Study of plasmonic nano-arrays for the development of cost-effective SERS substrate

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NANYANG
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**Study of Plasmonic Nano-arrays for the
Development of Cost-effective SERS Substrate**

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Contents

Acknowledgement	i
Contents	ii
List of Tables	v
List of Figures	vi
Abstract	2
Chapter 1 : Introduction	4
1.1 Motivation	4
1.2 Objective	7
1.3 Major Contributions	8
1.4 Thesis Organization	9
Chapter 2 : Literature Review	11
2.1 Introduction	11
2.2 Mechanism of Surface Enhanced Raman Scattering	11
2.2.1 Localized Surface Plasmon Resonance	15
2.2.2 Electro-magnetic Enhancement	16
2.3 Evaluation of SERS Substrate	18
2.3.1 Sensitivity	18
2.3.2 Uniformity and Reproducibility	21
2.3.3 Scalability	23
2.3.4 Stability	24
2.4 SERS Substrate Fabrication Strategies	25
2.4.1 Random and Non-uniform Roughened Surfaces	26
2.4.1.1 Electrochemical Oxidation and Reduction Cycles (EC-ORC)	26
2.4.1.2 Post-deposition Annealing	29
2.4.1.3 De-alloying of Bimetallic Composite	31
2.4.2 Chemical Synthesis of Well-dispersed Nano-particles	32
2.4.3 Well-ordered Periodic Nano-arrays	37
2.4.3.1 Optical Photo-lithography	38
2.4.3.2 Electron-beam Lithography	41
2.4.3.3 Focused Ion Beam	43

2.4.3.4 Nanosphere-Lithography	46
2.4.3.5 Ultra-thin AAO Membrane (UTAM).....	49
2.4.4 Shell-isolated Nano-particle-enhanced Raman Spectroscopy.....	53
2.6 Conclusion	55
Chapter 3 : Investigation of SERS Mechanism.	57
3.1 Introduction	57
3.2 Experimental Details	58
3.2.1 Gold Sputtered Substrate	59
3.2.2 AuFON	59
3.2.3 AAO Substrate	60
3.2.4 Characterization	61
3.2.5 FDTD Simulation.....	61
3.3 Results and Discussion	62
3.3.1 Structural Characterization	62
3.3.2 Optical and SERS Measurement.....	64
3.3.3 FDTD Simulation	69
3.3.4 SERS Substrate Comparison	72
3.3.5 Sputtered Gold on Textured Surfaces.....	75
3.4 Conclusion	78
Chapter 4 : Effect of Metallization on SERS Performance	80
4.1 Introduction	80
4.2 Experimental Details	81
4.3 Result and Discussion	83
4.3.1 Surface Characterization and SERS Measurement	83
4.3.2 FDTD Simulation	86
4.3.3 Hybrid Deposition Approach	89
4.4 Conclusion	92
Chapter 5 : Gold on PECVD SiO ₂ Nano-Pillar SERS Substrate	94
5.1 Introduction	94
5.2 Experimental Details	95
5.3 Results and Discussion	97

5.3.1 SERS Measurement	97
5.3.2 SEM and FDTD Simulation	100
5.3.3 Comparison of AuFON and Au-SiO ₂ -NP Substrates	103
5.3.4 Stability and Reusability	105
5.4 Conclusion	106
Chapter 6 : AAO-based SERS Substrate.....	107
6.1 Introduction	107
6.2 Experimental Details	108
6.2.1 Fabrication Technique	108
6.2.2. FDTD Simulation.....	109
6.3 Results and Discussion	110
6.3.1 Surface Morphology.....	110
6.3.2 SERS Characterization of AAO-based SERS Substrate.....	114
6.3.3. Resonance Study of the Optimized ANF Substrate.....	116
6.3.4. Evaluation of the Optimized ANF Substrate	119
6.3.4.1 Substrate Sensitivity.	119
6.3.4.2 SERS Signal Uniformity, Reproducibility, and Stability	121
6.3.4.3 Scalability.....	122
6.3.4. Label-Free Detection	123
6.4 Conclusions	125
Chapter 7 : Conclusion	127
7.1 Conclusion.....	127
7.2 Recommendations	130
Author Publications	132
References.....	133

List of Tables

Table 1: Estimated Size distribution of the gold nano-dots with deposition time of 18-36s	63
Table 2. Hybrid Deposition Gold on Nanosphere Substrate	90

List of Figures

Figure 2.1 Molecular energy diagram illustrating the different light scattering phenomena	12
Figure 2.2 Illustration of propagating surface plasmon on a metal film (left) and localized surface plasmon resonance of a metal sphere (right) in the presence of an incident EM wave	15
Figure 2.3 Schematic illustration of the EM field enhancement of SERS by considering a metal sphere as the SERS active substrate.....	16
Figure 2.4 (a) SERS spectra of a series of low-level malachite green and a blank reference collected on a triple processed gold nano-island array with 40 nm gold deposition. (b) Relationship between then Raman intensity at 1615cm^{-1} and the concentration of the analyte solution[42].....	20
Figure 2.5 (a) SERS spectra of 1 μM R6G aqueous solution collected from 10 different spots on Au-CuCl ₂ -AAO substrate. The inset is mean $\pm$ standard deviation of the Raman intensity at each characteristic peak [43] (b) 2D scanning SERS intensity distribution for 1573 cm^{-1} Raman peak-distribution (mean of 890 ± 137) [44].	22
Figure 2.6 Scalability of AAO membrane method; (a) and (b) show the square-type AAO free-standing membrane with 200 nm thickness and the transferred membrane on the Si substrate; (c) and (d) show the circle-type AAO free-standing membrane with 200 nm thickness and the transferred membrane on 3 inch Si wafer. (e) Shows 4 inch AAO free-standing membrane [45].	23
Figure 2.7 (a) SERS stability of Graphene Oxide-wrapped Gold Nano-Rod (GO@GNR)-based nano-probe [46] and (b) SERS stability of gold Nano-particles-Molybdenum Disulfide Quantum Dots (NPs@MoS ₂ QD) [47]	25
Figure 2.8 Rhodamine 6G (R6G) SERS spectrum and the corresponding SEM images of EC-ORC roughened silver substrates treated at temperatures of (a) 25 °C, (b) 100 °C, (c) 125 °C, and (d) 150 °C [57].	28

Figure 2.9 Schematic of the repeated deposition and post-annealing fabrication cycle [42]. 30

Figure 2.10 Microstructure characterizations of wrinkled nano-porous films. (a) SEM micrographs of the flat nano-porous film with a characteristic length of 20 and 25 nm. (b) Microstructure of a wrinkled nano-porous film with a quasi-periodic wavelength of 10 and 15 μm . (c) Chemical composition of a nano-porous film measured by energy dispersive X-ray spectroscopy. (d) Microstructure of wrinkle ridges showing nano-gaps, interleaving broken ligaments and linear chains of self-similar nano-cavities [59] 32

Figure 2.11 TEM (inset SEM) images of Au nano-particles synthesized under different conditions. Ascorbic Acid (AA) concentration increases from A to B; and seed concentration increases from C to D. (E, F, G, H): Transformation from cubic to rod-shaped gold particles at low AA concentrations in the presence of a small quantity of silver nitrate. Different dimension obtain with different relative concentration of CTAB, seed and Au^{3+} . Scale bar:100nm [65]. 34

Figure 2.12 SEM images of gold nano-particle arrays obtained with deposition times of (a) 2 days, (b) 4 days, (c) 6 days, and (d) 8 days. The corresponding extinction spectra (inset shows the photograph of the four samples) and SERS spectra of 10^{-6}M R6G under 514 nm excitation are also shown [66]. 35

Figure 2.13 (a) UV-Vis spectra of different sizes of silver nano-particle colloids made by seeded growth with ascorbic acid and diluted to a 1:30 ratio of colloid/water. (b) Plot correlating the calculated SERS enhancement factor, G , with AgNP size for two separate sample series (blue and red dots) [67] 36

Figure 2.14 (a) SEM images and (b) extinction spectrum of silver nano-pillars SERS substrate deposited with 80 nm of silver. (c) Normal Raman and SERS spectrum of benzenethiol. (d) Probability distribution showing the enhancement factor across the substrate [44]. 39

Figure 2.15 SEM image of Ag nano-particle decorated Si/ZnO nano-trees and SERS spectra of R6G molecule(10^{-5}M) at 532nm excitation collected from (a) Si/ZnO nano-trees; (b) 2-D Ag nano-particles substrate prepared by sputtering method; (c) Ag nano-particle decorated Si/ZnO nano-trees. [77] 40

Figure 2.16 Schematic illustration of the two possible approach using the EBL method for fabricating nano-structures as SERS substrate [78].....	41
Figure 2.17 SEM images of gold nano-structures fabricated with EBL combined with plasma etching or lift-off process.[78]	42
Figure 2.18 schematic representation and SEM images of the Au-SiO ₂ -Au tandem truncated nano-cones comprises (60 nm radius array with period 200 nm) Scale bar: 300 nm.[79]	43
Figure 2.19 FE-SEM images and the corresponding SERS measurement on rose Bengal molecules on the acute-angle gold nano-rod tilted at different angle fabricated by focused ion-beam [80].....	45
Figure 2.20 Schematic illustration of the self-assembly of polymeric sphere by (a) spin coating (b) capillary action assisted scooping and (c) the Langmuir Blodgett self-assembly technique.	46
Figure 2.21 SEM images of BMFON substrates with underlying silver layer of 160 nm and upper gold layer of (a) 0 nm, (b) 120 nm, and (c) 320 nm thickness [84]..	47
Figure 2.22 Schematic of the porous silicon nanopillar fabrication procedure: (a) formation of a monolayer of polystyrene (PS) nanospheres, (b) nanosphere etching via reactive ion etching, (c) gold deposition, (d) bead removal, and (e) catalytic chemical etch of the underlying silicon substrate. (f) Tilted SEM image of a porous silicon nano-pillar array. SERS spectrum of the silver silicon nanopillar substrates incubated in R6G [85].....	48
Figure 2.23 (a) Schematic illustration of an AAO structure prepared by electrochemical anodization of aluminium substrate. (b) Summary of the anodizing voltage and corresponding inter-pore distance and pore diameter of the AAO fabricated in the three well-known regimes of electrolytes (sulphuric, oxalic and phosphoric).[86].....	50
Figure 2.24 SEM images of Au/Ni hybrid NPs arrays with fixed size Ni NP at 25nm and increasing gold deposition of (a) 0nm, (b) 5nm, (c)10nm, (d) 15nm, (e) 20nm and (f) 25nm and the corresponding (g) SERS spectrum of crystal violet (10 ⁻⁷ M)	

and the (h) SERS intensity vs Au thickness at 1618 cm^{-1} , 1372 cm^{-1} and 1177 cm^{-1} [33] 52

Figure 2.25 (a) - (c) A schematic illustration of the fabrication process for the Au quasi-3D nano-hole arrays for flexible SERS substrates. SEM images of (d) nano-patterned cPS film and (e) Au quasi-3D nano-holes. (f) SERS spectrum of Au quasi-3D nano-holes at different concentration of thiolated polyethylene glycol (Cy5) Raman dye molecules [87] 53

Figure 2.26 (a) In situ probing of biological structures by SHINERS. Curves I, II and III, SHINERS spectra obtained from different spots at the wall of a yeast cell incubated with Au/SiO₂ nano-particles. Curve IV, the spectrum recorded from a substrate coated with Au/SiO₂ but without a yeast cell; curve V, a normal Raman spectrum for yeast cells. The peaks marked with red asterisks are closely related to mannoprotein. (b) In situ inspection of pesticide residues on food/fruit. Normal Raman spectra on fresh citrus fruits (Curve I), with clean pericarps (curve II), contaminated by parathion (Curve III), SHINERS spectrum of contaminated orange modified by Au/SiO₂ nano-particles (Curve IV) [88] 54

Figure 3.1 Schematic diagram illustrating the preparation of AuFON substrate in the order of (a),(b), (c) and (d) 59

Figure 3.2 Schematic diagram illustrating the preparation of AAO by two-step anodization of aluminium in the order of (a), (b), (c) and (d) 60

Figure 3.3 FDTD simulation model of the gold sputtered substrate..... 61

Figure 3.4 SEM images of the glass substrates after (a) 18s, (b) 36s, (c) 54s, (d) 72s, (e) 90s and (f) 108s of gold sputtering with a fine coater. Scale bar: 1000Å .. 63

Figure 3.5 (a) Measured optical transmittances of the gold sputtered substrates and the (b) corresponding estimated extinction spectra..... 66

Figure 3.6 (left) 2NT SERS spectrum ($\lambda_{\text{ext}} = 633\text{nm}$) of the gold sputtered substrate fabricated with sputtering deposition time of $\Delta t = 18\text{-}90\text{s}$ and (right) the corresponding comparison of the 1380cm^{-1} 2NT peak illustrating transition from LSPR coupling mode (discretized gold nano-particles) to propagating surface plasmon mode (quasi-continuous gold film). The SERS spectrum of $\Delta t = 108\text{s}$

sample has similar response as the $\Delta t = 72\text{s}$ and 90s sample and not plotted to maintain clarity. The SERS measurement at 532nm laser excitation did not show any signal. 67

Figure 3.7 Illustration of the self-assembly process of 2-Naphthalenethiol (2-NT) on the gold surface.....68

Figure 3.8 FDTD simulation of the extinction (blue solid), absorbance (blue dashed), scattering cross-section (blue dotted) and field enhancement (red) spectra of the cylindrical disk with increasing diameters of (a) 6nm , (b) 7nm , (c) 8nm , (d) 9nm and (e) 10nm . (f) The corresponding resonance wavelength (λ) of the extinction (*), absorbance (●), scattering cross-section (▲) and field enhancement (X) 71

Figure 3.9 (a) SEM image of the AuFON substrate and the (b) comparison of the simulated electric field enhancement spectrum of the AuFON and the gold sputtered substrate with deposition time of $\Delta t = 36\text{s}$. The red window demarcates the 633nm excited Raman band corresponding to a Raman shift range of $0\text{-}1800\text{cm}^{-1}$ 73

Figure 3.10 SERS spectrum comparison of the AuFON and gold sputtered substrate with deposition time of $\Delta t = 36\text{s}$ using 2NT Raman probe and 633nm wavelength laser excitation..... 74

Figure 3.11 FDTD simulation of the extinction (solid line), absorbance (dashed line) and scattering cross-section (dotted line) spectra of (a) the gold sputtered sample with deposition time of $\Delta t = 36\text{s}$ and (b) the AuFON substrate with 60nm of sputtered gold. The red window demarcates the 633nm excited Raman band corresponding to a Raman shift of $0\text{-}1800\text{cm}^{-1}$ 75

Figure 3.12 (a) SEM images of gold sputtered surfaces ($\Delta t = 36\text{s}$) of AAO and wrinkled PDMS and the (b) SERS spectra of gold sputtered ($\Delta t = 36\text{s}$) on AAO, Al, PDMS, glass and Si surfaces. 76

Figure 3.13 Simulated scattering cross-section of the gold sputtered particles ($\Delta t = 36\text{s}$) on gold, aluminium, silicon dioxide and alumina planar substrate 77

Figure 4.1 Schematic illustration of experimental details..... 82

Figure 4.2 SEM images of various gold thickness (10-60nm) deposited onto silicon substrate by (a) E-beam evaporation and (b) DC sputtering. Scale bar: 200nm ... 83

Figure 4.3 SEM images of AuFON coated with gold thicknesses of 20 - 60nm (a) by E-beam evaporation and (b) DC sputtering. Scale bar: 200nm 85

Figure 4.4 (a) The 1062cm^{-1} and 1380cm^{-1} SERS intensity peak of 2NT Raman molecules on AuFON substrate, metalized with the DC sputtering and E-beam evaporation techniques plotted as a function of gold thicknesses (b) 2NT SERS spectrum of the AuFON coated with 40nm and 60nm of gold using E-beam evaporation and DC sputtering techniques. 86

Figure 4.5 Distribution of electric field enhancement along the x-z plane of the (a) smooth and (b) roughened gold nanosphere array under 633nm wavelength laser excitation 87

Figure 4.6 SERS spectra of (a) AAO (20min etched in 5% H_3PO_4) and (b) AAO (40min etched in 5% H_3PO_4) coated with 20nm of gold using E-beam evaporation and DC sputtering. The corresponding SEM images of the substrates are shown on the right of the graphs. Scale bar: 200nm 88

Figure 4.7 SERS spectra of 2NT on AuFON coated with (A) 30nm E-beam gold (red), (B) 15nm sputtered gold and 15nm E-beam gold (green), (C) 15nm E-beam gold and 15nm sputter gold (blue) and (D) 30nm sputtered gold (cyan). Samples are excited with a laser wavelength of 633nm. 90

Figure 4.8 The 2NT SERS intensity of the 1380cm^{-1} peak from AuFON substrate coated with gold (blue), sputtered gold with 10nm E-beam gold (green) and sputtered gold with 15nm E-beam gold plotted as a function of sputtered gold thicknesses ranging from 20-60nm. The standard deviation (%) of the intensity of the 1380cm^{-1} peak is shown at each data point. 92

Figure 5.1 Preparation of PECVD Au-SiO₂ NP substrate 96

Figure 5.2 SEM images of (a) silicon nano-pillars after etching 300nm of silicon before sonication and (b) Au-SiO₂-NPs after depositing 50nm of PECVD SiO₂ on silicon nano-pillars followed by 80nm of E-beam gold 98

Figure 5.3. SERS intensity peak (1062cm^{-1} and 1380cm^{-1}) of 2NT on Au-SiO ₂ -NP substrates with various thicknesses of PECVD SiO ₂ (40-70nm). 80nm of gold is deposited by sputtering (square) and electron-beam evaporation (circle).	99
Figure 5.4 SEM images of Au-SiO ₂ -NP with (a) 40nm, (b) 50nm and (c) 70nm PECVD deposition of SiO ₂ . and (d-f) the corresponding simulated wavelength dependent electric field enhancement (red) and scattering cross-section(blue) ..	102
Figure 5.5 SERS spectra of the optimized Au-SiO ₂ -NP substrates (50nm PECVD SiO ₂) coated with 80nm E-beam gold (blue) and sputtered gold (green) and the AuFON coated with 80nm E-beam gold.....	103
Figure 5.6. (a) Simulated wavelength dependent electric field enhancement of the AuFON and the optimized Au-SiO ₂ -NP (50nm PECVD SiO ₂) substrates. Simulated electric field distribution of (b) the AuFON and (c) the optimized Au-SiO ₂ -NP (50nm PECVD SiO ₂) at 633nm wavelength excitation (x-z plane, y=0).....	104
Figure 6.1 Schematic diagram illustrating the formation of different types of AAO-based structures by varying the wet etching time.....	109
Figure 6.2 FDTD simulation model of the ANF substrate.	110
Figure 6.3 (a) AAO height in the nano-holes regime plotted as a function of anodization time (Δt_A). (b) Height of AAO fabricated with second step anodization time of 10s (T10), 20s (T20), 30s (T30), 40s (T40), 50s (T50) plotted as a function of wet etching time (Δt_E).	111
Figure 6.4 Pore diameter of the AAO plotted as a function of wet etching time (Δt_E) in 5% H ₃ PO ₄ solution with the corresponding SEM image of the AAO surface. Scale bar: 200nm	111
Figure 6.5 SEM images of AAO surface after etching in 5% H ₃ PO ₄ for (a) 0 min and (b) 50 min (scale bar: 200 nm).....	113
Figure 6.6. Wet etching time for ANF formation plotted as a function of starting AAO thicknesses/second anodization time.	113

Figure 6.7 SEM images of standing ANF fabricated from AAO with a second anodization time of (a) 30 s, (c) 60 s and bundled ANF fabricated from AAO with a second anodization time of (e) 90 s, (g) 150 s. The respective ANF substrates coated with 60 nm of electron beam evaporated gold are shown in (b), (d), (f) and (h)..... 114

Figure 6.8 (a) Measured SERS intensity of the 1380 cm^{-1} characteristic peak (2NT) plotted as a function of the second anodization and wet etching time. (b) SERS spectra of ANF, nano-hole and nan o-cavity substrates using $0.1\text{ }\mu\text{M}$ 2NT..... 115

Figure 6.9 (a) SERS spectrum comparison of the 60nm gold-coated ANF substrate excited with wavelengths of 532 nm, 633 nm and 785 nm. (b) SERS intensity of the ANF substrate at the 1062 cm^{-1} and 1378 cm^{-1} 2NT peak, measured with excitation wavelengths of 532 nm, 633 nm and 785 nm, plotted as a function of gold thickness..... 117

Figure 6.10 Theoretical scattering cross section of the ANF substrates with gold deposition thickness of (i) 20 nm, (ii) 40 nm and (iii) 60 nm calculated using a Lumerical 3D-FDTD simulation tool. 118

Figure 6.11 (a) SERS spectrum of the 2NT probe solution with concentrations varying from 0.1 nM to 1 μM absorbed onto ANF substrate and the (b) corresponding signal-to-noise ratio of the 1062 cm^{-1} (♦) and 1380 cm^{-1} (■) 2NT characteristic peak..... 120

Figure 6.12 SERS spectrum comparison between the optimized ANF and AuFON substrates with a 2NT concentration of 0.1 mM. 121

Figure 6.13 Boxplot of the SERS intensity of the 1378 cm^{-1} 2NT peak measured in (a) three different batches of ANF substrates and (b) plotted as a function of substrate storage time before measurement..... 122

Figure 6.14 SERS spectrum of Guanine and Cytosine with a concentration 0.1mM 124

Abstract

Due to its excellent signal uniformity and reproducibility, well-ordered plasmonic nano-arrays demonstrate great potential as practical SERS probe for real-time in-situ analysis of molecules. A basic study of SERS mechanism was carried out to elucidate relevant factors that can affect SERS enhancement. The results revealed that the rarely discussed scattering cross-section can have substantial influence on SERS enhancement and should be considered for a complete understanding and optimization of the performance of SERS substrates. Two commonly used metallization techniques, DC sputtering and electron-beam (E-beam) evaporation, was investigated to quantify their effect on SERS performance. It was found that the E-beam evaporation technique produces inherently roughened gold film which serve as additional EM hotspots favourable for SERS. A hybrid metal deposition approach to exploit the advantages of both the sputtering and E-beam technique is proposed and verified. Two well-ordered and cost-effective SERS substrates were proposed and investigated. The first is a Au-SiO₂ nano-pillar substrate fabricated by performing an additional plasma enhanced chemical vapour deposition of SiO₂ on nanosphere-lithography fabricated Si pillars to tune the plasmonic resonance of the nano-structures. The optimized Au-SiO₂ nano-pillar exhibits comparable SERS performance as the well-established gold-film-over-nanosphere substrate and has the added advantage of better stability and reusability due to its inherently rigid structure. The second SERS substrate developed in this work comprises non-lithographic anodized aluminium oxide (AAO)-based nano-fibres (ANF) that are fabricated by optimizing the second anodization and incorporating an additional chemical wet-etching step. The optimized ANF substrate exhibits better sensitivity, signal-to-noise ratio, signal uniformity and stability than those of

conventional substrates. The fabrication process of the ANF SERS substrate is also highly reproducible and scalable. Label-free detection of two DNA and RNA nucleobases are also demonstrated with the ANF SERS substrate.

