



DEVELOPMENT OF ADVANCED MICRO-NANOTOPOLOGICAL PLATFORMS FOR POINT-OF-CARE DIAGNOSTICS

By

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ABSTRACT

Topologically designed nano- and micro-structures have emerged as powerful tools for enhancing the detection of trace molecules in point-of-care (P-o-C) diagnostic devices, particularly for diseases such as traumatic brain injury (TBI). These structures, often fabricated using photolithography that includes photoresists on silicon wafers, offer precise control over certain parameters and significantly improve the detection of low-concentration biomarkers.

A key application of these structures is surface-enhanced Raman scattering (SERS), which amplifies the Raman signals by several orders of magnitude. By carefully designing the topology of metal-coated structures, it is possible to create highly localised electric fields that enhance the Raman scattering of molecules adsorbed on their surfaces. This enables the detection of trace biomarkers, providing early diagnosis and intervention for conditions such as TBI.

The research presented here explores the optimisation of topological nano- and micro-structures *via* photolithography to maximise SERS signal enhancement. By analysing various design parameters, including the shape, size, and arrangement of structures, optimal configurations were identified, which led to significant improvements in the detection of benzenethiol molecules and some TBI biomarkers. The impact of different metallic coatings, such as gold and silver, on SERS performance and the choice of laser wavelength to obtain high-quality Raman spectra was investigated.

The developed structures have the potential to revolutionise P-o-C diagnostics by enabling rapid, accurate, and sensitive detection of a wide range of biomarkers. This rapid fabrication method using photolithography holds promise for improving healthcare outcomes and facilitating early intervention in various diseases, including TBI.

DEDICATION

To ReX;

We did this together.

Your joyous smile and curiosity got us through

We persevered, and we got to the end

We calmed ourselves, and the storm passed

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'In complexity, find simplicity.
From discord, find harmony.
In the middle of difficulty lies opportunity.'

Author unknown

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Acronyms

<i>2D</i>	Two dimensional. 70	<i>MBP</i>	Myelin basic protein. 92
<i>3D</i>	Three dimensional. 32	<i>MCI</i>	Mild cognitive impairment. 6
<i>AAO</i>	Anodic aluminium oxide. 96	<i>MEMS</i>	Micro-electro-mechanical system. 22
<i>AChE</i>	Acetylcholinesterase. 91	<i>NA</i>	Numerical aperture. 51
<i>AD</i>	Alzheimer's disease. 6	<i>NADPH</i>	Nicotinamide adenine dinucleotide phosphate. 91
<i>AFM</i>	Atomic force microscopy/microscope. 19	<i>NF – H</i>	Neurofilament heavy chain protein. 92
<i>ASC</i>	Apoptosis-associated speck-like protein. 92	<i>NFL</i>	Neurofilament light. 91
<i>BT</i>	Benzenethiol. 23	<i>NLR</i>	Nucleotide-binding oligomerization domain (NOD)- like receptor. 92
<i>CAD</i>	Computer-aided design. 47	<i>NLRP3</i>	Nucleotide-binding oligomerization (NOD)-like receptors(NLRs) family pyrin domain-containing 3 inflammasomes. 92
<i>CCD</i>	Charge-coupled device. 45	<i>NMR</i>	Nuclear magnetic resonance. 94
<i>CSF</i>	Cerebrospinal fluid. 91	<i>NOD</i>	Nucleotide-binding oligomerization domain. 92
<i>CTE</i>	Chronic traumatic encephalopathy. 7	<i>NOX₂</i>	Nicotinamide adenine dinucleotide phosphate ox- idases. 91
<i>DNQ</i>	Diazo naphthoquinone-sulphonate. 33	<i>NSE</i>	Neuron-specific enolase. 92
<i>EF</i>	Enhancement factor. 57	<i>Nm</i>	Newtonmetre. 86
<i>EM</i>	Electromagnetic theory. 56	<i>OM</i>	Optical microscopy/microscope. 19
<i>GFAP</i>	Glial acidic fibrillary protein. 92	<i>P – o – C</i>	Point-of-Care. 15
<i>H – FABP</i>	Heart fatty acid-binding protein. 92	<i>PDMS</i>	Poly (dimethyl siloxane). 96
<i>I</i>	Intensity. 37		
<i>IL</i>	Interleukin. 92		
<i>LSPR</i>	Localised surface plasmon resonance. 56		
<i>MAP2</i>	Microtubule-associated protein-2. 92		

<i>PEB</i> Post-exposure bake. 32	<i>mW</i> Milliwatts. 45
<i>PGMEA</i> 2-methoxy-1-methyl ethyl acetate. 28	<i>min</i> Minute. 76
<i>PL</i> Photolithography. 22	<i>mm</i> Millimetre. 23
<i>PMMA</i> Poly(methylmethacrylate). 96	<i>nm</i> Nanometre. 30
<i>RRS</i> Resonance Raman spectroscopy. 94	<i>rpm</i> Rotations per minute. 29
<i>RS</i> Raman spectroscopy. 52	<i>s</i> Seconds. 37
<i>S/N</i> Signal-to-Noise. 17	<i>tau</i> Tubulin associated unit. 91
<i>SCD</i> Spray carbon dioxide. 24	<i>v/v</i> Volume by volume. 85
<i>SEM</i> Scanning electron microscopy/microscope. 43	$\alpha - syn$ Alpha-synuclein. 91
<i>SERS</i> Surface-enhanced Raman scattering. 10	λ Wavelength. 51
<i>SPM</i> Statistical parametric mapping. 43	μL Microlitre. 29
<i>SPR</i> Surface plasmon resonance. 12	μW Microwatts. 39
<i>Si</i> Silicon. 23	μm Micrometre/micron. 23
<i>TBI</i> Traumatic brain injury. 6	
<i>TERS</i> Tip-enhanced Raman spectroscopy. 82	
<i>UCH – L1</i> Ubiquitin C-terminal hydrolase-L1. 92	
<i>UV</i> Ultraviolet. 25	
<i>WHO</i> World Health Organisation. 121	
<i>XPS</i> X-ray photoelectron spectroscopy. 94	
Ω Ohm. 23	
<i>cm</i> Centimetre. 23	
<i>kHz</i> Kilohertz. 86	
<i>kV</i> Kilovolt. 44	
<i>mJ</i> Millijoule. 37	
<i>mM</i> Millimolar. 23	

List of Publications

Journal Articles

1. **Odetade, D. F., Rickard, J. J. S. & Goldberg Oppenheimer, P. Development and Optimization of Micro-Nanotopographical Platforms for Surface Enhanced Raman Scattering Biomolecular Detection.** Adv. Mater. Interfaces 2400352, (2024).
2. Stokes, K., Clark, K., **Odetade, D.**, Hardy, M. & Goldberg Oppenheimer, P. **Advances in lithographic techniques for precision nanostructure fabrication in biomedical applications.** Discov. nano 18, 153 (2023).

Oral Presentations

1. **International Congress on Analytical Nanoscience and Nanotechnology**, Santiago de Compostela, Spain. September 2024 – ‘**Topologically-fabricated nanostructures for surface-enhanced Raman spectroscopy**’.
2. **ANMSA Symposium, University of Birmingham**, United Kingdom. March 2024 – ‘**... on the path to solving macro problems using surface-enhanced Raman spectroscopy (SERS) through micro-engineering of materials**’.

Poster Presentations

1. **International Congress on Analytical Nanoscience and Nanotechnology**, Santiago de Compostela, Spain. September 2024 – ‘**Topologically-fabricated nanostructures for surface-enhanced Raman spectroscopy**’.
2. **2nd International Conference on Advance Nanomaterials and Nanotechnology**, Vienna, Austria. November 2023 – ‘**Topological design of nanostructured SERS substrates for early detection of traumatic brain injury**’.

CHAPTER ONE

INTRODUCTION

1.1 A WORLD OF PATTERNS

Nature has a cornucopia of patterns. Over the years, it has always been a treasure trove of inspiration for engineering designs, with patterns evident at all scales. Intricate geometries, biomimicry in cell membranes, hierarchical organisation, self-assemblies, fractals, tessellations, spirals, lattices, and Fibonacci sequences are just some of the few examples where patterns play essential roles, with specific functions wherever they appear^{1–7}.

In all these patterns, efficiency has always been the watchword. In its designs, evolution has ensured that everything is reasonable and in the right place, with less being preferable and when it is just enough to get a job done, as shown in the fascinating examples of flight patterns of birds and the movement of other animals, with wings shaped to optimise movement and reduce the energy expended in undertaking those tasks^{8–10}. It has often inspired most engineering designs on the macro scale and, later, on the micro and nanoscale. These natural patterns provide efficient, robust, and adaptive solutions that inspire innovative engineering applications^{11,12}, with the hierarchical structures and materials in the skeletons and shells of animals that show remarkable strength and lightweight properties that have inspired engineers to create biomaterials mimicking natural occurrences, translating these macro patterns into tailored mechanical properties and functionalities in innovative biochemical engineering designs^{13,14}. These patterns are inherent in different scales with specific functionality, as shown in Figure 1.1.

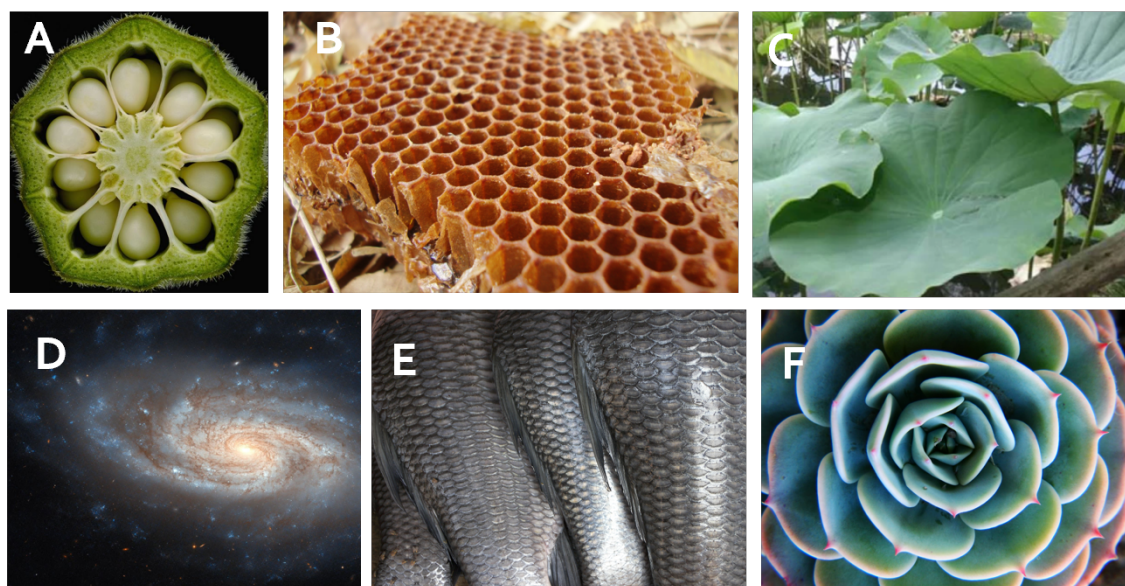


Figure 1.1: Patterns in nature showing how they perform specific functions, such as (A) protective layer for seeds in okra against predators and environmental hazards, (B) structural support for the hive in a honeycomb, (C) superhydrophobic surface in lotus leaf for self-cleaning, (D) Spiral galaxy NGC 3430, where gravitational pull holds the stars, gas and dust together, (E) protection offered by fish scales against abrasion, parasites, and predators, and, (F) adaptation of cactus echeveria in arid environments through storage of water and reflection of sunlight as a result of the waxy coating on their leaves. Images adopted via ^{15–20}, respectively.

Branching in organisms, waves, lightning, and periodic structures are all examples of natural patterns which provide insights into efficient designs and innovative solutions in biochemical engineering. These large-scale patterns are precious in the design of systems that require scalability, adaptability, and robustness^{21–23}, with replications of these in lab-on-a-chip devices of microfluidic devices with branching channels for efficient fluid distribution and mixing of reagents and disease modelling inspired by the microvasculature of organs^{4,24}; tissue scaffolds that support cell growth and tissue engineering using periodic structures adapted from the natural phenomenon of extracellular matrices²⁵, leaf arrangements²⁶, wave patterns used to enhance signal detection and amplification in the design of biosensors, and structures used to improve flux and reduce fouling in filtration membranes^{27–31}. The well-orchestrated pattern exhibited in plants to capture sunlight energy ensures light-harvesting pigments are arranged within chloroplasts to maximise the absorption of light for photosynthesis, which is the process of energy transfer and carbon

dioxide fixation, and other examples where patterns are influential for certain processes, such as the efficient utilisation of light for biofuels and other biochemical production which follows these natural designs^{32,33}. Spiral patterns, as seen in galaxies, nautilus shells, and hurricane formations, are employed in some drug delivery systems to control the release profiles of encapsulated drugs for a sustained and targeted delivery as well as to ensure efficient and thorough mixing of reactants in micro-mixers, vital for many biochemical processes^{34–39}. The tessellations in honeycomb patterns, the eyes of insects, and the shells of turtles are some examples of patterns adopted in designs such as tessellated electrodes to increase the surface area for microbial colonisation, thereby enhancing the efficiency of electric generation in microbial fuel cells^{40,41}.

The micro- and nanostructures found in nature took millions of years to evolve to their present state, and the intricate topologies exhibited are marvellous in their inherent functions. These appear in hierarchical structures such as in shells, wood, spider silks, and bones, whereby flexibility, toughness and strength are part of the optimised mechanical properties^{42,43}. The tiny, repetitive patterns found on materials and organisms, often because of environmental interactions, arise from several natural processes to produce particular characteristics in the organisms and materials^{44–48}. These micropatterns, such as the wing scales of butterflies, cause light to diffract and scatter at specific wavelengths to create the vibrant and beautiful iridescence observed; microscopic ridges on the skin of sharks that reduce drag, with dermal denticles that allow sharks to be more efficient swimmers; the super-hydrophobicity of some leaves that enables self-cleaning due to the tiny structures on the leaf's surface which has a waxy cuticle coating^{49–53}. Other examples include the self-similarity across different length scales that characterise fractal geometries, and these patterns offer higher surface area-to-volume ratios, multi-functionality, adaptability, efficiency in material use, and improved performance. Butterfly wings are prime examples of this. It has multi-functional properties such as repelling water, adding vibrant colours, and strength to the butterfly, which are possible due to the nanostructures that make up the wings. Due to the features of its nano- and microstructures, the lotus leaf repels water, while fern leaves, snowflakes, blood

vessels, and lung bronchi are other examples of these multifunctional properties. The engineering applications of these natural patterns are seen in the enhancement of mass transfer and efficient mixing in bioreactors, promoting better tissue integration and vascularisation in medical implants using fractal structures to mimic the natural patterns found in blood vessels or leaf veins that optimise efficient transport of nutrients and waste products^{1,3–7,14,21}.

Some of these natural patterns appear at much lower scales as well. The presence of nanocrystals within the silk fibres to give exceptional strength and elasticity, as noticed in spider silk, is due to the nanopatterns in these nanocrystals, which act as reference points, preventing the fibres from disintegrating under stress^{43,54,55}. The gecko's sticky feet, renowned for their incredible ability to climb and defy gravity on vertical surfaces, are natural examples of patterning, thanks to millions of setae (microscopic hairs). Furthermore, smaller spatulae (spatula-shaped structures) cover the setae - nanopatterns that allow the interaction with the surfaces due to Van der Waals forces, which enables the geckos to cling effortlessly^{56,57}. While engineering designs strive to replicate this phenomenon (albeit only sometimes successfully), natural improvements over millennia and centuries have taught us to streamline designs, eliminate excesses, and, most importantly, achieve efficiencies as little and quickly as possible. Learning from nature's intricate design principles has enabled the creation of sustainable, scalable, robust, efficient, and biocompatible technologies for our everyday use^{44,58–61}, which is achieved mainly by topologically designing structures based on these natural patterns to incorporate these multi-functionalities into engineering applications^{62,63}.

While regular patterns occur in nature to achieve a certain objective, replicating the regularity of these patterns in engineering designs is essential for accurate diagnosis of certain ailments when used in the design of medical devices, especially in biosensors. The use of patterned structures in the design of substrates for biosensors can enable the detection of *minute* concentrations of biomolecules, which can indicate neurological diseases, such as dementia and traumatic brain injury. Therefore, it is pertinent to design biosensors that can easily and accurately detect the presence of biomolecules in samples. However, as some diseases are confirmed by the presence of

several biomolecules, often with a very short shelf life, it is imperative to have tuneable biosensors that can detect more than one type of biomolecule.

In this research, a novel fabrication method was used to make tuneable 3D micro-nanostructure substrates used as biosensors, with the structures easily scalable and reproducible to detect the presence of biomolecules that can indicate the onset of traumatic brain injury in a blind sample.

Although it is possible to design metallic nanoparticles that can detect these biomarkers in medical devices with the potential to achieve high enhancement, they suffer from size inconsistencies and poor reproducibility. This makes it difficult to accurately and consistently reproduce the hotspots required for signal detection using these devices. The short shelf-life of these biomarkers also makes timely intervention crucial.

1.2 DEMENTIA, TRAUMATIC BRAIN INJURY AND OTHER NEUROLOGICAL DISEASES

Dementia refers to symptoms linked to decreased memory, reasoning and other thinking skills. It is a general term whose severity could affect the daily life of patients due to a decline of mental abilities and memory loss, with noticeable physical changes in the brain of patients affected⁶⁴. There is a ubiquitous reference to dementia among the older population (above 65 years of age). Still, there are slight differences in distinguishing the forms and advancement of the disease in all age groups, with a projection of 153 million people globally living with dementia by 2050⁶⁵. There are different types of dementia, such as Creutzfeldt-Jakob disease, Lewy body dementia, Frontotemporal dementia, Huntington's disease, Parkinson's disease dementia, Alzheimer's disease, Vascular dementia, and Korsakoff syndrome, amongst others⁶⁶.

Alzheimer's disease (AD) is the most common type of dementia, accounting for about 60 – 80% of cases⁶⁴. It is mainly caused by cell damage leading to complex brain changes, and it is often referred to as a degenerative brain disease. Some symptoms include disorientation, confusion, and changes in behaviours, leading to difficulty swallowing, walking, and speaking as the condition advances. An early sign of AD is the presence of antioxidant glutathione negatively correlated with the brain's pH value. The antioxidant neutralises reactive oxygen species that generate oxidative stress in the brain, thereby depleting glutathione. However, the level of oxidative stress increases with the patient's age. Some of the measurements for changes to the brain for AD include abnormal levels of beta-amyloid and a decrease in glucose metabolism, with the brain compensating for these deficiencies so that the patients can continue to function normally. Another one is accumulating an abnormal form of the protein tau. All these happen in the preclinical stage of the AD continuum – where it progresses from no symptoms or detection to severe AD. Future treatment is geared toward the preclinical or mild cognitive impairment (MCI) stage of the AD continuum for successful treatment of the disease. Therefore, biomarkers such as these will play an important role in treating and monitoring the disease as it is defined in vivo by biomarkers and post-mortem examination of aggregation of neuropathological changes, not clinical symptoms. Understanding the role of biomarkers in disease detection and treatment is crucial for healthcare professionals, and it is a field that continues to evolve and expand. Traumatic brain injury (TBI) is one of the pathways through which injuries to the brain increase the risk of developing dementia, including Alzheimer's disease, as neuroinflammation caused by TBI may persist for years⁶⁷. The release of some biomarkers, like the accumulation of Tau and protein and amyloid-beta, indicates the high-risk factor of AD^{68–71}.

Traumatic brain injury involves mechanical tissue damage followed by the injured cells' release of proteins and extracellular vesicles. These extracellular vesicles include lipid bilayer membranes called exosomes, which cross the blood-brain barrier, and their contents can be measured after isolation from the peripheral circulation^{72–74}. These protein biomarkers can inform the extent

of damage and severity of TBI in the sufferers of symptomatic patients⁷⁵, and it is one of the attributable factors that lead to dementia, making it be classed as a modifiable risk factor (during midlife) to prevent the development of dementia^{65,70,76}. The risk of dementia increases with the number of TBIs sustained, with mild TBI leading to a two-fold increase in the risk of a diagnosis of dementia. Also, patients with a history of TBI develop AD at a younger age than those without a history of TBI, although the direct link between TBI and AD (and dementia as a whole) is still being investigated⁶⁴. However, some long-term implications of repeated mild TBIs are the potential to lead to chronic traumatic encephalopathy (CTE), AD, Parkinson's disease, depression and anxiety⁷⁷. Annual diagnosis puts it at about 2 – 2.5 million Europeans with about 60,000 – 80,000 fatalities^{72,78,79}, and its effects can last years after the injury, leading to deterioration in the health of the sufferers. Worldwide, about 50 – 60 million people are diagnosed with TBI each year, costing the global economy more than £300 billion annually^{73,80}. These injuries are more common in military personnel in conflict zones, contact sports athletes, and very common among kids under the age of 4, which is the high-risk age group. A lot of mild TBI (mTBI) is left undiagnosed until the health of the sufferers deteriorates due to missed and difficult diagnosis.

However, early diagnosis is increasingly becoming possible due to detecting specific biomarkers such as s100 β , neuron-specific enolase, GFAP, and tau, amongst others. A combination of biomarkers is increasingly becoming an alternative approach to studying the progression of the disease for better diagnosis and treatment^{81,82}. The problem with these biomarkers is that they are proteins with a relatively short serum elimination half-life, so early detection, while very helpful in accurately diagnosing the illness, is quite challenging to obtain. The survival rate of those who suffer TBI can be as high as 75% during the 'golden hour' for severe TBI, up to 90% for moderate TBI, and almost 100% for mild TBI^{72,80,83}. All these also depend on other contributing factors such as speed of medical intervention, other health comorbidities, and patient age.

This makes early detection and expedient intervention crucial in its treatment. The effects can be

in the form of post-traumatic epilepsy, brain atrophy, AD, and CTE years after the onset of the disease⁸⁴. The presence of chronic microglial activation and progressive neurodegeneration have both been observed as part of the long-term clinical effects of TBI, both of which are seen in the white matter tracts affected by the injury⁸⁴. Increases in fluid biomarkers are due to damage to neurons and glial cells after TBI, which can prove clinically informative, using analytical techniques such as Raman spectroscopy for qualitative detection of chemical substances.

1.3 RAMAN SPECTROSCOPY

Raman spectroscopy is an analytical technique that provides both qualitative and quantitative information on a molecular level of blind samples based on inelastic scattering of light^{85,86}. Improving the spatial resolution, sample preparation, and signal collection through patterning enhances Raman spectroscopy, and the surfaced-enhanced Raman spectroscopy technique has become one of the most powerful analytical tools in the detection and analysis of blind samples for trace amounts of molecules because of its high sensitivity and high specificity in the detection of analyte molecules in these samples. This detection is possible because of the technique's unique ability to identify molecules at extremely low concentrations^{87–89} and because of rough metallic structured surfaces that significantly increase the otherwise weak Raman signals of the analyte molecules.

Raman spectroscopy has advantages over other analytical techniques for signal detection, including fluorescence spectroscopy, mass spectrometry, surface plasmon resonance, and Fourier transform infrared spectroscopy, because of its ability to analyse samples in an aqueous medium, reduced interference of background fluorescence, label-free detection of samples, molecular fingerprint detection necessary for complex samples, and the non-invasiveness of the technique^{86,89,90}. Some of these properties make it ideal for the analytical component of point-of-care diagnostic

devices, as the non-destructive nature of samples and minimal sample preparation make it ideal for deployment in out-of-clinic settings. The specificity, versatility, and portability make it suitable for these devices, which tends to be some of the challenges point-of-care device addresses. Other challenges, such as reproducibility issues, high cost of fabrication, reliability, interference from other biological samples such as urine, blood, or saliva, portability, integration with other medical devices, and the need for standardised protocols for validation of results, can be addressed during the design and fabrication of these devices through advancements in the fabrication process, and obtaining regulatory approval after clinical validation and standardised protocols are established. The Raman spectroscopy analytical technique is discussed in more detail in Section 3.3 of this thesis.

Over the past few years, many studies have shown how innovative methods enhance the Raman signals further and produce higher enhancement factors and reproducibility, using a combination of different micro- and nanostructure designs and methods to produce these designs. The topological design of these nanostructures to create better hotspots remains the goal, with control of specific tuneable parameters to enhance the Raman signals obtained from analytes based on the designs and improve the accuracy and reproducibility of these designs.

Various applications of this phenomenon have been used in biosensors for the detection of *minute* concentrations of analytes to predict the onset of traumatic brain injuries and dementia, which is caused by the release of specific biomarkers that are otherwise difficult to detect and diagnose at the early stage of the illnesses^{91,92,93–100,101}. These enhancements ensure surfaces are primed for improved sensitivity and selectivity, making the Raman spectroscopy technique ubiquitous in biosensors through the surface-enhanced Raman scattering effect^{102–104}.

1.3.1 Surface-Enhanced Raman Scattering

Surface-enhanced Raman scattering (SERS), based on the principle of surface-enhanced Raman spectroscopy, whereby the Raman signals of molecules are significantly enhanced (about the order of magnitude of up to 6 or more) when absorbed on the surface of rough metallic nanostructures such as gold or silver, making it possible to detect trace amounts of molecules with high sensitivity¹⁰⁵, and in some other cases, the combination of both gold and silver to optimise their different properties in the detection of certain drugs¹⁰⁶.

The enhancement of the Raman signals is due to the excitation of surface plasmons, which are collective oscillations of electrons on the surface of gold or silver (or other metals) that can be excited by incident light (through the electromagnetic interaction of light with metals)^{102,107}. The excited plasmons create an enhanced electric field near the surface of the metal, which can interact with the analyte molecules of the tested samples and enhance their Raman scattering signals. Molecules to be detected get absorbed on the surface of the metal surface or substrate – a significant point for applying SERS.

SERS and the theories governing it are further discussed in Section 3.4 to show its unique analytical qualities that differentiate it from others, making it suitable for point-of-care devices that use biosensors for signal detection.

Raman spectroscopy is suitable for miniaturisation and out-of-laboratory settings for accurately identifying components in blind samples typically present in *minute* concentrations (such as proteins released during concussions). It makes incorporating the technique into point-of-care diagnostic devices an excellent proposition. However, SERS offers higher sensitivity with a significant increase in the enhancement of signals compared to normal Raman spectroscopy, with SERS enhancement reported in the range of 10^5 – 10^{14} . It is also more suitable for point-of-care devices because of the portability of its sensors and the possibility of multiplex detection of several

analytes. Its single-molecule detection capability ensures that it has a much lower detection limit than Raman spectroscopy (normally in the micromolar to millimolar range for Raman spectroscopy compared to the attomolar range for SERS), making it possible to detect single molecules of compounds in blind samples^{89,108}.

Patterned substrates are possible platforms for multiplexed detection of signature biomolecules in samples using SERS substrates. SERS enhancement increases with an increase in the aspect ratio of pillars on the substrate and various morphologies, producing variations in SERS enhancement¹⁰⁹. The build-up of spatially non-homogenous oscillating electric fields in the vicinity of the nanostructures characterises surface plasmon resonance (SPR), producing locations called hotspots that enhance Raman signals. This SPR can be used to tune the optical properties of these nanostructures, with some examples seen in the extinction spectra of silver nanowires (hexagonal shape), silver spheres, and silver cubes^{110,111}.

SERS relies on molecules' inelastic scattering of light on plasmonic surfaces, enhancing the Raman signal by a factor of up to about an order of magnitude 12 in some cases, with the enhancement due to surface roughness and fine-tuning of the nanometric gaps between nanoparticles¹¹². Although there are some issues with SERS, such as the debate over the magnitude of enhancement due to different calculations employed, leading to the presentation of different values in the literature with wide differences in the order of magnitude of enhancements reported¹¹³, one of its main advantages is its non-destructive nature and single-molecule detection ability, especially for disease biomarkers^{104,112}. Some of the characteristics of SERS signals include molecules exhibiting the same SERS spectrum that is very similar to their typical Raman spectrum at the same excitation wavelength, with the Raman peaks being easily identifiable. One slight difference which highlights SERS is that the Raman spectrum under SERS conditions can be affected by the plasmon resonances (which produce the enhancement) being wavelength-dependent. Other advantages of SERS, especially in some point-of-care diagnostics over other methods, are its ability to achieve multiplex detection, low background signals, high sensitivity,

high selectivity, and, more importantly, the ability for single-molecule detection in analytes¹¹⁴. SERS' label-free detection of proteins has simplicity, rapidness, sensitivity, and reproducibility advantages that can be incorporated into point-of-care diagnostics^{115–122}.

To further enhance the Raman signals of the absorbed molecules on metallic nanostructures, SERS substrate designs have been typically designed to improve the creation of SERS hotspots on mainly gold and silver nanostructured surfaces (due to their strong plasmon resonances in the visible and near-infrared regions) because the slight topological changes in these designs play a huge role in the enhancement of the Raman signals produced. Some of the general designs for these nanostructures use techniques such as electrochemical deposition, chemical reduction, and lithography to produce nanoparticles, nanorods, nanowires and nanoholes, with the application and specific requirements of the SERS substrate influencing the type of nanostructures produced. For example, despite being easy to synthesise and providing a large surface area for analyte adsorption, nanoparticles have low reproducibility due to the random distribution of SERS hotspots.

The size and shape of the hotspots depend on the shape of the metal nanostructures and the wavelength and polarisation of the incident light^{123–125}. Despite producing more hotspots, nanorods and nanowires are more challenging to synthesise, requiring more advanced fabrication techniques, even though they have much better reproducibility. This challenge informs the combination of methods, such as lithography, with better control of specific parameters, such as size, topology, and patterns, to achieve desired results and reproducibility. Nanostructures obtained through the coating of SERS substrates with metals are essential in specific practical devices due to the intrinsic properties of the metal in question¹²⁶.

The practical purpose of these metallic coatings taps into some of its optical advantages, such as the significant enhancement of Raman signals that can indicate the presence of analytes (even in blind samples), which is paramount in the detection of the trace biomarkers to indicate the

onset in traumatic brain injury in patients. These enhancements are possible because the optical properties observed on these structures are enhanced by the strong localised plasmon resonance and substantial field enhancements, which are necessary for SERS applications and are essential for the accurate diagnosis of trace characteristic biomarkers that often mark the onset of TBI in patients whose symptoms might not manifest until years later when it becomes too late to have meaningful care to reverse or halt the progression. These biomarkers in the form of proteins are often present in *minute* concentrations and have a very short half-life, rendering them difficult to identify when they first appear in patients. The early detection by carefully designed topologically patterned biosensors for accurately diagnosing these ailments through disease-indicative biomarkers forms the basis of this research study.

1.4 TOPOLOGICAL DESIGN

While patterns involve arrangement within spaces, boundaries, and layout, understanding this is essential when adopting some of these in designs. Therefore, our understanding of topology in nature becomes relevant. However, the topological interpretation differs slightly between the pure mathematical definition and its engineering understanding, with engineering interpretation often involving the use of the term interchangeably with geometric design and optimisation¹²⁷. Although topology and geometry both describe the structure of objects, engineering views topology in terms of the optimisation of advanced design and manufacturing.

Topology is the study of invariant shapes or spaces under continuous deformation, with engineering applications involving the analysis of the stability of structures to optimise designs and help in spatial relationships, which is crucial in functional designs^{6,63}. Topology is all around us, as seen in the geometric and spatial arrangement of elements within a system that is abundant in nature, where they perform specific functions in organisms¹²⁸. Over millions of years, these

evolutionary intricacies form complex structures of different scales through hierarchical, self-assembly patterns, morphological adaptations, and more^{1,10,13,26,42,45,48,52,56}. The patterning observed in nature is a clear example of how topological design optimises the properties of certain parts in organisms for specific applications. Unlike other designs that focus on particular shapes and sizes of components, topological design is about the configuration and interrelationships between elements to optimise the efficiency, robustness and functionality, *à la* nature. The topological design also focuses on material distribution, the arrangement and stability of systems, functionality, and optimisation, which are the basis for evolutionary trends in nature. These properties are in the functionality and design of bird wings, including the arrangement of feathers and the skeletal structure to reduce drag and increase lift for gliding and flapping; bone structures for strength and lightweight, with the structure providing strength and flexibility while minimising weight; the intricate and porous structure of coral reefs to support a wide variety of marine life; hierarchical network in leaf venation, for efficient distribution, maximising photosynthesis and minimising use of resources^{129–132}. By studying and adopting these natural topologies, we can develop innovative solutions to produce sustainable, effective, and efficient devices in various fields, for example, devices that are particularly useful in medical engineering, as these topologically patterned surfaces can significantly enhance the detection of signals to indicate certain illnesses and diseases when used in biosensors for medical devices^{133–135}.

1.5 BIOSENSORS

Topological design is crucial in the design, by and large, of biomedical engineering and biosensors. It allows for developing intelligent, selective, sensitive, and robust devices for detecting analyte molecules and monitoring specific physiological parameters that give indications of ailments, both at the advanced stages or early detection^{133,136}. The consideration in the design is the arrangement of patterns at the nano and micro scale for sensor optimisation, and

by drawing inspiration from nature, topologically-designed biosensors enable diverse applications in healthcare, allowing point-of-care (P-o-C) diagnostics, for example, to become more common^{134,135,137}. These can be incorporated into imparting the flexibility and adaptability of biosensors to conform to biological environments and physiological conditions, such as the design of wearable and implantable platforms for continuous monitoring of biomarkers and vital signs in real-time at the nanoscale by designing specific binding sites, high selectivity for target molecules can be achieved, allowing biosensors to discriminate between closely related analytes with high accuracy, enabling detection in complex biological samples^{134,138,139}; the enhanced surface area that can be achieved by topologically-designed structures such as nanowires, and nanotubes, for example, can facilitate the efficient capture and identification of target analytes to improve the performance of biosensors for early detection and diagnosis of diseases.

All these are done by modifying the electronic properties, optical properties, materials, mechanical properties, and flow^{136,137,140,141}. The detection element in the sensors in some point-of-care devices has two main parts – the detection element that holds the target analyte and the transducer that converts the signal that can be detected as mechanical, electrochemical or optical energy^{142,143}. This phenomenon enables the design and fabrication of nanopatterned diagnostic devices directly interacting with analytes to provide signal¹⁴⁴. High sensitivity through miniaturisation of these sensors into micro-and nanostructures produces devices with a high signal-to-noise ratio, enabling the detection of analytes with extremely low concentrations in a reasonable time, with the sensitivity of these sensors primarily limited by how quickly analytes can diffuse to the sensor's surface^{145–147}. For a given topological-designed sensor, the time required for sufficient analytes to reach the receptor area and produce a detectable signal is critical to overall sensitivity.

A new generation of early-stage diagnostic techniques has been developed due to the ability of nanomaterials and nanopatterned devices to interact with specific analytes directly, converting the interactions directly to amplified signals that can be measured¹⁴⁷. The miniaturisation of

the critical dimensions, especially of the receptor area, because of the signal-to-noise (S/N) ratio gain leads to higher sensitivity in micro- and nanosensors. However, a key parameter is the ability to detect analyte molecules of very low concentrations in a reasonable time. Therefore, the specific geometry adopted in the sensor becomes paramount to achieve accurate and reliable results^{144,147}, especially in the early diagnosis of specific ailments such as traumatic brain injury and other neurological diseases, where the early detection of the symptoms in patients can lead to a significantly improved quality of life with early treatment and therapies. However, these devices can result from other factors, with their success or otherwise dependent on factors from the clinical pull or industrial push, as shown in Figure 1.2. A good example is the expedited actions taken during the COVID-19 crisis, when government regulations around the world were relaxed to allow for the rapid production of essential medical devices for testing and managing the epidemic. Such situations sometimes dictate that, apart from the rapid production of these devices, there must be ease of scalability and reproducibility while maintaining accuracy and sensitivity to encourage fabrication for successful deployment in other parts of the world. Other factors include the costs of the medical device and treatment associated with the illness, making it impossible to be publicly funded, or sometimes public health bodies having to make complex arrangements to fund treatments for patients.

The commercialisation and protection of the intellectual property of the device can influence manufacturing considerations, with various government regulations in different parts of the world accounting for the decision to proceed with a medical device manufactured by private companies^{113,150–154}. The reproducibility, scalability, costs, and deployment of these devices to different parts of the world while navigating the different government regulations is also a factor that is always considered, as the cost could potentially become prohibitive to patients in some countries if it is too expensive to justify or pay the costs without any government or philanthropic support. This research work aims to address these, and other challenges highlighted using tuneable 3D micro-nano structure substrates that can be incorporated into a point-of-care

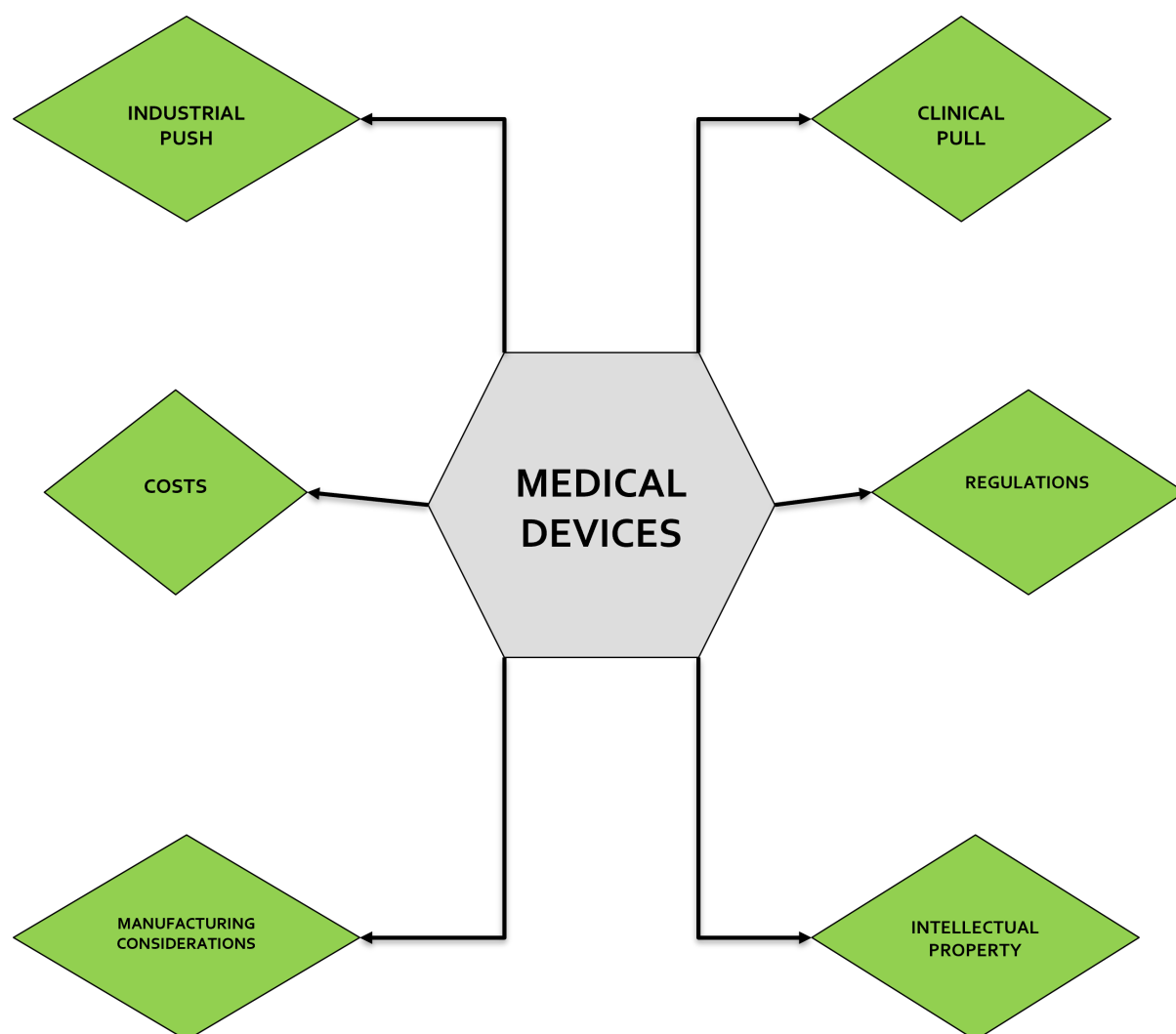


Figure 1.2: Some of the factors that drive medical diagnostic device innovation.

optofluidic diagnostic device to detect similar trace biomolecules present in patients with traumatic brain injury.

1.6 THESIS OUTLINE

The thesis starts with an introductory Chapter One, which discusses patterning in nature and its adoption in several engineering endeavours. It outlines the aims and objectives of this

thesis, which is to fabricate easily scalable and reproducible 3D patterned substrates that can be used as biosensors to accurately detect trace biomolecules indicative of traumatic brain injury. It will also cover how topological design is incorporated into patterning to develop biosensors used for early detection of disease-indicative biomarkers of diseases such as traumatic brain injury and other forms of dementia using the surface-enhanced Raman spectroscopy technique.

Following the introduction, Chapter Two will cover the experimental methods and analytical techniques used throughout the research. It will also cover the materials and fabrication process to make the SERS substrates. The characterisation techniques, including Raman spectroscopy, optical microscopy (OM), and atomic force microscopy (AFM), will be described in detail. This chapter will also discuss the simulation methods used for data analysis, such as COMSOL, Python, and others.

Chapter Three discusses the lithographic technique in greater detail, emphasising photolithography. This chapter will cover the different types of lithographic techniques and their pros and cons, as well as highlight photolithography, Raman spectroscopy, and surface-enhanced Raman scattering, which are the basis of this research. Some of this was part of a review article published in *Discover Nano*.

Chapter Four will cover the fabrication of different topological designs, highlighting the different enhancement factors and advantages of specific nano-topographical platforms for SERS molecular detection. The chapter will also discuss COMSOL simulation's role in obtaining optimal structures for Raman signal enhancement. This chapter has been published in *Advanced Materials Interfaces* journal.

Chapter Five covers other patterned designs based on simulation results, focusing on materials and other issues identified in Chapter 4. Here, tweaks to the fabrication process and different materials are highlighted to obtain better enhancement factor values.

Finally, Chapter 6 concludes the thesis by summarising the work done, achieving the objective of the research study, which is to fabricate easily scalable and reproducible substrates that can be used as biosensors, and future outlooks. It highlights the challenges and possible future processes to improve the biosensor for integration into a platform that can be used as a point-of-care diagnostic device. This chapter will also highlight some projects that were started but not completed due to low-quality results, which can be improved with modifications.

