Advanced SERS Sensing System With Magneto-Controlled Manipulation Of Plasmonic Nanoprobes

by

Christopher G. Khoury

Department of Biomedical Engineering
Duke University

Date: ______________________

Approved:

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Tuan Vo-Dinh, Advisor

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Nirmala Ramanujam

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Gregg E. Trahey

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Benjamin J. Wiley

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Benjamin B. Yellen

Dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Biomedical Engineering in the Graduate School of Duke University 2012
ABSTRACT
(Biomedical Engineering)

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Abstract

There is an urgent need to develop practical and effective systems to detect diseases, such as cancer, infectious diseases and cardiovascular diseases. Nanotechnology is a new, maturing field that employs specialized techniques to detect and diagnose infectious diseases. To this end, there have been a wealth of techniques that have shown promising results, with fluorescence and surface-enhanced Raman scattering being two important optical modalities that are utilized extensively. The progress in this specialized niche is staggering and many research groups in academia, as well as governmental and corporate organizations, are avidly pursuing leads which have demonstrated optimistic results.

Although much fundamental science is still in the pipeline under the guise of both ex-vivo and in-vivo testing, it is ultimately necessary to develop diagnostic devices that are able to impact the greatest number of people possible, in a given population. Such systems make state-of-the-art technology platforms accessible to a large population pool. The development of such technologies provide opportunities for better screening of at-risk patients, more efficient monitoring of disease treatment and tighter surveillance of recurrence. These technologies are also intrinsically low cost, facilitating the large scale screening for disease prevention.

Fluorescence has long been established as the optical transduction method of choice, because of its wealth of available dyes, simple optical system, and long heritage from pathology. The intrinsic limitations of this technique, however, have given rise to a complementary, and more recent, modality: surface-enhanced Raman scattering (SERS).
has been an explosive interest in this technique for the wealth of information it provides without compromising its narrow spectral width.

A number of novel studies and advances are successively presented throughout this study, which culminate to an advanced SERS-based platform in the last chapter.

The finite element method algorithm is critically evaluated against analytical solutions as a potential tool for the numerical modeling of complex, three-dimensional nanostructured geometries. When compared to both the multipole expansion for plane wave excitation, and the Mie-theory with dipole excitation, this algorithm proves to provide more spatially and spectrally accurate results than its alternative, the finite-difference time domain algorithm.

Extensive studies, both experimental and numerical, on the gold nanostar and Nanowave substrate for determining their potential as SERS substrates, constituted the second part of this thesis. The tuning of the gold nanostar geometry and plasmon band to optimize its SERS properties were demonstrated, and significant 3-D modeling was performed on this exotic shape to correlate its geometry to the solution’s exhibited plasmon band peak position and large FWHM. The Nanowave substrate was experimentally revived and its periodic array of E-field hotspots, which was until recently only inferred, was finally demonstrated via complex modeling.

Novel gold- and silver- coated magnetic nanoparticles were synthesized after extensive tinkering of the experimental conditions. These plasmonics-active magnetic nanoparticles were small and displayed high stability, were easy to synthesize, exhibited a homogeneous distribution, and were easily functionalizable with Raman dye or thiolated molecules.

Finally, bowtie-shaped cobalt micromagnets were designed, modeled and fabricated to allow the controllable and reproducible concentrating of plasmonics-active magnetic nanoparticles. The external application of an oscillating magnetic field was accompanied by a cycling of the detected SERS signal as the nanoparticles were concentrated and re-dispersed in the laser focal spot. This constituted the first demonstration of magnetic-
field modulated SERS; its simplicity of design, fabrication and operation opens doors for its integration into diagnostic devices, such as a digital microfluidic platform, which is another novel concept that is touched upon as the final section of this thesis.
To my parents, who supported me throughout this incredible journey, and without whom it would not have been possible.
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- $SI_{1\times} = 177/10$ (assuming $I_{\text{blue}}=10$), $SI_{10\times} = 4300/240 = 17.9$, $SI_{20\times} = 5825/700$, $SI_{50\times} = 8640/1400 = 6.2$, $SI_{100\times} = 1100/2170 = 5.1$. The standard deviation error bars indicate the variation in detected SERS at 3 adjacent micromagnet junctions along the y-direction. The two curves on the right document the relationship between SERS intensity and MNPs@Au@Ag concentration in the junction and in bulk solution.

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List of Abbreviations and Symbols

Abbreviations

SNR      Signal-to-Noise Ratio
POC      Point of Care
FEM      Finite Element Method
FDTD     Finite Difference Time Domain
ME       Multipole Expansion
DoF      Degrees of Freedom
NP       Nanoparticle
NPs      Nanoparticles
MNP      Magnetic Nanoparticle
MNP@Au   Gold-coated Magnetic NP
MNP@Au@Ag Silver & gold coated Magnetic NP
SERS     Surface-enhanced Raman scattering
PDT      Photodynamic therapy
PTT      Photothermal therapy
EtOH     Ethanol
NaOH     Sodium Hydroxide
OA       Oleic acid
OAm      Oleylamine
Fe(acac) Iron acetate
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Name</th>
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<tbody>
<tr>
<td>Au(acac)</td>
<td>Gold acetate</td>
</tr>
<tr>
<td>APTES</td>
<td>3-Aminopropyltriethoxysilane</td>
</tr>
<tr>
<td>TEOS</td>
<td>Tetraethyl Orthosilicate</td>
</tr>
<tr>
<td>HAuCl₄</td>
<td>Chloroauric acid</td>
</tr>
<tr>
<td>NaBH₄</td>
<td>Sodium borohydride</td>
</tr>
<tr>
<td>MUA</td>
<td>11-mercaptoundecanoic acid</td>
</tr>
<tr>
<td>PL-PEG-COOH</td>
<td>Lipid-PEG-carboxylic Acid (MW2000)</td>
</tr>
<tr>
<td>PLH</td>
<td>poly-L-hystidine</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission Electron Microscope</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning Electron Microscope</td>
</tr>
<tr>
<td>DTDC</td>
<td>3′-diethylthiadiacarbocyanine iodine</td>
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Introduction

There is an urgent need to develop practical and effective systems to detect diseases, such as cancer, infectious diseases and cardiovascular diseases. For example, cancer will claim the lives of over 1500 Americans each day this year, as it is now one of the leading causes of death for adults in the United States. In spite of the exponential increase in new technology platforms and biomarkers that have been discovered and reported in the literature for the diagnostics, prognostics and therapeutics of such diseases, only few have made it to the clinical world. With a number of agencies continually contributing significant investment to fund ongoing research in this complex field, it is in the interest of mankind as a whole to tackle such life-threatening diseases by any means necessary.

1.1 The need for advanced diagnostic systems

The critical step in any diagnostic assay is the ability to sensitively and selectively recognize the “target” or biomarker in a mixed population of species. A biomarker is defined as an in vivo, biologically derived molecule that indicates the progress or status of a disease. Using cancer as an example, biomarkers must not only signal the presence of a tumor or cancer, but should also predict the stage of tumorigenesis. Biomarkers for cancer diagnosis
can be tumor associated antigens, such as cellular molecules that can be detected in tumor
cells, blood or urine, or other body fluids that are over-expressed due to cancer onset and
growth. Most cancer biomarkers are discovered by molecular profiling studies, or omics
technologies, that are based on a correlation between a molecular signature and cancer be-
havior. By understanding the interrelationships between a defining set of biomarkers, the
diagnosis and prognosis of cancer based on a patient’s molecular profile could be possible,
which hints towards personalized and predictive medicine. The biomarkers that have been
deemed reliable for the diagnosis and prognosis of their corresponding diseases are listed
in several reference cancer databases (NCBI) and in the literature.\textsuperscript{1–3}

The detection and diagnosis of cancer typically makes use of biosensors. A biosensor
is defined as a bioanalytical device incorporating a \textit{bioreceptor} that is integrated with a
\textit{transducer}, whose generated signal magnitude is proportional to the analyte concentration
being detected. These two concepts are discussed below.

\subsection*{1.1.1 Bioreceptors}

Traditionally, the bioreceptor of choice for detecting proteins has been the antibody, which
is employed in the enzyme-linked immunosorbent assay (ELISA),\textsuperscript{4} and its “sandwich as-
say” variations.\textsuperscript{5} The biomolecule to be detected is sandwiched between a surface-bound
primary antibody (bioreceptor) and a fluorescence-labeled secondary antibody (transducer).
ELISA assays are usually carried out in multiwell plates, with one type of protein detected
per well, and the high affinity of the primary antibody yields a limit of detection in the
picomolar concentration regime. Nevertheless, ELISA has a number of shortcomings:

1. It is single-protein detection method, although extendable to multi-well detection

2. The dynamic range of detection is $\sim 10^2$, limited by the signal-to-noise ratio (SNR)
   and fluophore photobleaching

3. The use of 2 antibodies per biomolecule makes this procedure expensive, time-
consuming and non-trivial in terms of isolating the correct antibodies for a given biomolecule of interest

4. The lack of stability of antibody molecules makes long-term storage unfeasible and hinders its wider application and commercialization

As such, replacing these natural biomolecules with artificially produced receptors or biomimics, such as aptamers and peptides, has become a new thrust in the research arena. Compared to antibodies, these synthetic ligands are robust structures that do not lose their specificity in diverse environments, are synthesized via the conventional “wet” chemistry techniques and can easily be structurally modified to support the addition of reporters or allow for immobilization to sensing elements.

Peptides are smaller than antibodies, can be chemically synthesized and derivatized in large batches. Peptide sequences with affinity towards target analytes are being identified by the use of phage display technology, which has led to the successful synthesis of peptide ligands that target specific cancer biomarkers, such as HER2/neu receptor, ErbB-2 and ICAM-1. Peptides have relatively lower affinity than antibodies, however, so more effort in designing their assays is required and further research into obtaining higher affinity peptides is also desirable.

Aptamers are synthetic nucleic acids ligands (DNA or RNA) which, when carefully selected from a combinatorial library of synthetic nucleic acids, can be processed via synthetic evolution of ligands (SELEX) to target molecules such as cancer biomarkers. Although aptamers may have comparable affinity to antibodies, they have a lower specificity in most cases.

1.1.2 Transducers

Once the bioreceptor targets the analyte, the transducer, which has an intimate association with the bioreceptor, generates a physically measurable signal that can be electrochemical,
calorimetric, mass change, magnetic, or optical in nature. Although all these methods have their own advantages and show great promise as sensitive transducers, this discussion will focus exclusively on optical transducers because they have been an integral part of the research conducted in the Vo-Dinh lab.

Optical transducers that are employed for biosensing include fluorescence spectroscopy, interferometry and spectroscopy of guided modes of optical waveguides, surface plasmons and surface-enhanced Raman scattering (SERS). The mainstream optical transducer has been fluorescence due to the wide selection of fluorophores that cover the UV-Vis spectral range of interest and have many different functional groups, the relatively simple optical setup required for its use, and the possibility to achieve single molecule detection sensitivity. Additionally, with the widespread use of quantum dots (QDs) that offer advantages over fluorophores, including narrower emission spectra and the absence of photobleaching, fluorescence continues to be a major transduction modality for biosensing in the optical arena.

Nevertheless, alternative technologies are evolving as well; in particular, sensors based on spectroscopy of surface plasmons, or surface plasmon resonance (SPR) sensors, can be considered label-free measurement technologies and have been proposed and progressively developed for more than a decade.

Another surface plasmon based transduction technique, which now occupies a sweet spot in the author’s heart, is surface-enhanced Raman scattering (SERS) that was originally discovered in 1974 by Fleischmann et al., but has now become a prominent transduction technique in a wide array of biosensor applications.

In the optical regime, the availability of these more recent alternatives to fluorophore-based ELISA assays, such as QDs, SPR and SERS, are largely due to significant advances in the field of nanotechnology. Indeed, these three systems display phenomena that originate from their interaction with light, or more specifically electromagnetic fields, at the nanoscale. QDs are semiconductor nanoparticles whose excitons are confined in three di-
mensions such that their electronic properties lie between those of bulk semiconductors and discrete molecules. SPR and SERS systems consist of nanostructured surfaces that are designed such that their nanoscaled features couple incident electromagnetic energy to yield a plasmonic response that can used either for direct sensing (in the case of label-free SPR detection) or indirect sensing (via local electromagnetic field enhancements in the case of SERS). Moreover, the incorporation of nanotechnology into traditional sandwich-type assays, such as ELISA, has also led to improvements in their sensitivity, suggesting that the field of nanotechnology is becoming a important thrust in the biomedical arena that might have widespread uses in many aspects and disciplines.

1.2 Nanotechnology for medical diagnostics

Recently, cancer nanotechnology has become a prominent and promising field of interdisciplinary research, incorporating the disciplines of biology, chemistry, engineering and medicine. It is projected to lead to significant advances in the detection, diagnosis and treatment of diseases, such as cancer, because it hinges on the use of metal, semiconductor and polymeric nanoparticles that possess unique optical, electronic, magnetic and structural properties that are absent in individual molecules or bulk materials. To this end, the functionalization of various nanoparticles with biological molecules has recently become a significant focus.

Due to the versatility of nanoparticle probes, nanotechnology can impact crucial areas in the fight against deadly diseases. For the case of cancer, nanotechnology is impacting four distinct phases: (1) Molecular cancer imaging, (2) Molecular cancer diagnosis, (3) Early cancer detection, and (4) Targeted cancer therapy. Each one of these areas is an extensive and elaborate research field in itself, and because a comprehensive review cannot be contained in the few pages of this thesis, the author would like to redirect the interested reader to a number of excellent reviews in the literature by Nie et al. and Heath and
Davis.\textsuperscript{46} Instead, to lead the discussion toward the focus of this thesis, a brief overview of the use of nanoparticle probes, specifically QDs and SERS probes, in the diagnosis of cancer will now be presented.

1.3 Nanoparticle probes for optical detection

Compared to the majority of micro- and macroparticles, nanoparticles possess characteristic properties that make them ideal for combating cancer: (a) small size and correspondingly large surface-to-volume ratio, (b) chemically-tunable physical properties that directly depend on their size, composition and shape, (c) unusual target-binding properties, and (d) overall structural robustness.

Moreover, targeted nanoparticles are distinguished from other targeting or therapeutic entities by at least four features:

1. Nanoparticles can carry a very large “payload”. A 70-nm nanoparticle can contain \(\sim 2000\) siRNA drug molecules,\textsuperscript{47} whereas antibody conjugates have less than ten.\textsuperscript{48} This effectively concentrates the density of targeting or therapeutic molecules in a given volume of solution.

2. Nanoparticles are sufficiently large to contain multiple targeting ligands that can provide a multiplexing capability, as well as multivalent binding to cell surface receptors.\textsuperscript{49} Because binding to target cells can be tuned by both the affinity and density of the targeting moiety, the repertoire of molecules that can be used as targeting agents is greatly expanded.

3. Nanoparticles are also sufficiently large to accommodate multiple types of drug molecules, which offers the option of controlled, simultaneous therapeutic interventions.

4. Nanoparticles enter cells by endocytosis and therefore bypass multidrug resistance
mechanisms.

In general, nanoparticles can be classified into two distinct types: nanoparticles that are built mainly from organic molecules (such as liposomes, dendrimers and carbon nanotubes), and those that comprise inorganic elements (such as QDs, metal NPs, oxide NPs). Although organic NPs have already produced exciting results in fields such as breast cancer diagnosis and treatment, the two NPs structures of interest that exploit optical detection - QDs and SERS probes - fall under the latter group. Most inorganic nanoparticles comprise a central core that defines the fluorescence, optical, magnetic, and electronic properties of the particle, and a protective organic coating for stability and/or further surface functionalization.

1.3.1 Quantum dots

Quantum dots are fluorescent nanoparticles, ranging from $2 - 10$ nm, that comprise of atoms of group II and VI elements, or group III and V elements. This structure allows QDs to generate powerful fluorescence that originates from the so-called quantum confinement effect and is thus different in nature from fluorescent organic dyes that possess important limitations. The narrow symmetrical emission peaks of QDs can be tuned from 450 nm to 850 nm by adjusting the QD size or chemical composition, and can be visualized simultaneously with a single light source. Moreover, they have exceptional photostability, enabling their fluorescence to be reliably quantified over time. These critical advantages over organic dyes enable them to be used for multiplexed labeling of molecular targets, both \textit{in vitro} and \textit{in vivo}, although their imaging and therapeutic capabilities \textit{in vivo} are limited by the toxic effects of their heavy-metal core, and an alternative is desired to this end.
1.3.2 SERS probes

SERS is a sensitive method for the spectroscopic detection of multiple targets. It relies on SERS probes consisting of gold or silver nanoparticles (NPs) that host a Raman molecule for spectroscopic signature and an organic or silica layer for surface protection and functionalization. The huge electromagnetic enhancements generated in the vicinity of plasmonics-active nanosurfaces has enabled Raman scattering, an intrinsically weak scattering phenomenon, to become an important detection methodology that provides narrow spectral resolution, a wealth of molecular information and the potential sensitivity for single molecular detection.

Typically, gold and silver are the metals of choice for SERS because of the abundance of their salts and their ability to be easily synthesized into homogeneous NPs of varying sizes; they are also chemically functionalizable by well-established methods and boast special optical properties that make them ideal for use as SERS transducers.

Interestingly, the use of colloidal gold dates back to the antiquity in China and Egypt, where it was used for therapeutic and decorative purposes. A famous example is the Lycurgus cup, originating from the 4th century, which is housed at the British Museum in London. However, it was not until 1857 that Faraday reported the first scientific article on gold NPs, attributing the red color of the colloidal nature of gold: Mie then rationalized their absorption properties using Maxwell’s electromagnetic equations in 1908. The red color exhibited by gold nanoparticles occurs because of their interaction with light, a phenomenon that is termed surface plasmon resonance (SPR). SPR is the collective oscillation of free electrons at the surface of nanostructured conductors, that is induced by an incident electromagnetic field impinging on the structure. This process is resonant at a particular frequency of the light and is referred to as localized surface plasmon resonance (LSPR). The surface plasmon oscillation decays by radiating its energy in the form of light scattering, or by non-radiative decay via conversion of absorbed light to heat. At the LSPR
frequency, the electric field intensity and the scattering and absorption cross-sections are significantly enhanced;\textsuperscript{72} for gold and silver NPs, this interaction gives rise to absorption spectra that lie in the visible region.\textsuperscript{69,70} Furthermore, it is precisely this strong electric field enhancement, pertaining to the vicinity of metal nanoparticles, that is exploited for SERS measurements.

Nanoparticles are an attractive alternative to the photoabsorbing and fluorescent dyes that have been the standard biological labels and stains for the last few decades.\textsuperscript{73} Not only do their nanosized dimensions allow them to be incorporated into biological systems, which are of similar magnitude,\textsuperscript{42} but they can be conveniently conjugated with small molecules or recognition ligands to achieve molecular specificity.\textsuperscript{74} Moreover, their large surface to volume ratio creates a high density of these adhered molecules, which increases their binding effectiveness, and potentially accommodates multiple targeting ligands, as well as multiple optical probes. This opens the doors to the possibility of multiplexed detection. In terms of sensitivity, the surface plasmon enhancement experienced by metal NPs ranging from 10 to 100nm boosts their optical cross-sections to 5 orders of magnitude or more greater than those of dye molecules,\textsuperscript{71} effectively making each individual NP equivalent to up to a million dye molecules. In addition, metal NPs are photostable and do not undergo photobleaching, unlike dyes, which allows for higher light excitation energies and longer probing times.\textsuperscript{75} Another unique property of LSPR is that its resonant frequency can be conveniently tuned by changing the nanostructure size, shape, composition or environment\textsuperscript{68,71,76,77} in order to maximize either its radiative or non-radiative attributes, or combining a mix of both, thereby better suiting the bioapplication at hand. For example, elongating the shape of a nanosphere to a nanorod introduces a new long-wavelength plasmon band that is attributed to the SPR along the longitudinal axis. This shift of the plasmon band to the near-IR regions allows access to the optical window in biological tissue, where water absorption is at a minimum, allowing a deeper penetration into living tissues and exciting less background fluorescence.
Although fluorescence has long been the optical technique of choice due to its simplicity and widely available dyes, the explosive growth of SERS in the research community has recently elevated SERS as a promising contender for use in disease detection. Compared to fluorescence that has a broad emission spectrum, and is prone to photobleaching (fluorophores) or is toxic (QDs), SERS offers the potential of multiplexed detection due to its characteristic narrow vibrational peaks. Moreover, the surface binding of Raman dyes to metallic nanoparticles quenches fluorescence, thereby minimizing background noise and photobleaching. Finally, in addition to being inert, gold nanoparticles can be intricately customized by adjusting their size and shape, to ultimately optimize their maximal absorption wavelength and SERS enhancement factor.

Since these major publications in the field, there is wealth of publications in the literature that detail the study of SERS and methods used to maximize its signal. The SERS enhancement factor is subdivided into an electromagnetic enhancement component and a chemical enhancement component, whose magnitudes are dictated by the plasmonic properties of the metallic nanostructure substrate and chemical properties of the Raman dye, respectively. Briefly, the Raman dye can either be resonant or non-resonant with the excitation wavelength; the former generates a much stronger signal than the latter, and additional overlap of the dyes’ absorption spectrum with the plasmon band of the nanostructured substrate and incident excitation wavelength yields further enhancement. This requires the intricate control of NP shape, size and surface, for which there has been an incredible number of publications in the last decade. Rather than detail each one, the author would like to encourage the interested reader to examine Kneipp et al.’s and Nie and Emory’s papers, which paint a nice picture of where the field has been heading for the last decade.
1.4 SERS probes for medical diagnostics

The ability of SERS to be integrated as the optical transducer on disease-targeting probes has been documented by several groups, including the Vo-Dinh group. Recently, SERS has been employed for the detection of nucleic acid targets of interest by functionalizing metallic nanoparticles with Raman dye-tagged DNA oligonucleotides.\textsuperscript{79–83} These probes utilize unique optical and plasmonic properties of the metal nanoparticles as transducers for reporting oligonucleotide hybridization events. Together with the remarkably sharp melting profiles exhibited by oligonucleotide-functionalized nanoparticles,\textsuperscript{84} the sensitivity of DNA detection has been significantly improved.\textsuperscript{80,81,85}

Since our first report on the practical analytical use of the SERS techniques for the trace analysis of a variety of chemicals, including several homocyclic and heterocyclic polyaromatic compounds in 1984,\textsuperscript{86} the Vo-Dinh laboratory has been involved in the development of SERS technologies for applications in chemical sensing, biological analysis and medical diagnostics,\textsuperscript{83,87–90} specifically cancer detection.\textsuperscript{91,92} In addition to the novel molecular sentinel (MS) nanoprobe that was developed to detect the presence of the HIV gene in a homogeneous assay,\textsuperscript{83} our lab has also recently successfully detected single-nucleotide polymorphosm (SNP) and microRNA sequences involved in breast cancers by using the plasmonic coupling interference (PCI) mechanism.\textsuperscript{93} The potential of SERS for detecting and semi-quantifying the presence of crucial disease biomarkers is thus already being exploited as this technique shows great promise for the early diagnostic of diseases.

One of the most important limitations of SERS-based sensing is the inability to properly quantify the SERS signal being detected: due to the extreme sensitivity of SERS to inter-particle plasmon coupling, it is oftentimes challenging to directly correlate the detected signal intensity to the number of target biomarker molecules in the sample, due to uncontrollable aggregation. When aggregation occurs, due, for example, to changes in solution pH, temperature, surface charge neutralization, addition, etc., the nanoparti-
cles uncontrollably collapse such that the surface-bound Raman dye becomes sandwiched between adjacent nanoparticles - this results in inter-particle plasmon coupling, which increases the detected SERS signal many fold, such that it is no longer linearly proportional to the concentration of target biomarkers in the sample. This, in turn, leads to data reproducibility problems, since aggregation is a random event. Attempting to address this problem by reducing the sample volume and ensuring more controllable detection environment by means of microfluidic devices has been attempted by several research groups, which will be discussed next.

1.5 SERS Signal Enhancement & Quantification

The largest obstacle in the application of SERS for quantitative analyses is the difficulties associated with reliably reproducing SERS enhancements. The problem stems from the lack of control over critical factors that dramatically affect the obtained SERS signal, such as degree of NP aggregation, NP size and homogeneity and inhomogeneous distribution of analyte molecules on the NP surfaces.

A number of research groups have implemented diverse microfluidics techniques to address some of these issues, and have been able to demonstrate quantitative SERS-based analyses. 94–97

SERS-based microfluidic chips can be categorized into two broad groups: nanocolloid-based microfluidic systems that manipulate metallic nanoparticles (gold or silver) within a liquid, or colloid-based systems, and, metal nanostructure-embedded microfluidic systems that provide identical SERS-active sites for detection, or substrate-based systems.

1.5.1 Colloid-based systems

When dealing with NPs in solution, hotspots are formed when NPs are in close proximity to each other such that the plasmon coupling between them enhances the local electric field, which in turn amplifies the SERS signal. Quantifying the detected SERS signal can
be achieved by controllably inducing aggregation of NPs, such as to reproducibly create uniform hotspots. Once such platform consists of a NP trapping device that contains a pinched and step microfluidic-nanochannel junction.\textsuperscript{98,99} This structure reproducibly traps metallic NPs and analyte molecules through capillary forces within the channel, providing a quantitative and sensitive signal; this design was employed for the trace detection of an Alzheimer’s disease biomarker.\textsuperscript{100}

More recently, an optofluidic SERS compact disc platform, capable of high throughput biological analysis with additional signal sensitivity, was demonstrated by Choi et al..\textsuperscript{101} This technique also provided a wide range of flow rates depending on disc geometry, which translated to important microfluidic functions such as valving, decanting, mixing, metering, sample splitting and separation. Both the above microfluidic devices demonstrate enhanced SERS signals, but their biggest limitations is the fabrication complexity of these chips and the fact that they are one-time use devices. This would increase the fabrication costs and ultimately device costs. High throughput screening would also be limited for the first device since it comprises only a single channel.

External stimuli to control the aggregation of plasmonic NPs have also been proposed, such as optical tweezers, which trap and control colloidal aggregation where an axial gradient force dominates,\textsuperscript{102} or electrokinetic effects, that have been used to enhance solution-phase mixing of NPs and analytes, and concentrate them into microwells.\textsuperscript{103,104} Unfortunately, these also add to fabrication complexity and cost.