Chapter 1 : Introduction

1.1 Motivation

In modern time, bio-molecular and chemical sensing capability plays a significant role in numerous applications such as medical diagnostic tool, food and environmental analysis, forensic science, medical imaging and defence science. Advancement in technology and the increasing need for improved quality of living resulted in the demand for accurate and reliable sensing methods to be incorporated into practical devices. For example, early diagnosis of diseases in the premature state allows immediate delivery of drugs and effective treatment protocol to be implemented which effectively increases patient survivability. Typical diagnostic procedure involves the examination of biological samples such as urine, stools, blood, saliva and tissue which are complex mixtures and cross interference of signal is inevitable. Filtering technique has been extensively developed to sieve out subject of interest, but its procedure is complex and selectivity is still limited [1, 2]. In food and environmental analysis, the early detection of bacterial contaminations can possibly prevent the onset of epidemic especially in the third world countries. Similarly, in defence science, the ability to detect extremely low concentration of airborne contaminants could potentially put off the danger of a chemical attack.

Conventional non-spectroscopy sensing methods using metal-oxide semiconductor and electrochemical sensor lack specificity and hence require supplementary filtering to improve selectivity [3]. By contrast, spectroscopy technique which utilizes the specific interaction between matter and electro-magnetic (EM) radiation to provide molecular information has been identified as one of the suitable analytical methods for the reliable detection of specific analytes [4, 5].

Different nature of light-matter interactions, such as reflection, absorption, emission and scattering of light can leave characteristic imprints in the electro-magnetic (EM) spectrum. The resulting spectroscopic data is typically presented and analyzed in the spectral domain plotted as a function of wavelength or frequency. Over the past decades, spectroscopic analytical technique has extended its application beyond the field of basic research into developing innovative and practical diagnostic methods for diseases and therapeutic drug control [6-8]. Among all, vibrational Raman spectroscopy technique are of particular interest because of its non-invasive and label-free acquisition of the characteristic spectral signature of a sample arising from the in-elastic scattering of light [9].

A typical Raman system can simply be constructed using an unsophisticated optical setup comprising a laser source, a notch filter and a diffraction grating to channel the scattered light to a charge-coupled device (CCD) sensor. This is of great advantage for realizing portable sensor devices for the reliable detection of unknown compounds. Raman scattering cross-section, however is extremely small and detecting quantifiable signal is particularly challenging, limiting its potential use in practical applications [10]. This constraint was subsequently overcome by the discovery of surface enhanced Raman spectroscopy (SERS) that utilizes a nano-textured plasmonic surface to significantly amplify the Raman scattering signal through the enhancement of the local EM field and the charge-transfer interaction between molecules and substrate [11]. This breakthrough opens up numerous possibilities in the practical application of SERS [12-16]. R. Goodacre et al has demonstrated rapid discrimination of bacteria using SERS. By means of silver colloid SERS substrate, discrimination to strain level for a sample group of *Escherichia coli*

was achieved [17]. In more recent work, label-free detection of protease activity using gold nanodome was also demonstrated [18]. The successful SERS detection of biological entities requires high performing substrates that can provide reliable and repeatable SERS measurements. The improved sensitivity also value-add to the multiplexing detection capability of Raman spectroscopy as the unique Raman peak can be clearly distinguished in a single spectra acquisition [19].

SERS enhancement is highly dependable on the geometry of the nano-textures surfaces [20]. Nano-gaps and crevices formed by adjacent nano-particles are excellent SERS hotspots that are responsible for amplifying the incident and scattered field by several orders of magnitude to yield detectable Raman signal [21]. Raman signal can be significantly enhanced by narrowing the gap towards the sub-10nm region where intensive local field enhancement takes places [22, 23]. Coinage metals such as gold, silver and copper which exhibit surface plasmon resonance over the visible and near infrared wavelength range where Raman scattering takes place, are classic choice of SERS-active materials [24, 25]. Over the years, research attention has been focusing on the development of novel fabrication strategies and identifying SERS efficient structures and materials.

Several classes of SERS substrates, such as metal colloid, random roughened surfaces and well-ordered periodic nano-arrays have been developed [26]. Among them, the well-ordered periodic nano-arrays have demonstrated significantly higher degree of signal uniformity and reproducibility and are therefore more suitable for practical applications. However, achieving highly reproducible and sensitivity SERS signal in a cost-effective manner still remains a challenge owing to process variation and resolution constraint. Electron-beam lithography (EBL) [27]

and focus ion-beam (FIB) [28, 29] are the most direct approach in achieving high resolution nano-structures. These techniques involve the direct writing of nano-patterns on the substrate and has the disadvantage of low throughput. It is also a challenge to achieve large scale fabrication with such techniques. Alternatively, self-assembly methods such as nanosphere-lithography (NSL) [30, 31] and anodized aluminium oxide (AAO) template assisted fabrication [32, 33] can potentially provide a more cost-effective approach in realizing nano-textured surfaces. Therefore, this work focuses on a detailed understanding of the SERS mechanism and the research on new fabrication processes to achieve improved SERS performance and signal reliability that can meet the increasing demand for high performance sensing devices.

1.2 Objective

This work aims to develop and demonstrate high performing SERS substrates that are suitable for demanding practical applications through the following objectives:

1. Basic study of the SERS mechanism to elucidate the underlying factors affecting SERS performance.
2. To investigate the effect of metal surface roughness on SERS performance.
3. To develop an NSL-based fabrication approach using semiconductor processes to obtain a cost-effective SERS substrate.
4. To develop an AAO-assisted fabrication process to achieve highly sensitive and reproducible SERS substrates for label free detection of analytes.

1.3 Major Contributions

The major contributions to the thesis are described below:

1. Basic study of the SERS mechanism shows that the near-field coupling of the LSPR excitation is accompanied by a resonating field enhancement and also an increase in the scattering cross-section of the SERS substrate. The findings in this work suggest that other than the commonly recognized factor of field enhancement, scattering cross-section of the substrate also plays an important role in SERS enhancement. This factor should be carefully considered when designing SERS substrate to maximise its performance
2. Investigation of the two metallization techniques namely, DC sputtering and electron-beam evaporation, shows that the latter produce nano-scale roughness that is favourable for SERS. The state change in the electron beam evaporation process causes large contact angle between molten gold and the target substrate which result in a non-conformal coating. A cost-effective hybrid metallization technique was developed, which uses sputtering to narrow inter-particle gap and a final electron beam evaporation coating to introduce additional SERS hotspots through the induce roughness.
3. A cost-effective substrate fabrication strategy was developed by incorporating PECVD deposition of SiO_2 onto NSL fabricated silicon pillars to optimize the SERS resonance condition. The optimized Au- SiO_2 -nano-pillars possess a SERS enhancement factor in excess of $\sim 4.90 \times 10^6$ which is comparable to those of the well-established AuFON substrates. In addition, the Au- SiO_2 -

nano-pillars substrates possess better stability and are suitable for post-fabrication treatment to extend its sensing capability.

4. A scalable fabrication strategy for developing highly sensitive and reproducible AAO-based nano-fibre (ANF) SERS substrates is demonstrated. The optimized ANF substrate achieves a SERS enhancement factor of $\sim 9.28 \times 10^6$ and exhibits nano-molar detection limit with a signal-to-noise ratio of at least 3 using a low laser excitation power of 0.1mW. SERS signal deviation is well within 8% and shows no deterioration with time. The ANF substrate demonstrates excellent traits that have potential for commercial applications.

1.4 Thesis Organization

1. Chapter 1 discusses the motivation, objective and major contributions of this thesis.
2. In chapter 2, a literature review of the SERS enhancement mechanism, substrate evaluation parameters and fabrication strategies is presented to identify relevant considerations for improving the SERS performance.
3. Chapter 3 reports the basic study of the SERS mechanism using both FDTD simulation and carefully designed experimental measurements. The factors affecting SERS performance are presented and discussed
4. Chapter 4 investigated the effect of sputtering and electron-beam evaporation of gold on the performance of SERS substrate.

5. In chapter 5, the fabrication process for the development of Au-SiO₂ nano-pillars array is described. The theoretical simulation and characterization results of the fabricated nano-pillar array are presented and discussed.
6. Chapter 6 details the development of an AAO fabrication process to achieve nano-fibre arrays on a substrate. The performance of the ANF SERS substrate determined from experimental characterization is discussed. The application of the fabricated ANF substrate for label-free detection is also demonstrated in this chapter.
7. Finally, chapter 7 summarizes the results obtained in this thesis and present a list of recommendations for future work and improvement.

Chapter 2 : Literature Review

2.1 Introduction

In this chapter, the SERS technique is reviewed. The principle of Raman scattering and the associated electro-magnetic (EM) field enhancement and localized surface plasmon resonance (LSPR) excitation in SERS substrates are discussed. The metrics for quantifying the SERS performance of a substrate are also highlighted in this chapter. Existing SERS fabrication techniques for the various classes of SERS substrate are reviewed and discussed. This includes the random roughened metal surfaces, metal nano-particles colloid and the well-ordered periodic nano-arrays.

2.2 Mechanism of Surface Enhanced Raman Scattering

The scattering of light by a molecule occurs elastically as Rayleigh scattering when the incident and scattered photons experience no change in energy. An extremely small portion of the light is scattered in-elastically as Raman signal where the energy of the scattered photon is modulated. Figure 2.1 shows the energy profile of a molecule to illustrate the different types of light scattering effects. The molecule-of-interest absorbs the incident photon and re-emits a photon with a different energy. This results in a change in the wavelength of the re-emitted photon and the effect is known as Raman shift. The phenomenon can be modelled as a scattering interaction between a photon and a molecule (Figure 2.1)

Figure 2.1 Molecular energy diagram illustrating the different light scattering phenomena

During interaction, the incident photon $h\nu_i$ is in-elastically scattered by a molecule and it experiences a frequency shift corresponding to the characteristic molecular vibrations $h\nu_M$. Consequently, the energy of the scattered photon either increases or decreases relative to the incident photon depending on the initial state of the molecule. If the molecule is in the ground state, the scattered photon loses energy due to excitation of the vibrational mode and the scattered signal appears at a lower frequency ν_s , resulting in Stokes-scattering. When the molecule is in an excited vibrational state, the photon gain energy from the excited molecular vibrations and the scattered signal appears at higher frequency ν_{as} , resulting in anti-Stokes scattering [34, 35].

The mechanism behind the shift in frequency can be explained by considering the effect of induced dipole moment in the molecules cause by the EM wave. The polarizability of the molecule is a function of the vibrational coordinate and therefore the induced dipole moment is modulated by the molecular vibrations. This

causes the oscillation to occur not only at the frequency of the incoming field, but also at side bands determined by the sum (anti-Stokes) and difference (Stokes) of frequencies between the incoming light and the molecular vibrations. The Raman spectroscopy probes vibrational levels of the molecule, which provides information of specific atomic structure and arrangement as well as bond strength. The resulting Raman spectrum is often regarded as a spectral fingerprint of the molecule.

The intensity of Raman scattering, $RS(\nu_S)$, is proportional to the Raman cross-section, σ_{free}^R , the excitation laser intensity $I(\nu_L)$ and the number of molecules in the probe volume, N , as described by equation (1) [36]:

$$RS(\nu_S) = N\sigma_{free}^R I(\nu_L) \quad (1)$$

Typically, the Raman cross-section of a molecule is 10-12 orders weaker than that of fluorescence. Consequently, the characteristic peaks of the Raman signal are masked by strong fluorescence background. The inherently weak Raman signal can be significantly enhanced by attaching the molecule-of-interest on a nano-textured surface. This serves to provide significant amplification to the Raman signal through the phenomenon of surface enhanced Raman scattering. It is broadly recognized that the enhancement of the Raman signal is due to the EM enhancement generated from the plasmonic interaction of the metallic nano-structures as well as the chemical enhancement (CE) due to the charge transfer interaction between the metallic surface and the molecule as described by equation (2) [36]:

$$SERS(\nu_S) = N'\sigma_{ads}^R |A(\nu_L)|^2 |A(\nu_S)|^2 I(\nu_L) \quad (2)$$

While the EM and CE enhancements are the two commonly reported factors, there exist other mechanisms such as the scattering efficiency of a substrate, that may also affect the SERS enhancement such. In equation (2), the number of molecules involved in the SERS process is denoted as N' which could be smaller than the number of molecules in the probe volume N . The CE enhancement due to the chemical interaction between the molecule and the metal surfaces, typically of the order of 10-100 in magnitude, gives rise to a new effective Raman cross-section as described by σ_{ads}^R .

The chemical interaction is a result of charge transfer between the absorbed surface and the target molecule which is dependent on the surface material in relation to the intrinsic nature of the molecule. It therefore offers limited opportunity for considerable enhancement to the overall SERS effect. Furthermore, the choice of SERS active materials is limited to the rare-earth metal, of which the most established are gold and silver, which further limits the ability to optimize SERS through the CE mechanism. By contrast the EM enhancement, which has demonstrated more than 10^3 enhancement, offers more potential for enhancement. Various strategy and innovative approaches have been demonstrated to produce SERS substrate that exhibit tremendous local field enhancement.

The major contribution to SERS comes from the LSPR induced EM enhancement of the incident and scattered field which is represented by the gain factors $|A(v_L)|^2$ and $|A(v_S)|^2$ respectively.

2.2.1 Localized Surface Plasmon Resonance

Materials that possess a negative real and small positive imaginary dielectric constant exhibit surface plasmon resonance (SPR). This resonance, as illustrated in Figure 2.2, is the effect of coherent oscillation of the surface electrons against the restoring force of positive nuclei in the presence of an EM wave. Typically, SPR occurs in two characteristic form: (a) the Surface Plasmon Polariton (SPP) in the case of continuous metallic film and (b) the Localized Surface Plasmon Resonance (LSPR) in the case of discretized metallic nano-particles. SPPs are excited plasmons that propagate similarly to an EM wave over a distance of 10-100 μ m in the x - and y -directions along the metal-dielectric interface, and decay evanescently in the z -direction with $1/e$ decay length of the order of 200nm. The propagating surface plasmon interacts with the attached molecule-of-interest and leads to modulation of the plasmon resonance condition. This can be observed by measuring the reflectivity as a function of either the angle-of-incidence (at a constant wavelength) or wavelength (at a constant angle-of-incidence). Two-dimensional spectra mapping of the reflectivity can also be computed at a constant wavelength or angle-of-incidence.

Figure 2.2 Illustration of propagating surface plasmon on a metal film (left) and localized surface plasmon resonance of a metal sphere (right) in the presence of an incident EM wave

On the other hand, LSPRs are localized excitation of surface plasmon at a specific frequency that exist in discretized metallic nano-particles where the dimension is comparable or even smaller relative to the incident wavelength. This phenomenon can be explained by considering the electron cloud behaviour of a metal sphere in the presence of an EM wave as shown in Figure 2.2. Under resonance condition, the electron cloud experience a maximum displacement from the nucleus which results in a strong absorption and scattering of light. The resonance wavelength for the occurrence of LSPR is strongly dependent on the sizes, shapes, materials and the local dielectric environments of the metallic nano-particle or nano-structure.

2.2.2 Electro-magnetic Enhancement

The excitation of LSPR is the primary mechanism that accounts for the strong EM enhancement occurring in SERS. Upon laser excitation, a region of intense field enhancement, commonly known as SERS hotspots are generated in gaps and crevices of roughened plasmonic surfaces or nano-structures. The EM enhancement mechanism can be modelled by considering the electric field experienced by a molecule in the vicinity of a metal sphere as shown in Figure 2.3.

Figure 2.3 Schematic illustration of the EM field enhancement of SERS by considering a metal sphere as the SERS active substrate

A small sphere with a complex dielectric constant $\varepsilon(\nu)$ in a surrounding medium of dielectric constant ε_0 is used to represent the metallic nano-structure. The diameter of the sphere is set to be small as compared to the wavelength of light so as to limit Rayleigh scattering. The target molecule is placed in close proximity to the sphere and is exposed to the field, $E_m(\nu)$, corresponding to the superposition of the incoming field, $E_0(\nu)$, and the field, $E_{sp}(\nu)$, resulting from the induced dipole in the metal sphere. The EM enhancement factor, $A(\nu)$, can be estimated as the ratio of the field at the position of the molecule and the incoming field as described by equation (3) [36]:

$$A(\nu) = \frac{E_m(\nu)}{E_0(\nu)} \approx \frac{\varepsilon(\nu) - \varepsilon_0}{\varepsilon(\nu) + 2\varepsilon_0} \left(\frac{r}{r+d} \right)^3 \quad (3)$$

When the real part of $\varepsilon(\nu)$ is equal to $-2\varepsilon_0$, the EM enhancement factor is particularly strong. In addition, $A(\nu)$ can be maximized by reducing the imaginary part of the dielectric constant to attain the resonant condition for surface plasmon excitation. By considering the enhancement of the incident field as well as the scattered fields, the overall EM enhancement factor $G(\nu_S)$ can be express as:

$$G(\nu_S) = |A(\nu_L)|^2 |A(\nu_S)|^2 \approx \left| \frac{\varepsilon(\nu_L) - \varepsilon_0}{\varepsilon(\nu_L) + 2\varepsilon_0} \right|^2 \left| \frac{\varepsilon(\nu_S) - \varepsilon_0}{\varepsilon(\nu_S) + 2\varepsilon_0} \right|^2 \left(\frac{r}{r+d} \right)^{12} \quad (4)$$

Equation (4) [36] provides a simplistic model to estimate the SERS enhancement factor contributed by the EM enhancement mechanism. It shows that the enhancement of the local field by the metallic nano-structure is approximately

scaled to a power of four and that the enhancement is optimized when the incident field and the scattered fields matches the resonance of the surface plasmons. This is typically observed in low-frequency Raman modes where both the Raman mode and laser excitation are close to the resonance peak. It also suggests that the scattering powers of different Raman bands in a spectrum decreases with increasing vibrational energy, indicating that the enhancement of the Raman band is not uniform throughout the entire spectrum [36].

Direct contact between the molecule and the metal surface may not be necessary for SERS to take place. However, the field of the induced dipole decays with growing distance, d , between the molecule and the metal sphere (Figure 2.3). This cause the EM enhancement to decrease by a factor of $1/d$, leading to significant reduction in SERS. Theoretical studies have shown that sharp features and curvature on gold and silver nano-structures exhibit stronger EM enhancement. SERS enhancement factor can be increased to nearly 10^{11} by sharpening one of the edges on a metallic sphere [37]. Additional field enhancement can be achieved by decreasing the gap between nano-structures to increase the near-field coupling interactions [38]. An estimated EM enhancement factor of up to 10^{11} was predicted with a 1nm separation between two gold or silver spheres [48].

2.3 Evaluation of SERS Substrate

2.3.1 Sensitivity

The sensitivity of a SERS substrate can generally be improved by increasing the density of SERS hot spots to increase the average SERS enhancement factor (EF). Sensitivity comparison across all substrates are typically made by determining the average SERS EF or by the direct measurement of the lowest detection limit.

EFs has been extensively used for comparing the sensitivity across different class of SERS substrates. The most widely used definition for the average SERS is:

$$EF = \frac{I_{SERS}/N_{Surf}}{I_{RS}/N_{Vol}} \quad (5)$$

where N_{Vol} and N_{Surf} represent the average number of molecules in the scattering volume that participates in the Raman and SERS measurement respectively, and I_{RS} and I_{SERS} represent the intensity of the Raman and SERS signal respectively [39]. It is important to note that an accurate determination of EF is challenging. The uncertainty arises from the difficulty in determining the average number of molecules, N_{Surf} , adsorbed onto the substrate that actually contributes to SERS and in obtaining the reference Raman signal, I_{RS} , of the target molecule. In most cases, EFs are estimated with several approximations and assumptions to simplify calculation.