CHAPTER TWO

EXPERIMENTAL METHODS AND ANALYTICAL TECHNIQUES

This chapter discusses the materials, experimental methods, and techniques for designing and fabricating the patterned substrates for surface-enhanced Raman scattering (SERS). It also discusses the computational tools for designing the structures, including other software used for data analyses.

2.1 EXPERIMENTAL SETUP FOR PHOTOLITHOGRAPHY

Photolithography (PL) is one of the top-down fabrication techniques that uses a photoresist to design and make specialised high-precision patterning of micro and nanostructures upon exposure to light in certain regions. It is preferred to other lithographic techniques for miniaturisation and mass production due to its ability to transfer intricate tiny designs onto thin films or the surface of a substrate, usually a silicon wafer, which can be used in biosensors and as micro-electro-mechanical system (MEMS) which are used for automotive, medical, and microfluidic devices^{153–156}.

The substrates used in this research work were fabricated using PL to make micro/nano patterns, with the setup process shown in Figure 2.1. The process starts with cutting and cleaning the silicon wafers, then spin-coating the photoresist to form a uniform layer, followed by fabrication, development and finally, metallic coating for use as a SERS substrate. PL is used to make the patterned structures before undergoing post-fabrication processes, such as development in solution for a few seconds to remove the exposed or unexposed part of the photoresist during exposure to light in certain regions, depending on the type of photoresist used. Other post-fabrication processes include post-exposure baking for negative photoresist before sputter-coating with uniform metallic coating, ready to be used as a SERS substrate.

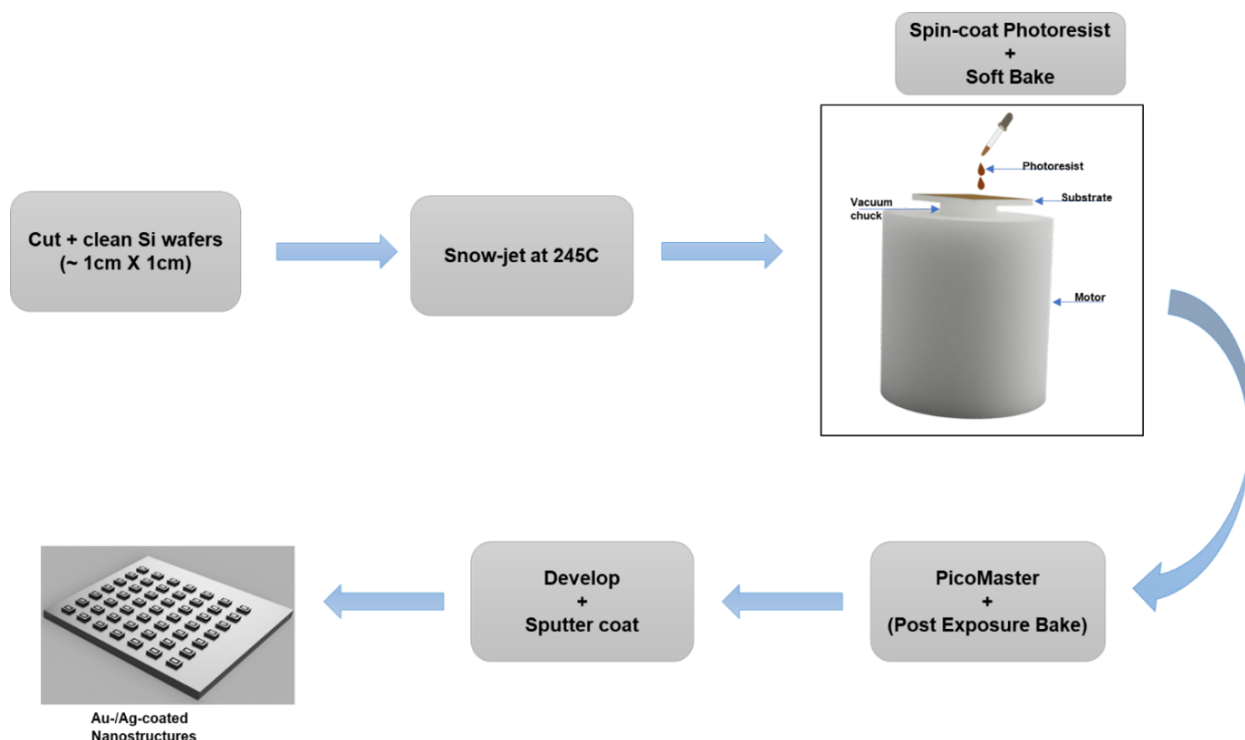


Figure 2.1: Schematic representation of the fabrication process of the substrates using photolithography.

2.1.1 Materials

The substrates for the nanostructure patterning are made from test-grade silicon (Si) wafers (n-type, crystal orientation $\langle 100 \rangle$), with one side polished and having a resistivity of 0 – 100 Ωcm (WaferNet GmbH, Germany). The Si wafers have a thickness of $500 \pm 25 \mu\text{m}$. The wafers were cut using a diamond scribe into $13 \times 13 \text{ mm}^2$ to act as the base for the deposition of photoresists before printing. The solvents used are HPLC-grade ethanol, acetone, and methanol (Sigma-Aldrich, Dorset, UK) to clean the silicon wafers and prepare 10 mM benzenethiol (BT) (Sigma-Aldrich, Dorset, UK) to calculate enhancement factors in the fabricated structures. Both the positive photoresist AZ1514H (MicroChemicals GmbH, Germany) and the negative photoresist AZ nLOF 2020 (MicroChemicals GmbH, Germany) were used as purchased, without dilution, for the fabrication process. The developers used for the post-fabrication process to develop the structures include AZ351B (MicroChemicals GmbH, Germany) for the positive

photoresist and AZ326 MiF (MicroChemicals GmbH, Germany) for the negative photoresists. Another negative photoresist used was AR-N-4340 (Allresist GmbH, Germany). TBI-indicative biomarkers used in this study include Sphingomyelin (1051-25 mg, Cambridge Bioscience, Cambridge, UK), Cholesterol (C8667-1G, Generon, Slough, UK), Myoinositol (A13586.22, VWR International, Lutterworth, UK), and S100 β (HY-P70659, Cambridge Bioscience, Cambridge, UK). These biomarkers were used in the purest form without additives to create Raman spectroscopy fingerprints to detect TBI. The importance of these biomarkers in diagnosing and assessing the severity of TBI is discussed in sections 4.2 and 5.2 of this thesis, and an inexhaustive list of other biomarkers is presented in Table 5.2.

2.1.2 Substrate Preparation

A clean surface is paramount, as the surfaces to be used are usually contaminated by microscopic particles. Clean surfaces were achieved by removing surface contaminants that might affect the spin coating of a uniform photoresist layer before printing the structures (Figure 2.2). The non-uniformity of the resist layer caused by impurities on the surface of the silicon wafers also inhibits the effectiveness of the sensitive fabrication printing carried out during PL.

The wafers were cleaned with cleanroom wipes before snow jet cleaning using an in-house-built spray carbon dioxide (SCD) system (Figure 2.3). Snow jet cleaning is a system that uses a nozzle to spray a jet of high-pressure and high-velocity carbon dioxide ice crystals onto the surface to be cleaned.

Depending on their intended use, certain surfaces require a plasma cleaner to ensure they are cleaned. It can also modify the surface and activate ionic and electron charges. These charges can neutralise positive charges or contribute to a net negative charge on the surface (together with the negative or positive charges deposited by the positive and negative ions in the plasma).



Figure 2.2: It shows the optical microscope images of silicon wafers before cleaning (A), after cleaning with solvents and a plasma cleaner (B) and using cleanroom wipes and the snow jet cleaning process (C). The scale bar is 500 μm .



Figure 2.3: An in-house-designed spray carbon dioxide system for snow jet cleaning.

Additionally, the plasma generates neutral radicals ultraviolet (UV) radiation, which can modify the surface and break molecular bonds, rendering the surface more hydrophilic^{157,158}. However, using the Harrick Plasma PGC 32G-2 (Harrick Plasma, USA) (Figure 2.4), whereby air is used,

introduces a combination of positive and negative ions and free electrons to the substrates' surfaces, contributing to more reactive and unstable fabricated patterns on the hydrophilic substrates and causing delamination of structures on the substrates (Figure 2.5).



Figure 2.4: Harrick Plasma Cleaner is used to clean the surface of wafers for use as substrates.

The delamination was resolved by using cleanroom wipes to clean the surfaces of the silicon wafers before the snow jet, which involved using the SCD to make eight blasts of CO₂ crystals on a heated hotplate at 245 °C for 120 seconds.

The interactions between the 'snow' particles and contaminants lead to the removal of hydrocarbons from the surface of the silicon substrates, with the method being 99.99% effective in removing contaminants, with the cleaning process being residue-free, non-abrasive and non-destructive¹⁵⁹. The stream of carbon dioxide's rapid expansion through the small orifice helps create a snow-like matter that interacts with the surface particulate matter and hydrocarbon contaminant, which are then removed from the surface as a result of the pressure generated from the orifice to leave a clean surface ready for photoresist deposition.

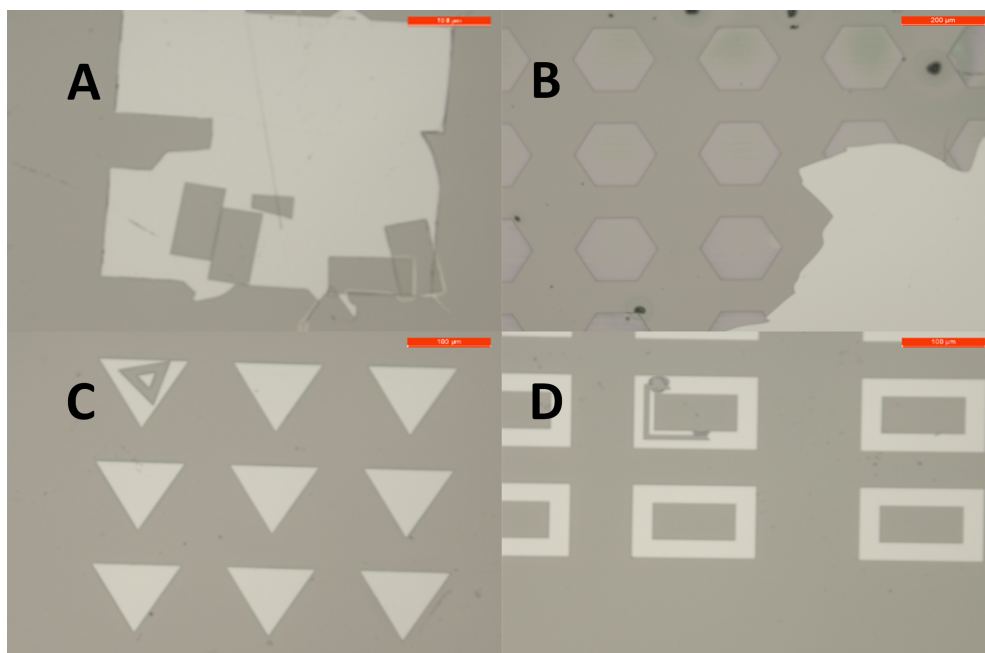


Figure 2.5: Delaminated substrates after using plasma cleaner during the cleaning process, showing the effect of delamination of the (A) rectangular, (B) hexagonal, (C) concentric triangular, and (D) concentric rectangular structures. The scale bar is 100 μm .

2.1.3 Photoresist

Photoresists change chemically upon exposure to certain wavelengths of light, with the thermal baking and other post-processing steps carried out to enable the exposed and unexposed resist area to have different dissolution rates in the solution used to develop it, thereby creating a structured pattern on the surface^{160–162}. Depending on their solubility during the developing stage and exposure, the photoresist can be positive or negative resist. The positive photoresist becomes soluble in the developer solution on exposure to UV light, while the negative becomes insoluble. The positive photoresist AZ1514H used in this research work contains Novolak, a polymerised phenolic resin made of formaldehyde and phenol (Figure 2.6), with the chain length of the resin molecules determining the relevant properties such as the stability against thermal rounding, while also reducing the removal rate of unexposed and exposed photoresists during development.

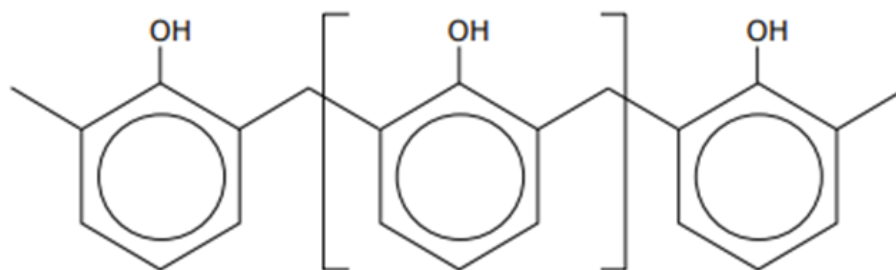


Figure 2.6: The structural formula of phenolic resin forms the AZ photoresist matrix.

The negative photoresist AZ nLOF 2020 is based on phenolic resin with a cross-linker (melamine). Another negative photoresist, AR-N-4340, was also used to fabricate the structures, similar to AZ nLOF 2020 in properties¹⁶³, to check if better micro-nanostructures can be fabricated due to the potential to produce different structure heights when compared to AZ nLOF 2020 (AR-N-4340 can produce up to 1.4 μm , while AZ nLOF 2020 can produce up to 1.5 – 3.0 μm , under the same spin-coating conditions). A carboxylic acid is formed upon the exposure of the negative photoresist, which cross-links the resin during the baking step, making it insoluble in the developer, while the unexposed areas remain soluble in the developer. The photoresists (both the positive and negative photoresists) already contain and can be further mixed with the solvent 2-methoxy-1-methyl ethyl acetate (PGMEA), which enables them to be sufficiently low-viscous during the spin-coating process due to the high boiling point of the solvent (148 °C), with most of this evaporating during the spin-coating process. The photoresist to be used for fabrication depends on the outcome desired. Still, some advantages of the negative photoresists include good adhesion to silicon and lower costs. In contrast, the positive photoresist offers faster processing time, as it does not require the post-exposure bake, which is a necessary step for negative photoresists. The positive photoresist also provides better resolution and thermal stability¹⁶⁴. Some of the other advantages of both positive and negative photoresists are shown in Table 2.1

Table 2.1: The advantages and disadvantages of positive and negative photoresists for the fabrication of structures.

PHOTORESIST TYPE	ADVANTAGES	DISADVANTAGES
Positive	• Better resolution and pattern fidelity of fabricated structures, allowing for a wide range of applications, including semiconductors • Faster processing time – no post-exposure bake necessary • Better contrast between exposed and unexposed areas, leading to better definition of edges during development	• Lower mechanical strength compared to negative photoresists • Lower chemical resistance
Negative	• Better adhesion and higher mechanical strength of structures • Lower costs compared to positive photoresists • Suitable for different feature sizes due to the wide range of heights of structures that can be produced (up to $150\mu\text{m}$)	• Slower processing time due to post-exposure baking step • Limited applications, as positive photoresists are better for when high resolution is crucial

2.1.4 Spin-Coating for Photoresist Deposition

Spin-coating is the uniform application of a thin layer of the photoresist solution onto a solid surface using centrifugal force to produce uniform films of about $1 - 10\ \mu\text{m}$. The photoresist ($15 - 20\ \mu\text{L}$) was deposited on the cut silicon substrate using a pipette. The spin-coater used was a Laurell WS-650MZ-23NPPB (Laurell Technologies Corporation, USA) (Figure 2.7). The substrate is held in place by a vacuum chuck, with the spin-coater speed set to between $1000 - 6000$ rotations per minute (rpm) for 60 seconds. This can be changed depending on the height of the desired layer deposition and the type of photoresist used. Table 2.2 shows the height

that can be obtained based on the suggested settings by the photoresist manufacturers with 4000 rpm at room temperature. The spin-coating time can also be altered to achieve the desired film thickness for fabricating the structures. Higher rotation speeds produce lower film heights and a more uniform film, and *vice versa*.

Table 2.2: The thickness obtained based on the type of photoresists used. The film thickness is achieved under standard conditions, and some resists can be diluted for lower film thickness. AR-N-4340 thickness was obtained under standard conditions for 60 seconds and 4000 rpm of the spin-coater^{162,163}.

PHOTORESISTS	FILM THICKNESS (μm)	DEVELOPERS
AZ 1514H	$\approx 1.2 - 2.0$	AZ 351B, AZ 326 MIF
AZ nLOF 2020	$\approx 1.5 - 3.0$	AZ 326 MIF
AR-N-4340	1.4	AZ 326 MIF

The spin-coater is placed in a fume cupboard to ensure no spillages or hazardous substances are evaporated. The centrifugal force spreads the solution on the surface to produce a homogenous film, and the excess solution is spun off the surface. While the evaporation process, in conjunction with the centrifugal force, determines the film's homogeneity, it is also heavily influenced by the type of solvent and photoresist used, with dilution of the photoresists with solvents such as PGMEA a consideration for changing the thickness of photoresist layer after spin-coating. The spin-coating was carried out under darkroom conditions using only light with a wavelength of 580 nm.

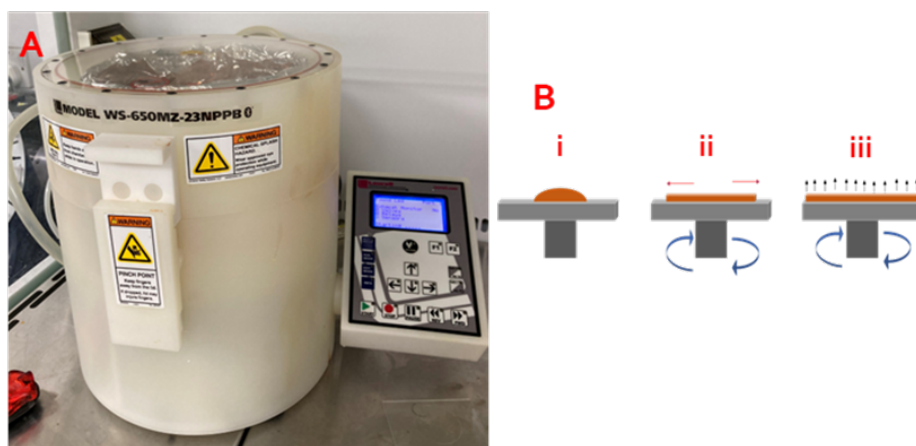


Figure 2.7: (A) Spin-coater used to add photoresist to surfaces homogeneously, (B)(i) Photoresist pipetted onto the wafer, (ii) spin-coater spreading photoresist evenly, (iii) evaporation and a thin homogeneous layer formed.

2.1.5 Softbake

A softbake process is a pre-fabrication step when photoresists are used in PL¹⁶². The photoresist-coated surfaces were put through a softbake process to improve the photoresist adhesion to the surface. It involves placing the photoresist-coated surface on a hotplate (Corning PC-4200, Corning Inc., USA) at 100 °C for ninety seconds for a film thickness of 1.5 μm (1 minute/ μm resist film thickness) for the positive photoresist and sixty seconds for a film thickness of 2 μm for the negative photoresist (Figure 2.8). The typical softbake process for negative photoresists is performed at a temperature range of 90 – 110 °C, with the process optimised depending on the lithographic technique used, pattern profile, and adhesion performance. Depending on the adjusted viscosity, the solvent makes up about 55 - 85% of the photoresist but is reduced to 2 – 5% after the softbake.



Figure 2.8: The hotplate was used during the softbake process to improve photoresist adhesion to the surface and for the post-exposure bake process for cross-linking when the negative photoresists were used.

2.1.6 Fabrication using PicoMaster

The photolithographic fabrication process used in this study was done using a PicoMaster 100 (PicoMaster XF, Raith Lasers Systems B.V., Netherlands) (Figure 2.9). It is a direct laser writer and versatile UV laser high-resolution equipment for printing micro/nanostructures of 3D optical and surface structures as small as 300 nm on a photoresist layer using a 405 nm laser.

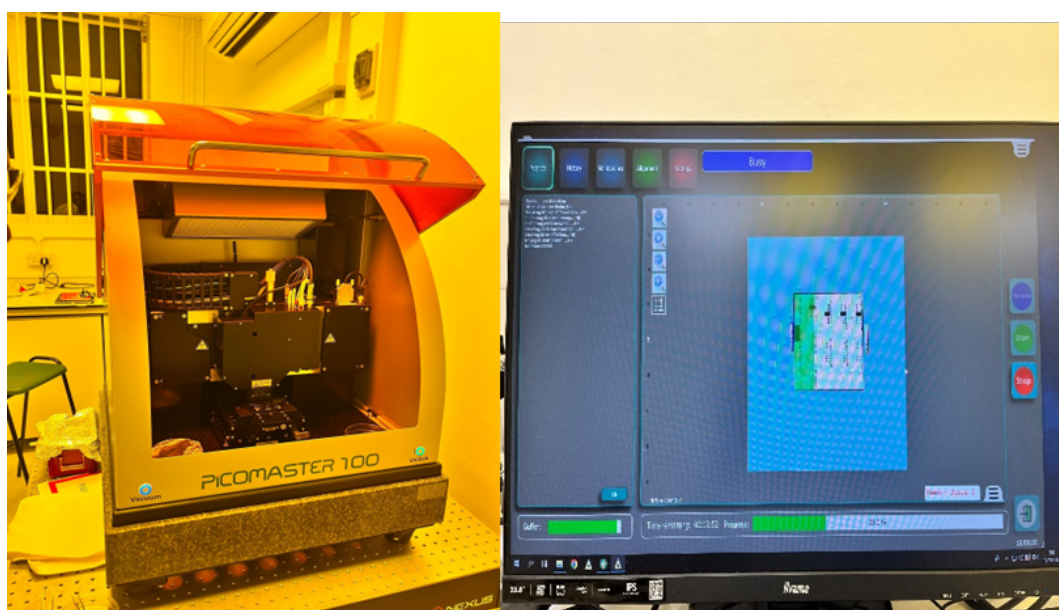


Figure 2.9: The PicoMaster is used to fabricate nanostructures using photolithography. The Project Manager, a software from PicoMaster or Klayout (another imaging software), transfers the designs from AutoCAD onto the PicoMaster software (shown on the screen) by converting the format from AutoCAD to a format that is edited on the PicoMaster software before the final set-up operations and subsequent printing.

2.1.7 Post-Exposure Bake

Post-exposure bake (PEB) is a necessary post-fabrication step when the negative photoresists are used to fabricate the structures. The baking step follows the exposure (printing) of the structures before the resist is developed. It completes the photoreaction initiated during exposure necessary for chemically amplified photoresists (negative photoresists) (Figure 2.10)¹⁶².

Without this PEB step, the resist films (and the structures made) will develop extremely slowly.

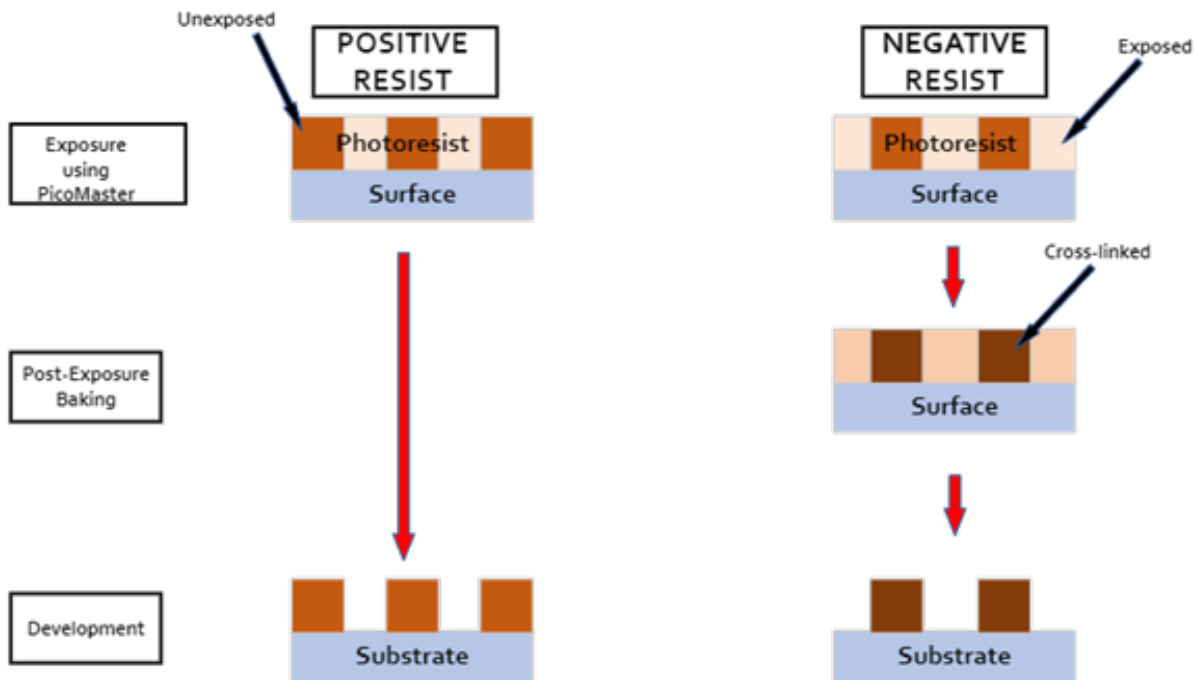


Figure 2.10: The post-exposure baking step is necessary for negative photoresists¹⁶⁵.

However, the time and temperature it takes to develop do not depend on the size of the structures but on the type of photoresist used^{160,162}.

2.1.8 Development of Printed Structures

Developing the printed substrates as quickly as possible is a good practice, as this eliminates the slow build-up of carbonates, which might otherwise affect the rate of development. It is necessary to develop the exposed areas from the unexposed area of the printed photoresist (by dissolving the exposed resist areas for the positive photoresist) to produce the desired printed nanostructures using AZ351B developer for the positive photoresist and deionised water, and AZ326MiF developer for the negative photoresist (with the unexposed areas dissolving in the developer for the negative photoresist). The dissolution of the exposed areas is based on the conversion of the diazo naphthoquinone-sulphonate (DNQ) based photoinitiator in an indene

carboxylic acid by changing from the hydrophobic to the hydrophilic side of the phenol resin chain where OH chains are located for the positive photoresist. The solubility of the resin molecules is increased in the developer due to the indene carboxylic acid splitting the hydrogen ion to cause the resin molecule to be electrically charged. For the negative photoresists, it forms a carboxylic acid, which cross-links the resin during the baking step to make it insoluble in the developer. At the same time, the unexposed areas become soluble in the developer. The dilution of the AZ351B developer with water in a ratio of 1:4 (developer: water) is due to the rate of dissolution of the exposed resist to that of the rate of dissolution of the unexposed resist that would remain on the surface as the nanostructure, and this was observed to produce well-developed structures upon immersion for twenty seconds and the same immersion time also produced well-developed structures for the negative photoresists but without any dilution of the developer used for negative photoresists (AZ 326 MiF)¹⁶².

2.1.9 Sputter-Coating of Structures

To enhance the Raman signal of the printed substrates, the printed surface must be covered with a metal such as gold or silver. An agar auto sputter-coater (Agar Scientific, Rotherham, UK) (Figure 2.11) was used to coat 30 - 40 nm of either gold or silver on the printed and developed patterned structures. The rough metallic coating enhanced the Raman signals, which are necessary for SERS, and the coatings of the patterned photoresists were necessary to metallise the structures for use as SERS substrates. Using the method described in this thesis, patterned photoresists can be easily scaled and different geometries fabricated with better control of the morphologies, compared to patterning gold directly. Coating the structures with 30 – 40 nm of gold also allows better surface roughness control. Even though rough surfaces are needed for SERS, uneven gold coatings will create inconsistent hotspots, leading to challenges in reproducibility. The coated nanostructured surfaces maintained the fidelity and designs of the

photoresists (as shown by the AFM images in Figure 4.3 and Figure 5.1), avoiding issues with uncontrolled and uneven surface roughness.

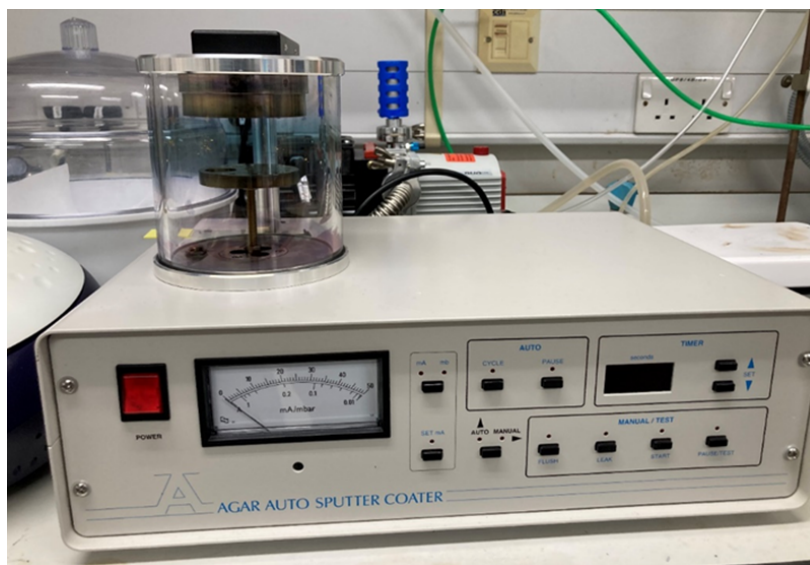


Figure 2.11: Agar Sputter Coater is used to coat the fabricated nanostructures with gold or silver.

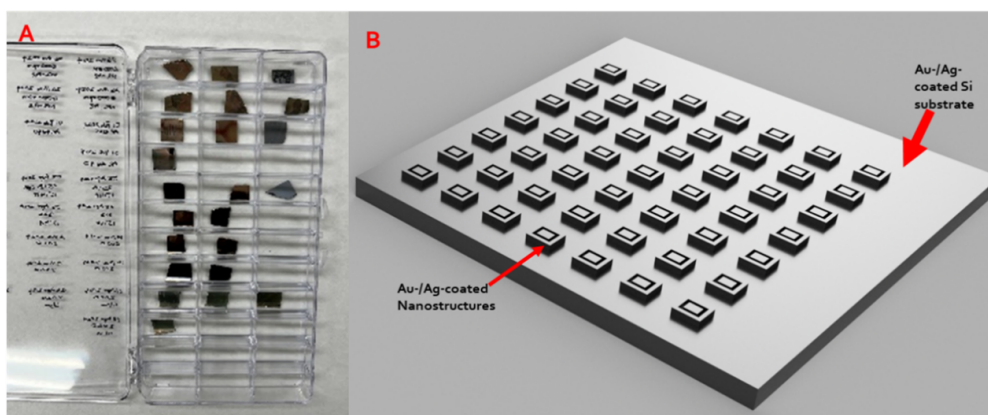


Figure 2.12: (A) Fabricated substrates ready to be used. (B) Conceptual design of structures using Fusion360.

2.2 PICOMASTER FOR FABRICATION OF PATTERNED STRUCTURES

PicoMaster is a high-precision laser lithography system that creates intricate micro- and nanostructures with a high resolution. It is a versatile direct-write lithography system that operates with laser-based technology using a focused laser with a wavelength in the ultraviolet range (typically 375 or 405 nm) as the light source¹⁶⁶. It can be used to create substrates, including channels and chambers in microfluidic devices, sensors, and other microdevices in MEMS, lab-on-a-chip devices, and other biomedical devices. The resolution of the structures produced can be as fine as 300 nm.

2.2.1 How it differs from other PL Techniques

PicoMaster differs from traditional photolithography because it is a maskless photolithography system. It does not require a physical mask to define the patterns, eliminating the need for intermediate and expensive mask fabrication and alignment processes. This makes it more flexible and cost-effective for custom design. Direct writing of the photoresists by the laser can achieve sub-micron resolution, enabling the design and patterning of highly detailed structures. 3D structures with varying heights and depths can also be fabricated by varying laser intensity and exposure time. These properties make it ideal for rapid prototyping, iterative design, and low-volume production.

PicoMaster works by directing the laser onto a photoresist-coated surface, exposing the patterns directly. Its high-precision optics, autofocus, and motion-control systems ensure accuracy even on uneven surfaces (usually determined during the scanning process while setting up the fabrication process).

However, PicoMaster has some limitations. Although the choice of photoresist (either negative or positive) depends on the desired resolution and specific application, the thickness of the resist plays a significant role in determining the resolution and fidelity of the pattern produced. A thinner photoresist can provide higher resolution but may be susceptible to defects during post-processing, whereas a thicker photoresist can produce deeper structures but with compromised resolution. Another limitation of direct-write lithography systems such as PicoMaster is their resolution limits. They are also slower for mass production because of the serial nature of patterning compared with the parallel patterning obtained in other photolithography systems, compared to other photolithography techniques such as extreme ultraviolet, which has a resolution limit of 30 – 40 nm, electron beam lithography with a limit of 2 – 5 nm, focused ion beam lithography with a limit of 10 nm^{167,168}. The resolution limit of the PicoMaster, therefore, affects the SERS performance, as the Raman signal enhancement is significantly amplified by structures in the nanometre region (less than 100 nm), and the technique cannot fabricate structures in this size region without modifications.

The PicoMaster100 used in this study for the fabrication process produced three different types of resolution¹⁶⁶; depending on the spot size used, the highest resolution was obtained with a spot size (i.e. the diameter of the laser on the substrate) of 280 nm and a depth of focus of 300 nm. The applied laser intensity (power), called the energy dose, d in mJ/cm² during a certain time, is given by the following equation:

$$d = \frac{I \times t}{A} \quad (2.1)$$

where I is the laser intensity, t is the time (s), and A is the area (cm²). The laser intensity set point is calculated based on the exposure energy chosen during the setup of the printing equipment, the scan speed v , and the step size, given by the equation:

$$I = v \times d \times \text{stepsize} \quad (2.2)$$

The step size, which can be set to 50% or 100% during the setup of parameters on the equipment, determines the extent to which the step axis moves between every line of the scan axis. For the PicoMaster 100 used in this study, there are three options for the spot size: 280 nm, 450 nm, and 880 nm, with corresponding values of 300 nm, 870 nm, and 2.4 μm for the depth of focus. These values correspond to high-, mid-resolution, and low-resolution. The optical module of the equipment has a focal length of 0.6 mm.

The PicoMaster100 was operated in a vector writing mode to eliminate stitching issues and optimise the focus depth, allowing lateral fabrication of each surface. The patterning is performed with single laser writing to produce selected structures to optimise the patterning speed, spacing, array, and structural dimensions for cost-effectiveness and capacity.

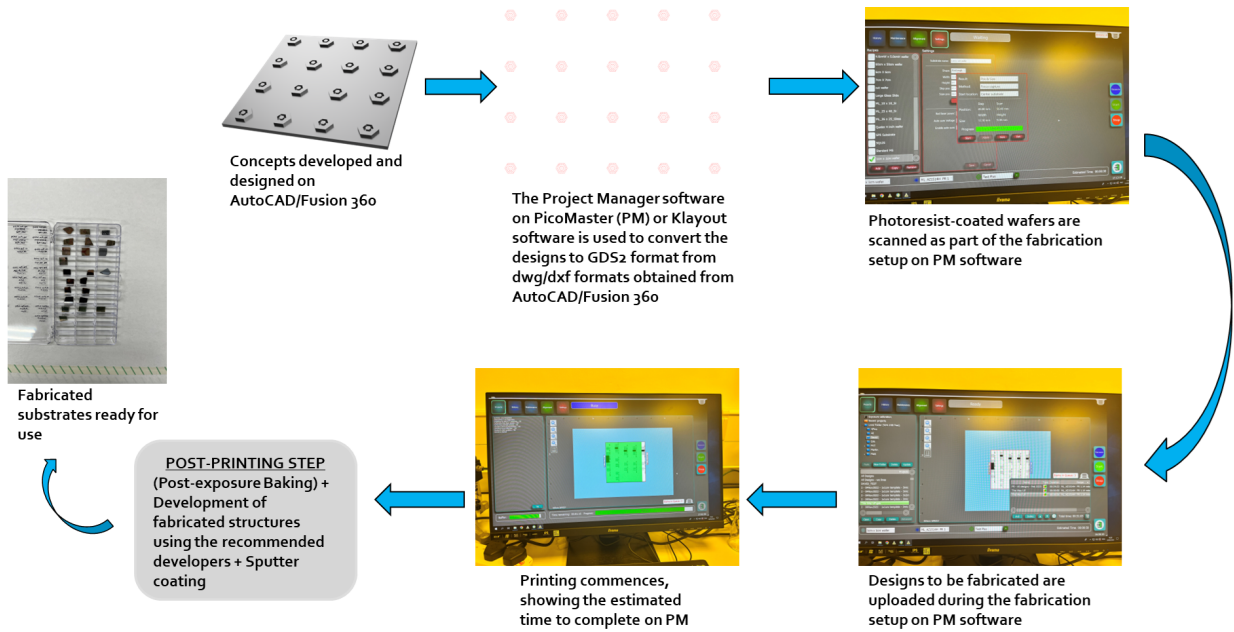


Figure 2.13: The schematic of the fabrication process on the PicoMaster 100, showing an example of the setup of the photoresist-coated wafer where the patterns are printed. The process also includes the setup of substrate, exposure, and alignment recipes¹⁶⁶.

A smooth, thin film of positive or negative photoresist was spin-coated onto a clean silicon wafer. The scan speed set to an optimal value of 70 mm/s (this could be set to a maximum of 200 mm/s or minimum of 50 mm/s speed values, affecting the speed with which it takes to complete a project). The laser power was set to between 120 and 250 μW , adjusted appropriately, depending on the surface detection by the PicoMaster before printing. The exposure energy (the energy dose representing the laser intensity applied to an area for a certain time) was set to an optimal value of 700 mJ/cm^2 for the negative photoresists and 200 mJ/cm^2 for the positive photoresist. PicoMaster100 has three options for the spot size, which refers to the diameter of the laser on the substrate: 280 nm, 450 nm and 880 nm. The optimal value used was 880 nm to obtain a depth of focus up to 2.4 μm , while for step size, 50% of the spot size was used as recommended by the manufacturers (the step size is the amount by which the step axis moves while scanning). These optimal conditions were achieved via a series of experimental iterations with a range of values for both material and equipment settings, as discussed in section 5.3 of this thesis and are presented in the images of the fabricated structures in Figure 5.1.

2.3 CHARACTERISATION TECHNIQUES

It is essential to view and capture images of the fabrication and printing of the structures on the substrates. The experimental analysis depends on the ability to see the images through optical, atomic force, and scanning electron microscopy, which will be adopted throughout this research.

2.3.1 Optical Microscopy

Optical microscopy gives a snapshot of the structural information of the patterns to produce micrographs. It was also used to check the effectiveness of the cleaning process, the design, printing by the PicoMaster, and the post-fabrication processes, such as the sputter-coating of metals on printed structures. Although optical microscopy is limited by the resolution it can achieve, it still provides information about pattern formation on the thin film. The resolution is the minimum separation between two points that can be resolved, and the maximal resolution is limited by the optical components' numerical aperture and the light's wavelength. Some of these properties have ensured the application of optical microscopy in various research fields^{169–171}.

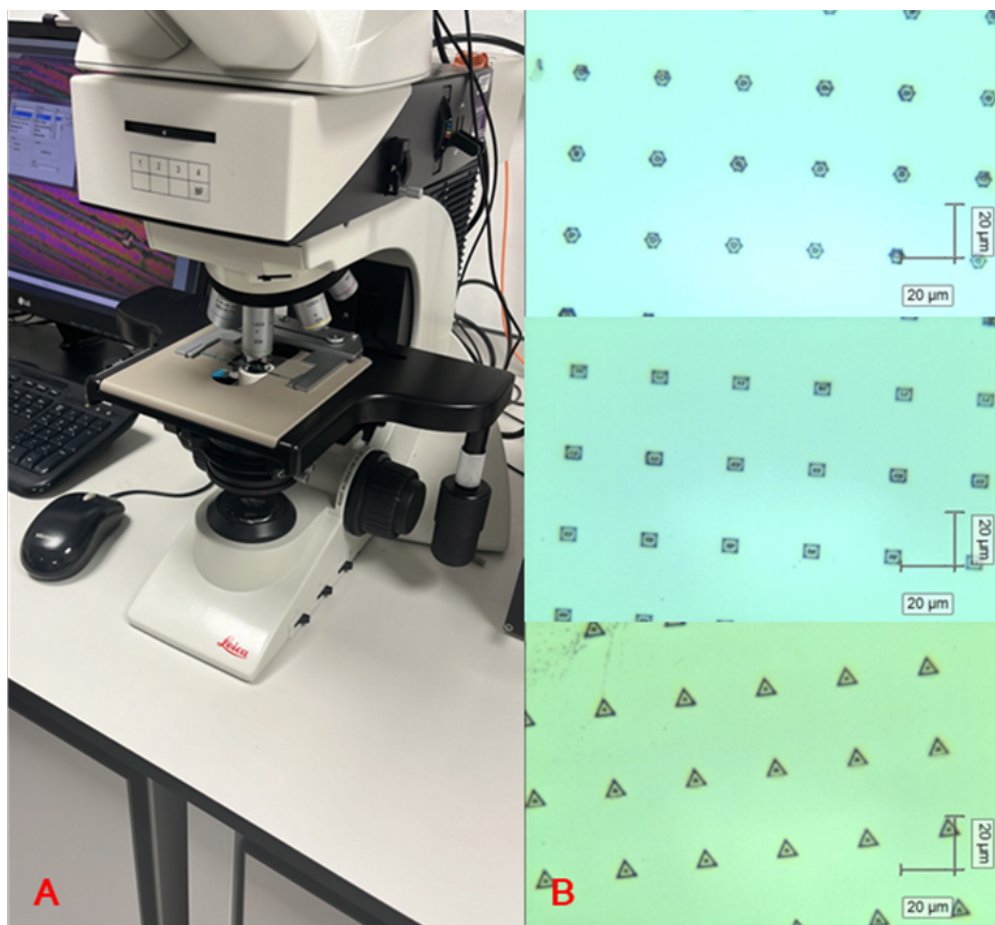


Figure 2.14: (A) The optical microscope Leica DM2700M used for imaging the structures fabricated, (B) micrographs of different SERS substrate patterns fabricated with a periodicity of 30 μm .

To qualitatively analyse the substrates, including structural information and cleanliness, a Leica DM2500 microscope (Leica Microsystems, Milton Keynes, UK) with universal white light LED illumination was used to view submicrometric features in the InVia Qontor Raman system (Renishaw, Wotton-under-Edge, UK), and a Leica DM2700M (Leica Microsystems, Milton Keynes, UK) to produce micrographs of the patterned substrates (Figure 2.14B).