1.5.2 Substrate-based systems

Substrate-based SERS systems comprise of metallic nanostructured surfaces that are integrated into microfluidic devices, and have been developed with the aim of alleviating some of the critical issues experienced by colloid-based systems. The premise is that these immobilized, SERS substrates should be more robust in biofluid, compared to their colloid-based counterparts, due to variations in biofluid ionic strength. Nanowell-based
silver SERS substrates, fabricated by soft lithography to enable mass production, were proposed and more recently, a periodic SERS-active nanostructure was demonstrated to be efficient for microfluidic detection. Nanoholes, which locally enhance the electric field, have been constructed into periodic arrays for the same purpose, but their lower enhancement factor relative to other substrates meant that antenna structures had to be integrated in their vicinity to improve device sensitivity. Although high-throughput detection can be achieved with substrate-based SERS systems, their biggest drawback is their fabrication complexity with respect to designing and implementing nanostructured surfaces, which in turn suggests high fabrication expenses and ultimate device cost. Moreover, these chips are for single use purposes, since once analytes adhere to their SERS-active surfaces, washing becomes futile; this also adds to the final diagnostic costs.

![Figure 1.1: Schematic illustrating two subdivision of SERS platforms and their related pros and cons.](image)

Given the research performed thus far, it is clear that the ideal SERS system has yet to be demonstrated. To give the reader a flavor of where this thesis is headed, Figure 1.1 depicts the two categories into which SERS platforms can be allocated - colloidal systems...
and substrate systems - and their pros and cons.

The goal of the research presented herein is to design and implement a hybrid system that combines the pros of both systems, while avoiding their cons.

1.6 Specific Aims

The specific aims of this dissertation are listed below:

**Aim 1:** Numerical investigation of the plasmonic response and E-field enhancement of plasmonics-active nanoplatforms for SERS detection

The qualitative and quantitative accuracy of the finite element method (FEM) for solving near-field electromagnetics in the vicinity of 3-D plasmonic nanoparticles will be investigated. The accuracy of the solutions will be bench-marked against analytical theories that are widely accepted as the norm in the field: the multipole expansion and the Mie theory. The FEM algorithm will be tested by solving the E-field enhancement around single and dimer nanoshell geometries that are excited by a plane wave or an oscillating dipole. The solution accuracy and resolution in both the spatial and frequency domains will be demonstrated, and the potential of FEM to be employed as a proficient tool for elucidating the plasmonic behavior of nanostructures will be confirmed.

**Aim 2:** Synthesis, characterization and optimization of plasmonics-active nanoplatforms for SERS detection

The gold nanostar, a novel nanoparticle shape, will be thoroughly investigated and optimized such as to evaluate its potential as a promising SERS nanoplatform. The growth kinetics will be studied and complex numerical studies on its plasmonic behavior will be undertaken to understand how it compares to the popular gold nanorod for SERS detection in the NIR. Specifically, the goal is to understand the relationship between nanostar shape, plasmon band characteristics and local E-field enhancement.
The “Nanowave” SERS substrate, an inexpensive and efficient spin-coated assembly of silica nanoparticles with a coated layer of silver or gold of varying thickness, will be fabricated and modeled to determine the optimal metal thickness that yields the strongest SERS enhancement. The numerical model will employ periodic boundaries around a unit cell of the substrate to simulate an infinite plane of particles, thereby accounting for long-range plasmonic effects and smoothed junctions will be forced to produce more realistic solutions.

**Aim 3: Synthesis and functionalization of novel plasmonics-active, magnetic nanoparticles for SERS**

Plasmonics-active magnetic nanoparticles, specifically gold-coated iron oxide nanoparticles, will be synthesized via a newly developed protocol. The ability of these nanoparticles to be effectively controlled by a magnetic field requires them to adhere to strict conditions; they need to be stable, small and magnetically responsive. Moreover, their potential as SERS nanoplatforms requires them to be easily functionalizable, and thus the protocol will be designed to synthesize nanoparticles with these crucial features. Following synthesis, these nanoparticles will be functionalized such that they remain stable while exhibiting a SERS signature.

**Aim 4: Integration of plasmonics-active magnetic nanoparticles with a micromagnet platform to demonstrate SERS detection enhancement and quantification**

A novel concept, combining the colloidal system of SERS-active magnetic nanoparticles and a micromagnet-based substrate system, will be demonstrated that modulates the detected SERS signal via an externally applied magnetic field, in a reproducible manner. Specifically, a novel micromagnet shape will be designed, numerically modeled and fabricated on quartz via photolithography. The ability of the intense magnetic hotspots produced by the micromagnets to concentrate the nanoparticles and enhance the detected
SERS signal will be quantified. The spatial reproducibility of the hybrid substrate will also be investigated, and finally, its versatility for easily controlling the aggregation state of the plasmonics-active magnetic nanoparticles will be demonstrated by measuring their SERS response to a cyclical, applied magnetic field. The integration of this hybrid substrate into a digital microfluidic platform will be discussed and its initial implementation revealed.

1.7 Outline

The remainder of this thesis is divided into 4 chapters, which guide the reader through the flow of how the research was conducted over the author’s Ph.D. career. As concisely summarized above, the first chapter discusses the context of the complex field of nanobiotechnology that attempts to address the plight of deadly diseases.

Chapter 2 presents an investigation of the accuracy of numerical simulation based on the finite element method (FEM), when compared to analytical solutions. The first study details the analysis of the interesting dimer nanoshell problem, for different parameter variations such as shell thickness, nanoparticle separation and overall diameter. This is compared against the multipole expansion algorithm for a plane wave excitation. The second study employs an oscillating dipole as the excitation source to model the radiation of a fluorescence or Raman molecule when it is located inside or outside of a silver nanoshell structure.

Chapter 3 details novel studies on SERS substrates, both colloidal and wafer-based. The synthesis, characterization and optimization of gold nanostars for SERS is first presented. These exotic 3D shapes were then modeled numerically to unravel the relationship between nanostar shape and plasmon band tunability in the visible region. The extensive plasmon broadening of nanostar solutions that was experimentally observed was also modeled by constructing 3D models with higher degree of complexity. In terms of wafer-
based SERS substrates, the simple spin-coating method that was used to produce sensitive SERS substrates by Vo-Dinh et al. in 1984 was revisited experimentally; this substrate was then numerically modeled using a complex 3D structure that was made as realistic as possible by employing three-dimensional rounding and periodic boundaries. This wafer was coined “Nanowave”.

Chapter 4 describes the synthesis, characterization and functionalization of novel plasmonics - active magnetic nanoparticles that were developed for the purpose of achieving a quantifiable and reproducible SERS signal; these nanoparticles therefore need to adhere to very stringent conditions. The difficulties in synthesizing such multifunctional nanoparticles is addressed by reproducing the major protocols in the literature and discovering their significant limitations when attempting to manipulate them in a controllable fashion. Finally, a novel synthesis is presented that overcomes most of their drawbacks, to yield small, stable, and SERS-active magnetic nanoparticles that could possibly be utilized as versatile SERS substrates in a digital microfluidic system.

Chapter 5 presents a novel concept to manipulate the plasmonics-active magnetic nanoparticles developed in Chapter 4 to yield an enhanced and reproducible SERS signal via a novel magnetic field modulation technique. This study involved the design and simulation of newly-shaped micromagnets to optimize their ability to generate extremely high magnetic field gradients that concentrate magnetic nanoparticles; this is followed by the demonstration of magnetic field modulated SERS.

Chapter 6 concludes this thesis by providing the major results and contributions to the field and a section that proposes future work that could build on the results presented herein.
The research described in this chapter fulfills Aim 1

2.1 Introduction

The field of plasmonics, more specifically nanoparticle surface plasmons, has experienced a recent surge in interest following the discovery that surface plasmons are responsible for enhancements of the local E-field in the vicinity of metallic, nanostructured surfaces. Such enormous enhancements, up to a factor of $10^{14}$ reported by Kneipp et al. have enabled Raman scattering, an intrinsically weak scattering phenomenon, to become an important detection methodology that provides narrow spectral resolution, wealth of molecular information and potential sensitivity for single molecular detection; this is known as the field of Surface Enhanced Raman Scattering (SERS).

Plasmon resonances arise within a metallic nanoparticle from the collective oscillation of free electrons driven by an incident optical field. The plasmonic response of nanopar-
articles have played a role in a growing number of applications, including SERS, chemical sensing, drug delivery, photothermal cancer therapy and new photonic devices. Our laboratory has been involved in the investigation and application of plasmonics nanosubstrates for SERS detection for over two decades. Since our first report on the practical analytical use of the SERS techniques for the trace analysis of a variety of chemicals including several homocyclic and heterocyclic polyaromatic compounds in 1984, \cite{86} our laboratory has been involved in the development of SERS technologies for applications in chemical sensing, biological analysis and medical diagnostics. \cite{83,87–92}

The wealth of experimental protocols and data in the literature for synthesizing SERS substrates, both via wet chemistry \cite{90,116–119} or on fixed substrates, \cite{120–122} necessitates numerical methods that confidently solve nanoscale electromagnetics of 3-D geometries with high spatial and spectral resolution; this is essential for understanding the fundamentals of the already available SERS substrates, as well as for further exploring and optimizing future substrate designs.

The Mie theory provides the exact analytical description of the behavior of the E-field surrounding isolated spherical nanoparticles, but is unfortunately strictly limited to the spherical geometry. It cannot be employed to solve more interesting and realistic problem, consisting of irregularly-shaped structures that are arbitrarily positioned in space. The analysis of such geometries require numerical methods to solve Maxwell’s equation in the computational domain via iterative procedures. The literature details two important computational electrodynamics modeling techniques to this end: the Finite Difference Time Domain (FDTD) and the Finite Element Method (FEM). Due to the nature of its discretizing algorithm, however, FDTD possesses intrinsic limitations for solving the electromagnetics problem at boundaries. \cite{123–125} A recent report evaluating FDTD as a method for solving the electromagnetic fields around metallic dimer nanoparticles demonstrated only a fair ability to do so, conveying large inconsistencies in field amplitude and plasmon peak position. \cite{125} This would suggest FEM as a more suitable approach when near-field optics
are of interest.

COMSOL Multiphysics is numerical simulation package based on FEM that allows accurate resolving of nanoscale electromagnetics in the vicinity of irregular nanostructures. Its accuracy relative to analytical theory is therefore of utmost importance for accurate characterization and design of such geometries. COMSOL has recently been used by several groups for modeling the plasmonics of nanoshells,\textsuperscript{126–128} as well as other nanoscale geometries,\textsuperscript{129–131} but these groups have focused exclusively on 2-D geometries. Ehrhold \textit{et al.}\textsuperscript{132} only briefly discussed the use of COMSOL for modeling the plasmonic properties of 3-D bimetallic nanoshells. The lack of 3-D modeling in COMSOL is a result of the significant time and computational power required to solve even a trivial problem in 3-D space. Nevertheless, there is significant discrepancy between 2-D and 3-D solutions, so much so that 2-D models are unable to reconcile experimental observation and numerical analysis.

Although there exists a need for the accurate numerical modeling of complex 3-D nanostructures to elucidate their plasmonic behavior (that cannot be resolved analytically), the lack of confidence in using the FEM algorithm for 3-D modeling has left a void in this domain that needs to be filled. Thus, this chapter presents two studies that comprehensively investigate the FEM's accuracy for solving the electromagnetics in the vicinity of 3-D nanostructures. The first benchmarks COMSOL’s numerical algorithm based on FEM to the ME analytical method for solving a 3-D nanoshell dimer geometry, while the second investigates the dipole oscillation-induced plasmonics of a silver nanoshell compared to the Mie Theory.

2.2 Plasmonics of 3-D Nanoshell Dimers Using Multipole Expansion and Finite Element Method

This section comprehensively investigates the behavior of the fields around gold and silver nanoshell dimers in 3-D space, solved using the analytical ME and the numerical FEM.
Clusters of nanoparticles under optical illumination have become the subject of recent analytical studies because of the potentially large field enhancements between the particles arising from surface plasmon resonances. Spherical dimers composed of pairs of solid nanospheres as well as linear chains of nanospheres have been analytically derived by several researchers.\textsuperscript{133,134} The nanoshell dimer, however, represents a more versatile geometry that exhibits stronger field enhancements in its gap, relative to an isolated nanoshell, while boasting plasmon tuning capabilities by variations in shell thickness; both properties are important criteria of a good SERS substrate. Nanoshell dimers have recently been investigated both theoretically, using the plasmon hybridization method,\textsuperscript{135} and numerically, by employing the FDTD method.\textsuperscript{124} The plasmon hybridization method expresses the nanoshell dimer plasmon as a linear combination of the primitive plasmons, associated with the individual nanoshells, that electromagnetically interact and effectively ‘hybridize’. The second report used the FDTD method to analyze the optical properties of silver nanoshell dimers, and unraveled the FDTD as being prone to inherent numerical staircasing errors when attempting to map a curved surface on a Cartesian grid. Considering that the synthesis and intricate control of the nanoshell dimer configuration has already been achieved,\textsuperscript{136} it is of interest to identify other promising analytical and numerical methods that are able to accurately model this geometry. This study extends Norton \textit{et al.}’s ME analysis of a solid dimer\textsuperscript{134} to a nanoshell dimer, and complements the two aforementioned reports by comparing the spatial and spectral responses of the E-field between ME and FEM as a function of dimer size, shell thickness and inter-particle separation. The study concludes with a final section that discusses the effects brought about by the incorporation of the size-effect factor in the dielectric function, which arises when the particle size becomes comparable to the electron scattering mean-free path length.
2.2.1 Methods

The numerical simulations were performed with the commercial software package COMSOL Multiphysics (Version 3.4 with incorporated RF Module, installed on a dual-Quad Core 32GB RAM Workstation), which comprises electromagnetic code based on the Finite Element Method (FEM) (http://www.comsol.com).

The computational domain containing the nanoparticle system of interest was delimited by Perfectly Matched Layers (PMLs) that efficiently absorb any scattering off the particles, thereby preventing any unwanted reflections in the domain. Adequate meshing of the geometry of interest is a critical step in a FEM simulation, since the spatial resolution in the computational domain needs to be high enough to capture fast changing geometries, which directly translates to solution accuracy. A new spatial mesh was interactively constructed for each unique variable combination ND\(_{\left[D, d, s\right]}\) to ensure that the E-field was properly solved at the position (0, 0, 0). The meshing was considered sufficiently dense when little variation in the 2nd decimal place of the solved \(|E_z|\) was observed. In 3-D space, meshes usually consisted of between 180k to 350k points, representing between 1M to 2.7M Degrees of Freedom (DoF). This number decreased significantly in 2-D to between 10 – 20k, corresponding to tens of thousands of DoF.

The spectral domain was sampled finely enough to ensure any rapid change in the spectrum would be captured with sufficient resolution to accurately compare both computational methods. This was especially critical around the plasmon resonance peak in all cases, and finer sampling was also required in regions where the trend was uncertain, such as the 400 – 500nm wavelength range for the silver dimers. The ME produced \(F_E(\lambda)\) sampled at 2.5nm wavelength intervals, whereas the spectral resolution in COMSOL was dynamically selected according to the important features observed in \(F_E(\lambda)\), thus varying between 5nm and 50nm wavelength intervals.

The complete derivation of the Multipole Expansion can be found in the supplementary
information of the published version of the manuscript, authored by Khoury et al.. The presented data was assembled using MatLab and COMSOL’s in-house postprocessing environment.

### 2.2.2 Initial discussion and model setup

The versatility of gold and silver nanoshells for use in plasmonics originates from the fact that their plasmon resonance peaks can be controllably tuned throughout the visible region by adjusting the ratio of core to shell radii. The dimer configuration results in plasmon coupling between the two nanoparticles, effectively concentrating the local E-field relative to that induced by a single nanoparticle, or monomer. The tunability of dimers is expected to follow a similar trend as for monomers, with possible differences stemming from additional plasmonic coupling behavior due to the particles’ close proximity.

From a simulation perspective, the nanoshell dimer provides several degrees of freedom to test COMSOL’s ability to mesh and solve at nanoscale resolutions: shell thickness \(s = 1 - r_1/r_2\) in \%, inter-particle separation \(d\) in (nm) and particle diameter \(D = 2r_2\) (nm). These are depicted in schematic of the 3-D nanoshell dimer model employed throughout the study, Figure 2.1. For conciseness, unique geometry combinations that define the nanoshell dimer geometry are represented using the “ND\([D, d, s]\)” format, such that “ND\([20\text{nm}, 5\text{nm}, \Delta s]\)” indicates the study of a Nanoshell Dimer of diameter \(D = 20\text{nm}\) and inter-particle separation \(d = 5\text{nm}\) as a function of shell-thickness \(s\). Due to the polarization selectivity of the plasmon excitation, the incident source was polarized with the electric field parallel to the bisphere axis, which effectively excites the dominant ‘longitudinal’ plasmon of the system. This is the optimal polarization for creating the largest field enhancement in the gap between the particles. As such, the incident source was represented by a normalized z-polarized TM wave, propagating in the x-direction.

Although the corresponding SERS enhancement would be approximately proportional to \(|E_z|^4\), the E-field magnitude \(|E_z|\) is employed in this report for visualization pur-
FIGURE 2.1: 3-D Nanoshell Dimer schematic

poses. Since the incident wavelength was set such that \(|E_z|_{\text{inc}} = 1\), the computed \(|E_z|\) is in fact representative of E-field enhancement: Enhancement = \(|E_z|/|E_z|_{\text{inc}} = |E_z|\). In this report, we are particularly interested in computing the maximum E-field enhancement in the gap between the two nanoshells, at point (0,0,0). Throughout the remainder of the report, the variation of \(|E_z|\) as a function of wavelength will be represented by the function \(F_E(\lambda)\).

The gold and silver dielectric functions were modeled using the Lorentz-Drude dispersion model:\textsuperscript{140}

\[
\epsilon(\omega) = 1 + \sum_{k=1}^{6} \frac{\Delta \epsilon_k}{-a_k \omega^2 - b_k \omega + c_k}, \tag{2.1}
\]

where \(\Delta \epsilon_k\), \(a_k\), \(b_k\) and \(c_k\) are constants that provide the best fit for either metal.\textsuperscript{140} The graphical representation of \(\epsilon(\omega)\) for both gold and silver is depicted in Figure 2.2. The refractive index of the silica comprising the core of each particle was taken as 1.45 and assumed to be wavelength independent.\textsuperscript{141,142} The surrounding medium was defined as air.

The ME employed in our study is based on the quasi-static approximation for which the particles are assumed much smaller than an optical wavelength, such that the incident
FIGURE 2.2: Real and imaginary parts of the permittivity $\epsilon(\omega)$ of gold and silver metal as defined by the Lorentz-Drude dispersion model.

The electric field may be assumed uniform over the dimensions of the particle. The basic approach can be extended to the full-wave problem in which retardation affects are accounted for. The reduced complexity of the quasi-static approximation greatly simplifies the solution to the nanoshell dimer geometry, compared to that involving a full expansion of the Mie scattering coefficients, while boasting a similar accuracy for small nanoparticles. This size limitation is been claimed to be $\sim \lambda/20$, beyond which the approximation progressively breaks down and the ME loses validity. In treating a dimer or cluster of spheres, the ME method is sometimes called the superposition method since the scattered field can be expressed as a superposition of the field scattered from each particle in the cluster. An advantage of this approach is that its accuracy can be easily evaluated by checking the convergence of the multipole expansion, undertaken by adding additional higher order terms until convergence is evident. The ME method can be very accurate and is often used as a benchmark against which other algorithms may be evaluated and its derivation is detailed in the Supporting Information.

COMSOL Multiphysics is a FEM-based numerical simulation package, which inter-
faces with MatLab and provides a number of modules for physics and engineering applications. The RF-module was employed to characterize the electromagnetic fields in the computation domain comprising the nanoshell dimer. The Maxwell’s equations are simplified according to the selected application mode, which in this case is TM incidence, and are encompassed in a system of matrices. For 2-D geometries with a small number of DoF, a direct solver is able to solve the inverse problem, but for 3-D problems, an iterative solver iterates through the system until convergence is reached.

2.2.3 Accuracy of ME relative to Mie Theory

The exact Mie theory solution for an isolated sphere is represented as an expansion of coefficients of the form \((a/\lambda)^{2n+1}\) with alternating sign, which converges very rapidly when \(a \ll \lambda\) where \(a\) is the particle radius. Thus, the first term in the Mie expansion is proportional to \((a/\lambda)^3\) and the second term to \((a/\lambda)^5\). The magnitude of the first two terms are given by

\[
|a_1| = \frac{2}{3} \left| \frac{\epsilon(\omega) - \epsilon_0}{\epsilon(\omega) + 2\epsilon_0} \right| \left( \frac{2\pi a}{\lambda} \right)^3 \tag{2.2}
\]

\[
|a_2| = \frac{1}{15} \left| \frac{\epsilon(\omega) - \epsilon_0}{2\epsilon(\omega) + 3\epsilon_0} \right| \left( \frac{2\pi a}{\lambda} \right)^5 \tag{2.3}
\]

The ratio of these terms is

\[
\frac{|a_2|}{|a_1|} = \frac{1}{10} \left| \frac{\epsilon(\omega) + 2\epsilon_0}{2\epsilon(\omega) + 3\epsilon_0} \right| \left( \frac{2\pi a}{\lambda} \right)^2 \tag{2.4}
\]

The quasi-static approximation retains only \(a_1\), such that the resonance plasmon peak is predicted to occur a frequency determined by the condition \(\text{Re}\{\epsilon(\omega) + 2\epsilon_0\} = 0\).

As an illustration, the value of the ratio (2.4) using the Lorentz-Drude dispersion model (2.1) is computed. Using the parameters for silver, the quasi-static approximation predicts
a resonance peak at a wavelength of 370nm. Evaluating Eq. (2.4) at this wavelength and for a particle radius of 10nm results in a ratio of 0.001. For a solid sphere, the quasi-static approximation is equivalent to assuming a spatially uniform incident electric field (i.e., the limit as $\lambda \to \infty$), in which case the external field is equivalent to that of a dipole residing at the sphere’s center. The second term, given by Eq. (2.3), corresponds to a quadrupole response and is maximum when the real part of the denominator of Eq. (2.3) vanishes. This defines a quadrupole resonance and at this wavelength one might expect that the quasi-static approximation would fare poorly. However, using the above parameters for silver and evaluating Eq. (2.4) at the wavelength of the quadrupole resonance ($\lambda = 357$nm), the ratio evaluates to 0.002, which is still very small. It is also worth noting that the second term in an infinite series with alternating sign, such as the Mie series, provides an upper bound to the error that results from dropping all terms in the series except the first. The above example for a solid sphere illustrates the dominance of the first term of the Mie series for small particles and may be regarded as providing further support for the validity of the quasi-static approximation under these conditions. Although the above results hold for an isolated spherical particle, similar arguments should hold for more complex objects, such as dimers.

The small divergence between the solutions of the ME and the Mie Theory for 20nm nanoparticles suggest that this diameter small enough for the quasi-static approximation to hold; it is therefore employed as the nominal size for comparisons between ME and COMSOL.

2.2.4 Analysis of the E-Field as a Function of Wavelength

Effect of Shell Thickness, $\Delta s$

The plasmon resonance tunability was investigated for 20nm nanoshell dimers of gold and silver, by varying the shell thickness $s$ while keeping the inter-particle distance fixed at 5nm (or 25% of the particle diameter) and evaluating $|E_z|$ at the origin $(0,0,0)$ for the
wavelength range 300 – 1000nm. This separation was chosen to test COMSOL’s ability to spatially resolve a narrow gap in a medium with a real, positive dielectric constant (free space), while varying the adjacent subdomains (shells) that are modeled by a complex dielectric function with a negative real part. The computed $F_E(\lambda)$ are plotted in Figure 2.3.

![Figure 2.3: $F_E(\lambda)$ v.s. shell thickness $s$ for ND[20nm, 5nm, $\Delta s$] of (a) Au (b) Ag.](image)

The figures clearly depict a red-shifting of the resonance bands, as well as an increase in $|E_z|$ in the gap, with decreasing shell thickness; both trends become more pronounced in the limit of the shell thickness tending to zero. Interestingly, there exists a wide dynamic range in $F_E(\lambda)$, from $\sim 7$ to a maximum of $\sim 27$ for gold but only from $\sim 24$ to $\sim 37$ in the case of silver, implying that a gold nanoshell has a greater impact on improving potential E-field enhancement than for silver. It should also be noted that the $F_E(\lambda)$ curves for gold are generally broader than for silver, for all shell thicknesses.

The COMSOL-generated $F_E(\lambda)$ curves are very slightly offset compared to the ME results, but the correlation between the two methods is qualitatively and quantitatively sound. With regards to the magnitude differences, both methods produce $F_E(\lambda)$ that are nicely superimposed on both sides of the resonance band where the error stabilizes around zero, for both silver and gold, and the greatest differences occur close to the resonance
peaks. A possible explanation is that, as a result of the resonance phenomenon, the surface plasmons that are excited around the dimer’s inner and outer surfaces in this wavelength range expose and amplify any differences between the two methods.

Effect of Dimer Separation, $\Delta d$

The effect of the dimer separation on the error between the two methods was investigated by fixing $D$ and $s$, and solving for $|E_z|$ as a function of separation distance $d$, Figure 2.4. The separations were decreased from $d = D = 20\text{nm}$ (100% of particle diameter), deemed as sufficiently far, down to $d = 1\text{nm}$, which, even though possibly too small to realistically synthesize, was interesting from a meshing point of view. It should be noted that $\sim 4$ mesh elements were fitted along the $z$-axis in the $1\text{nm}$ gap to ensure an adequate spatial sampling of the inter-particle space. This represents a spatial resolution of $\sim 0.25\text{nm}$ and is the highest resolution necessary throughout this study.

As the dimer separation increases, the $F_E(\lambda)$ resonance bands of both gold and silver blue-shift, tending towards the peak position of an isolated nanoshell as plasmon coupling between the dimers tends to zero. As a result of this reduced coupling with increasing separation, the $|E_z|$ magnitudes are also affected and dramatically decrease, requiring the $F_E(\lambda)$ curves to be plotted with a logarithmic y-axis for visualization purposes. The insets represent the original unscaled curves, for reference. Once again, COMSOL generates $F_E(\lambda)$ curves that very closely resemble those of the ME, in that they superimpose about the resonance band, have a closely correlated $|E_z|$ values and are consistently red-shifted by a few nanometers.