For example, it is often assumed that the target molecule forms monolayer coverage over the substrate surface and that every molecule in the diameter of the laser spot contributes to SERS. In reality, only molecules in the vicinity of the intensely amplified EM field experiences SERS. SERS EF tends to be higher on silver coated nano-structures than gold due to the fact that silver material has a higher scattering efficiency of EM radiation [40]. Thus, EF may not be a fair metric for comparing substrates with different SERS-active coating. Caution has to be taken to ensure biased assumptions and approximations are not made when comparing the sensitivity of SERS substrates using the EF. Several considerations has been proposed for the accurate determination of EF [41, 42]. These include the normalization of I_{SERS} and I_{RS} based on the instrument used, ensuring a complete

coverage of the substrate surface with the target molecules to avoid random contributions from hot spots and the usage of standard molecules such as Rhodamine 6G (R6G) and crystal violet (CV) for a well-defined SERS spectrum.

Figure 2.4 (a) SERS spectra of a series of low-level malachite green and a blank reference collected on a triple processed gold nano-island array with 40 nm gold deposition. **(b)** Relationship between then Raman intensity at 1615cm⁻¹ and the concentration of the analyte solution[43]

The limit of detection (LOD) measures the lowest detectable concentration and provides a more direct and practical quantification of the SERS sensitivity for comparison across various substrates. The LOD is typically quantified by the concentration of the probe solution that corresponds to the smallest SERS signal that is still distinguishable from the background noise. It is often estimated by visually observing the SERS signal-to-noise ratio becoming close to unity as the concentration of the probe solution gradually decreases. A more scientific approach in determining the LOD can be achieved by measuring the SERS signal of a solution as a function of decreasing concentration and plotting the SERS intensity at a particular Raman peak vs concentration curve as illustrated in Figure 2.4(b). The

LOD can then be determined from the SERS intensity-concentration curve using the following relation:

$$C_{LOD} = \frac{3 \sigma_y}{m} \quad (6)$$

where σ_y is the standard deviation of the lowest concentration and m is the gradient of the calibration curve [44].

The general accepted rule is that the signal must be at least two or three times greater than the background noise depending on the stringency of the application. This can be achieved by comparing the ratio of the measured signals of a known low concentration with those of blank samples and establish the minimum concentration at which the analyte can be reliably detected. The significance of LOD for a given type of sample, instrument, and measurement method is that it expresses the lowest concentration of an analyte that is detectable. This value establishes the limit of the instrument and measuring any concentration below the LOD is a state of uncertainty.

2.3.2 Uniformity and Reproducibility

Considerations for the reproducibility of a substrate include spatial and sample-to-sample variations of the SERS signals. The spatial variation is affected by the structure uniformity of the substrate and the sample-to-sample variation is an indication of the stability and repeatability of the fabrication process. These two figures-of-merit are important considerations for the commercialization of substrates especially in the field of clinical SERS where signal reliability is extremely crucial. The spatial intensity variations of a SERS substrate is quantified using the relative

percentage standard deviation (RSD %) of 20-50 random spots on the substrate. Figure 2.5 illustrates two examples on the determination of spatial signal uniformity in the form of histograms.

Figure 2.5 (a) SERS spectra of 1 μM R6G aqueous solution collected from 10 different spots on Au-CuCl₂-AAO substrate. The inset is mean $\pm$ standard deviation of the Raman intensity at each characteristic peak [45] (b) 2D scanning SERS intensity distribution for 1573 cm^{-1} Raman peak-distribution (mean of 890 ± 137) [46].

Randomly chosen spots should be sufficiently far apart and covers all domain of the substrate. In addition, the signal uniformity of a given area of the substrate can be evaluated by carrying out a 2-dimensional (2D) scan to produce a Raman map of SERS signal distribution. However, such result may be unreliable as it does not take into account the effect of non-planar surfaces. The distance between the objective and the substrate may also not be optimized to collect the maximum SERS signal. This process can be performed on a few isolated batches of samples to obtain the sample-to-sample variation. A box plot is useful to represent the statistical mean and distribution of the data which includes the median and the inter-quartile range.

2.3.3 Scalability

Scalability is a measure of production throughput and cost-effectiveness of the fabrication process and is one of the key considerations for SERS substrate commercialization. To date, a suitable metric for quantifying the scalability of a SERS substrate has yet to be established. The scalability of a substrate can generally be determined by quantifying the throughput of the fabrication process per unit resources. This may include major factors such as cost and time of the fabrication process and is predominantly affected by the nature of the fabrication process. Nano-fabrication process such as EBL and FIB which writes individual pattern one at a time are considered low-yielding and hence low scalability.

Figure 2.6 Scalability of AAO membrane method; **(a)** and **(b)** show the square-type AAO free-standing membrane with 200 nm thickness and the transferred membrane on the Si substrate; **(c)** and **(d)** show the circle-type AAO free-standing membrane with 200 nm thickness and the transferred membrane on 3 inch Si wafer. **(e)** Shows 4 inch AAO free-standing membrane [47].

Template assisted fabrication technique such as the nanosphere-lithography (NSL) and nano-patterning through ultra-thin alumina mask (UTAM) utilizes a

template to fabricate large area of nano-structures in a single process step. These are considered high-yielding and hence are highly scalable. In some cases, scalability is determined by the area of the fabricated substrate. In one work, a facile and scalable patterning approach was realized by using ultra-thin AAO free-standing membrane as patterning template to obtain nano-wire array. A rigid aluminium frame, obtained by partial etching of the supporting bulk aluminium, is used to provide mechanical support to the thin AAO membrane. This method successfully realized a 4-inch wafer size ultra-thin (sub 100nm) AAO patterning membrane (Figure 2.6), which demonstrates the scalability of the technique.

2.3.4 Stability

The stability of SERS substrate is evaluated by determining the rate of deterioration of SERS signal over time. This is typically achieved by measuring the SERS spectrum over a period of time to observe for signal degeneration. Figure 2.7 illustrates two examples evaluating the SERS stability of substrates reported in the literature. In Figure 2.7(a), the SERS signal of graphene oxide-wrapped gold nano-rod is found to show no sign of deterioration for up to 60 minutes. In another study, the stability of gold nano-particles molybdenum disulfide quantum dots is evaluated for up to 60 days with no sign of deterioration as shown in Figure 2.7 (b). It is observed that the length of investigation varies from minutes to days and even to weeks in some of the reported substrates. In general, the period of investigation depends on the application of the substrate. For one-time usage disposable SERS substrates, the period of investigation can be as short as a few minutes.

Figure 2.7 (a) SERS stability of Graphene Oxide-wrapped Gold Nano-Rod (GO@GNR)-based nano-probe [48] and (b) SERS stability of gold Nano-particles-Molybdenum Disulfide Quantum Dots (NPs@MoS₂ QD) [49]

2.4 SERS Substrate Fabrication Strategies

The advancement in SERS is greatly dependent on the ability to fabricate highly uniform plasmonic nano-features that exhibit consistent and highly sensitive SERS signal. Sensitivity, signal uniformity and substrate reproducibility are the key elements for evaluating the performance of SERS substrates. Nano-gaps and crevices of sub-100nm have been demonstrated to be highly efficient optical cavity for facilitating near-field coupling of the LSPR excitation which contributes significantly to the overall SERS enhancement. The optical resonance of the substrate is strongly dependent on the incident laser excitation wavelength with respect to the surface morphology of the substrate and the surrounding medium. By adjusting the geometry, inter-particle spacing and arrangement of the nano-features, the LSPR can be specifically tuned to optimize SERS enhancement under desirable laser wavelength [50]. Research emphasis on cost-effective fabrication process and optimization of nano-structures offers tremendous possibilities and potential towards the commercialisation of SERS devices.

SERS substrates are mainly classified into the following three categories: (1) random and non-uniform roughened surfaces, (2) well-dispersed nano-particles on planar substrates or colloid suspensions and (3) well-ordered periodic nano-arrays. The following section review the sensitivity, signal uniformity and substrate reproducibility of the various reported SERS substrates and fabrication techniques. In addition, an unconventional SERS approach known as shell-isolated nano-particle-enhanced Raman spectroscopy (SHINERS) which uses “smart dust” as SERS reporters for the characterization of bio-cells is also discussed.

2.4.1 Random and Non-uniform Roughened Surfaces

Randomly arranged or roughened surfaces possesses a high density of cost-effective hot spots [51, 52]. This is, however, at the expense of poor reproducibility and limited optical tenability, due to the limited control of the fabrication process. This section discusses three commonly fabricated roughened surfaces for the study of SERS: (1) electrochemical oxidation and reduction cycles (EC-ORC) roughen metal electrode, (2) post-deposition annealing gold nano-island and (3) selective dissolution of metallic alloy.

2.4.1.1 Electrochemical Oxidation and Reduction Cycles (EC-ORC)

Roughened silver electrode prepared by electrochemical oxidation and reduction cycles (EC-ORC) is the first reported SERS substrate [53]. An oxidation potential is first applied to the silver electrode to form soluble compounds or surface complexes. Subsequently, a reduction potential is applied to dissolve the species on the surface resulting in the formation of nano-structures. The ensuring SERS

enhancement is attributed to the EM hotspots localized in the nano-gaps formed by the surface complex of the roughened metal electrode

The roughening of the surface by the EC-ORC process is entirely random. Consequently, signals uniformity and substrate reproducibility is extremely poor. To improve the reproducibility of the substrate, a mechanical polishing and ultra-sound cleaning step is often incorporated prior to processing to remove defects and surface impurities [54]. Potassium chloride and sulphuric acid that produce specific waveform potential are preferred choice of electrolyte for a better control of the oxidation and reduction cycle in the EC-ORC process. Chloride containing electrolytes also facilitates the metal dissolution-deposition process that is essential for producing SERS active roughened surfaces. In general, enhanced controllability and reproducibility of surface roughness is achieved using triangular waveform over square waveform in the EC-ORC process [55, 56]. Other preparation conditions which affects the surface roughness of EC-ORC electrode includes the cathodic and anodic potential, scan rate and number of scan cycle [57, 58]. For example, it was demonstrated that SERS enhancement of roughened Ag electrode can be optimized by cycling with cathodic and anodic potential from -0.3 to +0.2 V at 25mVs^{-1} for a total of five cycle [59].

Typical enhancement factor of 10^4 - 10^6 was reported on EC-ORC roughened gold and silver electrodes. Lower enhancement factor of 10 - 10^4 was reported on roughened transition metal substrates including iron, cobalt, nickel, rhodium, palladium and platinum that exhibit a relatively low scattering efficiency in the visible wavelength range [60]. The EC-ORC process provides a simple fabrication approach in achieving good stability SERS substrate with low probability of contamination. In

addition, the potential dependent SERS properties of the EC-ORC roughened electrodes provides a suitable platform for studying SERS mechanism, in particular, the study of CE factor. Due to the limited control of the EC-ORC roughening process, sizes and shapes of the nano-features is non-uniform which give rise to a broad distribution of EFs across the substrate. Therefore, a common practice in ensuring reliable and reproducible SERS signals is to specifically select the spectra acquisition spot that is most representative of the true Raman measurement.

Figure 2.8 Rhodamine 6G (R6G) SERS spectrum and the corresponding SEM images of EC-ORC roughened silver substrates treated at temperatures of **(a)** 25 °C, **(b)** 100 °C, **(c)** 125 °C, and **(d)** 150 °C [59].

In a comparative study shown in Figure 2.8, the optimum electrochemically roughened silver electrode demonstrates a 9-fold improvement in SERS intensity over the typical electrochemically roughened silver electrode reported in the literature. Atomic force microscope measurements show that the optimally roughened silver electrode possesses a higher mean surface roughness of 143nm while the non-optimally roughened counterpart has a mean surface roughness of

only 40nm. The increase in surface roughness contributes to a larger density of EM hotspots and an improved SERS enhancement. Improved thermal stability was also demonstrated with the non-optimally roughened silver electrode showing diminishing Raman peaks at 125°C while the optimally roughened silver electrode pushes the envelope to 200°C. The elevated temperature caused the silver particles on the electrode to merge into larger and smoother clusters resulting in a reduced surface roughness and a lower SERS hotspot density.

2.4.1.2 Post-deposition Annealing

Roughened metallic gold film or discontinuous gold nano-islands can be alternatively fabricated onto planar substrates (eg, Si wafer, microscopic glass, graphite) or even nano-textured surfaces using electron-beam evaporator. A post-deposition annealing step is incorporated to migrate the gold atoms and cause it to agglomerate into island structure. Different size and shape of the nano-islands can be realized by controlling the deposition rate and thickness of the precursor gold film, and the temperature of the post-deposition annealing step. The gap between adjacent gold islands provide the essential confinement of the surface plasmon. This feature promotes near-field coupling of the LSPR mode and enhancement of the local EM field which collectively leads to SERS. Similar to the EC-ORC roughened electrodes, there is limited control over the formation of the discontinuous gold islands. The migration and accumulation of gold at elevated temperature is a random process. Consequently, there is a large distribution in the size and shape of the nano-islands which results in poor signal uniformity and substrate reproducibility.

Several innovative methods have been proposed to improve control over the formation of the nano-island substrates. It was demonstrated that well-controlled

growth of the gold nano-island array can be achieved by repeating the gold deposition and post-annealing process for up to 3 cycles as illustrated in Figure 2.9. With each deposition, the gold material pile onto the preceding gold nano-islands and the subsequent annealing step merge the gold materials to grow the nano-islands. The triple deposition/post-annealing cycle produces gold nano-islands with a diameter of approximately 60nm. These optimized nano-islands exhibit the strongest SERS signal using Malachite green as the probe molecule. A final gold film is deposited over the triple processed nano-islands to further enhance the SERS sensitivity and the signal deviation is improved to approximately 7% over a 5cm x 5cm domain.

Figure 2.9 Schematic of the repeated deposition and post-annealing fabrication cycle [43].

2.4.1.3 De-alloying of Bimetallic Composite

Bi-continuous network of nano-porous metallic films synthesized by de-alloying method were proposed to realize cost-effective SERS substrates. Active plasmonic hotspots that resides in the large curvatures of the nano-sized ligament and nano-pores promotes strong coupling of the EM fields leading to SERS. Figure 2.10 shows the SEM images of the roughened surface by de-alloying bimetallic composite. In order to further improve SERS performance, 3D quasi-periodic wrinkles obtained from the thermal contraction of pre-strained polymer material was introduced as the supporting substrate. This was accomplished by the selective dissolution of silver from a silver/gold alloy to form bi-metallic nano-porous film which is then bonded onto a pre-strained amorphous polystyrene substrate. The as-formed assembly is baked and subsequently annealed to significantly shrink the underlying polystyrene substrate. The annealing process introduces wrinkles in the conforming nano-porous film and deformations along the ridges were created as illustrated in Figure 2.10(b). The resulting cracks vary in sizes from one nano-metre to tens of nano-metres and are found to be SERS active when tested with R6G probe molecule.

A typical enhancement factor of 10^9 is obtained for most hotspots on such substrates. Up to 10% of the hotspots exhibit an enhancement factor as high as 10^{10} - 10^{11} which enabled single molecular detection even in the case of non-resonating SERS excitation. However, a large distribution in spatial SERS enhancement factor is expected in substrate fabricated through the de-alloying technique due to the heterogeneous structure which consist of a mixture of nano-pores, nano-tips, nano-gaps and super lenses architecture.

Figure 2.10 Microstructure characterizations of wrinkled nano-porous films. **(a)** SEM micrographs of the flat nano-porous film with a characteristic length of 20 and 25 nm. **(b)** Microstructure of a wrinkled nano-porous film with a quasi-periodic wavelength of 10 and 15 μm. **(c)** Chemical composition of a nano-porous film measured by energy dispersive X-ray spectroscopy. **(d)** Microstructure of wrinkle ridges showing nano-gaps, interleaving broken ligaments and linear chains of self-similar nano-cavities [61]

2.4.2 Chemical Synthesis of Well-dispersed Nano-particles

Metallic nano-particles colloid is one of the simplest forms of SERS substrates that can be synthesized by a variety of chemical procedures such as chemical reduction, chemical replacement, electrochemical reduction, photochemical, thermal decomposition, and ultrasonic decomposition [62]. The chemical reduction method is by far the simplest and the most widely used technique for fabricating gold or silver nano-particles colloid SERS substrates. A starting metal salt is reduced in aqueous solution by a reducing agent (eg. sodium citrate, alcohol, hydrogen) [63-66]

and a surfactants such as sodium dodecyl sulfate (SDS), cetyl trimethylammonium bromide (CTAB), poly vinyl pyrrolidone (PVP), to produce colloidal suspensions of nano-particles with tuneable diameter of 10-80nm. The surfactant acts as a capping agent to reduce aggregation or oxidation of the synthesized nano-particles and also serve to control the final shape and size of the nano-particles. In addition, the geometrical distribution and the aggregation state of the synthesized nano-particles can be controlled by other parameters such as the type of metal salt, the reducing agent, the surfactant and also their relative concentrations used in the process. The temperature and pH value of the solution are also found to influence the quality of the synthesized nano-particles.

Sau et al. [62] demonstrated a high yielding solution-based seed-mediated growth method to produce gold nano-particles with controllable morphology and dimension by manipulating the relative ratio of the reagent (Figure 2.11). The formation of various shapes is likely the outcome of the interplay between the faceting tendency of the stabilizing agent and the growth kinetics. The solution contains the chloroauric acid (HAuCl_4) which supply the gold seed, CTAB surfactant and ascorbic acid (AA) as reducing agent. A vast variety of nano-particle architecture such as rod, rectangle, hexagon, cube, triangle, star-like outlines and branched gold particles were successfully synthesized in the experimental study by varying the ratio of the reagent.

Figure 2.11 TEM (inset SEM) images of Au nano-particles synthesized under different conditions. Ascorbic Acid (AA) concentration increases from A to B; and seed concentration increases from C to D. (E, F, G, H): Transformation from cubic to rod-shaped gold particles at low AA concentrations in the presence of a small quantity of silver nitrate. Different dimension obtain with different relative concentration of CTAB, seed and Au^{3+} . Scale bar:100nm [67]

In another study, a similar seed-mediated approach was employed to synthesis gold nano-particles of 43nm average size which are immobilized onto ITO/glass substrate using a self-assembly strategy [68]. The treated ITO/glass substrate is immersed in a PVP and AA modified gold nano-particle solution to allow the self-assembly of the gold nano-particles.. Figure 2.12 shows the SEM images of the resulting gold nano-particles arrays and the corresponding extinction and SERS spectra of R6G Raman molecules.

Figure 2.12 SEM images of gold nano-particle arrays obtained with deposition times of **(a)** 2 days, **(b)** 4 days, **(c)** 6 days, and **(d)** 8 days. The corresponding extinction spectra (inset shows the photograph of the four samples) and SERS spectra of 10^{-6} M R6G under 514 nm excitation are also shown [68].

The density of the gold nano-particles was found to increase with incubation time. Agglomeration of the gold nano-particles into nano-chains occurs as the deposition time increases beyond 6 days. The change in structural architecture caused the extinction spectrum to be blue-shifted, the LSPR peak to increase and two additional bands near the 600nm wavelength to be induced. The increased in the density of the gold nano-particles shows improvement in SERS sensitivity of the substrate. Under 514nm laser wavelength excitation, the agglomerated gold nano-chains are found to exhibit SERS enhancement factor of 5.83×10^6 which is approximately two-fold higher than that of the isolated gold nano-particles (enhancement factor of 9.32×10^5).

Figure 2.13 (a) UV-Vis spectra of different sizes of silver nano-particle colloids made by seeded growth with ascorbic acid and diluted to a 1:30 ratio of colloid/water. **(b)** Plot correlating the calculated SERS enhancement factor, G , with AgNP size for two separate sample series (blue and red dots) [69]

In another work, the seed growth method was used to synthesize spherical silver nano-particles (Ag-NP) to investigate the particle size for optimizing SERS enhancement. The final size of the Ag-NP can be controlled by the initial concentration of the Ag-NP seeds, where higher concentration of the seed produces smaller particle size. The Ag-NP is characterized using UV-Vis spectroscopy and a diminishing, broadening and red-shifting of the absorbance peak in the 400nm wavelength range is observed as depicted in Figure 2.13 (a). Consequently, the SERS enhancement factor, G , was found to increase linearly under an off-resonance excitation wavelength of 785nm. SERS-active nano-particles colloids can have monometallic, alloy, or bimetallic composition.

The advantages of metal nano-particles colloid as SERS substrate over the EC-ORC roughened electrode and post-deposition annealing induced nano-islands are its cost effective fabrication approach, a relatively better size control and shapes to achieve the desirable optical properties and an extremely high enhancement factor that enable single molecular detection [70, 71]. Silver and gold nano-particles

with a diameter of about 200 nm and 60 nm respectively are reported to exhibit the highest SERS enhancement under 647nm wavelength excitation [72, 73]. Theoretically, SERS enhancement factor of 10^3 - 10^4 can be achieved on single gold nano-particle while even higher enhancement factor of 10^6 - 10^7 is possible with single silver nano-particle. With the aggregation of two or multiple nano-particles, the resulted near-field coupling of the local EM field is capable of producing exceptionally large enhancement factor as high as 10^{14} that enable single-molecular detection by SERS [74, 75].

However, due to the large size and shape distributions in of the nano-particles from aggregation, the spectra reproducibility is generally poor. Chemical degradation of the nano-particles can also be expected due to the change in surface charge or the desorption or decomposition of the polymeric molecules. This makes long term storage of the substrate challenging. Fresh substrate has to be fabricated prior to its use to ensure functionality. Furthermore, the addition of sample molecules and salt to the nano-particles colloid may form precipitation that can quench SERS signal within minutes [76]. Hence, reliability of the measurement is greatly affected.

