). Localized surface plasmon resonance biosensing: Current challenges and approaches. *Sensors*, 15(15684-15716).
12. Babicheva, V. E. (2023). Optical processes behind plasmonic applications. *Nanomaterials*, 13(1270).
13. Meira, D. I., Rodrigues, M. S., Borges, J., & Vaz, F. (2023). Enhancing the extinction efficiency and plasmonic response of bimetallic nanoparticles of Au-Ag in robust thin film sensing platforms. *Sensors*, 23(9618).
14. Kim, D. M., Park, J. S., Jung, S.-W., Yeom, J., & Yoo, S. M. (2021). Biosensing applications using nanostructure-based localized surface plasmon resonance sensors. *Sensors*, 21(3191).
15. Nguyen, D. D., Lee, S., & Kim, I. (2023). Recent advances in metaphotonic biosensors. *Biosensors*, 13(631).
16. Choi, H. K., & Yoon, J. (2023). Nanotechnology-assisted biosensors for the detection of viral nucleic acids: An overview. *Biosensors*, 13(208).
17. Mousavi, M. Z., Chen, H.-Y., Hou, H.-S., Chang, C.-Y.-Y., Roffler, S., Wei, P.-K., & Cheng, J.-Y. (2015). Label-free detection of rare cell in human blood using gold nano slit surface plasmon resonance. *Biosensors*, 5(98-117).
18. Zhang, T., Quan, X., Cao, N., Zhang, Z., & Li, Y. (2022). Label-free detection of DNA via surface-enhanced Raman spectroscopy using Au@Ag nanoparticles. *Nanomaterials*, 12(3119).
19. Lee, K.-L., Wu, T.-Y., Hsu, H.-Y., Yang, S.-Y., & Wei, P.-K. (2017). Low-cost and rapid fabrication of metallic nanostructures for sensitive biosensors using hot-embossing and dielectric-heating nanoimprint methods. *Sensors*, 17(1548).
20. Mauriz, E. (2020). Recent progress in plasmonic biosensing schemes for virus detection. *Sensors*, 20(4745).
21. Chung, T., Lee, S.-Y., Song, E. Y., Chun, H., & Lee, B. (2011). Plasmonic nanostructures for nano-scale bio-sensing. *Sensors*, 11(10907-10929).
22. Dong, C., Wang, Y., Zhao, X., Bian, J., & Zhang, W. (2023). Chemical sensing and analysis with optical nanostructures. *Chemosensors*, 11(497).
23. Long, F., Zhu, A., & Shi, H. (2013). Recent advances in optical biosensors for environmental monitoring and early warning. *Sensors*, 13(13928-13948).
24. Qiao, X., He, J., Yang, R., Li, Y., Chen, G., Xiao, S., Huang, B., Yuan, Y., Sheng, Q., & Yue, T. (2022). Recent advances in nanomaterial-based sensing for food safety analysis. *Processes*, 10(2576).
25. Quintanilla-Villanueva, G. E., Maldonado, J., Luna-Moreno, D., Rodríguez-Delgado, J. M., Villarreal-Chiu, J. F., & Rodríguez-Delgado, M. M. (2023). Progress in plasmonic sensors as monitoring tools for aquaculture quality control. *Biosensors*, 13(90).
26. Lazarević-Pašti, T., Tasić, T., Milanković, V., & Potkonjak, N. (2023). Molecularly imprinted plasmonic-based sensors for environmental contaminants—current state and future perspectives. *Chemosensors*, 11(35).
27. Tomassetti, M., Martini, E., Campanella, L., Favero, G., Sanzón, G., & Mazzei, F. (2015). A new

- surface plasmon resonance immunosensor for triazine pesticide determination in bovine milk: A comparison with conventional amperometric and screen-printed immunodevices. *Sensors*, 15(10255-10270).
28. Berger, L., Jurczyk, J., Madajska, K., Szymańska, I. B., & Hoffmann, P. (2021). Room temperature direct electron beam lithography in a condensed copper carboxylate. *Micromachines*, 12(580).