Effect of Dimer Size, $\Delta D$

The following test was performed to investigate the reliability of COMSOL to mesh the space, and solve the electromagnetics problem, in a narrow gap that is surrounded by two increasingly large objects. The dimers were increased from $D = 20\text{nm}$ to $D = 150\text{nm}$, representing an experimentally relevant range of sizes, while keeping $d = 5\text{nm}$
and $s = 20\%$, Figure 2.5. The 3-D surface plots, generated by postprocessing in MatLab via a 2-D spline interpolation, highlight the progression of the $F_E(\lambda)$ resonance bands with increasing size. The divergence of the shape and position of $F_E(\lambda)$ between COMSOL and ME is obvious, with COMSOL producing a red-shift over approximately a few hundred nanometers for both gold and silver in the former case, compared to about 50nm for the two metals in the ME case. The rapidly increasing $F_E(\lambda)_{ME}$ diverges from $F_E(\lambda)_{COMSOL}$, which appears to plateau at $\sim 50$ for dimer sizes. The ME seems unable to properly solve for dimer nanoshells that start exceeding 20nm, for which the quasi-static approximation appears to break down, due to the increasing influence of phase retardation effects.

**Figure 2.4:** $F_E(\lambda)$ v.s. dimer separation $d$ for ND$[20\text{nm}, \Delta d, 20\%]$ of (a) Au (b) Ag.

**Figure 2.5:** $F_E(\lambda)$ v.s. nanoshell diameter $D$ for ND$[\Delta D, 5\text{nm}, 20\%]$ of (a) Au (b) Ag.
A quantitative comparison between COMSOL and ME for each explored dimer scenario is concisely summarized in Figure 2.6, which focuses on the error between 3 variables associated with the plasmon peak: $|E_z|$ relative error, $\Delta |E_z|$, resonance $\lambda_{max}$ shift, $\Delta \lambda_{peak}$, and FWHM relative error, $\Delta \text{FWHM}$. These properties help characterize the plasmon resonance band and are usually the features of greatest importance when developing and tuning nanostructures. The first, second and third column convey scenarios $\text{ND}[20nm, 5nm, \Delta s]$, $\text{ND}[20nm, \Delta d, 20\%]$ and $\text{ND}[\Delta D, 5nm, 20\%]$, respectively. In each case, the errors were computed by evaluating $|E_z|_{\text{COMSOL}}$, FWHM$_{\text{COMSOL}}$ and $\lambda_{max-\text{COMSOL}}$ relative to $|E_z|_{\text{ME}}$, FWHM$_{\text{ME}}$ and $\lambda_{max-\text{ME}}$, respectively,

i.e. $\Delta |E_z(\lambda)| = |E_z(\lambda)|_{\text{COMSOL}} - |E_z(\lambda)|_{\text{ME}}$

As such, positive values indicate an overestimation by COMSOL and negative values suggest underestimation.

The shell-thickness analysis is dealt with in the first column, which depicts a fairly stable, slightly-increasing $\Delta |E_z|$ with increasing shell thickness, with $\Delta |E_z|$ averaging approximately 5% in the range (2%, 6%) for gold, and 4% in the range (0.5%, −6%) for silver. It is interesting to note that $|E_z|$ is overestimated for gold but underestimated for silver. The $\lambda_{max}$ position is overestimated for both metals and decreases with increasing shell thickness in both cases, $\Delta \lambda_{peak}$ ranging (2%, 7%) and (6%, 9%) for gold and silver, respectively. The third graph of $\Delta \text{FWHM}$ as a function of shell thickness conveys that gold starts with a $\sim 6\%$ error for the smallest shell thickness, and decreases to stabilize around zero, implying that as the nanoshell core shrinks, the plasmon bands become more similar. Interestingly, the opposite occurs for silver, with $\Delta \text{FWHM}$ starting around $\sim 7\%$ and stabilizing close to 16%. It is crucial to remark, however, that this is a relative error, and considering the the narrow widths of the thick-shelled silver dimers (Figure 2.3), such a high percentage only maps to $\Delta \text{FWHM}$ of only a few nanometers.

The second column of Figure 2.6 details this quantitative error analysis as a function of separation distance. Again, a fairly stable, slightly-increasing $\Delta |E_z|$ with increasing sepa-
Figure 2.6: Matrix of Error plots between COMSOL and ME for 3 scenarios of interest: ND[20nm, 5nm, Δs], ND[20nm, Δd, 20%] and ND[ΔD, 5nm, 20%]. Error computed as \[\Delta var = \text{var}_{\text{COMSOL}} - \text{var}_{\text{ME}},\] where \(\text{var}\) represents variables \(|E_z|, \Delta \lambda_{\text{max}}\) or FWHM.

A ratio is seen for gold, with an average \(\sim 4\%\) in the range \((0\%, 5\%)\), whereas silver starts underestimated around \(-8\%\) and increases to an overshoot of \(2\%\). Both gold and silver follow a decreasing trend in \(\Delta \lambda_{\text{peak}}\) with increasing separation, suggesting that accuracy improves as the particles become more isolated from each other. The last graph of the column conveys a stable, overestimated \(\Delta\text{FWHM}\) for both metals, averaging \(5\%\) and \(12\%\) for gold and silver, respectively. This last result suggests that the plasmon FWHM_{COMSOL} is not critically affected by coupling between the two particles as the software is able to solve the geometries with a consistent offset from its analytical counterpart.

The third column of Figure 2.6 demonstrates the diverging behavior of the error variables between both methods, with increasing dimer size. In this case, however, the COMSOL results are used as the trusted baseline against which the ME is compared. In that regard, the ME increasingly overshoots \(\Delta |E_z|\) for both metals (except for gold dimers \(D <\))
50nm), and differences in $\lambda_{max}$ and FWHM become more prominent as the dimers grow, really emphasizing the ME as suitably accurate for nanoshell dimer sizes $D \leq 20$nm.

The discrepancy between an overestimated $|E_z|$ for gold but underestimated $|E_z|$ for silver, as observed across the first row of Figure 2.6, is intriguing since both silver and gold dimers were solved with identical meshing that yielded sufficiently converged solutions in both cases; further mesh refinement did not improve the obtained results. This effectively disqualifies meshing inconsistencies as the potential source of the observed difference. Interestingly, the discrepancy appears to be consistent across dimer geometry, ND[$D, d, s$], and is therefore most probably material-specific in nature, arising from differences in the metal dielectric functions. The plasmon resonance peaks of gold and silver, at which $|E_z|$ is measured in each case, are effectively dictated by their corresponding dielectric function and occur at different wavelengths in the optical spectrum; this difference in resonance position could ostensibly affect the accuracy with which the fields in the vicinity of the dimer are solved by Comsol’s FEM algorithm. This would then result in over-, or under-, estimation. Although further investigation into this phenomenon is beyond the scope of this manuscript, it is noteworthy that as long as the overshoot in either direction is consistent and small, as demonstrated here, Comsol’s FEM algorithm can still be trusted to generate reliable solutions.

The interesting $\lambda_{peak}$ offsets of the $F_E(\lambda)$ curves seen in Figure 2.3, Figure 2.4 and Figure 2.5 between ME and COMSOL was further investigated by comparing the local E-field around a single nanoshell (monomer), to categorize it as a possible discrepancy introduced by the dimer configuration or an intrinsic difference related to the fundamental algorithms of each model. The E-field for the nanoshell monomer geometry was probed at a distance of 2.5nm from the shell surface, effectively co-registering with the interrogation point in the dimer case. In the monomer case, Figure 2.7(a), the two methods generated $F_E(\lambda)$ curves that are in good agreement with each other, yet an evident offset in resonance peak position and E-field magnitude is still present. The relative error between COMSOL
Figure 2.7: Analysis of 20nm Au Nanoshell Monomer v.s. shell thickness \( s \), interrogated at 2.5nm from shell surface (a) \( F_E(\lambda) \) (b) Relative Error between ME and COMSOL

and ME is presented in Figure 2.7(b).

Very briefly, \( \Delta |E_z| \) and \( \Delta \text{FWHM} \) do not display obvious trends, the former roaming around \( \sim 3\% \) and the latter in the range \( \sim (2\%, 3\%) \). The shift in \( \lambda_{max} \), however, decreases with increasing shell thickness and stabilizes around 1nm in the limit \( s \rightarrow r_2 \). This analysis suggests that the observed \( F_E(\lambda) \) offset potentially arises from algorithm-related, rather than dimer geometry-related, differences.

As aforementioned, most of the literature dealing with the simulation of nanosphere electromagnetics using COMSOL describes the use of 2-D, rather than 3-D, models to investigate resonance band width, amplitude and peak position. Although the former consumes a fraction of the time and computational power of the latter, the results are significantly different. Figure 2.8(a) illustrates this important comparison, from which it is evident that the 2-D resonance band is narrower, red-shifted and dampened relative to the 3-D resonance band.

Figure 2.8(b) quantitatively suggests that as \( s \) decreases, \( \Delta \text{FWHM} \) and \( \Delta \lambda_{max} \) both increase, but interestingly, \( \Delta |E_z| \) tends to zero. This analysis implies that 2-D modeling is
Analysis of the E-Field as a Function of Spatial Coordinate

For each selected wavelength, COMSOL solves the electromagnetic problem at every mesh node in 3-D space, which, although computationally intensive, enables 3-D spatial maps of the desired variable to be assembled and conveniently visualized. One such map for ND[20nm, 5nm, 20%] @ $\lambda_{\text{peak}} = 660\text{ nm}$ is shown in Figure 2.9. The core and shell boundaries of the nanoshell dimer are delimited with dashed-lines on either side of the separating junction. The 3-D surface contours represent isosurfaces of constant $|E_z|$, and the red and white arrows indicate the direction of the vector $E_z$ on the x-z and y-z planes, respectively, and are proportional to the E-field strength at their position in 3-D.
space. This map depicts the concentrating and propagating behavior of the E-field in the vicinity of the dimers, as well as its rate of change away from the same. It is noteworthy that the arrows passing through the gap in between the two nanoshells are purposefully withheld for better visual clarity; they would point from the left nanoshell to the right nanoshell.

**Figure 2.9**: 3-D illustration of E-field behavior surrounding Gold Dimer ND[20 nm, 5 nm, 20%] @ $\lambda_{\text{peak}} = 660$ nm. Arrows depict the E-field direction and the nanoshell dimers are delineated with dashed circles.

For any method lacking a closed-form solution, spatial resolution is a critical measure and is dictated by the extent to which space is discretized into meshes for numerical solving. Figure 2.10(a) shows the corresponding $x - z$ slice plot, with $|E_z|$ represented as the third dimension, and effectively conveys COMSOL’s ability to smoothly mesh and solve the E-field around 3-D geometrical edges that bound domains of differing dielectric functions. This plot also conveys that the maximum $|E_z|$ occurs at the metal/air interface, on either side of the gap, along the z-axis. The spatial resolution was investigated for
ND\([D = 20nm, s = 5nm]\) as a function of the shell thickness, evaluated at their corresponding plasmon peak wavelength, Figure 2.10(b). The excellent agreement between the line profiles confirms that the 3-D meshing was dense enough, for all shell thicknesses, to effectively capture the features of interest, especially the abrupt E-field discontinuity at the dielectric boundaries. Unlike FDTD, which exhibits large errors at the nanoshell’s interior or exterior boundaries\(^{123,125}\), FEM allows for a more versatile meshing algorithm that employs tetrahedrons to effectively discretize the shell’s curved surface, such that the shell geometry is accurately maintained and solved for.

2.2.5 Computational requirements

For completeness, it is appropriate to briefly discuss computational power and time differences between the two methods, as well as between COMSOL models solved in 2-D and 3-D space. This is illustrated in Table 2.1.

<table>
<thead>
<tr>
<th></th>
<th>ME</th>
<th>COMSOL 2-D</th>
<th>COMSOL 3-D</th>
</tr>
</thead>
<tbody>
<tr>
<td>time</td>
<td>&lt; 1 min</td>
<td>5 – 10 min</td>
<td>12 – 24 hours</td>
</tr>
<tr>
<td>RAM (Gb)</td>
<td>&lt; 1</td>
<td>~ 1</td>
<td>4 – 15</td>
</tr>
</tbody>
</table>

The results convey a huge range of times and powers that originates mostly from differences in the intrinsic problem-solving algorithms.

Several properties of the ME algorithm in this geometry resulted in exceptionally fast execution. In a calculation with many frequencies, the frequency-independent matrix elements computed only once. For each frequency, the linear system of equations are then solved for the ME coefficients, which takes little time since the system is relatively small. This system comprises \(2N\) equations, where \(N\) is the number of multipole terms. In our calculations, we used \(N = 20\) for all but the smallest dimer gaps; in the latter case \(N\) was increased to 40. The relatively small number of terms in the multipole expansion is a
Figure 2.10: Spatial plots for Au ND [20nm, 5nm, 20%]: (a) Slice plot along x-z plane, represented in 3-D with $|E_z|$ as the third dimension. Inset is 2-D slice plot for reference. (b) Spatial resolution comparison of x- and z-line plots as a function of shell thickness $s$.

A direct result of the axial symmetry of the problem. Incrementing through 400 frequencies, the entire calculation in MatLab took typically less than a minute.

The generation of 3-D plots using COMSOL, however, are significantly more chal-
lenging. The extreme RAM loads for 3-D geometries in COMSOL are strongly dependent on the required meshing density for the particular geometry. The computational time, however, is affected not only by the meshing density, but also more critically by the number of desired wavelength iterations; the sampling resolution of \( F_E(\lambda) \) demonstrated in the above graphs, i.e. 25 – 50 spectral points in the wavelength range 300-1000nm, requires 12 – 24 hours. COMSOL meshes the geometry, obtaining the number of DoF required to solve for, and uses very large matrices in the 3D case to solve the inverse problem for each electromagnetic quantity that appears in the original Maxwell’s equations, whether or not they are desired by the user for the problem at hand. This is repeated for each wavelength of interest. As such, COMSOL effectively generates a 4-D volume of data for each electromagnetic variable \((\mathbf{E}, \mathbf{H}, etc)\), consisting of a field distribution in 3-D space with wavelength occupying the fourth dimension; although this routinely utilizes a couple of Gigabytes of hard drive storage, it is particularly convenient for probing electromagnetic quantities at any position in computational domain and at any solved wavelength, once the solution is achieved, allowing plots such as Figure 2.9 to be built and studied.

Given the long processing times and huge RAM utilized for 3-D geometries, there exists a memory limitation on the achievable meshing density (spatial sampling), as well as a compromise between spectral sampling and processing time, both which need consideration when solving complex geometries. Evidently, a numerical solver such as COMSOL would not be used for spherical geometries, where analytical methods such as the ME provide very accurate solutions for small particles, but instead for an arbitrarily-shaped geometry which the Mie scattering theory, and thus any Mie theory-derived approximation, are unable to accommodate.

**Investigation of Small size effects**

The aforementioned analysis used the Lorentz-Drude model for the dielectric function of both silver and gold, and excluded any correction to account for a more experimentally
valid analysis of the electromagnetic behavior of such small particles. Although several factors contribute towards tweaking the shape of the plasmon band,\textsuperscript{146,147} the most prominent is the size-effect, which results in its damping and broadening. The small-size effect due to electron surface scattering at the particle boundaries becomes prominent as the metal thickness becomes smaller than the electron mean free path of a specific material. The electron mean free path for gold and silver are approximately 50nm,\textsuperscript{148–150} and the size-limiting effect is encompassed in the “effective mean free path” variable, $L_{eff}$. Detailed comparison between experimental and calculated colloidal gold spectra have demonstrated that this size-correction need only be applied to the imaginary part of the bulk metal dielectric function;\textsuperscript{146,151} as such, $L_{eff}$ only appears in $\epsilon''$ (2.5) and accounts for any additional loss incurred by the finite thickness of the nanoshell relative to the overall particle size. Here, $\lambda_p$ is the wavelength of plasma oscillations, $v_F$ and $c$ are the Fermi velocity of electrons and the light velocity in vacuum, respectively. The dimensionless parameter $A$ detailing the electron scattering process is taken as unity.\textsuperscript{147,152}

Moroz\textsuperscript{153} recently employed the nanoshell geometry to revisit a $L_{eff}$ model, $L_{effKP}$ (2.6), proposed by Kachan et al.,\textsuperscript{154} and further proposed two models that are suggested to be more accurate: $L_{effDiff}$ (2.7), derived by considering diffusive scattering, and $L_{effBill}$ (2.8), derived from the billiard scattering model. These three equations are plotted as a function of relative shell thickness in Figure 2.11. Note that Eqs (2.6) and (2.7) differ only by the sign of the $(1 - q)$ term in front of the Lognormal function.

\begin{equation}
\epsilon'' = \epsilon''_{bulk} + \epsilon''_{correction} = \epsilon''_{bulk} + A \frac{\lambda_p}{L_{eff}} \frac{v_c}{2\pi c} \left(\frac{\lambda_0}{\lambda_p}\right)^3 \tag{2.5}
\end{equation}

\begin{equation}
L_{effKP} = R \left[\frac{1}{1 + q^2} - \frac{q}{2} - \frac{1}{4} \frac{1 - q^2}{1 + q^2} (1 - q) \ln \frac{1 - q}{1 + q}\right] \tag{2.6}
\end{equation}
\[ L_{\text{effDiff}} = R \left[ \frac{1}{1+q^2} - \frac{q}{2} - \frac{1}{4(1+q^2)}(1+q) \ln \frac{1-q}{1+q} \right] \] 

(2.7)

\[ L_{\text{effBill}} = \frac{4}{3} R \frac{1-q^2}{1+q^2} \] 

(2.8)

**Figure 2.11:** Comparison between the size-correction models \( L_{\text{effKP}} \), \( L_{\text{effDiff}} \) and \( L_{\text{effBill}} \) for Au ND[20nm, 5nm, 20%]; (a) \( L_{\text{eff}} \) v.s. shell thickness \( s \), (b) Au Dielectric Function, \( \epsilon_{\text{Au}}(\lambda) \) and (c) Comparison of three \( L_{\text{eff}} \) models

The variable \( L_{\text{eff}} \) is a real positive number that estimates the physical confinement experienced by electrons, and is therefore inversely proportional to the constricting ef-
ffects of the shell thickness. Its incorporation ultimately broadens the plasmon band and leads to a diminishing of the overall intensity of the local E-field in the vicinity of metal nanoparticles, effects that disappear only in the limit \( L_{\text{eff}} \to \infty \) (2.5). This implies that the size-effect is to be accounted for regardless of the particle size, although the effects will rapidly fade away with increasing size as will be demonstrated shortly.

Figure 2.11(a) depicts the dependence of \( L_{\text{eff}}/r^2 \) on relative shell thickness, starting at the origin at increasing to 1 for \( L_{\text{effKP}} \) and \( L_{\text{effDiff}} \), but increasing beyond 1 for \( L_{\text{effBull}} \). This translates to corrections to the dielectric function of Gold displayed in Figure 2.11(b), which were evaluated for a shell thickness \( s = 20\% \). The real part \( \epsilon' \) is unaffected but \( \epsilon'' \) increases with decreasing \( L_{\text{eff}} \), for all \( \lambda \). Figure 2.11(c) is a comparison between the \( |E_z| \) spectra of the 3 models for ND\([20\text{ nm}, 5\text{ nm}, \Delta s]\) nanoshell dimers, as a function of shell thickness. This plot clearly emphasizes significant difference between them for thin shells, but they converge towards a similar E-field spectral profile in the limit \( r_1 \to 0 \).

In the remainder of the report, size-effects are investigated using the \( L_{\text{effKP}} \) model, firstly because it introduces the greatest loss of the three, enabling the study of the greatest impact size-correction has on nanoparticle electromagnetics, and secondly due to the fact that it has already appeared as a size-correction model in the literature.\(^{155}\) Evidently, since all three boast similar behaviors, the trends depicted by \( L_{\text{effKP}} \) will apply to the other two. The following section presents a quantitative comparison between uncorrected and size-corrected E-field spectra, using \( L_{\text{effKP}} \), for both silver and gold as a function of shell thickness and size.

Figure 2.12(a) and (b) compare \( F_E(\lambda) \) curves of ME (size-corrected) and COMSOL (uncorrected and size-corrected), for gold and silver, respectively. Thus far, it has been established that COMSOL is both qualitatively and quantitatively up to par with ME, for small nanoparticles. The additional loss component in the dielectric function appears not to disrupt their correlation as seen by their good agreement in both graphs.

The most striking feature is the significant \( F_E(\lambda) \) damping and broadening experienced
by the particles with thin shells, for both gold and silver. For $s = 10\%$, gold experiences a $\sim 5 \times$ decrease in $|E_z|$ and a $\sim 65\%$ FWHM broadening, whereas silver conveys $\sim 6 \times$ decrease in $|E_z|$ and a $\sim 75\%$ broadening. With increasing shell thickness, both damping and broadening diminish as $L_{eff}$ reaches its maximum value for $s = r_2$.

The effect of the size-correction on $F_E(\lambda)$ as a function of dimer size is presented in Figure 2.13, for which ND[$\Delta D, 5nm, 20\%$] were employed. As observed throughout this analysis, the correction term affects the plasmon characteristics, rather than the
off-resonance behavior of the particles. Indeed, in the range $300 - 500\text{nm}$ for gold, and $300 - 400\text{nm}$ for silver, the uncorrected and corrected versions as a function of shell thickness and dimer size are practically identical. They also converge in the long-wavelength limit. In addition, it is noteworthy than there still exists substantial error for the $150\text{nm}$ nanoshells, which suggests that that the correction term cannot be ignored even at these sizes and relative shell thicknesses.

Finally, these results are summarized in Figure 2.14. The first column unanimously shows that the difference between uncorrected and size-corrected $F_E(\lambda)$ tends towards zero as shell thickness increases. In addition to significant damping and broadening for thin shells, a substantial red-shift of the size-corrected curves also takes place, which is more pronounced for gold ($\lambda_{max} = 50\text{nm}$) than for silver ($\lambda_{max} = 27\text{nm}$). The $\Delta|E_z|$ and $\Delta\text{FWHM}$ variables show greater deviation for silver than for gold; however, the error seems to plateau for thicknesses greater than $40\%$ in all three cases.

The second column reiterate the trends observed with the data in Figure 2.12, with resonance damping, broadening and red-shifting all being greatest for small particles and reducing with increasing particle size. Interestingly, both metals appear to be more consistent with each other.

As a final note, it is of experimental interest to mention the impact of variations in the nanoshell dimer geometry on the SERS enhancement factor, $SERS_{EF}$, which is afore-mentioned to be approximately proportional to $|E_z|^4$. The maximum SERS enhancement factors achieved in the dimer gap using $\epsilon(\lambda)_\text{uncorrected}$ are extracted from Figure 2.3, Figure 2.4 and Figure 2.5. The thinnest shell of $10\%$ yielded $SERS_{EF} \sim 27^4 = 5.3 \times 10^5$ and $\sim 37^4 = 1.9 \times 10^6$ for Au and Ag, respectively. The smallest interparticle distance of $1\text{nm}$ produced $SERS_{EF} \sim 118^4 = 1.9 \times 10^8$ and $\sim 200^4 = 1.6 \times 10^9$ for Au and Ag, respectively. With respect to dimer size, the $90\text{nm}$ and $50\text{nm}$ dimers were the strongest for Au.
Figure 2.14: Error between models employing $\epsilon(\lambda)_{uncorrected}$ and $\epsilon(\lambda)_{corrected}$. The error is evaluated as follows:
\[ \Delta \text{var} = \text{var}_{\text{COMSOL-uncorrected}} - \text{var}_{\text{COMSOL-corrected}}, \]
where var represents variables $|E_z|$, $\Delta \lambda_{\text{max}}$ or FWHM.

and Ag, respectively, both interestingly generate $SERS_{EF} \sim 56^4 = 9.8 \times 10^6$. These are very large $SERS_{EF}$ and would suggest that synthesizing extremely thin-shelled dimers that are very close to each other would yield even stronger enhancements. This is not the case, unfortunately, since the $SERS_{EF}$ are dramatically reduced with the incorporation of the size-effect, reflected in Figure 2.12 and Figure 2.13. For the same shell thickness, the $SERS_{EF}$ of both Au and Ag are reduced to a mere $\sim 5^4 = 625$! The dramatic changes brought about by the forth power factor, used to estimate the SERS enhancement, emphasizes the importance of considering the size-effect to yield accurate models, especially for the design and characterization of small particles for use in SERS applications.
The numerical simulation package COMSOL Multiphysics is a versatile tool that can be confidently used for nanoscale electromagnetics of 3-D geometries. Although it is memory-intensive, the obtained result is accurate, as a function of both wavelength and space, to within a few percent of its theoretical counterpart; it additionally provides the ability to truly investigate the plasmonic behavior within and around a nanostructure of interest, which can then be reconciled with experimental analysis. In order to achieve this, however, it is noteworthy to emphasize that the size-dependence is of crucial importance, especially when dealing with the nanoshell geometry, for which electron mean free path models have been derived. COMSOL’s agile meshing algorithm and resulting solution accuracy would suggest it to be a more promising computational electrodynamics modeling tool than FDTD-based packages for use in the plasmonics arena.

The complete aforementioned study was published by the author in 2009 in the journal ACSNano.137

After discovering that the FEM algorithm accurately solves the electromagnetic response of lossy, metallic nanostructures when excited by a plane wave incidence, it is worth investigating whether the accuracy is maintained when the plane wave excitation is replaced by a radiating dipole. This situation is of particular interest when dealing with single, radiating molecules, such as fluorophores or Raman dyes, which can be effectively modeled by radiating dipoles that emit an oscillating signal. This interesting problem is comprehensively investigated in the following section.
2.3 Investigating the Plasmonics of a Dipole-Excited Silver Nanoshell: Mie Theory v.s. Finite Element Method

2.3.1 Introduction

Since the advent of the Mie Theory in 1908 describing the analytical solution for a spherical geometry,\textsuperscript{66} a number of computational electrodynamics modeling techniques have been developed for the investigation and analysis of irregular geometries; the two methods that have found widespread use are the Finite Difference Time Domain (FDTD) and the Finite Element Method (FEM). Another approach involves solving the EM problem by transformation of the volume integral equation to a surface integral equation.\textsuperscript{156} Previous FDTD studies in the literature\textsuperscript{123–125} have shown that this technique may be non-ideal for dealing with curved structures because its discretization algorithm effectively decomposes the 3-D geometrical space into cubic subunits. Although the FDTD method has its own merits for various applications, the FEM is suitable for investigating curved boundaries such as sphere surfaces, since its meshing algorithm employs tetrahedra to smoothly follow curved contours thereby providing higher spatial resolution and quantitative accuracy; this feature is especially important when investigating near-field electromagnetics, and suggests FEM is a promising modeling technique that has the potential to reconcile theory and experiment.