2.3.2 Atomic Force Microscopy

Atomic force microscopy (AFM) is a high-resolution imaging technique that provides detailed topographical, chemical, and magnetic information on the structures of a sample without destroying the sample in a high-resolution medium such as air, liquid or vacuum. AFM is based on scanning a sharp tip made from silicon or silicon nitride attached to a flexible cantilever with a spring constant over a sample surface that feeds back information to the AFM system optically. The movement of the AFM tip over structural features of the sample deflects the AFM cantilever, which is tracked by the laser beam and directed to a position-sensitive photodetector. 3D topographic images are generated by the coordinates of the AFM tip tracked during the scans to provide structural images of the sample analysed. The scanning is based on two standard modes of operation, the contact mode and the tapping mode, with the main difference being the level of contact the tip makes with the sample^{172–174}.

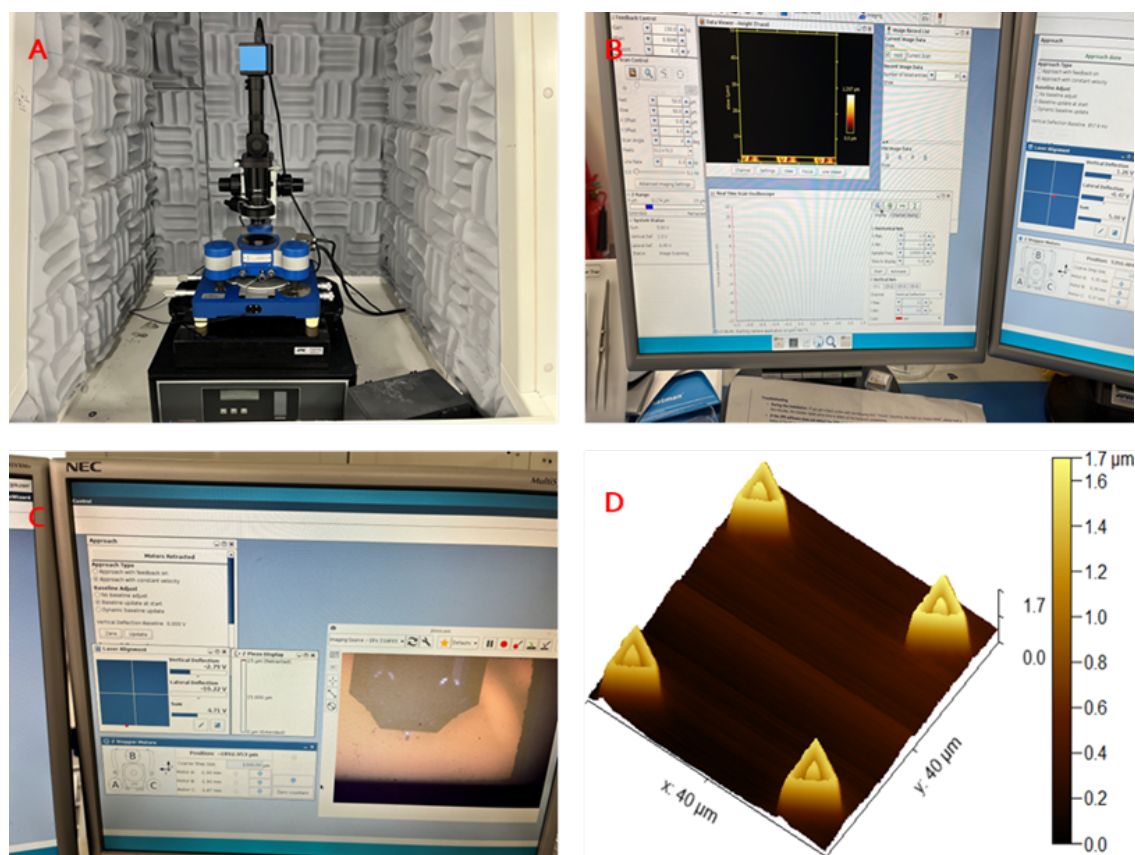


Figure 2.15: (A) JPK Nano Wizard 4 AFM, (B) Capturing the optical microscope image during sample analysis, (C) Set up of the equipment with cantilever and laser alignment, and (D) Processed AFM image of a sample using Gwyddion.

The AFM provided more detailed structural information than optical microscopy. Measurements were carried out on JPK NanoWizard 4, with image processing and analysis carried out on the instrument software JPK Instruments NanoWizard3 version 4.2 and Gwyddion version 2.59, a Statistical Parametric Mapping (SPM) data visualisation and analysis tool.

2.3.3 Scanning Electron Microscopy

A scanning electron microscope (SEM) uses a focused beam of electrons to produce images of a sample by scanning its surface in a raster pattern. This is achieved by the interactions between the sample and the electron beam to produce different signals, such as secondary and

backscattered electrons and characteristic X-rays, which are detected and processed to create high-resolution images to reveal the topography and composition of the sample. The SEM uses an electron gun to produce the focused beam of electrons, which is directed onto the sample, leading to interactions with the atoms in the material. The secondary electrons are commonly used to produce the topographical images, as they are sensitive to the changes in the topography of the sample's surface. SEMs provide detailed visualisation of surfaces as they produce much higher resolution images than light microscopes and a large depth of field to give a 3-dimensional image. They are not limited by the wavelength of light, which limits light microscopy^{175,176}. The characteristic X-rays obtained in SEMs can also provide more information about the sample's elemental composition. Due to image quality, they are mainly used in materials science, nanotechnology, biological and medical analyses, and forensic science, including sample modifications to ensure detailed image capture^{175,177–179}. For this study, the SEM measurements were performed using a Hitachi SU5000 at acceleration voltages of 0.5 - 5 kV with a 2 - 5 nm lateral resolution.

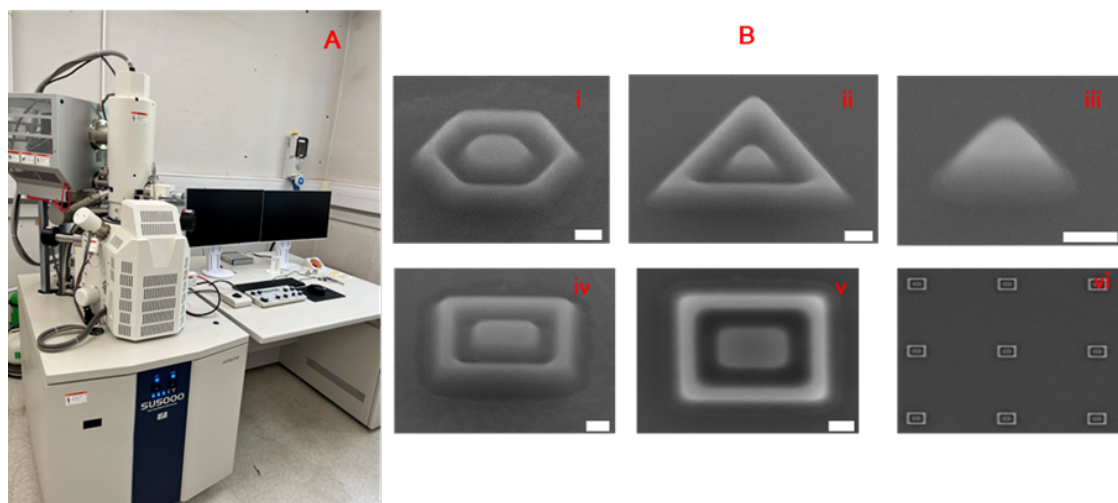


Figure 2.16: (A) Hitachi SU5000 SEM, (B) SEM images of some of the fabricated structures (i) concentric hexagonal pillar, (ii) concentric triangular pillar, (iii) solid triangular pillar, (iv) concentric rectangular pillar, (v) concentric rectangular pillar (bird view), and (vi) an array of concentric rectangular pillars with 30 μm periodicity. Scale bars = 1 μm .

2.3.4 Surface-Enhanced Raman Spectroscopy

Raman spectroscopy is a robust analytical technique that uses the inelastic scattering of light to study rotational, vibrational and other low-frequency modes in molecules to provide information about the chemical composition and molecular structure of the molecules in samples^{180–182}. Surface-enhanced Raman spectroscopy is a variation of Raman spectroscopy that enhances the Raman signals of molecules adsorbed on coated metal rough surfaces. The enhancement is achieved via the coated metal's localised surface plasmon resonance for trace-level molecule analysis with increased sensitivity and detection^{102,116,183}. Figure 2.17A shows a schematic representation of the InVia Qontor Raman spectrometer by Renishaw (Renishaw, Wotton-under-Edge, UK), with the beamsplitter separating the beams used to measure Raman spectra of samples. The spectrometer measures samples using 514 nm, 633 nm, 785 nm, and 830 nm lasers. It is equipped with a Leica confocal microscope DM2500 with an objective lens of 50x magnification and a 0.75 numerical aperture, which aims at the sample with the scattered radiation going through a set of mirrors, lenses, filters and grating that cleans the scattered radiation into the Raman scattering, which is resolved in the charge-coupled device (CCD) and analysed using the Renishaw WiRE software (Renishaw, Wotton-under-Edge, UK) for the Raman spectra obtained.

The samples were measured with an acquisition time range of 0.1 - 10 seconds for each point in a map scan and three accumulations, excitation laser power of 30 - 40 mW, and a grating of 1200 l/mm with scans recorded in the range of 500 – 2000 cm^{-1} to produce 100 scans per sample.

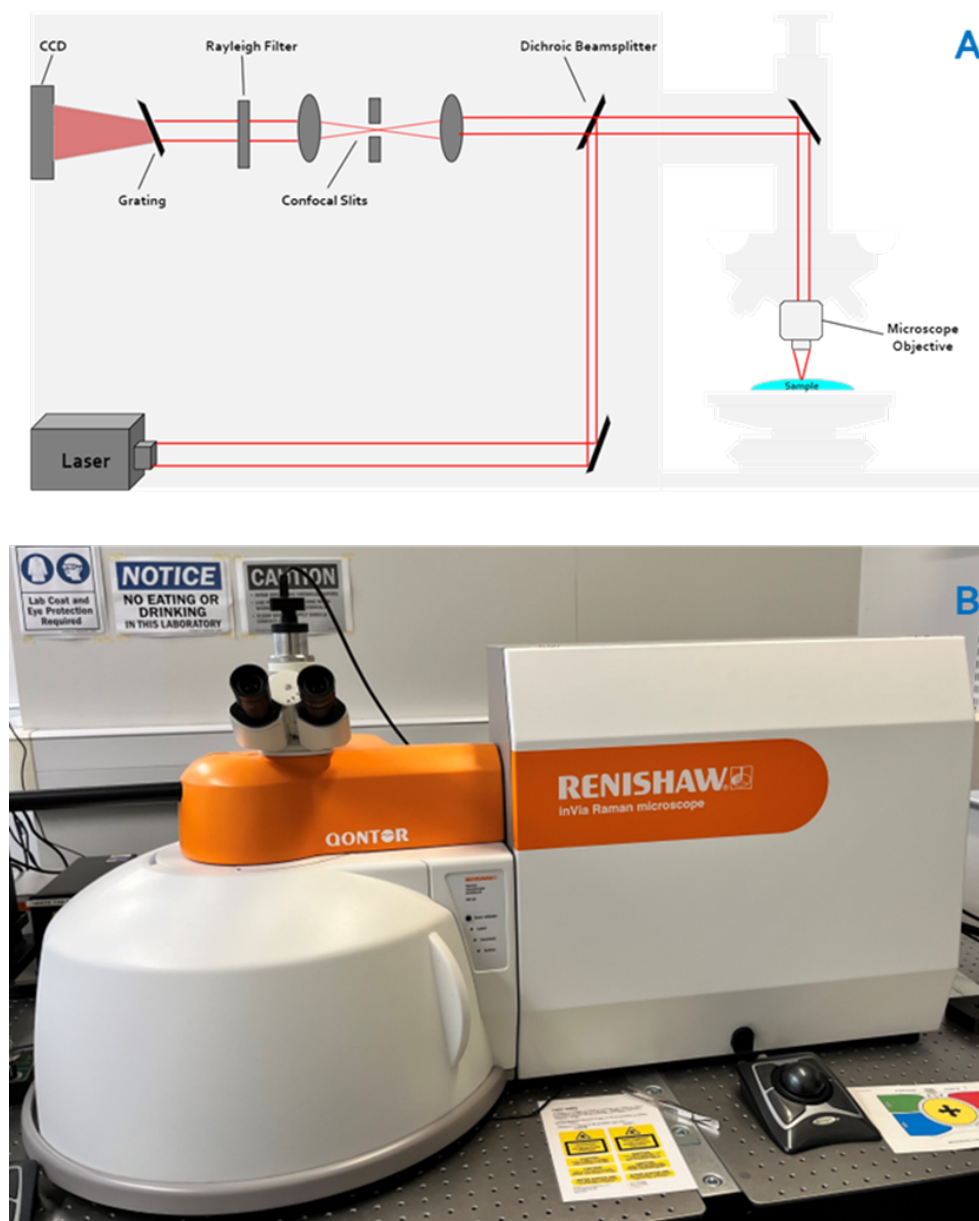


Figure 2.17: (A) Schematic representation of the Raman spectrometer showing the main components, (B) The InVia Qontor Raman spectrometer from Renishaw.

2.4 SOFTWARE

For this research study, several software packages were used for data analyses, simulations of optimal structures before fabrication, image processing, schematic drawings, and other processes to present results.

2.4.1 COMSOL Multiphysics

COMSOL Multiphysics software (COMSOL Ltd, Cambridge, UK) is a versatile tool that simulates designs, processes, devices, and other engineering aspects. It provides fully coupled single and Multiphysics modelling capabilities and an understanding of the behaviour of complex systems involving multiple phenomena. COMSOL Multiphysics 5.5 with wave optics module and frequency domain was used to simulate structures to determine the optimal structure for Raman signal enhancement¹⁸⁴. This was done with topologically designed structures on AutoCAD 2021 (Autodesk Ltd, Birmingham, UK) before being imported into and simulated with the finite-element solver COMSOL via CAD module.

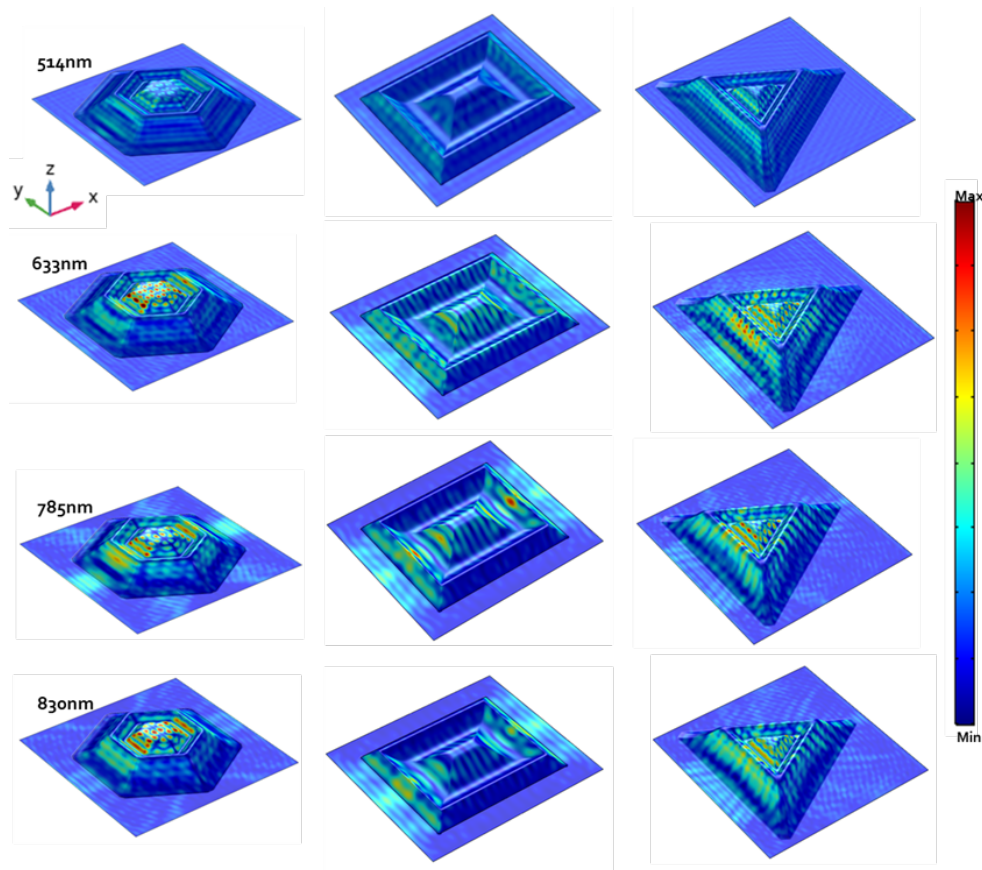


Figure 2.18: COMSOL simulations of various topological patterns using different lasers to identify the optimal structure for Raman signal enhancement. The electric field is polarised along the x direction, the magnetic field is polarised along the y direction, and incoming wave is propagating in the z direction.

2.4.2 Python

Python is a high-level programming language widely used in data analysis due to its ease of learning, versatility, extensive libraries, and community support¹⁸⁵. It is used in this research project for data cleaning, statistical analysis, visualisation and processing of large data sets.

2.4.3 Other Software

The other software used include Mendeley (Mendeley Ltd, London, UK) for reference organisation and presentation; Microsoft Visio (Microsoft Ltd, Reading, UK), Microsoft PowerPoint (Microsoft Ltd, Reading, UK) and Inkscape (open-source software, online) for schematic diagrams; and Renishaw WiRE 5.6 for Raman spectra data analysis, which includes baseline subtraction, cosmic ray removal and normalisation of spectra. Other software used includes Gwyddion (open-source software, online), which was used for processing AFM data and images of fabricated structures; AutoCAD, AutoCAD Mechanical, and Fusion 360 (Autodesk Ltd, Birmingham, UK) were used for the design of structures that were transferred to the PicoMaster software via Klayout (open-source software, online) to convert to a different data format for fabrication of the structures to produce the substrates; and OriginPro (Silverdale Scientific Ltd, Bucks, UK) for Raman data processing and presentation in the form of charts.

CHAPTER THREE

EXPLOITED RESEARCH
TECHNIQUES: THEORETICAL
BACKGROUND

Part of this chapter was covered as a co-author in a published journal article, Discover Nano, as a review article titled 'Advances in lithographic techniques for precision nanostructure fabrication in biomedical applications', Stokes *et al.*, 2023. This chapter further highlights the photolithographic technique used in this research work, including the theoretical background used to fabricate patterned SERS substrates. The chapter also covers Raman spectroscopy and the modified Raman-enhanced Raman scattering to achieve higher Raman signal enhancements in substrates.

3.1 LITHOGRAPHY TECHNIQUES

Lithography is a fundamental technique used in the microfabrication of substrates through patterning to produce intricate designs on the micro and nanoscale^{25,186–189}. It is a top-down approach that involves the selective removal of materials to produce patterned substrates, and is particularly common in the semiconductor industry in manufacturing integrated circuits and MEMS, such as those found in computer processing devices, biosensors, and other biotechnological devices used in the health industry^{25,145,190–192}. There are different types of lithographic techniques broadly classified as conventional, such as photolithography (PL), scanning beam lithography (electron beam and focused ion beam), ultraviolet (UV) lithography, X-ray lithography, and unconventional techniques, such as scanning probe lithography, solvent-assisted micromolding, nanoimprint lithography, and edge lithography^{167,168,186,187,193–197}. PL is the most commonly used of these techniques due to the stability of the pattern produced, lateral fidelity and high resolution^{23,187,198}.

3.2 PHOTOLITHOGRAPHY

Photolithography is a top-down lithographic technique that uses light to fabricate geometric patterns using photoresists on a surface^{168,199–201}. The distinguishing feature of PL compared to other lithographic techniques is the use of light to fabricate patterns on the surface containing the photoresist layer, which causes chemical changes to the photoresist upon exposure to light. This allows for selective removal of the photoresist's exposed or unexposed regions, depending on the type of photoresist used^{187,193,202,203}. The scalability, precision, high resolution, and batch processing also ensure that PL is a leading method for producing devices that rely on sensors and other microelectronic devices and offers a good balance between costs and resolution^{133,198}. Photoresists are sensitive to light and play essential roles in patterned structures based on the pre-fabrication and post-fabrication steps undertaken^{153,160,161,204}. Another distinctive feature of PL is its ability to produce extremely fine patterns, with the resolution limited by the wavelength of the light used. A shorter wavelength is typically used to fabricate finer patterns of structures to be fabricated¹⁶⁰.

The resolution of an optical imaging system is generally determined using Equation 3.1.

$$k \times \frac{NA}{\lambda} \quad (3.1)$$

where NA is the numerical aperture of the imaging lens, k is the resolution scaling factor, and λ is the wavelength^{160,204}. Initially, k , which indicates the relative resolution based on a given NA/λ ratio, was set in the industry to 0.8 or higher until patterned corners suffered rounding issues, and it was dropped to 0.35 for memory circuits with resolution enhancement techniques, such as phase-shifting mask. This adds to costs as it introduces more processing steps, masks, and higher resolution tools, which led to the introduction of other lithographic techniques as well as

PL being used with other techniques to leverage the advantages of two or more techniques, such as nanoimprint lithography, multi-layer deposition, and etching^{195,201,205,206}. An optical imaging system also relies on another parameter, depth of focus, to determine its performance²⁰⁴.

The basic PL steps involved in preparing the substrates (already covered in Section 2.1) can be summarised as cleaning the silicon wafer to remove contaminants that could affect the photoresists spin-coated on the surface to form the structures; the photoresist is then exposed to light from the laser in the PicoMaster machine, causing chemical changes in the photoresist; and the development process, whereby a developer solution is used to dissolve the exposed or unexposed regions, depending on the type of photoresist used. Other processes involved include the soft-baking immediately after the spin-coating of the photoresist on the surface; an additional post-baking step is necessary for the negative photoresist to improve adhesion (done immediately after exposure) before the development process^{156,161,164,167}. The patterning of the substrates *via* PL can allow the use of Raman spectroscopy for fingerprint detection of the characteristic Raman signals of molecules used to detect diseases such as TBI, as discussed in Section 1.2.

3.3 RAMAN SPECTROSCOPY

Raman spectroscopy (RS) is a technique based on the inelastic scattering of photons (known as the Raman effect) used to observe rotational, vibrational, and other low-frequency modes in a sample that provides important information about lattice dynamics, crystallinity, and inter-atomic or inter-molecular forces. This technique uses monochromatic light, often in the ultraviolet (UV), visible, or near-infrared range, to understand the inelastic scattering of light. This phenomenon was first discovered in 1922 by (and named after) the Indian physicist C.V. Raman, while Smekal theoretically predicted inelastic light scattering in 1923. Still, Raman, alongside his colleague K.S. Krishnan, further experimentally observed the phenomenon in 1928

by inelastic scattering with sunlight, a cross filter, and a narrow photographic filter^{183,207}. He predicted that a small fraction of the scattered light would undergo a change in wavelength when light interacted with matter, for which he was awarded the Nobel Prize in Physics in 1930²⁰⁷. Further advancements in technology, such as improved detectors, optics, and data processing, have made Raman spectroscopy an excellent, non-destructive analytical tool for identifying the composition of samples.

The Raman effect occurs when a monochromatic light source, such as a laser, is incident on a sample, making the photons interact with the molecules in the sample and causing some of them to be absorbed and re-emitted. While most of them are scattered elastically without wavelength changes (known as Rayleigh scattering), a small fraction of the scattered light changes the wavelength, which is referred to as Stokes Raman scattering (for those that increase in wavelength) or anti-Stokes Raman scattering (for those that decrease in wavelength)⁸⁶. This shift in the wavelength of the Raman scattered light from molecular or lattice vibrations can provide valuable information about the chemical composition of the samples, including the type of chemical bonds and vibrational frequencies. The monochromatic light source is used to check the shift of the scattered wavelengths⁸⁵, whereby, according to the Planck-Einstein equation,

$$E = h \times \nu \quad (3.2)$$

where h is Planck's constant and ν is the frequency of the radiation; no energy, E , is lost or gained for Rayleigh scattering, and the frequency is maintained. However, the energy is transferred from the photon to the sample for Stokes scattering, with part of the initial energy transformed to vibratory energy for the elastically scattered emitted photons (Figure 3.1), and *vice versa* for anti-Stokes scattering.

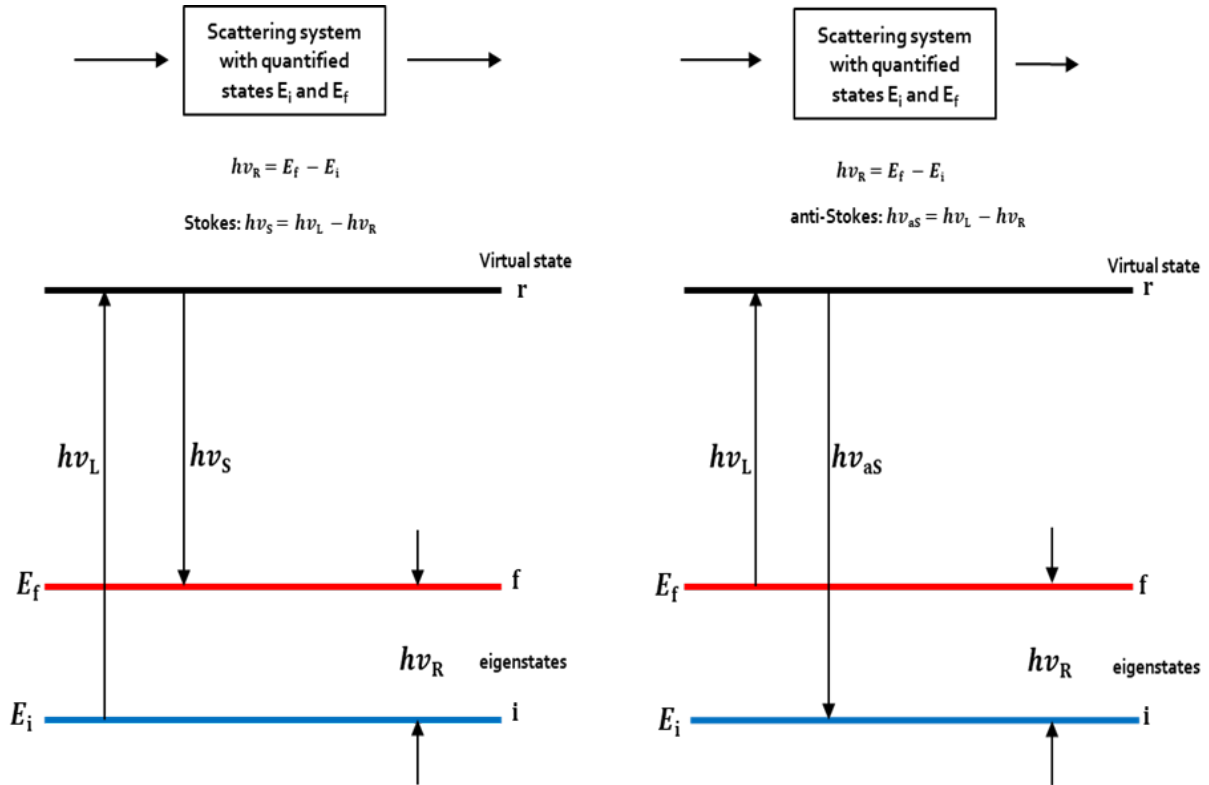


Figure 3.1: Schematic illustration of the Stokes Raman and anti-Stokes Raman scattering, where ν_L is the laser frequency, ν_S is the frequency of the Stokes scattered light, ν_{aS} is the frequency of the anti-Stokes scattered light, ν_R is the final frequency of the system, h is the Planck's constant, E_f and E_i are the energy level of two quantum states i and f respectively of a photon, and r is the virtual state⁸⁶.

The information from the wavelength shift obtained from the differences associated with the molecular vibrations of the sample, also known as the Raman shift, correlates to the wavenumber of the vibrations, with the same information obtained from both the Stokes and anti-Stokes modes⁸⁵. The Raman scattering intensity is inversely proportional to the fourth power of the exciting wavelength ($\propto 1/\lambda^4$), with the Raman spectra typically shown with the horizontal axis denoting Raman shift, with the unit of inverse centimetres, cm^{-1} (or wavenumber). This relationship explains why specific laser wavelengths produce better Raman peaks than others; this is further described in Section 3.4.3 in the choice of laser used for qualitative measurement. The spatial resolution of the laser spot on the sample, which in Raman microscopy, is estimated as $1.22\lambda/\text{NA}$, showing that the shorter the wavelength λ of the laser, the smaller the focused laser spot. NA is the numerical aperture of the microscope objective, with this given consideration in

the choice of laser to use⁸⁵.

Although Raman spectroscopy provides both qualitative and quantitative analyses of samples, detecting the bonds between the atoms is very poor, especially in samples with low concentrations, with only 10^{-6} to 10^{-8} of the laser photons being converted to Raman photons. For some biological samples, high fluorescence inhibits the detection of the inherently poor Raman signals, making techniques that can enhance the Raman signal detection, such as surface-enhanced Raman scattering, essential, as it significantly enhances the intensity of Raman signals by several orders of magnitude^{86,90,181,207}.

3.4 SURFACE-ENHANCED RAMAN SCATTERING

The use of an electrochemically-roughened silver electrode surface by Fleischman *et al.*²⁰⁸, to try to increase the number of adsorbed molecules of pyridine, led to a Raman intensity enhancement that kickstarted the phenomenon known as surface-enhanced Raman scattering (SERS). The high enhancements achieved through SERS transformed RS from a structural analytical tool to a structurally sensitive, single-molecule probe used for fingerprint identification of molecules in samples and for disease identification^{102,116,191,209,210}. This is the only detection technique that can be deployed in out-of-laboratory settings without significant performance loss.

It is pertinent to design rough SERS surfaces that can produce consistently reliable results, which is lacking with the use of nanoparticles to fabricate SERS substrates¹¹⁶. The most common metals used as SER surfaces are gold, silver, and copper¹⁸⁶, but when these are in the form of metallic nanoparticles, even though they produce high enhancements (via hotspots), it is challenging to reproduce consistent enhancement due to the randomness of these hotspots, making them unsuitable for use as biosensors, as reliability, reproducibility, and accuracy are paramount. Therefore, planar nanostructured surfaces are more suitable for SERS biosensors because of their

consistent hotspots, batch-to-batch reproducibility, and ease of fabrication¹⁸⁶. Initially, it was hypothesised that the large surface area causes these enhancements, with the chemical theory suggesting that charge transfers for molecules that form chemical bonds with the surface²¹¹. However, this was later clarified with the electromagnetic theory¹⁸², which proposed that the increased local electrical fields due to the excitation of collective electron oscillations contribute significantly to the enhancement.

3.4.1 Raman Spectroscopic Theories

While SERS requires more metal nanostructures than other Raman spectroscopic techniques, two theories (enhancement methods) primarily drive it: electromagnetic and chemical theories. Both are influenced by the topology of the rough surface, which plays a vital role in Raman signal enhancement of the substrates^{102,108,117,182,211,212}.

Electromagnetic theory (EM), based on nanometre-scale gaps between the metallic structures or particles used, commonly referred to as hotspots, generates localised surface plasmon resonance (LSPR). An LSPR occurs when the light frequency matches the oscillation frequency of the electrons, creating an enhanced electrical field near the particle's surface^{181,202,213}. This LSPR enables the enhancement of the Raman signals between the hotspots, and the enhancement relies significantly on the optical resonance of the noble metal nanostructures, which in turn enhances the EM field through the excitation of the LSPR^{115,186}. Some of the common surface metals that are used include gold (*Au*), silver (*Ag*), copper (*Cu*), platinum (*Pt*), palladium (*Pd*), titanium (*Ti*), and aluminium (*Al*). The shape, concentration, and size of the metal nanoparticles contribute significantly to the enhancement, as these properties influence the ratio of absorption and scattering properties^{108,214–216}.

Chemical theory is based on intermolecular and intramolecular charge transfer to enhance Raman

spectrum peaks. Although considerably smaller in terms of its enhancement capabilities compared to EM enhancement, it is sometimes seen as a modification of the probe's electronic polarisability, which can induce resonant-Raman scattering at wavelengths where the non-absorbed molecules would not be resonant, thereby enhancing the signals^{186,217}.

SERS is based on the LSPR phenomenon, whereby rough metallic surfaces greatly enhance the Raman scattering signals of molecules adsorbed on or near these roughened surfaces^{186,218–220}. According to Jiang *et al.*, surface plasmon resonance is the electromagnetic wave-induced collective oscillation of charge carriers at the interface between materials with positive and negative permittivities, typically a dielectric and a metal²²¹. Different shapes of structures were fabricated, such as concentric triangular, rectangular, hexagonal, and cylindrical pillars, as well as the solid versions of these structures, to utilise LSPR for signal enhancement.

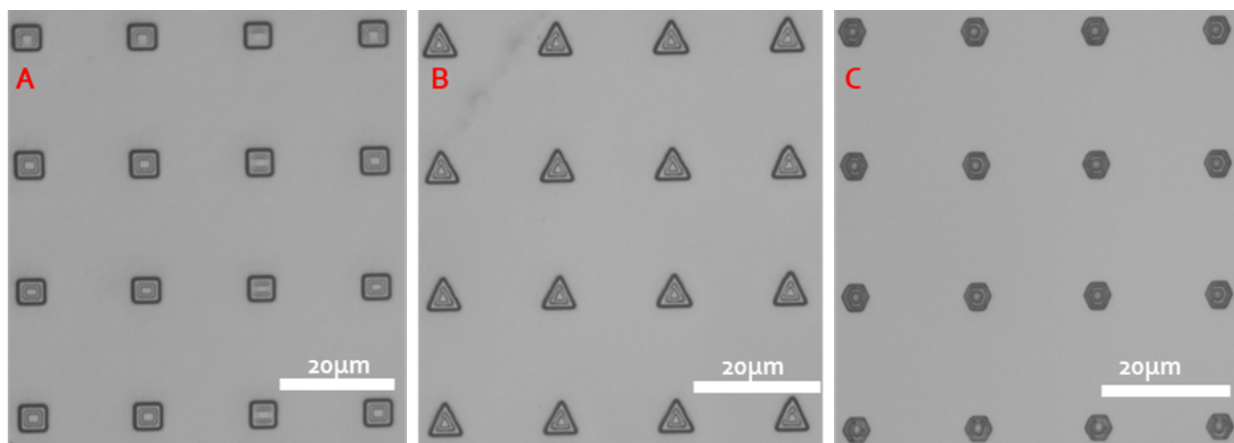


Figure 3.2: Optical micrographs examples of different patterned concentric SERS substrates (A) rectangular, (B) triangular, and (C) hexagonal structures using photolithography for Raman signal enhancement.

The enhancement factors (EF) of the fabricated structures (Section 5.3) were determined using the Raman spectra produced by a layer of benzenethiol (BT) deposited on the patterned surfaces. The SERS substrate enhancement factor measures the difference in the Raman signals of an analyte due to the presence of certain phenomena (such as the type of patterned structure and metallic coating properties) compared to the unenhanced reference sample. While there is significant variation due to the enhancement reference methods and enhancement factor

calculations in the reporting of SERS substrate performance^{108,222}. The enhancement factor used in this study is shown in Equation 3.3 below:

$$EF = \left[\frac{I_{SERS}}{\rho_S A} \right] / \left[\frac{I_{RAMAN}}{\rho_V V_f} \right] \quad (3.3)$$

where I_{RAMAN} = SERS intensity of the unenhanced Raman scattering peak at 1070 cm^{-1} of pure benzenethiol,

I_{SERS} = corresponding SERS signal of the SERS substrate containing the nanostructures,

ρ_S = surface density of the absorbed BT molecules on the nanostructure, taken as $3.3 \text{ molecules/nm}^2$ ^{223,224},

A = enhanced area, which is defined as the diffraction-limited spot size ($\pi(0.4\lambda/2NA)^2$), with NA being the numerical aperture, λ is the wavelength,

ρ_V = surface density of molecules in the detection volume and

V_f = the detection volume of the solution-phase BT sample calculated using the following relation $V_f = \text{depth of focus} \times \text{focus area} = (1.4n\lambda/NA^2) \times \pi(0.4\lambda/2NA)^2$, where n is the refractive index.

The substrates were designed to create patterned metallic surfaces capable of creating better hotspots, as the structures play a critical role in enhancing Raman scattering signals. Among the designs fabricated (discussed in more detail in Section 4.3), it was observed that the concentric triangular pillars and concentric hexagonal pillars produced better signal enhancement (from the enhancement factor calculated), with absolute enhancement of 5.0×10^6 and 1.2×10^6 , respectively. Hexagonal shapes are particularly effective as arrays for creating SERS hotspots because they have six vertices and edges, creating a regular pattern of hotspots on the surface

of the substrate.

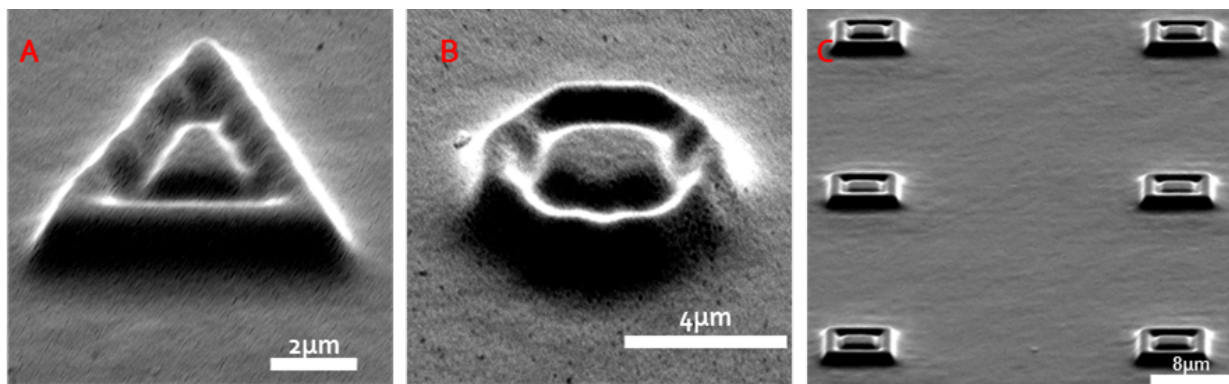


Figure 3.3: SEM images examples of photolithographically patterned SERS substrates used for the detection of molecules showing concentric structures: (A) triangular, (B) hexagonal, and (C) array of concentric rectangular structures.

The same phenomenon was observed in the concentric triangular pillars, with the regularity of the pattern allowing for the optimisation of the SERS enhancement by controlling the size and spacing of the hotspots. Because LSPR is highly dependent on the shape and size of the metallic nanoparticles, the design with sharp corners and edges tends to exhibit stronger LSPR and, thus, more significant enhancement of the Raman signals. The shapes with various corners and edges provide multiple enhancement hotspots for the electromagnetic field, and the symmetric designs allow for a uniform distribution of the field enhancement, resulting in a more reproducible and predictable signal enhancement.

The ability to fabricate these designs in arrays and patterns (examples shown in Figure 3-4) makes them suitable for high-throughput SERS applications, as it allows for a more uniform distribution of localised electromagnetic fields around the structures, especially with the hexagonal and triangular designs, to make reproducible hotspots. The properties associated with concentric structures, such as their optical properties, efficient plasmonic coupling, and large surface area, make them more appealing for enhancing the Raman signals in surface-enhanced Raman scattering²²⁵.

The example dataset of results obtained from concentric and solid structures (discussed in more

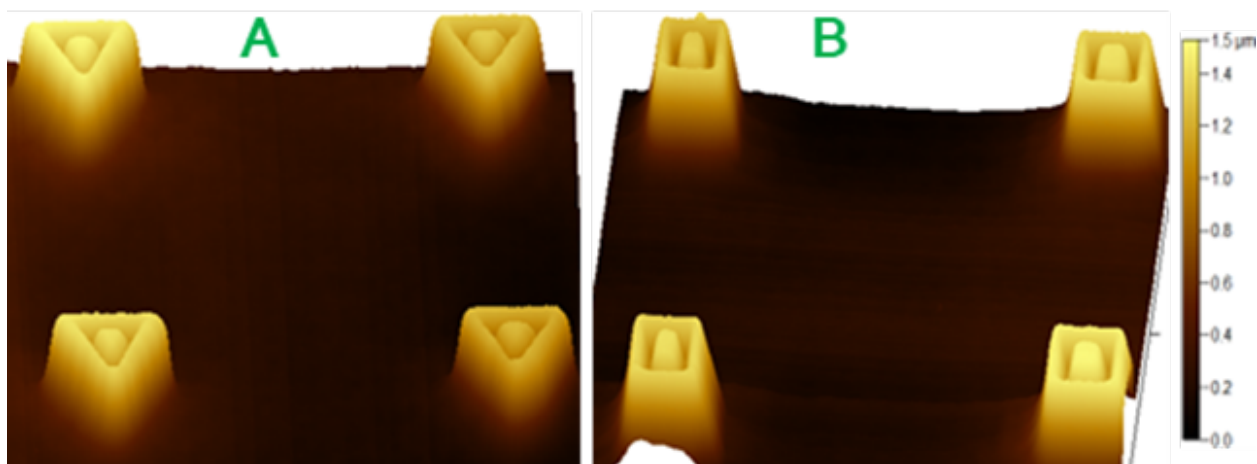


Figure 3.4: AFM image examples of fabricated concentric arrays of patterns – (A) Triangular and (B) Rectangular.

detail in Section 4.3) showed that concentric structures can provide enhancement up to three times more than their solid equivalent, as the hollow interior of these structures provides a confined space, resulting in stronger local field enhancements, thereby significantly enhancing the Raman scattering signals, compared to their solid counterparts. This was corroborated by the simulation results in Figure 4.2h due to the hollow interior that provides pathways for the diffusion of analyte molecules, granting access to the plasmonic hotspots on the inner surfaces. Owing to the ability to adjust the geometric parameters of the concentric structures, such as the wall thickness and size, the resonance wavelength can be tuned, further enhancing the local electromagnetic field near the coaxial structure, leading to improved Raman signals.

Controlling the size, shape, and structure contributes to the enhancement of the signals and other desired properties^{226,227}. Precise control of the wall thickness in concentric structures is crucial for achieving optimal resonance effects and plasmonic coupling. The plasmonic resonance can be finely tuned to match the excitation wavelength to achieve an enhanced Raman signal by adjusting the wall thickness of the concentric structure, which is impossible in solid structures. While it has been reported that nanopillars have high enhancement capability and signal reproducibility, they can be otherwise insensitive to size and spatial orders²²⁸. The large surface area associated with

concentric structures, compared to solid structures with the same volume, enables more sites for analyte adsorption, contributing to a more significant number of Raman-active molecules within the proximity of the plasmonic field, further enhancing the Raman signals. The tuneability of these structures can ensure engineering of various shapes and sizes, such as nanowells, nanorods, nanotubes, and nanocages. This phenomenon also offers flexibility in material selection for these structures, leading to the fabrication of a wide range of topological designs with the desired optical properties and surface chemistry designed for specific applications, thereby increasing the Raman signal enhancement^{229–231}.