29. Ko, T., Kumar, S., Shin, S., Seo, D., & Seo, S. (2023). Colloidal quantum dot nanolithography: Direct patterning via electron beam lithography. *Nanomaterials*, 13(2111).
30. Baracu, A. M., Avram, M. A., Breazu, C., Bunea, M.-C., Socol, M., Stanculescu, A., Matei, E., Thrane, P. C. V., Dirdal, C. A., & Dinescu, A. (2021). Silicon metalens fabrication from electron beam to UV-nanoimprint lithography. *Nanomaterials*, 11(2329).
31. Wen, Q., Wei, X., Jiang, F., Lu, J., & Xu, X. (2020). Focused ion beam milling of single-crystal sapphire with A-, C-, and M-orientations. *Materials*, 13(2871).
32. Caruso, R., Camino, F., Gu, G., Tranquada, J. M., Han, M.-G., Zhu, Y., Bollinger, A. T., & Božović, I. (2023). Effects of focused ion beam lithography on $\text{La}_{2-x}\text{Sr}_x\text{CuO}_4$ single crystals. *Condens. Matter*, 8(35).
33. Fürjes, P. (2019). Controlled focused ion beam milling of composite solid state nanopore arrays for molecule sensing. *Micromachines*, 10(774).
34. Butt, M. A. (2023). Integrated optics: Platforms and fabrication methods. *Encyclopedia*, 3(824-838).
35. Zuo, F., Ma, S., Zhao, W., Yang, C., Li, Z., Zhang, C., & Bai, J. (2023). An ultraviolet-lithography-assisted sintering method for glass microlens array fabrication. *Micromachines*, 14(2055).
36. Thanner, C., & Eibelhuber, M. (2021). UV nanoimprint lithography: Geometrical impact on filling properties of nanoscale patterns. *Nanomaterials*, 11(822).
37. Muehlberger, M. (2022). Nanoimprinting of biomimetic nanostructures. *Nanomanufacturing*, 2(17-40).
38. Mühlberger, M. M., Kopp, S., Deyett, A. A., Pribyl, M., Haslinger, M. J., Siegel, A. M., Taus, P., Guillén, E., Torres-Caballero, A., Baltov, B., et al. (2023). Nanoimprinted hierarchical micro-/nanostructured substrates for the growth of cardiomyocyte fibers. *Nanomanufacturing*, 3(416-433).
39. Yin, M., Sun, H., & Wang, H. (2018). Resist filling study for UV nanoimprint lithography using stamps with various micro/nano ratios. *Micromachines*, 9(335).
40. Grigoriev, F. V., & Sulimov, V. B. (2023). Atomistic simulation of physical vapor deposition of optical thin films. *Nanomaterials*, 13(1717).
41. Ebrahimzadeh Esfahani, N., Kováč, J., Jr., Kováčová, S., & Feiler, M. (2023). Plasmonic properties of the metal nanoparticles (NPs) on a metal mirror separated by an ultrathin oxide layer. *Photonics*, 10(78).
42. Badshah, M. A., Koh, N. Y., Zia, A. W., Abbas, N., Zahra, Z., & Saleem, M. W. (2020). Recent developments in plasmonic nanostructures for metal enhanced fluorescence-based biosensing. *Nanomaterials*, 10(1749).
43. Taghipour, A., & Heidarzadeh, H. (2022). Design and analysis of highly sensitive LSPR-based metal-insulator-metal nano-discs as a biosensor for fast detection of SARS-CoV-2. *Photonics*, 9(542).
44. Mamonova, D. V., Vasileva, A. A., Petrov, Y. V., Koroleva, A. V., Danilov, D. V., Kolesnikov, I. E., Bikbaeva, G. I., Bachmann, J., & Manshina, A. A. (2022). Single step laser-induced deposition of plasmonic Au, Ag, Pt mono-, bi- and tri-metallic nanoparticles. *Nanomaterials*, 12(146).