This study presents a comprehensive comparison between the Mie Theory and the FEM numerical simulation of a dipole-excited silver nanoshell. The novelty lies in the excitation source of the nanoshell system: an oscillating electric dipole is frequently used as a model for both molecular scattering and molecular fluorescence. Moreover, a common classical model of atomic or molecular spontaneous emission is a decaying electric dipole\textsuperscript{157}. From a modeling perspective, a point dipole source is a more challenging problem to solve, both analytically and numerically, than the planewave excitation attributed to the classical Mie problem. This is the case for several reasons: the Mie solution for
the dipole excitation is expressed as an infinite sum of spherical harmonics that must be truncated at some point, and the rate of convergence of this series varies with the distance from the dipole to each interface of the nanoparticle’s shell, which is not the case for a planewave source. The FEM dipole problem is also more numerically challenging than the planewave problem since modeling an infinitesimal point dipole involves dipole length optimization and discretization issues that do not arise in the planewave case. The latter two reasons render the dipole problem more interesting and challenging than the classical Mie problem of a planewave incident on a sphere. In addition, the ability to position the dipole both inside and outside the shell adds additional degrees of the freedom to the problem, such as location of the dipole relative to the shell interface and shell thickness, which are not present in the planewave case.

In this study, we extend our analysis of the FEM algorithm by developing the exact analytical solution of a dipole-excited nanoshell, based on the Mie theory, and using it as the benchmark to investigate the solution accuracy for 3-D silica@silver nanoshells of size spanning the range of interest: 20nm, 60nm, and 100nm. The nanoshell encompasses several material and geometrical properties that make it an elegant geometry for such a comparison. The nanoshell is illuminated via a dipole excitation source, and the analysis of each geometrical layout is performed both in the spectral domain, by evaluating the nanoparticle’s radiated power and normalized power, as well as in the spatial domain, via polar and line profile plots of the E-field in the vicinity of the nanoshell. Despite these issues, excellent agreement was achieved for all the cases studied, confirming that FEM is an accurate numerical technique for modeling molecular scattering, molecular fluorescence and general near-field electromagnetics.
2.3.2 Initial discussion and model setup

The nanoshell, a term defining a dielectric core surrounded by a metal shell, has become a popular nanoparticle geometry in the field of plasmonics, embedding itself in a number of medical applications such as optical coherence tomography\textsuperscript{158} and photodynamic therapy.\textsuperscript{159} The strength of this geometry arises from the tunability of its plasmon resonance peak, which can be shifted throughout the visible region by adjusting the ratio of its core to shell radii.\textsuperscript{137,138} Silver was chosen as the metal layer for two reasons. Firstly, this material is known to exhibit a strong plasmon resonance and thus SERS enhancement, both which are of interest in the plasmonics field, and secondly silver nanoshell dimers were demonstrated to yield larger quantitative errors between the Multipole Expansion Method and FEM,\textsuperscript{137} relative to gold, which suggests it would constitute the upper limit on any observed error between Mie and FEM.

Rather than exciting the surface plasmons with the traditional planar wave, a more general oscillating dipole source was employed, enabling it to be positioned within and without the nanoshell thereby providing greater analytical and geometrical versatility; additionally, a point electric dipole source is of interest because it is quite often employed as a classical model of molecular emission processes. The geometry of the generic silica@silver nanoshell is depicted in the 3-D schematic, Figure 2.15. The ratio of the nanoshells core to shell radii, $1 - b/a$, was fixed to 20\% for all three nanoshell sizes under investigation, such that the shell thicknesses are 2\text{nm}, 6\text{nm} and 10\text{nm} for the 20-\text{nm}, 60-\text{nm} and 100-\text{nm} nanoshells, respectively. Here, $a$ and $b$ are the radii of the outer and inner shell boundaries, respectively.

The dielectric function of the silver shell was again modeled via the Lorentz-Drude dispersion model and the wavelength- independent refractive index of the silica core was set to 1.45. The nanoshell was placed in a surrounding medium of air.

A more realistic nanoshell model would include an important geometric correction,
termed the “small size effect”, which is due to electron surface scattering at the particle boundary. This effect is particularly significant when the metal shell thickness becomes smaller than the metal’s electron mean free path, which, for silver, is approximately 50nm.\textsuperscript{148–150,153} This ultimately results in a damping and broadening of the nanoparticle’s plasmon band, which in turn impacts the achievable E-field enhancement in the vicinity of the nanoparticle; this is theoretically modeled by introducing additional loss to the bulk silver dielectric function.\textsuperscript{146,151} We have previously shown, however, that such a modeling adjustment does not enhance the differential error between the FEM and the Multipole Expansion for a nanoshell dimer,\textsuperscript{137} and it is therefore not expected to skew the trends presented in this study.

### 2.3.3 Mie Theory: Formulation

In contrast to evaluating the E-field at a single point in 3-D space, as a function of wavelength as undertaken in the first part of this chapter, the Mie and FEM algorithms were investigated by integration of the Poynting vector around the nanoshell, such as to obtain the radiated power at a given wavelength; this provides a spatial average that is a more
meaningful assessment of the EM field behavior and consistency in the vicinity of the particle. The corresponding power spectrum is then assembled by concatenating such point measurements across the wavelength range of interest.

The Mie Theory formulation begins by expressing the electric field emitted by the dipole and scattered by the nanoshell as expansions in radial Hertz potentials.\textsuperscript{160,161} For a radially-directed dipole, the fields are purely transverse-magnetic and thus only TM Hertz potentials are required to define the problem. This formulation is equivalent to, but considerably simpler than, expansions in vector spherical harmonics.

A point electric dipole at \( \mathbf{r}_d \) oscillating at frequency \( \omega \) and directed radially inward with moment \( p \) will give rise to a radial current density \( \mathbf{J} = J_r \hat{r} \), where \( \hat{r} \) is the radial unit vector and

\[
J_r = i\omega p \delta(\mathbf{r} - \mathbf{r}_d) e^{-i\omega t}
\]  

(2.9)

The associated transverse-magnetic Hertz potential \( \psi \) generated by this dipole is a solution to\textsuperscript{161}

\[
(\nabla^2 + k^2)\psi = -\frac{1}{i\omega\varepsilon_0} \frac{J_r}{r}
\]  

(2.10)

where \( k = \sqrt{\varepsilon\omega/c} \), \( c \) is the free space speed of light, \( \varepsilon \) is the dielectric constant of the medium and \( \varepsilon_0 \) is the permittivity of free space. Using (2.9), (2.10) may be written

\[
(\nabla^2 + k^2)\psi = -\frac{p}{\varepsilon_0\varepsilon R} \delta(\mathbf{r} - \mathbf{r}_d);
\]  

(2.11)

where we have suppressed the harmonic time dependence \( \exp(-i\omega t) \) and \( R \) is the radial distance of the dipole from the origin. In spherical coordinates, \( \mathbf{r}_d = (R, 0, 0) \). In our current geometry, only the TM field components are required, which are derived from the potential \( \psi \) as follows:
Due to axial symmetry, all other field components vanish. The potential associated with the field emitted by the point dipole is a solution to (2.11) and is given by

$$\psi(r) = \left( \frac{p}{\varepsilon_0 \epsilon R} \right) \frac{e^{i k |r - r_d|}}{4 \pi |r - r_d|}$$

We now employ the following expansions in a system centered on the shell

$$e^{ik|r - r_d|} \left| \frac{r}{|r - r_d|} \right| = \sum_{n=0}^{\infty} p_n \frac{\hat{\psi}_n(kr)}{r} P_n(\cos \theta), \quad r > R \quad (2.16)$$

$$e^{ik|r - r_d|} \left| \frac{r}{|r - r_d|} \right| = \sum_{n=0}^{\infty} q_n \frac{\hat{\zeta}_n(kr)}{r} P_n(\cos \theta), \quad r < R \quad (2.17)$$

where $P_n(\cos \theta)$ are Legendre polynomials and

$$p_n = i(2n+1) \frac{\hat{\psi}_n(kR)}{kR} \quad (2.18)$$

$$q_n = i(2n+1) \frac{\hat{\zeta}_n(kR)}{kR} \quad (2.19)$$

In the latter equations, $\hat{\psi}_n(z) \equiv z j_n(z)$ and $\hat{\zeta}_n(z) \equiv z h_n^{(1)}(z)$, where $j_n(z)$ and $h_n^{(1)}(z)$ are spherical Bessel functions of order $n$. 

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Dipole outside the shell

When the dipole is outside the shell, the potentials associated, respectively, with the fields emitted by the dipole, scattered from the shell, in the shell layer and in the shell core, are given by

\[
\psi_0 = \frac{p}{4\pi\epsilon_0\tilde{\epsilon}_0 R} \sum_{n=0}^{\infty} p_n \frac{\hat{\zeta}_n(k_0 r)}{r} P_n(\cos \theta), \quad r \geq R
\] (2.20)

\[
\psi_0 = \frac{p}{4\pi\epsilon_0\tilde{\epsilon}_0 R} \sum_{n=0}^{\infty} q_n \frac{\hat{\psi}_n(k_0 r)}{r} P_n(\cos \theta), \quad a \leq r \leq R
\] (2.21)

\[
\psi_s = \frac{p}{4\pi\epsilon_0\tilde{\epsilon}_0 R} \sum_{n=0}^{\infty} q_n a_n \frac{\hat{\zeta}_n(k_0 r)}{r} P_n(\cos \theta), \quad r \geq a
\] (2.22)

\[
\psi_1 = \frac{p}{4\pi\epsilon_0\tilde{\epsilon}_1 R} \sum_{n=0}^{\infty} q_n \left[ b_n \frac{\hat{\psi}_n(k_1 r)}{r} + c_n \frac{\hat{\zeta}_n(k_1 r)}{r} \right] P_n(\cos \theta), \quad b \leq r \leq a
\] (2.23)

\[
\psi_2 = \frac{p}{4\pi\epsilon_0\tilde{\epsilon}_2 R} \sum_{n=0}^{\infty} q_n d_n \frac{\hat{\psi}_n(k_2 r)}{r} P_n(\cos \theta), \quad r \leq b
\] (2.24)

where \( k_j = \sqrt{\tilde{\epsilon}_j} \omega / c \). Equations (2.20) and (2.21) for the incident-field potentials follow from (2.15)-(2.17). The coefficients \( a_n, b_n, c_n \) and \( d_n \) are determined by the boundary conditions at the interfaces of the shell.

Dipole inside the shell

When the dipole resides within the core, the fields are calculated from the following potentials:
\begin{equation}
\psi_s = \frac{p}{4\pi\epsilon_0\epsilon_0} \sum_{n=0}^{\infty} p_n \frac{\hat{\zeta}_n(k_0 r)}{r} P_n(\cos \theta), \quad r \geq a \tag{2.25}
\end{equation}

\begin{equation}
\psi_1 = \frac{p}{4\pi\epsilon_0\epsilon_1} \sum_{n=0}^{\infty} p_n \left[ b_n \frac{\hat{\psi}_n(k_1 r)}{r} + c_n \frac{\hat{\zeta}_n(k_1 r)}{r} \right] P_n(\cos \theta), \quad b \leq r \leq a \tag{2.26}
\end{equation}

\begin{equation}
\psi_2 = \frac{p}{4\pi\epsilon_0\epsilon_2 R} \sum_{n=0}^{\infty} p_n \left[ d_n \frac{\hat{\psi}_n(k_2 r)}{r} + \hat{\zeta}_n(k_2 r) \right] P_n(\cos \theta), \quad R \leq r \leq b \tag{2.27}
\end{equation}

\begin{equation}
\psi_2 = \frac{p}{4\pi\epsilon_0\epsilon_2 R} \sum_{n=0}^{\infty} (p_n d_n + q_n) \frac{\hat{\psi}_n(k_2 r)}{r} P_n(\cos \theta), \quad r \leq R \tag{2.28}
\end{equation}

2.3.4 Power & Normalized Power

Mie Theory Formulation

The total power emitted by a dipole-particle system is obtained by integrating the normal component of the Poynting vector \( S = \frac{1}{2} \text{Re} \{ \mathbf{E} \times \mathbf{H}^* \} \) over the surface of a sphere that encloses the system, as given below:

\begin{equation}
P = r^2 \frac{1}{2} \text{Re} \left[ \oint_{4\pi} \mathbf{E} \times \mathbf{H}^* \cdot \hat{r} \, d\Omega \right] \tag{2.29}
\end{equation}

To evaluate the total power, we substitute \( \mathbf{E} \times \mathbf{H}^* = E_\theta H_\phi^* \hat{\phi} \) in (2.29), where \( E_\theta = E_{0\theta} + E_{s\theta} \) and \( H_\phi = H_{0\phi} + H_{s\phi} \). Noting that the problem is axially symmetric, (2.29) becomes:

\begin{equation}
P = r^2 \frac{1}{2} \text{Re} 2\pi \int_0^\pi (E_{0\theta} + E_{s\theta})(H_{0\phi} + H_{s\phi})^* \sin \theta \, d\theta \tag{2.30}
\end{equation}
In this equation,

\[ E_{0\theta} + E_{s\theta} = \frac{p}{4\pi\varepsilon_0\tilde{\varepsilon}_0 R} \sum_{n=1}^{\infty} (p_n + q_n a_n) \frac{1}{r} \frac{d\hat{\zeta}_n(k_0 r)}{dr} P_n^1(\cos \theta) \]  

(2.31)

\[ H_{0\phi} + H_{s\phi} = i\frac{\omega p}{4\pi R} \sum_{n=1}^{\infty} (p_n + q_n a_n) \frac{1}{r} \hat{\zeta}_n(k_0 r) P_n^1(\cos \theta). \]  

(2.32)

Substituting into (2.30) and using the orthogonality relation of the Legendre functions, we find

\[ P = -\frac{\pi \omega |p|^2}{\varepsilon_0\tilde{\varepsilon}_0 (4\pi R)^2} \sum_{n=1}^{\infty} |p_n + q_n a_n|^2 \text{Re}\left\{ i\hat{\zeta}_n(k_0 r)^* \frac{d\hat{\zeta}_n(k_0 r)}{dr} \right\} \frac{2n(n+1)}{2n+1} \]  

(2.33)

We can show that

\[ \text{Re}\left\{ i\hat{\zeta}_n(k_0 r)^* \frac{d\hat{\zeta}_n(k_0 r)}{dr} \right\} = -k_0 \]  

(2.34)

independent of \( r \) as follows. We write

\[ \text{Re}\left\{ i\hat{\zeta}_n(k_0 r)^* \frac{d\hat{\zeta}_n(k_0 r)}{dr} \right\} = \frac{i}{2} \left[ \hat{\zeta}_n(k_0 r)^* \frac{d\hat{\zeta}_n(k_0 r)}{dr} - \hat{\zeta}_n(k_0 r) \frac{d\hat{\zeta}_n(k_0 r)^*}{dr} \right] \]  

(2.35)

Recalling that \( \hat{\zeta}_n(z) = z[j_n(z) + iy_n(z)], \) where \( j_n \) and \( y_n \) are spherical Bessel functions of the first and second kind, and using the Wronskian \( j_n(z)y_n'(z) - j_n'(z)y_n(z) = 1/z^2, \) equation (2.34) follows. Using (2.34) in (2.33), we find

\[ P = \frac{\omega |p|^2 k_0}{8\pi\varepsilon_0\tilde{\varepsilon}_0 R^2} \sum_{n=1}^{\infty} \frac{n(n+1)}{(2n+1)} |p_n + q_n a_n|^2. \]  

(2.36)

The power from an isolated dipole is

\[ P_0 = r^2 \frac{1}{2} \text{Re} \oint_{4\pi} \mathbf{E}_0 \times \mathbf{H}_0^* \cdot \hat{r} \, d\Omega. \]  

(2.37)
This can be evaluated analytically to give

\[
P_0 = \frac{k_0^2 \omega |p|^2}{12 \pi \varepsilon_0 \varepsilon_0}.
\]  
(2.38)

Normalizing (2.36) by \( P_0 \), we obtain the normalized power, \( \overline{P} \)

\[
\overline{P} = \frac{P}{P_0} = \frac{3}{2(k_0 R)^2} \sum_{n=1}^{\infty} \frac{n(n+1)}{(2n+1)} |p_n + q_n a_n|^2
\]  
(2.39)

If we wish to compute only the power scattered from the shell, (2.39) becomes

\[
\overline{P}_{\text{out}} = \frac{P}{P_0} = \frac{3}{2(k_0 R)^2} \sum_{n=1}^{\infty} \frac{n(n+1)}{(2n+1)} |q_n a_n|^2
\]  
(2.40)

This above formulas hold for the dipole outside the shell. When the dipole resides inside the core a similar derivation for the total power results in

\[
\overline{P}_{\text{in}} = \frac{P}{P_0} = \frac{3}{2(k_0 R)^2} \sum_{n=1}^{\infty} \frac{n(n+1)}{(2n+1)} |p_n a_n|^2
\]  
(2.41)

The Mie solution was programmed in MatLab, which is convenient because MatLab provides built-in Bessel functions and Legendre functions of any order. In computing the Mie series, a finite number of terms is used and this number is determined by examining how rapidly the terms converge. Typically, 20 terms was found to be sufficient. The execution time of the MatLab program was quite fast. For example, the power calculation computed for 100 wavelengths using 20 terms in the Mie series executed in about 5 seconds.

**Quasistatic Approximation**

When a particle’s size is much smaller than the incident wavelength, the incident electric field may be regarded as spatially uniform over the extent of the particle; this is referred
to as the quasistatic (QS) approximation and its validity has been discussed in detail in the literature. The advantage of this approximation is that the equations simplify considerably and spherical Bessel functions need not be computed.

Expressions for the electric field in the QS approximation are obtained by letting the wavelength tend to infinity relative to the particle size, or equivalently, by letting the wavenumber \( k_0 \) tend to zero in our equations. In this limit, the magnetic field vanishes, as expected, although the electric field does not.

2.3.5 Finite Element Method: Formulation

The numerical simulations were performed with the COMSOL Multiphysics software package (v.3.4 with RF Module, installed on a dual-Quad Core 32GB RAM Workstation), which comprises electromagnetic code based on the FEM.

The 3-D nanoshells were positioned at the coordinate system origin, and were embedded within a spherical computational domain that was bounded by a Perfectly Matched Layer (PML) to prevent unwanted reflections. The simulation employed quadratic elements and a unique mesh was compiled for each nanoshell size. The accuracy of the FEM solutions was ensured by refining the meshing density such that only minimal variations in the \( 2^{nd} \) decimal place of the solved E-fields were observed. The meshes usually consisted of between 150k to 250k points, representing between 1-2 million DoF, and the problem was solved with the iterative GMRES solver.

The power calculation required exporting the EM solution into MatLab and numerically integrating the Poynting vector (2.29) around a circle of radius \( R_0 \), depicted in Figure 2.15, to obtain the power at each wavelength. Axial symmetry allowed us to integrate around a circle in the x-z plane rather than over the surface of a sphere. This was performed after transforming the E-field and H-field Cartesian components of interest to spherical components using \( E_\theta = E_x \cos \theta - E_z \sin \theta \) and \( H_\phi = H_y \); it is noteworthy that the integration space was sampled to the extent that increasing the sampling density

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did not increase the solution accuracy.

The spectral domain was sampled finely enough to ensure rapid spectral fluctuations would be captured with sufficient resolution to accurately compare Mie theory and FEM. The Mie theory generated solutions at 5nm wavelength intervals, whereas the spectral resolution in COMSOL was dynamically selected according to the important features observed in the spectra, thus varying between 5nm and 50nm wavelength intervals.

In this dipole-nanoshell system, it is important to properly interpret the fields that manifest in the vicinity of the nanoshell, which depend on the dipole position \( r_d \) and observation radius \( R_0 \) relative to the nanoshell radius \( a \). As aforementioned in the Mie Theory formulation section, the total fields \( E_t \) and \( H_t \) comprise the addition of the scattered fields \( E_s \) and \( H_s \) to the fields emitted by an isolated dipole, \( E_0 \) and \( H_0 \), i.e. \( E_t = E_s + E_0 \) and \( H_t = H_s + H_0 \). When both the observation point and dipole position are outside the nanoshell, \( R_0 > a \) and \( R > a \), the relevant measured fields would be the scattered fields \( \{E_s, H_s\} \). In all other cases, the measured fields comprise the total fields \( \{E_t, H_t\} \).

It is noteworthy that the Mie Theory algorithm calculates \( \{E_s, H_s\} \) directly, and obtains the total fields \( \{E_t, H_t\} \) by adding the fields generated by the isolated dipole, \( \{E_0, H_0\} \); the FEM algorithm, however, calculates \( \{E_t, H_t\} \), from which \( \{E_0, H_0\} \), evaluated in the absence of the nanoshell, are subtracted to yield \( \{E_s, H_s\} \).

**Dipole model**

The dipole model, used as the excitation source, was established by considering the E-field generated by an electric dipole moment oriented along the z-axis. For an electric dipole of moment \( p \), the radial component of the near-field electric field is given by:

\[
E_r = \frac{p}{2\pi} \frac{\cos \theta}{j\omega \varepsilon_0 r^3} \tag{2.42}
\]
which, along the z-axis ($\theta = 0$), simplifies to

$$ E_r = \frac{p}{2\pi} \frac{1}{j\omega \varepsilon_0 r^3} $$

(2.43)

Selecting a dipole moment that yields a normalized E-field $E_r = 1$ results in

$$ |p| = 2\pi \omega \varepsilon_0 r^3 = \frac{(2\pi)^2 \varepsilon_0 r^3}{\lambda} $$

(2.44)

The dipole moment was evaluated for a dipole that is positioned at $r = R = 60$nm and excites a 100-nm nanoshell ($a = 50$nm, $b = 25$nm) at a frequency $\omega = 2\pi/\lambda$, where $\lambda = 480$nm is the approximate plasmon resonance peak of this nanoshell system. This combination yields a current dipole moment of $|p| = 4.7157 \times 10^{-17}$ Am. Ideally, the dipole should have an infinitesimal length such as to model a delta function; numerically, however, a finite length is required for discretization purposes. A practical dipole length of 1nm was selected following a comparison of its power spectrum relative to theory. Figure 2.16. Letting $p = Id$ for a dipole length $d = 1$nm suggests a line current of $I = 4.7157 \times 10^{-8}$ A.

**Figure 2.16:** Theoretical and FEM power spectra of an isolated radiating dipole
The plots convey a decreasing dipole power with increasing wavelength for a theoretical dipole, a 1 Å and 1 nm dipole, and although the latter two are not exactly superimposed onto the theory, their emitted power oscillates around the theoretical benchmark with a marginal error, approaching a maximum of \( \sim 5\% \) in the 400 – 500 nm interval. Additionally, it is interesting that no noticeable difference exists between the power spectra of the 1 Å and 1 nm dipoles; as such, it is computationally less expensive, in terms of mesh elements and thus memory, to employ the longer of the two.

Varying the dipole position along the z-axis, while keeping the dipole moment constant, did not significantly affect its power spectrum. This behavior is expected since the power of the isolated dipole, obtained by integrating the normal Poynting vector around a sphere that completely encompasses it, should be invariant; this analysis demonstrates spatial and spectral consistency of the incident source that is used in the remaining investigation.

This aforementioned pair of \( \{I, d\} \) values was employed throughout the FEM analysis for consistency. It is noteworthy, however, that fixing the two values \( \{I, d\} \) results in a varying E-field as a function of wavelength \( \lambda \) and dipole distance \( R \), which prevents a meaningful quantitative comparison between different dipole-nanoshell geometries; nevertheless, the nature of this report did not require such a normalization.

2.3.6 Spectral Comparison

Power & Normalized Power Spectra

Nanoshell sizes of 20 nm, 60 nm and 100 nm were chosen to investigate the performance of the FEM algorithm as a function of nanoparticle size, given that the Mie theory is analytically exact and can therefore be used as the benchmark to which COMSOL’s FEM is compared. We have previously demonstrated that, although the QS approximation is in very good agreement with the Mie theory for nanoshell sizes below 20 nm, it rapidly breaks down as nanoparticle size increases. The data presented in this section, however,
demonstrates that COMSOL’s FEM is able to accurately reproduce the Mie theory spectra, both qualitatively and quantitatively, even for large nanoshell sizes.

The power spectra of the nanoshells for an excitation dipole source positioned in the nanoshell core and in the surrounding air medium are presented in Figures 2.17(a) and 2.18(a), respectively. Their corresponding geometrical specifications, in terms of dipole position, $R_{in}$ and $R_{out}$, and observation radius, $R_0$, are conveyed in Table 2.2. The terms $R_{in}$ and $R_{out}$ are defined such that $R_{in} = R$ when the dipole is positioned inside the nanoshell core ($R < b$), and $R_{out} = R$ when the dipole is outside the nanoshell ($R > a$). The spectra in Figures 2.17(b) and 2.18(b) were calculated by normalizing the power spectra to the isolated dipole power spectra.

<table>
<thead>
<tr>
<th>Diameter (nm)</th>
<th>Radius (nm)</th>
<th>Dipole distance (nm)</th>
<th>Observ. Radius (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D$</td>
<td>$a$</td>
<td>$b$</td>
<td>$R_{in}$</td>
</tr>
<tr>
<td>20</td>
<td>10</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>60</td>
<td>30</td>
<td>24</td>
<td>12</td>
</tr>
<tr>
<td>100</td>
<td>50</td>
<td>40</td>
<td>20</td>
</tr>
</tbody>
</table>

![Figure 2.17: Spectra of (a) Total Power $P_t$ (b) Total Normalized Power $P_t^*$ of 20nm, 60nm and 100nm Nanoshells, for a dipole positioned inside the core @ $R_{in} = 4$nm, 12nm and 20nm, respectively](image_url)
The Mie spectra display the typical red-shifting and broadening of their plasmon resonance that occurs with increasing nanoshell size. When the dipole is lodged inside the core, the resonance peak at $\lambda = 535\text{nm}$ for the 20-nm nanoshell shifts to $\lambda = 567.5\text{nm}$ for the 100-nm nanoshell, as conveyed by the figure insets. The peak positions for an outside dipole are further red-shifted relative to the inside dipole to $\lambda = 540\text{nm}$ and $\lambda = 582.5\text{nm}$ for the 20-nm and 100-nm nanoshells, respectively. The FEM spectra were computed by sampling the frequency space such as to capture the rapid transitions of the resonance peak as well as other inflection points that appeared in the shorter wavelength region $\lambda = 300 - 450\text{nm}$. It should be reiterated that using the same dipole moment $|p|$ for the 3 particle sizes prevents quantitative comparisons between them, since $p$ is dependent on the dipole distance $R$, seen in equations (2.39-2.41), and thus varies with dipole position. As such, direct comparison of their spectra amplitude is meaningless. The FEM solutions in Figures 2.17 and 2.18 boast an excellent agreement with the Mie spectra, conveying no relative shift of the plasmon resonance peak, and little error, even at the resonance peak wavelength, which is usually where the largest error occurs. The full width half maximum (FWHM) values of the resonance peak are nearly identical and the FEM accurately captures smaller inflections points in the high-frequency end of the spectrum. It is noteworthy that the QS approximation cannot be employed to undertake such power calculations because the QS analysis is essentially electrostatic; no propagation of energy is predicted and the power is essentially zero.