3.4.2 Metallic Coatings of Structures

In all fabricated designs, the substrates coated with silver enhanced the Raman scattering signals better than gold (approximately 14 times more in the case of concentric hexagonal structures). The silver-coated substrates produced an enhancement factor one order of magnitude greater than that of the gold-coated designs. This is as expected, given the intrinsic optical properties and surface plasmon resonances of silver, giving it an advantage over gold. Silver has a higher polarisability than gold in the visible and near-infrared regions, with silver nanoparticles interacting more strongly with the incident light, leading to enhanced electromagnetic field confinement, and therefore, more significant Raman signal enhancement.

The surface plasmon resonance (SPR) wavelength is determined by the size, shape, and composition of the nanoparticles, with silver having an SPR peak in the visible region (400 – 500 nm), which overlaps with the standard Raman laser excitation wavelengths leading to a stronger interaction between the incident light and the metallic-coated structures⁸⁹. This enables the electromagnetic field around the silver-coated structures to be more intensely concentrated near the surface, creating a more significant field enhancement and enhanced Raman scattering signals, thereby giving it an edge over gold. Another reason why silver-coated

patterns performed better than gold is their strong affinity for many analyte molecules compared to gold. These affinities result in stronger chemical interactions between the silver-coated surfaces and analyte molecules, leading to chemical enhancement and, in turn, additional Raman signal enhancement^{229,232}.

A key element of SERS is the surface roughness of the coated metallic substrate^{180,212,233}; their biocompatibility, excellent photostability, and long emissive lifetimes make them suitable for biosensing^{234–237}. Silver-coated structures often show rougher surfaces than gold nanoparticles because of their growth mechanisms, which produce more localised hotspots and increase Raman signal enhancement. These hotspots are due to surface roughness, which leads to the formation of gaps, edges, or junctions between nanoparticles, creating a more substantial electromagnetic field enhancement and a more efficient blend of analyte molecules with the incident light. Although *Ag* produces better enhancements than *Au*, *Au* is still preferred over *Ag* for specific SERS applications for several reasons. This is because gold is chemically more stable than silver, as silver is prone to oxidation and can be easily affected by certain chemicals which cause its oxidation.

Both gold and silver nanomaterials exhibit naturally high affinities for molecules such as oxygen, nitrogen, and sulphur, which have highly electronegative or charged atoms, with many biomarkers such as metabolites, nucleic acids, and proteins with one or more electronegative atoms that have strong SERS activity²⁰⁹. This oxidation can affect the stability and degrade the SERS activity of the silver-coated substrates over time, making gold suitable for long-term and reproducible SERS measurements. In addition, the strong affinity of silver for certain analytes and biomolecules can lead to interference in SERS measurements. Simultaneously, gold exhibits a lower propensity for non-specific interactions with proteins, reducing the chances of interfering with the Raman signals. Gold-coated structures are more biocompatible and less toxic than silver-coated ones. This biocompatibility is particularly important in biomedical applications for analysing biological samples and sensing platforms *in vivo*, with gold-coated surfaces exhibiting increased SERS

sensitivity and improved detection limits. This makes gold a preferred and safe choice when designing these devices and for such applications.

Gold nanoparticles show a more uniform size distribution than silver nanoparticles, allowing for better reproducibility in SERS experiments and devices, and this uniformity and reproducibility are essential in detecting certain molecules to predict the onset of illnesses whereby very low concentrations of the analytes are released; therefore, accuracy is paramount. This ability to produce a uniform size distribution allows for tuneability, adapts the plasmonic properties of gold to match the specific excitation wavelengths, and optimises SERS enhancement^{91,237,238}. As previously mentioned, silver exhibits SPR in the visible region (400 – 500 nm), whereas gold exhibits strong plasmon resonances in the near-infrared region (around 700 – 900 nm), which is often the preferred spectral range for biomedical applications because it offers deeper tissue penetration and reduced background fluorescence. This enables *Au* to provide excellent SERS performance while minimising unwanted background signals. The choice between gold and silver (as well as other metallic coatings) often depends on the specific requirements of the target analytes, experimental conditions, and desired outcomes.

3.4.3 Choice of Laser Wavelength

The quality of the Raman spectra obtained, especially for structures coated with gold or silver in SERS substrates, is significantly affected by the laser wavelength. Figure 3.5 highlights this by showing the Raman spectra of benzenethiol obtained using four different lasers: 514 nm, 633 nm, 785 nm, and 830 nm for concentric rectangular-patterned substrates coated with gold. The 633 nm and 785 nm laser excitation wavelengths produced distinct characteristic Raman peaks compared to the 514 nm and 830 nm laser excitation owing to the 633 nm and 785 nm laser wavelengths corresponding to the maximum absorption of the plasmon resonance of the gold nanoparticles, which enhances the Raman signals.

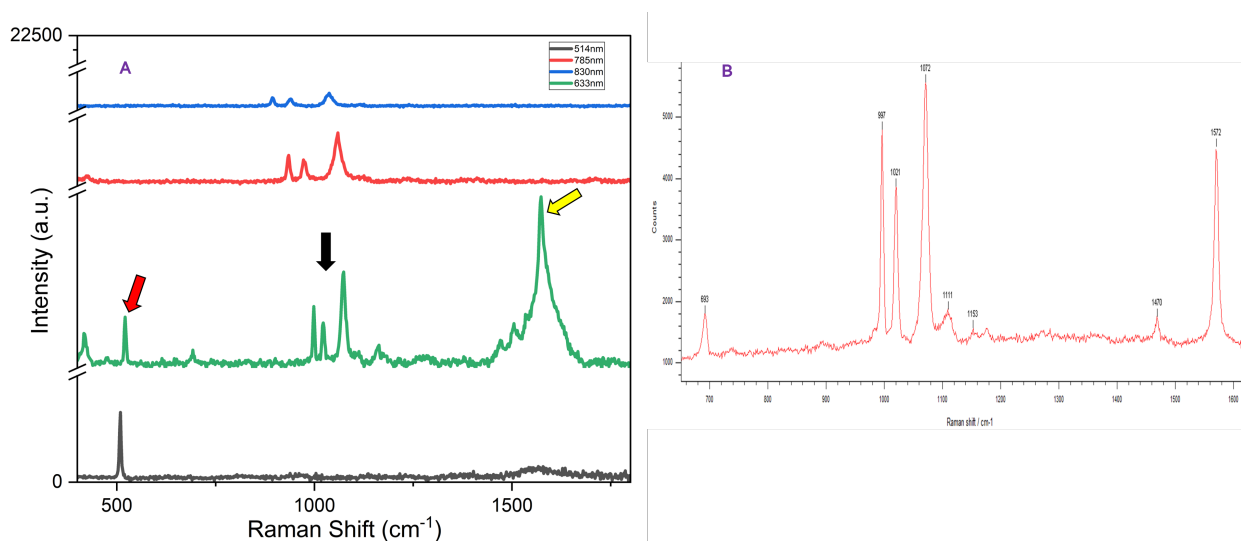


Figure 3.5: The Raman spectra of BT - (A) on SERS substrates using four different lasers, 514 nm, 633 nm, 785 nm, and 830 nm, (B) showing distinct Raman peaks from a laser excitation wavelength of 633 nm. Background subtraction was performed on all the Raman spectra shown. The red arrow indicates the silicon peak at $\approx 520 \text{ cm}^{-1}$, the black arrow indicates the C-H bending peaks at $\approx 1000 - 1200 \text{ cm}^{-1}$ (in-plane and out-of-plane), and the yellow arrow indicates the aromatic ring stretching C=C peak at $\approx 1570 \text{ cm}^{-1}$ (these peaks are minimised in other spectra due to vertical axis scaling to accommodate all spectra).

Fluorescence can interfere with Raman signals, impacting the quality of the spectra produced (evidenced in both the 514 nm and 830 nm lasers), but this is reduced significantly in the spectra produced by 633 nm and 785 nm laser excitation wavelengths (and to a lesser extent, 830 nm). An increased fluorescence background owing to the laser excitation wavelength of 830 nm can overlap with the fluorescence emission of certain molecules, interfering with the detection of the Raman signal and reducing the signal-to-noise ratio. The resolution of the Raman spectrum is also affected by the linewidth of the 830 nm laser because it is broader than that of the 785 nm laser, resulting in a poorer spectral resolution. The 785 nm wavelength is preferred for biomedical applications because of reduced sample damage due to the lower energy of the laser when compared to 514 nm and 633 nm. It also reduces the likelihood of photodamage to the sample due to increased heating of the sample when compared to the 830 nm laser, as the 785 nm laser produces less heat (on average, based on the laser system design used in this research, the laser power of the 830 nm produces more than 150 mW at 100% laser power compared to the 75 mW produced at 100% laser power for the 785 nm laser), making it more suitable

for sensitive samples, because the shorter the wavelength, the more intense the Raman signal obtained. The sensitivity refers to the intensity of the Raman characteristic band used to detect the analyte through its measured signal and gives qualitative information about the sample being measured⁸⁵.

CHAPTER FOUR

DEVELOPMENT AND
OPTIMISATION OF
MICRO-NANOTOPOGRAPHICAL
PLATFORMS FOR
SURFACE-ENHANCED RAMAN
SCATTERING BIOMOLECULAR
DETECTION

This work was published by 'Odetade, D. F., Rickard, J. J. S., & Goldberg Oppenheimer, P. (2024). Development and Optimization of Micro-Nanotopographical Platforms for Surface Enhanced Raman Scattering Biomolecular Detection. *Advanced Materials Interfaces*, 2400352. <https://doi.org/10.1002/admi.202400352>', with my contribution as the first author for all the experimental work and some data analysis; SEM analysis by JJSR, analysis by PGO, with all COMSOL simulation carried out by Dr Zoltan Sztranyovszky.

This chapter highlights the successful novel biosensor fabrication method and testing with characteristic traumatic brain injury biomarkers to validate its effectiveness.

4.1 ABSTRACT

Topologically designed micro- and nanostructured surface-enhanced Raman scattering (SERS) substrates propel the advancements of innovative applications including, environmental and forensic point-of-care miniaturised devices via enhancing the localised electric fields for accurate analyte sensing. Herein, a method for designing, optimising and fabricating fine-tuneable, concentric hexagonal, triangular and rectangular SERS-active micronano-substrates is developed, with each unit yielding significant signal enhancement. Numerical simulations of the 3D near-field electric field guided the optimal design process. While the coaxial SERS substrates consistently outperformed their solid counterparts, the hexagonal micro-nano topologies exhibited $\times 21$ higher signal than coaxial square arrays and a 12-15-fold increase over the triangle structures. Alternating the topological designs from square to triangle lattice yielded more uniform plasmonic modes propagating along the 60° -directions with various resonance modes playing key roles in light reflectance. This enables the engineering of platforms with tailor-enhanced signals by changing the arrangement of micro-nano patterned coaxial arrays. The fabricated SERS substrates are validated by detecting traumatic brain injury indicative biomarkers, effectively yielding the characteristic fingerprint spectra of each neuro-molecule.

The straightforward development of sub-micrometre tuneable SERS-active architectures enables an elegant route for high-throughput biochemical sensing, laying a platform for amplified biomolecular detection of disease biomarkers and integration in bioanalytical systems.

4.2 INTRODUCTION

Surface-enhanced Raman spectroscopy is a highly sensitive spectroscopic and rapid sensing technique, enabling detection down to single molecule levels^{104,105}. In the past decades, SERS has emerged as a powerful analytical tool for a breadth of applications. It is particularly ubiquitous in medical diagnostics^{81,94,96–101,239,240} due to its unique ability to detect trace-level concentrations of disease-indicative biomarkers without specific antibodies, labelling or requiring complex sample preparation. It is also one of the not many spectroscopic techniques, which enables the ability to be deployed out of the laboratory settings without significant loss in performance. While high-enhancement is possible using metallic nanoparticles, due to their poor batch-to-batch reproducibility, only a *minute* fraction of these exhibits the desired SERS-activity, i.e., consistent “hot-spots”. This substantially affects the achievable signals and sensitivity^{92,241–244}.

The substrate on which SERS is performed is often the critical component for successful, consistent, and reproducible detection^{109,219,229,232,245}. Sub-microstructured SERS substrates have been evolving, however, the micro- and nano texturing of plasmonic structures inside micro-devices remains challenging and complicated, especially for low-cost fabrication. Most synthetic routes to generate SERS structures are based on conventional photolithographic techniques. These are costly, time-consuming, cumbersome, and require precise integration of multi-step processes^{88,167,206,210,220,239,246–249}, thus limiting the scalability of the resulting SERS substrates. The limited resolution, inevitable defects, poor mechanical stability of the mold, and pattern distortion are the further limiting factors of the employed lithographic techniques.

The precise control of features, prevention of defects and reproducibility, therefore, remain a challenge, particularly in the sub-micrometre regimes.

A considerable signal enhancement in SERS is typically achieved *via* the designed and fabricated metallic surface micro-nano topologies^{89,106,233} arising from the surface plasmons, which are collective oscillations of electrons on the metallic surfaces, excitable by the incident light^{102,107}. These yield an increased electric field near the surface of the metallic structures, which interacts with the target molecules, effectively augmenting the Raman scattering signal. The SERS enhancement factor is dependent on the local electric field enhanced at both the incident frequency ($E_{Incident}^2$) as well as the Raman scattered ($E_{Scattered}^2$) frequency, resulting in an overall E^4 electromagnetic enhancement. Hence, the overall enhancement is maximised when surface plasmon resonances are generated at both the frequencies of the in-going and outgoing photons, leading to high SERS signals^{226,227}. Lithographically defined, periodic SERS substrates typically sacrifice a degree of sensitivity due to larger surface features. Nevertheless, these enable better spot-to-spot uniformity for a given sample and higher reproducibility across multiple samples. The design and fabrication of the optimal structures are typically focused on achieving more consistent substrates. These comprise reproducible areas of significant local electric field enhancement, i.e., the “hot spot” to improve the accuracy and analytical sensitivity of detection.

Slight topological changes *via* tuneable parameters in SERS designs play an important role in the enhancement of the Raman signals produced. However, various techniques for fabricating SERS-active substrates capable of fulfilling a multitude of criteria remain challenging. For instance, despite a larger number of hotspots, nanorods and nanowires require advanced and cumbersome fabrication techniques^{226,250,251}. Metallic solid nano-pillars, whilst exhibiting high enhancement and consistency, are often insensitive to spatial orders and periodicity variations²²⁸. Ring cavity-enclosed bimetallic SERS “nano stars” fabricated via electron beam lithography have been shown to provide good control of hotspot location and homogeneity. However, these are time-consuming and the typical fabrication of $\approx \text{cm}^2$ area with dimensions in the 20 nm range takes

several days to complete. It is also of a high-cost and requires trained specialist personnel, limiting the scalability of such substrates^{252–255}. The choice of surface metal is also dictated by the plasmon resonance frequency. Whilst silver constitutes a common metal for SERS substrate production, it is highly prone to oxidation in air. Gold-based structures, on the other hand, are more biocompatible and less toxic, providing large enhancements, chemical robustness, biocompatibility, and straightforward functionalisation chemistry^{91,256–261}. Recently, coaxial metallic micro and nano-architectures have been gaining popularity as SERS-active structures due to the increased tunability through the topological design alterations. This allows various engineering shapes and sizes as well as the fabrication of larger hot spot areas thus, enabling a larger number of molecules to attach in these regions²²⁵. The control of wall thickness in concentric metallic structures is crucial in achieving optimal resonance effects, which can be fine-tuned to match the excitation wavelength and optimise SERS enhancement^{91,225,237}. These architectures also allow the modification of the sharpness of the resonance curve, increasing the achievable SERS intensity at the Raman wavelengths near the resonance.

Herein, lithographically defined concentric micro- and nanostructures were systematically designed and fabricated as reproducible arrays for SERS. These were subsequently tested for their performance as analytical substrates and validated for proof-of-concept detection of neurological biomarkers. For achieving the optimal signal enhancements of the to-be-fabricated topologically structured platforms, the various morphologies were simulated via 2D and 3D near-field electric field distribution. This revealed the predominant localisation of the enhanced electromagnetic fields within the noble metal micro-nanostructures, which guided the fabrication of the SERS substrates. These included concentric and solid hexagonal, triangular and rectangular morphologies with increased surface area and hot spots. In turn, it enables a larger number of molecule interactions in each region and a broader bandwidth, yielding augmented SERS signal intensity. The fabricated substrates were tested for detection of four representative brain-specific biomolecules released during cellular damage, which are complementary indicators for diagnosing

traumatic brain injury (TBI) and assessing its severity. These included: (i) sphingomyelin, which is known for brain myelination and regulation of inflammatory response vital in the regulation of cellular growth rate, differentiation and cell death in the central nervous system²⁶²; (ii) S100 β , a calcium-binding protein synthesised in glial cell subtype, which modulates long-term synaptic plasticity²⁶³; (iii) myoinositol, a sugar-derivative produced in glial cells found to be elevated in proportion to the severity of TBI, which also regulates the glial and neuronal activity and intracellular [Ca²⁺] as well as participates in intracellular signalling pathways²⁶⁴; and (iv) cholesterol, a key component of the cellular membrane, known to increase due to the cellular damage, building up following dysregulation and causing cellular toxicity²⁶⁵.

Overall, the ease of fabrication and scalability of these structures could enable tuneable engineering of a broad range of SERS-active topological designs, including, for instance, concentric nanowells and nanopillars. These, combined with the flexibility of plasmonic metal selection, allow the optical properties and surface chemistry to be tailored for excitation laser, signal enhancement and specific applications^{229–231}. The developed topological surfaces structured on micro and nanoscales further lay the platform for achieving improved sensing capabilities, which in the longer term, can be integrated into miniaturised lab-on-a-chip technologies.

4.3 RESULTS AND DISCUSSION

An array of concentric topological substrate designs with a range of geometries, including hexagonal, triangular, and rectangular coaxial architectures, were modelled and optimised via COMSOL simulations (Figure 4.1) and CAD Autodesk Fusion-360 (Figure 4.2d–f), respectively. The simulated maximum achievable local electric field enhancement for each metallic structure is shown in Figure 4.2h. The numerical calculations were carried out in COMSOL using a plane wave illumination to model the incident laser light. This wave had a transverse field component to

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the direction of propagation (z -axis) with the relative permittivity of Au as a function of λ . The designed arrays of concentric topological hexagonal, triangular, and rectangular substrates were systematically analysed for a range of inner and outer diameters, gap and wall widths and periodicity. These revealed that the relative enhancement correlates with the gap-to-width parameters (Figure 4.1), allowing to optimise and guide the subsequent SERS substrates fabrication.

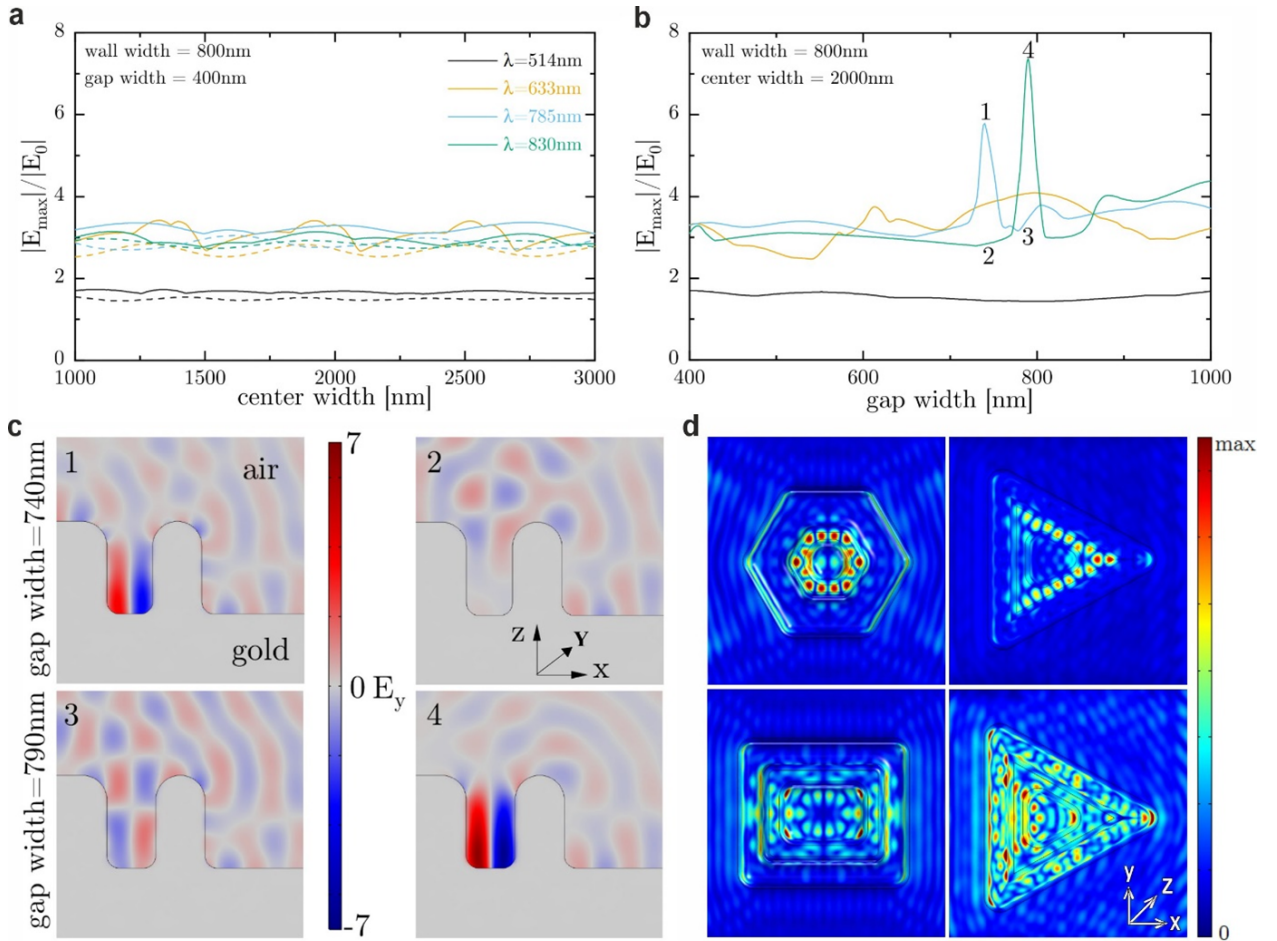


Figure 4.1: a) Maximum field enhancement of 2D concentric structures (solid lines) and solid structures (dashed lines) with varying width of centre. b) Maximum field enhancement of 2D concentric structures versus the gap width. The simulations were carried out for x and y polarised incident waves and the maximum value of the field on the surface of the structure was taken. c) Electric field profile of 2D concentric structures for the 785 nm (left) and the 830 nm (right) excitation wavelengths with the (c, 2) incoming wave propagating in z direction, the electric field polarised along the x direction and the magnetic field polarised in the y direction. d) 3D near-field simulations of the electric field distribution for hexagonal (650 nm gap), square (800 nm gap) and triangle (750 nm gap) coaxial micronano-structures with the visible confined and scattered field components for 785 nm (left column and bottom row) and 830 nm (top right) excitation. The incoming wave is propagating in z direction, the electric field is polarised along x direction and the magnetic field is polarised in y direction.

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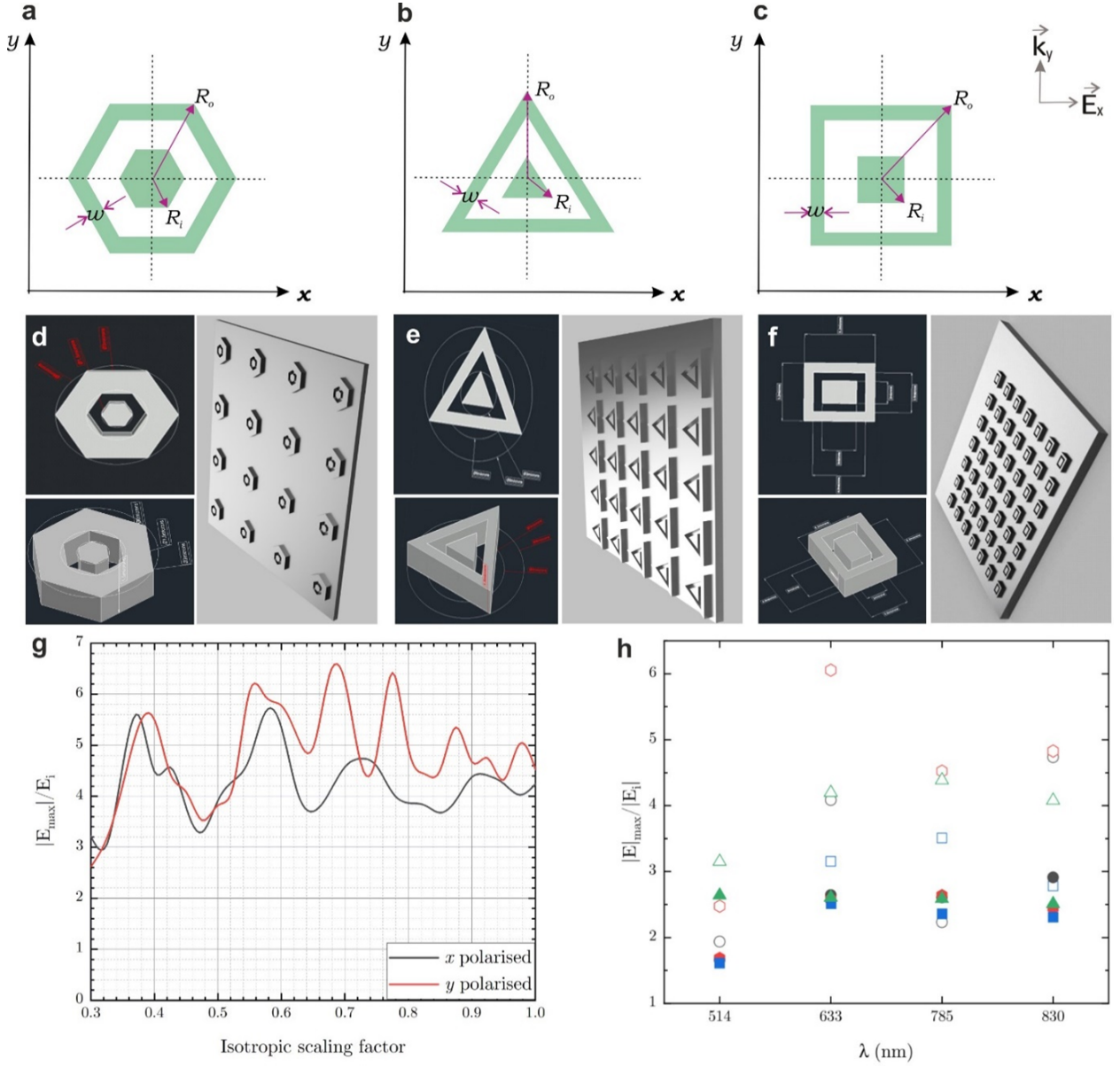


Figure 4.2: Concentric topological substrate design for SERS including a) hexagonal, b) triangular and c) rectangular geometries inscribed in the three circles (for hexagonal and triangular), with the d–f) corresponding CAD and Fusion 360 designs top view (top left) and 3D view (bottom left) and 3D periodic arrays (right-hand side). g) Maximum calculated field enhancement under isotropic scaling for x and y polarised excitation. h) Comparison of the theoretical simulated maximum achievable field enhancement for each metallic structure, including the concentric hexagonal, rectangular, and triangular and their corresponding solid counterparts at excitation wavelengths of 514, 633, 785, and 830 nm, where $|E|_{max}$ calculated for x and y polarised plane wave.

To study the SERS response of the fabricated coaxial micronano-structured substrates, the variation of peak wavelength resonance and the intensity enhancement for the resonance as a function of gap width in the concentric arrays, the generated E_f of the gold-coated arcatures was

numerically simulated via a 3D model. A finite-element method was implemented where a plane wave is incident normal to the substrate with a linear polarisation perpendicular to the top edge, with the simulation being infinite in the third lateral direction (i.e., infinite ridges with cross-sections).

Since the full 3D simulations for the larger micro-nano structures are computationally expensive, the dependence of the maximum field enhancement with the centre, gap and wall widths was investigated using simplified 2D topologies (Figure 4.1a–c). Here, a 400 nm edge rounding was applied at the top (for 2D and 3D) and on the side (for 3D) micro nanostructures and a 200 nm edge rounding at the bottom (limited by other geometric parameters). This collectively enabled removing the edge impacts and highlighting the cavity effects. For the solid structures without an external wall, the centre width has a negligible effect (Figure 4.1a) since the plasmons are excited along the sides and propagate away from the structure, which is independent of the centre width in this parameter range. For the concentric structures (Figure 4.1a), the effect is also minimal. However, it is found to be more prominent, most probably due to the plasmons launching on the inner side of the wall, subsequently propagating across the centre and interfering.

Similarly, varying the wall width exhibited an even smaller effect on the concentric micronano-structures. From 2D simulations, the maximum field enhancement for the fabricated concentric structures was found to be most sensitive to the gap width parameter (Figure 4.1b,c). From a modal perspective, this parameter determines the formation of the localised plasmon mode at the bottom of the well. From the excitation perspective, it determines which wavelength couples most efficiently to the plasmon. While both 785 and 830 nm excitation wavelengths exhibit a strong and narrow resonance peak, these are found at different gap widths. The 633 nm laser outperformed these for a wide variety of gap widths, albeit with the maximum achievable field enhancement being lower than for the other two excitation lasers (Figure 4.1b). From the electric field profiles for the 785 and the 830 nm excitation wavelengths, high-quality localised plasmon mode is evident for the 740 nm and 790 nm gap width, respectively (Figure 4.1c). When the

gap width or excitation differ from these, the coupling strength is reduced, and other modes are excited. This effect also leads to the 633 nm excitation having an overall good performance across a wide parameter range (i.e., 600–850 nm), with the maximum achievable enhancement, however, lower than for 785 or 830 nm lasers. The 514 nm excitation demonstrates a poor enhancement across all scenarios due to the combination of a small refractive index and large extinction coefficient of gold at this wavelength, with the cavity not supporting a localised high-quality mode at such frequencies. This highlights the gap width as a key parameter for the design of optimised coaxial SERS-active substrates whilst indicating the detailed structural properties coming into play for optimised EFs. 3D near-field simulations of the electric field distribution (Figure 4.1d) exhibit the highest EF at 785 nm excitation (left column and bottom row) for the triangular architecture, correlating with the 2D simulations (Figure 4.1b). It is evident that the topology of the structures significantly influences the distribution of hotspots with the edges, despite the significant rounding, guiding the plasmon formation. This is manifested:

(i) in two hotspot areas for the concentric triangle, extending up and down and aligned with the edges of the centre triangle column; (ii) small, localised hotspots in the centre column of the concentric square and (iii) uniformly distributed hotspots across the whole cavity in the concentric hexagonal structure, which has the most uniform and closely packed edges and the highest degree of symmetry. To note, the field enhancement at the corners is not visible in square and hexagonal structures on the outer walls. Therefore, the formation of the hotspots in the edges of the centre column, particularly for the square structure, is not just an edge effect. It is a consequence of the concentric structure and the cavity, further highlighting the enhanced SERS performance of coaxial topologies. For the 830 nm excitation laser, the strongest enhancement occurs between the tip and wedge in the gap toward the right and plasmonic enhancement is observed to propagate along the 60° side cavities. Overall, varying the gap distance width leads to the formation of a plasmon mode inside the coaxial structures' cavity, with the E_f excited on the gold micronano-structures initially increasing with decreasing gap-to-width ratios. While the periodic grating couples light into plasmon polariton and propagating plasmon resonances

by diffraction, these are noted to couple with each other if the gap between the structures is large. The coupling for smaller gaps leads to increased localisation between the architecture with increased E_f and, thus, higher SERS enhancements in the gaps. The increase in SERS with decreased interstitial gap due to resonant excitation of propagating plasmons diffractively coupled into the structure can be excited with the various laser wavelengths, depending on the cavity width. However, a considerable shrinking of the interstitial gap results in almost a complete elimination of the cavity plasmon mode with no manifestable resonance.

Subsequent to the numerical modelling, eXTRA fast maskless lithography with parallel multi-beam writing, speed up to $280 \text{ mm}^2/\text{min}$ and resolution down to 300 nm was employed to fabricate high precision and throughput platforms. While the fabrication builds upon the numerical simulations and CAD designs to guide the optimal structures, the lithographic route enables the ease with which these could be scaled and tuned isotropically. Therefore, during the fabrication stages, it is possible to rapidly attain other desirable dimensions, rendering fabrication flexibility a key part of the design and iterations. The structures' up- and down scalability and tunability enabled us to further optimise the fabrication dimensions for optimal signal enhancement via isotopically scaling dimensions. The simulations were carried out for x and y polarised incident waves and the maximum value of the field on the surface of the structure was taken. When the structures are downscaled, the maximum field enhancement oscillates (Figure 4.2g), from a 27% lower value than the original scaling up to a 43.5% improvement. This is due to constructive and destructive interference of the plasmon resonance occurring in the structure and either the former or the latter dominating at different dimensions. This could be beneficial in further scaling the design structures to achieve a higher EF for a given polarisation. The different dimensions resulting from the scaling of the structures affect the surface plasmon resonance and the constructive interference will be a valuable parameter for directing the fabrication of future features when scaling for y -polarised excitation. A scaling factor (a number ratio by which the fabricated structure dimension can be changed with respect to its original size) of $\times 0.56$ was identified to

exhibit an increased enhancement for both x and y incident polarisation waves. This produced the highest Raman signals with a nearly twofold increase in enhancement relative to the original hollow concentric hexagonal architecture. The simulation and scalability optimisation led to the fabrication of a range of optimal structured surfaces for the projected highest SERS enhancement (Appendix One, Figures A1 and A2). These included concentric hexagons with outer and inner radii $R_o = 3000$ nm and $R_i = 750$ nm, respectively and wall width, $w = 1.35$ μ m (Figure 4.2a) generating an inner gap size of 650 nm as well as concentric rectangles and triangles with wall widths of $w = 800$ nm and $w = 750$ nm, respectively (Figure 4.2b,c). The substrates were typically arranged in 15×12 periodic arrays with a fixed period of 30 μ m. The waveguide approximation provides further useful insights for a circular coaxial waveguide filled with air in the gap with the cutoff wavelength expressed as $\lambda = \pi(R_o + R_i)$, where R_o and R_i are the radii of the outer and inner surfaces²⁶⁶. With the latter being on the order of 1 μ m for the studied structures, the cutoff wavelength is $\lambda \approx 6$ μ m. This is well above the maximum wavelength used for excitation, suggesting that the field can couple and propagate inside the structures, exciting the cavities. The fabricated topological substrates were characterised *via* an array of optical (Figure 4.3a–c), scanning electron (Figure 4.3d–f) and atomic force (Figure 4.3g–i) microscopy (OM), (SEM), and (AFM), respectively.

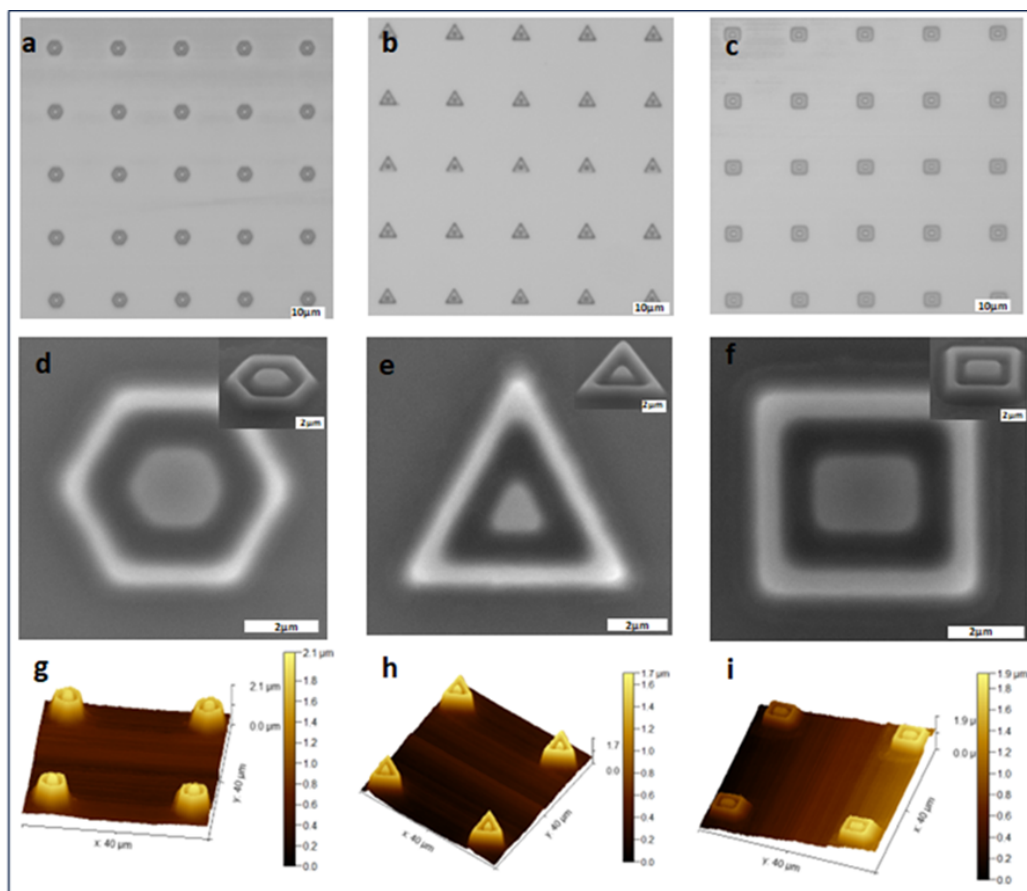


Figure 4.3: Representative OM top (a–c) and SEM (with 3D inset) (d–f) images of pattern formation, generating a range of SERS-active substrates including the hexagonal (a,d), triangular (b,e), and rectangular (c,f) structures with the corresponding 3D AFM height images (g–i).

To evaluate the SERS performance of the concentric patterned structures shown in Figure 4.3, these were coated with 30 ± 12 nm thick gold layer (Appendix Figures A.5 and A.6), followed by adsorption of a benzenethiol monolayer *via* immersing the gold-coated surfaces in 10 mM ethanolic solution. Representative average SERS spectra recorded for these metallic structures are shown in Figure 4.4b. The hexagonal and triangular hollow concentric structures produce better signal enhancement compared to their solid counterparts. Particularly, the hexagonal structures are found to be more effective in creating hotspots due to their six vertices and edges on the substrate surfaces. A similar, albeit to a lower degree, phenomenon was observed in the hollow concentric triangular structures with the regularity of the pattern allowing for the optimisation of the SERS enhancement by controlling the size and spacing of the hotspots.

By changing the arrangement of apertures from rectangular to triangular lattice, a more uniform mode resonance is formed in the plasmonic structures. This is due to the hollow concentric structures with sharp edges and crevices concentrating the target analyte molecules in the larger hotspot areas, yielding an effective Raman signal enhancement. These findings are in correspondence with previous studies, where the sharp corners and edges were found to exhibit stronger localised surface plasmon resonance (LSPR)^{226,231,267}.

Further to the averaged spectroscopic data, SERS mapping was carried out for each array. The representative SERS map overlaid over the corresponding optical microscopy images in Figure 4.4a shows the SERS signals arising exclusively from the concentric structures and no signal was observed from the flat gold-coated areas. This indicates that the adjacent structures are not plasmonically coupled and, therefore, can function as isolated detection centres, rendering these substrates as possible platforms for multiplexed SERS of diverse biochemical analytes.

Different morphologies were found to yield substantial variations in SERS enhancements (Figure 4.4b). The concentric hexagonal structures showed a $21\times$ enhancement compared to coaxial square arrays and a 12–15-fold increase in signal over the triangle structure at an excitation wavelength of 633 nm (Figure 4.4a). The absolute enhancement for the concentric hexagons was $\approx 1.2 \times 10^6$ at 633 nm and 3.9×10^5 at 785 nm. The 95% confidence intervals were $(8.40 \times 10^5, 1.30 \times 10^6)$ and $(3.0 \times 10^5, 3.96 \times 10^5)$, respectively. An EF of 0.8×10^5 with a 0.95 confidence interval of $(0.75 \times 10^5, 0.84 \times 10^5)$ was found for the hollow coaxial triangular structures at 633 nm. The hollow concentric SERS structures were found to exhibit up to three times higher EFs than the solid equivalents due to the confined areas resulting in stronger local fields and larger surface area-to-volume ratio. The latter contributes to a larger number of the SERS-active molecules within the proximity of the plasmonic field, enhancing the Raman signal.

For a given micro- nanostructure, when an additional inner or outer similar architecture is added to make a concentric topology, it enables a larger bandwidth of electric field enhancement.