2.4.3 Well-ordered Periodic Nano-arrays

In contrast to the random and non-uniform roughened surfaces and nano-particles colloid, well-ordered periodic nano-arrays exhibit a more homogenous distribution of SERS hotspots with good reproducibility due to a better control of the fabrication process. Well-ordered structures fabricated via photo-lithography patterning method is cost effective, highly scalable and reproducible. However, fabrication cost increases significantly with smaller features as high resolution photo-mask are costly, especially in low-volume fabrication requirement. Direct writing

approaches such as, sub-wavelength EBL and FIB lithography are suitable choices for high resolution structures (sub-10nm) and low-volume fabrication requirement. To extend its cost-effectiveness for high-volume fabrication requirement, a soft-lithography approach can be employed to transfer the pattern repeatedly from the master stamp fabricated by the EBL or FIB technique onto flexible substrates [77, 78]. Challenges lies in the pattern transfer integrity and resolution, aspect ratio limit and the reusability of the master stamps.

In recent development, NSL and UTAM assisted patterning technique has become popular due to the self-assembly nature of the patterning template which promotes high scalability and cost-effectiveness. The following section discuss the various techniques used to fabricate well-ordered periodic nano-array SERS substrate and their inherent challenges and limitations.

2.4.3.1 Optical Photo-lithography

Current optical photolithography technique has enable the fabrication of reproducible structures with resolution down to 32 nm. Highly ordered and periodic SERS-active silver nano-pillar (Figure 2.14) is realized by silver metallization of silicon dioxide nano-pillar fabricated using photo-lithography [46]. A photo-resist coated silicon dioxide wafer is first patterned by light exposure through an optical mask. Deep reactive-ion-etching (RIE) is applied through the patterned photo-resist to produce the silicon dioxide nano-pillars. Due to the anisotropic nature of the RIE process, the fabricated pillars tapered towards the tip resulting in a conical structure with a diameter of 150nm and an inter-structural spacing of 350nm. After coating with electron-beam evaporated silver, the nano-pillar array exhibit a broad extinction resonance of approximately 500-700nm and a SERS enhancement factor of

approximately 5×10^7 is measured using benzene-thiol as the Raman probe. An improved in SERS signal-to-noise ratio was demonstrated using the optical lithography approach to fabricate silver coated nano-pillars.

Figure 2.14 (a) SEM images and (b) extinction spectrum of silver nano-pillars SERS substrate deposited with 80 nm of silver. (c) Normal Raman and SERS spectrum of benzenethiol. (d) Probability distribution showing the enhancement factor across the substrate [46]

AgNP-Si-ZnO Nanotrees

Figure 2.15 SEM image of Ag nano-particle decorated Si/ZnO nano-trees and SERS spectra of R6G molecule(10^{-5} M) at 532nm excitation collected from (a) Si/ZnO nano-trees; (b) 2-D Ag nano-particles substrate prepared by sputtering method; (c) Ag nano-particle decorated Si/ZnO nano-trees. [79]

Several innovative surface modifications has been demonstrated to further improve the SERS sensitivity of conventional nano-pillars. In general, such approaches aim to increase the density of SERS hot-spots by increasing the surface roughness of the nano-pillars through additional nano-scale features. For example, *Cheng et al* uses a bottom-up hydrothermal growth of zinc oxide nano-rods to introduce 3-D hierarchical tree-like nano-structures onto photo-lithographic fabricated silicon nano-pillars [79]. The growth of the ZnO nano-rods onto high aspect ratio Si pillars increases active surface area. When decorated with silver nano-particles, the substrate exhibit a SERS enhancement factor of approximately 10^6 and demonstrates a significant improvement in signal-to-noise ratio (Figure 2.15). The improved SERS enhancement is attributed to the large density of EM enhancement hotspots as a result of depositing silver nano-particles over the hierarchical nano-tree morphology. SERS spectra of R6G molecules over 15 random chosen spots on the substrate demonstrate decent signal reproducibility. Slight fluctuations in the

signal can be expected due to the non-homogenous adsorption of the silver nano-particles onto the nano-tree structure.

2.4.3.2 Electron-beam Lithography

Electron-beam lithography (EBL) is a highly versatile and controllable fabrication technique for producing high resolution and reproducible 2-D array of nano-structures. Geometry and separation of nano-structures can be precisely controlled down to the nano-metre scale. In a typical EBL process, an electron sensitive resist such as poly (methyl methacrylate) (PMMA) is spin coated onto a silicon or silicon dioxide wafer. The pattern is programmed into an EBL system which controls the electron gun to write directly onto the resist. The resist is subsequently developed and the patterned resist can be used as an etching mask or subject to a lift-off process by depositing a layer of titanium/gold to produces the required nano-structures

Figure 2.16 Schematic illustration of the two possible approach using the EBL method for fabricating nano-structures as SERS substrate [80]

Figure 2.16 illustrates the possible fabrication route using the EBL techniques to produce nano-arrays. Several arbitrarily-shaped nano-structures such as rhombus-shaped nano-holes, circular nano-holes, nano-grid, nano-gratings, nano-triangles, nano-discs and split-ring resonators can be realized using the EBL technique (Figure 2.17). The resulted nano-arrays exhibit SERS enhancement after deposition with a layer of gold material [80].

Figure 2.17 SEM images of gold nano-structures fabricated with EBL combined with plasma etching or lift-off process.[80]

The SERS properties of the nano-structures are highly dependent on their geometry and arrangement. It was demonstrated that the SERS intensity of the nano-gratings can be enhanced by reducing the period of gratings while fixing the line width at 60nm [80]. This effectively decreases the gap size and also increases the density of nano-gaps of the structure. Similar trend is observed on the nano-discs where the SERS intensity increases with decreasing period. The high-resolution EBL technique can realize such precise modifications of the structure geometry.

Figure 2.18 schematic representation and SEM images of the Au-SiO₂-Au tandem truncated nano-cones comprises (60 nm radius array with period 200 nm) Scale bar: 300 nm.[81]

The highly controllable EBL technique also enables the fabrication of tuneable tandem truncated nano-cones with an Au-SiO₂-Au layers, as shown in Figure 2.18. The individual Au-SiO₂-Au layer was specifically tuned to achieve double resonance effect at both the laser excitation and also the Raman scattered frequencies. A maximum SERS enhancement factor of 3.86×10^7 was achieved. This is 10-fold higher than that of the single resonance structure consisting only of gold material. The SERS enhancement can be improved by one order of magnitude by increasing the radius of the nano-cones from 50nm to 60nm. This is attributed to the near-field coupling effect that enhances the localized electric field at the significantly reduced inter-particle gap between adjacent nano-cones.

2.4.3.3 Focused Ion Beam

Similar to the EBL technique, focused ion beam (FIB) direct milling is a highly controllable nano-fabrication technique for producing high homogeneity nano-structures with precise geometry and inter-structure spacing. In FIB, instead of

transferring the pattern onto the resist, the pattern is directly etched onto the target material deposited on a substrate using highly energetic ion bombardment. Such direct writing technique has enabled the fabrication of nano-structures of arbitrary shapes and sizes.

Sivashanmugan *et al* fabricated well-ordered acute-angled gold nano-rod (NR) arrays tilted at an angle of 30–90° on a silicon wafer using the FIB directing milling method [82]. The initial thin gold film is deposited onto polished (100) silicon wafers primed with an adhesion layer of titanium. The pattern is written directly onto the gold material using highly focused 30kV gallium ion beam. Various acute angle gold nano-rods were fabricated by adjusting the substrate tilted angle, the working current and the exposure time as illustrated in Figure 2.19. The tilted gold nano-rods were found to exhibit higher SERS enhancement than the vertically upright geometry and the largest tilted morphology from the vertical exhibiting the highest enhancement factor of 7.781×10^7 . The origin of the enhancement is attributed to the strong local EM enhancement effect contributed by the increased number of SERS active site when the tilt angle of the gold nano-rod is large. Molecules near the strongly enhanced EM field experience a significant amplification in SERS signal. The application of the optimized acute angle gold nano-rods in the field of food analysis was demonstrated by identifying cyanuric acid in milk solution with decent signal reproducibility.

Figure 2.19 FE-SEM images and the corresponding SERS measurement on rose Bengal molecules on the acute-angle gold nano-rod tilted at different angle fabricated by focused ion-beam [82].

Advanced complex geometrical structures such plasmonic nano-antennae with ultra-small inter-particle separations and different outlines can also be realized by direct FIB milling lithography [83]. The densely packed plasmonic nano-antennae with ultra-small gaps enable strong field enhancement. Different modifications of the array parameters can be realized by adjusting the geometric variables of the milling process. The tuneable optical properties and SERS capability of the nano-antennae has promising potential in the applications of plasmonic sensors.

2.4.3.4 Nanosphere-Lithography

FIB and EBL lithographic techniques possess the advantage of high reproducibility and controllability, allowing nano-structures with arbitrary shape, size and inter-particle spacing to be easily fabricated. The high resolution of both processes also enable fabrication of ultra-small nano-features which exhibit unique optical and plasmonic properties. However, the fabrication throughput is extremely low due to the direct writing process and they are not cost-effective especially for high-volume fabrication.

Figure 2.20 Schematic illustration of the self-assembly of polymeric sphere by **(a)** spin coating **(b)** capillary action assisted scooping and **(c)** the Langmuir Blodgett self-assembly technique.

Nanosphere-lithography (NSL) is a potentially cost-effective large scale patterning approach for fabricating highly ordered and uniform SERS nano-structures. It involves the self-assembly of a layer of mono-disperse spherical polymeric spheres which serves as the template for metal deposition. The self-assembly of the nanosphere array is typically accomplished by three commonly used techniques: (1) spin-coating and drying of polystyrene beads suspension, (2) capillary action assisted oblique angle scooping and (3) Langmuir-Blodgett self-assembly which involves the pulling of the substrates off a polystyrene nanosphere suspension assisted by a barrier. With the aid of surfactant, the hydrophobicity of the polystyrene nanosphere, suspended at the gas-liquid interface, is enhanced [84].

This led to the formation of a mono-layer of nanospheres, which by means of a barrier, is compressed into a closely packed hexagonally arranged lattice. Simultaneously, the barrier is compressed further as the substrate is pulled upward from the nanosphere suspension at a controlled rate. The rate of barrier compression and substrate withdrawal is carefully designed to ensure pressure acting on the sphere and on the substrate is within equilibrium spreading pressure to obtain mono-layer of deposited sphere [83]. After depositing a layer of metal, a low-cost metallic film over nanosphere (MFON) SERS substrates that demonstrates excellent SERS sensitivity and signal uniformity can be fabricated.

Gold-film-over-nanosphere (AuFON) and silver-film-over-nanosphere (AgFON) are the two established types of MFON SERS substrate that can be easily fabricated using the NSL technique. The nano-gap at the curvature of the metallic sphere junction serves as plasmonic hotspot which produces intense near-coupling of the LSPR mode under appropriate laser excitation. This generates enormous EM enhancement of the local field responsible for the significant enhancement of the Raman signal.

Figure 2.21 SEM images of BMFON substrates with underlying silver layer of 160 nm and upper gold layer of (a) 0 nm, (b) 120 nm, and (c) 320 nm thickness [85].

While different sizes of nanosphere suspension are available, additional nano-fabrication processes are required to achieve specific tuning of the inter-particle gap. A simple and widely used approach is to increase the SERS-active metal layer to reduce the inter-particle gap. Fu *et al* compared the SERS performance of singularly coated AuFON and AgFON with a hierarchical bimetallic film over nanosphere (BMFON) fabricated by depositing a layer of gold film over silver film (Figure 2.21) [85]. It was reported that there exists an optimal thickness for maximum SERS enhancement. Furthermore, excessive deposition of metal materials cause the discrete metal particles to coalesce into larger grains unfavourable for SERS due to the diminished surface roughness. The bimetallic configuration has the advantages of high SERS enhancement arising from the underlying silver film and an improved stability provided by the outer gold film. The optimized BMFON exhibit a SERS enhancement factor of 2×10^7 and showed minimal degradation in SERS signal after a week.

Figure 2.22 Schematic of the porous silicon nanopillar fabrication procedure: **(a)** formation of a monolayer of polystyrene (PS) nanospheres, **(b)** nanosphere etching via reactive ion etching, **(c)** gold deposition, **(d)** bead removal, and **(e)** catalytic chemical etch of the underlying silicon substrate. **(f)** Tilted SEM image of a porous silicon nano-pillar array. SERS spectrum of the silver silicon nanopillar substrates incubated in R6G [86].

The polymeric spheres (PS) can also be used as a patterning mask for fabricating nano-pillars. Kiraly *et al* fabricated multifunctional silicon nano-pillar arrays with anti-reflection, super-hydrophobicity, photoluminescence and SERS properties by incorporating a catalysis etching step after the NSL process [86], as shown in Figure 2.22. Self-assembly of the polymeric sphere is achieved through a combination of spin-coating and capillary action assisted 60° oblique angle scooping. The stabilized mono-layer of PS beads is transfer onto a piece of silicon wafer with the assistance of capillary action. This 2-step transfer process allows the PS bead to attain a close hexagonal packing with reduced dislocation and discontinuity on the final silicon substrate.

Reactive Ion Etching (RIE) was performed on the PS assembly to reduce the diameter followed by a subsequent gold deposition and PS removal step to fabricate hexagonal gold nano-hole array. A metal-catalytic etching step, where the metal protected silicon experience a significantly higher etching rate than the unprotected to produce silicon, was performed using a mixture of hydrofluoric acid (HF) and hydrogen peroxide (H₂O₂) to produce nano-pillar array. Metallization of the nano-pillar for SERS is accomplished in two deposition steps: electrochemical deposition of silver as the intermediate layer and DC sputtering of thin gold film as the final layer. The metalized nano-pillars are annealed at 500°C to redistribute the metal to the sidewall to improve conformity.

2.4.3.5 Ultra-thin AAO Membrane (UTAM)

Template-assisted fabrication techniques have been extensively used to create large area of cost effective nano-structures for SERS applications. Ultra-thin

alumina mask (UTAM) derived from standard anodized aluminium oxide (AAO), is the most popular hard templates used due to its high scalability and ease of production. The AAO template obtained from the anodization of aluminium comprises of hexagonally arranged alumina nano-tubes which appear as two dimensional nano-hole array from the top (Figure 2.23). Three well characterized growth regime of the AAO was demonstrated using sulphuric, oxalic and phosphoric acid as the electrolyte. The required anodization voltage also varies with the electrolyte used which resulted in different pore diameter of the AAO. The geometry of the AAO template can be easily controlled by varying the applied potential, the choice of electrolyte, the electrolyte pH and concentration, the anodization duration and pre-patterning of the aluminium surface. Further modification of the dimension such as widening the pore diameter can be achieved by additional wet-etching step.

Figure 2.23 (a) Schematic illustration of an AAO structure prepared by electrochemical anodization of aluminium substrate. **(b)** Summary of the anodizing voltage and corresponding inter-pore distance and pore diameter of the AAO fabricated in the three well-known regimes of electrolytes (sulphuric, oxalic and phosphoric).[87]

To utilize the template as a patterning mask, a through-hole membrane configuration is required. This is accomplished by selectively removing the bulk aluminium and the barrier layer of the AAO before transferring it onto the desired

substrate surfaces. Subsequently, the pore is wet-etched to ensure that the opening is sufficiently large to prevent mask shadowing effect which can affect the fidelity of the pattern transfer. Hexagonally arranged periodic nano-dot and nano-rod array can be realized using direct decomposition from metallic salts, thermal or electron-beam evaporation and electro-chemical deposition through the UTAM membrane.

In the work of Fu. *et al*, the UTAM template is used as a deposition mask to fabricate large-area of nickel/gold hybrid nano-particle array as depicted in Figure 2.24 [33]. Nickel (Ni) is first deposited as the fundamental building block through the UTAM to form nano-hole array on which the gold nano-particles is subsequently deposited as the SERS activation particles. By using nickel as the building block, lesser gold material is needed to achieve similar SERS enhancement from those attained from single gold NP arrays. The resulting regular pattern of the Ni/Au NP array exhibits a SERS enhancement factor of 10^8 and a detection limit of 10^{-10} M on crystal violet (CV) molecule. It was also demonstrated that excellent SERS signal reproducibility with a relative standard deviation (RSD) as low as 6–7% is achieved, and the substrate has a long-term stability of 10 months.

Figure 2.24 SEM images of Au/Ni hybrid NPs arrays with fixed size Ni NP at 25nm and increasing gold deposition of (a) 0nm, (b) 5nm, (c) 10nm, (d) 15nm, (e) 20nm and (f) 25nm and the corresponding (g) SERS spectrum of crystal violet (10^{-7} M) and the (h) SERS intensity vs Au thickness at 1618 cm^{-1} , 1372 cm^{-1} and 1177 cm^{-1} [33]

The UTAM template can also be used as an etching mask for patterning nanostructures on SERS substrates. Lee *et al* demonstrated a flexible SERS substrate consisting of sub-100nm quasi-3D metallic nano-holes fabricated by the UTAM assisted etching process [88]. The fabrication process consists of the direct pattern transfer of the nano-holes array onto a polymer film coated polydimethylsiloxane (PDMS) substrates by plasma etching through the UTAM, followed by SERS activation of the substrate by gold deposition (Figure 2.25). Before metallization of the nano-hole array, the UTAM etching mask can be conveniently removed via an adhesive tape. The well-ordered and periodic quasi-3D metallic nano-holes exhibit a SERS detection limit of 10^{-6} M on thiolated polyethylene glycol(Cy5) Raman dye molecules.

Figure 2.25 (a) - (c) A schematic illustration of the fabrication process for the Au quasi-3D nano-hole arrays for flexible SERS substrates. SEM images of **(d)** nano-patterned cPS film and **(e)** Au quasi-3D nano-holes . **(f)** SERS spectrum of Au quasi-3D nano-holes at different concentration of thiolated polyethylene glycol (Cy5) Raman dye molecules [88]

This top down fabrication approach has the major advantage of enabling selective patterning of curved surfaces of flexible polymeric substrate. It was demonstrated that by simply using an additional circular shadowing mask, selective patterning of the metallic nano-holes can be achieved on the polystyrene substrate. The resulting metallic nano-holes substrate showed good adhesion on glass surfaces and is an advantage for developing into a practical SERS fibre probe which convention patterning technique has difficulties fulfilling.

2.4.4 Shell-isolated Nano-particle-enhanced Raman Spectroscopy

Shell-isolated nano-particle-enhanced Raman spectroscopy (SHINERS) provides a non-conventional approach in acquiring SERS spectrum. Traditionally, the analyte is absorbed onto the substrate and SERS signal is collected by an objective lens. In SHINERS, the Raman enhancing surface exists in the form of ultra-

thin silica or alumina encapsulated gold nano-particles that are mono-disperse over the probe surface as "smart dust". These spherical SERS reporters have demonstrated to be an effective and flexible SERS substrate in the experimental studies of yeast cells and pesticide residues on food as illustrated in Figure 2.26.

In the yeast cells study, additional Raman peaks related to the bioactivity of living cells are detectable by the SHINERS approach which are absent in the normal Raman spectra. These results demonstrate the advantage of SHINERS for in-situ detection of cell wall proteins and as a possible probe of the dynamics and mechanisms of living system. In the study of pesticide residues on food, the SHINERS approach is found to be able to identify methyl parathion contamination on orange by detecting the $1,108\text{ cm}^{-1}$ and $1,341\text{ cm}^{-1}$ Raman band which are absent in the normal Raman spectrum (Figure 2.26 (b)).

Figure 2.26 (a) In situ probing of biological structures by SHINERS. Curves I, II and III, SHINERS spectra obtained from different spots at the wall of a yeast cell incubated with Au/SiO₂ nano-particles. Curve IV, the spectrum recorded from a substrate coated with Au/SiO₂ but without a yeast cell; curve V, a normal Raman spectrum for yeast cells. The peaks marked with red asterisks are closely related to mannoprotein. **(b)** In situ inspection of pesticide residues on food/fruit. Normal Raman spectra on fresh citrus fruits (Curve I), with clean pericarps (curve II), contaminated by parathion (Curve III), SHINERS spectrum of contaminated orange modified by Au/SiO₂ nano-particles (Curve IV) [89]

The chemically inert alumina/silica ultrathin shell prevents direct interaction between the gold nano-particles and the probed surface which can possibly distort the true vibrational information of the spectrum. It also prevents agglomeration of the gold nano-particles and improves the surface conformity which is beneficial for the characterization and imaging of biological cells. Each isolated gold nano-particle serves as a SERS hotspot for amplifying the Raman signal. In order to ensure SERS enhancement, the alumina/silica shells need to be ultra-thin (2-4nm) to allow the strong EM field from the gold core to propagate to the probe molecule. It was found that shell thickness of 20nm completely attenuates the Raman signal.