### 2.3.7 Spatial Comparison

Validation of the spatial accuracy of the FEM algorithm is as important as the spectral validation, especially if investigating the spatial behavior of the fields at a single wavelength.

To aid in conceptualizing the dipole-nanoshell system, a typical 3-D geometry solved for $|E_z|$ in COMSOL is depicted in Figure 2.19, in which the radiating dipole fields are seen to be axially symmetric about $z$ and in turn excite the nanoshell that generates fields
that are also axially symmetric, as expected. The cutaway exposes the location of the dipole inside the core as well as the field behavior inside both the core and shell.

More specifically, focusing explicitly on the two dipole scenarios of the 100-nm nanoshell,
the E-field activity in the vicinity of the investigated dipole-nanoshell systems is conveyed by 3-D the FEM solutions in Figures 2.20 and Figure 2.21, for a dipole in the nanoshell core and in the air medium, respectively. By use of isosurface plots, boundary plots and arrow plots, these figures elegantly summarize the behavior of the E-field components $|E_z|$ and $|E_x|$ and provide further insight into the more detailed spatial plots in the following section.

**Figure 2.20:** 3-D solution plots of the E-field components in the vicinity of the 100-nm nanoshell, with dipole @ $R_{in} = 20$nm, and at the plasmon resonance wavelength $\lambda = 567.5$nm (a) $|E_x|$ (b) $|E_z|$. The displayed solution contains elements $y > 0$, and is conveyed by the $|E|$ isosurfaces, $|E|$ at the nanoshell boundaries, and the red arrows, which depict the direction and magnitude of $E$ in the x-z plane. The white dashed line represents the nanoshell core and shell boundaries.

For visualization purposes, the displayed solution only contains elements in the space $y > 0$; this translates to the dipole-nanoshell system being sliced along the $x-z$ plane such that only half of the 3-D solution is effectively depicted. Axial symmetry along the $z$-axis, however, implies that it completely describes the E-field behavior in all space. The fields were solved at the plasmon resonance wavelength of the dipole-nanoshell systems, as determined by their corresponding spectra in Figures 2.17 and 2.18, and summarized in Table 2.2. The $|E|$ isosurfaces and $E$ arrows show that the E-field emanating from the
Figure 2.21: 3-D solution plots of the E-field components in the vicinity of the 100-nm nanoshell, with dipole @ \( R_{\text{out}} = 75 \) nm, and at the plasmon resonance wavelength \( \lambda = 582.5 \) nm (a) \( |E_x| \) (b) \( |E_z| \). The displayed solution contains elements \( y > 0 \), and is conveyed by the \(|E|\) isosurfaces, \(|E|\) at the nanoshell boundaries and the red arrows, which depict the direction and magnitude of \( E \) in the \( x-z \) plane. The white dashed line represents the nanoshell core and shell boundaries.

dipole interacts with the nanoshell and diminishes while diverging away from the dipole-nanoshell system. The dipole source has a field pattern that is typical of a radiating dipole, and its interaction with the nanoshell generates a plasmonic activity with a field pattern of four lobes for the \( |E_x| \) components and two main lobes along the \( z \)-axis for the \( |E_z| \) components. It is important to note that the displayed E-field magnitude of the isosurfaces is limited to 500 V/m, but was fixed at 15V/m on the nanoshell boundaries to help distinguish the shell. The direction of the arrows show that dipole is directed along, and radiating in, the \( -z \) direction. It is also noteworthy that when the dipole is lodged inside the core, the E-field in the core aligns in the \( -z \) direction, but when the dipole is positioned outside the particle, the field lines are oriented in the \( +z \) direction.
**Spatial Resolution**

The spatial resolution of this particular system was investigated using two methods. The first part involved calculating the E-field as a function of the polar angle $\theta$ for a fixed observation radius $R_0$, such that the circular locus in the x-z plane traced by $R_0$ as $\theta$ increases from $-\pi$ to $\pi$ always lies in the same medium. The second part consists of calculating the E-field line profile along the x-axis, which effectively traverses the air/shell and shell/core boundaries on either side of the nanoshell; as such, this provides a comprehensive understanding of the capability and consistency of the FEM algorithm when challenged with small spatial features and E-field discontinuities at the shell boundaries. The meshing density was selected such that the differential error became negligible as the density was increased. These calculations were performed at the plasmon resonance wavelength of the corresponding dipole-nanoshell system, which consistently incurs the largest deviation between Mie and FEM for all nanoshell sizes and dipole positions. While exploring the following spatial analysis, it is critical to keep in mind that the solved fields for the dipole-nanoshell system with the dipole inside the core at $R_{in}$ and outside the nanoshell at $R_{out}$ are the total fields and scattered fields, respectively. For conciseness, however, they will both be labeled $E$ in their respective sections.

This analysis was performed for a 20-nm and 100-nm nanoshell, Figures 2.22 and 2.23, respectively, to test the behavior of the FEM algorithm and QS approximation for both a relatively small and large nanoparticle dimension. Parts (a) and (b) depict the case for a dipole lodged inside the core and positioned in the air medium, respectively, according to Table 2.3. The fields, described in their polar forms with $\theta$ increasing clockwise relative to the positive z-axis, were solved at each dipole-nanoparticle plasmon resonance wavelength and measured at the observation point detailed in Table 2.3. The plasmon resonance peaks in Table 2.3 were evaluated by spline interpolation between Mie sample points, and it is noteworthy that the ratio of the dipole position $R$ relative to the nanoshell radius $a$ is
constant for consistency, and the observation radius obeys $R_0 = R_{out} + 5\text{nm}$. The first row compares the complex components of the E-fields solved via the Mie and FEM techniques, and the second row concentrates on the viability of the QS approximation relative to Mie and FEM.

<table>
<thead>
<tr>
<th>Nanoshell D (\text{nm})</th>
<th>Dipole Distance (\text{nm})</th>
<th>Peak $\lambda$ (\text{nm})</th>
<th>Observ. Radius, $R_0$ (\text{nm})</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>$R_{in} = 4$</td>
<td>535.0</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>$R_{out} = 15$</td>
<td>540.0</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>$R_{in} = 12$</td>
<td>547.5</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>$R_{out} = 45$</td>
<td>555.0</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>$R_{in} = 20$</td>
<td>567.5</td>
<td>30 45 80</td>
</tr>
<tr>
<td></td>
<td>$R_{out} = 75$</td>
<td>582.5</td>
<td></td>
</tr>
</tbody>
</table>

For the case of the 20-nm nanoshell in Figure 2.22, the FEM is in excellent agreement with the Mie for all E-field components and at all angles $\theta$. It is important that the real and imaginary parts of the E-field components are plotted and compared individually, since comparison of the Mie- and FEM-generated field magnitudes alone could in fact hide possible discrepancies between their complex parts; if, for example, $\Re[E_r] >> \Im[E_r]$ but the $\Im[E_r]$ solution generated via FEM is significantly different to its Mie counterpart, comparing $|E_r|$ alone would not expose this inaccuracy.

It is interesting to note that $\Re[E_\theta]$ and $\Im[E_\theta]$ are both zero at $\theta = 0$ and $\theta = \pi$, which is expected since the dipole only exhibits $E_r$ components along the z-axis, due to symmetry. On a more subtle note, the $\Re[E_r]$ curve is a global maximum for $R_{in} = 4\text{nm}$ at $\theta = 0$ but a global minimum for $R_{out} = 15\text{nm}$ at $\theta = 0$. This inverted nature of one curve relative to the other about the x-axis can be intuitively understood by noting that the fields in both the inside and outside dipole cases are measured at the same observation radius $R_0$, which is located in the air medium such that the inequality $R_0 > R_{out} > a > b > R_{in}$ always holds. Restricting this discussion to the z-axis and recalling the convention that the dipole radiates
in the -z direction, the E-field vectors described by the arrows in Figure 2.21 convey that a dipole inside the core produces an E-field in the air medium at \( r_d = (20, 0, 0) \) that is directed along +z, translating to a positive \( E_r \), and directed along -z at \( r_d = (-20, 0, 0) \), which translates to a negative \( E_r \). Since \( E_r \) is strongest along the z-axis, this correlates to the maximum and minimum inflection points of \( \Re[E_r] \) at \( \theta = 0 \) and \( \theta = (-\pi, \pi) \) in Figure 2.22(a), respectively. The transition between these extremes behaves as expected as \( \Re[E_r] \) decreases smoothly between these angles.

Conversely, a dipole radiating in the air medium, Figure 2.22(b), incurs an E-field that is also directed along -z at \( r_d = (20, 0, 0) \) but along +z at \( r_d = (-20, 0, 0) \), which translates to the observed \( \Re[E_r] \) curve. The behavior of the \( \Re[E_\theta] \) curves in Figure 2.22 can also be accounted for by a similar explanation.

The polar plots are elegant displays of the E-field behavior around this symmetric geometry. The dual circular lobes for each E-field component demonstrate both the phase change that occurs at \( \theta = \pi/2 \) and \( \theta = -\pi/2 \), as well as the symmetry along the z-axis. They confirm that, as anticipated, \( |E_r| \) and \( |E_\theta| \) both reach maxima in their corresponding lobes at \( \theta = 0, -\pi, \pi \) and \( \theta = -\pi/2, \pi/2 \), respectively. The QS approximation is relatively accurate for the 20-nm nanoshell for both E-field components, as expected, although still conveys some error relative to the Mie theory even at this size. The good agreement stems from the fact that the E-field variation across this small-sized particle is more or less constant at this excitation wavelength, suggesting that the QS approximation should hold fairly well. The error increases slightly when the dipole is positioned outside the nanoshell.

Figure 2.23 presents the fields for a 100-nm nanoshell excited by a dipole positioned at \( R_{\text{in}} = 12 \text{nm} \) and \( R_{\text{out}} = 75 \text{nm} \), with the observation radius fixed at \( R_0 = 80 \text{nm} \). The FEM solution demonstrates a very strong agreement with the Mie solution, even at this particle size, which confirms that FEM is truly able to solve nanoparticle EM for nanoparticle sizes that are of practical interest. The behaviors of the real curves follow a similar trend
as those in Figure 2.22. The QS approximation, however, breaks down at this particle size for which there is significant error between the Mie or FEM and the QS solution.

The spatial comparison was extended in Figure 2.24 by investigating the accuracy of the FEM solution for a 100-nm nanoshell as a function of varying observation radius $R_0$. The E-field behavior in the core, shell and surrounding air medium was observed by setting $R_0$ to 30nm, 45nm and 80nm, respectively, while exciting with the dipole at $R_{in} = 20nm$ and $R_{in} = 75nm$. The FEM solution in all 3 media is in excellent agreement with the Mie solution, which confirms that the FEM is accurate in a dielectric, in a lossy material (metal) and in air. The peaks and troughs of the curves are intuitive following the aforementioned analysis of the dipole and observation point positions relative to the nanoshell. In all
Figure 2.23: Spatial Resolution of 100-nm Nanoshell as a function of \( \theta \) (a) Complex components of \( E \) & polar plots of \( |E| \) comparing Mie, QS and FEM, for \( \lambda = 567.5\text{nm}, R_{in} = 20\text{nm}, R_0 = 80\text{nm} \) (b) Complex components of \( E \) & polar plots of \( |E| \) comparing Mie, QS and FEM, for \( \lambda = 582.5\text{nm}, R_{out} = 75\text{nm}, R_0 = 80\text{nm} \)

In cases, except for \( R_{in} \) with \( R_0 = 30\text{nm} \), the QS solution is unable to reproduce the spatial behavior of the E-field displayed by the Mie or FEM. Interestingly, the QS has excellent agreement with the Mie for the exceptional case, even though this particle has a diameter of 100nm: this can be understood by considering the short distance between the dipole and the observation point, \( R_0 - R_{in} \), in the nanoshell core. Since they are relatively close, \( 5\text{nm} < R_0 - R_{in} < 15\text{nm} \) for \( 0 < \theta < \pi/3 \), the E-field across this distance is relatively constant at this wavelength, such that QS is a very good approximation for the E-field behavior in the core. However, an increase in the observation radius, which also increases its separation from the dipole, is accompanied by a deterioration of the QS approximation, clearly seen for the cases \( R_0 = 45\text{nm}, 80\text{nm} \).
Figure 2.24: Spatial Resolution of $|E|$ in the vicinity of a 100-nm Nanoshell as a function of $\theta$, comparing Mie, QS and FEM for observation in the core, shell and air media @ $R_0 = 30\text{nm}$, $45\text{nm}$, $80\text{nm}$, respectively (a) $|E|$ solution for $\lambda = 567.5\text{nm}$, $R_{in} = 20\text{nm}$, (b) $|E|$ for $\lambda = 582.5\text{nm}$, $R_{out} = 75\text{nm}$

The following line profiles describe the E-fields across material boundaries, which are usually where field discontinuities occur when solving the boundary conditions, especially at metal/dielectric interfaces where surface charge accumulates. Figures 2.25(a) and (b) present the fields in the vicinity of a 100-nm nanoshell excited by a dipole, lodged inside the core and the air medium, respectively, that is radiating at a wavelength corresponding to each system’s resonance peak. The x-axis is now the observation line along which the E-field is measured. The FEM performs very well, even at the SiO$_2$/Ag/Air boundaries where discontinuities occur, such as for $E_x$. The intensity and rate of change of the fields along the x-axis are dependent on the dipole distance relative to the x-axis: the maximum $|E_z|$ occurs at $x = 0$, where the dipole is situated, and decays away on either side of this
origin. The rate of decay is higher when the dipole is close to the x-axis in Figure 2.25(a), particularly when $x$ is small, but is similar for both dipole positions when $x$ is large. The $E_x$ curve drops to zero at $x = 0$, since the $E_x$ components cancel due to symmetry, and also approaches zero inside the shell, for both dipole positions.

The QS solution, even though inaccurate in magnitude, follows the Mie solution shape fairly well, even in the air/shell/core transition. The $E_x$ curve generally displays less error than $E_z$, although the agreement of both curves is particularly good for an inner dipole, in the region $|x| < 15$ nm, due to the proximity of the dipole to the x-axis observation line as previously discussed.

2.3.8 Conclusion

The excellent agreement between the FEM algorithm and the Mie Theory indicate that COMSOL is able to accurately solve electromagnetic problems at the nanoscale level, even when dealing with metals such as silver. Indeed, in both the spectral and spatial domains, the FEM deviates minimally from the Mie Theory for nanoshell sizes of practical importance, ranging from 20 nm to 100 nm. It is particularly important to stress that the

Figure 2.25: Line profile plots of $|E_x|$ and $|E_z|$ along the x-axis, computed via Mie Theory, FEM and QS, for a 100-nm nanoshell at plasmon resonance wavelength. E-fields evaluated with (a) dipole @ $R_{in} = 20$ nm and $\lambda = 567.5$ nm (b) dipole @ $R_{out} = 75$ nm and $\lambda = 582.5$ nm
tetrahedral mesh elements enable curved boundaries to be discretized such that potential field discontinuities across boundaries are reliably accounted for. The comparative study performed in this work indicates that FEM is a robust algorithm for solving more complex, interesting and realistic geometrical models for further advancing the field of plasmonics.

This study was published in the journal Nanotechnology in 2010.\textsuperscript{164}

The next chapter discusses the synthesis of novel-shaped gold nanostars and their potential as SERS substrates, as well as imaging contrast agents due to their strong two-photon luminescence. The confidence established in the FEM algorithm for solving the electromagnetic field in the vicinity of 3D nanostructures will be employed to study the gold nanostar’s exotic shape, which consists of sharp protruding branches, and demonstrate the creation of strong local E-field enhancements at the branch tips. The correlation between nanostar geometry and the resonance wavelength and width of its plasmon band will also be investigated.
Investigation, Development & Optimization of SERS Nanoplatforms

The research described in this chapter fulfills Aim 2

3.1 Introduction

The widespread use of Surface-Enhanced Raman Scattering, SERS, as a spectroscopic technique for the detection and identification of molecules in today’s biomedical arena has been achieved in a progressive, stepwise fashion since the discovery of Raman scattering, named after Sir Chandrasekhra Venkata Raman, who discovered the phenomenon in 1928. The simplicity of the instrumentation employed for this feat make its discovery all the more impressive: Sir Raman used the sunlight as the source, a telescope as the collector and his eye as the detector. Gradually, improvements in the various components of Raman instrumentation took place with research into sensitive detectors and improved optical trains, as well as the development of laser sources for Raman spectroscopy in 1962, all culminating in the the state of the art commercial instruments available today.
This chapter introduces background on Raman scattering and surface-enhanced Raman scattering that is required for appreciation of the research presented in this dissertation.

3.1.1 Raman scattering & surface-enhanced Raman scattering (SERS)

Surface-enhanced Raman scattering (SERS) is a powerful tool for the analysis of vibrational information of analyte molecules, sufficiently sensitive to achieve single molecule detection.\(^{63,64}\) SERS is the result of two main types of enhancement: electromagnetic
and chemical, where the former stems from the local electromagnetic (EM) field enhancement, and the latter results from the electronic resonance-charge transfer between the molecule and metal surface. Recently, much emphasis has been put on controlling the shape of the metallic substrate as this process has been shown to significantly alter the local field enhancement experienced by the adsorbed analyte, and thus the enhancement factor (EF). Our laboratory has been involved in the development of SERS-active nanostructures for chemical and biomedical sensing.

Recently, SERS measurements for gold and silver substrates have been reported for several other particle morphologies, including dendrites, rods, cubes and colloids. The synthesis of exotic nanostars has been reported in low yield and more recently in high yield. However, the SERS effect, has not been demonstrated for this intriguing shape. Additionally, the size control of these particles has not yet, to the best of our knowledge, been reported.

This first section discusses the synthesis, characterization and optimization of gold nanostars for SERS.

3.2 Gold Nanostars for SERS: Synthesis, Characterization and Optimization

3.2.1 Introduction

This section reports a thorough analysis and comparison of the morphology, optical characteristics, and SERS efficiency of high yield, synthesized stars as a function of size. We detail the modification of the protocol detailed by Kumar et al., and further extend this procedure to simultaneously control star size, branch lengths and branching density, which translate to the tuning of the longer plasmon peak in the near infrared (NIR) region. A simple model describing the interrelation of several parameters that constitute the shape of a generic nanostar is proposed; based on this model, statistical studies of the various nanostar structures are performed to assess the reliability in controlling their morphology,
as well as investigate any subtle parameter interdependencies which may exist. The paper closes by the investigation and comparison of the SERS efficiencies of such star-shaped gold nanoparticles in solution for their potential use as SERS-substrates in detection applications.

3.2.2 Experimental Procedures

Preparation and Characterization of Gold Nanostars

Gold nanostars were synthesized by the protocol presented by Liz-Marzan et al.. We further extended this protocol to enable size-control of the stars from approximately 45nm to 116nm in size, which translates to tuning capabilities of the longitudinal plasmon peak in the NIR region from around 725nm to over 850nm.

Briefly, 20nm PVP-coated Au seeds in ethanol were prepared according to a modified version of the protocol by Graf et al., resulting in a colloidal suspension containing [Au] = 1.75mM. A 10mM solution of PVP in 15mL DMF was prepared, into which 84μL of an aqueous solution of 50mM HAuCl₄ were added. This solution is termed ‘growth solution’. This step was followed by the injection, under vigorous stirring, of Au seed volumes ranging from 45μL to 700μL, which yielded stars of 116nm down to 45nm, respectively. Within 15 – 30 minutes of synthesis, each solution experienced a three-color transition starting with pink, then colorless and finally purple/blue for small stars, around 15min, to deep green for larger stars, past 25 minutes.

Gold nanostar growth as a function of time was investigated during an ongoing star synthesis, firstly, by analyzing the time-progression of the star’s absorption spectrum, and secondly, by imaging the star’s morphological changes over time, via TEM. The first procedure utilized fast spectral scan rates of approximately 45 seconds, enabling spectra to be taken in ‘real-time’ at two-minute intervals throughout the entire synthesis. The second study required further post-processing for the delicate preparation of TEM samples, and was therefore performed by successively extracting and quenching reaction mixture...
aliquots with sodium sulfide, where the sulfide acts as a scavenger for Au ions. Briefly, a 4.2mM solution of Na₂S in 15mL DMF was prepared, followed by immersion in an ultrasonic cleaning bath for efficient dispersing. A rack of fifteen 10mL-glass test tubes was placed close to the reaction vessel, and into each test tube was pipetted 1mL of the Na₂S ‘quenching solution’. A star synthesis was initiated at t = 0 minutes and, at two-minute intervals, 1mL reaction mixture aliquots were successively extracted, injected into the corresponding quenching solutions, which were gently mixed and rested for 1 minute prior to spectral analysis. Once sampling complete, the samples were washed 5 times with ethanol to remove excess PVP and DMF and imaged via TEM.

The gold nanostar samples were characterized by UV-Visible spectroscopy, transmission electron microscopy (TEM) and scanning electron microscopy (SEM). UV-Visible absorption spectra were measured using a Beckmann Coulter 530 spectrophotometer. TEM and SEM samples were prepared by drying ethanolic suspensions of the particles on carbon-formvar coated 300-mesh copper grids and copper tape, respectively. TEM images were acquired with a FEI Tecnai G2 Twin system operated at 160kV and SEM images were obtained with a FEI XL30 SEM-FEG system at 29kV. Particle dimensions were measured using the ImageJ software and the data shown in Table 3.1 are averages for 100 particles.

Preparation of SERS solutions

Nanostars are synthesized by the addition of pre-formed seeds into ‘growth solutions’, which enables calculation of their concentration by estimating the concentration of Au seeds added to the DMF growth solution. The concentration of citrate-capped Au seeds was estimated as 3.5 \times 10^{11} particles/mL, prior to PVP functionalization and re-suspension in ethanol. Varying volumes of the subsequently functionalized seeds were then added to the DMF growth solution to yield the final star solutions of different size and concentration. These solutions were centrifuged five times in ethanol to wash away excess PVP
and DMF solvent. Solutions containing a normalized surface-area of Au nanostars (2.560 m²/L) were then incubated with 20.0µM p-MBA in ethanol and gently stirred overnight (~ 15 hours). Assuming a thiol molecular footprint of ~ 0.22nm² this p-MBA concentration would be just sufficient to ensure monolayer coverage. The p-MBA-tagged nanostars were then washed three times with ethanol to remove displaced PVP and then transferred into a DI solution with EtOH 5% v/v. Small customized sample cells were prepared by affixing cover slips (0.17 – 0.18 mm thickness) onto 1.5mL cleaved, plastic microcentrifuge tubes with epoxy. Cleaving was achieved by horizontally slicing the tip of the tube with a utility knife, at the 100µL mark, forming an aperture of ~ 4mm in diameter. Epoxy was spread along the exposed wall-ends, and care was taken to ensure the aperture, through which sample detection would occur, was not compromised by excess epoxy. The cleaved tube was then vertically affixed, cleaved end and epoxy facing downwards, onto a cover slip and left to dry at room temperature for 24 hours. These customized cuvettes allowed for the use of higher NA objectives and prevented sample evaporation by capping with their original caps. A 50µL volume of ample solution was then pipetted into these containers for Raman detection, and the solution pH was adjusted to approximately pH10 by addition of 2µL 10mM aqueous NaOH. The cuvettes were positioned on the inverted microscope stage and the laser was focused 200µm above the top surface of the cover slip to ensure identical sampling volumes. The Raman detection was performed using an in-Via Renishaw Raman Microscope with a linearly-polarized 633nm laser line, with a 20×0.4NA Leica objective, 20mW power and a 60 second acquisition time.

3.2.3 Results and Discussion

Nanoparticle characterization

For the reported synthesis, the size of nanostars was controlled by varying the concentration of Au seed suspended in the DMF growth solution. The higher the seed concentration, the lower the available Au atoms per seed and thus the smaller the resulting nanostar. Ev-
identically, a negative correlation also exists between seed concentration and synthesis time: the higher the seed concentration, the thinner the deposition layer of Au atoms per seed and thus the shorter the time until depletion of Au atoms in the growth solution. It is also hypothetically possible to control the star size by varying the HAuCl\textsubscript{4} concentration in the growth solution, with a larger concentration of HAuCl\textsubscript{4} yielding larger stars, but this pathway would require impractical PVP concentrations to maintain the large [Au]:[PVP] ratio required for the reduction of HAuCl\textsubscript{4} and formation of the branches, as previously pointed out.\textsuperscript{168} The latter method was explored as an alternative, but no further particle growth was observed when the concentration of HAuCl\textsubscript{4} was increased whilst that of PVP was kept constant. Another possible route for controlling star size would be to inject Au seeds of varying sizes into the DMF growth solution, but this process requires the extra complication of synthesizing several seed batches and recalculating reagent concentrations to yield the desired star size.

TEM images of a typical batch of synthesized nanostars, extracted from samples originally injected with 45µL and 135µL of seed solution, are shown in Figure 3.2. The high-yield nature of the stars, as well as their complex morphology and homogeneous distribution, can be clearly interpreted. In the ensuing analysis, a thorough investigation of the nanostar’s growth kinetics and final morphology is undertaken, by characterizing nanostar growth, for which the evolution of the star’s morphology is studied over the synthesis duration, and nanostar size, which involves the study of the nanostar’s final size as a function of added seed volume.