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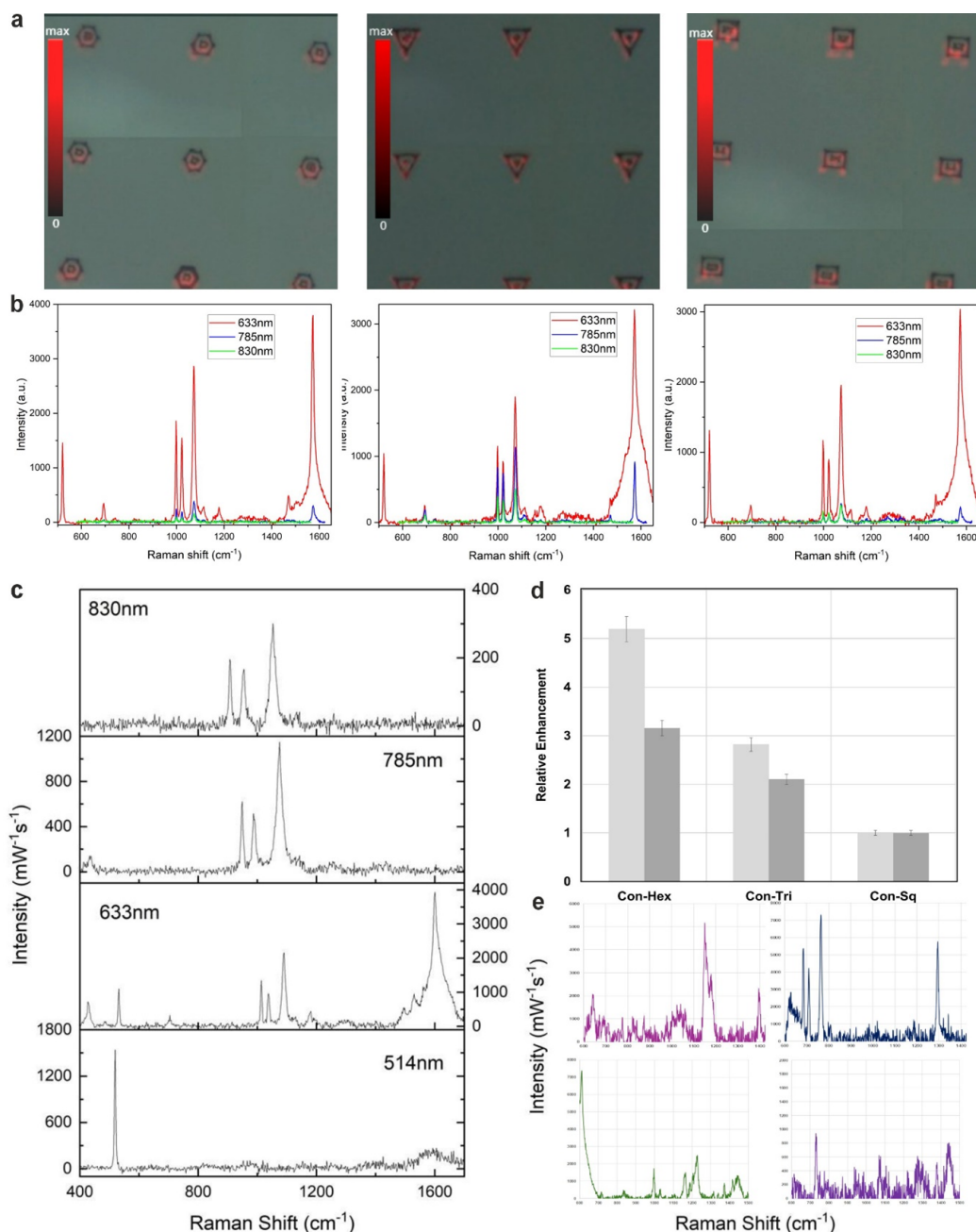


Figure 4.4: a) SERS maps overlaid over an optical image of the patterned area, revealing the localised nature of the SERS activity extracted for the benzenethiol peak at 1070 cm^{-1} . b) SERS spectra of benzenethiol recorded from the three structured surfaces excited with 633, 785, and 830 nm lasers. c) SERS enhancement as a function of excitation wavelengths with the highest signal enhancement obtained for the hexagonal concentric structures with 633 nm followed by 785, 830, and 514 nm. d) Relative SERS enhancement of the 1000 cm^{-1} and 1070 cm^{-1} peaks for each concentric micro-nano topology. e) Average SERS spectra of representative TBI biomarker's fingerprint, including the sphingomyelin (purple), cholesterol (plum), myoinositol (green), and S100 β (navy) deposited on hollow hexagonal coaxial SERS-active substrates and excited with a 785 nm laser.

This is due to a second resonance arising from the interaction of the inner and outer structural units, yielding a higher overall SERS enhancement. For all three fabricated topologies, the relative SERS enhancement of benzenethiol molecules was found to be the highest for the 633 nm excitation laser versus the 785 nm, with the 830 nm and the 514 nm excitations yielding negligible signal enhancement. Furthermore, the coaxial hexagonal SERS surface demonstrated the biggest increase in enhancement using the 633 nm excitation laser versus the 785 nm, exceeding the other two substrates. However, the concentric triangle surfaces exhibited the highest EF at 785 nm, $\times 3.3$ and $\times 4.2$ higher relative to the hexagonal and square morphologies, respectively, with the latter showing the lowest EF amongst the fabricated substrates (1.2×10^6 compared to 9.0×10^4) (Figure 4.4b–d).

Fluorescence was found to be significantly reduced in the spectra for all the excitation wavelengths except the 514 nm. The 785 and the 830 nm are typically preferred excitation lasers for biomedical applications, yielding reduced sample damage due to the lower photon energy^{268–272}. The ultimate choice of excitation wavelength is often dictated by the plasmonic properties of the SERS substrates (or *vice versa*) as well as the analytes' resonance if a narrow range of compounds or similar physiological bodies are being targeted^{222,273,274}. Since the SERS enhancement is dependent on the λ_i , mainly arising from the LSPRs of the metallic architectures, a particular substrate can yield the highest electromagnetic enhancement at a specified excitation. It is also strongly dependent on the surface morphology and precise shape of the features at the metal surface. Our results indicate that the hexagonal topology is the optimal structure to be exploited with the 633 nm excitation laser to yield the highest enhancement. Whereas the coaxial triangular morphology presents itself as a promising and viable candidate as an enhancing substrate with the 785 nm laser. The fabricated SERS-active substrates were subsequently used to profile four representative biomarkers signposted to correlate with TBI, successfully yielding the fingerprint spectra of sphingomyelin, cholesterol, myoinositol and S100 β , characterising the enhanced characteristic peaks of each biomarker (Figure 4.4e).

The experimental data was found to correspond with the numerical modelling, with the hexagonal coaxial structures providing the highest field enhancement across nearly all excitation wavelengths and concentric structures yielding higher enhancement compared to their solid counterparts. In comparison to results based on the fabricated substrates, higher enhancement factors are found for the ideal models based on simulations and CAD designs. This is attributed to neglecting minor fabrication imperfections such as inclined walls or *minute* depth variations. Furthermore, while the concentric triangular structures exhibited the highest enhancement in the ideal models, these are highly sensitive to topological deformations of the tip and the wedge. Whereas the concentric hexagons, with no similar tip, are less sensitive to defects and, thus, perform better experimentally than the modelled counterparts. This could further be considered in conjunction with the effects of tip-enhanced Raman spectroscopy (TERS), which combines SERS with scanning probe microscopy such as the AFM. TERS decouples molecular adsorption and enhancing processes, and the tip serves as an external enhancer. Localised surface plasmons can also be excited in the tip apex, providing a high near-field enhancement. Via theoretical simulations confirmed by the experimental investigations, the field enhancement properties of TERS have shown that plasmon coupling effects between the metal tip and the metal substrate play the key role in field enhancement, and the latter drops dramatically as the distance between the tip and the substrate increases. The spatial resolution was found to mainly be dependent on the radius of curvature of the tip end, with a sharp tip dramatically increasing the spatial resolution^{275–279}. Correlation with the experiment could further be improved by applying a Gaussian beam as excitation instead of the plane wave, which is also more applicable for a small spot size. However, this is computationally unfeasible and therefore, symmetries could not be implemented to reduce the simulation domain.

From analysis of the coaxial geometries, it is noticeable from the simulation and the experimental outputs that when the topology of the designed structures is altered from square to triangle lattice, the plasmonic resonance modes are more uniform propagating along the 60° directions.

This not only implies that the side-to-side, side-to-corner, and corner-to-corner resonance modes play key roles in light reflectance but also indicates that the SERS signal can be tailor-enhanced by changing the arrangement of arrays from square to triangle lattice.

4.4 CONCLUSIONS

In conclusion, a range of coaxial topologically designed and optimised 3D micro- and nanostructured substrates have been engineered as reproducible and tuneable arrays for SERS that can be used as biosensors. The morphologies were simulated *via* 2D and 3D near-field electric field distributions, revealing the predominant localisation of the enhanced electromagnetic fields within the noble metal micro-nanostructures. In the coaxial geometries, the cutoff wavelength is found to be governed by the gap size and diameter of the structures. The tuneable resonance available in these architectures couples and stores a significant amount of optical energy in the generated concentric cavities. Since the resonance wavelength independently of the cavity length dramatically red-shifts as a function of the gap size, this can enable a further construction of large-area arrays with $\lambda_{resonance} \ll \text{period}$. The fabricated substrates were subsequently validated for the detection of a range of neurological biomarkers, exhibiting an average signal enhancement of $\times 10^6$. The fabricated concentric hexagonal, triangular, and rectangular morphologies with a broader bandwidth, a larger surface area and hot spots, increasing the molecular interactions in each region, show structure-dependent SERS performance. The structural and fabrication tunability allows the lithographically defined substrates to be optimised for plasmon-dependent phenomena. The ease of fabrication enables the micro nanostructures to be isotopically scaled up or down to the desired topological dimensions whilst various micronano-architectures can be fabricated on the same substrate simultaneously. This enables to tailor-make specific topologies to achieve the optimal signal enhancement compatible with the chosen excitation wavelength. SERS enhancement requires a delicate balance between the excited and scattered wavelengths

with the plasmon peak of the metallic micro-nanostructure. The plasmons excited at the scattered wavelength out-couple the Raman scattered radiation more efficiently. This is particularly important in the near-infrared, where the laser excitation and the scattered radiation can be significantly different in terms of wavelength. A range of SERS applicable laser excitation wavelengths, including the 514, 633, 785, and 830 nm, were applied for each fabricated substrate. The highest signal enhancement is found to arise from the hexagonal concentric micro-nanostructures with a 633 nm excitation laser, considerably outperforming the 514 nm and highlighting the consideration to be given to the choice of the excitation laser to detect the target bio-analytes. In the long-term, the fabrication of optimised concentric plasmonic micro- and nanostructured substrates with inherent tunability opens avenues for a strong analytical platform. These can be suitable for a range of applications, for instance, multiplexed detection of trace level disease indicative biomarkers, photocatalysis, environmental and forensic *on-site* detection, as well as miniaturised integrative sensing platforms, such as point-of-care diagnostic devices.

4.5 EXPERIMENTAL SECTION

4.5.1 Materials

Silicon (Si) wafers (n-type, crystal orientation <100>) with a polished side and resistivity of 0–100 ohm-cm (WaferNet, GmbH Germany) were used as an underlying surface during substrate fabrication, acting as the base for the deposition of photoresists. HPLC-grade ethanol (Sigma-Aldrich, Dorset, UK) was used for the preparation of 10 mM Benzenethiol (BT) solution^{88,167}.

4.5.2 SERS Substrates Fabrication

The positive and negative photoresists for the fabrication included AZ1514H and AZnLOF2020 (MicroChemicals, GmbH Germany), used without further dilution. Si wafers were initially thoroughly cleaned *via* physical techniques, including snow-jet, removing 99.99% of contaminants¹⁵⁹. The photoresists (20 μ L) were subsequently deposited on the clean Si wafers *via* spin-coating (Laurell, WS-650MZ-23NPPB) at 4 000 rotations per minute (rpm) for 60 s in a controlled humidity environment. Subsequently, photoresist-coated substrates underwent a softbake process to improve the adhesion of the photoresist to the wafer. This included heating at 100 °C for 90 s for a film thickness of 1.5 μ m (1 min/ μ m resist film thickness) for the positive photoresist AZ1514H and 60 s for a film thickness of 2 μ m for the negative photoresist AZnLOF2020. A range of micro and nanostructures were fabricated *via* a direct laser writer (PicoMaster XF, Raith Lasers Systems B.V., Netherlands) eXTRA fast maskless lithography system using a 405 nm laser. The eXTRA maskless lithography was operated in a vector writing mode performance to avoid stitching problems and optimise the depth of focus, laterally fabricating each surface. The established engineering of SERS platforms *via* eXTRA lithography in a single laser writing, for patterning the selected structures and dimensions, the optimal grid array organisation included 15 and 12 structures in each line and row, respectively. This grid array of 15 $\times$ 12 ensured the optimal structural dimensions, optimal speed of patterning and spacing between the structures, maximising both the capacity to enhance signal as well as ease and cost-effectiveness of the fabrication. For the negative photoresist, a post-exposure bake was necessary to complete the photoreaction initiated during exposure, prior to development, where, for the positive resist the exposed areas were dissolved using the AZ351B (MicroChemicals, GmbH Germany) and deionised water at 1:4 ratio (developer: water v/v)¹⁶² and for the negative resist, the unexposed areas were removed using the AZ326MiF developer (MicroChemicals, GmbH Germany). The dissolution of the exposed areas was based on the conversion of the diazo naphthoquinone-sulphonate-based

photoinitiator in indene carboxylic acid. The dilution of the AZ351B in water occurs due to the rate of dissolution of the exposed resist to the rate of dissolution of the unexposed resist, which remains on the substrate as the nanostructure¹⁶². The fabricated nanostructures were sputter-coated with a thin gold layer.

4.5.3 Optical Microscopy

Images of the fabricated nanostructures were acquired using Olympus Optical Microscope (GX61, GX2) with a Leica DM2700 M camera.

4.5.4 Scanning Electron Microscopy

The scanning electron microscopy (SEM) measurements were performed using a Hitachi SU5000 at acceleration voltages of 0.5 - 5 kV with a lateral resolution of 2 - 5 nm.

4.5.5 Atomic Force Microscopy

The atomic force microscopy (AFM) measurements were performed using JPK NanoWizard II atomic force microscope. The measurements were done using tapping mode *via* an intermittent contact mode of the cantilever tip with the sample in ambient conditions. NCHV-A cantilevers with a resonance of 320 kHz and stiffness of 42 Nm⁻¹ were used. The software used to analyse the height and phase of the images was Gwyddion, version 2.59. The size of the nanostructures was measured by scanning 50 × 50 μm² of each sample, with the regions chosen to represent different topologies obtained during fabrication.

4.5.6 SERS Acquisition

SERS measurements were carried out using micro-Raman system *via* an inVia Qontor confocal Raman spectrometer (Renishaw, Wotton-under-Edge, UK) and four excitation lasers, including the 514, 633, 785, and 830 nm. To avoid photochemical effects, degradation or sample damage, SERS maps spectral scans were typically acquired for 100 data points over a range of 500 – 2000 cm^{-1} with the laser power of 30 – 40 mW, 50x objective lens, the numerical aperture of 0.75, the acquisition time of 0.1 seconds with 3 accumulations. All Raman spectra were collected at the ambient temperature. SERS enhancement factor (EF) of the fabricated structures was calculated *via* the: $\text{EF} = [I_{\text{SERS}}/N_{\text{SERS}}]/[I_{\text{Normal}}/N_{\text{Normal}}]$, where I_{Normal} is the intensity of the Raman scattering peak, I_{SERS} is the signal of the SERS substrate containing the fabricated structures, N_{SERS} and N_{Normal} are the numbers of adsorbed molecules on SERS active substrates with the surface density of the molecules taken as 3.3 molecules/nm (with the assumption that a monolayer of benzenethiol was obtained during preparation) and the number of molecules probed for a normal Raman setting, respectively²¹⁰. A reference silicon wafer was used to measure the fluctuation of the intensity of the Raman signals each time the Raman spectrometer was used for measurement, which enabled the monitoring of analyte decay and instrument drift to ensure that calibration was performed as necessary. The spectra obtained were processed using the Renishaw WiRE software to remove cosmic rays and perform baseline subtraction on the 100 datasets collected for each scan. Baseline subtraction was necessary to mitigate the overestimation of the contribution to the sample Raman intensity, and this was performed consistently for all processed data. The data were analysed using Python scripts (Appendix Two) to identify the highest-intensity peaks before calculating the enhancement factors. Integrated peaks were used because of the variations in their shapes and intensity distributions. The peak height can also be used when the peaks are well-defined and have a high signal-to-noise ratio.

4.5.7 Numerical Modelling

The computational simulations were conducted using the finite-element solver COMSOL Multiphysics 5.5¹⁸⁴, with the Wave Optics module and frequency domain study. The topologies were modelled based on images in Fusion 360 and imported into COMSOL *via* CAD module. The silica substrate had a refractive index $n = 1.45$, the structure, which was made of photoresist, had a refractive index of $n = 1.58$, the top layer was air with $n = 1$, and the thin gold layer was modelled using transition boundary conditions with thickness $t = 40$ nm and gold permittivity from Johnson and Christy²⁸⁰. The excitation at each of the employed laser wavelengths was taken as a plane wave, with wavelength λ propagating along the z -axis (Figure 4.1). Separate x and y polarised wave studies were conducted. An element size of $\lambda/5$ was used^{281,282}. A mesh independence study was not conducted, which is a limitation of this study. The symmetry of the structures was taken into account to reduce the computational domain, which was truncated approximately one λ from the structure and surrounded with scattering boundary conditions.

CHAPTER FIVE

MULTIPLEXED PATTERNED
DESIGNS – DEVELOPMENT AND
OPTIMISATION STUDIES

This chapter is a follow-up to the previous chapter, 'Development and Optimisation of Micro-Nanotopographical Platforms for Surface-Enhanced Raman Scattering Biomolecular Detection'. It discusses alternative patterned substrates fabricated using photolithography, covering various engineering designs and the stability of the substrates over a 13-month period. The fabrication was guided by COMSOL Multiphysics simulations (also covered in the previous chapter), with the enhancement of each micro-nanostructure determined to assess their use as SERS substrates for detecting benzenethiol molecules.

5.1 ABSTRACT

Multiplexed surface-enhanced Raman scattering (SERS) substrates are essential for point-of-care detection, enhanced biosensors, and miniaturised medical devices for identifying biomolecular samples containing disease-indicative biomarkers. These substrates focus on enhancing local electric fields, which are crucial for sensitive and accurate analyte sensing, where early detection and intervention are critical. Herein, patterned micro-nanostructures were easily designed, optimised, and fabricated using photolithography to produce fine-tuneable, reproducible topological structures such as concentric and solid hexagonal, triangular, rectangular, and circular (or a combination of these) substrates to obtain a significant enhancement of the Raman signals detected on these substrates. Each structural unit significantly enhanced the signal detection, guided by 3D near-field electric-field distribution simulations. The fabricated structures demonstrate scalability, achieving a two-fold increase in signal enhancement compared with the initial designs for certain isotropic scaling of the designs. These engineered SERS platforms enable tailor-enhanced signal detection by changing the arrangement of the micro nano-patterned coaxial arrays from square to hexagonal and triangular lattices, as well as the periodicity to obtain a 200% increase in Raman signal detection.

5.2 INTRODUCTION

Traumatic brain injury (TBI) is a contributing injury associated with Alzheimer's disease (AD) onset, accounting for approximately 60 – 80% of dementia cases, with TBI significantly increasing the development risk of AD^{64,67}. The onset of AD could be as great as 22 years before the first symptoms are observed for people with rare genetic mutations that cause AD. Research suggests glucose metabolism, which is an indication of a healthy brain, begins to decrease 18 years before the onset of symptoms, while brain atrophy may be notable some 13 years before the appearance of symptoms⁶⁴.

Biomarkers released to indicate the presence of diseases have been identified and suggested, however, few are utilised in routine clinical practice²⁸³, with a diagnostic biomarker objectively useful as a measurable characteristic to help with the accurate detection and treatment of certain illnesses in patients^{82,284}. Using these diagnostic biomarkers increases accuracy⁸² and improves treatment options, especially when diagnosis is performed promptly. One method to detect these biomarkers is by using a multiplexed detection system via topologically designed biosensors in optofluidic platforms that can serve as a point-of-care diagnostic device^{134,135,236}. Common AD biomarkers, such as NADPH oxidases (Nicotinamide adenine dinucleotide phosphate, NOX₂) - a biomarker for several neurodegenerative diseases, including AD and TBI^{285,286}, are listed in Table 5.1, which indicates the biofluid to which they can be measured in.

Table 5.1: Proposed Alzheimer's disease biomarkers and where they are prominent in biofluids.

	Biomarker	Biofluid
1	A β 42	Cerebrospinal fluid (CSF) ^{287,288} and blood/plasma biomarker ²⁸⁹
2	A β 40	CSF ²⁹⁰ and blood/plasma biomarker ²⁸⁹
3	Total tubulin associated unit (tau)	CSF ^{287,288} and plasma ²⁸⁹
4	Phosphorylated tau 181 p (p-tau)	CSF ^{287,288}
5	Neurofilament light chain (NFL)	Blood/plasma biomarker ²⁸⁹
6	Acetylcholinesterase (AChE)	Saliva ²⁹¹
7	Lactoferrin	Saliva ²⁹¹
8	Alpha-synuclein (α -syn)	Saliva ²⁹¹
9	NADPH oxidases (NOX ₂)	Blood ²⁸⁶

Some of these biomarkers can also be used as indicators of TBI. Elevated levels of neurofilament light chain (NFL) have been shown to indicate a variety of brain injuries, including mild TBI²⁹². Other diagnostic biofluids where biomarkers can be obtained include tears (eyes), semen, and cerebrospinal fluid; some of the biomarkers that have been assessed to act as informative parameters for TBI include tau, ubiquitin C-terminal hydrolase L1 (UCH-L1), and glial fibrillary acidic protein (GFAP)^{292,293}. S100 β and neuron-specific enolase (NSE) have also been indicated as biomarkers in patients with TBI, with automated assays available for both, thereby making them faster and more practical to use²⁹⁴, primarily due to limitations with proteins of relatively short serum elimination half-life (as short as 25 minutes in patients with ongoing brain injury). S100 β is the most common and is considered superior to NFL in detecting secondary harmful events when monitoring patients with TBI²⁹⁴.

Table 5.2: Inexhaustive lists of biomarkers for detection of TBI^{71,77,82,292,295–297}.

Biomarkers	
1	Ubiquitin C-terminal hydrolase-L1 (UCH-L1)
2	Glial acidic fibrillary protein (GFAP)
3	Neuron-specific enolase (NSE)
4	Microtubule-associated protein-2 (MAP2)
5	Myelin basic protein (MBP)
6	Tau
7	S100 β
8	Neurofilament heavy chain protein (NF-H)
9	Neurofilament light (NFL)
10	Heart fatty acid-binding protein (H-FABP)
11	Inflammasome protein, apoptosis-associated speck-like protein (ASC)
12	Caspase-1
13	Interleukin IL-1 β (IL-1 β)
14	Nucleotide-binding oligomerisation domain (NOD)-like receptors (NLRs) Family Pyrin domain-containing 3 inflammasomes (NLRP3)
15	Cholesterol
16	Myoinositol
17	Sphingomyelin

Inflammasomes are large multiprotein signalling complexes that regulate immune system cells, which mainly include microglial cells in the brain. NFL and tau peak at different times post-TBI, with tau, like most biomarkers in TBI, peaking around the first day post-injury, and then rapidly falling. NFL peaks some 20 days post-TBI, and it is relatively specific, i.e., it is much more

elevated in patients who have had head injuries than in those with extracranial injuries^{298,299}. This makes it vital to detect these biomarkers to accurately obtain necessary information about potential diagnoses. The ever-increasing need for a faster turnaround time for the detection and diagnosis of these types of illnesses has inadvertently increased the burden placed on clinical practices, whereby the premise of similar lab-on-a-chip analysis for the rapid detection of symptoms for other medical conditions has increased the strain on healthcare services in attempting to keep up with treatments administered to patients.

However, despite the large set of point-of-care diagnostic devices, only a few have translated into clinical practice. This is because of several factors that the device must satisfy. Notably, for devices that contain sensors (the fundamental component of a measurement system), the sensor must have high sensitivity, reproducibility, high resolution, linearity, and low power consumption to enable deployment in out-of-clinic settings. To achieve this, innovative solutions are required in the design of these medical devices. Driving these innovative medical devices are topological principles that revolutionises our understanding of the design and manufacture of new materials, devices, and data structures.

Numerical approaches to topological optimisation in the biomedical field can improve the performance of medical devices, such as reducing their mass or volume^{300,301}. An improvement, for example, to the local electric field enhancement, optical cross-sections, and refractive index sensitivity has been reported to contribute to the better plasmonic properties of gold nanobipyramids when compared to gold nanorods as an example that provides promising biomedical applications in the areas of sensing and spectroscopy²⁵⁰. A further example of these principles is the reported topologically designed fabrication of functionally graded porous biomaterials as a viable substitute for orthopaedic implants and as bone substitutes for resolving the uncertainties of the mechanical behaviour of previous porous biomaterials³⁰².

Topological design and optimisation offer a route to improving medical device efficiency, effectiveness, and other desirable properties before fabrication and deployment in out-of-clinic settings and point-of-care diagnostic devices. Some of the techniques that have been used for measuring these biomarkers include infrared absorption, mass spectrometry, nuclear magnetic resonance (NMR), X-ray photoelectron spectroscopy (XPS) and Raman spectroscopy (which includes resonance Raman spectroscopy (RRS), surface-enhanced Raman spectroscopy, nonlinear Raman spectroscopy and 'Offset' Raman spectroscopy). Raman spectroscopy is widely adopted because of some of its characteristics in both qualitative and quantitative analysis of samples²²².

Raman spectroscopy is an analytical technique ubiquitous because of its advantages. It is a powerful analytical tool that offers qualitative and quantitative detection of molecular fingerprints^{86,269,273}. The acquisition of high-quality spectra at sub-cellular resolution and non-invasively obtaining valuable information, label-free, and without interference from water, makes Raman spectroscopy an ideal approach for in vivo biological investigations. However, because of the inherent nature of Raman signals, which have an exceptionally low probability of occurrence (approximately 1 in 10^8), it is a rare phenomenon when compared to Rayleigh scattering^{183,269}, significant enhancements are needed to accurately detect the presence of biomarkers, as these biomarkers appear in *minute* concentrations.

Surface-enhanced Raman spectroscopy techniques use nanostructures (coated with certain metals such as gold, silver, copper, and aluminium) to significantly increase the detection of molecular bonds indicative of biomarkers in a sample. SERS is when the presence of these metallic coatings is used to significantly enhance Raman signals, with the metallic coatings enhancing the Raman signals of molecular biomarkers that are detected, as the Raman scattering is a change in the wavelength of scattered light, which is molecule-specific.

When Raman-active molecules are indirectly adsorbed on or in very close vicinity to the surface of metallic-coated surfaces, the Raman signal intensity is significantly amplified by a factor of 10^5

or more, which allows the development of ultrasensitive methods for the quantification of small molecules and biomarkers²⁴⁵. This is possible because of plasma waves which occur on the surface of the metallic coating (called surface plasmons); when polarised light is incident at the resonance angle to the surface, it causes changes to the energy absorbed by these plasmons, producing a change in the reflection angle, which is the signal that is measured to provide qualitative and quantitative information to describe the chemical composition of the sample being measured.

Surface plasmon resonance (SPR) is characterised by the build-up of spatially non-homogenous oscillating electric fields in the neighbourhood of nanostructures, producing locations called hotspots, which enhance Raman signals. SERS greatly enhances Raman signals owing to SPR, whereby the coated surfaces with nanoscale roughness significantly increase the electric field when the photons from a laser are excited because of the adsorption of biomolecules on rough surfaces. These effects are associated with the interaction of electromagnetic radiation with metals that appear as metallic nanostructures owing to the localised surface plasmons and the charge transfer between the two materials, making it even more essential to have more selective designs to further improve the interactions. Often determined by the morphology of the substrates, the orientation of molecules, and the polarisation of the laser²⁰², the enhancement factor indicates the effectiveness of the SERS substrate.

The enhancement of SERS signals is effective with tweaks to the nanostructures, with an aspect ratio (the ratio of the width to the height of pillars in a nanostructure) of 0.79 of a nanostructure shown to produce 30 times more enhancement than a nanostructure with an aspect ratio of 0.61^{109,303}. While these are the principles used in producing SERS substrates, obtaining even greater enhancements has made the use of unconventional solutions to be considered for SERS substrates, including plasmonic paper²¹⁸, the use of graphene as a SERS substrate³⁰⁴, and gold-coated zirconium-based metallic glass used as a 3-D metallic nanostructure¹¹⁵.

These solutions are also driven partly by the need to cut the costs of a substrate while maintaining the effectiveness and efficiency of the platforms when these substrates are used in the design of point-of-care diagnostics. Other examples involve the use of nanoparticles to produce an enhancement factor of 1×10^6 via silicon nanowire arrays coated with 20 weight percent silver nanoparticles to produce a 3-D SERS substrate¹¹⁸; a ternary film containing poly(methyl methacrylate) (PMMA) using a silver-coated gold nanoparticle plasmonic array fabricated as a SERS chip¹¹⁹; SERS substrate, which involves the fabrication of a monolayer of gold nanoparticles at the air-water interface¹²⁰. Further examples include roll-to-roll ultraviolet nanoimprint lithography that was used to produce an enhancement factor of 1.21×10^7 via a polymer nanostructure array based on anodic aluminium oxide (AAO) mold, subsequently deposited onto a layer of gold film to produce a flexible SERS substrate¹²¹; an enhancement factor of 1.9×10^8 was achieved by the deposition of a layer of poly (dimethyl siloxane) (PDMS) film on a self-assembled gold nanostar array to obtain a flexible SERS substrate¹²²; and a SERS substrate obtained an enhancement factor of 1.49×10^8 which was made from a periodic metallic nanostructure array with gold nanoparticles¹¹⁵.

Although these nanoparticle-based SERS substrates produce high Raman signal enhancements, the problem with using nanoparticles is that they suffer from sample variability and poor batch-to-batch reproducibility, leading to a lack of consistent hotspots for accurate signal detection. Designing a SERS substrate to mitigate these issues while retaining high-enhancement Raman signal capabilities for accurate sample detection is vital. One way to make an effective SERS substrate is to produce patterned micro-nanostructures via photolithography and topological design.

The topological design of silicon-based micro-nanostructures involves the manipulation of their geometrical and electronic properties using techniques such as lithography, etching, and deposition. The goal is to create structures with specific topological properties that can be used in various applications such as electronics, photonics, quantum computing, and medical

devices. The surface area is another important characteristic of a SERS substrate, and a larger surface area increases the potential number of molecules that can produce SERS and often improves sensitivity³⁰⁵. The structural arrangement and topological design of the nanostructure can contribute as high as 50% more to the total SERS signal, as this is crucial for the detection of single molecules (especially in samples with very low concentrations) because of the extremely strong electromagnetic field in the volume of the nanostructures³⁰⁶.

As the geometrical and morphological properties of SERS substrates affect the amount of enhancement obtained, other issues, such as economic costs, make it essential to consider other properties, such as the high oxidation of silver-coated surfaces, limiting their longevity compared to gold-coated surfaces, even though they produce better enhancements. Ideally, suitable substrates support the 'strongest' plasmon resonances and provide the largest enhancement or amplification. A given SERS substrate exhibits good enhancements in a limited excitation wavelength range, as they are typically strongly wavelength dependent. Gold and silver are metals mainly used for SERS and plasmonic applications because they sustain good plasmon resonances in the visible/near-infrared range (400 – 1000 nm). This property, especially with gold, which lasts much longer than silver, makes it more desirable for biological applications.

Successful Raman scattering and enhancement depend on gold and silver structures because of their optical properties that sustain plasmon resonances. It also depends on structures with dimensions typically smaller than 100 nm, although these conditions are not strictly exclusive. A slight adjustment to the surface roughness of a silver nanoparticle-reduced graphene oxide SERS substrate has been shown, for example, to enhance the capture and characterisation of breast cancer cells efficiently and about four times more than the silver nanowire-graphene oxide films³⁰⁷.

Patterned substrates can act as possible platforms for multiplexed SERS of different substrates, with enhancement that can be increased by varying the morphologies¹⁰⁹. SPR can also be used to

tune the optical properties of these nanostructures, with some examples observed in the extinction spectra of silver nanowires (hexagonal shape), silver spheres, and silver cubes^{110,111}.

5.3 RESULTS AND DISCUSSION

Various geometries for patterned SERS substrates, including hexagonal, triangular, and rectangular coaxial and solid structures with dimensions ranging from 300 nm to 10 μ m, 700 nm to 10 μ m, and 700 nm $\times$ 600 nm to 60 μ m $\times$ 400 μ m, respectively (Figure 2.5 in Chapter 2) were fabricated in an array. This was subsequently scaled to a few microns (2 – 10 μ m), guided by the simulation results, to produce stable, reproducible structures that significantly enhanced the Raman signals. The optimal design of the substrates were arranged in 15 $\times$ 12 periodic arrays with a fixed periodicity of 30 μ m. Some of the geometries considered, owing to ease of fabrication and fidelity of structures produced, are shown in Figure 5.1, where AutoCAD 2021 was used to generate the designs before using KLayout, which is an open-source software for viewing, editing, and manipulating geometric shapes, to convert the files to a suitable format from .dwg and .dxf obtained in AutoCAD to GDS2, which is compatible with the PicoMaster software used for photolithographic fabrication of the substrates. A cursory check is necessary using KLayout to ensure that the correct designs have been converted (Figure 5.2) before the start of the fabrication process.

MULTIPLEXED PATTERNED DESIGNS – DEVELOPMENT AND OPTIMISATION STUDIES

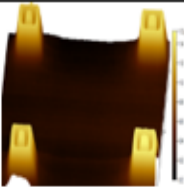
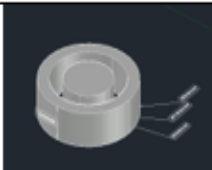
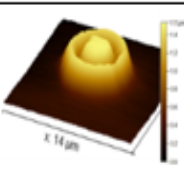
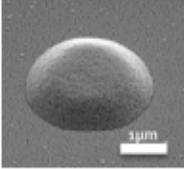
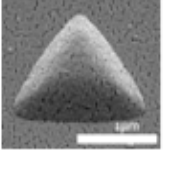
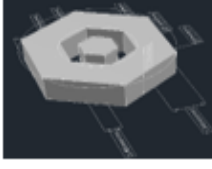
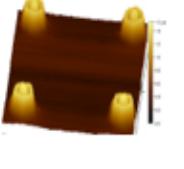
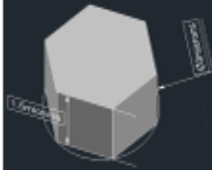
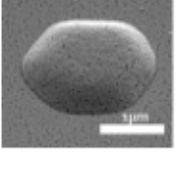
3D SHAPE	BIRDEYE VIEW	AFM/SEM IMAGES OF FABRICATED STRUCTURES	DIMENSIONS (OPTIMAL STRUCTURES)
			Height = 1.5 μm Inner pillar = 3 μm x 2 μm Middle rectangular = 4.6 μm x 3.6 μm Outer rectangular = 6.2 μm x 5.2 μm
			Height = 1.5 μm Outermost diameter = 6 μm Middle diameter = 4.6 μm Inner diameter = 3 μm
			Height = 1.5 μm Diameter = 3 μm
			Height = 1.5 μm The innermost triangle inscribed in a circle of diameter = 3 μm Middle triangle inscribed in a circle of diameter = 6 μm Outer triangle inscribed in a circle of diameter = 9 μm
			Height = 1.5 μm Triangle inscribed in a circle of diameter = 3 μm
			Inner hexagon inscribed in a circle of diameter = 1.5 μm Middle hexagon inscribed in a circle of diameter = 3 μm Outer hexagon inscribed in a circle of diameter = 6 μm
			Height = 1.5 μm Hexagon inscribed in a circle of diameter = 3 μm

Figure 5.1: AutoCAD-designed images of the topologies for patterned substrates, showing the dimensions of the optimal structures used as SERS substrates. Enlarged images are shown in Appendix One, A1.1 - A1.4.

The ease of scalability and reproducibility (with high fidelity based on AutoCAD designs) of the structures makes the process suitable for fabricating SERS substrates while offering flexibility in the material selection of the surface layer, as SERS adoption in sensing platforms requires that reproducibility is given utmost consideration³⁰⁸.

These designs can be easily altered just before fabrication, and multiple designs can be added to a single substrate for multiplexed detection of different molecules, with the process taking 20 minutes to fabricate multiple arrays of structures over an area of 100 μm^2 on the silicon wafer.

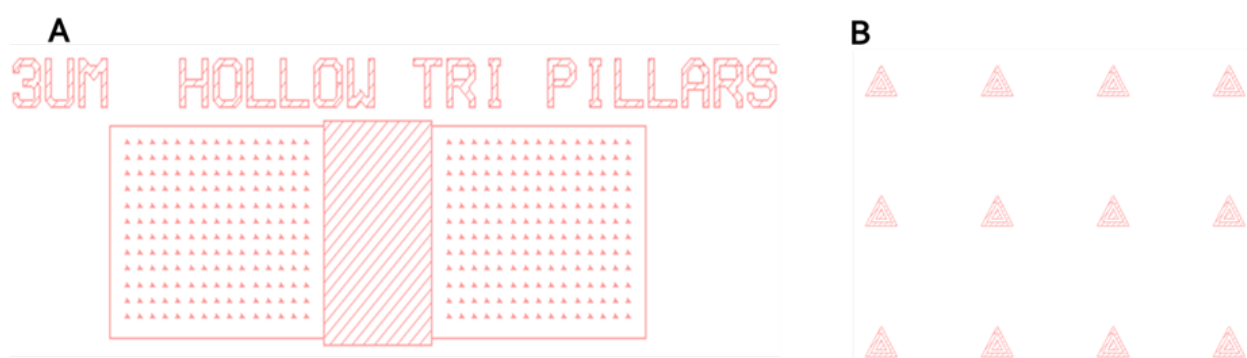


Figure 5.2: Example of the obtained KLayout image (A shows the whole array of patterned structures, while B shows the zoomed in structures). The KLayout software converts the patterns from the AutoCAD format .dwg and .dxf files to GDS2 format before fabrication on the photolithographic equipment.

The shapes and sizes of the patterned nanostructures significantly impact the SERS-active areas of the substrates; therefore, the simulation of specific topologies provides clarity for a better chance of congregating the SERS hotspots in localised regions where the Raman signals are considerably enhanced because of the interaction between the analyte molecules and the local surface plasmon resonance (LSPR) of the metallic-covered nanostructures.

The polarisation and wavelength of the incident light also play a crucial role in the hotspots; as such, using COMSOL for 3D near-field simulation of the electric field distribution of various shapes highlights the impact of slight adjustments in the dimensions of micro-nano structures on the enhancement obtained from the SERS substrates. The source of high sensitivity in SERS is also the leading cause of poor reproducibility, because SERS field enhancement is proportional

to $|E/E_0|^4$, the ratio of the local electric field at the surface of the metal nanostructure to the incident electric field, small changes in the in- and out-coupling, or perturbations in the near-field enhancement (E/E_0) drastically alter the SERS intensities³⁰⁸. For example, a 50 nm increase in the width of the wells of the hollow rectangle showed enhancements (Figure 5.3), while the distribution of the hotspots was along the centre structures and cavity of the concentric structures. This highlights the advantages of the hollow structures, as the hollow interiors provide confined spaces for electromagnetic field enhancements, as well as the large surface area compared to their solid counterparts with the same volume, enabling more analyte adsorption, leading to a more Raman-active molecule, and in turn, boosting Raman signals.

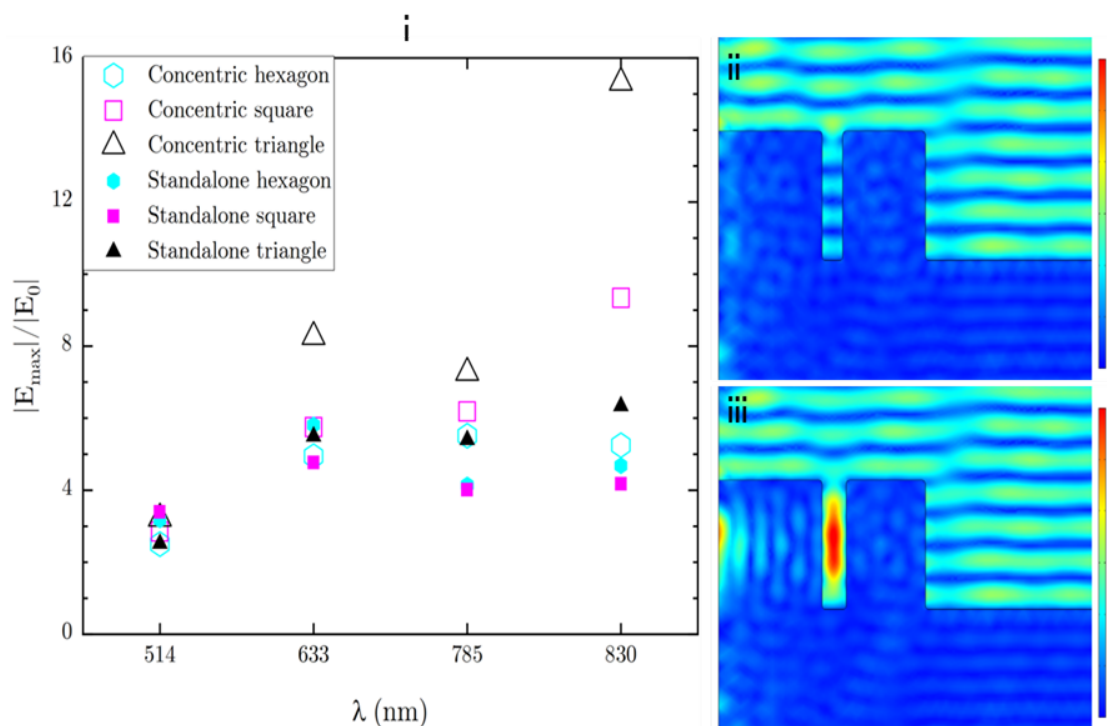


Figure 5.3: COMSOL simulation of (i) 3D field enhancement of different morphological structures, (ii) field enhancement using a laser excitation wavelength of 785 nm, with a 40 nm gold coating and well width of structures = 300 nm, and (iii) well width = 350 nm.

COMSOL simulation of the localised electric field distribution and the maximum field enhancement of the isotropically scaled-down version of these structures produced higher enhancements of the Raman signals, which were confirmed experimentally, with the highest values obtained with x0.56 scaling of the initial size of hexagonal concentric structures (Figure 5.4).

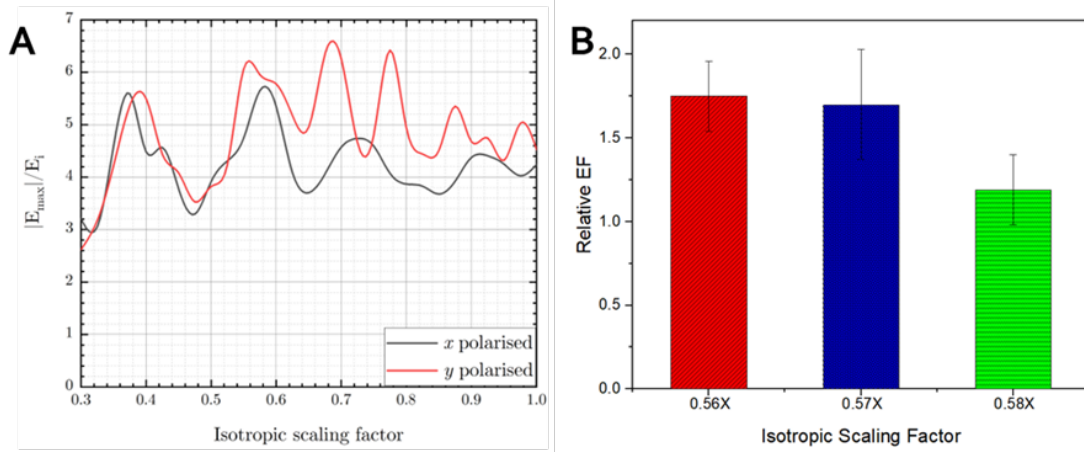


Figure 5.4: (A) Computational study and (B) Experimental results of the change in maximum field enhancement of isotropically scaled concentric hexagonal structure showing optimal structures for Raman signal enhancement was obtained with a scaling factor of 0.56 of the previous size of the concentric hexagonal structures producing the most enhancement of the signals.