2.6 Conclusion

The SERS enhancement mechanism is highly dependent on the geometrical structure of the SERS substrate. The near-field coupling of the LSRP excitation localized at the nano-gap between adjacent metallic structures generate intense EM field enhancement under resonance condition which amplifies Raman signal by several orders of magnitude. SERS substrates can be classified into three main categories: random roughened surfaces, nano-particles colloid suspension and well-ordered periodic array. The choice of SERS substrate depends on the application requirements. Nano-particles suspension that are specifically functionalized to bind onto target analyte are appropriate choice for in-vivo SERS analysis or cell imaging application due to its high conformity. They are also suitable for pre-mature disease detection due to the extremely high SERS enhancement factor which enables single molecule detection. In qualitative analysis, random roughness surfaces offer a more cost-effective option as they can be simply fabricated in large scale. In the case of

qualitative analysis where spatial signal uniformity and substrates reproducibility are of concern, highly ordered and periodic plasmonic nano-structures has demonstrated to be an excellent choice of sensing surface especially in developing real-time in-situ SERS probe. Highly precise and controllable EBL or FIB process can be utilized as an indirect lithographic or direct writing technique to produce high resolution (sub-10nm) nano-features that are suitable for SERS sensing. However, due to the extremely low process throughput and the requirement of expensive and complex EBL and FIB system, such technique may not be economical in large scale production. Alternatively, self-assembled NSL technique and AAO template assisted fabrication method offer cost effective approaches for achieving large area of well-ordered and periodic nano-structures that exhibit excellent SERS sensitivity and reproducibility. These two techniques are extensively studied in this thesis to evaluate and realize highly scalable and reproducible SERS sensing surface for real-time in-situ application

Chapter 3 : Investigation of SERS Mechanism.

3.1 Introduction

The enhancement of Raman signal was first demonstrated using pyridine absorbed roughened silver electrode which led to the discovery of surface enhanced Raman spectroscopy (SERS) [53]. Since then, SERS has developed rapidly as a sensitive and specific label-free spectroscopy technique in biological and chemical sensing applications. Its multiplexing detection capability and non-invasive approach has benefited various applications such as complex chemical system analysis, and food and environmental analysis [90, 91]. The SERS enhancement mechanism has broadly been studied to identify design parameters for optimizing the performance of SERS substrates. The widely recognized factor contributing to SERS is the electromagnetic (EM) field enhancement of the local excitation and the Raman scattered fields induced by the collective oscillation of electrons, known as surface plasmon (SP) [92, 93] .

SP preferentially resides at the metal-dielectric interface where surface electrons oscillate coherently against the restoring force of the positive nuclei under the influence of an alternating incident EM radiation. In continuous metallic film, the oscillation of the surface electrons produce an EM wave that propagates along the metal-dielectric interface. In the discretized metal configuration, the surface electrons oscillate locally about the metallic nano-particle resulting in localized surface plasmon resonance (LSPR). Consequently, this generates an enormous local enhancement of the incident and scattered fields under resonance condition. To contribute to effective SERS signal, the enhanced EM field needs to also radiate out-of-plane. The factor affecting the efficient out-of-plane radiation of the enhanced field

has not been widely investigated or discussed. This warrants a detailed study of the effect of the associated factor namely the scattering cross-section, on the performance of SERS substrates.

In this work, the influence of the substrate scattering cross-section on SERS enhancement is investigated. The relation between LSPR excitation and SERS mechanism is studied by observing the attenuation in SERS intensity as the SERS metal layer evolved from a discretized (LSPR) to a quasi-continuous morphology. FDTD simulation is carried out to quantify how the scattering cross-section and absorbance changes when the near-field coupling of the plasmonic structure changes. SERS measurements are also performed on a gold-film-over-nanosphere (AuFON) substrate and a gold sputtered substrate to substantiate the importance of scattering cross-section on SERS enhancement. Based on the results obtained in this work, methods to improve the SERS performance of the gold sputtered substrates is proposed and verified.

3.2 Experimental Details

This section describes the preparation procedures of the three different types of SERS substrate used in this study: (1) discretized and quasi-continuous gold sputtered substrates for studying the surface plasmon modes in SERS enhancement, (2) gold film over nanosphere (AuFON) substrate for SERS comparison with the discretized gold nano-particle substrate, and (3) anodized-aluminium-oxide (AAO) nano-hole array as a 3-dimensional surface for increasing the scattering cross-section of deposited SERS particles.

3.2.1 Gold Sputtered Substrate

Gold (99.99% purity, JEOL) is sputtered onto 2cm x 2cm microscopic glass substrate (ThermoFisher Scientific) at a deposition rate of 0.11nm/s using a fine coater operating at a current of 10 mA. The sputtering duration is varied from 0-108s to obtain morphology ranging from discretized gold nano-particles to quasi-continuous gold configurations.

3.2.2 AuFON

Figure 3.1 Schematic diagram illustrating the preparation of AuFON substrate in the order of (a),(b), (c) and (d)

Polystyrene (PS) microsphere (diameter of 370nm) suspension and sodium dodecyl sulfate (SDS) were purchased from Polyscience and Merck respectively. The preparation process of the AuFON substrate is illustrated in Figure 3.1. A 1cm x 1cm silicon substrate is first treated with oxygen plasma to achieve high hydrophilicity. Next, 10 μL mixture of 2.36% PS beads suspension and 0.085% SDS is spin-coated onto the silicon substrate at 3000rpm with an acceleration of 250rpm/s for 30s. After spinning off the excess solvent, a mono-layer of hexagonally arranged

PS assembly is self-arranged on the silicon substrate. Metallization is performed by sputtering gold over the nanosphere template at a rate of 0.11 nm/s.

3.2.3 AAO Substrate

Figure 3.2 Schematic diagram illustrating the preparation of AAO by two-step anodization of aluminium in the order of (a), (b), (c) and (d)

Aluminium foil (99.99%) and oxalic acid ($\text{C}_2\text{H}_2\text{O}_4$) were purchased from Alfa Aesar. Ethanol ($\text{C}_2\text{H}_6\text{O}$), chromic acid (H_2CrO_4), phosphoric acid (H_3PO_4) and nitric acid (HNO_3) were obtained from Fisher Scientific. The AAO nano-hole substrate is fabricated by a standard two-step anodization of the high purity aluminium foil in an oxalic growth regime as shown in Figure 3.2 [94]. Prior to anodization, the high purity aluminium sheet is chemically polished in a mixture of H_3PO_4 and HNO_3 at temperature of 80°C follow by immersion in NaOH solution to remove native oxides and surface impurities. The aluminium is then copiously rinsed with de-ionized water to remove excess reagent. Anodization of the aluminium foil is carried out at room temperature in 0.3M $\text{C}_2\text{H}_2\text{O}_4$ electrolyte using a voltage of 40 V. The aluminium is anodized for 15min and the first oxide layer is subsequently removed by chemical

etching in a mixture of H_3PO_4 and H_2CrO_4 at 60°C . A second anodization step is then performed for duration varying from 10s to 210s to grow the final AAO layer.

3.2.4 Characterization

Surface morphologies of the substrates were characterized using a field emission scanning electron microscope (FE-SEM, SU8010, and Hitachi) operated at 5 kV. Optical transmission spectrum was measured using a Shimadzu UV-2450 spectrophotometer. SERS spectra measurements were carried out using an Alpha 500R confocal Raman microscopy system (WITec, Germany) equipped with a 532nm and a 633nm lasers (beam size: $\sim 0.5\mu\text{m}$ with a 50x, 0.8NA objective) and an EMCCD (UHTS300). The spectra of the 2-Naphthalenethiol ($\text{C}_{10}\text{H}_7\text{SH}$, Sigma-Aldrich) Raman probe was measured in the Raman shift range of 0 to 1800 cm^{-1} using a 600 g/mm grating. An excitation laser power of 0.1mW and an integration time of 10s were used. Prior to measurement, a silicon substrate was used to calibrate the system.

3.2.5 FDTD Simulation

Figure 3.3 FDTD simulation model of the gold sputtered substrate.

Three-dimensional finite-difference time-domain (3D-FDTD) simulation of the extinction cross-sections was obtained using the Lumerical Solution, Inc software. Figure 3.3 illustrates the schematic of the simulation model. The discretized gold nano-dots substrate is constructed using periodic array of gold cylindrical disks positioned on a glass substrate. The permittivity of gold and glass were obtained from the Johnson and Christy model and Palik model respectively. A total-field-scattered field (TFSF) excitation was injected at normal incidence onto the structure in the z-direction. Periodic boundary condition (PBC) is applied to the x and y-axes and perfectly matched layer (PML) condition is applied to the z-axis. The mesh size is set to 0.1nm for all three dimensions. The field analysis domain is constructed within and outside the TFSF source to monitor the absorbance and scattering cross-section, respectively.

3.3 Results and Discussion

3.3.1 Structural Characterization

The nominal thickness of the gold sputtered substrate is deduced from a calibration curve provided by the manufacturer. No adhesion layer was used prior to the gold deposition in order to have the flexibility of making non-continuous gold film at a very small thickness and quasi-continuous gold film at much larger thickness. The fast deposition rate of 0.11 nm/s using 10mA current was chosen to facilitate the formation of coarse sputtered gold particles on the glass substrate. This simple modification to the sputtering process prohibits the complete coverage of the substrate when the deposition thickness is extremely thin, particularly at the atomic scale. The resulting structural change in the gold film with thickness serves as a platform for investigating the intrinsic mechanism of SERS enhancement as the

surface plasmon mode transit from LSPR (discretized structure) to propagating surface plasmon mode (quasi-continuous layer).

Figure 3.4 SEM images of the glass substrates after (a) 18s, (b) 36s, (c) 54s, (d) 72s, (e) 90s and (f) 108s of gold sputtering with a fine coater. Scale bar: 1000Å

Table 1: Estimated Size distribution of the gold nano-dots with deposition time of 18-36s

Deposition time (s)	Nominal gold thickness (Å)	Estimated Period (Å)	Estimated Mean diameter (Å)
18	20	124	56 $\pm$ 10 (17.8%)
36	40	146	98 $\pm$ 15.6(15.9%)
54	60	181	151 $\pm$ 56(37.0%)

Figure 3.4 shows the SEM images of the structural evolution of the gold sputtered substrate as deposition time (Δt) varies from 18-108s. At low deposition time of $\Delta t = 18-36s$, discretized gold nano-particles were observed to form on the substrate. As the deposition time increased to 54s, individual gold nano-particles grew in size and eventually coalesce with neighbouring particles into larger gold

island resulting in an increased in the spatial period of the islands. Beyond 54s deposition time ($\Delta t = 72-108s$), the gold islands grew even larger and merged to form a quasi-continuous gold film on the glass substrate. In the discontinuous regime ($\Delta t = 18-54s$), the gold nano-particle can be approximated as an ellipsoidal disk with size distribution of approximately 50-200Å. Table 1 presents the size distributions of the gold nano-particles from different deposition time, where the period and mean diameter are estimated by Fourier transforming the SEM images (ImageJ software).

The coalescence of the gold particles with sputtering duration is a random process and hence a large distribution of particles size can be expected. Prior to quasi-continuous gold film formation, the gold sputtered particles first aggregate into larger islands ($\Delta t = 54s$), which resulted in an increase in the periodicity and larger particle size deviation (standard deviation = 37%). In the quasi-continuous regime ($\Delta t = 72-108s$), the gold islands become connected across the glass substrate. Nano-gaps exist in the form of elongated fissures on the substrate which substantially diminished as the gold film progressively became more continuous at $\Delta t = 108s$.

3.3.2 Optical and SERS Measurement

The optical properties of the gold sputtered substrates were characterized by measuring the optical transmittance, shown in Figure 3.5 (a) over the visible to near infrared range (400-800nm) where typical Raman scattering takes place. Transparent microscopic glass substrate is deliberately chosen as the supporting substrate in this study to minimize any form of optical noise interfering with the intrinsic property of the gold film. In such a setup, the measured transmittance can be attributed entirely to the plasmonic characteristic of the gold nano-particles. The extinction cross-section, σ_{ext} is estimated using:

$$1 - T = \sigma_{ext}N \quad (7)$$

where N is the 2-dimensional particle density and $T = I/I_0$ is the measured transmittance of the substrate [95]. Figure 3.5 (b) shows the extinction spectra derived from the measured optical transmittance of the gold sputtered substrates. It is evident from Figure 3.5 (a) that an initial resonance dip in the transmittance of the discretized gold nano-particles on substrates ($\Delta t = 18-54s$) changes to a non-resonance broadband transmittance as the gold layer become quasi-continuous ($\Delta t = 72-108s$). The resonance dip occurring at $\Delta t = 18-54s$ corresponds to a strong resonance extinction of light across the visible range as depicted in Figure 3.5 (b). The existence of this pronounced resonance effect is due to the excitation of LSPR which is characteristically size-dependent and functions as optical nano-antenna that radiates EM radiation into free space.

On substrate deposited with gold for $\Delta t = 18s$, the near-field coupling of LSPR excitation occurs at the confined gaps between closely spaced gold nano-particles. This near-field coupling results in a constructive interference of the electric field excited under resonance condition. As the gold deposition time increase to $\Delta t = 36s$, the resulting gold nano-particles increased in size and the inter-particle spacing decreases. The reduced gaps between nano-particles produced a nano-confinement effect which increases the plasmonic coupling of the LSPR excitation. Consequently, the extinction cross-section (σ_{ext}) increases and a red-shift in the resonance position is observed, as shown in Figure 3.5 (b). This is in good agreement with the results reported on various architecture of plasmonic nano-arrays [96].

Figure 3.5 (a) Measured optical transmittances of the gold sputtered substrates and the (b) corresponding estimated extinction spectra.

As the gold nano-particles grew further with a longer deposition time of $\Delta t = 54$ s, the extinction resonance experiences an even larger red-shift. Red-shifting of the resonance position is a typical effect from increasing the size of the nano-particles on SERS substrates [97]. In addition, a substantial reduction in the extinction peak is also observed. The drop in the extinction peak is due to the coalescence of the gold nano-particles in certain region of the substrate which delocalizes the surface plasmon resonance effect. The competing effect between the coupling of the LSPR excitation and the propagating surface plasmon due to connected islands causes the extinction peak to decrease. As a result of the large distribution of the gold nano-particle sizes, broadening of the extinction spectrum is also observed. The condition on substrate with gold deposition time of $\Delta t = 54$ s can therefore be considered as a quasi-plasmonic state which marks the onset of the transition from the LSPR coupling mode to a propagating surface plasmon mode.

Further increase in the gold deposition time to $\Delta t > 72\text{s}$ results in a quasi-continuous gold film which significantly de-coupled the LSPR effect. The continuous gold film now serves as a conductive path that facilitates in-plane propagation of EM wave along the metal-dielectric interface which translate to a radiative loss of the resonance effect. As a result, a broad dip is produced in the extinction spectrum, across the Raman scattering band of 400-800nm as shown in Figure 3.5 (b).

Figure 3.6 (left) 2NT SERS spectrum ($\lambda_{\text{ext}} = 633\text{nm}$) of the gold sputtered substrate fabricated with sputtering deposition time of $\Delta t = 18\text{-}90\text{s}$ and (right) the corresponding comparison of the 1380cm^{-1} 2NT peak illustrating transition from LSPR coupling mode (discretized gold nano-particles) to propagating surface plasmon mode (quasi-continuous gold film). The SERS spectrum of $\Delta t = 108\text{s}$ sample has similar response as the $\Delta t = 72\text{s}$ and 90s sample and not plotted to maintain clarity. The SERS measurement at 532nm laser excitation did not show any signal.

SERS measurement using 2-Naphthalenethiol (2-NT) probe molecule is conducted on the gold sputtered substrates to investigate the SERS intensity characteristic as the substrate transit from the LSPR coupling mode to a propagating

surface plasmon mode. 2-NT is chosen as the SERS probe due to its strong affinity to gold as a result of the distinction thiol group (-SH functional group) which consist of a sulfur atom bonded to a hydrogen atom. As illustrated in Figure 3.7, sulfur, having a strong affinity to gold, immediately loses the hydrogen atom, and self-assembled on the gold surface via a strong covalent bond.

Figure 3.7 Illustration of the self-assembly process of 2-Naphthalenethiol (2-NT) on the gold surface

The substrates are excited using 532nm and 633nm laser wavelengths. SERS activity was not detected with the 532nm laser excitation. Noticeable SERS signal is only measured on the $\Delta t = 18s$, 36s and 54s samples with 633nm laser excitation as shown in Figure 3.6. The quasi-continuous gold film substrates, whose surface plasmon operates in the propagating mode did not produce any measurable SERS. The largest SERS intensity is observed on the $\Delta t = 36s$ substrate, and the strongest extinction resonance occurs at $\lambda \sim 580nm$. The $\Delta t = 18s$ sample with a significantly weaker extinction and a moderately blue-shifted resonance position ($\lambda \sim 551nm$), exhibits a considerably weaker SERS signal. Despite having a nearer resonance position to the 633nm laser excitation, the $\Delta t = 54s$ sample, produces a weaker SERS signal than the $\Delta t = 36s$ sample. This is attributed to the presence of a

quasi-plasmonic state where the conductivity of large gold islands de-couple the LSPR excitation effect and reduces the overall SERS signal.

The extinction response of the substrate as the gold surface structure transits from a discretized to a quasi-continuous configuration shows a strong correlation between SERS enhancement and the near-field coupling of the LSPR excitation. Furthermore, the strength of the near-field coupling, as quantified by the magnitude of the extinction resonance, is found to be directly correlated to the SERS enhancement.

3.3.3 FDTD Simulation

It is generally accepted that the local enhancement of the EM field occurring at the inter-particle gap is the dominant mechanism of SERS enhancement. The two stage field enhancement that occur at the incident field and the Raman scattered field amplifies the Raman signal by an enhancement factor to the power of four [98]. The results in the previous section has also indicated that near-field coupling of the LSPR excitation has a strong correlation to SERS enhancement.

To better understand the dependency of SERS enhancement on LSPR excitation, the extinction and field enhancement spectra under increasing near-field coupling are compared using FDTD simulation. This is achieved by modelling the near-field coupling of the LSPR excitation with a periodic array of cylindrical disk that progressively increases in diameter to simulate the narrowing of inter-particle spacing. Using the $\Delta t = 18\text{s}$ sample as a starting reference, a periodicity of $120\text{\AA}$ (refer to Table 1) is used in the simulation model. The simulated extinction and field enhancement spectra for disk diameters ranging from 6nm to 10nm are shown in

Figure 3.8. The field enhancement spectrum is obtained by using the electric field enhancement factor, $E(\lambda)/E_0$, at the center of the disk where the field enhancement is the greatest.

As shown in Figure 3.8(a), the extinction spectrum of the 6nm disk structure is mainly contributed by the absorbance. As the disk diameter increases, the contribution by the scattering cross-section to the extinction spectrum is observed to increase. While the absorbance of the disk also increases with disk diameter, the increment is notably smaller. As the disk diameter increases towards 10nm, the contribution from the scattering cross-section becomes comparable to that of the absorbance as depicted in Figure 3.8(e). These results indicate that the near-field coupling of the LSPR excitation under resonance condition is accompanied by a significant increase in the scattering cross-section. Figure 3.8(f) shows the resonance wavelength of the extinction, absorbance, scattering cross-section and field enhancement spectra plotted as a function of increasing disk diameter.

Figure 3.8 FDTD simulation of the extinction (blue solid), absorbance (blue dashed), scattering cross-section (blue dotted) and field enhancement (red) spectra of the cylindrical disk with increasing diameters of (a) 6nm, (b) 7nm, (c) 8nm, (d) 9nm and (e) 10nm. (f) The corresponding resonance wavelength (λ) of the extinction (*), absorbance (●), scattering cross-section (▲) and field enhancement (×)

It is observed that the increasing contribution from the scattering cross-section cause the resonance of the extinction spectrum to progressively red-shift towards the resonance of the field enhancement as the disk diameter increases from 6nm to 10nm. Since electric field enhancement is the main contributing factor to SERS, the agreement in the resonance position suggest the relevance of the scattering cross-section in influencing the phenomenon of SERS. In a typical explanation of SERS mechanism, only the EM enhancement and the significantly weaker CE enhancement are considered. However, the simulation results revealed that another factor, the substrate scattering cross-section, should also be taken into account to better describe the phenomenon. It can be deduced that the scattering cross-section which represents the likelihood of a scattering event, affects the amount of electric field enhanced light that can be radiated into free space as quantifiable SERS signal.

3.3.4 SERS Substrate Comparison

The FDTD simulation of cylindrical disk periodic arrays in Section 3.3.3 demonstrates that the near-field coupling of the LSPR excitation is accompanied by an increase scattering cross-section, whose resonance wavelength is in much closer agreement with the corresponding electric field enhancement. The result indicate that the increased scattering cross-section plays an important role in facilitating the out-of-plane radiation of the enhanced EM field localized at the inter-particle spacing. To verify the significance of scattering cross-section on SERS enhancement, comparative SERS measurements were carried out on two substrates with widely differing scattering cross-section: a gold-film-over-nanosphere (AuFON) substrate having a larger scattering cross-section and a sputtered gold substrate ($\Delta t = 36s$

sample) with a small scattering cross-section. The AuFON with a diameter of 370nm is fabricated using the spin-coating technique followed by sputtering of 60nm gold film as described in Section 3.2.2. Figure 3.9 (a) shows the SEM image of the AuFON substrate and Figure 3.9 (b) compares the simulated electric field enhancement of the AuFON and the sputtered gold substrate with $\Delta t = 36s$ over the wavelength range of 500-800nm.

Figure 3.9 (a) SEM image of the AuFON substrate and the **(b)** comparison of the simulated electric field enhancement spectrum of the AuFON and the gold sputtered substrate with deposition time of $\Delta t = 36s$. The red window demarcates the 633nm excited Raman band corresponding to a Raman shift range of 0-1800 cm^{-1} .