**Star Growth Characterization**

The growth mechanism of a nanostar has not been discussed extensively in the nanostar literature thus far. Nonetheless, such a study is important for elucidating nanostar growth kinetics after seed-injection, or ‘nucleation’, enabling a correlation between nanostructure and optical resonance, as well as for developing a simple geometrical model that would
Figure 3.2: Typical TEM images of gold nanostars, synthesized via addition of 135 µL Au seed (main image) and 45 µL Au seed (inset). The inset is presented at this magnification to detail the star morphology for an enhanced perspective of their 3-dimensional structure.

reliably represent the synthesized nanostar. The nanostar synthesis was sampled at two-minute intervals by treating reaction mixture aliquots with sodium sulfide. Sulfide ions have a strong affinity for heavy metals in their ionic and metallic states, and sulfide treatment has previously been demonstrated to successfully stabilize the long-plasmon drift of gold nanorods by immediately arresting nanorod growth.\textsuperscript{170} For quenching the nanostar synthesis, a mole ratio of S to the total Au content of 4 : 1 was utilized, established as
optimal in the nanorod growth arrest experiment. Given the thirty-minute synthesis duration, quenching and spectrally analyzing the reaction mixture at two-minute intervals was deemed a compromise between excessive, uninformative spectral data and lack of time-resolution for capturing important spectral transitions. These superimposed spectra are presented in Figures 3.3(a,b), for nanostars formed by injecting 135\(\mu\)L and 540\(\mu\)L Au seed, respectively. This ‘spectral’ growth study was performed on these two star sizes as a means of comparing the growth mechanism of both small and large nanostars.

![Figure 3.3: Nanostar growth spectral study for nanostars synthesized via injection of (A) 135\(\mu\)L (B) 540\(\mu\)L Au seed. Red curve represents spectrum of reaction mixture at \(t = 0\) min, and spectra are graphed at two-min intervals until synthesis completion. Dotted arrows are loci of short and long plasmon band peaks as they evolve over time. The labeled time values are the times at which TEM imaging of the reaction mixture was performed.](image)

Surface plasmons (SP), which involve collective oscillations of electrons confined to a metal/dielectric boundary such as a metal nanoparticle surface, can interact strongly with incident electromagnetic fields. The SP behavior is directly influenced by several parameters including nanoparticle structure, shape, size and material, all of which dictate the electron confinement by the metal. This ultimately affects the optical and SERS properties of the nanoparticle, which can be observed via its UV-Visible absorption and SERS
spectra, respectively. In Figure 3.3, the star spectra exhibit a ‘short’ plasmon band in the mid-Visible region and a ‘long’ plasmon band, which appears following nucleation and evolves over time, in the NIR region. Interestingly, FDTD calculations on a nanostar have shown that the plasmons of such a particle result from hybridization of plasmons associated its core and individual tips, and generate bonding and anti-bonding states of lower and higher energy levels compared to those of core and tips, respectively. Nevertheless, in order to simplify the interpretation of the growth mechanism, the short and long plasmon peaks are attributed to the plasmon modes associated with the inner core and branch tips, respectively.

The two dashed arrows on each graph represent the loci of the peaks of both the short and long plasmon bands, as the spectra evolve in time, and allow monitoring of the rate of change of the peak’s position: indeed, since the synthesis was sampled at regular intervals, the shallower the gradient between two consecutive spectra, the faster the shift in peak position. Additionally, the rate of change of the peak’s intensity can be monitored by evaluating the intensity difference between two consecutive spectra: the larger this difference, the faster the intensity increases. Considering Figure 3.3(a), the short plasmon peak is observed to red-shift throughout the synthesis, with the greatest rate of change being between \( t = 0 \) and \( t = 8 \) min. This suggests that the core grows the fastest during the initial stages of the synthesis and continues to grow steadily until synthesis completion, which occurs at approximately \( t = 30 \) min, as judged spectrophotometrically. The long plasmon band starts appearing around \( \lambda = 700 \) nm very early in the synthesis, at \( t = 2 \) min, and rapidly red-shifts to approximately \( \lambda = 880 \) nm by \( t = 10 \) min, following which it slowly progresses further towards the NIR, reaching \( \lambda = 900 \) nm at \( t = 16 \) min. The long plasmon peak becomes dominant over the short plasmon at \( t = 8 \) min. Interestingly, the long plasmon band then steadily blue-shifts back towards \( \lambda = 850 \) nm as the synthesis terminates. These observations imply that the branches start growing from the core surface very shortly following nucleation, and lengthen rapidly in the first 10 minutes. The
blue-shift would indicate that the branches, which are geometrically comparable to conical nanorods, decrease in ‘aspect ratio’ in the later stages of the synthesis, a phenomenon also observed during the growth of short nanorods.\(^{175}\) It is noteworthy that both core and branches simultaneously grow throughout the synthesis, with their fastest growth rate being at the synthesis initiation, followed by a steady growth until synthesis termination. Additionally, the above trends, analyzed for large nanostars, are also observed for smaller stars as depicted in Figure 3.3(b), suggesting that the growth mechanism is independent of the star size (and therefore injected seed volume) and only one set of TEM images of a given star solution is sufficient for elucidating the growth mechanism of stars synthesized using this particular protocol.

Following spectral analysis, those samples which corresponded to spectra displaying critical changes were selected for TEM imaging. Since the greatest spectral changes appeared to occur at the beginning of the reaction, more emphasis was placed on imaging the initial samples. A total of ten samples of the large nanostars, corresponding to the time-labeled spectra in Figure 3.3(a), were selected. These samples are chronologically ordered in Figure 3.4, and effectively depict the color changes that occur during nanostar synthesis. Their corresponding TEM images are also sequentially ordered in Figure 3.5 and confirm the observations made in the above spectral analysis of the growth mechanism: the nanostar core increases rapidly between \(t = 0\) and \(t = 8\) min, during which time surface protrusions, which appear at \(t = 2\) min, develop into distinct star branches by \(t = 6\) min. It is important to note that the appearance of protrusions at \(t = 2\) min and \(t = 4\) min correlates to the appearance of the long plasmon band at those same times, which suggests that simplifying the plasmon analysis by bypassing plasmon hybridization theory and attributing this plasmon band to the plasmon mode associated with star branches still yields a reliable analysis. Between \(t = 8\) min and synthesis termination, both the core and branches are observed to gradually grow, most likely by gold depositing onto branches
while filling up the fractal spaces at the base of the branches, which leads to an increase in the core size, the number, length and width of branches. The blue-shift phenomenon is not obvious from the TEM images, which is expected since the spectral shift is small and rather gradual.

Figure 3.4: Photograph of quenched nanostar reaction mixture nanostar (synthesized by injection of 135 µL Au Seed), corresponding to the times labeled in Figure 3.3(a).

Figure 3.5: TEM images monitoring the nanostar evolution over time, and corresponding to the samples in Figure 3.4: (A) \(t = 0\) min (B) \(t = 2\) min (C) \(t = 4\) min (D) \(t = 6\) min (E) \(t = 8\) min (F) \(t = 12\) min (G) \(t = 16\) min (H) \(t = 20\) min (I) \(t = 24\) min (J) \(t = 28\) min. The scale bars represent a distance of 100 nm.

Together, the spectral data and TEM images provide strong evidence with regards to the growth mechanism of nanostars. We propose that the nanostar growth mechanism can be divided into two stages: firstly, a rapid growth following nucleation, where gold begins depositing onto the seeds, increasing their diameter and forming shallow protrusions that emanate from their surface and develop into distinct branches; secondly, a gradual growth, whereby the continual deposition of gold serves to simultaneously grow the branches and
fill up the fractal spaces at their base, which effectively further increases the star’s core diameter. As the core surface area increases, additional protrusions are able to form and develop into branches, which increase the star branch count. Synthesis termination occurs when the all the gold in solution is depleted.

It is noteworthy that attempting to halt the nanostar synthesis by simply centrifuging and diluting the reaction mixture aliquots, without sulfide-treatment, proved not to be effective: by the time the synthesis reached completion, the aliquots that were extracted and diluted at the initial stages of the reaction had already changed color from pinkish to deep blue, typifying nanostar formation. A likely explanation for this evolution is that the unreacted Au ions were not freely suspended in solution, but rather adsorbed onto the surfaces of the PVP, which co-precipitated with the nanostars upon centrifugation and redispersion, as observed for CTAB-stabilized nanorods. In comparison, sulfide treatment immediately arrested the nanostar synthesis at the desired point in time, and the solution remained stable for days.

**Star Size Characterization**

Size characterization involved the study of final nanostar size as a function of added seed volume. Nanoparticle sizing was performed using TEM images such as those in Figure 3.6, which also highlights the controllability of the protocol for synthesizing stars of desired size. TEM was chosen over SEM for its enhanced resolution and visualization clarity, especially when dealing with nanostars capped with a non-conductive PVP-layer that prevents the capturing of well-resolved SEM images; nevertheless, SEM images are included as insets in Figure 3.6 for a three-dimensional (3D) perspective of the nanoparticles.

For more accurate size characterization, as well as for SERS analysis purposes, a 3D nanostar model is proposed in Figure 3.7, where a nanostar of overall size $D_s$ is interpreted as a spherical core of diameter $D_c$, from which originates a number, $N$, of round-end cone
FIGURE 3.6: Transmission and scanning (insets) electron microscopy images of the nanostars in order of increasing size (A-F). Their synthesis in high-yield is evident. The samples A-F were synthesized by addition of the following volumes of Au seed: (A) 700µL, (B) 540µL, (C) 400µL, (D) 270µL, (E) 135µL, (F) 45µL. Scale bars correspond to 200nm for main panels and 100nm for insets and the TEM or SEM images are acquired at the same magnification.

'branches'. Here, the core is measured as the central sphere on top of which shallow protrusions (surface roughness) and branches are formed. The branch-length threshold, \( L_{\text{thresh}} \), quantifies the surface roughness thickness above which protrusions are considered branches, and subsequent to TEM analysis, was established as \( \sim 7 \) nm for modeling purposes; as such, a branch is defined as any well-formed protrusion that is more elevated (>\( L_{\text{thresh}} \)) than the underlying surface roughness at its base. It is governed by the parameters of branch base \( L_b \), branch length \( L_a \) and a \( \sim 3 \) nm radius of curvature at the branch tip. The model’s dimensions for samples A-F are estimated in Table 3.1.

These 5-parameter definitions were set up to enable fair nanostar modeling across all sizes and were derived with the previously analyzed nanostar growth under consideration: branches begin their growth by the localized deposition of gold atoms, which is controlled by the reduction kinetics of PVP. At the initial growth stages, the growth kinetics are such
Table 3.1: Average nanostar dimensions, estimated by measuring 100 nanoparticles imaged under TEM and SEM. Samples A-F correspond to images A-F in Figure 3.6.

<table>
<thead>
<tr>
<th>Star Sample</th>
<th>(N)</th>
<th>(L_a) (nm)</th>
<th>(L_b) (nm)</th>
<th>(D_c) (nm)</th>
<th>(D_S) (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>7 ± 1</td>
<td>12 ± 2</td>
<td>9 ± 2</td>
<td>27 ± 2</td>
<td>45 ± 4</td>
</tr>
<tr>
<td>B</td>
<td>7 ± 1</td>
<td>13 ± 3</td>
<td>10 ± 2</td>
<td>27 ± 2</td>
<td>52 ± 4</td>
</tr>
<tr>
<td>C</td>
<td>8 ± 1</td>
<td>15 ± 3</td>
<td>13 ± 2</td>
<td>31 ± 3</td>
<td>57 ± 5</td>
</tr>
<tr>
<td>D</td>
<td>10 ± 1</td>
<td>19 ± 4</td>
<td>13 ± 2</td>
<td>39 ± 3</td>
<td>72 ± 6</td>
</tr>
<tr>
<td>E</td>
<td>12 ± 1</td>
<td>22 ± 5</td>
<td>15 ± 2</td>
<td>46 ± 4</td>
<td>94 ± 9</td>
</tr>
<tr>
<td>F</td>
<td>14 ± 1</td>
<td>28 ± 7</td>
<td>15 ± 3</td>
<td>57 ± 5</td>
<td>116 ± 11</td>
</tr>
</tbody>
</table>

that small, scattered protrusions would form relatively uniformly on the gold seed surface, at which point they would be considered part of the surface roughness and not as branches per se. It is only when some protrusions start preferentially outgrowing the others (along various crystal facets) as a result of continued metal deposition that the formation of well-formed branches occurs.

The ensuing analysis refers to Figure 3.8 and the statistics of Table 3.1 to provide a comprehensive understanding of the trends of these two plasmon bands, as the star size increases. As aforementioned in the nanostar growth investigation, although plasmon hybridization has been shown to occur between nanostar core and branch plasmons, the short and long plasmon peaks in Figure 3.8 are be attributed, for simplification purposes, to the plasmon modes associated with the inner core and branch tips, respectively.

It is also noteworthy that Figure 3.8 conveys the spectra of the as-synthesized, non-normalized star solutions, purposefully done to provide transparency to the nanostar syn-
FiguRe 3.8: Absorption spectra of as-synthesized nanostars of increasing size from A to F, corresponding to samples (A-F) in Table 3.1.

thesis. Since samples A-F are synthesized by injecting different volumes of Au seed, their final concentrations are all different and directly proportional to the volume of added seed. This implies that intensity comparisons across samples A-F are meaningless and not deemed of great importance. The positions and widths of the short and long plasmon peaks across samples, however, are comparable and of critical importance. The relative intensities, or intensity ratio, of the two peaks within a sample is also an important quantity whose trend is also depicted here. The nanostar solution color transitions as a function of size, depicted in Figure 3.9, may also be inferred from their corresponding spectra.

FiguRe 3.9: Photograph of PVP-functionalized seed and as-synthesized nanostar dispersions in DMF of samples A-F, corresponding to curves A-F in Figure 3.8. Samples were illuminated from the left for better color identification.

As the star size increases from samples A to F, the short plasmon band centered around
550 nm, usually assigned to the plasmon band of spherical particles, becomes increasingly red-shifted as the spherical core diameter increases in size from 27 to 57 nm. At the same time, the long plasmon band, associated with the rod-like star branches, also undergoes red-shifting due to lengthening branches as well as an overall increase in star size.

The long plasmon band experiences an important broadening as the nanostars increase in size, which is a result of the increasingly inhomogeneous distributions of either particle sizes and/or shapes, which may both result in overall spectral broadening. Similarly to how size heterogeneity yields broad plasmons by the spectral superposition of plasmons associated with the different sized particles, shape heterogeneity could also result in broad plasmons by the spectral superposition of plasmons associated with the differently shaped particles. Interestingly, Table 3.1 reveals that the overall size distribution of the stars remains homogeneous from samples A to F, reflected by the standard deviations of $D_s$ not exceeding 10% (generally accepted as the threshold for homogeneity). Additionally, the same observation is made for the core size, $D_c$. This statistical evidence implies that the extreme broadening of the long plasmon band originates from the increasingly inhomogeneous distributions of star shapes: since the distributions of both $D_c$ and $D_s$ remain homogeneous, the shape heterogeneity must originate from the increasing randomness of the star branch morphology, which comprises branch dimensional parameters, packing density and orientation. This hypothesis is further emphasized when analyzing parameters $L_a$ and $L_b$ in Table 3.1. Indeed, the parameters La and Lb both convey an increase in average length from A to F, but more importantly $L_a$ shows a significant increase in its standard deviation, which translates to a larger distribution of branch lengths. As such, the branch length distribution becomes increasingly heterogeneous with increasing star size. The above analysis of the distributions of $L_a$, $D_c$ and $D_s$ as a function of increasing star size, proposes that the stars in a particular sample solution have similar average size (homogeneous $D_s$) but varying degrees of randomness in their branch morphology.
As star size increases from A to F, the branch morphology, and subsequently nanostar shape, both become increasingly random, translating to the observed broadening of the long plasmon band. Finally, it is noteworthy that this broadening could also be due to increase plasmon hybridization between the core and branches, as the overall star size increases from samples A to F. The complexity of the nanostar morphology makes a simple plasmon analysis using the hybridization method virtually impossible and will not be attempted in this investigation.

On the other hand, the short plasmon band, associated with the star core, is well defined for sample A, but slowly becomes engulfed by the overwhelming intensity and width of the long plasmon band, from A to F. As such, these spectra alone do not provide sufficient information to infer any dependence between increasing star size and short plasmon band behavior; nevertheless, by reverting to Table 3.1, it can be concluded that the core diameter $D_c$ maintains distribution uniformity from samples A to F, which is indicated by the $D_c$ having a standard deviation inferior to 10%. The consistency of the standard deviations suggests that the short plasmon band width is likely unaffected by the increasing heterogeneous distributions of branch lengths, as star size increases. As such, the short plasmon band is not expected to broaden significantly, as in the case of the long plasmon band, and specifically not as a result of increasing star shape heterogeneity. Rather, the short plasmon band experiences slight broadening as $D_c$ increases with increasing star size $D_s$. Again, it should be emphasized that the above analysis was simplified by ignoring the effects of plasmon band hybridization, which could introduce unaccounted contributions towards plasmon broadening and/or shifting; nevertheless, the general trends discussed should still be valid. Finally, the decreasing relative intensities of the short to long plasmon peaks is a similar trend observed when increasing the aspect ratio of gold nanorods, and is attributed to the increasing branch length and AR from A to F, a trend also demonstrated by Nordlander’s FDTD calculations. The increasing branch AR is analogous to increasing...
nanorod AR, and results in stronger confinement of plasmon oscillations at the tips. This translates to an increasingly intense longitudinal plasmon band.

While Table 3.1 depicts numerical entities from which general trends may be understood, further insight can be obtained by studying these parameters from a purely graphical standpoint. Figure 3.10 conveys a graphical representation of interdependencies between parameters in Table 3.1. A pair of parameters, (X, Y), are plotted as clusters of data points in 2D space. Each cluster represents data points from one sample, and therefore each presented graph comprises all samples, for an elegant visual comparison. It is noteworthy that for each sample A to F, only 30 randomly chosen data points per parameter (out of total of 100) are shown for clarity, but the parameter averages and standard deviations, $X_{\text{average}}$ & $Y_{\text{average}}$ and $X_{\text{std}}$ & $Y_{\text{std}}$, respectively, are extracted from Table 3.1. The graphs were thus plotted from a data cube of dimensions $6 \times 5 \times 30$ (Sample, Parameter, Data point). For each sample A to F, the drawn ellipses are centered at the point $(X_{\text{average}}, Y_{\text{average}})$, join the four standard deviation boundaries $(X_{\text{average}} \pm X_{\text{std}}, Y_{\text{average}} \pm Y_{\text{std}})$, and represent the standard deviation locus in $2D$ parameter space. The width of the ellipse along each axis determines the variability of that specific parameter, from sample to sample; the greater the width, the more heterogeneous the parameter along that specific axis. As the parameter becomes increasingly homogeneous, the corresponding ellipse width would decrease until it collapses into an infinitesimal point, in the ideal case, at which point the parameter would be statistically constant. It is critical to note that, since the plotted parameters are not normalized with respect to each other, visual comparisons and deductions based on the ellipse characteristics (axes length and eccentricity) for a single, isolated sample are meaningless; rather, reliable intuition can be inferred from comparing ellipse transitions across the samples in a particular graph.

The cluster plots depict parameter dependencies on two different scales: \textit{intra-sample}
parameter dependencies within the same sample of stars, and *inter-sample* parameter dependencies across samples of varying-sized stars. Evaluating the parameter cross-correlations in Figures 3.10(a-d) yielded a relatively low value of $\pm 0.1$, suggesting negligible intra-sample parameter dependence. This negligible dependence enables non-oblique ellipses to be used for the representation of variance loci.

The lines of best fit in each graph are fitted through the ellipse centers for the sole
purpose of qualitative evaluation of inter-sample parameter trends; as such, they should not be considered quantitative evidence of the trends they suggest. Figure 3.10(a, b, d, e) were established using a least-squares algorithm, whereas the disposition of ellipses in Figure 3.10(c) lead to a gentle curving of the line of best fit. For the purpose of analysis simplification, the injected seed solution is assumed ideally monodisperse with a size of 20nm, which, given their actual size distribution of 20.1 ± 1.6nm, is a fair assumption.

Figure 3.10(a) displays the relationship between star core and overall size, whose line of best fit is remarkably linear throughout. Their relative distributions remain narrow with increasing size, as indicated by uniformly growing ellipses of similar eccentricity. Extrapolating the line of best fit down to the minimum value $D_c = 20$nm hypothetically suggests a minimum overall size $D_s \sim 34$nm. Under ideal conditions of infinitesimal star growth, the minimum star size, $\max(D_s)$, should equal the minimum core size, $\min(D_c)$, which is in turn defined by the 20nm size of the injected gold seed; this should entail a line of best fit through the origin (20, 20). Nevertheless, the 14nm offset along the $D_s$-axis shows consistency with the proposed nanostar model, since the employed ‘branch’ and ‘core’ definitions imply an unaccounted surface roughness ‘shell’, of maximum thickness $L_{thresh}$, surrounding the whole core and contributing to $D_s$, when both of these are at their minimum. In other words, $\min(D_s) = \min(D_c) + 2 \times L_{thresh}$.

Figure 3.10(b) clearly conveys that as overall size $D_s$ increases, so does branch length $L_a$. While the overall size remains quite homogenous, indicated by narrow ellipses from sample A to F, the branch length becomes more heterogeneous, depicted by increasingly elongated ellipses (increasing eccentricity). On a more subtle note, it appears that the ellipsoid centers are positioned along a line of best fit that is mostly linear, except at $D_s = 34$nm, where a singularity is forced. This interesting result suggests proportionality between $L_a$ and $D_s$ for the synthesized nanostars A-F. The singularity was introduced to validate the model’s consistency with other parameters: as such, simultaneous comparison between Figure 3.10(a) and Figure 3.10(b) depict that branching below $L_{thresh}$ has no
contribution towards the overall star size. Branching only starts contributing as the branch length exceeds the surrounding surface roughness. A value of $L_a \sim L_{\text{thresh}}$ when $D_s = 34\text{nm}$ also shows consistency with the star model used for measurement purposes.

Figure 3.10(c) demonstrates the correlation between $L_a$ and $L_b$, for which there is significantly more scattering of the data points. For consistency with the well-established nanorod literature and terminology, the quantity $L_a/L_b$ may be defined as the branch ‘aspect ratio’, AR, although it should be noted that the branches are conical in shape, rather than cylindrical, as implied by the original term describing the nanorods. Interestingly, the curve represents the locus of points $(L_b, L_a)$, whose gradient conveys the local rate of change of branch AR with dimension $L_b$, and tends to zero as the curve approaches the horizontal asymptote at a value $L_a$ of approximately 7nm, attributed to $L_{\text{thresh}}$. The graph depicts more heterogeneous branching accompanied by a sharpening of the star branches with increasing branch length.

Figure 3.10(d) shows the trend between seed volume and overall star size. Only the averages and standard deviation loci are plotted here, for clarity. The linear line of best fit extrapolates towards a threshold $N = 5$, as $D_s$ tends to its predetermined minimum size of 34nm. Finally, Figure 3.10(e) is plotted to calibrate the resulting star size as a function of injected seed volume. The inversely proportional relationship is expected since the overall star size should decrease as the total seed surface area (SA) available for gold deposition increases. The singularity at $V_{\text{seed}} = 0\mu\text{L}$ was introduced to ensure consistency with the idea that star growth does not occur in the absence of added seed.

3.2.4 SERS Spectra and Enhancement Factors

A fair comparison between particles of different morphology is only achievable if the number of sampled analyte molecules is kept constant between the particle shapes. The nanostars are synthesized with a thick coating of long PVP chains (MW 10,000), which prevents any dye from accessing the highest enhancement regions on the gold surface. PVP is an
organic polymer, whose polyvinyl skeleton serves as a hydrophobic tail group, and its polar pyrrolidone group serves as a hydrophilic head group, giving PVP an amphiphilic feature.\textsuperscript{177} The head groups interact with Au or Ag particle surfaces via physisorption, whereas the tail groups provide a physical barrier that prevents particle aggregation. The nanostars comprise the same protective sheath which helps them remain in suspension in organic solvents such as DMF and ethanol. When incubated with a dye that comprises a thiol group (-SH), such as p-MBA, the very strong affinity of the thiol for the gold surface triggers a ligand-exchange process.\textsuperscript{178} The thiol ends of p-MBA bond to the gold surface via chemisorption that is generated from the redox reaction:

\[ Au_n + mRSH = Au_n(SR)_m + 1/2mH_2, \quad (3.1) \]

As such, the physically adsorbed nucleophilic groups of PVP are replaced by the thiol group of p-MBA, bringing the latter within close proximity of the enhanced E-fields.

Although this protocol generates stars in very high yield, the complex nanostar geometry makes estimation of the p-MBA surface coverage significantly more challenging than for particles with a defined symmetry. Table 3.1 enabled the estimation of average surface areas of 3.7, 3.9, 5.4, 9.0, 12.9 and 20.0 \( \times 10^3 \) nm\(^2\) for a generic nanostar in samples A-F, respectively, from which it was possible to keep the concentration of analyte molecules fixed across all star sizes.

It is noteworthy that due to the star’s outward radiating branches and sub-wavelength size (evidently depicted by the star TEMs in Figure 3.6 and model in Figure 3.7) and the linear polarization of the incident laser excitation, not all branches can be aligned with the E-field’s direction of polarization at any one instant in time; indeed, the local surface plasmons (LSPs) oscillating in the direction of the branch and confined at the tips would mainly be excited by components of the incident E-field in the same direction. It is therefore anticipated that the tips would exhibit varying enhancements for any given excitation.
snapshot, even for the ideally symmetric model shown in Figure 3.7. For SERS measurements in aqueous solutions, however, nanostars are randomly oriented and as such, the SERS intensities would be representative of all the possible nanostar branch orientations in the probed volume averaged over the acquisition period. This effectively removes any polarization-dependence of the SERS measurements and further increases comparison fairness across star sizes.

Valid comparisons also necessitate minimal particle aggregation, which leads to the localized formation of extremely high E-field enhancements as a result of plasmon coupling effects, or “hotspots”. The randomness of particle aggregation is the reason these SERS experiments were done in aqueous solutions under dilute conditions. Nevertheless, even in solution, particles may still aggregate depending on their surface chemistry as well as the ionic strength of the solvent. Self-Assembled Monolayers (SAMs) of p-MBA prepared via incubation in pure ethanol have been shown to form multilayers owing to the formation of hydrogen bonds between the carboxyl head groups of the p-MBA molecules. Acetic acid and trifluoroacetic acid have been used by several authors as a substitute reagent for hydrogen bonding, but these experiments were successful for synthesizing p-MBA SAMs on gold substrates, not on nanoparticles in solution. The addition of trace amounts of acetic acid to the synthesized nanostar solutions resulted in aggregation most probably due to neutralization of the charge shielding surrounding the particles. Even if successful, this procedure, involving the injection of new reagents to undergo sacrificial H-bonding, would result in additional Raman peaks pertaining to their intrinsic vibrations; more importantly, if possessing similar bonds to those in the dye of interest, unwanted contributions to extant Raman peaks could deceptively increase the SERS spectra intensities, which would invalidate any attempted comparison. This method also lacks simple, real-time calibration capabilities to indicate whether, and how much, H-bonding between p-MBA and acetic acid molecules has successfully taken place. As a more effective alternative, aggregation was prevented, post-incubation with p-MBA, by adjusting the so-
solution to basic conditions of $\text{pH } \sim 10$ which deprotonates the COOH head groups of the chemisorbed p-MBA, yielding a negative, repelling surface charge on each nanostar.\textsuperscript{183–185} A pH of 10 was chosen as a compromise to ensure sufficient COOH deprotonation while maintaining an ionic strength low enough to keep the particles in suspension. Typical SERS spectra of the p-MBA-nanostar system are shown in Figure 3.11, before and after pH adjustment.