The simulated and experimental data were compared, with a similar trend observed for the simulated theoretical maximum achievable field enhancement of the structures and the calculated absolute enhancement of the fabricated structures as shown in Figure 5.5. The hollow structures outperformed their solid counterparts, with the differences observed owing to the polarised light used for the simulation, unpolarised light for the experimental values, and other experimental errors during the fabrication process. However, these differences can be mitigated for direct comparison using the same conditions as much as possible and by minimising experimental errors.

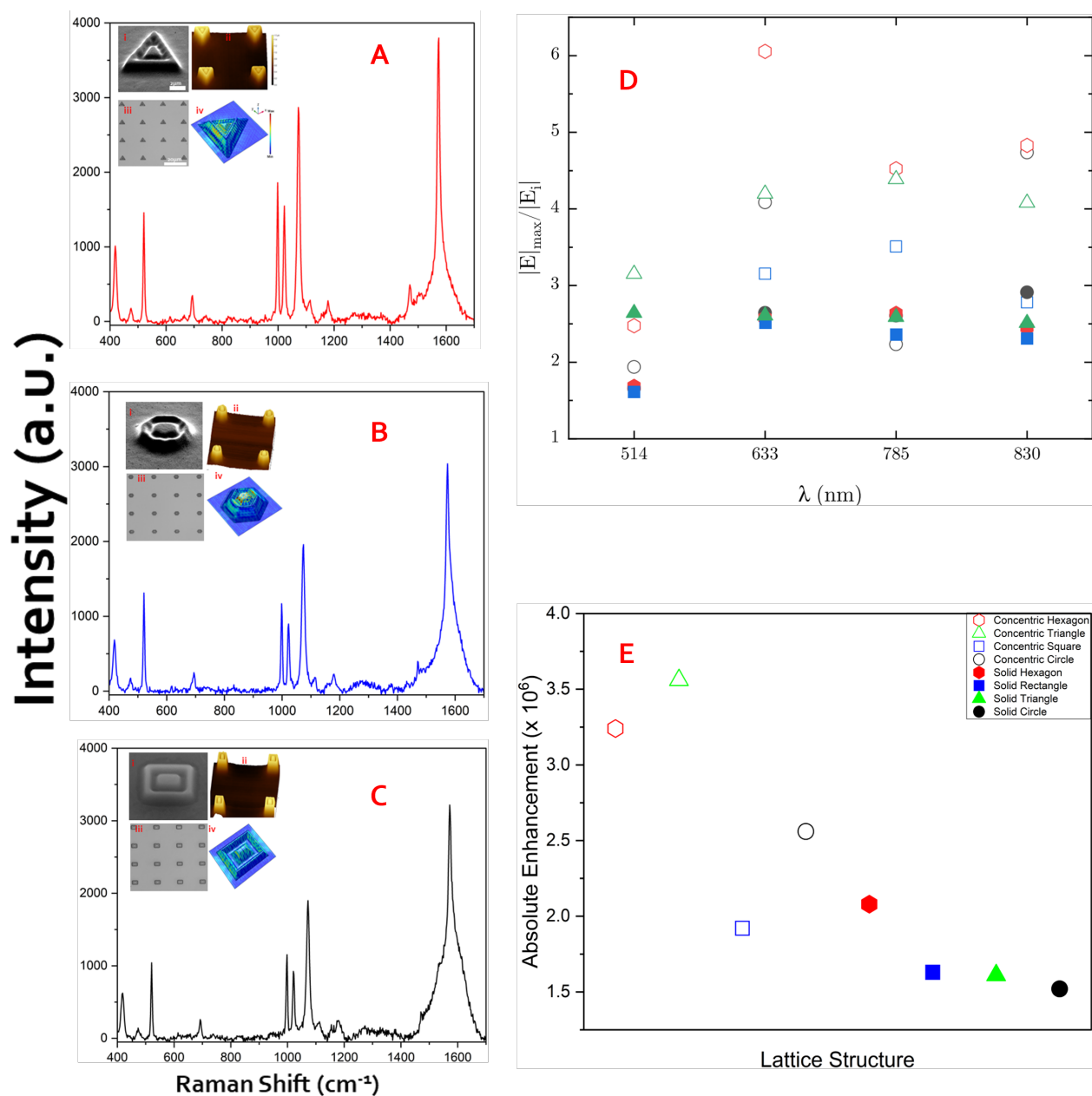


Figure 5.5: Raman spectrum of benzenethiol on differently patterned SERS substrates (A) Concentric triangular, (B) Concentric hexagonal, and (C) Concentric rectangular topologies. The inset shows the (i) SEM images, (ii) AFM images, (iii) optical microscope images, and (iv) COMSOL simulations of field enhancements of the respective structures. (D) The simulated maximum field enhancement for each lattice structure compared to the calculated absolute enhancement of the experimentally fabricated structures (E) shows a similar trend for 633 nm excitation wavelength.

Topology optimisation can be seen as the ‘lightweighting’ of a design by reducing its material (matter) component while retaining all or improving its functionality and structural integrity. It has been applied to SERS substrates and integrated nanostructures to improve efficiency, reduce costs, and create multiplexed designs^{300,301,309–311} for the detection of a combination of biomarkers, which is increasingly becoming an alternative approach to studying the progression of the disease for better diagnosis and treatment^{81,82}. The enhancement factors obtained by changing the lattices of the fabricated structures are given in Table 5.3, showing how slight changes can contribute to higher values of the enhancements, as indicated in the simulation results (Figure 5.4). The enhancement factor, the ratio between the Raman signals from a given number of molecules in the presence and absence of nanostructures, was calculated using Equation 3.5 (Chapter 3). The enhancement depends strongly on the size and shape of the nanostructures, which gives rise to this effect³¹².

Table 5.3: Enhancement factors (EF) of selected patterned SERS substrates at 633 nm excitation wavelength.

Lattice Structure	Absolute Enhancement ($\times 10^6$)	95% Confidence Interval ($\times 10^6$)
Concentric Rectangular	1.92	1.47, 2.44
Concentric Circular	2.56	2.47, 2.61
Concentric Triangular	3.56	3.36, 3.76
Concentric Hexagonal	3.24	2.86, 3.71
Solid Rectangular	1.63	1.56, 1.79
Solid Hexagonal	2.08	1.93, 2.24

Changing the topological structure of the designs from concentric circular structures to concentric hexagonal structures yielded an almost twofold increase in absolute enhancement (Table 5.3), and the variations in the topology of the substrates yielded different absolute enhancements, with shapes with vertices and edges, such as hexagonal and triangular substrates, producing higher enhancement values because of the regularity of the structures to create consistent hotspots. The designs containing sharp edges and corners exhibited stronger LSPR because of the symmetry, allowing for a uniform distribution of the localised field enhancement, resulting in a more reproducible and predictable signal enhancement. As reported by Yu *et al.*, an increase in

SERS performance was observed when nanostructures were fabricated closer to each other³¹³.

Similarly, further modification in the fabrication of the SERS substrates by reducing the periodicity between the structures to 10 μm from 30 μm and positioning the hexagonal and triangular structures next to each other in the array (Figure 5.6) resulted in a more than 200% increase in the absolute enhancement of the signals by the concentric triangular SERS substrates at an excitation wavelength of 633 nm.

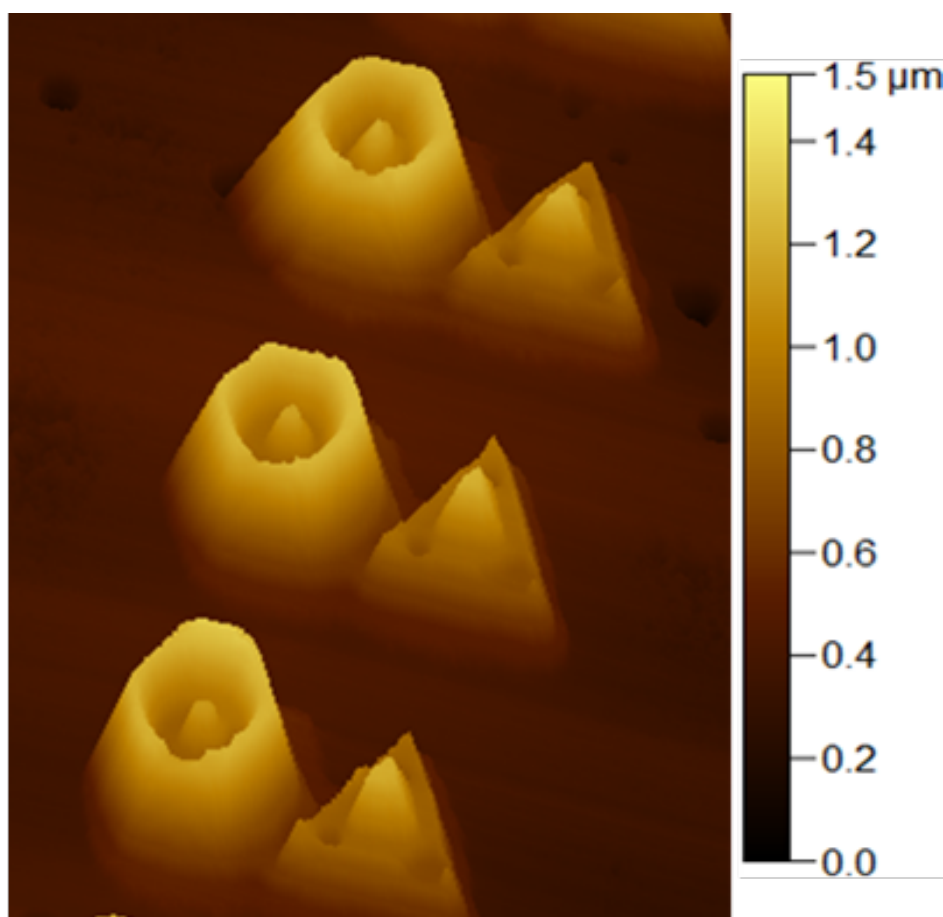


Figure 5.6: SERS hexagonal and triangular patterned structures with periodicity reduced to 10 μm .

The absolute enhancement for structures with 30 μm periodicity was 3.56×10^6 , with a 95% confidence interval of 3.36×10^6 and 3.76×10^6 . In comparison, the absolute enhancement for the modified structures with 10 μm periodicity was 1.09×10^7 , with 95% confidence interval of 6.47×10^6 and 1.83×10^7 .

The long-term stability of a thin layer of photoresist deposited on substrates is crucial for the efficiency and desired output of substrates used in point-of-care (P-o-C) diagnostic devices.

Ideally, smooth, non-defective thin layers and photoresists with metallic coatings are essential for substrates designed to enhance signals for detecting diseases^{314–318}. Stability depends on several factors, such as the interfacial energy between the liquid and solid (or air), as a typical thin film is the deposition of a liquid layer on a solid substrate. Other factors include surface tension forces, van der Waals forces (attractive and repulsive intermolecular forces between atoms, molecules, and surfaces), externally applied forces such as centrifugal force, electric and magnetic fields, and temperature gradients between the phases. The stability of the SERS substrates produced showed consistent enhancement of the Raman signals of benzenethiol over four weeks, with a minor variation of 2% within the first 200 hours and stable substrates over 13 months (within experimental errors), showing distinct peaks of representative Raman spectra of BT on the substrates (Figure 5.7).

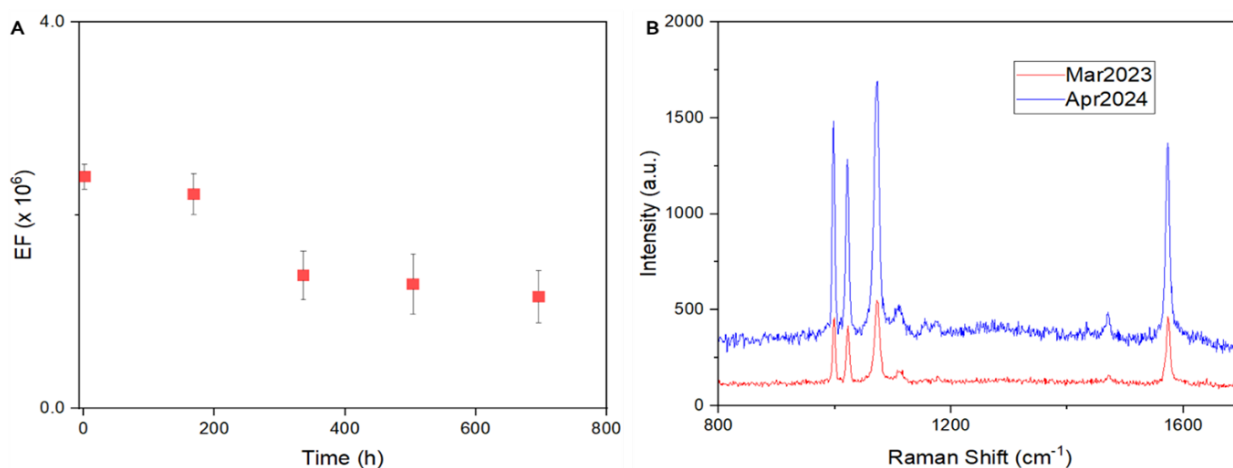


Figure 5.7: (A) The enhancement factor of the SERS substrate over one month shows the consistency of the substrate in detecting and enhancing the Raman signals of the BT molecules over time. (B) The characteristic Raman spectra of BT detected on the SERS substrate over 13 months.

5.4 CONCLUSIONS

In conclusion, this chapter examined the potential of multiplexed SERS substrates for the rapid and sensitive detection of BT molecules. The micro-nanostructures were systematically designed, optimised, and fabricated to produce tuneable structures, which were informed by field enhancement simulations of different topological designs. This enabled the identification of optimal structures for improving the Raman signal enhancement (with an average enhancement of $\times 10^6$), notably among structures with increased vertices and edges, with further modifications yielding signal improvements of more than 200%. The substrates were analysed for stability, showing their stability over 13 months, including a minor 2% variability of enhancement after the first 200 hours. The ease of fabrication, scalability, and multiplexing on a single substrate makes this approach suitable for developing engineered SERS platforms with excellent potential, which could be integrated into sensors for P-o-C diagnostic devices for multiplexed detection of molecular fingerprints of biomarkers associated with traumatic brain injury.

5.5 METHODOLOGY

5.5.1 Materials

The substrates used for the nanostructure patterning were test-grade silicon (Si) wafers (n-type, crystal orientation $\langle 100 \rangle$), with one side polished and having a resistivity of 0 – 100 ohm-cm (WaferNet, GmbH Germany). The wafer was $500 \pm 25 \mu\text{m}$ thick and cut into $1.3 \times 1.3 \text{ cm}^2$. The substrate acted as a base for the deposition of the photoresist prior to printing.

5.5.2 Substrates Fabrication

An ultra-clean substrate is essential for a reproducible and accurate substrate, and this was performed to remove surface contaminants that might affect the effectiveness of the substrate. As discussed in Section 2.1, the substrates were fabricated by cleaning the silicon wafer, spin-coating the photoresist, photolithographic printing the patterns, and sputter-coating the metallic coatings on the fabricated patterns. Further modifications were made to the periodicity of the structures (the gaps between the rows and columns of the structures in the array), with different morphologies placed next to each other to determine their influence on signal enhancement.

5.5.3 Optical Image Acquisition

A universal white light LED illumination Leica DM2700 M microscope was used to view submicrometric features, including structural information and cleanliness, for the qualitative analysis of the substrates.

5.5.4 Surface-Enhanced Raman Scattering Acquisition

Near-infrared lasers are commonly used in biological applications, especially at 633 nm, 785 nm, and 830 nm, owing to their relatively low photon energy and limited photodamage compared to others⁹⁰. However, the resolution of the Raman spectrum is affected by the linewidth of the 830 nm laser because it is broader than that of the 785 nm laser, resulting in a poorer spectral resolution. The 785 nm laser is preferred for biomedical applications owing to reduced sample damage, compared to the increased heating of the sample by the 830 nm laser, making it more suitable for sensitive samples. While the total laser power used on a sample is crucial to the quality of the obtained results, less than 30mW is recommended for shorter visible wavelengths and more

than 100mW for longer wavelengths, although the optimised illumination power delivered to the sample is dependent on the laser spot size (sampling area) and magnification, both of which determine the laser intensity and thus have a significant impact on laser exposure on the sample.

To obtain high-quality results, the choice of the microscope objective is crucial. The numerical aperture (NA), magnification, and solid angle of light collection of the objective's throughput indicate that a high magnification/NA provides higher axial spatial resolution but a shorter working distance (better suited for thin samples). Low magnification provides a lower axial spatial resolution but has a longer working distance, which is suitable for bulky samples to prevent the objective from touching the samples. High magnification is better for samples in which biological architectures may be examined because of their higher spatial resolution. Metal-coated slides, such as aluminium- or gold-coated slides, show that the effect of the slide signals is eliminated, removing slides that produce strong background fluorescence⁹⁰. The SERS measurements were performed using a micro-Raman system via an inVia Qontor confocal Raman spectrometer and at two excitation lasers: 633 nm and 785 nm. A laser power of 30 - 40 mW was used to avoid photochemical effects, degradation or sample damage, with SERS maps spectral scans typically acquired for 100 data points over a range of 500 – 2000 cm^{-1} , 50x objective lens, with a numerical aperture of 0.75, the acquisition time range of 0.1 - 10 seconds with three accumulations. The scans were performed with the beam covering a single pattern each time, with the data collected analysed to determine where most of the signals were observed, particularly for the hollow structures, and the location of the highest enhancements within each structure. All the Raman spectra were recorded at ambient temperature of 20 °C, and the integrated peaks from the spectra were used for enhancement factor calculations. Acquisition time, wavelength, and laser power were selected to avoid photochemical effects and sample damage. The choice of the laser wavelength follows the quality of the spectra obtained for benzenethiol using four different methods, as shown in Figure 4.4c, and the aforementioned damage caused to the sensitive samples. However, the potential sources of errors include the adsorption uniformity to obtain a monolayer and spot size

mismatches when adding benzenethiol to be tested to the substrates.

5.5.5 Scanning Electron Microscope Image Acquisition

The scanning electron microscopy (SEM) measurements were performed using a Hitachi SU5000 at acceleration voltages of 0.5 - 5 kV with a lateral resolution of 2 - 5 nm.

5.5.6 Atomic Force Microscope Image Acquisition

Atomic force microscopy (AFM) provided more detailed structural information than optical microscopy, and measurements were carried out on JPK NanoWizard 4, with image processing and analysis carried out using the instrument software JPK Instruments NanoWizard3 version 4.2, and Gwyddion version 2.59, a Statistical Parametric Mapping (SPM) data visualisation and analysis tool.

5.5.7 Numerical Modelling

All the COMSOL Multiphysics simulations were performed by Dr Zoltan Sztranyovszky using the software's finite-element solver in version 5.5¹⁸⁴, through the wave optics module and frequency domain study. The structures were modelled based on AutoCAD, SEM, and AFM images with an assumption of 45° wall angles and imported via the CAD module. The geometry of the structures was assumed to be well-defined. The field enhancements were exported from COMSOL on the surface of the structures, with the illumination in two plane waves, polarised in the 'x' and 'y' directions, with quarter and half spaces simulated to reduce computation time, and using $\lambda/5$ element size^{281,282}.

The silica substrate had a refractive index of $n = 1.45$, the structure, made of a photoresist

coated with gold, had a refractive index of $n = 1.58$, and the top layer was air with $n = 1$. The thin gold layer was modelled using transition boundary conditions with a thickness $t = 40$ nm (with the assumption that the surface was uniformly coated) and gold permittivity from Johnson and Christy²⁸⁰. The excitation at each of the employed laser wavelengths was taken as a plane wave, with wavelength λ propagating along the z axis. A mesh independence study was not conducted, which is a limitation of the simulation process. The simulations were performed using polarised light to obtain a better spectra quality and ease of calculation and analysis, whereas the experimental conditions only allowed unpolarised light.

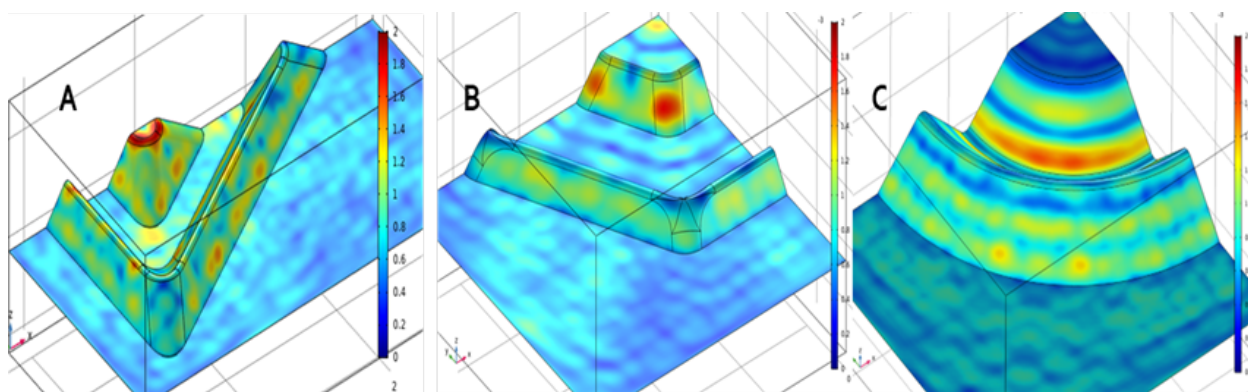


Figure 5.8: COMSOL simulation of the average enhancement of the 'x' and 'y' polarisation of the field enhancements based on the structures using the laser excitation wavelength of 785 nm. The height of the structures is $1.5 \mu\text{m}$, and the outermost structures were inscribed in circles of diameter $9 \mu\text{m}$, $6 \mu\text{m}$, and $6 \mu\text{m}$, respectively. Quarter structures were simulated to reduce computation times.

5.5.8 Stability Study

The stability study was performed using the fabricated concentric triangular structures described in Section 4.3, with the optimal dimensions given in Figure 5.1, to determine the effectiveness of the substrates after a period by checking the enhancement of the Raman signals of benzenethiol. By checking the enhancement produced (via the Raman intensity produced by the substrate from a monolayer of benzenethiol over the structure) after a period while maintaining the same conditions and parameters of measurement (the same laser wavelength, acquisition time, laser power, and objective lens), using the same batch of fabricated substrates, reproducible

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information was obtained to check the degradation and effectiveness of the substrates over time. The effects of laser wavelength and choice of metallic coating were not investigated.

CHAPTER SIX

SUMMARY AND FUTURE OUTLOOK

6.1 SUMMARY

This doctoral thesis showed the use of patterned substrates to significantly enhance the Raman signals of biomarker molecules in samples that indicate the presence of certain neurological diseases. The introductory chapter described the ubiquitous patterning observed in nature and how it is incorporated into biomedical devices, using topological design to produce innovative solutions for sustainable, effective, and efficient devices for improving the quality of life through rapid and accurate detection of diseases, thereby initiating appropriate and timely care for patients. This is used in biosensor designs to detect disease-indicative molecular bonds of biomarkers in complex biological samples to accurately detect symptoms of diseases, such as traumatic brain injury, which may persist for years, leading to dementia.

Chapter 2 discussed in more detail the experimental and analytical techniques used in the design and fabrication process, summarised in Figure 2.1, which includes the step-by-step photolithographic process involved (and the equipment used), such as cleaning of the silicon wafer, spin coating of the photoresist, fabrication of the pattern, and post-fabrication processes to obtain the SERS substrate. The chapter also discussed the characterisation techniques used to present the results of the fabricated patterns while also covering the COMSOL simulation software used to obtain the optimal structure sizes for better enhancement of Raman signals and other software such as Python and Renishaw WiRE, which were used for statistical analysis, data cleaning and processing, and visualisation.

Part of Chapter 3 is briefly covered as a co-author in a published review article in the *Discover Nano* journal titled 'Advances in lithographic techniques for precision nanostructure fabrication in biomedical applications', Stokes et al., 2023. Further details of the photolithographic technique used in this research were highlighted in this chapter, including the theoretical background of patterned structures using photolithography, Raman spectroscopy, and modified surface-enhanced

Raman scattering, which relies on modifying the surfaces to achieve significantly higher signal enhancements. The choice of materials used for these enhancements, such as the type of metallic coating and laser wavelength, were also covered in this chapter.

The patterned substrates were easily and rapidly fabricated using photolithography and other experimental techniques to produce novel designs that can be easily scaled to the desired sizes. These designs were presented in Chapter 4 and show significant enhancements in the characteristic Raman signals of benzenethiol and representative TBI biomarkers. This chapter was published in the *Advances Materials Interfaces* journal article as a first author, titled 'Development and Optimization of Micro-Nanotopographical Platforms for Surface Enhanced Raman Scattering Biomolecular Detection', Odetade et al., 2024.

In Chapters 4 and 5, COMSOL simulation was used to guide the sizes of the fabricated structures to obtain optimal sizes for Raman signal enhancements using various topological designs. The experimental data corresponded to the numerical modelling in Chapter 4, showing the structures which produced the best enhancements. This was further explored in Chapter 5 by combining some of the structures and adjusting their periodicity to obtain even better enhancements while maintaining the fidelity of the structures produced. The versatility and scalability of the process, leveraging topological designs to fabricate the substrates, were shown in how the changes in the size of structures can be obtained for tailored topologies, making it a cost-effective method for the large-scale production of multiplexed SERS substrates that can be used as biosensors and other bioanalytical systems for the rapid and sensitive trace-level detection of biomarkers indicative of traumatic brain injury and other similar diseases.

The fidelity of the fabricated structures can be measured by comparing them to the design using characterisation techniques, such as AFM, SEM, and OM. These can be used to compare the dimensions of the CAD designs and check the structural integrity, surface quality, and performance of the fabricated structures. Interferometry and profilometry are other characterisation techniques

that can be used to check dimensional accuracy.

Using the SEM and AFM images of the fabricated structures, the height of the structures was $1.5 \pm 0.2 \mu\text{m}$ (10 different structures), with similar deviations observed in the other dimensions compared to the CAD designs.

A stability study to determine the viability of the substrates as biosensors showed the effectiveness of the substrates in detecting benzenethiol after a 13-month period, showing its enhancement of Raman signals, with a minor variation of 2% over more than 200 h and stability over a longer period.

However, some of the limitations of this study are the potential storage of the substrates in a stable and clean environment and the changes to the micro-Raman system used for measurement, as each day produces a different value during its calibration using a pure silicon wafer before use. These limitations also apply to the experimental part of the research, including working in a cleanroom to minimise contamination during storage and testing, and measuring Raman signals while maintaining constant parameters.

Other limitations include the assumption of a uniform monolayer of benzenethiol during preparation, with a constant number of molecules used to determine the enhancement factors of the substrates, as well as the unpolarised light used to obtain the Raman spectra. In contrast, polarised light was used for COMSOL simulations.

These limitations can affect the reported enhancement factors and, therefore, the specificities of biosensors.

Some of these limitations can be minimised by comparing the intensity values obtained during the calibration to ensure that the variations are low and within the acceptable margin and storing the substrates in a cleanroom.

Point-of-care diagnostic devices should be able to detect biomolecules in a sample rapidly, accurately, and sensitively while being portable and stable in various environments, especially in out-of-clinic settings. The substrates designed in this research study have these properties, including versatility and scalability, and can be used as biosensors when incorporated into a point-of-care device to detect biomolecules that indicate traumatic brain injury in patients. It is stable with very low limits of detection for identifying trace molecules in blind samples.

6.2 FUTURE OUTLOOK

While the research work herein conveyed the possibility of fabricating SERS substrates that can significantly enhance the detection of the Raman signals of molecules, further modifications were identified that could prove cost-effective while maintaining the effectiveness of the fabricated substrates. One is to substitute the metallic coating of gold or silver with graphene.

6.2.1 Graphene-Coated Substrates

Gold substrates are more stable than silver substrates; however, the SERS enhancement is weaker than that of silver substrates¹¹⁶. Other factors, such as overall cost, practicality, and ease of fabrication, are considered when selecting substrates and metals for use as coating substances for SERS substrates. Graphene has been considered a substrate for SERS with varying degrees of success, as its enhancement factors depend on the type of graphene layer used. Monolayer graphene offers the best SERS enhancement and is much stronger than SiO₂/Si substrate³⁰⁴. The use of graphene is partly due to its reduced costs and biocompatibility. Gold (*Au*) and silver (*Ag*) are dominant in the production of SERS substrates owing to their strong LSPRs in the visible region, which is the most important phenomenon for electromagnetic enhancement in SERS^{221,319}. A cheaper and readily available alternative that is equally effective and efficient

in the enhancement of Raman signals is desirable, especially for the production of affordable point-of-care diagnostic devices.

However, care must be taken to ensure that it provides high specificity and selectivity for analytes, comparable to *Au* and *Ag*, for it to be viable. Different orientations of silicon substrates with graphene have been explored for possible applications in various electronic devices. This method is potentially feasible for improving Raman signals and, possibly, better-detecting fingerprint molecules of biomarkers using an integrated optofluidic device for early detection of analytes of particular neurological disorders³²⁰. However, for the photolithographic technique adopted for this research, there continued to be chemical reactions between the photoresists and graphene coatings, making it impractical for reproducible patterned substrates. One suggestion would be to find the right balance of the thin-layer coating of gold to avoid initiating the chemical reaction before coating it with graphene. Thus, a combination of gold and graphene can be explored to enable the adoption of both properties for signal enhancement.

6.2.2 Oblique Angle Substrates

The design, patterning, and ease of fabrication of the different structures, as shown in Chapters 4 and 5, highlight the possibility of using this photolithographic technique. However, some limitations exist, such as the fabrication of pyramids, cones, stars, and other complex structures, which is a result of the PicoMaster equipment printing the structures on a flat silicon wafer containing the spin-coated photoresist that makes up the structures (Figure 2.7). Spin-coating different layers of photoresist and oblique-angle fabrication could offer a route to produce complex structures, significantly enhancing the Raman signals of molecules. Platonic solids such as tetrahedrons, octahedrons, icosahedra, and dodecahedra can be explored for SERS substrate patterns, taking advantage of their congruent regular polygons to enhance signals. Structures with regular patterns have been shown to produce better signal enhancements owing to their vertices

and edges compared to structures with fewer sharp edges and vertices³²¹, for example, pyramid nanoparticles have been used for the accurate diagnosis of Alzheimer's disease³²². Successful fabrication of these structures can lead to other modifications that can increase the enhancements of signals, with such modifications based on the creation of materials or structures that can change their shapes, properties, or functionality over time in response to external factors such as moisture, light, magnetic fields, chemicals, or heat; *à la* 4-D printing^{323,324}. Alternatively referred to as auxetic shapes (similar to the honeycomb structures)^{325,326}, these structures respond to stimuli and can be described as 'memory shapes' that can return to their primary states, with the substrates harnessing functionality from the geometry and materials from which they are made³²⁷. Some of the factors currently limiting the production of these structures, apart from those previously mentioned, include cost, the development of smart materials, and precision control.

6.2.3 Polarised Raman Spectroscopy

Another modification to consider is the changes in the resonance conditions using purpose-designed multiwavelength SERS substrates, whereby polarised light is used instead of unpolarised light to provide quality information about the orientation of the molecules and the symmetry of the vibrational modes to be obtained. Polarised light offers a deeper understanding of the structure of a sample. The intensity of Raman scattering depends on several factors, including the polarisation of the incident light and the orientation of the molecules being examined. If the proper orientation of the molecule is matched with the incident light, the intensity of the polarised light will be higher than that of unpolarised light. The Raman scattering efficiency is dependent on the alignment between the vibrational mode of the analyte molecule and the electric field of the light, making the Raman intensity of unpolarised light more significant than that of polarised light most of the time, as the molecules will be aligned favourably with some of the electric

field directions. However, for polarised light, selective excitation of specific vibrational modes can be achieved using the symmetry of the molecules. The enhancement factor of the electric field of SERS is based on the enhancement of the excitation laser and the enhancement of the Raman-scattered light, where the intensity of each is proportional to the square of the electric field strength; therefore, the overall SERS enhancement is proportional to the fourth power of the normalised electric field intensity³²⁸. As such, a better quality of information can be obtained using polarised light (using a polariser) when acquiring Raman data of molecules for analyses.

6.2.4 Integrated Platform

Integrating the optimal SERS substrate design into an optofluidic platform capable of rapidly detecting biomarkers indicative of traumatic brain injury and other neurological diseases is the next stage of this research. The aim was to fabricate and develop advanced, low-cost, yet highly effective substrates that will ultimately facilitate the quick and accurate detection of biomarkers in patients with TBI that can be deployed in out-of-clinic settings, such as the back of an ambulance or other easily accessible spots.

6.3 FINAL REMARKS

While the variations in SERS and Raman spectroscopy and their application have shown that they will continue to be a force for good, the challenge remains to find a revolutionary substrate that can significantly improve Raman signal detection and stand apart from the already available pool. Isolated efforts are rare, although there is the possibility of them being abandoned altogether; however, the choice of material and its use in making structures that are currently complicated will likely play a crucial role in making this leap to a higher magnitude of enhancements not yet obtained, regardless of how the enhancements are calculated.

The World Health Organisation (WHO) gave recommendations for biomedical devices, called the **'ASSURED'** recommendation for point-of-care testing diagnostics, which are some criteria a device should have. This includes affordability, sensitivity, specificity, user-friendliness, reliability, robustness, equipment-free, and delivered easily to those who need it^{329,330}. Compliance with these criteria by SERS substrates offers considerable challenges, as sourcing for a cheaper alternative that will work just as effectively as the already established ones can prove difficult, as evidenced by the challenges faced in this research while trying to substitute gold-coated substrates with graphene, which could have acted as a cheaper alternative. However, a promising option is to combine these two, similar to the gold-silver combination used in bimetallic nanorods as a platform for SERS detection of drugs¹⁰⁶, but this can inadvertently increase manufacturing costs. Ensuring affordability without compromising other qualities such as the sensitivity and specificity of the SERS substrate could prove challenging, especially in avoiding false positives or negatives.

Designing an intuitive, simple, and user-friendly device, especially for those without technical training, can be challenging because some biosensors require calibration, precise handling, and storage. Fluctuations in conditions can affect the performance of the substrates, especially as cold-chain storage for those that deal with enzymes and antibodies can complicate distribution in resource-constrained parts of the world. This can also prove challenging with the short shelf-lives of some biosensors, limiting their viability.

Making a robust biosensor is possible with innovative and continuous improvements through simpler designs and manufacturing processes that lead to the production of substrates that align with ASSURED standards to overcome these limitations.

CHAPTER SEVEN

REFERENCES

LIST OF REFERENCES

1. Weibel, E. R. Fractal geometry: A design principle for living organisms. *Am. J. Physiol. - Lung Cell. Mol. Physiol.* 261, (1991).
2. Soylu, E. et al. Fractal analysis as a useful predictor for determining osseointegration of dental implant? A retrospective study. *Int. J. Implant Dent.* 7, 1–8 (2021).
3. Korolj, A., Wu, H. T. & Radisic, M. A healthy dose of chaos: Using fractal frameworks for engineering higher-fidelity biomedical systems. *Biomaterials* vol. 219 119363 (2019).
4. Herrmann, N., Neubauer, P. & Birkholz, M. Spiral microfluidic devices for cell separation and sorting in bioprocesses. *Biomicrofluidics* 13, 61501 (2019).
5. Tempra, C., La Rosa, C. & Lolicato, F. The role of alpha-helix on the structure-targeting drug design of amyloidogenic proteins. *Chem. Phys. Lipids* 236, 105061 (2021).
6. Pan, C., Han, Y. & Lu, J. Design and Optimization of Lattice Structures: A Review. *Appl. Sci.* 2020, Vol. 10, Page 6374 10, 1–36 (2020).
7. Lenzi, E. et al. SERS and Fluorescence-Active Multimodal Tessellated Scaffolds for Three-Dimensional Bioimaging. *ACS Appl. Mater. Interfaces* 14, 20708–20719 (2022).
8. Norberg, U. M. Vertebrate Flight. 27, (1990).
9. Rayner, J. M. V. Form and Function in Avian Flight. in *Current Ornithology* 1–66 (Springer, Boston, MA, 1988). https://doi.org/10.1007/978-1-4615-6787-5_1.
10. Tobalske, B. W. Biomechanics of bird flight. *Journal of Experimental Biology* vol. 210 3135–3146 (2007).
11. Shyy, W., Berg, M. & Ljungqvist, D. Flapping and flexible wings for biological and micro air vehicles. *Progress in Aerospace Sciences* vol. 35 455–505 (1999).
12. Liu, H. & Kawachi, K. A Numerical Study of Insect Flight. *J. Comput. Phys.* 146, 124–156 (1998).
13. Lowenstam, H. A. (Heinz A. On biomineralization / Heinz A. Lowenstam, Stephen Weiner. at (1989).
14. Velasco, M. A., Narváez-Tovar, C. A. & Garzón-Alvarado, D. A. Design, materials, and mechanobiology of biodegradable scaffolds for bone tissue engineering. *Biomed Res. Int.* 2015, (2015).
15. Patterns in Okra. <https://www.pinterest.co.uk/pin/146437425360960539/visual-search/?x=0&y=55&w=564&h=585&cropSource=4&surfaceType=flashlight%0A>. Accessed: 01/08/2024.
16. Honeycomb Patterns. https://commons.wikimedia.org/wiki/File:Honeycomb_15_03_2012.jpg%0A. Accessed: 01/08/2024.
17. Ebihara, Y., Ota, R., Noriki, T., Shimojo, M. & Kajikawa, K. Biometamaterials: Black Ultrathin Gold Film Fabricated on Lotus Leaf. *Sci. Rep.* 5, 1–6 (2015).
18. Spiral Galaxy NGC 3430. <https://www.nasa.gov/images/>. Accessed: 01/08/2024.

19. Fish Scales. https://commons.wikimedia.org/wiki/File:Fish_scales.jpg%0A. Accessed: 01/08/2024.
20. Lotus Leaf. https://commons.wikimedia.org/wiki/File:Phi_-_*.JPG%0A. Accessed: 01/08/2024.
21. Astor, T. L. & Borenstein, J. T. The microfluidic artificial lung: Mimicking nature's blood path design to solve the biocompatibility paradox. *Artificial Organs* vol. 46 1227–1239 (2022).
22. Isenberg, B. C. et al. A Clinical-Scale Microfluidic Respiratory Assist Device with 3D Branching Vascular Networks. *Adv. Sci.* 10, 2207455 (2023).
23. Saraei, N. et al. Scaled-up Microfluidic Lung Assist Device for Artificial Placenta Application with High Gas Exchange Capacity. *ACS Biomater. Sci. Eng.* 10, 4612–4625 (2024).
24. Noblin, X. et al. Optimal vein density in artificial and real leaves. *Proc. Natl. Acad. Sci. U. S. A.* 105, 9140–9144 (2008).
25. Sharifi, F. et al. Polycaprolactone Microfibrous Scaffolds to Navigate Neural Stem Cells. *Biomacromolecules* 17, 3287–3297 (2016).
26. Barthlott, W. & Neinhuis, C. Purity of the sacred lotus, or escape from contamination in biological surfaces. *Planta* 202, 1–8 (1997).
27. Ramesh, M., Janani, R., Deepa, C. & Rajeshkumar, L. Nanotechnology-Enabled Biosensors: A Review of Fundamentals, Design Principles, Materials, and Applications. *Biosensors* vol. 13 (2023).
28. Naresh, V. & Lee, N. A review on biosensors and recent development of nanostructured materials-enabled biosensors. *Sensors (Switzerland)* vol. 21 1–35 (2021).
29. Nair, M. P., Teo, A. J. T. & Li, K. H. H. Acoustic biosensors and microfluidic devices in the decennium: Principles and applications. *Micromachines* vol. 13 (2022).
30. Zhang, J. et al. Recent advances in acoustic wave biosensors for the detection of disease-related biomarkers: A review. *Analytica Chimica Acta* vol. 1164 (2021).
31. Goosen, M. F. A. et al. Fouling of reverse osmosis and ultrafiltration membranes: A critical review. *Separation Science and Technology* vol. 39 2261–2297 (2004).
32. Markvart, T. & Castañer, L. *Practical Handbook of Photovoltaics: Fundamentals and Applications*. Practical Handbook of Photovoltaics: Fundamentals and Applications (Elsevier Inc., 2003).
33. Hwang, S. & Yasuda, T. Indoor photovoltaic energy harvesting based on semiconducting π -conjugated polymers and oligomeric materials toward future IoT applications. *Polymer Journal* vol. 55 297–316 (2023).
34. Yin, B. et al. Micromixer with Fine-Tuned Mathematical Spiral Structures. *ACS Omega* 6, 30779–30789 (2021).
35. Lee, C. Y., Wang, W. T., Liu, C. C. & Fu, L. M. Passive mixers in microfluidic systems: A review. *Chemical Engineering Journal* vol. 288 146–160 (2016).
36. Chen, D. L., Gerdt, G. J. & Ismagilov, R. F. Using microfluidics to observe the effect of mixing on nucleation of protein crystals. *J. Am. Chem. Soc.* 127, 9672–9673 (2005).