It is observed that the electric field enhancement, $E(\lambda)/E_0$, of the gold sputtered substrate is higher than that of the AuFON over the wavelength range of 633-714nm, which corresponds to a Raman shift of 0-1800 cm^{-1} . One would reasonably expect this larger electric field enhancement to translate to a larger SERS signal from the gold sputtered substrate. Instead, it can be seen from Figure 3.10 that the AuFON substrate, having a lower field enhancement in the associated Raman band, produces a larger SERS signal as compared to the gold sputtered substrate. The presence of background noise in the AuFON spectrum is attributed to

the fluorescence emission of the polystyrene beads that are not covered by the sputtered gold material.

Figure 3.10 SERS spectrum comparison of the AuFON and gold sputtered substrate with deposition time of $\Delta t = 36s$ using 2NT Raman probe and 633nm wavelength laser excitation

A comparison of the simulated extinction spectrum of the gold sputtered sample and the AuFON substrate is shown in Figure 3.11. The nanosphere of the AuFON substrate (diameter of 370nm) is approximately 30 time larger than the gold nano-particles on the sputtered sample. The larger particle size translates to a significantly larger scattering cross-section as observed in the extinction spectrum of the AuFON. Within the associated Raman band, the scattering cross-section of the AuFON contributes nearly 100% to the extinction spectrum while the scattering cross-section of the gold sputtered sample contributes much lesser to the extinction spectrum due to the competing absorbance component. This indicates that the AuFON possess a significantly higher scattering efficiency of the EM radiation than that of the gold sputtered sample in the associated Raman band. This is more than

enough to compensate the lower electric field enhancement obtained in the AuFON to give a larger SERS signal as compared to that of the gold sputtered sample. This study shows that while the strong EM field enhancement is a key consideration, the scattering of the enhanced field also plays a substantial role in achieving SERS enhancement.

Figure 3.11 FDTD simulation of the extinction (solid line), absorbance (dashed line) and scattering cross-section (dotted line) spectra of **(a)** the gold sputtered sample with deposition time of $\Delta t = 36\text{s}$ and **(b)** the AuFON substrate with 60 nm of sputtered gold. The red window demarcates the 633 nm excited Raman band corresponding to a Raman shift of 0-1800 cm^{-1} .

3.3.5 Sputtered Gold on Textured Surfaces

The previous sections demonstrated that although the gold sputtered nanoparticles produce a high electric field enhancement, an inherently low scattering cross-section within the associated Raman band can reduce the out-of-plane EM radiation and the resulting SERS enhancement. In this section, a method to increase the substrate scattering cross-section by sputtering gold particles for $\Delta t = 36\text{s}$ onto 3-dimensional textured surfaces such as wrinkled PDMS and AAO nano-hole array is

investigated. The sputtered gold particles are also deposited on other planar substrate such as glass, silicon and aluminium for comparison. Figure 3.12 shows the SEM images of the resulting gold sputtered AAO and wrinkled PDMS surfaces and the SERS spectra measured on the different substrates coated with sputtered gold particles.

Figure 3.12 (b) clearly shows that the gold sputtered wrinkled PDMS surface and the AAO template respectively demonstrates two-fold and three-fold improvements in the SERS signal as compared to the planar glass substrate. These results indicate that the low scattering cross-section of the gold sputtered sample can be compensated by introducing 3-dimensional surfaces as the supporting substrate to effectively increase the deposition area, hence the overall scattering cross-section of the substrate. In addition, the PDMS substrate offers flexibility and optical transparency which are positive attributes for cell imaging application. The alumina composite of the AAO is biocompatible to skin tissue and cell which makes them suitable material for biological sensing application.

Figure 3.13 Simulated scattering cross-section of the gold sputtered particles ($\Delta t = 36s$) on gold, aluminium, silicon dioxide and alumina planar substrate

The gold sputtered particles deposited on aluminium substrate are observed to not produce any SERS signal. From the FDTD simulation of the scattering cross-

section of the substrate (Figure 3.13), an absence of LSPR excitation is observed when the gold sputtered particles are deposited on conductive surfaces such as aluminium and gold. This is attributed to the conductivity of the underlying metallic surface which facilitates in-plane propagation of the EM wave and thus de-couples the LSPR excitation of the gold particles. Strong LSPR characteristic are observed on dielectric surface such as silicon dioxide and aluminium oxide which facilitates out-of-plane radiation of the EM wave that leads to SERS.

3.4 Conclusion

The mechanism for SERS enhancement is investigated using a range of gold sputtered substrates where the deposited gold layer varies from a discretized to a quasi-continuous morphology. The SERS signal of the gold layer is found to be attenuated when it transits to a quasi-continuous morphology. This suggests a strong correlation between SERS enhancement and the near-field coupling of the LSPR excitation. FDTD simulations show that the intensifying near-field coupling of the LSPR excitation is accompanied by the local electric field enhancement and an increase in the scattering cross-section of the structure. In addition, the scattering cross-section is found to increase more than the absorbance resulting in an increasing contribution to the extinction spectrum. This observation indicates that increasing the scattering cross-section enhances the scattering efficiency of the substrate, which in turn affects the amount of EM field enhanced light that can be radiated into free space as SERS signal. It is also shown that the AuFON substrate which has a lower electric field enhancement but a larger scattering cross-section in the associated Raman band gave a higher SERS intensity than a gold sputtered sample with a higher electric field enhancement but a smaller scattering cross-

section. Up to three-fold enhancement in the SERS signal were demonstrated by depositing the same gold sputtered particles on textured substrate to effectively increase the scattering cross-section via the increased nano-particle density.

Chapter 4 : Effect of Metallization on SERS Performance

4.1 Introduction

Intense amplification of the EM field, induced by the excitation of LSPR is widely recognized as the dominating mechanism of SERS enhancement. While the CE enhancement also contributes to SERS, the order of magnitude is several times lower than the EM enhancement factor. Furthermore, CE enhancement is limited to the chemical nature of the molecule-of-interest. Thus, SERS optimization approach is often based on enhancing the EM enhancement factor which has a larger potential for improvement. The enhancement of the EM field through LSPR excitation is preferentially found in crevices and gaps of plasmonic structures. Such structures, usually based on gold and silver, possess LSPR bandwidth that span the visible and near-infrared bands where Raman scattering typically takes place. Consequently, solid substrates fabricated from non-gold or non-silver materials requires a gold or silver metallization step to achieve SERS activation. The resonance frequency and magnitude of the LSPR excitation is heavily dependent on the geometrical parameters of the nano-structures. Therefore, structural modifications by the final metallization step has an effect on the LSPR response which in turn has an influence on the overall SERS enhancement.

Sputtering and electron-beam (E-beam) evaporation are two commonly used semiconductor deposition techniques for coating metals on SERS substrates. Sputtering involves the bombardment of the target material by highly energetic plasma excited by a strong electric field. This causes particles to eject from the target material and deposit on the substrate. The sputtering process only requires low vacuum condition and can achieve a high rate of metal deposition. In E-beam

evaporation, the target material undergoes a sublimation process under high vacuum by means of an electron gun. The evaporated material is then carried to the substrate where the growth occurs. A high vacuum environment is needed to ensure sufficiently long mean-free-path for the target material to deposit efficiently on the substrates. The need for high vacuum environment and the inherently low deposition rate are some of the disadvantages of the E-beam evaporation process.

The morphology of the metal deposited by the two techniques on a substrate can vary notably due to the difference in deposition mechanisms. The difference can have some impact on the SERS performance of the coated substrate. In this work, the morphological effect of gold material deposited by DC sputtering and E-beam evaporation techniques on the SERS performance of coated substrates are investigated. The two metal deposition techniques are applied on a nanosphere array to fabricate AuFON SERS substrate. A cost-effective hybrid deposition approach using DC sputtering to narrow the inter-structural gap and the subsequent application of an E-beam evaporated gold layer to introduce roughness is proposed to fabricate high performance AuFON SERS substrates.

4.2 Experimental Details

The DC sputtering and E-beam evaporation techniques were used to deposit gold (99.99% purity) of 20-60nm thickness onto various substrates comprising of planar silicon, nanosphere array fabricated by NSL and AAO nano-holes array fabricated by a 2-step anodization of aluminum. A schematic illustration of the experimental details is depicted in Figure 4.1. The DC sputtering and E-beam evaporation technique is first applied on the planar silicon substrate to study the

intrinsic morphology of the coated film. Both techniques are then applied to nano-textured surfaces of AuFON and AAO nano-hole array to study its effect on their SERS performance. For the AAO nano-hole array, two different pore diameters are used in the study. This is achieved by etching the AAO in 5% H_3PO_4 solutions for 20min and 40min respectively. Base on the initial findings, a hybrid deposition approach using the DC sputtering to narrow the inter-structural gap, followed by a final E-beam evaporation layer to introduce roughness is applied onto the AuFON substrate to evaluate its SERS performance.

Figure 4.1 Schematic illustration of experimental details

The sputtering of gold is performed at a rate of 0.11nm/s, which is the lowest achievable rate of the fine coater using a current of 10mA. E-beam evaporation of gold is performed at a rate of 0.02nm/s with a current of 75mA. To ensure consistency in the comparison, the purity of the gold foil used in the DC sputtering and the gold target used in the E-beam evaporation are kept the same.

4.3 Result and Discussion

4.3.1 Surface Characterization and SERS Measurement

Figure 4.2 shows the morphology of the gold layers deposited by the DC sputtering and E-beam evaporation technique on planar silicon substrates. It is observed that the E-beam evaporated gold on silicon forms a quasi-continuous film when the deposition thickness is 20nm. As the deposition thickness increases, a complete coverage of the substrate is achieved and the resulting gold layer possesses some degree of roughness resulting from the presence of gold clumps on its surface.

a) Planar Si substrate (Electron-beam evaporation)

b) Planar Si substrate (DC sputtering)

Figure 4.2 SEM images of various gold thickness (10-60nm) deposited onto silicon substrate by **(a)** E-beam evaporation and **(b)** DC sputtering. Scale bar: 200nm

In the E-beam evaporation process, the gold materials are transferred via a thermal vaporization process by heating the gold source with an electron gun which

eventually turn the solid gold material into gaseous phase. Upon contact with the silicon substrate, the vaporised gold material condenses into molten state at a large contact angle. This leads to the formation of gold clumps upon solidification and, gives the coated surface a roughened finishing. In contrast, the delivery of gold in DC sputtering is a non-thermal vaporisation process whereby the gold atoms are physically ejected from the source by the bombardment of highly energetic plasma. This produces a smoother gold coating on the silicon substrate with a relatively higher degree of conformity to the planar surface, as seen in the SEM images of Figure 4.2 (b) [99].

It has been reported that surface roughness of metallic film, in addition to its thickness, plays an significant role in SERS activity [100]. The increase in surface roughness on a SERS substrate is found to increase the density of field enhancement hotspots leading to a larger average SERS signal [101]. Hence, the roughness introduced by the E-beam evaporation technique when a sufficiently thin metal layer is deposited has the potential to improve the SERS signal of nano-structures as compared to the sputtering technique. To evaluate the effect of surface roughness on SERS performance, measurements were performed on AuFON coated with gold using the two metallization techniques. Figure 4.3 shows the SEM images of the AuFON coated with gold ranging from 20-60nm by the E-beam evaporation and DC-sputtering techniques. Surface roughness, similar to those seen on planar silicon substrate, is observed on the AuFON coated with E-beam evaporated gold. The gold sputtered AuFON, on the other hand, acquired a smooth finishing as a result of the isotropic deposition process

a) AuFON (Electron-beam evaporation)

b) AuFON (DC sputtering)

Figure 4.3 SEM images of AuFON coated with gold thicknesses of 20 - 60nm **(a)** by E-beam evaporation and **(b)** DC sputtering. Scale bar: 200nm

The comparison of the 1062cm^{-1} and 1380cm^{-1} Raman peaks of 2NT on the substrates in Figure 4.4 (a) showed that the SERS intensity increases with gold thickness for both DC sputtered and E-beam deposited substrate. This is attributed to the enhanced near-field coupling of the LSPR excitation arising from the reduced inter-particle gap when gold thickness is increased. As mentioned in Chapter 3, this near-field coupling of the LSPR excitation is accompanied by an increase in the scattering cross-section and also intensifies the localized electric field enhancement at the curvature of the sphere junction which collectively leads to a larger SERS enhancement. Figure 4.4 (a) also revealed that the AuFON coated using the E-beam evaporation technique produced a significantly larger SERS signal (as much as ~ 10 times higher with 60nm gold thickness) than those coated using DC sputtering technique. Since gold of 99.99% purity is used in both metallization techniques, this

factor cannot account for the difference in the measured SERS signals. Thus, the improved SERS signal observed in the E-beam evaporated AuFON must be due to the presence of surface roughness on the gold layer. It is obvious from Figure 4.4 (b) that the SERS spectra of the AuFON coated using the E-beam evaporation technique demonstrate consistent improvement in the SERS intensity across the entire 2NT spectrum of 200-1800 cm^{-1} .

Figure 4.4 (a) The 1062 cm^{-1} and 1380 cm^{-1} SERS intensity peak of 2NT Raman molecules on AuFON substrate, metalized with the DC sputtering and E-beam evaporation techniques plotted as a function of gold thicknesses **(b)** 2NT SERS spectrum of the AuFON coated with 40nm and 60nm of gold using E-beam evaporation and DC sputtering techniques.

4.3.2 FDTD Simulation

To quantify the effect of surface roughness on SERS performance, the FDTD-simulated field enhancement distribution of smooth and roughened nanospheres under 633nm incident field are compared in Figure 4.5. The smooth AuFON is constructed using a 3-dimensional gold nanosphere (370 diameter) array on a silicon dioxide substrate. For the roughened nanosphere, small 3-dimensional hemispheres of 60nm diameter are incorporated along the upper surface of the

370nm diameter nanosphere to represent surface roughness. The nanospheres are placed on the x-y plane with a total-field-scattered-field (TFSF) incident source injected in the z-direction. The distribution of field enhancement in the x-z plane obtained from the simulation is shown in Figure 4.5.

Figure 4.5 Distribution of electric field enhancement along the x-z plane of the (a) smooth and (b) roughened gold nanosphere array under 633nm wavelength laser excitation

It is observed that the strongest field enhancement is localized at the curvature between adjacent nanospheres for both smooth and roughened morphologies. Additional regions of field enhancement are also found along the undulating surface of the roughened nanosphere as depicted in Figure 4.5(b). To quantify the difference in the electric field enhancement of the two types of nanosphere, the average field enhancement per pixel (0.5nm) is calculated. The roughened configuration produces approximately 20% more average field enhancement per pixel as compared to that of the smooth nanosphere configuration.

It is widely recognized that the electric field enhancement in SERS contributes to an enhancement factor of approximately E^4 [102]. Therefore, the

~1.2x improvement in the average field enhancement per pixel of the roughened nanospheres over the smooth nanospheres will potentially result in an approximately two-fold improvement in SERS signal. It should be noted that this estimation assumes that all field enhancement hotspots contribute to the measured SERS signal. In cases where the molecules-of-interest do not attach to the nano-scale gaps, especially in the sub-10nm dimension that produces the strongest electric field enhancement, the additional SERS improvement is expected to be less than two-fold.

Figure 4.6 SERS spectra of (a) AAO (20min etched in 5% H_3PO_4) and (b) AAO (40min etched in 5% H_3PO_4) coated with 20nm of gold using E-beam evaporation and DC sputtering. The corresponding SEM images of the substrates are shown on the right of the graphs. Scale bar: 200nm

To ensure that the roughness introduced by the E-beam evaporation technique is not caused by the interaction between the molten gold and the

polystyrene material of the nanosphere, both metallization techniques are also evaluated using different substrate materials. The AAO nano-holes, made of porous alumina, is chosen because it has been demonstrated to be a cost-effective substrate for numerous SERS applications [103-105]. 20nm of gold is deposited onto the AAO nano-hole array with two different pore diameters using DC sputtering and E-beam evaporation technique (Figure 4.6).

The presence of surface roughness is similarly observed on the E-beam coated AAO substrates which distorted the circularity of the nano-holes. In contrast, the DC sputtered AAO substrates retain nano-hole circularity due to the higher conformity of the deposited gold film. As seen in Figure 4.6, the E-beam coated substrates exhibit significantly larger SERS intensity peak as compared to the DC sputtered AAO for both diameters. This confirms that the improvement in SERS signal originates from the roughness of the E-beam evaporated gold and is substrate-independent. The finding suggest that the E-beam metallization technique can be used to maximize SERS performance by forming a roughened layer on SERS substrates.

4.3.3 Hybrid Deposition Approach

The DC sputtering technique, being more cost-effective and can achieve a high deposition rate, has clear advantages over the E-beam evaporation technique in metallizing of SERS substrates. However, the results obtained in this work indicates that substrates metalized with the E-beam evaporation technique possess improved SERS signal sensitivity due to the inherent roughness of the deposited film.

To exploit the advantages of both techniques, a hybrid deposition approach for SERS metallization is investigated.

Table 2. Hybrid Deposition Gold on Nanosphere Substrate

Sample	First Desposition	Second Deposition
A	30nm (E-beam)	Nil
B	15nm (DC Sputtering)	15nm (E-beam)
C	15nm (E-beam)	15nm (DC Sputtering)
D	30nm (DC Sputtering)	Nil

Figure 4.7 SERS spectra of 2NT on AuFON coated with (A) 30nm E-beam gold (red), (B) 15nm sputtered gold and 15nm E-beam gold (green), (C) 15nm E-beam gold and 15nm sputter gold (blue) and (D) 30nm sputtered gold (cyan). Samples are excited with a laser wavelength of 633nm.

Gold of 30nm total thickness is first deposited on the nanospheres substrate using 4 combinations of DC sputtering and E-beam evaporation processes as shown in Table 2 to establish a suitable hybrid deposition scheme. SERS measurement of 2NT on each sample is performed and the results are shown in Figure 4.7. It is worth noting that both samples A and B which has E-beam evaporated gold as the final coating, exhibit comparable SERS signal. In comparison, samples C and D with sputtered gold as the final coating exhibit mediocre SERS signal which pales in comparison with those obtained from samples A and B. The weaker SERS signal measured in sample C is attributed to the diminution of the initial nano-scale roughness introduced by the first E-beam deposition technique when the second gold sputtered layer is deposited. Consequently, the EM hotspots found in the initial E-beam metallized layer are greatly reduced by the second smoother gold sputtered layer. These results indicate that while the sputtering technique can be used to efficiently deposit a reasonably thick layer of gold on a SERS substrate to reduce the gaps between nano-structures for enhanced LSPR near-field coupling, an E-beam evaporated gold layer is found to be more suitable as a final coating on SERS substrate to maximize SERS enhancement.

The hybrid deposition approach is also tested on a series of AuFON substrates. Gold of 20-60nm thickness is first deposited using DC sputtering and is followed by depositing additional 10nm and 15nm of E-beam evaporated gold as the finishing coating. SERS measurement of 2NT is conducted on the substrates and the comparison of the intensity peak at 1380cm^{-1} in Figure 4.8 shows that the addition of the roughened gold layer significantly improves SERS signal of the AuFON substrates.

Figure 4.8 The 2NT SERS intensity of the 1380cm⁻¹ peak from AuFON substrate coated with gold (blue), sputtered gold with 10nm E-beam gold (green) and sputtered gold with 15nm E-beam gold plotted as a function of sputtered gold thicknesses ranging from 20-60nm. The standard deviation (%) of the intensity of the 1380cm⁻¹ peak is shown at each data point.

4.4 Conclusion

In conclusion, the effect of metallization on SERS enhancement was evaluated using DC sputtering and E-beam evaporation of gold. When coated onto planar silicon substrate, the E-beam evaporated gold film show nano-scale roughness while the DC sputtered gold produce a smooth film. Similar roughness is also observed when E-beam evaporated gold is coated onto AuFON and AAO nano-hole array. This inherent roughness of E-beam deposited gold is found to significantly increase the SERS signal from the substrate. FDTD simulation shows that the improved SERS signal is due to the increase in the average EM field enhancement as a result of additional EM hotspots contributed by the roughness of

the E-beam deposited gold surface. A hybrid metallization approach utilizing the DC sputtering process to narrow the inter-structural gaps and the E-beam evaporation process to introduce a final layer with roughness is proposed and evaluated. Promising results are demonstrated on AuFON substrates metalized using hybrid approach. The proposed approach exploits the high deposition rate and high conformity nature of the DC sputtering and the inherent roughness of E-beam metallization to realise cost-effective and high performance SERS substrates.

Chapter 5 : Gold on PECVD SiO₂ Nano-Pillar SERS Substrate

5.1 Introduction

EM field enhancement is the dominating factor contributing to overall SERS enhancement of a SERS substrate. Although, CE exists in SERS due to the charge transfer between molecules and substrate, it only contributes a small fraction of the total enhancement. On the other hand, the major EM enhancement factor increases exponentially with increasing inter-particle proximity especially in the sub-10nm regime. As discussed in Chapter 3, the highly-confined space between nano-structures facilitates near-field dipolar coupling of the LSPR excitation, leading to an increase in the scattering cross-section as well as the EM field enhancement. The magnitude of SERS enhancement is largely related to the geometrical properties of the nano-structures on SERS substrates. Well-ordered periodic nano-array that exhibit high structural consistency are excellent SERS substrate in practical applications with stringent demand for high signal uniformity and reproducibility. The high precision electron-beam lithography (EBL) and focused ion beam (FIB) techniques are the most direct approach for fabricating such substrates due to the high process controllability and the high resolution advantage. However, these techniques suffer from low process throughput, making them ineffective for large scale production.