The deprotonation was verified by the disappearance of the $1710\text{ cm}^{-1}$ peak and intensity increase of the $1415\text{ cm}^{-1}$ peak in the nanostar Raman spectra in Figure 3.11, corresponding to the C=O stretching vibrations $\nu$(C=O) of non-dissociated COOH groups, and $\nu_s$(COO$^-$), respectively.\textsuperscript{186} Interestingly, an intensity decrease of around $5 - 10\%$ was observed in the Raman spectra following addition of NaOH, suggesting a possible minor contribution to the detected intensity from E-field enhancements induced by interparticle E-field coupling in the original solution. This intensity decrease may also be in part to slight sample dilution, but this effect was observed not to be the controlling factor.
factor in this case. It should be noted that the $v_{s}$(COO-) vibration at 1415 cm$^{-1}$ in this study is slightly lower than previously reported values$^{185,186}$ of 1430 cm$^{-1}$ and 1423 cm$^{-1}$, but is nevertheless reasonable considering the different experimental conditions. Shifts of Raman peaks are usually associated with variations in local environments surrounding the probed molecules, such as substrate material, surface chemistry (capping/protective agents) and solvent; these ultimately alter the local dielectric constant, as well as any substrate-adsorbate chemical interactions which may exist, and lead to shifts in Raman peaks. Similarly, the same argument can be used to explain the observed shift of the same band from 1415 cm$^{-1}$ to 1384 cm$^{-1}$ for the normal Raman spectrum.

The two strong bands at about 1590 and 1080 cm$^{-1}$ are assigned to $v_{8a}$ and $v_{12}$ aromatic ring vibrations, respectively$^{186}$. The SERS efficiencies of all star sizes were investigated and their spectra are displayed in Figure 3.12. The slight right shift of the 1080 cm$^{-1}$ peak to a higher wavenumber in the normal p-MBA Raman spectrum is also in accordance with that observed under similar conditions by the same authors.

The SERS spectra convey limited correlation between intensity and star size, which, considering the fact that star morphology also varies with increasing star size, implies a more complicated relationship between the two. The decreasing overlap of the leading edge of the longitudinal plasmon band and the 633 nm excitation source, as star size increases, would hypothetically also be accompanied by an observable decrease in intensity; however, this feature was not observed. Given the star morphology, the local field enhancement is expected to be inhomogeneous on the particle surface, with greater enhancements occurring in close proximity of sharp edges or corner.$^{68}$ As such, the branch tips should boast significantly higher plasmonic enhancement intensities than on the flatter parts of the core surface.$^{168}$ Assuming a branch tip radius of curvature of $\pm 3$ nm, the branch AR generally increases with increasing star size, from 1.3 to 1.9, but no relationship to intensity may be inferred from this trend since other parameters ($N, D_c$ and $D_s$) also increase
simultaneously. If these other parameters were constant, however, it would be reasonable to expect intensity to generally increase with branch AR. Another possible factor for variations in intensity among stars of different sizes is the ratio of the number of p-MBA molecules adsorbed to the branch tips to the total number of p-MBA molecules adsorbed to the star; the larger this fraction, the greater the anticipated intensity since more molecules would be located in the stronger field-enhanced region. Assuming a similar packing density of p-MBA molecules on the tip as everywhere else on the star, this ratio was estimated as 0.11 down to 0.039 for samples A-F, respectively. Again, the intensities of the SERS spectra indicate otherwise, which is to be expected considering this parameter is one of several variables affecting the overall detected intensity. For the stars described here, the strongest substrate (i.e. that which exhibits the most intense SERS signal) is sample B, stars of 52nm in size, and the weakest is sample A, stars of 45nm, with a peak intensity difference of approximately $2.5 \times$ between the two.

**Figure 3.12:** The surface-enhanced Raman scattering (SERS) spectra of nanostars in an aqueous solution with 5% EtOH v/v at pH 10, for samples A-F. The last spectrum is the ordinary Raman spectrum of 0.1M p-MBA in 1M aqueous NaOH, for reference.
The surface enhancement factors of the stars were estimated by considering the \( v_{8a} \) Raman band, since it is the strongest and shift-free peak of all bands in the spectra. The surface enhancement factor (EF) can be defined as

\[
EF = \frac{I_{SERS}}{I_{Bulk}} \times \frac{N_{Bulk}}{N_{SERS}},
\]

(3.2)

where \( I_{SERS} \) and \( I_{Bulk} \) are intensities of the same band in the SERS and bulk spectra, \( N_{SERS} \) is the number of adsorbed molecules sampled on the SERS-active substrate, \( N_{Bulk} \) is the number of molecules sampled in the bulk. The \( I_{Bulk} \) was determined from the normal Raman spectrum, which was measured by preparing p-MBA in a strong basic solvent (serving the purpose of dissolution) and keeping the ethanol concentration identical to the star solutions minimal shifts of, and fair comparisons between, peak intensities. The intense, shifted band at 1384 cm\(^{-1}\), corresponding to \( v(C=O) \), reflects the saturated pH of the solution. However, since this band is independent of \( v_{8a} \) in that this particle-less solution is immune to aggregation or precipitation as a result of extremely low or high pH, respectively, direct comparison to \( v_{8a} \) in samples A-F is still possible.

The sampled volume was estimated by noting that the objective is characterized by a 5.8 µm depth of field, and produces a measured 633 nm laser spot size of approximately 3.2 µm in diameter. This yields a sampled volume of about \( 4.7 \times 10^{-11} \) mL, although any marginal error associated with this calculation does not affect the final EF since the experimental setup was identical for all measurements (implying the probed volume cancels out in equation (3.2)). With the particle SA fixed at 2.650 m²/L across star samples, assuming p-MBA monolayer coverage and a thiol footprint of 0.22 nm², \( N_{SERS} \) was estimated as \( 5.62 \times 10^5 \) in the probed volume. The 0.1 M p-MBA solution comprises \( 2.81 \times 10^9 \) analyte molecules in the same probed volume. Table 3.2 summarizes the EF approximations for the six star samples A-F.

The enhancement factors were found to range from a minimum of \( 2.02 \times 10^3 \) to \( 5.00 \times 10^3 \) for star sizes 45 and 52 nm, respectively. It should be noted that the assumed
Table 3.2: Enhancement factors for the $v_{8a}$ vibrational mode of p-MBA on different star samples, using a 633nm laser line.

<table>
<thead>
<tr>
<th>Star Sample</th>
<th>Diameter (nm)</th>
<th>Enhancement Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>45</td>
<td>$2.02 \times 10^4$</td>
</tr>
<tr>
<td>B</td>
<td>52</td>
<td>$5.00 \times 10^3$</td>
</tr>
<tr>
<td>C</td>
<td>57</td>
<td>$3.69 \times 10^3$</td>
</tr>
<tr>
<td>D</td>
<td>72</td>
<td>$2.86 \times 10^3$</td>
</tr>
<tr>
<td>E</td>
<td>94</td>
<td>$3.27 \times 10^3$</td>
</tr>
<tr>
<td>F</td>
<td>116</td>
<td>$3.63 \times 10^3$</td>
</tr>
</tbody>
</table>

Complete monolayer coverage of the adsorbate, p-MBA, on the stars is the theoretical maximum calculated under ideal conditions. Experimentally, the structure of the resulting SAM and rate of formation is based on several critical factors such as adsorbate purity and molecular structure, adsorbate incubation time and concentration, nanostar surface cleanliness, nanostar surface radius of curvature, non-specific binding (H-bonding), all of which would increase the effective thiol molecular footprint of p-MBA from the ideal $0.22 \text{nm}^2$. This suggests that the EF values in Table 3.2 are surely underestimates. The proposed simplistic star model, designed to estimate star SA was consistently used across all star sizes, which implies a fair qualitative EF comparison among them. A quantitative EF comparison with other shapes in the literature, however, is insignificant due to the large intrinsic error associated with this model in particular. Stars have a complex morphology that varies with size, and whose description is attempted with the aid of five interdependent parameters, or Degrees of Freedom, in Table 3.1. Evidently, increasing degrees of freedom in a model lead to greater potential inaccuracies. Specifically, the likelihood of, and errors associated with, under or over estimates in the p-MBA coverage are significantly higher: if the SA is overestimated, the concentration of added p-MBA would result in an excess of p-MBA molecules, NSERS, which would be removed from the sample solution via washing post-incubation. The effective NSERS would be lower than that
used in the EF calculation, leading to an underestimated EF. If, on the other hand, the SA is underestimated, the added p-MBA would be insufficient to ensure complete monolayer coverage. This would result in detected SERS intensities that are lower than the nanostar’s true enhancing potential. Again, the EF would be underestimated. Adding an excess p-MBA could improve the problem of underestimating the star SA, but this would introduce further variables into the nanoparticle-adsorbate system, such as uncontrolled non-specific H-bonding. Furthermore, this would not resolve inaccuracies originating from the model’s simplicity. As such, quantitative EF estimates are extremely hard to attain.

It is important to note that the reported values are for isolated particles in solution, and care has been taken to prevent aggregation leading to hotspots, so that they are significantly lower than for particles where aggregation is promoted.

Furthermore, greatest SERS enhancements are expected to occur when the spectral overlap between the incident wavelength and the absorption peaks, representative of the wavelengths at which LSPs are excited, is maximized. However, since SERS intensity is also proportional to $1/\lambda^4$, a compromise exists between red-shifting the incident wavelength to maximize spectral overlap and ensuring minimal negative contributions originating from the $1/\lambda^4$ factor. With reference to Figure 3.8, the use of a 633nm laser excitation source, which was available in our laboratory, does not maximize the overlap with the SP bands (or absorption bands) of any sample, which implies that the EF can be further increased by shifting the excitation source to a wavelength of 780 – 820nm, where the overlap is nearly twice as large in some cases. The factor should not play a critical role for such a red-shift, with a $\sim 3 \times$ decrease from $6.22 \times 10^{-12}\text{nm}^{-4}$ to $2.21 \times 10^{-12}\text{nm}^{-4}$ for $\lambda = 633\text{nm}$ and $\lambda = 820\text{nm}$, respectively.

It is noteworthy that although each nanostar comprises several branches, with the greatest E-field enhancement in the vicinity of their tips, the detected SERS is effectively a spatial average of all the E-field interactions occurring over the surface of the particles, integrated over the acquisition period (this is assuming no coupling interaction between
particles, since solutions were dilute and particle surface were repelling). As aforementioned, the branch tip to total SA was estimated as being inferior to 10%, in the best case scenario (sample A). This implies that at least 90% of the remaining p-MBA molecules are adsorbed on the sides of the branches or on the central core surface, where the E-field enhancement is significantly lower. This weighted spatial average would effectively hamper the overall EF, providing insight into the reported range of EF values. Incidentally, if normalization was effectuated with respect to particles per unit volume, rather than to area per unit volume, the EF values are anticipated to be significantly higher since each nanostar comprises several branch tips.

The reported SERS experiments were performed to enable a more accurate, qualitative comparison between different star sizes, with the intention of determining that which provides the best SERS efficiency. The nanostar E-field enhancement, and thus SERS efficiency, appears to originate from an intricate interplay of star size, spectral overlap between surface plasmon peaks and excitation source, excitation wavelength, the number of branches (i.e., proportion of branch tip to total SA), branch AR, branch length and general star morphology.

3.2.5 Conclusion

The synthesis of high-yield gold nanostars of varying sizes, the growth mechanism, their physical modeling, optical response and use as SERS substrates were investigated and reported for the first time. The results indicated that it is possible to vary the size and morphology of the nanostar structures in a controlled fashion, by carefully adjusting the volume of preformed Au seed added to the growth solution. Detailed statistical analysis of the synthesized samples, based on TEM and SEM measurements, was performed and unraveled unique parameter interdependences across samples, even though intra-sample analysis revealed little correlation. The homogeneously-sized star samples conveyed interesting absorption spectra comprising a short and long plasmon band, with the latter
becoming increasingly broad and red-shifted with enlarging nanostar, indicating a heterogeneous distribution of morphologies within a given sample. Solution-based SERS measurements were performed using the Raman-active dye p-MBA, demonstrating the use of this new nanostructure as a useful SERS-active substrate.

This research was published in the Journal of Physical Chemistry C in 2008 by Khoury and Vo-Dinh.90

3.3 Gold nanostars: surfactant-free synthesis, 3D numerical modeling and two-photon luminescence

The initial development of gold nanostars for SERS was limited by the nature of the synthesis protocol. Although nanostars were expected to yield strong electromagnetic enhancements due to their sharp, protruding branches, the dense coverage of their surface by the PVP polymer prevented the efficient surface binding of Raman dyes to exploit the high local E-fields.

Recently, a novel, polymer-free synthesis protocol was developed in our lab that allowed the growth of nanostars by only using small citrate molecules as the stabilizing surfactant188. This renewed the interest in gold nanostars as potential SERS substrates, and also opened new opportunities for their use in areas such as two-photon luminescence, photodynamic therapy, and photothermal therapy. Indeed, the plasmons of nanoshells, nanorods, nanocages, nanostars, and hollow nanospheres can be tuned to the near infrared (NIR) region,189–194 which is advantageous for in vivo application due to superior tissue penetration at that spectral range.195,196 Uniquely shaped nanoparticles have been used as contrast agents in optical bioimaging techniques,197–199 and as photothermal transducers for cancer treatment.191,200–203 To date, due to the increased demand in custom-designed bioapplication, tailoring AuNP’s plasmon for a specific application remains an active area of research.

The numerical modeling of these intricately shaped nanoparticles is also an important
research area that demands further elucidating. Gold nanostars contain multiple sharp branches that act as “lightning rods” to greatly enhance the local EM-field.\(^{90,204-207}\) According to previous calculations in 2-D models, the plasmon resonant wavelength correlates with the branching.\(^{168,174,193}\) The nanostar plasmon results from hybridization of the plasmon from each branch, while the plasmon peak intensity depends on the polarization angle.\(^{174}\) The branch angle and radius are the major factors that determine the plasmon shift in a simplistic two-branch model.\(^{168}\) However, understanding the plasmon behavior of realistic nanostars consisting of \(8 - 10\) branches requires a more elaborate multi-branched model that accounts for the random incident polarization. To date, such extensive 3-D modeling of nanostars has not been reported.

One bioapplication of nanostars is optical imaging. Lately, efficient plasmon-enhanced two-photon photoluminescence (TPL) from non-spherical gold nanoparticles has been used as a contrast mechanism in several reports.\(^{190,208-214}\) On metal nanoparticles, the resonant coupling of the plasmon band with the incident laser greatly amplifies the nanoparticles’ TPL, which originates from the recombination of an electron-hole pairs.\(^{69,215}\) Typically, plasmon resonance increases the two-photon action cross sections (TPACS) of NIR-absorbing nanoparticles greatly above those of organic fluorophores\(^ {213}\) TPL can therefore be applied to multiphoton microscopy, offering a convenient way to visualize NIR-absorbing gold nanoparticles using NIR excitation, which is preferable for in vivo imaging.\(^ {196}\) To date, the TPACS of gold nanostars remains uncharacterized and exploiting this feature for in vivo imaging has not been reported.

This section briefly discusses the 3D simulation of gold nanostars and their comparison to experimental results, and touches on the TPL potential of gold nanostars. More comprehensive detail on the synthesis and experimental setup for TPL can be found in the recent publication by Yuan et al..\(^ {188}\)
3.3.1 Results and Discussion

We report, for the first time, a surfactant-free synthesis of nanostars that achieves high monodispersity and plasmon tunability, which have not been demonstrated before. The synthesis is extremely simple and the reaction is completed in less than 30 seconds at room temperature, resulting in particles of around 60nm diameters with narrow size distribution, Figure 3.13.

Plasmon tunability was achieved by adjusting the Ag$^+$ concentration using a constant seed amount in our study. Previously, plasmon tunability was achieved by adjusting the ratio of auric chloride to seed hence forming nanostars of different sizes. In our study, similar to the growth of nanorods, adding higher concentrations of Ag$^+$ progressively red-shifted the plasmon band by forming longer, sharper, and more numerous branches with small overall size variation, Figure 3.13. This is accompanied by a visible change...
in the solution color from dark blue to dark grey as the plasmon red-shifts and broadens. Both the plasmon peak position and spectral width followed a linear trend with increasing Ag⁺ concentration.

**Figure 3.14:** (a) Extinction spectra of the star solutions (∼ 0.1nM) in DI. (b) The corresponding calculated absorption spectra of nanostars embedded in water. The data points (±1SD) were interpolated with a spline fit. (c) The scatter plots of polarization-averaged absorption against aspect ratio (AR) tuned by varying branch height while keeping the base width, core and tip diameters, and branch number, constant. Their corresponding 3-D geometry is on top, where the blue one is the original model for S30 nanostars. (inset) The linear relationship between the plasmon peak position and AR, which is tuned by varying branch height (red, R² = 0.997) or base width (blue, R² = 0.987) while keeping all other parameters constant.

This section investigates the modeling and comparison of the optical properties of different nanostars (S5-S30). Instead of modeling the plasmon of a single polarization, our analysis features polarization-averaging over space as the nanostars were discretely rotated at six angles, a feature that has not been addressed so far. The 3-D nanostar simulations were performed using the finite element method (FEM), which yields solutions to the local E-field around 3-D metallic nanostructures that are in excellent agreement with the theory. Figure 3.13 depicts that for each of the four nanostars, the local E-field is most greatly enhanced at the tips of those branches that are aligned at least partially parallel to the incident polarization. The enhancement was greatest when the particle’s plasmon matches the incident energy. S20, which plasmon peak matches the 800nm laser line, has
the strongest field enhancement among other nanostars. Also, the E-field along the surface of the branch is enhanced to a value of at least between 1 and 4, suggesting that these surfaces also contribute to the total E-field enhancement around the nanostar.

Table 3.3: Structural features of nanostars produced under different Ag+ concentrations.

<table>
<thead>
<tr>
<th>Ag⁺ (µM)</th>
<th>Hydro. size (nm)</th>
<th>Core Dia. (nm)</th>
<th>Branch Length (nm)</th>
<th>Base (nm)</th>
<th>Tip Dia. (nm)</th>
<th>#</th>
<th>Surface Area (nm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>50 ± 24</td>
<td>28.4 ± 2.6</td>
<td>13.1 ± 5.0</td>
<td>18.5 ± 3.1</td>
<td>11.1 ± 1.5</td>
<td>4.2 ± 1.9</td>
<td>3416</td>
</tr>
<tr>
<td>10</td>
<td>51 ± 21</td>
<td>28.2 ± 3.1</td>
<td>14.7 ± 4.3</td>
<td>16.4 ± 3.3</td>
<td>9.4 ± 1.4</td>
<td>6.4 ± 1.4</td>
<td>4665</td>
</tr>
<tr>
<td>20</td>
<td>59 ± 21</td>
<td>23.7 ± 2.4</td>
<td>19.0 ± 5.1</td>
<td>13.5 ± 2.4</td>
<td>7.8 ± 1.3</td>
<td>7.8 ± 1.1</td>
<td>5202</td>
</tr>
<tr>
<td>30</td>
<td>67 ± 24</td>
<td>21.7 ± 3.6</td>
<td>20.9 ± 5.1</td>
<td>10.1 ± 2.0</td>
<td>6.5 ± 1.4</td>
<td>9.9 ± 2.4</td>
<td>5924</td>
</tr>
</tbody>
</table>

The experimental absorption peak shifts can be properly modeled using a 3-D nanostar geometry designed according to the parameters in supporting information Table 3.3, namely, core diameter, branch base width, branch length and tip radius. Figure 3.14(b) conveys that the modeled absorption peaks of the various nanostars align well with the experimentally-measured spectra, and reproduce experimentally observed peaks for each nanostar solution sample. Similar to previous findings that the plasmon of a nanostar results from hybridization of plasmons of the core and the branches, the plasmon spectrum here shows a weak absorption around 520nm attributed to the plasmon resonance of the nanostar’s core, and a dominant plasmon band at longer wavelengths due to the resonance supported by the nanostar branches. As the number of branches increases from 4 to 10 for S5 to S30, respectively, the plasmon red-shifts, peak absorption cross-section increases and the SD of the averaged spectrum data points decrease.

This study showed that the plasmon shift is controlled mainly by the branch aspect ratio (AR): branch length divided by base width. Figure 3.14(c) shows the progression of absorption peak position and intensity along with AR. A linear relationship was demonstrated between the absorption peak position and AR, either by adjusting branch length while keeping branch width constant, or vice versa. Tip angle, however, does not correlate as linearly as AR to the peak position. Branches with varying tip radius or angle
but the same aspect ratio result in the same peak position. Consistent with Hao et al.’s finding, the polarization only affects the peak intensity but not the peak position.\(^{174}\) Also, we found that the peak intensity increases with increasing branch number, branch length, and the core size. It is noteworthy that the core size only contributes to the 520nm peak, whereas the branch geometry determines the plasmon position and intensity in the NIR. Meanwhile, the geometrical parameters that do not significantly affect the plasmon shift include nanostar core size, branch length (assuming constant AR) and branch number beyond at least two oppositely positioned branches. All the results that are reported here but not presented can be found in the manuscript’s supplementary information section.\(^{188}\)

It is clear, however, that the calculated plasmon resonance widths are significantly narrower than the experimental data. This experimental broadening is likely due to the nonuniform nanostar geometries (e.g. wide distribution of AR), as well as the finite-size effects, which broaden and dampen the resonance but do not influence the plasmon band position\(^{137,217}\). This will be investigated in the next section.

Finally, these gold nanostars were injected into a biological medium to demonstrate their potential for TPL imaging. For cells studies, the gold nanostars were functionalized with wheat-germ agglutinin (WGA), which has a high affinity to glycoproteins and glycolipids on the cell membrane. Paraformaldehyde-fixed BT549 cancer cells were employed. For in-vivo studies, the nanostars were PEGylated and concentrated to 50nM in PBS after two centrifugal washes, and TPL imaging was performed through a dorsal window chamber mounted on female CD\(^ {31} \) nu/nu mice.

The overlap of the gold nanostar plasmon band with NIR laser excitation allows these nanoparticles to exhibit greatly enhanced TPL. A quadratic dependence of TPL intensity on excitation power (below 10mW) suggests the existence of an underlying non-linear two-photon upconverting process on nanostars, which was not observed for 60nm gold or silver nanospheres.

Polymer-free nanostars with such a high TPACS can be used as a strong contrast agent.
in TPL imaging in biological samples. Figure 3.15(a) shows a preferential binding of WGA-coated nanostars on the cell membrane. In contrast, PEG-coated nanostars bound poorly, Figure 3.15(b). Numerous white spots representing the nanostars were observed to preferentially cover the cell membrane rather than the cytoplasm. The observed white color was a composite signal originating from the three different detection channels of the two-photon microscope, indicating a broad TPL emission spectrum from nanostars. The nanostars emitted strongly without photobleaching under low laser power (4mW), which is in the typical working range for organic fluorophores. In contrast, the signal from
WGA-coated nanospheres could only be observed under a much higher excitation power.

Imaging the presence and movement of nanoparticles in vivo is an attractive feature that is desired in the research community. The optical tracking of gold nanoparticles in vivo has always been challenging to achieve. Illumination of fluorophores that are bound to the nanoparticle surface via fluorescence microscopy is limited due to surface-quenching, and thus requires the intricate manipulating of the distance between the fluorophore and the metal surface. Dark field microscopy and differential interference contrast microscopy can both visualize AuNPs in cells but cannot penetrate deep enough into tissue. The strong TPACS of nanostars enable their imaging and tracking without the need of fluorophores. This was demonstrated by undertaking TPL imaging of PEGylated nanostars through a dorsal window chamber on nude mice. Within 5 minutes after injection, PEGylated nanostars traveled along the blood vessel where the tissue vasculature became clearly visible at rather low excitation power, at which the background tissue autofluorescence was minimal, Figure 3.15(c). PEGylation was employed as a strongly hydrophilic surfactant on the nanostars to prevent their aggregation in physiological condition and to extend their serum half-life from immunoclearance. Impressively, motion tracking of PEGylated nanostars in the blood vessel could also be obtained. Without nanostars or dyes, a much higher excitation power was required to detect the autofluorescence from blood vessel and tissue, Figure 3.15(d).

It is noteworthy that no apparent toxicity from PEGylated nanostars was observed throughout the study period. The ability of using optical microscopy to visualize metal nanoparticles greatly simplifies tracking and possibly quantifying gold nanostars for both in vitro and in vivo applications. Gold nanostars offer a promise for use as contrast agents with TPL microscopy.
3.4 Investigating the plasmonic broadening of Gold Nanostars

Although the plasmon band peak wavelength and relative intensities of the synthesized nanostar samples S5-S30 were accurately reproduced numerically, these numerical plasmon bands were much narrower than their experimental counterparts; in other words, the numerical model was not complex enough to reproduce the plasmon broadening that was experimentally observed.

Intuitively, the origin of plasmon broadening can be narrowed down by considering several potential variables. The orientation of nanostar branches relative to the incident excitation wavelength was found to only contribute to variations in plasmon intensity, and not plasmon shift. Thus, branch orientation is unlikely to be the main cause. It is well known to experts in the field that broad plasmons are normally attributed to either an inhomogeneous nanoparticle distribution or nanoparticle aggregation, or a combination of both; the synthesized gold nanostars, however, were relatively homogeneous on a per-particle basis and thus these two factors are unlikely candidates. Small size effects are known to broaden and dampen plasmon bands when the nanoparticle size is very small, however, the extent of the experimentally observed broadening is unlikely to be caused mainly by small size effects that are unaccounted for numerically. This conclusion is further supported by the comparison between nanorods and nanostars: compared to high aspect-ratio nanorods that exhibit a relatively narrow plasmon band in the NIR (attributed to an E-field excitation along their longitudinal axes) the plasmon band of NIR-absorbing nanostars is very broad. This suggests that narrow plasmon band of rods is a result of their well-controlled geometrical shape; more specifically, it can be inferred that their narrow plasmon band is guaranteed by a narrow distribution of rod aspect ratios (ARs) in a given sample. To extend this notion to nanostars, it is hypothesized that the plasmon broadening originates from the inhomogeneity of the branches that fuse to make up each nanostar. More specifically, the root cause of such extensive broadening is the wide distribution of
branch ARs that is inherent to the nanostar formation.