-
37. Krishnan, M., Agrawal, N., Burns, M. A. & Ugaz, V. M. Reactions and fluidics in miniaturized natural convection systems. *Anal. Chem.* 76, 6254–6265 (2004).
38. Sudarsan, A. P. & Ugaz, V. M. Fluid mixing in planar spiral microchannels. *Lab Chip* 6, 74–82 (2006).
39. Lee, J. H. & Yeo, Y. Controlled drug release from pharmaceutical nanocarriers. *Chem. Eng. Sci.* 125, 75–84 (2015).
40. Logan, B. E. & Regan, J. M. Electricity-producing bacterial communities in microbial fuel cells. *Trends in Microbiology* vol. 14 512–518 (2006).
41. Zorlutuna, P. et al. Microfabricated biomaterials for engineering 3D tissues. *Advanced Materials* vol. 24 1782–1804 (2012).
42. Vollrath, F. & Selden, P. The role of behavior in the evolution of spiders, silks, and webs. *Annual Review of Ecology, Evolution, and Systematics* vol. 38 819–846 (2007).
43. Seijsing, F. et al. Recombinant spider silk coatings functionalized with enzymes targeting bacteria and biofilms. *Microbiologyopen* 9, (2020).
44. Hurbain, I. et al. Microvilli-derived extracellular vesicles carry Hedgehog morphogenic signals for *Drosophila* wing imaginal disc development. *Curr. Biol.* 32, 361–373.e6 (2022).
45. Satir, P. & Christensen, S. T. Overview of structure and function of mammalian cilia. *Annual Review of Physiology* vol. 69 377–400 (2007).
46. Vincent, J. F. V. & Wegst, U. G. K. Design and mechanical properties of insect cuticle. *Arthropod Struct. Dev.* 33, 187–199 (2004).
47. Casson, S. & Gray, J. E. Influence of environmental factors on stomatal development. *New Phytologist* vol. 178 9–23 (2008).
48. Alibardi, L. Adaptation to the Land: The Skin of Reptiles in Comparison to That of Amphibians and Endotherm Amniotes. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution* vol. 298 12–41 (2003).
49. Maan, A. M. C., Hofman, A. H., de Vos, W. M. & Kamperman, M. Recent Developments and Practical Feasibility of Polymer-Based Antifouling Coatings. *Advanced Functional Materials* vol. 30 2000936 (2020).
50. Bechert, D. W., Bruse, M. & Hage, W. Experiments with three-dimensional riblets as an idealized model of shark skin. *Exp. Fluids* 28, 403–412 (2000).
51. Chung, K. K. et al. Impact of engineered surface microtopography on biofilm formation of *Staphylococcus aureus*. *Biointerphases* 2, 89–94 (2007).
52. Bixler, G. D. & Bhushan, B. Review article: Biofouling: Lessons from nature. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences* vol. 370 2381–2417 (2012).
53. Wang, L. & Zhou, Q. Surface hydrophobicity of slippery zones in the pitchers of two *Nepenthes* species and a hybrid. *Sci. Rep.* 6, (2016).
54. Widhe, M. et al. Recombinant spider silk as matrices for cell culture. *Biomaterials* 31, 9575–9585 (2010).

55. Altman, G. H. et al. Silk-based biomaterials. *Biomaterials* 24, 401–416 (2003).
56. Autumn, K. et al. Evidence for van der Waals adhesion in gecko setae. *Proc. Natl. Acad. Sci. U. S. A.* 99, 12252–12256 (2002).
57. Puthoff, J. B., Prowse, M. S., Wilkinson, M. & Autumn, K. Changes in materials properties explain the effects of humidity on gecko adhesion. *J. Exp. Biol.* 213, 3699–3704 (2010).
58. Li, Y., Zhang, L. Y., Zhang, C., Zhang, Z. R. & Liu, L. Bioinspired antifouling Fe-based amorphous coating via killing-resisting dual surface modifications. *Sci. Rep.* 12, 1–10 (2022).
59. Song, W., Lu, H., Kawazoe, N. & Chen, G. Adipogenic differentiation of individual mesenchymal stem cell on different geometric micropatterns. *Langmuir* 27, 6155–6162 (2011).
60. Falconnet, D., Csucs, G., Michelle Grandin, H. & Textor, M. Surface engineering approaches to micropattern surfaces for cell-based assays. *Biomaterials* vol. 27 3044–3063 (2006).
61. Théry, M. Micropatterning as a tool to decipher cell morphogenesis and functions. *Journal of Cell Science* vol. 123 4201–4213 (2010).
62. LaValle, S. M. Planning algorithms. *Planning Algorithms* vol. 9780521862 (Cambridge University Press, 2006).
63. Bendsoe, M. P. & Sigmund, O. Topology Optimization. *Topology Optimization* (Springer Berlin Heidelberg, 2004).
64. 2020 Alzheimer's disease facts and figures. *Alzheimer's Dement.* 16, 391–460 (2020).
65. Nichols, E. et al. Estimation of the global prevalence of dementia in 2019 and forecasted prevalence in 2050: an analysis for the Global Burden of Disease Study 2019. *Lancet Public Heal.* 7, e105–e125 (2022).
66. Alzheimer's Association. What is Alzheimer's Disease? Symptoms & Causes | alz.org. Alzheimer's Association <https://www.alz.org/alzheimers-dementia/what-is-alzheimers> (2020). Accessed: 25/04/2022.
67. Fann, J. R. et al. Long-term risk of dementia among people with traumatic brain injury in Denmark: a population-based observational cohort study. *The Lancet Psychiatry* 5, 424–431 (2018).
68. Fleminger, S., Oliver, D. L., Lovestone, S., Rabe-Hesketh, S. & Giora, A. Head injury as a risk factor for Alzheimer's disease: The evidence 10 years on; a partial replication. *J. Neurol. Neurosurg. Psychiatry* 74, 857–862 (2003).
69. Yokobori, S. et al. Biomarkers for the clinical differential diagnosis in traumatic brain injury - A systematic review. *CNS Neuroscience and Therapeutics* vol. 19 556–565. <https://doi.org/10.1111/cns.12127> (2013).
70. Gu, D., Ou, S. & Liu, G. Traumatic Brain Injury and Risk of Dementia and Alzheimer's Disease: A Systematic Review and Meta-Analysis. *Neuroepidemiology* vol. 56 4–16 (2022).
71. Zetterberg, H., Smith, D. H. & Blennow, K. Biomarkers of mild traumatic brain injury in cerebrospinal fluid and blood. *Nat. Rev. Neurol.* 9, 201–210 (2013).
72. Maas, A. I. R. et al. Traumatic brain injury: integrated approaches to improve prevention, clinical care, and research. *Lancet Neurol.* 16, 987–1048 (2017).

73. Berwick, D., Bowman, K. & Matney, C. Traumatic Brain Injury: A Roadmap for Accelerating Progress (2022). Traumatic Brain Injury (National Academies Press, 2022). <https://doi.org/10.17226/25394>.
74. Brazinova, A. et al. Epidemiology of Traumatic Brain Injury in Europe: A Living Systematic Review. *J. Neurotrauma* (2018).
75. Guedes, V. A. et al. Exosomal neurofilament light: A prognostic biomarker for remote symptoms after mild traumatic brain injury? *Neurology* 94, e2412–e2423 (2020).
76. Raj, R., Kaprio, J., Jousilahti, P., Korja, M. & Siironen, J. Risk of Dementia After Hospitalization Due to Traumatic Brain Injury: A Longitudinal Population-Based Study. *Neurology* 98, E2377–E2386 (2022).
77. O'Brien, W. T. et al. The NLRP3 inflammasome in traumatic brain injury: Potential as a biomarker and therapeutic target. *Journal of Neuroinflammation* vol. 17 1–12 (2020).
78. Luo, Z., Chang, Y. C., Lam, C. S., Lau, K. C. & Ng, C. Y. Two-Color Pulsed-Field Ionization-Photoelectron Spectroscopy: A Quest to Benchmark State-of-the-Art ab initio Quantum Electronic Structure Calculations of Spectroscopic and Energetic Properties for Transition Metal-Containing Species. *Frontiers and Advances in Molecular Spectroscopy* (Elsevier Inc., 2018).
79. Tong, Q., Malachosky, E. W., Raybin, J., Guyot-Sionnest, P. & Sibener, S. J. End-to-end alignment of gold nanorods on topographically enhanced, cylinder forming diblock copolymer templates and their surface enhanced Raman scattering properties. *J. Phys. Chem. C* 118, 19259–19265 (2014).
80. Maas, A. I. R. et al. Traumatic brain injury: progress and challenges in prevention, clinical care, and research. *Lancet Neurol.* 21, 1004–1060 (2022).
81. Chandra, A. et al. Applications of amyloid, tau, and neuroinflammation PET imaging to Alzheimer's disease and mild cognitive impairment. *Human Brain Mapping* vol. 40 5424–5442 (2019).
82. Andersen, E. et al. Diagnostic biomarkers in Alzheimer's disease. *Biomarkers in Neuropsychiatry* 5, 100041 (2021).
83. Rosenfeld, J. V. et al. Early management of severe traumatic brain injury. *Lancet* 380, 1088–1098 (2012).
84. Scott, G. et al. Minocycline reduces chronic microglial activation after brain trauma but increases neurodegeneration. *Brain* 141, 459–471 (2018).
85. Zapata, F., López-Fernández, A., Ortega-Ojeda, F., Montalvo, G. & García-Ruiz, C. A practical beginner's guide to Raman microscopy. *Applied Spectroscopy Reviews* vol. 56 439–462 (2021).
86. Petry, R., Schmitt, M. & Popp, J. Raman spectroscopy - A prospective tool in the life sciences. *ChemPhysChem* vol. 4 14–30 (2003).
87. Tang, J. et al. Silver-coated Si nanograss as highly sensitive surface-enhanced Raman spectroscopy substrates. *Appl. Phys. A Mater. Sci. Process.* 96, 793–797 (2009).
88. Abu Hatab, N. A., Oran, J. M. & Sepaniak, M. J. Surface-enhanced Raman spectroscopy substrates created via electron beam lithography and nanotransfer printing. *ACS Nano* 2, 377–385 (2008).
89. Moskovits, M. Surface-enhanced Raman spectroscopy: A brief retrospective. *J. Raman Spectrosc.* 36, 485–496 (2005).

90. Butler, H. J. et al. Using Raman spectroscopy to characterize biological materials. *Nat. Protoc.* 11, 664–687 (2016).
91. Qian, X. et al. In vivo tumor targeting and spectroscopic detection with surface-enhanced Raman nanoparticle tags. *Nat. Biotechnol.* 26, 83–90 (2008).
92. Leng, C. et al. Design and Fabrication of Plasmonic Nanostructures with DNA for Surface-Enhanced Raman Spectroscopy Applications. *Chinese Journal of Chemistry* vol. 34 273–282 (2016).
93. Lyandres, O. et al. Real-time glucose sensing by surface-enhanced Raman spectroscopy in bovine plasma facilitated by a mixed decanethiol/mercaptohexanol partition layer. *Anal. Chem.* 77, 6134–6139 (2005).
94. Vander Ende, E. et al. Physicochemical trapping of neurotransmitters in polymer-mediated gold nanoparticle aggregates for surface-enhanced Raman spectroscopy. *Anal. Chem.* 91, 9554–9562 (2019).
95. Brasiliense, V., Park, J. E., Berns, E. J., Van Duyne, R. P. & Mrksich, M. Surface potential modulation as a tool for mitigating challenges in SERS-based microneedle sensors. *Sci. Rep.* 12, 1–9 (2022).
96. Wang, P. et al. Label-Free SERS Selective Detection of Dopamine and Serotonin Using Graphene-Au Nanopyramid Heterostructure. *Anal. Chem.* 87, 10255–10261 (2015).
97. Hanif, S. et al. Nanopipette-Based SERS Aptasensor for Subcellular Localization of Cancer Biomarker in Single Cells. *Anal. Chem.* 89, 9911–9917 (2017).
98. Wang, L. et al. Electrochemistry-Regulated Recyclable SERS Sensor for Sensitive and Selective Detection of Tyrosinase Activity. *Anal. Chem.* 91, 6507–6513 (2019).
99. Lim, D. K., Jeon, K. S., Kim, H. M., Nam, J. M. & Suh, Y. D. Nanogap-engineerable raman-active nanodumbbells for single-molecule detection. *Nat. Mater.* 9, 60–67 (2010).
100. Candeloro, P. et al. Plasmonic nanoholes as SERS devices for biosensing applications: An easy route for nanostructures fabrication on glass substrates. *Microelectron. Eng.* 175, 30–33 (2017).
101. Li, C. et al. Towards practical and sustainable SERS: a review of recent developments in the construction of multifunctional enhancing substrates. *J. Mater. Chem. C* 9, 11517–11552 (2021).
102. Kneipp, K., Kneipp, H. & Kneipp, J. Surface-enhanced Raman scattering in local optical fields of silver and gold nanoaggregates - From single-molecule Raman spectroscopy to ultrasensitive probing in live cells. *Acc. Chem. Res.* 39, 443–450 (2006).
103. Santoro, G. et al. Silver substrates for surface enhanced Raman scattering: Correlation between nanostructure and Raman scattering enhancement. *Appl. Phys. Lett.* 104, 243107 (2014).
104. Kneipp, K. et al. Single Molecule Detection Using Surface-Enhanced Raman Scattering (SERS). *Phys. Rev. Lett.* 78, 1667 (1997).
105. Nie, S. & Emory, S. R. Probing single molecules and single nanoparticles by surface-enhanced Raman scattering. *Science* (80). 275, 1102–1106 (1997).
106. Gambucci, M., Cambiotti, E., Sassi, P. & Latterini, L. Multilayer gold-silver bimetallic nanostructures to enhance SERS detection of drugs. *Molecules* 25, (2020).

107. Li, M., Cushing, S. K. & Wu, N. Plasmon-enhanced optical sensors: A review. *Analyst* vol. 140 386–406 (2015).
108. Le Ru, E. C., Blackie, E., Meyer, M. & Etchegoint, P. G. Surface enhanced Raman scattering enhancement factors: A comprehensive study. *J. Phys. Chem. C* 111, 13794–13803 (2007).
109. Goldberg-Oppeneheimer, P., Mahajan, S. & Steiner, U. Hierarchical electrohydrodynamic structures for surface-enhanced Raman scattering. *Adv. Mater.* 24, OP175–OP180 (2012).
110. Linic, S., Christopher, P. & Ingram, D. B. Plasmonic-metal nanostructures for efficient conversion of solar to chemical energy. *Nat. Mater.* 10, 911–921 (2011).
111. Lee, J. B., Choi, S., Kim, J. & Nam, Y. S. Plasmonically-assisted nanoarchitectures for solar water splitting: Obstacles and breakthroughs. *Nano Today* vol. 16 61–81 (2017).
112. Panikar, S. S., Cialla-May, D., De la Rosa, E., Salas, P. & Popp, J. Towards translation of surface-enhanced Raman spectroscopy (SERS) to clinical practice: Progress and trends. *TrAC - Trends Anal. Chem.* 134, 116122 (2021).
113. National Institute for Health and Clinical Excellence [NICE]. 4 Economic evaluation | NICE health technology evaluations: the manual | Guidance | NICE. www.Nice.Org.Uk (2022). Accessed 30/11/2024.
114. Wang, C. et al. Point-of-care diagnostics for infectious diseases: From methods to devices. *Nano Today* vol. 37 101092 (2021).
115. Yeh, Y.-J., Liu, C.-Y., Chu, J. P., Chiang, W.-H. & Tung, K.-L. Plasmonic Au loaded semiconductor-engineered large-scale metallic nanostructure arrays for SERS application. *Surf. Coatings Technol.* 128285 (2022)
116. Han, X. X., Zhao, B. & Ozaki, Y. Surface-enhanced Raman scattering for protein detection. *Analytical and Bioanalytical Chemistry* vol. 394 1719–1727 (2009).
117. Han, X. X., Ozaki, Y. & Zhao, B. Label-free detection in biological applications of surface-enhanced Raman scattering. *TrAC - Trends in Analytical Chemistry* vol. 38 67–78 (2012).
118. Wu, J. et al. Reusable and long-life 3D Ag nanoparticles coated Si nanowire array as sensitive SERS substrate. *Appl. Surf. Sci.* 494, 583–590 (2019).
119. Wang, K., Sun, D. W., Pu, H., Wei, Q. & Huang, L. Stable, Flexible, and High-Performance SERS Chip Enabled by a Ternary Film-Packaged Plasmonic Nanoparticle Array. *ACS Appl. Mater. Interfaces* 11, 29177–29186 (2019).
120. Guo, Q., Xu, M., Yuan, Y., Gu, R. & Yao, J. Self-assembled large-scale monolayer of Au nanoparticles at the air/water interface used as a SERS substrate. *Langmuir* 32, 4530–4537 (2016).
121. Zhang, C. et al. Continuous fabrication of nanostructure arrays for flexible surface enhanced Raman scattering substrate. *Sci. Rep.* 7, 1–9 (2017).
122. Park, S., Lee, J. & Ko, H. Transparent and Flexible Surface-Enhanced Raman Scattering (SERS) Sensors Based on Gold Nanostar Arrays Embedded in Silicon Rubber Film. *ACS Appl. Mater. Interfaces* 9, 44088–44095 (2017).

123. Singh, U. B. et al. Engineering of hydrophilic and plasmonic properties of Ag thin film by atom beam irradiation. *Appl. Surf. Sci.* 258, 1464–1469 (2011).
124. Kreibig, U. & Vollmer, M. Optical Properties of Metal Clusters. 25, (1995).
125. Sooraj, B. N. S. & Pradeep, T. Optical properties of metal clusters. in *Atomically Precise Metal Nanoclusters* 83–101 (Elsevier, 2022).
126. Gan, Z. et al. High-fidelity and clean nanotransfer lithography using structure-embedded and electrostatic-adhesive carriers. *Microsystems Nanoeng.* 9, 1–9 (2023).
127. Thomas-Seale, L. E. J. et al. Topological Analysis to Enhance the Understanding of Transdisciplinary Engineering. in *Advances in Transdisciplinary Engineering* vol. 60 299–308 (IOS Press, 2024).
128. Kaniewska, P., Anthony, K. R. N. & Hoegh-Guldberg, O. Variation in colony geometry modulates internal light levels in branching corals, *Acropora humilis* and *Stylophora pistillata*. *Mar. Biol.* 155, 649–660 (2008).
129. Katifori, E., Szöllösi, G. J. & Magnasco, M. O. Damage and fluctuations induce loops in optimal transport networks. *Phys. Rev. Lett.* 104, 048704 (2010).
130. Madin, J. S., Baird, A. H., Dornelas, M. & Connolly, S. R. Mechanical vulnerability explains size-dependent mortality of reef corals. *Ecol. Lett.* 17, 1008–1015 (2014).
131. Cranford, S. W., Tarakanova, A., Pugno, N. M. & Buehler, M. J. Nonlinear material behaviour of spider silk yields robust webs. *Nature* vol. 482 72–76 (2012).
132. Lentink, D. & Dickinson, M. H. Biofluiddynamic scaling of flapping, spinning and translating fins and wings. *J. Exp. Biol.* 212, 2691–2704 (2009).
133. Jahani, Y. et al. Imaging-based spectrometer-less optofluidic biosensors based on dielectric metasurfaces for detecting extracellular vesicles. *Nat. Commun.* 12, 1–10 (2021).
134. Townshend, B., Xiang, J. S., Manzanarez, G., Hayden, E. J. & Smolke, C. D. A multiplexed, automated evolution pipeline enables scalable discovery and characterization of biosensors. *Nat. Commun.* 12, 1–15 (2021).
135. Sekhon, H. et al. Adaptable, turn-on maturation (ATOM) fluorescent biosensors for multiplexed detection in cells. *Nat. Methods* 20, 1920–1929 (2023).
136. Mohammadpour-Haratbar, A., Zare, Y. & Rhee, K. Y. Electrochemical biosensors based on polymer nanocomposites for detecting breast cancer: Recent progress and future prospects. *Adv. Colloid Interface Sci.* 309, 102795 (2022).
137. Lang, X. Y. et al. Nanoporous gold supported cobalt oxide microelectrodes as high-performance electrochemical biosensors. *Nat. Commun.* 4, 1–8 (2013).
138. Liu, Y. et al. Reprogrammed tracrRNAs enable repurposing of RNAs as crRNAs and sequence-specific RNA biosensors. *Nat. Commun.* 13, 1–12 (2022).
139. Kim, J. et al. Intensiometric biosensors visualize the activity of multiple small GTPases in vivo. *Nat. Commun.* 10, 1–11 (2019).
140. Shobana, M. K. Nanoferrites in biosensors – A review. *Mater. Sci. Eng. B* 272, 115344 (2021).

141. Fletcher, L. Bringing biosensors to life. *Nat. Biotechnol.* 19, 100–100 (2001).
142. Koedrith, P. et al. Recent trends in rapid environmental monitoring of pathogens and toxicants: Potential of nanoparticle-based biosensor and applications. *Scientific World Journal* vol. 2015 (2015).
143. Grieshaber, D., MacKenzie, R., Vörös, J. & Reimhult, E. Electrochemical Biosensors - Sensor Principles and Architectures. *Sensors* 8, 1400–1458 (2008).
144. Hu, Y., Fine, D. H., Tasciotti, E., Bouamrani, A. & Ferrari, M. Nanodevices in diagnostics. *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology* vol. 3 11–32 (2011).
145. Hegner, M. & Arntz, Y. Advanced biosensing using micromechanical cantilever arrays. *Methods Mol. Biol.* 242, 39–49 (2004).
146. Nair, P. R. & Alam, M. A. Performance limits of nanobiosensors. *Appl. Phys. Lett.* 88, 233120 (2006).
147. Salvati, E., Stellacci, F. & Krol, S. Nanosensors for early cancer detection and for therapeutic drug monitoring. *Nanomedicine* vol. 10 3495–3512 (2015).
148. Medical devices given exceptional use authorisations during the COVID-19 pandemic - GOV.UK. <https://www.gov.uk/government/publications/medical-devices-given-exceptional-use-authorisations-during-the-covid-19-pandemic>. Accessed 30/11/2024.
149. Novack, G. D. Expedited regulatory product approval in the time of COVID-19. *Ocul. Surf.* 26, 345 (2022).
150. Li, D. et al. Genetically engineered T cells for cancer immunotherapy. *Signal Transduct. Target. Ther.* 2019 41 4, 1–17 (2019).
151. June, C. H. & Sadelain, M. Chimeric Antigen Receptor Therapy. *N. Engl. J. Med.* 379, 64–73 (2018).
152. Nuijten, M. Pricing Zolgensma – the world’s most expensive drug. *J. Mark. Access Heal. Policy* 10, 2022353 (2021).
153. Dill, F. H. Positive optical lithography. in *Digest of Technical Papers - IEEE International Solid-State Circuits Conference* vols 1975-Febru 54–55 (Institute of Electrical and Electronics Engineers Inc., 1975).
154. McDonald, J. C. & Whitesides, G. M. Poly(dimethylsiloxane) as a material for fabricating microfluidic devices. *Acc. Chem. Res.* 35, 491–499 (2002).
155. Soper, S. A. et al. Peer Reviewed: Polymeric Microelectromechanical Systems. *Anal. Chem.* 72, 642 A–651 A (2000).
156. Duffy, D. C., McDonald, J. C., Schueller, O. J. A. & Whitesides, G. M. Rapid prototyping of microfluidic systems in poly(dimethylsiloxane). *Anal. Chem.* 70, 4974–4984 (1998).
157. Li, Y. et al. Influence of reactive oxygen species concentration and ambient temperature on the evolution of chemical bonds during plasma cleaning: a molecular dynamics simulation. *RSC Adv.* 12, 30754–30763 (2022).
158. Norrman, K. & Al-Yaseri, A. The effects of air plasma and argon cluster ion cleaning on quartz and calcite surfaces – Implications for rock-water-gas wettability. *Int. J. Hydrogen Energy* 62, 617–627 (2024).
159. Sherman, R., Hirt, D. & Vane, R. Surface cleaning with the carbon dioxide snow jet. *J. Vac. Sci. Technol. A Vacuum, Surfaces, Film.* 12, 1876–1881 (1994).

160. Wallraff, G. M. & Hinsberg, W. D. Lithographic Imaging Techniques for the Formation of Nanoscopic Features. *Chem. Rev.* 99, 1801–1821 (1999).
161. Luo, C. et al. Review of recent advances in inorganic photoresists. *RSC Adv.* 10, 8385–8395 (2020).
162. MicroChemicals GmbH. Application Notes for photolithography with technical information for photoresists, solvents, developers and etchants. https://www.microchemicals.com/downloads/application_notes.html. Accessed: 28/05/2022.
163. Allresist GmbH. Negative Photoresist AR-N 4340 Material Data Sheet. (2014).
164. Quero, J. M., Perdigones, F. & Aracil, C. Microfabrication technologies used for creating smart devices for industrial applications. in *Smart Sensors and MEMS: Intelligent Sensing Devices and Microsystems for Industrial Applications: Second Edition* 291–311 (Woodhead Publishing, 2018).
165. MicroChemicals GmbH. Application notes - The Best-suited Photoresist. <https://www.microchemicals.com/DOWNLOADS/Application-Notes/>. Accessed: 28/05/2022
166. PicoMaster PM100 User Guide. <https://raith.com//products/picomaster/>. Accessed 27/08/2024.
167. Stokes, K., Clark, K., Odetade, D., Hardy, M. & Goldberg Oppenheimer, P. Advances in lithographic techniques for precision nanostructure fabrication in biomedical applications. *Discov. nano* 18, 153 (2023).
168. Sharma, E. et al. Evolution in Lithography Techniques: Microlithography to Nanolithography. *Nanomaterials* vol. 12 (2022).
169. Davidson, M. W. & Abramowitz, M. OPTICAL MICROSCOPY. <http://microscopy.fsu.edu>. Accessed 30/05/2022.
170. Warley, A. Microscopy | Biomedical applications. in *Encyclopedia of Analytical Science* 10–18 (Academic Press, 2019).
171. Grubb, D. T. Optical Microscopy. in *Polymer Science: A Comprehensive Reference*, 10 Volume Set vol. 2 465–478 (Elsevier, 2012).
172. What is AFM? Learn about Atomic Force Microscopy! - NanoAndMore. https://www.nanoandmore.com/what-is-atomic-force-microscopy?gclid=Cj0KCQjw1tGUBhDXARIsAIJx01IC8cBLY27VSzlgjNhAi5ojHqXjpFTPuvOeCPXbSGIBgNgwG-w-3oaAvCHEALw_wcB. Accessed: 30/05/2022.
173. Binnig, G., Quate, C. F. & Gerber, C. Atomic force microscope. *Phys. Rev. Lett.* 56, 930–933 (1986).
174. Marti, O., Drake, B. & Hansma, P. K. Atomic force microscopy of liquid-covered surfaces: Atomic resolution images. *Appl. Phys. Lett.* 51, 484–486 (1987).
175. Neděla, V., Tihlaříková, E., Cápal, P. & Doležel, J. Advanced environmental scanning electron microscopy reveals natural surface nano-morphology of condensed mitotic chromosomes in their native state. *Sci. Rep.* 14, 1–11 (2024).
176. Suga, M. et al. Recent progress in scanning electron microscopy for the characterization of fine structural details of nano materials. *Progress in Solid State Chemistry* vol. 42 1–21 (2014).
177. Wightman, R. An Overview of Cryo-Scanning Electron Microscopy Techniques for Plant Imaging. *Plants* vol. 11 (2022).

178. Thomas, G. Some applications of electron microscopy in materials science. *Ultramicroscopy* 20, 239–260 (1986).
179. Zhou, W. & Wang, Z. L. *Scanning microscopy for nanotechnology: Techniques and applications*. Scanning Microscopy for Nanotechnology: Techniques and Applications (Springer New York, 2007).
180. Schlücker, S. Surface-enhanced Raman spectroscopy: Concepts and chemical applications. *Angew. Chemie - Int. Ed.* 53, 4756–4795 (2014).
181. Han, X. X., Rodriguez, R. S., Haynes, C. L., Ozaki, Y. & Zhao, B. Surface-enhanced Raman spectroscopy. *Nat. Rev. Methods Prim.* 1, 1–17 (2021).
182. Jeanmaire, D. L. & Duynes, R. P. VAN. Surface Raman Spectroelectrochemistry Part1. Heterocyclic. *J. Electroanal Chem.* 84, 1 (1977).
183. Haynes, C. L., McFarland, A. D. & Van Duynes, R. P. Surface-enhanced: Raman spectroscopy. *Anal. Chem.* 77, (2005).
184. COMSOL Multiphysics. COMSOL - Software for Multiphysics Simulation. COMSOL Multiphysics/Heat Transfer Module 217–218 <https://www.comsol.com/> (2021). Accessed: 16/11/2023.
185. Python. General Python FAQ — Python 2.7.11 documentation. [Www.Python.Org](http://www.python.org) <https://docs.python.org/3/faq/general.html> (2006). Accessed: 20/08/2024.
186. Le Ru, E. & Etchegoin, P. *Principles of Surface Enhanced Raman Spectroscopy and related plasmonic effects*. Elsevier vol. 1 (2009).
187. Xia, Y., Rogers, J. A., Paul, K. E. & Whitesides, G. M. Unconventional Methods for Fabricating and Patterning Nanostructures. *Chem. Rev.* 99, 1823–1848 (1999).
188. Mack, C. A. Fifty years of Moore's law. in *IEEE Transactions on Semiconductor Manufacturing* vol. 24 202–207 (2011).
189. Oldham, W. G., Subramanian, S. & Neureuther, A. R. Optical requirements for projection lithography. *Solid State Electron.* 24, 975–980 (1981).
190. George, D. et al. Fabrication of patterned graphitized carbon wires using low voltage near-field electrospinning, pyrolysis, electrodeposition, and chemical vapor deposition. *Microsystems Nanoeng.* 6, 1–12 (2020).
191. Cottat, M. et al. Highly sensitive detection of paclitaxel by surface-enhanced Raman scattering. *J. Opt. (United Kingdom)* 17, 114019 (2015).
192. Ren, L. et al. Fabrication of flexible microneedle array electrodes for wearable bio-signal recording. *Sensors (Switzerland)* 18, (2018).
193. Gates, B. D., Xu, Q., Love, J. C., Wolfe, D. B. & Whitesides, G. M. Unconventional nanofabrication. *Annu. Rev. Mater. Res.* 34, 339–372 (2004).
194. Stephen, Y. C., Peter, R. K. & Preston, J. R. Imprint Lithography with 25-Nanometer Resolution. *Sci. (American Assoc. Adv. Sci.)* 272, 85–87 (1996).
195. Guo, L. J. Nanoimprint lithography: Methods and material requirements. *Adv. Mater.* 19, 495–513 (2007).

196. Guo, S. et al. The polarization modulation and fabrication method of two dimensional silica photonic crystals based on UV nanoimprint lithography and hot imprint. *Sci. Rep.* 6, 1–7 (2016).
197. Albisetti, E. et al. Nanopatterning reconfigurable magnetic landscapes via thermally assisted scanning probe lithography. *Nat. Nanotechnol.* 11, 545–551 (2016).
198. Htwe, Y. Z. N. & Mariatti, M. Printed graphene and hybrid conductive inks for flexible, stretchable, and wearable electronics: Progress, opportunities, and challenges. *Journal of Science: Advanced Materials and Devices* vol. 7 100435 (2022).
199. Rickard, J. J. S. et al. Rapid optofluidic detection of biomarkers for traumatic brain injury via surface-enhanced Raman spectroscopy. *Nat. Biomed. Eng.* 4, 610–623 (2020).
200. Bae, W. G. et al. 25th anniversary article: Scalable multiscale patterned structures inspired by nature: The role of hierarchy. *Adv. Mater.* 26, 675–700 (2014).
201. Kim, J. U., Lee, S. & Kim, T. II. Recent Advances in Unconventional Lithography for Challenging 3D Hierarchical Structures and Their Applications. *J. Nanomater.* 2016, (2016).
202. Lin, X. M., Cui, Y., Xu, Y. H., Ren, B. & Tian, Z. Q. Surface-enhanced Raman spectroscopy: Substrate-related issues. *Analytical and Bioanalytical Chemistry* vol. 394 1729–1745 (2009).
203. Jeong, J. W. et al. High-resolution nanotransfer printing applicable to diverse surfaces via interface-targeted adhesion switching. *Nat. Commun.* 5, (2014).
204. Lin, B. J. Optical lithography — present and future challenges. *Comptes Rendus Phys.* 7, 858–874 (2006).
205. Huang, D. et al. Recent advances on fabrication of microneedles on the flexible substrate. *Journal of Micromechanics and Microengineering* vol. 31 073001 (2021).
206. Sharma, H., Nguyen, D., Chen, A., Lew, V. & Khine, M. Unconventional low-cost fabrication and patterning techniques for point of care diagnostics. *Ann. Biomed. Eng.* 39, 1313–1327 (2011).
207. Mukhopadhyay, R. Raman flexes its muscles. *Anal. Chem.* 79, 3265–3270 (2007).
208. Fleischmann, M., Hendra, P. J. & McQuillan, A. J. Raman spectra of pyridine adsorbed at a silver electrode. *Chem. Phys. Lett.* 26, 163–166 (1974).
209. Chisanga, M., Muhamadali, H., Ellis, D. I. & Goodacre, R. Enhancing disease diagnosis: Biomedical applications of surface-enhanced Raman scattering. *Applied Sciences (Switzerland)* vol. 9 1163 (2019).
210. Dhawan, A. et al. Methodologies for developing surface-enhanced Raman scattering (SERS) substrates for detection of chemical and biological molecules. *IEEE Sens. J.* 10, 608–616 (2010).
211. Albrecht, M. G. & Creighton, J. A. Anomalous Intense Raman Spectra of Pyridine at a Silver Electrode. *J. Am. Chem. Soc.* 99, 5215–5217 (1977).
212. Gersten, J. I. The effect of surface roughness on surface enhanced Raman scattering. *J. Chem. Phys.* 72, 5779–5780 (1980).
213. Guler, U. et al. Local heating with lithographically fabricated plasmonic titanium nitride nanoparticles. *Nano Lett.* 13, 6078–6083 (2013).

214. Lu, H. et al. Seed-mediated Plasmon-driven Regrowth of Silver Nanodecahedrons (NDs). *Plasmonics* 7, 167–173 (2012).
215. Tsuneda, T., Iwasa, T. & Taketsugu, T. Roles of silver nanoclusters in surface-enhanced Raman spectroscopy. *J. Chem. Phys.* 151, 094102 (2019).
216. Ding, S. Y., You, E. M., Tian, Z. Q. & Moskovits, M. Electromagnetic theories of surface-enhanced Raman spectroscopy. *Chemical Society Reviews* vol. 46 4042–4076 (2017).
217. Wang, Y., Yan, B. & Chen, L. SERS Tags: Novel optical nanoprobe for bioanalysis. *Chemical Reviews* vol. 113 1391–1428 (2013).
218. Mekonnen, M. L., Workie, Y. A., Su, W. N. & Hwang, B. J. Plasmonic paper substrates for point-of-need applications: Recent developments and fabrication methods. *Sensors and Actuators, B: Chemical* vol. 345 130401 (2021).
219. Lee, T. et al. Label-free localized surface plasmon resonance biosensor composed of multi-functional DNA 3 way junction on hollow Au spike-like nanoparticles (HAuSN) for avian influenza virus detection. *Colloids Surfaces B Biointerfaces* 182, (2019).
220. Petti, L. et al. A plasmonic nanostructure fabricated by electron beam lithography as a sensitive and highly homogeneous SERS substrate for bio-sensing applications. *Vib. Spectrosc.* 82, 22–30 (2016).
221. Jiang, N., Zhuo, X. & Wang, J. Active Plasmonics: Principles, Structures, and Applications. *Chemical Reviews* vol. 118 3054–3099 (2018).
222. Hardy, M. et al. Methods in Raman spectroscopy for saliva studies—a review. *Applied Spectroscopy Reviews* vol. 57 177–233 (2022).
223. Mahajan, S., Hutter, T., Steiner, U. & Goldberg Oppenheimer, P. Tunable microstructured surface-enhanced Raman scattering substrates via electrohydrodynamic lithography. *J. Phys. Chem. Lett.* 4, 4153–4159 (2013).
224. Love, J. C., Estroff, L. A., Kriebel, J. K., Nuzzo, R. G. & Whitesides, G. M. Self-assembled monolayers of thiolates on metals as a form of nanotechnology. *Chemical Reviews* vol. 105 1103–1169 (2005).
225. Messina, G. C. et al. Hollow plasmonic antennas for broadband SERS spectroscopy. *Beilstein J. Nanotechnol.* 6, 492–498 (2015).
226. Sun, Y. & Xia, Y. Shape-controlled synthesis of gold and silver nanoparticles. *Science* (80). 298, 2176–2179 (2002).
227. Shenashen, M. A., El-Safty, S. A. & Elshehy, E. A. Synthesis, morphological control, and properties of silver nanoparticles in potential applications. *Particle and Particle Systems Characterization* vol. 31 293–316 (2014).
228. Yue, W. et al. Surface-enhanced Raman scattering with gold-coated silicon nanopillars arrays: The influence of size and spatial order. *Spectrochim. Acta - Part A Mol. Biomol. Spectrosc.* 267, 120582 (2022).
229. Anker, J. N. et al. Biosensing with plasmonic nanosensors. *Nature Materials* vol. 7 442–453 (2008).
230. Park, J. M., Choi, H. E., Kudaibergen, D., Kim, J. H. & Kim, K. S. Recent Advances in Hollow Gold Nanostructures for Biomedical Applications. *Frontiers in Chemistry* vol. 9 419 (2021).

231. Genç, A. et al. Hollow metal nanostructures for enhanced plasmonics: Synthesis, local plasmonic properties and applications. *Nanophotonics* vol. 6 193–213 (2017).
232. Khoury, C. G. & Vo-Dinh, T. Plasmonic nanowave substrates for SERS: Fabrication and numerical analysis. *J. Phys. Chem. C* 116, 7534–7545 (2012).
233. Stiles, P. L., Dieringer, J. A., Shah, N. C. & Van Duyne, R. P. Surface-enhanced Raman spectroscopy. *Annual Review of Analytical Chemistry* vol. 1 601–626 (2008).
234. Howes, P. D., Chandrawati, R. & Stevens, M. M. Colloidal nanoparticles as advanced biological sensors. *Science* vol. 346 (2014).
235. Oh, E. et al. PEGylated luminescent gold nanoclusters: Synthesis, characterization, bioconjugation, and application to one- and two-photon cellular imaging. *Part. Part. Syst. Charact.* 30, 453–466 (2013).
236. Zhang, M., Liu, Y. Q., Yu, C. Y., Yin, B. C. & Ye, B. C. Multiplexed detection of microRNAs by tuning DNA-scaffolded silver nanoclusters. *Analyst* 138, 4812–4817 (2013).
237. Austin, L. A., MacKey, M. A., Dreaden, E. C. & El-Sayed, M. A. The optical, photothermal, and facile surface chemical properties of gold and silver nanoparticles in biodiagnostics, therapy, and drug delivery. *Archives of Toxicology* vol. 88 1391–1417 (2014).
238. Sönnichsen, C. et al. Drastic reduction of plasmon damping in gold nanorods. *Phys. Rev. Lett.* 88, 774021–774024 (2002).
239. Zhao, Y. et al. Fabrication of a silicon oxide stamp by edge lithography reinforced with silicon nitride for nanoimprint lithography. *J. Micromechanics Microengineering* 18, (2008).
240. Li, J. et al. Printable two-dimensional superconducting monolayers. *Nat. Mater.* 20, 181–187 (2021).
241. Srichan, C. et al. Highly-Sensitive Surface-Enhanced Raman Spectroscopy (SERS)-based chemical sensor using 3D graphene foam decorated with silver nanoparticles as SERS substrate. *Sci. Rep.* 6, 1–9 (2016).
242. Wu, L. A., Li, W. E., Lin, D. Z. & Chen, Y. F. Three-Dimensional SERS Substrates Formed with Plasmonic Core-Satellite Nanostructures. *Sci. Rep.* 7, 1–11 (2017).
243. Liu, D. et al. Rapid Synthesis of Monodisperse Au Nanospheres through a Laser Irradiation-Induced Shape Conversion, Self-Assembly and Their Electromagnetic Coupling SERS Enhancement. *Sci. Rep.* 5, 1–9 (2015).
244. Navas-Moreno, M. et al. Nanoparticles for live cell microscopy: A surface-enhanced Raman scattering perspective. *Sci. Rep.* 7, 1–10 (2017).
245. Wang, Y., Zhao, P., Mao, L., Hou, Y. & Li, D. Determination of brain injury biomarkers by surface-enhanced Raman scattering using hollow gold nanospheres. *RSC Adv.* 8, 3143–3150 (2018).
246. Kwak, R., Jeong, H. E. & Suh, K. Y. Fabrication of monolithic bridge structures by vacuum-assisted capillary-force lithography. *Small* 5, 790–794 (2009).
247. Acikgoz, C., Hempenius, M. A., Huskens, J. & Vancso, G. J. Polymers in conventional and alternative lithography for the fabrication of nanostructures. *Eur. Polym. J.* 47, 2033–2052 (2011).
248. Cheung, C. L., Nikolić, R. J., Reinhardt, C. E. & Wang, T. F. Fabrication of nanopillars by nanosphere lithography. *Nanotechnology* 17, 1339–1343 (2006).

249. Desbiolles, B. X. E., Bertsch, A. & Renaud, P. Ion beam etching redeposition for 3D multimaterial nanostructure manufacturing. *Microsystems Nanoeng.* 5, (2019).
250. Chow, T. H. et al. Gold Nanobipyramids: An Emerging and Versatile Type of Plasmonic Nanoparticles. *Acc. Chem. Res.* 52, 2136–2146 (2019).
251. Sun, Y. Silver nanowires - Unique templates for functional nanostructures. *Nanoscale* 2, 1626–1642 (2010).
252. Kahl, M., Voges, E., Kostrewa, S., Viets, C. & Hill, W. Periodically structured metallic substrates for SERS. *Sensors Actuators, B Chem.* 51, 285–291 (1998).
253. Chirumamilla, M. et al. 3D nanostar dimers with a sub-10-nm gap for single-/few-molecule surface-enhanced Raman scattering. *Adv. Mater.* 26, 2353–2358 (2014).
254. Lafuente, M. et al. Exploring the surface-enhanced Raman scattering (SERS) activity of gold nanostructures embedded around nanogaps at wafer scale: Simulations and experiments. *Appl. Mater. Today* 35, 101929 (2023).
255. Gopalakrishnan, A. et al. Bimetallic 3D nanostar dimers in ring cavities: Recyclable and robust surface-enhanced Raman scattering substrates for signal detection from few molecules. *ACS Nano* 8, 7986–7994 (2014).
256. Xue, Y. et al. Shape controlled gold nanostructures on de-alloyed nanoporous gold with excellent SERS performance. *Chem. Phys. Lett.* 709, 46–51 (2018).
257. Song, X., Li, H., Al-Qadiri, H. M. & Lin, M. Detection of herbicides in drinking water by surface-enhanced Raman spectroscopy coupled with gold nanostructures. *J. Food Meas. Charact.* 7, 107–113 (2013).
258. Calis, B. & Yilmaz, M. Fabrication of gold nanostructure decorated polystyrene hybrid nanosystems via poly(L-DOPA) and their applications in surface-enhanced Raman Spectroscopy (SERS), and catalytic activity. *Colloids Surfaces A Physicochem. Eng. Asp.* 622, 126654 (2021).
259. Chao, B. K. et al. Gold-rich ligament nanostructure by dealloying Au-based metallic glass ribbon for surface-enhanced Raman scattering. *Sci. Rep.* 7, 1–7 (2017).
260. Zong, X., Zhu, R. & Guo, X. Nanostructured gold microelectrodes for SERS and EIS measurements by incorporating ZnO nanorod growth with electroplating. *Sci. Rep.* 5, 1–10 (2015).
261. Fortuni, B. et al. In situ synthesis of Au-shelled Ag nanoparticles on PDMS for flexible, long-life, and broad spectrum-sensitive SERS substrates. *Chem. Commun.* 53, 11298–11301 (2017).
262. Schneider, N. et al. Sphingomyelin in brain and cognitive development: Preliminary data. *eNeuro* 6, (2019).
263. Nishiyama, H., Knöpfel, T., Endo, S. & Itohara, S. Glial protein S100B modulates long-term neuronal synaptic plasticity. *Proc. Natl. Acad. Sci. U. S. A.* 99, 4037–4042 (2002).
264. Pascual, J. M. et al. Time course of early metabolic changes following diffuse traumatic brain injury in rats as detected by ¹H NMR spectroscopy. *J. Neurotrauma* 24, 944–959 (2007).
265. Xu, H. et al. Brain-specific loss of Abcg1 disturbs cholesterol metabolism and aggravates pyroptosis and neurological deficits after traumatic brain injury. *Brain Pathol.* 33, e13126 (2023).
266. Baida, F. I., Belkhir, A., Van Labeke, D. & Lamrous, O. Subwavelength metallic coaxial waveguides in the optical range: Role of the plasmonic modes. *Phys. Rev. B - Condens. Matter Mater. Phys.* 74, (2006).