An alternate approach to achieve low cost, large scale production of SERS substrate is the nanosphere-lithography (NSL) technique which uses a self-assembled spherical polystyrene (PS) mask as a patterning template to create nano-structures [106]. The resulting hexagonally arranged spherical mask can be converted into a cost-effective gold-film-over-nanosphere (AuFON) SERS substrates

using a simple gold deposition step. Regions of intense EM field enhancement are localized at the curvature of the nanosphere junction acting as SERS hotspots to amplify the weak Raman signals. However, the stability and reusability of AuFON are heavily affected by the imperceptibly immobilized nanospheres via the weak Van-der-Waal force. The overlying gold coating provides additional stability but, the nanospheres can still be easily detached from the substrate after long period of incubation in target solution or unintentional physical perturbation.

In this work, a SERS substrate based on the hexagonal array of Au-SiO₂ nano-pillars (Au-SiO₂-NP) is demonstrated by incorporating an optimization step using plasma enhanced chemical vapour deposition (PECVD) of SiO₂ on NSL fabricated silicon nano-pillars. The optimized Au-SiO₂-NP substrate is characterized to evaluate its SERS performance. FDTD simulation of the Au-SiO₂-NP is also carried out to study the resonance condition of the scattering cross-section and the electric field enhancement with respect to the associated Raman band. The performance of the optimized Au-SiO₂-NP and the AuFON substrate is compared and discussed.

5.2 Experimental Details

The results obtained in Chapter 3 demonstrates that SERS enhancement can be increased by enhancing the scattering cross-section as well as the EM field through reducing the inter-structural gap to promote near-field dipolar coupling. Therefore, the gaps between the silicon nano-pillars can, in principle, be expediently narrowed by reducing the duration of the oxygen plasma RIE step to obtain closer packing of the PS beads. However, the shadowing effect of the resulting high resolution PS mask can potentially affect the fidelity of the pattern transfer. This

effect becomes increasingly significant when the gap size is extremely small beyond the penetrating limit of the etchant. To overcome this constraint, a bottom-up growth approach is therefore adopted in this work to narrow the inter-pillar gaps by widening the pillar width using PECVD of SiO_2 on the NSL-fabricated silicon nano-pillars.

Figure 5.1 Preparation of PECVD Au-SiO₂ NP substrate

PS beads of 370nm diameter are first self-assembled onto a Si substrate by spin-coating the PS suspension as described in Section 3.2.2. Sodium dodecyl sulfate (SDS) is added as surfactant to reduce surface tension. After spinning off the excess solvent, the mono-layer of PS beads acquire a hexagonal arrangement which possess the lowest surface energy. The PS mask is reduced using oxygen plasma reactive-ion-etching (RIE) to create a top-down etching mask for the underlying Si substrate. Chlorine plasma RIE is then applied to etch the exposed silicon. The PS mask is then removed by sonicating the substrate in chloroform solution for 1min to reveal the underlying silicon nano-pillars. Plasma enhanced chemical vapour deposition (PECVD) of SiO_2 (40-70nm) is applied to obtain SiO_2 -NPs. Gold of 80nm

thickness is deposited over the SiO_2 -NPs by both sputtering and E-beam evaporation to obtain the final Au- SiO_2 -NP SERS substrate. Figure 5.1 shows the schematic illustration of the fabrication procedure for preparing PECVD Au- SiO_2 -NP substrate.

5.3 Results and Discussion

5.3.1 SERS Measurement

The diameter of the PS bead is substantially reduced from 370nm to 300nm by oxygen plasma RIE to ensure that the gap size ($\sim 70\text{nm}$) is sufficient to achieve effective etching of the underlying silicon. With this PS mask, chlorine plasma RIE produces silicon nano-pillars with a height of 300nm and a diameter of 300nm that are distributed across the substrate with a period of 370nm. Figure 5.2 (a) shows the SEM images of the resulting Si nano-pillars with the capping PS beads. The observed vertical sidewall profile of the Si nano-pillar indicates that the 70nm gap between adjacent PS beads is sufficiently large to prevent mask shadowing effect. The large gaps between the fabricated Si nano-pillars allow space for the subsequent deposition of the PECVD SiO_2 on the nano-pillars to optimize its dimension for SERS.

Figure 5.2 SEM images of **(a)** silicon nano-pillars after etching 300nm of silicon before sonication and **(b)** Au-SiO₂-NPs after depositing 50nm of PECVD SiO₂ on silicon nano-pillars followed by 80nm of E-beam gold

Figure 5.2(b) shows the SEM images of the Au-SiO₂-NP fabricated with 50nm of PECVD SiO₂. It is observed that the pillar width merely increases from 300nm to 329nm diameter, indicating that the deposited SiO₂ accumulates more on the top of the Si pillar than on the sidewall. This observation is expected due to the intrinsic nature of the PECVD process, where the chemical reaction takes place more rapidly on exposed surfaces. The sidewalls of the Si nano-pillar, being less exposed due to the presence of adjacent nano-pillar, inevitably accumulates less SiO₂ materials as the vapour reaches it at an oblique angle. PECVD of SiO₂ was performed to grow 40nm, 50nm, 60nm and 70nm of SiO₂ on the Si nano-pillars to evaluate the resonance condition of the structure for maximum SERS enhancement. The fabricated SiO₂-NPs are then coated with 80nm of gold as the SERS active layer to obtain an array of Au-SiO₂-NP by E-beam evaporation and DC sputtering metallization process.

Figure 5.3. SERS intensity peak (1062cm⁻¹ and 1380cm⁻¹) of 2NT on Au-SiO₂-NP substrates with various thicknesses of PECVD SiO₂ (40-70nm). 80nm of gold is deposited by sputtering (square) and electron-beam evaporation (circle).

SERS measurement using 2NT thiol-based molecules is performed on the Au-SiO₂-NP substrates deposited with different PECVD SiO₂ thicknesses. The comparison of the SERS signal intensity peak at 1062cm⁻¹ and 1380cm⁻¹ in Figure 5.3 reveals that the SERS performance of the Au-SiO₂-NP is optimized when it is coated with 50nm of PECVD SiO₂. This is consistently observed for both the DC sputtered and E-beam coated samples. In addition, it is also observed that the E-beam coated Au-SiO₂-NP substrate exhibit a relatively higher SERS intensity than those of the sputtered sample. The enhanced SERS signal is a result of the presence of additional EM hotspots arising from the inherent roughness of the E-beam evaporated gold film as previously discussed in Chapter 4. This increases the average EM field enhancement within a laser excitation spot and leads to a larger SERS signal. On the other hand, the DC sputtering deposition is isotropy in nature and produces a smooth gold film which does not possess any additional EM

hotspots. The observation of the improved SERS signal from the E-beam coated Au-SiO₂-NP substrates again demonstrates that the roughness-induced SERS enhancement is independent of substrate morphology.

5.3.2 SEM and FDTD Simulation

Figure 5.4 (a-c) shows the SEM images of the Au-SiO₂-NP fabricated with 40nm, 50nm and 70nm of PECVD SiO₂. It is observed that the height of the SiO₂ pillars stay consistently in the range of 298-307nm while the width of the pillars increases with PECVD SiO₂ thicknesses. This indicates that chemical vapour is able to penetrate the gap between adjacent structure and deposit as SiO₂ on the silicon substrate. Consequently, the simultaneous deposition of SiO₂ on the top of silicon nano-pillar and the silicon substrate in the gap resulted in a uniform pillar height regardless of SiO₂ deposition. The results showed that the PECVD deposition of SiO₂ can successfully narrow the gap of the silicon nano-pillars and optimize the near-field dipolar coupling of LSPR excitation without increasing the height of the pillars.

The E-beam metalized Au-SiO₂-NP structure is simulated in FDTD using cylindrical rod array with increasing diameter to investigate the resonance of the near-field dipolar coupling effect. The height of the pillar is consistently kept at 300 nm. The simulation results in Figure 5.4(d-f) shows that the resonance of the electric field enhancement and the scattering cross-section of the optimized Au-SiO₂-NPs with PECVD SiO₂ of 50 nm is well within the 633nm excited Raman band of 633-714nm. Under this condition, the incident and Raman scattered field are optimally enhanced by the structures and the enhanced field can effectively be radiated out-of-plane as quantifiable SERS signal through the large scattering cross-section. The

results are in good agreement with those presented in the previous chapter and further support the importance of scattering cross-section in SERS enhancement.

The Au-SiO₂-NPs with 40nm and 70nm of PECVD SiO₂ exhibit resonance peaks that are blue and red-shifted respectively from the centre of the excited Raman band and produced weaker SERS signals as compared to that of Au-SiO₂-NPs with 50 nm PECVD SiO₂. It is also noted that the Au-SiO₂-NPs with 70nm PECVD SiO₂ possess a larger field enhancement of ~20 while the Au-SiO₂-NPs with 40nm PECVD SiO₂ has a smaller field enhancement of ~10 at resonance. Since both structures exhibit similar scattering cross-section of approximately $4 \times 10^5 \text{ nm}^2$, the Au-SiO₂-NPs with 70 nm PECVD SiO₂ is expected to produce larger SERS signal. This is consistent with the SERS measurement results of the E-beam metalized substrate depicted in Figure 5.3 and indicates that optimization of the scattering cross-section and electric field enhancement is essential for yielding maximum SERS enhancement. The SERS enhancement factor of the optimized Au-SiO₂-NP with 50nm of PECVD SiO₂ is calculated to be approximately 4.90×10^6 .

Figure 5.4 SEM images of Au-SiO₂-NP with (a) 40nm, (b) 50nm and (c) 70nm PECVD deposition of SiO₂ and (d-f) the corresponding simulated wavelength dependent electric field enhancement (red) and scattering cross-section(blue)

5.3.3 Comparison of AuFON and Au-SiO₂-NP Substrates

The SERS performance of the Au-SiO₂-NP substrate is benchmarked by comparing to that of the well-established AuFON substrate. The AuFON is coated with a layer of E-beam evaporated gold of the same thickness (80nm) as that of the Au-SiO₂-NP substrate to ensure consistent comparison. Figure 5.5 shows the SERS spectra of 2NT on the respective substrates. It is observed that the optimized Au-SiO₂-NP (50nm PECVD SiO₂) substrate coated with E-beam evaporated gold, exhibits a comparable SERS intensity as that of the AuFON substrate over the entire 2NT spectrum. On the other hand, the gold sputtered Au-SiO₂-NP substrate, exhibits weaker SERS signal owing to the absence of extra SERS hotspots. This result clearly demonstrates that the sensitivity of the optimized Au-SiO₂-NP is comparable to those of well-established AuFON substrate.

Figure 5.5 SERS spectra of the optimized Au-SiO₂-NP substrates (50nm PECVD SiO₂) coated with 80nm E-beam gold (blue) and sputtered gold (green) and the AuFON coated with 80nm E-beam gold

Figure 5.6 compares the FDTD simulated wavelength dependant electric field enhancement and the respective electric field distributions of the optimized Au-SiO₂-NP and the AuFON substrates under 633nm wavelength excitation. It is interesting to note that the Au-SiO₂-NP substrate demonstrates comparable SERS signal as that of the AuFON substrate despite having a weaker electric field enhancement within the excited Raman band of 633-714nm. It can be seen in Figure 5.6(b) that intense field enhancement is localized around the contact regions between the spheres and around the contact regions between the sphere and the underlying substrate. However, the useful enhancement for SERS is limited to those at the upper contact regions between spheres where attachment of the Raman molecules are possible. The field enhancement located at the other contact regions therefore do not contribute to the overall SERS signal since Raman molecules are unable to attach to these unexposed regions.

Figure 5.6. (a) Simulated wavelength dependent electric field enhancement of the AuFON and the optimized Au-SiO₂-NP (50nm PECVD SiO₂) substrates. Simulated electric field distribution of (b) the AuFON and (c) the optimized Au-SiO₂-NP (50nm PECVD SiO₂) at 633nm wavelength excitation (x-z plane, y=0)

By contrast, the Au-SiO₂-NP substrate possess a larger region of SERS active site as illustrated in Figure 5.6 (c). Intense field enhancement are localized not

only at the top circumference of the Au-SiO₂-NP, but also along the lower third of the pillars. These exposed regions allow molecules to easily bind to them and to experience the full effect of the electric field enhancement. This possibly compensate for the inherently weaker electric field enhancement of the Au-SiO₂-NP substrate and thereby producing an overall SERS intensity that is comparable to those of the AuFON substrate.

5.3.4 Stability and Reusability

The Au-SiO₂-NP structure is fabricated on the substrate via NSL patterning and PECVD deposition processes and hence is more robust and stable as compared to the AuFON structure where the underlying PS spheres are immobilized on the substrate by a weak Van-der-Waal force. Under long period of incubation, the PS sphere structure of the AuFON can possibly detach from the substrate and hence loses its SERS capability. Other forms of perturbation may also disrupt the arrangement of the PS spheres and can affect the stability of SERS signal from the AuFON. This makes it challenging to further refine or develop the AuFON substrates. By contrast, the Au-SiO₂-NP substrates possess higher development potential as the inherently stable structure allows post-fabrication treatment and surface functionalization of the substrate to extend its sensing capability.

Reusability of the AuFON is also limited by the less stable PS sphere structure as cleaning of the substrate will risk disrupting and detaching the underlying PS spheres. In a typical SERS measurement, binding of the target molecules to the substrate surface is crucial in obtaining high signal-to-noise ratio. A piranha cleaning step is necessary after measurement to ensure complete removal of the bonded molecules from the substrate surface before reuse. In the case of the

Au-SiO₂-NP, the SERS structure is fabricated from the underlying substrate and is therefore more able to withstand the vigorous standard cleaning process. Consequently, the Au-SiO₂-NP SERS substrate demonstrated in this work has a higher reusability potential.

5.4 Conclusion

A resilient Au-SiO₂-NP SERS substrate is demonstrated by performing PECVD of SiO₂ on NSL fabricated Si-NPs. A SERS enhancement factor of approximately 4.90×10^6 is achieved by the optimized Au-SiO₂-NP substrate deposited with 50nm of PECVD SiO₂. The optimized Au-SiO₂-NP substrate exhibit comparable SERS signal to those of a well-established AuFON substrate. FDTD simulation of the electric field enhancement and the scattering cross-section spectra revealed that the best SERS performance occurs when the plasmon resonance of the structure is well-within the excited Raman band. The simulated electric field distribution of the structures under 633nm wavelength excitation shows that the Au-SiO₂-NP possess a larger effective field enhancement region. This possibly compensate for the weaker electric field enhancement found in the Au-SiO₂-NP structure and results in SERS performance that is comparable to those of the AuFON. As compared to the AuFON substrate, the Au-SiO₂-NP substrate possess better stability and reusability which allows for post-fabrication treatment to extend its sensing functionality. The successful demonstration of utilizing the PECVD deposition process to optimize SERS performance provides an alternative solution for fine-tuning SERS substrates.

Chapter 6 : AAO-based SERS Substrate

6.1 Introduction

The NSL technique is a cost-effective alternative for patterning periodic array of nano-structures for SERS applications. Without additional processing, the hexagonally PS beads are found to be excellent deposition template for obtaining low cost SERS substrates. However, achieving monolayer of un-interrupted PS packing [107] remains a challenge, especially when very small beads are used. Convective assembly [108], dip-coating method [109] and the Langmuir–Blodgett (LB) method [110, 111] are some proposed solutions for overcoming this constraint but they either have a long processing duration or require the use of expensive equipment. In particular, the use of a LB-trough system for facilitating the self-assembly of the PS beads is costly and has low throughput since the substrate has to be pulled at low rate. The spin-coating method is an economical alternative, but the resulting discontinuity and dislocation of the PS beads can greatly affect the scalability of the process.

In recent years, porous anodized aluminium oxide (AAO) assisted fabrication technique has become popular due to its simplicity and its capability to produce large area of highly ordered and periodic cylindrical nano-holes with excellent continuity throughout the entire anodization domain [87]. This porous layer has been further processed into an ultra-thin alumina mask (UTAM) for patterning nano-structures on SERS substrate [33]. Apart from functioning as a mask, the gaps localized in the nano-holes of the AAO are also found to be effective SERS hotspots that are suitable for various application [45, 112, 113]. In this work, the AAO process is exploited to fabricate a highly sensitive and scalable AAO-nano-fibre (ANF) SERS

substrate by using an optimized combination of the second anodization step and an additional wet etching process on the conventional AAO nano-hole array. AAO nano-hole array were transformed into large area nano-fibre arrays by sufficiently wet etching the AAO formed after the second step anodization. The optimized ANF SERS substrate are characterized to evaluate its SERS performance. The measured SERS performance of the ANF substrate is compared to those of AAO nano-hole substrate and AuFON substrate. The detection limit, signal uniformity, reproducibility and stability of the ANF substrate are measured and discussed. In addition, label free detection of two major RNA and DNA bases (guanine and cytosine) is evaluated using the ANF substrate.

6.2 Experimental Details

6.2.1 Fabrication Technique

AAO nano-hole array is prepared using the two-step anodization process of high purity aluminium foil as described in Section 3.2.3. The AAO starting templates of different thicknesses are fabricated by varying the second step anodization time from 10s to 210s. The anodized aluminium is then wet-etched in 5% H_3PO_4 solution for duration varying from 0 to 80min to modify the AAO structure. Figure 6.1 illustrates the different types of SERS substrate that are fabricated by selectively varying the wet etching time on the AAO template. The AAO-based substrates are SERS-activated by depositing a thin layer of gold using an E-beam evaporator. Surface morphologies are characterized using a field emission scanning electron microscope (FE-SEM, SU8010, Hitachi) at 5 kV.

Figure 6.1 Schematic diagram illustrating the formation of different types of AAO-based structures by varying the wet etching time.

6.2.2. FDTD Simulation

The simulated plasmonic resonance of the ANF substrate is obtained by employing the three-dimensional finite-difference time domain (3D-FDTD) method using the Lumerical Solution software. To calculate the scattering cross-section spectrum of the structure, a total-field-scattered field (TFSF) excitation is injected at normal incident onto structure in the z direction. The simulation domain is defined by a 100×100×500 nm cube (mesh size:1 nm) with a periodic boundary condition (PBC) applied to the x and y-axis and a perfect matching layer condition applied to the z-axis. The ANF substrate is modelled as a periodic array of gold rod on a 250 nm thick gold substrate as shown in Figure 6.2. The Johnson and Christy model is used to describe the dielectric permittivity of gold. The scattered field analysis domain is constructed outside the TFSF source to monitor the scattering cross section.

Figure 6.2 FDTD simulation model of the ANF substrate.

6.3 Results and Discussion

6.3.1 Surface Morphology

The first anodization step in the AAO fabrication process serves as a pre-patterning step to create a highly ordered nano-cavity growth site on the aluminium substrate for the subsequent anodization step [114]. The second anodization step defines the thickness of the AAO and a subsequent wet etching step can be used to tune the diameter of the nano-holes [115, 116]. A systematic study of different combinations of the second anodization and wet etching time durations were carried out to determine the optimal processing conditions for achieving the most effective AAO-based SERS substrate. The wet etching time was varied from 0–80 min on 100–1000nm thick AAO formed with a second anodization time of 10–210s. Figure 6.3 shows the characterization of the final AAO height as a function of the second anodization time (Δt_A) and the subsequent wet-etching duration (Δt_E). The diameter of the AAO nano-holes as a function of wet-etching time (Δt_E) is shown in Figure 6.4.

Figure 6.3 (a) AAO height in the nano-holes regime plotted as a function of anodization time (Δt_A). (b) Height of AAO fabricated with second step anodization time of 10s (T10), 20s (T20), 30s (T30), 40s (T40), 50s (T50) plotted as a function of wet etching time (Δt_E).

Figure 6.4 Pore diameter of the AAO plotted as a function of wet etching time (Δt_E) in 5% H_3PO_4 solution with the corresponding SEM image of the AAO surface. Scale bar: 200nm

It is clear from Figure 6.3 (a) that the AAO thickness increases linearly with the anodization time. In addition to linearly decreasing the height of the AAO as shown in Figure 6.3 (b), the subsequent wet-etching step also widens the pores

(refer to Figure 6.4). These results indicate that a linear isotropic etching of the alumina materials is achieved using 5% H_3PO_4 solution and demonstrate that the anodization time and wet-etching time are suitable parameters for controlling the geometry of the AAO nano-holes.

With the etching time of 0-50min, the AAO template retains its original hexagonally packed nano-hole structure. As the wet etching time extends to the 58–75 min range, breakdown of the pores occurs and the hexagonally arranged nano-hole array is found to be converted into high density of vertically aligned alumina nano-fibres across the entire anodization domain. The formation of the ANF is attributed to the presence of more material between any three adjacent nano-holes as compared to those between any two neighbouring nano-holes as illustrated in Figure 6.5. With a suitably selected wet etching time, the materials between any two neighbouring nano-holes can be completely removed while those between any three adjacent nano-holes are only partially etched. The remaining un-etched material between any three adjacent nano-holes consequently forms the nano-fibre array.

Figure 6.6 shows the experimentally determined wet etching time for nano-fibre formation plotted as a function of the starting AAO thicknesses (and the corresponding second anodization time). It is observed that the wet etching time required to achieve nano-fibre array formation increases linearly as the thickness of the starting AAO increases from 100–1000 nm. This is consistent with the volumetric increase of the alumina into the AAO nano-holes as the anodization time increases [117, 118]. As a result of the increased thickness between adjacent nano-holes, a longer wet etching duration is required to obtain nano-fibre formation on the substrate. The fabricated ANF substrates possess two distinct morphologies which

depend on the starting thickness of the AAO as shown in the SEM images of Figure 6.7.