45. Prosa, M., Bolognesi, M., Fornasari, L., Grasso, G., Lopez-Sanchez, L., Marabelli, F., & Toffanin, S. (2020). Nanostructured organic/hybrid materials and components in miniaturized

- optical and chemical sensors. *Nanomaterials*, 10(480).
46. Ganesh, K. M., Bhaskar, S., Cheerla, V. S. K., Battampara, P., Reddy, R., Neelakantan, S. C., Reddy, N., & Ramamurthy, S. S. (2024). Review of gold nanoparticles in surface plasmon-coupled emission technology: Effect of shape, hollow nanostructures, nano-assembly, metal-dielectric and heterometallic nanohybrids. *Nanomaterials*, 14(111).
47. Kim, M., Ahn, H.-J., Silalahi, V. C., Heo, D., Adhikari, S., Jang, Y., Lee, J., & Lee, D. (2023). Dual-dewetting process for self-assembled nanoparticle clusters in wafer scale. *Int. J. Mol. Sci.*, 24(13102).
48. Badán, J. A., Navarrete-Astorga, E., Henríquez, R., Jiménez, F. M., Ariosa, D., Ramos-Barrado, J. R., & Dalchiele, E. A. (2022). Silver nanoparticle arrays onto glass substrates obtained by solid-state thermal dewetting: A morphological, structural and surface chemical study. *Nanomaterials*, 12(617).
49. Sharipova, A., Zlotver, I., Sosnik, A., & Rabkin, E. (2023). Solid-state dewetting of thin Au films for surface functionalization of biomedical implants. *Materials*, 16(7524).
50. Li, G., Zhang, W., Luo, N., Xue, Z., Hu, Q., Zeng, W., & Xu, J. (2021). Bimetallic nanocrystals: Structure, controllable synthesis and applications in catalysis, energy and sensing. *Nanomaterials*, 11(1926).
51. Łapiński, M., Drózd, P., Gołębiowski, M., Okoczuk, P., Karczewski, J., Sobanska, M., Pietruczik, A., Zytewicz, Z. R., Zdyb, R., & Sadowski, W. (2023). Thermal instability of gold thin films. *Coatings*, 13(1306).
52. Wu, J. A., Huang, C.-Y., Wu, W.-W., & Chen, C. (2018). Fabrication of (111)-oriented nanotwinned Au films for Au-to-Au direct bonding. *Materials*, 11(2287).
53. Awada, C., & Ruffino, F. (2023). A study of the laser-assisted alloying effect on plasmonic properties of Au-Pd nanostructured film using surface-enhanced Raman spectroscopy. *Coatings*, 13(797).
54. Alaqarbeh, M., Adil, S. F., Ghrear, T., Khan, M., Bouachrine, M., & Al-Warthan, A. (2023). Recent progress in the application of palladium nanoparticles: A review. *Catalysts*, 13(1343).
55. Bruno, L., Scuderi, M., Priolo, F., Falcioia, L., & Mirabella, S. (2023). Enlightening the bimetallic effect of Au@Pd nanoparticles on Ni oxide nanostructures with enhanced catalytic activity. *Scientific Reports*, 13(3203).
56. Altomare, M., Nguyen, N. T., & Schmuki, P. (2016). Templated dewetting: Designing entirely self-organized platforms for photocatalysis. *Chemical Science*, 7(11), 6865-6886.
57. Liu, Y., Zhang, N., Li, P., Yu, L., Chen, S., Zhang, Y., Jing, Z., & Peng, W. (2019). Low-cost localized surface plasmon resonance biosensing platform with a response enhancement for protein detection. *Nanomaterials*, 9(7), 1019.
58. Zhang, H., Zhou, X., Li, X., Gong, P., Zhang, Y., & Zhao, Y. (2023). Recent advancements of LSPR fiber-optic biosensing: Combination methods, structure, and prospects. *Biosensors*, 13(3), 405.
59. Gao, F., & Goodman, D. W. (2012). Pd–Au bimetallic catalysts: Understanding alloy effects from planar models and (supported) nanoparticles. *Chemical Society Reviews*, 41(21), 8009-8020.