267. Rekha, C. R., Jiji, S. G., Nayar, V. U. & Gopchandran, K. G. Simultaneous SERS detection using hexagonal hollow Au-Ag nanoparticles with near infrared plasmon. *Vib. Spectrosc.* 114, 103233 (2021).
268. Creely, C. M., Volpe, G., Singh, G. P., Soler, M. & Petrov, D. V. Raman imaging of floating cells. *Opt. Express* 13, 6105 (2005).
269. Swain, R. J. & Stevens, M. M. Raman microspectroscopy for non-invasive biochemical analysis of single cells. in *Biochemical Society Transactions* vol. 35 544–549 (Portland Press, 2007).
270. Radzol, A. R. M., Lee, K. Y., Mansor, W. & Yahaya, S. R. Nano-scale characterization of Surface Enhanced Raman Spectroscopic substrates. in *Procedia Engineering* vol. 41 867–873 (No longer published by Elsevier, 2012). <https://doi.org/10.1016/j.proeng.2012.07.256>.
271. Zoladek, A., Pascut, F. C., Patel, P. & Notingher, I. Non-invasive time-course imaging of apoptotic cells by confocal Raman micro-spectroscopy. *J. Raman Spectrosc.* 42, 251–258 (2011).
272. Draux, F. et al. Raman spectral imaging of single living cancer cells: A preliminary study. *Analyst* 134, 542–548 (2009).
273. Movasaghi, Z., Rehman, S. & Rehman, I. U. Raman spectroscopy of biological tissues. *Appl. Spectrosc. Rev.* 42, 493–541 (2007).
274. Talari, A. C. S., Movasaghi, Z., Rehman, S. & Rehman, I. U. Raman spectroscopy of biological tissues. *Appl. Spectrosc. Rev.* 50, 46–111 (2015).
275. Picardi, G. Raman spectroscopy and light emission at metal surfaces enhanced by the optical near field of a scanning tunneling tip. (2003). <https://doi.org/10.17169/REFUBIUM-15803>.
276. Yang, Z., Aizpurua, J. & Xu, H. Electromagnetic field enhancement in TERS configurations. *J. Raman Spectrosc.* 40, 1343–1348 (2009).
277. Hayazawa, N., Inouye, Y., Sekkat, Z. & Kawata, S. Metallized tip amplification of near-field Raman scattering. *Opt. Commun.* 183, 333–336 (2000).
278. Pettinger, B., Ren, B., Picardi, G., Schuster, R. & Ertl, G. Nanoscale probing of adsorbed species by tip-enhanced Raman spectroscopy. *Phys. Rev. Lett.* 92, 8–11 (2004).
279. Ren, B., Picardi, G., Pettinger, B., Schuster, R. & Ertl, G. Tip-enhanced raman spectroscopy of benzenethiol adsorbed on Au and Pt single-crystal surfaces. *Angew. Chemie - Int. Ed.* 44, 139–142 (2004).
280. Johnson, P. B. & Christy, R. W. Optical constants of the noble metals. 6, (1972).
281. Griesmer, A. Size Parameters for Free Tetrahedral Meshing in COMSOL Multiphysics. <https://www.comsol.com/blogs/size-parameters-free-tetrahedral-meshing-comsol-multiphysics/>. (2014).
282. COMSOL, A. B. COMSOL Documentation. Reference Manual vol. COMSOL Mul 1–51 https://doc.comsol.com/6.0/docserver/#!/com.comsol.help.comsol/comsol_ref_mesh.20.73.html (2020).
283. Poste, G. Bring on the biomarkers. *Nature* vol. 469 156–157
284. Jack, C. R. et al. A/T/N: An unbiased descriptive classification scheme for Alzheimer disease biomarkers. *Neurology* vol. 87 539–547 (2016).

285. Galluzzi, S. et al. Supporting evidence for using biomarkers in the diagnosis of MCI due to AD. *J. Neurol.* 260, 640–650 (2013).
286. Sorce, S. et al. NADPH oxidases as drug targets and biomarkers in neurodegenerative diseases: What is the evidence? *Free Radical Biology and Medicine* vol. 112 387–396 (2017).
287. Haris, M. et al. T1rho MRI and CSF biomarkers in diagnosis of Alzheimer's disease. *NeuroImage Clin.* 7, 598–604 (2015).
288. Hansson, O. et al. Association between CSF biomarkers and incipient Alzheimer's disease in patients with mild cognitive impairment: A follow-up study. *Lancet Neurol.* 5, 228–234 (2006).
289. Jiao, B. et al. Associations of risk genes with onset age and plasma biomarkers of Alzheimer's disease: a large case-control study in mainland China. *Neuropsychopharmacology* 1–7 (2022).
290. Jack, C. R. et al. NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. *Alzheimer's and Dementia* vol. 14 535–562 (2018).
291. Ashton, N. J., Ide, M., Zetterberg, H. & Blennow, K. Salivary Biomarkers for Alzheimer's Disease and Related Disorders. *Neurology and Therapy* vol. 8 83–94 (2019).
292. Dickstein, D. L. et al. Brain and blood biomarkers of tauopathy and neuronal injury in humans and rats with neurobehavioral syndromes following blast exposure. *Mol. Psychiatry* (2020).
293. Joseph, J. R. et al. Elevated markers of brain injury as a result of clinically asymptomatic high-acceleration head impacts in high-school football athletes. *J. Neurosurg.* 130, 1642–1648 (2019).
294. Thelin, E. P. et al. Serial sampling of serum protein biomarkers for monitoring human traumatic brain injury dynamics: A systematic review. *Front. Neurol.* 8, 1–23 (2017).
295. Camporesi, E. et al. Fluid Biomarkers for Synaptic Dysfunction and Loss. *Biomark. Insights* 15, (2020).
296. Gafson, A. R. et al. Neurofilaments: Neurobiological foundations for biomarker applications. *Brain* 143, 1975–1998 (2020).
297. Inzaugarat, M. E. et al. NLR Family Pyrin Domain-Containing 3 Inflammasome Activation in Hepatic Stellate Cells Induces Liver Fibrosis in Mice. *Hepatology* 69, 845–859 (2019).
298. Heneka, M. T., McManus, R. M. & Latz, E. Inflammasome signalling in brain function and neurodegenerative disease. *Nature Reviews Neuroscience* vol. 19 610–621 (2018).
299. Kerr, N. et al. Inflammasome proteins as biomarkers of traumatic brain injury. *PLoS One* 13, e0210128 (2018).
300. Sutradhar, A., Paulino, G. H., Miller, M. J. & Nguyen, T. H. Topological optimization for designing patient-specific large craniofacial segmental bone replacements. *Proc. Natl. Acad. Sci. U. S. A.* 107, 13222–13227 (2010).
301. Didier, P., Piotrowski, B., Le Coz, G. & Laheurte, P. Topology optimization for the control of load transfer at the bone-implant interface: a preliminary numerical study. *Comput. Methods Biomech. Biomed. Engin.* 23, S82–S84 (2020).

302. Zhang, X. Y., Fang, G., Leeflang, S., Zadpoor, A. A. & Zhou, J. Topological design, permeability and mechanical behavior of additively manufactured functionally graded porous metallic biomaterials. *Acta Biomater.* 84, 437–452 (2019).
303. Gomes, P. D. C., Rickard, J. J. S. & Goldberg Oppenheimer, P. Electrofluidynamic Patterning of Tailorable Nanostructured Substrates for Surface-Enhanced Raman Scattering. *ACS Appl. Nano Mater.* 3, 6774–6784 (2020).
304. Ling, X. et al. Can graphene be used as a substrate for Raman enhancement? *Nano Lett.* 10, 553–561 (2010).
305. Zhao, M. et al. State-of-the-art nanotechnologies for the detection, recovery, analysis and elimination of liquid biopsy components in cancer. *Nano Today* vol. 42 (2022).
306. Ding, S. Y. et al. Nanostructure-based plasmon-enhanced Raman spectroscopy for surface analysis of materials. *Nature Reviews Materials* vol. 1 1–16 (2016).
307. Park, J. E. et al. Efficient capture and Raman analysis of circulating tumor cells by nano-undulated agnps-rgo composite sers substrates. *Sensors (Switzerland)* 20, 1–16 (2020).
308. Grys, D. B. et al. Eliminating irreproducibility in SERS substrates. *J. Raman Spectrosc.* 52, 412–419 (2021).
309. Rosinha, I. P., Gernaey, K. V., Woodley, J. M. & Krühne, U. Topology optimization for biocatalytic microreactor configurations. in *Computer Aided Chemical Engineering* vol. 37 1463–1468 (Elsevier, 2015).
310. Sun, Y., Liu, Y., Xu, L. & Lueth, T. C. Design of a disposable compliant medical forceps using topology optimization techniques. *IEEE Int. Conf. Robot. Biomimetics, ROBIO 2019* 924–929 (2019).
311. Generative Design Versus Topology Optimization | by 3DHEALS | 3DHEALS: Healthcare 3D Printing Facts, Opinions, and Stories | Medium. <https://medium.com/healthcare-3d-printing-stories/generative-design-versus-topology-optimization-ed549dbf19b4>. Accessed: 19/01/2022.
312. Fan, M., Andrade, G. F. S. & Brolo, A. G. A review on the fabrication of substrates for surface enhanced Raman spectroscopy and their applications in analytical chemistry. *Anal. Chim. Acta* 693, 7–25 (2011).
313. Yu, Q., Guan, P., Qin, D., Golden, G. & Wallace, P. M. Inverted size-dependence of surface-enhanced Raman scattering on gold nanohole and nanodisk arrays. *Nano Lett.* 8, 1923–1928 (2008).
314. Dregia, S. A., Banerjee, R. & Fraser, H. L. Polymorphic phase stability in thin multilayers. *Scr. Mater.* 39, 217–223 (1998).
315. Sinha, A. K., Marcus, R. B., Sheng, T. T. & Haszko, S. E. Thermal stability of thin PtSi films on silicon substrates. *J. Appl. Phys.* 43, 3637–3643 (1972).
316. Mukherjee, S. D., Palmstron, C. J. & Smith, J. G. Thermal stability of thin layer transition and refractory metallization on GaAs. in *Journal of vacuum science & technology* vol. 17 904–910 (1980).
317. Tiggelaar, R. M., Sanders, R. G. P., Groenland, A. W. & Gardeniers, J. G. E. Stability of thin platinum films implemented in high-temperature microdevices. *Sensors Actuators, A Phys.* 152, 39–47 (2009).
318. Craster, R. V. & Matar, O. K. Dynamics and stability of thin liquid films. *Rev. Mod. Phys.* 81, 1131–1198 (2009).

319. Sharma, B., Frontiera, R. R., Henry, A. I., Ringe, E. & Van Duyne, R. P. SERS: Materials, applications, and the future. *Materials Today* vol. 15 16–25 (2012).
320. Sun, Y. W. et al. Graphene on silicon: effects of the silicon surface orientation on the work function and carrier density of graphene. *arXiv Condens. Matter* 1–9 (2022).
321. Odetade, D. F., Rickard, J. J. S. & Goldberg Oppenheimer, P. Development and Optimization of Micro-Nanotopographical Platforms for Surface Enhanced Raman Scattering Biomolecular Detection. *Adv. Mater. Interfaces* 2400352, 2400352 (2024).
322. Yu, X. et al. The Feasibility of Early Alzheimer's Disease Diagnosis Using a Neural Network Hybrid Platform. *Biosensors* 12, 1–10 (2022).
323. Le Duigou, A., Chabaud, G., Scarpa, F. & Castro, M. Bioinspired Electro-Thermo-Hygro Reversible Shape-Changing Materials by 4D Printing. *Adv. Funct. Mater.* 29, 1903280 (2019).
324. Han, D. et al. 4D Printing of a Bioinspired Microneedle Array with Backward-Facing Barbs for Enhanced Tissue Adhesion. *Adv. Funct. Mater.* 30, 1909197 (2020).
325. Boldrin, L. et al. Dynamic behaviour of auxetic gradient composite hexagonal honeycombs. *Compos. Struct.* 149, 114–124 (2016).
326. Ingrole, A., Hao, A. & Liang, R. Design and modeling of auxetic and hybrid honeycomb structures for in-plane property enhancement. *Mater. Des.* 117, 72–83 (2017).
327. Ge, Q. et al. Multimaterial 4D Printing with Tailorable Shape Memory Polymers. *Sci. Rep.* 6, 1–11 (2016).
328. Kazemi-Zanjani, N. et al. Multiwavelength Surface-Enhanced Raman Spectroscopy Using Rainbow Trapping in Width-Graded Plasmonic Gratings. *Adv. Opt. Mater.* 6, 4–11 (2018).
329. Land, K. J., Boeras, D. I., Chen, X. S., Ramsay, A. R. & Peeling, R. W. REASSURED diagnostics to inform disease control strategies, strengthen health systems and improve patient outcomes. *Nature Microbiology* vol. 4 46–54 (2019).
330. Mabey, D., Peeling, R. W., Ustianowski, A. & Perkins, M. D. Diagnostics for the developing world. *Nature Reviews Microbiology* vol. 2 231–240 (2004).

APPENDIX ONE

SUPPORTING INFORMATION FOR
CHAPTERS FOUR AND FIVE

A.1 DESIGNS STRUCTURAL OVERVIEW

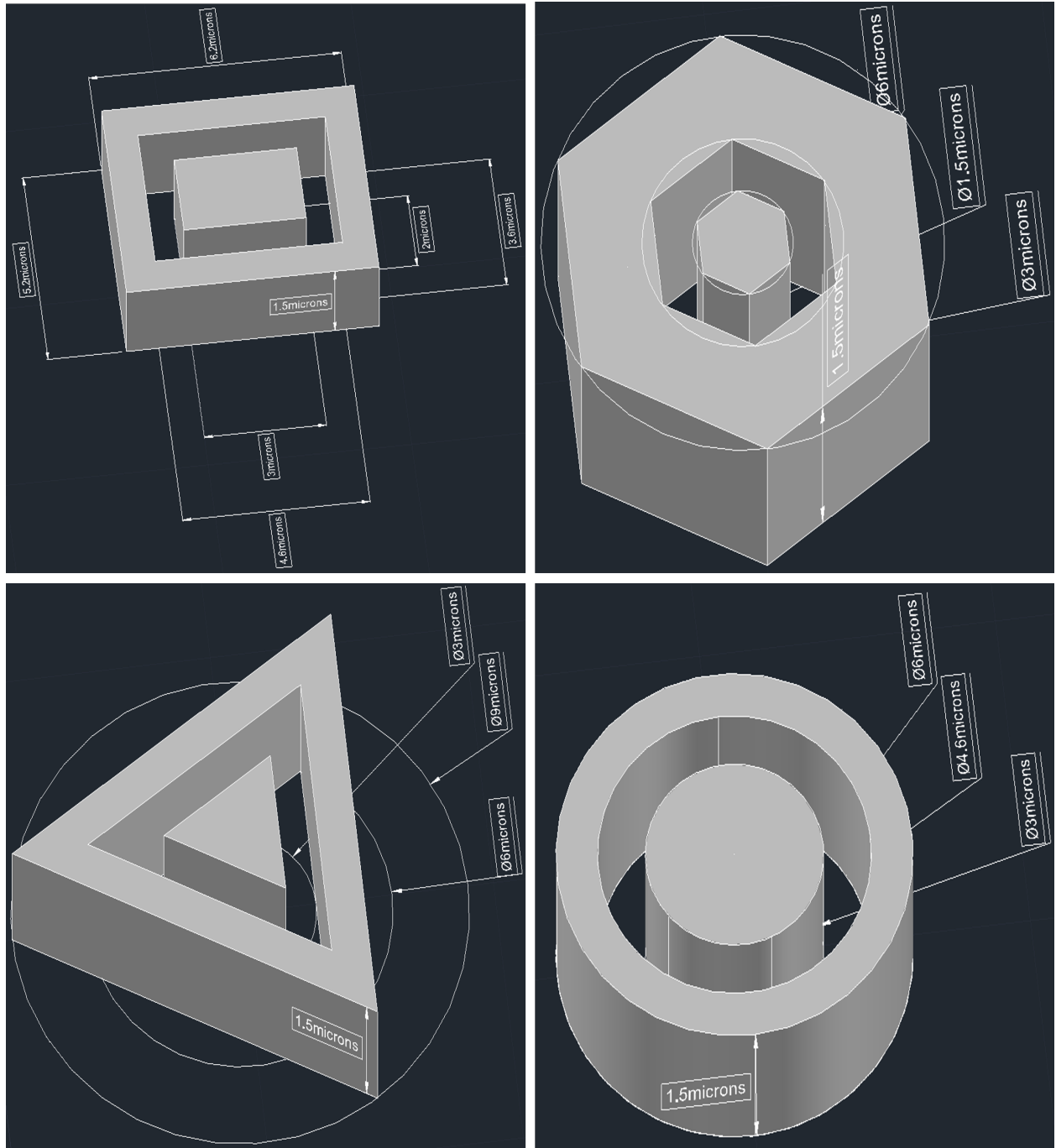


Figure A.1: Representative CAD designs of the coaxial structures with detailed dimensions.

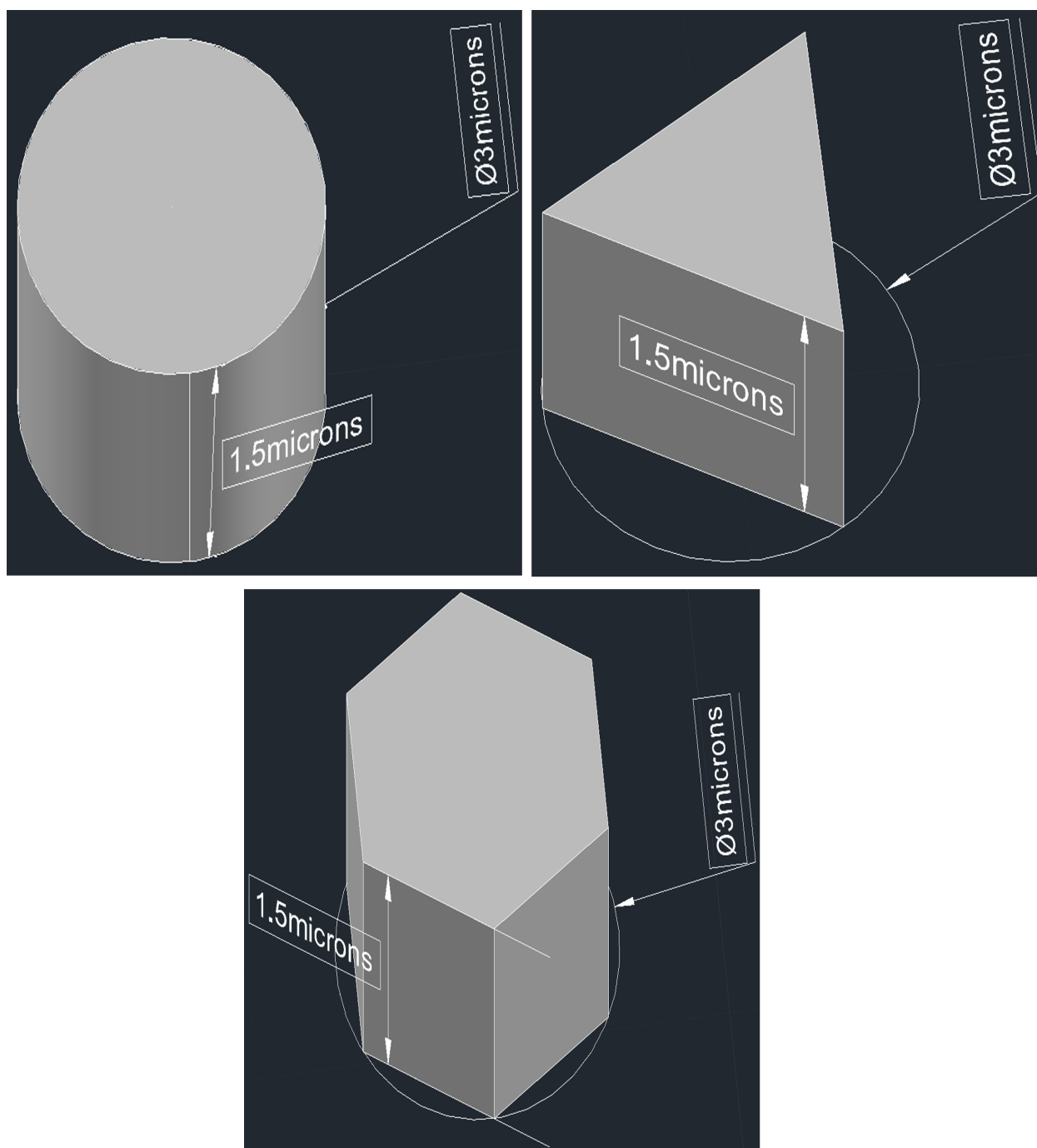


Figure A.2: Representative CAD designs of the solid structures with detailed dimensions.

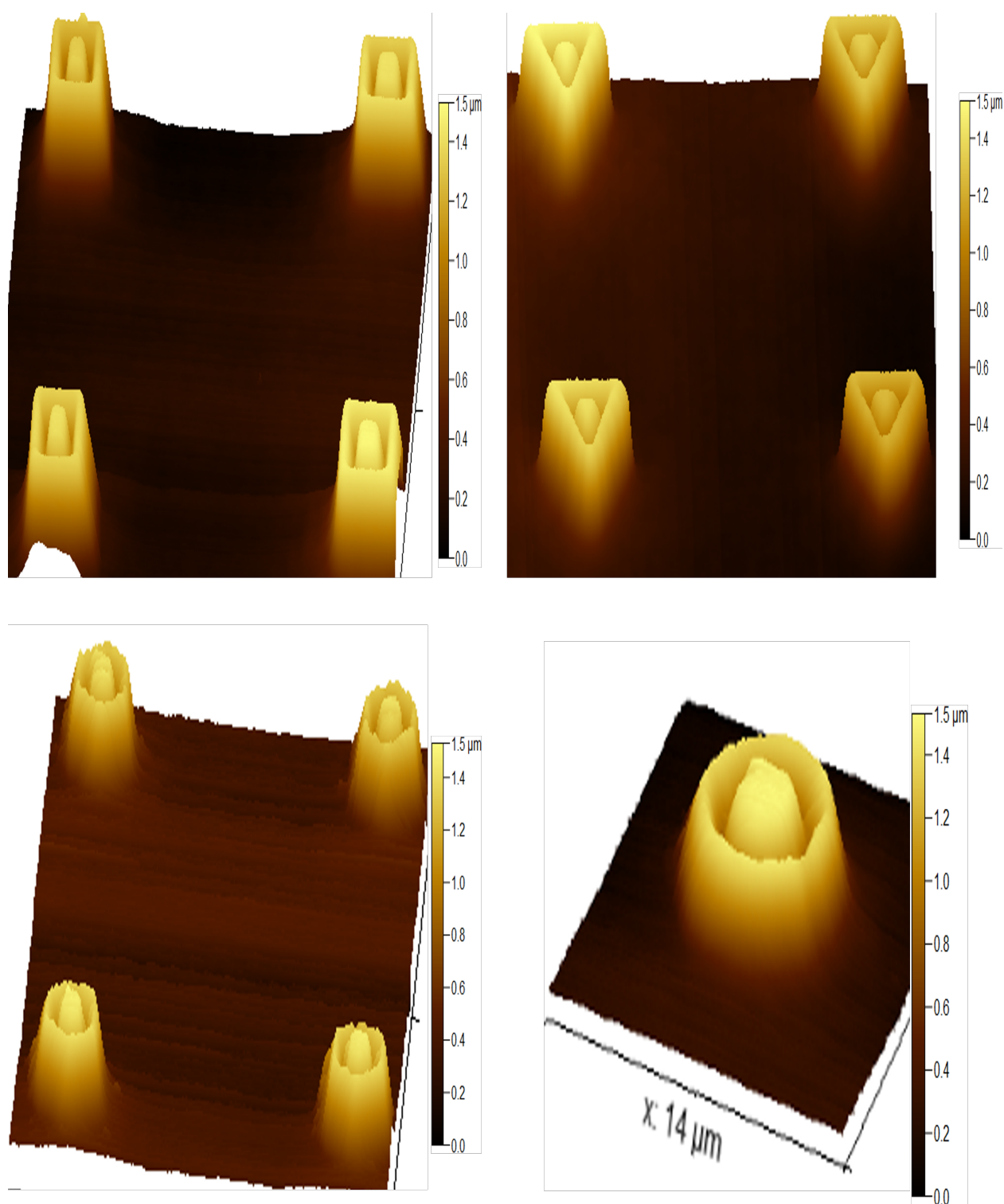


Figure A.3: AFM images of the fabricated coaxial structures.

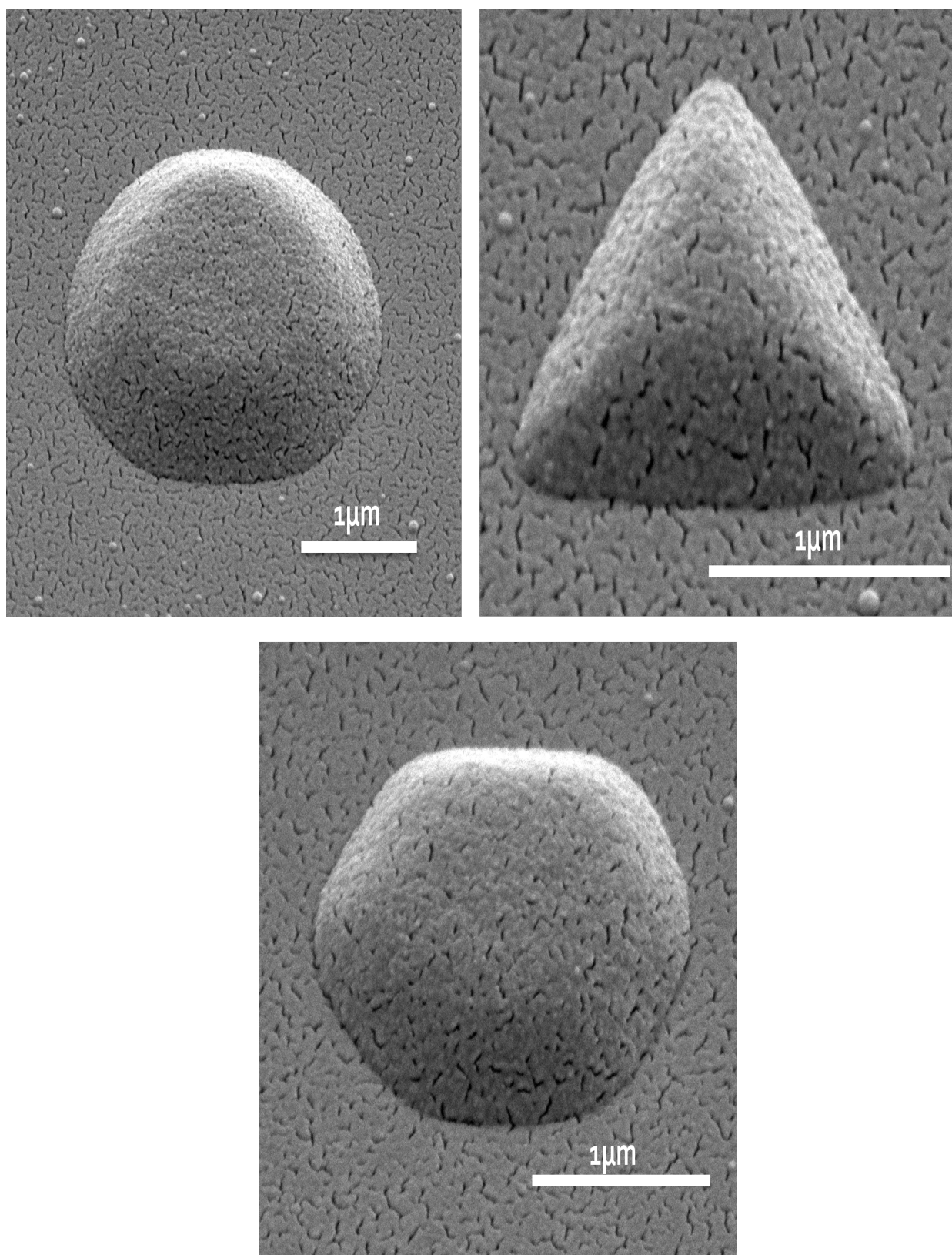


Figure A.4: SEM images of the fabricated solid structures.

A.2 GOLD METALLIZATION AND THICKNESS COVERING THE GENERATED STRUCTURES

SEM and cross-section TEM analysis of the generated structures was used to confirm gold nano-film morphology and covering of the fabricated structures. Figure A.5 shows smooth and conformal coverage of the deposited gold films, precisely following the eXTRA lithographically generated structures. For SEM characterisation, the formed structures were coated with 30 ± 12 nm gold and cross-sectioned before imaging. The contrast between the internal, prior to (dark) and following the *Au* coating (bright), clearly reveals the conformal gold layer on the structures (Figure A.5,a-b). The coating imperfections on the broken structures are a result of the cross-sectioning process. Cross-sectional TEM provides further confirmation of the conformal gold cover and thickness on a set of patterns (Figure A.5,c).

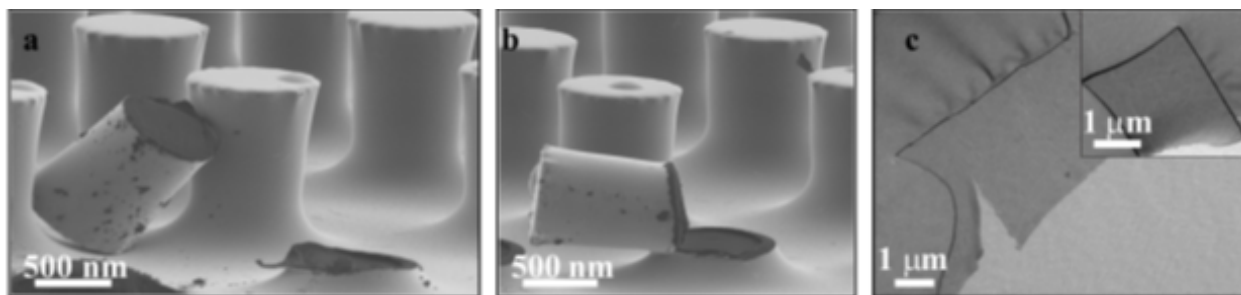


Figure A.5: (a-b). SEM images of the structures coated with gold confirm that the sputtered-on metal coating (bright) smoothly and conformally follows the generated morphologies (dark core). The conformal coating of gold is further confirmed by cross-sectional TEM analysis in (c) where both the top and the curved sides of the structures are homogeneously covered by a thin gold layer.

The gold thickness calibration was further confirmed by measuring the *Au* film thickness deposited on silicon substrates and making a scalpel scratch into the film followed by measuring the resulting step height via the AFM (Figure A.3).

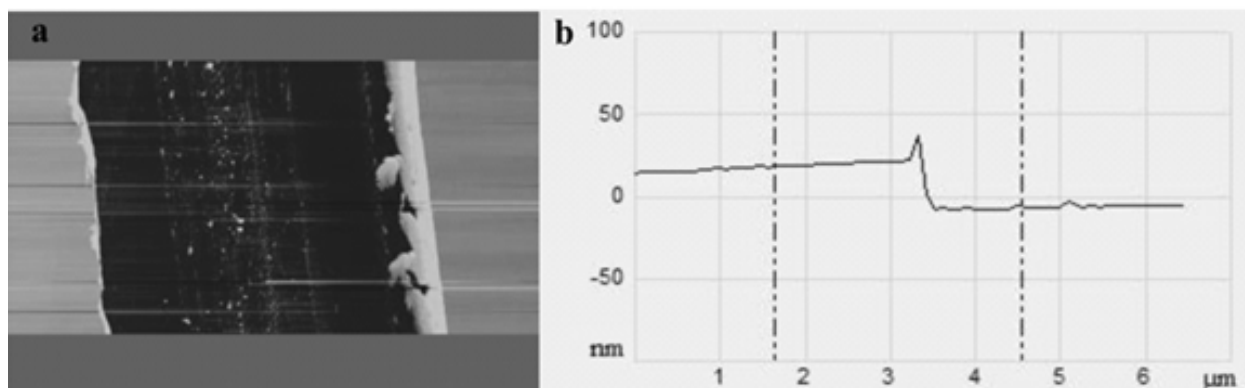


Figure A.6: (a). Representative AFM image of the gold film scratched fragment. (b). Cross-section of the corresponding AFM image in (a) reveals a gold layer with the calculated arithmetic average of the height deviations from the step in the central region on average of 30.0 ± 12.0 nm per $\approx 6 \times 6 \mu\text{m}^2$ substrate area.

APPENDIX TWO

PYTHON CODES

B.1 RAMAN PEAKS FROM LARGE DATASETS

Python codes were written to analyse large datasets (txt files in folders) obtained from Raman spectra. Each folder for a particular reading contained 100 files with several data points.

The code identifies the files with the highest Raman peaks at the stated point. Similar codes were written to analyse other spectra characteristics in conjunction with the Renishaw WiRe software used for the Raman spectroscopy characterisation.

An example of the code written to sift through the data to obtain the files with the highest peak at 1070 cm^{-1} (for BT) is given below:

```
# -*- coding: utf-8 -*-
"""
@author: david
"""

def find_highest_value_file(folder_path, target_value, column_index):
    """
    Searches for the file in the given folder with the highest value in the
    specified column, corresponding to the target value in another column.

    Args:
        folder_path (str): The path to the folder containing the txt files.
        target_value (float): The value to compare against in the specified column.
        column_index (int): The index of the column to check for the target value (0-based indexing).

    Returns:
        tuple: A tuple containing the path to the file with the highest value (or None if not found)
               and the highest value found.
    """

    highest_value_file = None
    highest_value = None

    for filename in os.listdir(folder_path):
        if filename.endswith(".txt"):
```

```
file_path = os.path.join(folder_path, filename)
try:
    # Open the file in read mode
    with open(file_path, 'r') as file:
        for line in file:
            # Split the line based on tabs
            data = line.strip().split('\t')
            if len(data) > column_index:
                try:
                    # Convert the target column value to float and compare
                    if float(data[column_index]) == target_value:
                        value = float(data[column_index + 1]) # Second column value
                        if highest_value is None or value > highest_value:
                            highest_value = value
                            highest_value_file = file_path
                except ValueError:
                    # Handle potential errors if the line cannot be converted to a float
                    pass
            except FileNotFoundError:
                # Handle potential file not found errors
                pass

    return highest_value_file, highest_value

# Example usage (assuming you have permission to access the folder)
folder_path = "C:/Users/david/OneDrive - University of Birmingham/CDT/ANMSA/"
# Replace with the actual folder path
target_value = 1070.758789
column_index = 0 # Assuming the target value is in the first column (0-based indexing)

highest_value_file, highest_value = find_highest_value_file(folder_path, target_value, column_index)

if highest_value_file:
    print("File with highest value:", highest_value_file)
    print("Highest value in second column:", highest_value)
else:
    print("No files found in the folder or no file has", target_value, "in the first column"
          "with a value in the second column.")
```


APPENDIX THREE

COMSOL SIMULATIONS

C.1 2D AND 3D MODELS

The COMSOL simulations were conducted using the finite-element solver COMSOL Multiphysics 5.5, with the Wave Optics module and frequency domain study. The structures were modelled based on experimental designs produced using AutoCAD and Fusion 360 and imported into COMSOL *via* a CAD module. The 2D and 3D files are located in:

1. <https://github.com/DavidTade/Documents/tree/2D-Models>
2. <https://github.com/DavidTade/Documents/tree/3D-Models>

COMSOL Multiphysics 5.5 or higher is needed to view these files.

APPENDIX FOUR

EXCERPT OF PUBLISHED
JOURNAL ARTICLE

RESEARCH ARTICLE



Development and Optimization of Micro-Nanotopographical Platforms for Surface Enhanced Raman Scattering Biomolecular Detection

David Femi Odetade, Jonathan J. S. Rickard, and Pola Goldberg Oppenheimer*

Topologically designed micro- and nanostructured surface-enhanced Raman scattering (SERS) substrates propel the advancements of innovative applications, including environmental and forensic point-of-care miniaturised devices via enhancing the localised electric fields for accurate analyte sensing. Herein, a method for designing, optimising and fabricating fine-tuneable concentric hexagonal, triangular and rectangular SERS-active micronano-substrates is developed, with each unit yielding significant enhancement. Numerical simulations of the 3D near-field electric field guided the optimal design process. While the coaxial SERS substrates consistently outperformed their solid counterparts, the hexagonal micro-nano topologies exhibited $\times 21$ higher signal than coaxial square arrays and a 12–15-fold increase over the triangle structures. Alternation of the topological designs from square to triangle lattice yielded more uniform plasmonic modes propagating along the 60°-directions with various resonance modes playing key roles in light reflectance. This enables the engineering of platforms with tailor-enhanced signals by changing the arrangement of micro-nano patterned coaxial arrays. The fabricated SERS substrates are validated by detecting traumatic brain injury biomarkers, effectively yielding the characteristic fingerprint spectra of each neuro-molecule. The straightforward development of sub-micrometre tuneable SERS-active architectures enables an elegant route for high-throughput biochemical sensing, laying a platform for amplified biomolecular detection of disease biomarkers and integration in bioanalytical systems.

to single molecule levels.^[1,2] In the past decades, SERS has emerged as a powerful analytical tool for a breadth of applications. It is particularly ubiquitous in medical diagnostics^[1–12] due to its unique ability to detect trace-level concentrations of disease-indicative biomarkers without specific antibodies, labeling or requiring complex sample preparation. It is also one of the not many spectroscopic techniques, which enables the ability to be deployed out of the laboratory settings without significant loss in performance. While high-enhancement is possible using metallic nanoparticles, due to their poor batch-to-batch reproducibility, only a minute fraction of these exhibit the desired SERS-activity, i.e., consistent “hot-spots”. This substantially affects the achievable signals and sensitivity.^[13–17]

The substrate on which SERS is performed is often the critical component for successful, consistent, and reproducible detection^[18–22]. Sub-microstructured SERS substrates have been evolving, however, the micro- and nano texturing of plasmonic structures inside micro-devices remains challenging and complicated, especially for low-cost

1. Introduction

Surface-enhanced Raman spectroscopy is a highly sensitive spectroscopic and rapid sensing technique, enabling detection down

fabrication. Most synthetic routes to generate SERS structures are based on conventional photolithographic techniques. These are costly, time-consuming, cumbersome, and require precise integration of multi-step processes,^[23–32] thus, limiting the

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APPENDIX FIVE

EXCERPT OF PUBLISHED REVIEW

ARTICLE

Discover Nano



Review

Advances in lithographic techniques for precision nanostructure fabrication in biomedical applicationsKate Stokes¹ · Kieran Clark¹ · David Odetade¹ · Mike Hardy^{2,3} · Pola Goldberg Oppenheimer^{1,4,5}

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© The Author(s) 2023 **OPEN****Abstract**

Nano-fabrication techniques have demonstrated their vital importance in technological innovation. However, low-throughput, high-cost and intrinsic resolution limits pose significant restrictions, it is, therefore, paramount to continue improving existing methods as well as developing new techniques to overcome these challenges. This is particularly applicable within the area of biomedical research, which focuses on sensing, increasingly at the point-of-care, as a way to improve patient outcomes. Within this context, this review focuses on the latest advances in the main emerging patterning methods including the two-photon, stereo, electrohydrodynamic, near-field electrospinning-assisted, magneto, magnetorheological drawing, nanoimprint, capillary force, nanosphere, edge, nano transfer printing and block copolymer lithographic technologies for micro- and nanofabrication. Emerging methods enabling structural and chemical nano fabrication are categorised along with prospective chemical and physical patterning techniques. Established lithographic techniques are briefly outlined and the novel lithographic technologies are compared to these, summarising the specific advantages and shortfalls alongside the current lateral resolution limits and the amenability to mass production, evaluated in terms of process scalability and cost. Particular attention is drawn to the potential breakthrough application areas, predominantly within biomedical studies, laying the platform for the tangible paths towards the adoption of alternative developing lithographic technologies or their combination with the established patterning techniques, which depends on the needs of the end-user including, for instance, tolerance of inherent limits, fidelity and reproducibility.

1 Introduction

The ability to manufacture nano- scale components has enabled the production of highly capable devices, generating technological advancements in many industries. Progress in lithographic techniques often centres around semiconductor research and the ever-decreasing size of transistors—the miniature components that can alter electrical signals—and consequently increasing number being squeezed into computer chips to form logical components to make decisions at the nano-level. The importance of continued transistor research has been underlined recently by spending in the US and EU, where the US Government signed off a \$280bn package, the CHIPS and Science Act, to stimulate Stateside chip growth, while the EU has proposed doubling their chip production by 2023 with a \$50bn spend. Elsewhere, the Taiwan Semiconductor Manufacturing Company have resolved to keep apace with Moore's law by opening a new microchip

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