Figure 6.5 SEM images of AAO surface after etching in 5% H_3PO_4 for (a) 0 min and (b) 50 min (scale bar: 200 nm).

Figure 6.6. Wet etching time for ANF formation plotted as a function of starting AAO thicknesses/second anodization time.

A thin starting AAO of 100–300 nm thick (anodization time of 10–60 s) produces short ANF that are sufficiently rigid to remain standing on the substrate after wet etching. A thicker starting AAO of 400–1000 nm thick (anodization time of

90–210 s) produces longer ANF that collapse towards one another to form networks of bundle-like structures. Significantly increasing the wet etching time will completely remove all alumina and expose the underlying nano-cavities on the aluminium substrate. For SERS activation of the substrate, 60 nm of gold is deposited onto the ANF substrate by the E-beam evaporation method. The resulting morphology of the ANF is observed to change from the initial fine tip structure to a finger-like structure with a rounded tip as shown in Figures 6.7(b), (d), (f) and (h).

Figure 6.7 SEM images of standing ANF fabricated from AAO with a second anodization time of (a) 30 s, (c) 60 s and bundled ANF fabricated from AAO with a second anodization time of (e) 90 s, (g) 150 s. The respective ANF substrates coated with 60 nm of electron beam evaporated gold are shown in (b), (d), (f) and (h).

6.3.2 SERS Characterization of AAO-based SERS Substrate

2NT Raman probe molecules are attached to the surfaces of the fabricated substrates for SERS measurement to evaluate their performance. Figure 6.8 (a) shows the measured intensity of the 1380 cm^{-1} characteristic peak plotted as a function of the second anodization and wet etching time. It is interesting to note that

the ANF substrates (58–75 min wet etching time) produce much higher SERS intensity as compared to the nano-hole (0–50 min wet etching time) and nano-cavity (80 min wet etching time) substrates. The significant improvement in the SERS signal is attributed to the EM hotspots found in the nano-gaps between adjacent structures of the ANF substrate. These nano-metric gaps serve as optical cavity that strongly confine the surface plasmon and produce an intense amplification of the localized EM field. The optimal ANF substrate is achieved with a second anodization time of 30 s and a wet etching time of 60 min

Figure 6.8 (a) Measured SERS intensity of the 1380 cm^{-1} characteristic peak (2NT) plotted as a function of the second anodization and wet etching time. (b) SERS spectra of ANF, nano-hole and nano-cavity substrates using $0.1\text{ }\mu\text{M}$ 2NT.

As shown in Figure 6.8 (b), consistent SERS improvement for all characteristic peaks of the 2NT is observed in the optimal ANF substrate. Among all the fabricated substrates, the vertical standing fibre morphology (second anodization time of 20–60s) gave a larger SERS enhancement as compared to that of the bundled fibre morphology (second anodization time of 90–210s). This can be explained by the considerable diminution of SERS hotspots as a result of fibre

collapsing to form fused structures. Potential SERS hotspots from underlying fibres in each fused structure are obscured and the resulting SERS signal from such substrates is greatly reduced.

6.3.3. Resonance Study of the Optimized ANF Substrate

To understand the origin of the strong SERS enhancement in the ANF substrate, the structural resonance of the optimized ANF substrate is investigated by measuring the SERS spectrum at the excitation wavelengths of 532 nm, 633 nm and 785 nm. The spectra comparison presented in Figure 6.9(a) reveals that the maximum enhancement occurs with the 633 nm wavelength. This indicates the presence of a strong resonance effect of the ANF substrate at 633 nm wavelength. At 532 nm excitation wavelength, plasmonic effect of gold is lossy and negligible signal enhancement is observed. While some SERS signal is measured with the 785 nm wavelength, the enhancement is not strong as this excitation wavelength and its associated scattered field is too far from the structural resonance peak.

The resonance peaks in the various types of plasmonic substrates can be red-shifted by reducing the inter-particle gaps [96]. The red-shifting of the resonance peak is typically accompanied by an increase in the magnitude of the EM enhancement factor. This is attributed to the increased in the near-field coupling of the LSPR as a result of the nano-confinement effect induced by closely spaced plasmonic structures, leading to a significantly amplified EM field. By sufficiently reducing the gap, the structural resonance can be specifically tuned to match the incident and scattered field wavelength to further optimized SERS enhancement.

Figure 6.9 (a) SERS spectrum comparison of the 60nm gold-coated ANF substrate excited with wavelengths of 532 nm, 633 nm and 785 nm. **(b)** SERS intensity of the ANF substrate at the 1062 cm⁻¹ and 1380 cm⁻¹ 2NT peak, measured with excitation wavelengths of 532 nm, 633 nm and 785 nm, plotted as a function of gold thickness

To elucidate the presence of this phenomenon in the ANF substrate, gold of thickness varying from 20–80 nm is deposited onto the ANF by E-beam evaporation. The evaporated gold material agglomerate on the surface of the AAO fibres which increases the width and the height of the structure. Figure 6.9(b) shows the SERS intensity of the two characteristic peaks of 2NT (1062cm⁻¹ and 1380cm⁻¹) measured with the excitation wavelengths of 532nm, 633nm and 785nm over the range of deposited gold thickness. The result shows that the ANF substrate consistently exhibit the strongest enhancement at the 633nm wavelength excitation across the range of gold thickness with the optimal enhancement achieved by depositing 60 nm of gold. This suggests that the intrinsic resonance of the gold nano-structures is in close vicinity to the 633 nm laser wavelength and that further optimization of the resonance condition can be realized by reducing the gap between adjacent ANF structures to increase the plasmonic coupling effect.

Figure 6.10 Theoretical scattering cross section of the ANF substrates with gold deposition thickness of (i) 20 nm, (ii) 40 nm and (iii) 60 nm calculated using a Lumerical 3D-FDTD simulation tool.

To obtain a theoretical validation of the plasmonic coupling phenomenon, the ANF structure is modelled as a periodic array of gold rod with varying diameter and height to simulate the accumulation of gold on the sidewall and on the tip of the ANF with increasing deposition thickness. Figure 6.10 shows the simulated scattering cross-section obtained from the simulation study. The resonance of the scattering cross-section is observed to consistently red-shift and its magnitude increases with increasing gold thickness, in good agreement with the results obtained in Chapter 3. The resonance peak is also observed to broaden which results in a higher scattering efficiency for higher Raman modes, thereby increasing the versatility of the ANF substrate.

6.3.4. Evaluation of the Optimized ANF Substrate

6.3.4.1 Substrate Sensitivity.

As discussed in Chapter 2, SERS sensitivity of the ANF substrate can be quantified using two metrics, namely, the enhancement factor (EF) and limit of detection (LOD). The average EF of the ANF substrate is calculated using the following equation (5):

$$EF = \left(\frac{I_{SERS}}{I_{RAMAN}} \right) \left(\frac{N_{RAMAN}}{N_{SERS}} \right) \quad (5)$$

where I_{SERS} and I_{RAMAN} are the Raman intensity of the SERS and Raman spectra of 2NT, respectively. N_{SERS} and N_{RAMAN} represent the number of molecules contributing to SERS signals and Raman signals, respectively. I_{Raman} is measured by drop casting 20 μ L of 0.1M 2NT probe solution onto a silicon substrate. The laser power is increased to 1 mW with the same integration time to obtain measurable signal. The determination of N_{SERS} is simplified by assuming monolayer coverage of the 2NT molecule which has been reported to be 0.244 nm²/molecule [119]. The optimized ANF substrate exhibits an EF of 9.28×10^6 . This value is comparable to those of solid-state nano-array SERS substrates reported in the literature which typically varies between of 10^6 – 10^8 [42].

The concentration of the 2NT solution is gradually decreased from 10^{-6} to 10^{-10} M to determine the LOD of the optimized ANF substrate. The spectrum comparison of the SERS signal presented in Figure 6.11(a) shows that all characteristic peaks of 2NT remain detectable down to a concentration of 0.1 μ M. As the concentration decreases further into the nano-molar range, the weaker peaks from 200–1000 cm⁻¹ disappear into the background noise. The 1064 cm⁻¹ and 1378

cm^{-1} characteristic peaks, however, remain detectable at the extreme low concentration of 0.1 nM.

Figure 6.11 (a) SERS spectrum of the 2NT probe solution with concentrations varying from 0.1 nM to 1 μM absorbed onto ANF substrate and the (b) corresponding signal-to-noise ratio of the 1062 cm^{-1} (♦) and 1380 cm^{-1} (■) 2NT characteristic peak

As discussed in Chapter 2, the LOD is commonly established by determining the minimum concentration of the Raman probe where the corresponding SERS SNR is at least 3. From Figure 6.11 (b), it can be seen that the SNR of the SERS signal is more than 3 for concentration down to 0.1 nM. This result indicates that the SERS sensitivity of the ANF substrates is sufficiently large for nano-molar detection of analytes. To further evaluate the sensitivity of the substrate, the SERS signal of the ANF substrate is compared with that of the well-established AuFON substrate. Using the same gold deposition thickness and measurement condition, the ANF substrates gave an approximately three-fold improvement in SERS signal over all characteristic peaks as depicted in Figure 6.12 (b). In addition, the high sensitivity of the ANF does not require high excitation power or long exposure time, routinely used in published SERS work to produce significant SERS signal. This advantage allows

the use of low laser power and short exposure time to avoid laser heating effects which can affect the stability of the structure.

Figure 6.12 SERS spectrum comparison between the optimized ANF and AuFON substrates with a 2NT concentration of 0.1 mM.

6.3.4.2 SERS Signal Uniformity, Reproducibility, and Stability

The signal uniformity of the optimized ANF substrate is evaluated by computing the standard deviation of the 1378 cm^{-1} characteristic peak of 2NT collected from 30 random locations on the substrate. The spots are chosen such that they covers all region of the ANF substrate to ensure that the measurement is unbiased. This procedure is repeated with two additional batches of ANF substrate to evaluate the sample to sample variation of the fabrication process. The resulting intensity distribution of the 1378 cm^{-1} 2NT peak in Figure 6.13 (a) indicates that the three different ANF substrates exhibit excellent signal standard deviation in the range of 6–8% which is well within the widely accepted standard of 10% [120]. In Figure 6.13(b), the substrate is found to possess excellent stability as evaluated by

measuring the sample 1 day, 1 week and 1 month after fabrication. The average signal strength and standard deviation remains in the range of 16500–17500 counts and 5–8%, respectively, with no major deterioration.

Figure 6.13 Boxplot of the SERS intensity of the 1378 cm⁻¹ 2NT peak measured in (a) three different batches of ANF substrates and (b) plotted as a function of substrate storage time before measurement.

6.3.4.3 Scalability

The other major advantage of the proposed ANF substrate is its relatively simple fabrication steps and the highly scalable process. Large area AAO substrates can be fabricated by placing aluminium sheets in a region of constant electric field using a platinum counter electrode and a voltage source. Hexagonally arranged nano-holes formed on the entire anodization domain can be easily transformed into ANF by a well-controlled wet etching step. With a precise control of the anodization and wet etching parameters, consistency in the dimension of the ANF structure can be easily achieved. Furthermore, the ANF structure is supported by the aluminium substrate which boosts the overall robustness while providing sufficient flexibility for further processing into customized sizes. The alumina composite of the AAO is

biocompatible to skin tissue and cell which makes them suitable material for biological sensing application

6.3.4. Label-Free Detection

Specific label-free detection is one of the numerous benefits of SERS as it allows the detection of bio-chemical molecules without functionalizing the surface with long chain of receptors and cross-linkers that could possibly affect the fidelity of the signal. In this section, the label-free detection capability of the ANF substrate is evaluated using two major DNA and RNA nucleobases: guanine ($C_5H_5N_5O$) and cytosine ($C_4H_5N_3O$) at a concentration of 0.1mM which is equivalent to 11.1ppm and 15.1ppm, respectively. Analytical grade cytosine and guanine (sigma Aldrich) is first dissolved in de-ionized water to achieve a concentration of 0.1mM before drop casting onto the ANF substrate. The ANF substrate with the nucleobase is then heated over a hotplate at a temperature of 50°C to accelerate the drying process. SERS measurement is then conducted using a 633nm wavelength laser excitation with a power of 0.1mW. A 10s integration time is used to obtain the SERS spectrum as shown in Figure 6.14.

The measured SERS signatures of guanine and cytosine shows distinguishable characteristic peaks despite their similarity in chemical compositions. The characteristic SERS peaks is attributed to the differences in the chemical structure and the bond vibration mode. The high sensitivity of the ANF substrate exhibit sufficient enhancement to produce detectable SERS signal without the need for surface functionalization to capture specific target molecules. Furthermore, the signal is acquired without noise-reduction filter which preserve the fidelity of the measurement. Across the Raman shift of 600-1600 cm^{-1} , the peaks are sufficiently

separated without significant overlapping. For example, the 669cm^{-1} and 863cm^{-1} guanine peak is separated by approximately 124cm^{-1} and 70cm^{-1} , respectively, from the 793cm^{-1} cytosine peak.

Figure 6.14 SERS spectrum of Guanine and Cytosine with a concentration 0.1mM

All characteristic peaks between guanine and cytosine have a minimum separation of 50cm^{-1} which can be easily distinguished by the Raman system used in this work which samples the wavelength at a resolution of 0.1nm . This preliminary result therefore demonstrates that the ANF substrate is capable of label-free detection of biological samples. In addition, the measurement is conducted without the need for over-night sample incubation and is highly suitable for the on-site detection of biological compounds. This experiment is only intended to highlight the sensitivity of the ANF structure, by demonstrating label-free SERS detection capability without the aid of surface functionalization. In cases where SERS

measurement of biological molecule in its natural environment has to be made, one then needs to consider the use of suitable receptor molecules that do not interfere with the measurement to bond the molecule-of-interest to the SERS substrate

6.4 Conclusions

A cost-effective AAO-based nano-fibre SERS substrate is realized by controlling the second anodization time and incorporating an additional wet etching step on the AAO substrate. The proposed approach transforms the standard nano-hole array into ANF structures with morphologies that are dependent on the starting AAO thickness and wet etching duration. These ANF substrates exhibit a higher SERS sensitivity than the nano-hole and the nano-cavity arrays. The optimized ANF substrate has an EF of 9.28×10^6 and its sensitivity surpasses that of the well-established AuFON SERS substrate by three-fold under the same measurement condition. The optimized ANF, coated with gold thickness ranging from 20–80 nm, exhibits a strong resonance at the 633 nm excitation wavelength. The ANF SERS substrate achieved a detection limit of 0.1 nM at an excitation laser power of 0.1 mW. SERS signal standard deviation from three different batches of ANF samples are found to be within 6–8% which indicates excellent signal uniformity and reproducibility. The substrate stability was also evaluated by demonstrating that the average SERS signal strength and relative standard deviation show no major deterioration after up to 1 month of storage. The label-free detection capability of the ANF substrate is also demonstrated by measuring the SERS spectrum of two major DNA and RNA nucleobases. Characteristic SERS peaks of both guanine and cytosine are clearly visible in the measured spectra. The fabrication process of the

ANF substrate is relatively simple and highly scalable, and is therefore very suitable for commercial applications.

Chapter 7 : Conclusion

7.1 Conclusion.

A basic study of SERS mechanism is carried out to investigate the modulation in SERS signal as a gold film progresses from a discretized to a quasi-continuous configuration. Strong SERS signal from the discretized gold is observed, which becomes significantly attenuated as the gold particles coalesce to form quasi-continuous gold film. This observation verifies that the dominating mechanism attributing to SERS is the effect of LSPR excitation. FDTD simulations of periodic gold cylindrical disk arrays with decreasing inter-particle gap were carried out to study the effect of near-field dipolar coupling. The result showed that other than the widely recognized local EM field enhancement, the near-field dipolar coupling is also accompanied by an increase in the scattering cross-section of the structure as the particle size increase. Further analysis of the extinction spectrum revealed that the scattering cross-section increases more than the absorbance and exhibits a resonance wavelength that is in closer agreement with the field enhancement. The observation indicates that the scattering cross-section increases the scattering efficiency of the substrate and affects the amount of EM enhanced light that can be radiated out-of-plane as SERS signal. This is in good agreement with the $\sim 2\times$ higher SERS signal measured on an AuFON substrate, with a larger scattering cross-section as compared to a gold sputtered substrate having a higher electric field enhancement. It was also demonstrated that by depositing gold sputtered particles on 3-dimensional textured surfaces such as PDMS and AAO template, the overall scattering cross-section can be increased to improve SERS enhancement. These findings highlight the importance of substrate scattering cross-section in achieving SERS enhancement.

The commonly used SERS Metallization techniques, E-beam evaporation and DC sputtering, were studied to evaluate their effect on SERS performance. The E-beam evaporation technique is found to produce gold film with an inherent roughness while a smooth film is obtained when DC sputtering technique is used. E-beam coated AuFON is observed to produce a significantly larger SERS signal than the sputtered AuFON due to the presence of multiple SERS hotspots arising from the nano-scale roughness of the E-beam gold film. Similar results are obtained when E-beam evaporation and DC sputtering gold is applied to AAO nano-holes substrate, indicating that the inherent roughness of the E-beam evaporated gold is substrate independent. The origin of the improved SERS enhancement is verified with FDTD simulation which shows additional EM hotspots along the roughened surface of the E-beam deposited gold nanosphere under 633nm excitation. A cost-effective hybrid deposition approach, which uses sputtering to first narrow the inter-particle gap to promote near-field LSPR coupling, and a subsequent thin layer of E-beam evaporated gold to introduce roughness, is evaluated to exploit the advantages of both the DC sputtering and E-beam deposition techniques. An improved SERS signal and a decent signal uniformity were achieved when the hybrid deposition approach is applied to AuFON substrate

Periodic array of Au-SiO₂-NP as cost effective SERS substrate is demonstrated by performing PECVD deposition of SiO₂ on NSL fabricated Si-NP. The optimized Au-SiO₂-NPs exhibit comparable SERS signal as the well-established AuFON substrate and has a SERS enhancement factor of $\sim 4.90 \times 10^6$. FDTD simulation of the electric field distribution showed that the Au-SiO₂-NPs possess a larger effective field enhancement region than the AuFON which compensates for its

weaker electric field enhancement. The NSL lithographic technique, using a layer of self-assembled polystyrene beads as the patterning mask offers a more cost-effective approach in attaining large area of periodic nano-structures as compared to direct writing methods. Furthermore, the Au-SiO₂-NPs developed in this work possess improved stability and reusability as compared to the AuFON. This allows post-fabrication treatment to further develop the substrate for practical applications. A complete coverage of Au-SiO₂-NPs across the substrate is still challenging due to the difficulties in achieving very large area of continuous PS packing.

The conventional approach to make SERS substrates using the porous anodized aluminium oxide assisted fabrication technique involves the processing of AAO into UTAM which is then transferred onto a substrate as a patterning mask. The multi-steps involve in the process could potentially introduce significant amount of process variation which can lead to poor signal uniformity. A simple fabrication approach to realize large area of AAO nano-fibres for SERS application is developed by controlling the second anodization time and incorporating an additional wet etching step on the AAO template. The optimized ANF SERS substrate has an EF of 9.28×10^6 and surpasses the sensitivity of the well-established AuFON substrate by three-fold under the same measurement condition. A detection limit of 0.1 nM was attained at a low laser power of 0.1 mW. The low power operation minimizes damage to the analyte and also increases its potential to be developed into portable devices since a small laser system is sufficient to obtain detectable signal. SERS signal standard deviation from three different batches of ANF samples are found to be within 6-8% indicating excellent signal uniformity and reproducibility. The ANF substrate also exhibit excellent stability and no major deterioration in signal is

observed after long period of storage. Label-free detection of cytosine and guanine using the ANF substrate demonstrates its potential for on-site analysis of biological compound. The results obtained in this work showed that the ANF substrate is highly suitable for commercial SERS application.

7.2 Recommendations

The recommendations for future work are as follows:

- a) Scattering cross-section of substrates has considerable contribution to SERS signal. EM field enhanced light needs to be scattered into free-space so that it can be captured as quantifiable SERS signal. Further in-depth studies can be conducted to develop an accurate representation of the scattering cross-section in the SERS equation to provide a more comprehensive description of SERS.
- b) Development of a suitable simulation model to consider the effect of nano-particle size distribution on the optical response and SERS performance.
- c) The ANF structure derived from the AAO template demonstrates excellent SERS performance in terms of signal sensitivity, uniformity, reproducibility, and stability. There is therefore great potential to fabricate the ANF structure onto the tip of an optical fibre to realize a real-time in-situ SERS probe with remote sensing capability.
- d) Surface functionalization of the ANF structure can be further studied extend its applications. For example, the ANF structure can be functionalized with phages, which are excellent bacteria receptors to

realize rapid detection of bacteria in the food and environmental industry. Bio-receptor can also be functionalized onto the ANF to tap on its high SERS sensitivity to realizes a highly specific biological SERS probe.

Author Publications

1. **Lim, Lu-Kai**, and Beng-Koon Ng. "Large scale AAO nano-fiber Substrate for SERS application." *Photonics North (PN)*, 2016. IEEE, 2016.
2. **Lim, L. K.**, et al. "Highly sensitive and scalable AAO-based nano-fibre SERS substrate for sensing application." *Nanotechnology* 28.23 (2017): 235302.
3. **L. K. Lim et al** "Investigation of the Role of Scattering Cross-section on SERS Enhancement" (*submitting to Journal of Applied physics*)
4. **L. K. Lim et al** "SERS Optimization of Au-SiO₂ Nano-pillar Array using Plasma Enhanced Chemical Vapour Deposition" (*submitting to Journal of Nano Research*)

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