This study investigates the origin of the plasmonic broadening of gold nanostars by increasing the degree of complexity of the 3-D nanostar geometry. In particular, the nanostar branches will be individually tweaked to yield a single nanostar with 10 branches of unique geometries. The analysis will go a step further by introducing hyperbranched-nanostars, which are experimentally observed in sample S30. Finally, the plasmon bands of gold nanorods will be investigated as a control, to confirm that such branching is not the sole result of small size effects.

3.4.1 Experimental and Numerical Section

The synthesis of nanorods was conducted by following Nikoobakht and El-Sayed’s method, but the reaction was quenched by the addition of Na$_2$S. The nanostars were synthesized by our, previously published method.

The theoretical simulation was performed using the RF module of COMSOL Multiphysics v3.4, and the gold nanorod and nanostar was analyzed in 3-D by modeling a typical nanorod and S30 nanostar whose average dimensions are reported in Table 3.4 and in Table 3.3, respectively.

3.4.2 Results and Discussion

An experimental and numerical side-by-side comparison is presented in Figure 3.16. Parts (a) and (d) present typical TEMs of the synthesized nanorods and nanostars, respectively.

Table 3.4: Geometrical dimensions used to simulate the Nanorods, averaged by measuring 100 nanoparticles under TEM

<table>
<thead>
<tr>
<th>Nanorod Sample</th>
<th>Length (nm)</th>
<th>Width (nm)</th>
<th>AR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>57</td>
<td>19</td>
<td>3.0</td>
</tr>
<tr>
<td>2</td>
<td>55</td>
<td>16.5</td>
<td>3.3</td>
</tr>
</tbody>
</table>


Two samples of nanorods were analyzed to demonstrate the effect of AR on their plasmon band position. As depicted by the TEMs and by their respective measurements, these nanoparticles have a uniform, homogeneous distribution. Figures 3.16(b) and (e) display the experimentally measured absorption spectra of the two nanorod samples and the S30 nanostar sample, respectively. As confirmed by the spectral red-shift of Nanorods #2 relative to Nanorods #1, a higher aspect ratio is accompanied by a red-shifting of the plasmon band, which is expected. More importantly, however, it is clear that the nanostar’s plasmon band FWHM is significantly larger than that of the nanorods, the understanding of which is at the heart of this study.

Finally, Figures 3.16(c) and (f) present the numerically simulated absorption spectra of the nanorods and nanostars, respectively. For anisotropic nanoparticles such as nanorods, nanocubes and nanostars, the orientation of the nanoparticle relative to the incident field polarization strongly influences the particle’s plasmon resonance: a nanorod possesses a transverse and longitudinal plasmon band, attributed to plasmon excitations along the nanorod’s width and length, respectively. These are evident in the experimental absorption spectra of 3.16(c). Here, the numerical model accounted for the orientation dependence of the incident E-field by averaging the absorption spectra of nanorods and nanostars that were incrementally rotated by 30 degrees in the \( x = y \) plane, adding a stochastic behavior to the orientation of the branches relative to the z-polarized incident field. This is more clearly conveyed by the 3D schematic inset in (c) and (f), that describe the rotation of the nanoparticles in space. Interestingly, the rotated absorption spectra of both nanorod and nanostar samples conveyed no relative peak shift, and only slight variation in amplitude that is conveyed by small error bars denoted on the spectra. Their peak positions are well aligned with the experimental ones, and reproduce the two experimentally observed peaks of both the nanorod and nanostar: a shallow, short wavelength peak and a dominant plasmon band at longer wavelengths.

There are a number of observation to be made by comparing these numerically simu-
FIGURE 3.16: Comparison of gold Nanorods and Nanostars; (a,d) TEM of gold Nanorods and Nanostars, whose dimensions are reported in Table 3.4 and Yuan et al.'s manuscript, respectively; (c,e) Experimental absorption spectra of gold nanorods and nanostars, respectively, dispersed in aqueous solution; (d,f) polarization-averaged absorption spectra of gold nanorods and nanostars, respectively, obtained via numerical simulation.

lated spectra with their respective experimental ones. First of all, their plasmon peaks are aligned in all three instances, which confirms that this numerical method is fairly accurate.
in being able to reproduce such an important trend. Secondly, the relative intensities of the Nanorod spectra are also mimicked quite effectively. There is a clear difference between the widths of the plasmon bands of It is clear, however, that the experimental absorption spectra of nanostars are significantly broader than the simulated ones, which is due to the aforementioned parameters. These critical variables - number of branches, branch randomness, the branch AR and hyperbranching - were analyzed and are conveyed in Figure 3.17, while the latter - branch length, branch tip radius of curvature, surface protrusions - are not reported here.

**Figure 3.17**: Absorption spectra of gold Nanostars obtained via numerical simulation, depicting the spectral broadening associated with geometrical variations of the nanostar shape: (a) number of branches; (b) Aspect Ratio (AR) of branches controlled by varying either branch length or branch width, and assembled into a star in two different configurations; (c) 10 branches of random and unique dimensions, assembled into a star in two different configurations; (d) partial and full-hyperbranched Nanostars.
From a simulation perspective, the accurate modeling of a multi-branched, 3-D nanosstructure is a challenge in itself, but tweaking each individual branch to investigate different geometrical parameters in order to understand their effect on plasmon broadening is intricate and time consuming.

We have previously shown that three geometrical variables do not significantly affect the nanostar’s plasmon shift: nanostar core size, branch length (assuming constant AR) and branch number beyond at least two oppositely positioned branches.

Figure 3.17(a) conveys the polarization-averaged absorption spectra as a function of number of branches, for a 1-, 4- and 10-branch S30 nanostar, with the standard deviation bars representing the effect of polarization on the resulting spectra: significant variation for a 1-branch star, but only minute differences for a 10-branch star. It is noteworthy that the absorption band experienced no shift with rotation. An increase in the number of branches is accompanied by an intensifying of the absorption bands as well as a significant red-shift of $\sim 100$ nm; the former observation is consistent with Kumar et al.’s report, however, the authors predicted no relation between shift and branch number. More generally, for a nanostar consisting of several identical branches, the position of the resulting plasmon band is unaffected by their individual orientation in space or by the incident polarization.

Figure 3.17(b) depicts a study of the branch AR, adjusted from $\sim 2.5$ to $\sim 5.7$ by varying, in turn, the branch base width or branch length, keeping all other parameters constant. The absorption spectrum consists of the linear superposition of ten individual peaks, whose relative intensities varied as a function of polarization to yield the broadened polarization-average absorption spectra. Branches with AR $< 3.7$ (original S30) generated absorption peaks that were blue-shifted relative to the original S30 absorption peak, whereas those with AR $> 3.7$ were red-shifted. It should be noted that plasmon broadening is accompanied by a decreased overall peak intensity as the plasmon band attributed to each individual branch occupies a different peak resonance along the wavelength axis. Interestingly, interchanging the relative position of the branches of a given nanostar geom-
etry alters the shape of the polarization-averaged absorption spectrum. This observation is more likely a result of the limited discretized polarization angles (only six) at which the spectra are evaluated, rather than the plasmon coupling between adjacent branches: as the number of polarization angles tends to infinity (effectively representative of a solution of randomly oriented nanostars) the polarization-averaged spectrum would conceivably tend to an equilibrium shape, regardless of the relative positions of its individual branches. This study was extended by reducing the tip radius of curvature while keeping the individual branch AR constant, for which no significant difference could be inferred. It is also noteworthy to contrast this study with one that solely focuses on tip angle, which is achieved by increasing the branch tip diameter, and fixing all other parameters, especially the branch AR. In this case, no plasmon shift was observed and only slight amplitude variation. Together, these two latter studies confirm that a distribution of aspect ratios, rather than merely branch tip angles, is the root cause for plasmon broadening in a nanostar.

Figure 3.17(c) illustrates the plasmon broadening from a nanostar with a more random set of 10 branches, whereby tip radius of curvature, branch length and width were all varied, making this the most general nanostar in this study.

Figure 3.17(d) demonstrates the effect of hyper-branching (branching off of existing branches), a geometrical feature that is apparent in the TEM of S30 in Figure 3.16. The hyperbranches were modeled by affixing two side-branches to the main branch (each comprised of scaled, rotated versions of the main branch). The fully hyperbranched star generated two absorption peaks on either side of the original S30 peak, suggesting that the side-branches effectively 'steal away' the plasmon resonance from the main branch; the 'bare' branches of the partially hyperbranched star contributed to blue-shifting the longest peak toward the S30 original spectrum. It is noteworthy, however, that the shift is not due to variations in the individual branch AR (kept constant) and implies more complex geometrical dependence beyond the scope of this paper. Variations of both branch length and core diameter showed no appreciable shift, except for a slight red-shifting with increasing...
length and core diameter, most likely due to the increasing overall size of the nanostars, rather than isolated effects of those geometrical tweaks. Nanostar core surface protrusions also generated additional side peaks, but were deemed relatively insignificant.

3.4.3 Conclusion

This study has provided two important results that contribute to the advancement of nanoparticle characterization, as well as to the more complete understanding of the plasmonic behavior of complex 3D nanostructures. Firstly, the simulation complexity was extended beyond any such result that has thus far been reported in the literature, to the best of the author’s knowledge. The most complex 3-D structure investigated in this study comprised of a 30-branch nanostar (hyperbranched), that was accurately meshed and solved to yield its absorption spectrum. Beyond the challenge in designing such a structure, the ability of the FEM algorithm to produce a solution is a feat in itself. Secondly, the broad absorption spectrum of gold nanostars was numerically analyzed to be the result of a combination of effects of key geometrical parameters: branch AR, that is determined either by varying the branch width for a given length, or by varying the branch length for a given width, and hyperbranching, a term that coins the appearance of small protrusions from the main branches. Large variations in these parameters were shown to induce extensive broadening of the nanostar plasmon, which correlate more strongly to the observed experimental spectra. It can be inferred that although nanostars are homogeneous on a particle-basis, each nanostar comprises of randomly formed branches of differing parameters, and whose combination produces large plasmon broadening both for single nanostars and nanostar ensembles. This suggests that each nanostar can be excited by a range of wavelengths, which makes them particularly attractive for SERS, PDT and PTT.
3.5 ‘Nanowave’ SERS Substrates: experimental fabrication and numerical analysis

The use of solution-based gold and silver nanoparticles for SERS has many advantages over dry substrates. Nanoparticles are inexpensive and easy to synthesize, simple to functionalized, and are versatile such that they can be mixed with cells or injected into tissue for ex-vivo and in-vivo SERS detection, respectively. Unfortunately, unless these nanoparticles are have been stabilized in solution via surface functionalization with the appropriate surfactant, they are prone to uncontrolled aggregation. Nanoparticles can become unstable as a result of varying solution ionic strengths, surfactant displacement, surface-charge neutralization, temperature fluctuations, pH-variations, etc. Due to the strong dependence of SERS on inter-particle plasmon coupling, uncontrolled aggregation leads, in turn, to unquantifiable SERS signals. To address this issue, there has been substantial research on the development of dry, metallic nanostructured substrates that can be used for SERS. In fact, SERS was first demonstrated on a dry substrate. Because dry substrates typically consist of closely packed nanostructures of silver or gold, they exploit the aforementioned inter-particle plasmon coupling to enhance the SERS signal by attempting to create controllable hot-spots. The use of dry SERS substrates thus allows the detected SERS signal to be quantified in a reproducible manner, which is key to advancing the widespread use of SERS as a detection mechanism throughout the field of cancer nanotechnology, and specifically for diagnostic devices, which is the focus of this thesis. This section investigates the formation of SERS hotspots on the Nanowave substrate, a term that was recently coined by the author, but that was first introduced by Vo-Dinh et al. in 1984. This study was just accepted to the Journal of Physical Chemistry 2012.

Following the discovery of the surface-enhanced Raman scattering (SERS) effect, our laboratory has first demonstrated the general applicability of the SERS effect for trace analysis using solid substrates having silver-coated nanospheres. In 1984 Vo-Dinh
et al. exploited the spin-coating and electron beam evaporation techniques to fabricate close-packed arrays of nanospheres, onto which a thin silver shell was deposited, effectively forming a controlled, reproducible substrate of ‘hotspots’,

here referred to as the Nanowave due to its analogy to a periodic waveform. Although this type of substrate produced large SERS effects and has been used in a wide variety of chemical and biological sensing applications,

the origin of this plasmonic behavior as a result of this topography has only been induced.

During the last two decades, there has been an explosive interest in both practical applications of SERS as sensing techniques, as well as fundamental studies of the plasmonic theory of the electromagnetic (EM) enhancement of SERS. In 1996, Garcia-Vidal et al.\textsuperscript{221} developed an implementation of Maxwell’s equation for modeling a rough silver surface as an array of half-cylinders of silver enclosed in a silver substrate. The authors demonstrated that the strongest E-field enhancement, up to $10^7$, occurred in the crevices or pores of the rough surface; the structure they analyzed was infinite in extent along the cylinder’s length, lacking confinement along that direction, thus effectively being analogous to the evaluation of a 2-D geometry. Moreover, the crevices were not ‘smoothened’ by rounding, which, although generating the extremely large enhancement factors reported, are unrealistic from an experimental perspective. Finally, the analyzed structure comprised only a single material, silver, such that the underlying structure of the substrate was intrinsically different to the Nanowave substrate synthesized by the Vo-Dinh group, and thus no analysis of the effect of varying metal thickness on the substrates’ plasmon band could be undertaken. Three-dimensional studies of nanoparticle structures have also been investigated in 2008 and 2009,\textsuperscript{126–128} but focus on nanocylinders or chains of nanoshells, which have significantly simpler geometries than that of the Nanowave substrate, which translates to a much reduced computational complexity.

In this study, the fabrication of the Nanowave substrate using 100nm silica spheres was undertaken, and then FEM was employed to perform numerical simulations of the spatial
and spectra behavior of such a model as a function of metal thickness. Both gold and silver metals are used as model metals in the simulation and analysis. The model is constructed with periodic boundary conditions to take into account the infinite nature of the substrate relative to a single particle, and 3-D smoothing of the sharp crevices is enforced to ensure both reliable results and a more realistic model.

3.5.1 Experimental Procedure

The substrate fabrication involved two separate steps. The first step involved washing glass cover slips by incubating them in nitric acid, followed by a careful rinsing process with ultrapure DI water. This ensured removal of organic residue on the glass surface and populated the surface with hydroxyl groups. The cover slips were dried in a stream of nitrogen and placed on a spin coater. Silica nanospheres (100nm diameter, Polysciences), dispersed in a ethanol:ethylene glycol solvent (85% : 15% v:v), were dropcast on the cover slip and spun at 6000 rpm for 5 seconds. The nanospheres adhered to the coverslip, producing a close-packed array of silica nanospheres. The second step entailed coating the sphere-covered slides with a metal (gold or silver), achieved by transfer to an E-beam evaporator. To ensure uniform metal coating the substrates were rotated above the silver source during deposition, which was conducted at a vacuum pressure below $5 \times 10^{-6}$ Torr. The desired thickness of metal was evaporated onto the array of nanospheres, producing an array of silica@metal half-nanoshells, referred to as the Nanowave. Characterization was done under the scanning electron microscope (SEM) at inclination angles ranging between 0 and 45 degrees.

3.5.2 Theoretical Simulation

The theoretical simulation was performed using the RF module of COMSOL Multiphysics v3.4, and the substrate was analyzed in 3-D by modeling a generic subset of particles as observed under the SEM and delimited by a contour in Figure 3.18; the detailed schematic
A unit cell model of the Nanowave substrate was built in four distinct steps: first, a model for the single metal-capped silica particle was designed. Seven of these were then positioned such as to form a hexagonally close-packed array. The sharp crevices formed by this assembly were then smoothed, in three dimensions, by the manual fusion of unique structures whose geometry and dimensions are detailed in Figure 3.19 and Table 3.5, respectively. Finally, the hexagonal structure was sliced through the 6-outer particles to form a cuboidal computational domain, such that the unit cell possessed symmetry in the x-y plane, Figure 3.19. Periodicity in the x-y plane was ensured by setting the boundaries along the x- and y-directions to Perfect Electric Conductors (PECs) and Perfect Magnetic Conductors (PMCs), respectively, and the PML boundaries along the z-direction prevented unwanted reflections onto the substrate.

The meshing of structures in FEM is particularly important to guarantee solution convergence as well as solution accuracy. The maximum mesh size in the metal shell was set to 5nm to ensure fine special meshing, to typically yield 1 to 2 million DoF, which corresponds to $7 - 20$ GB of RAM when solving using the direct PARDISO solver. Fine spectral sampling was ensured by solving the fields at wavelength intervals $\Delta \lambda = 10$nm to capture the rapid spectral changes or fluctuations that typically occur around peaks.

The unit cell substrate was placed in a surrounding medium of air. As in Chapter 2, the silica is described by a wavelength-independent refractive index of 1.45 and the metal coating was modeled by the Lorentz-Drude dispersion models for gold and silver.

The substrate was excited by an incident plane wave at 633nm, propagating perpendicular to the substrate along the negative z-direction (from above), and containing only x-polarized components; as such, the E-field has the form $\mathbf{E}(\mathbf{r}, t) = E_x e^{j(\omega t + k_z z)}$ and is schematically shown in Figure 3.19. The model was solved for the scattered fields for wavelengths ranging the $300 - 1200$nm region. For each of the five metallic shell thicknesses (10, 20, 30, 40 and 50nm) investigated in this study, a new unique model had to be
constructed, meshed and simulated.

This model does not take into account geometrical imperfections and further losses that dampen and broaden the surface plasmon of the close-packed NP network; these mainly include small size-effects, which are significant when the metal thickness is smaller or on the order of the electron mean free path.

3.5.3 Results and Discussion

The spin coating method is a rapid, inexpensive substrate fabrication technique that yields a reproducible, homogeneous thickness of polymer films or distribution of nanoparticles on substrate. The monolayer, close-packing of 100 nm silica nanospheres was achieved by optimizing the solvent composition and concentration, particle concentration, spin speed and solution volume drop cast on the glass slide. The spin duration did not affect the final distribution of nanospheres on the substrate; beyond the spin time at which the solvent evaporated, the particles remained immobile and adhered to the glass surface even with continued spinning.

Following evaporation of 50 nm of gold or silver onto the silica nanospheres, each individual NP could still be perceived, suggesting deposition of a uniform coating of metal on top of the underlying structure, Figure 3.18. Although a few cracks and irregularities are observed in the NP close-packing pattern, the overall distribution is ordered, predictable and therefore reproducible, which is of utmost importance when attempting to quantify SERS measurements.

The inset of Figure 3.18 is an enlarged view of a portion of the substrate, and depicts the repetitive unit of interest, consisting of seven particles arranged as a “ring”, and denoted by a white rectangle. This characteristic pattern is repeated throughout the entire substrate and is a practical model to investigate the plasmonic behavior of the Nanowave substrate when excited by a plane wave incident beam.

A typical SERS measurement on such a substrate is achieved, in our laboratory, by
delivering a $5\mu L$ sample of the analyte of interest onto the substrate surface allowing it to dry in air, and then placing it on an inverted Raman microscope, equipped with a linear polarizer (field polarized in the plane of the substrate), and excited by a 633nm He-Ne laser source oriented perpendicular to the substrate. To investigate the substrate’s plasmonic behavior, these experimental conditions were mimicked by designing a 3-D numerical structure that was modeled on the rectangular unit cell in Figure 3.18, a schematic of which is presented in Figure 3.19.

The geometry and orientation of this unit cell was purposefully chosen to ensure two lines of symmetry, one along the x-axis and the other along the y-axis. This enabled x-y plane periodicity to be enforced with PEC and PMC boundaries normal to those axes, rather than Comsol’s in-built Periodic Boundary Condition, which we found was unable to solve this particular geometry given the normal propagation vector of the incident excitation. With regards to unit cell size, it is noteworthy that the unit cell effectively comprises four nanoparticles (1 whole and 6 halves), and that the same result could be achieved at a lower computational cost by a four-time reduction of the unit cell in the x-y plane to...
one quarter of its current size, such that it effectively comprises only a single nanoparticle. However, for visualization purposes, due to the availability of RAM and to test the computational limits of the software, this cell size was employed as the generic Nanowave solution in Figure 3.21, but the subsequent analyses were performed with a two-particle unit cell (half the size along the x-axis) for solution conciseness while maintaining solution clarity.

As indicated, the incident plane wave $E(r, t) = E_x e^{j(\omega t + k_z z)}$ was defined to have amplitude of 1, a wavelength $\lambda = 633$ nm, propagate in the negative z-direction and contain only x-polarized E-field components. The latter condition was imposed to probe the surface plasmon formed in the two inter-particle crevices that are parallel to the x-axis. The values corresponding to the parameters in Figure 3.19 and presented in Table 3.5, for each shell thickness $\Delta z$. The smoothing thickness, $\delta$, was limited to a minimum of 2 nm for the 10 nm metal coating, since this value was assumed to be the smallest experimentally-achievable rounding in the crevices which, given the deposition rate of 0.5 nm/sec, is reasonable. Similarly to the experimental metal-deposition process, it is important for modeling purposes to ensure that, as the metal deposition thickness increases, the metal layer is indeed uniformly “deposited” over the silica nanoparticles. This means that although the thickest part of the shell will be directly above the center of the silica sphere (when looking top-down on the substrate), the inter-particle crevices should also have a significant layer of metal above it, albeit slightly thinner due to the larger effective area over which the metal is deposited. This concept is illustrated in Figure 3.20 with snippets of the actual geometrical structures solved in FEM: as the metal thickness increases, the triangular opening between any three adjacent nanoparticles gradually closes (top-view), and the height of the metal shell rises (side-view), both trends that are experimentally consistent and thus expected. It is critical to appreciate that this geometrical trend is only possible if the rounding thickness $\delta$ is also increased as the metal-shell is increased; if $\delta$ is maintained equal to 2 nm for all metal thicknesses, the metal-layer over the crevice would not thicken
FIGURE 3.19: Schematic of the geometry used for theoretical modeling of the Nanowave substrate. The unit cell, delineated in the SEM from Figure 3.18, is reproduced as a 3-D FEM model comprising 100nm silica nanospheres coated with metal of varying thicknesses. The top view conveys the rectangular unit cell, whose dimensions were selected such as to enclose one complete particle; the infinite extent of the unit cell in the x-y plane is modeled by employing periodic boundary conditions, consisting of PEC’s in the x-direction, and PMC’s in the y-direction. The incident, x-polarized E-field propagates perpendicular to the Nanowave array in the $-k_z$ direction. The x-z slice through $y = 0$ conveys that the structure is simulated in air and that the computational boundaries in the z-direction consist of PML’s that prevent any back reflections of the scattered field onto the structure. The smoothing in 3-D schematic illustrates the method employed to smooth the interstitial space between adjacent nanoparticles. Two orthogonal planes, $x-z$ and $y-z$, describe the flat smoothing of thickness that encircles the entire 3-D junction; this was achieved by forcing a combination of geometrical variables, heights $h$ and radii $r$, depicted here and detailed in Table 3.5.

much, and the triangular opening would remain unchanged for all metal thicknesses. Although this latter geometry would constitute a fairer theoretical comparison of the effect of metal thickness on the plasmonic behavior of Nanowave substrates, it would be experimentally inconsistent and was purposely avoided. Instead, the rounding thickness $\delta$ was increased by 0.5nm for every additional 10nm metal thickness, reaching a smoothing of
4nm for the 50nm shell. The five other parameters (rounding heights and widths) were adjusted such that the same $\delta$ was maintained three-dimensionally around the entire rounding junction.

Table 3.5: Geometrical properties of the Gold and Silver Nanowaves and Nanowires, corresponding to the parameters in Figure 3.19. The Nanowires are designed with the same parameters as the Nanowaves, except that they extend to infinity in the y-direction. The last column represents the position coordinates at which $|E|$ is measured in to plot the spectra in Figure 3.23. The measurement points are located in the inter-particle crevices and can be co-registered in 3-D with Figure 3.22.

| Shell $\Delta z$ (nm) | $r_{metal}$ (nm) | $r_{SiO_2}$ (nm) | $h_{metal}$ (nm) | $h_{SiO_2}$ (nm) | $w$ (nm) | $\delta$ (nm) | Top SA (nm$^2$) | $|E|$ meas. 1 (nm) |
|----------------------|------------------|------------------|------------------|------------------|---------|--------------|----------------|-------------------|
| 10                   | 9.0              | 9.0              | 0                | 10               | 18.0    | 2.0          | 3.02 x 10^{-14} | 2.44 x 10^{-14}   |
| 20                   | 11.0             | 11.0             | 6                | 14               | 22.0    | 2.5          | 3.20 x 10^{-14} | 2.38 x 10^{-14}   |
| 30                   | 12.0             | 12.0             | 16               | 14               | 24.0    | 3.0          | 3.53 x 10^{-14} | 2.35 x 10^{-14}   |
| 40                   | 13.0             | 13.0             | 26               | 14               | 26.0    | 3.5          | 3.83 x 10^{-14} | 2.33 x 10^{-14}   |
| 50                   | 13.5             | 13.5             | 36               | 14               | 27.0    | 4.0          | 4.13 x 10^{-14} | 2.31 x 10^{-14}   |

A typical simulation result is presented in Figure 3.21 and Figure 3.22, where isosurfaces of $|E_x|$ are plotted to illustrate the behavior of the electric field in the vicinity of a 50nm gold Nanowave substrate. The motivation behind fabricating such substrates over 25 years ago was based on the idea that the E-field would be particularly strong in the interstitial space between the metal covered nanospheres. Indeed, Figure 3.21(b) emphasizes
that the strongest E-field enhancement occurs at each x-oriented interstitial space between adjacent nanoparticles; more specifically, these fields are most intense at the gold surface on either side of the inter-particle junction, clearly seen in Figure 3.22(a,b), which is intuitively expected since the local geometry is similar to that of a dimer nanoshell, which also generates the strongest field at these locations. Figure 3.21 and Figure 3.22 also confirm that the periodicity in the $x - y$ plane is successfully enforced by the PEC-PMC boundaries, since the E-field in the six interstitial spaces that are cut by these boundaries (four junctions in the PMC/PEC corners and two junctions on the PMC boundaries) are identical to that in the two middle junctions, which suggests that any long-range plasmonic behavior that may be induced over the infinite extent of the substrate would be accounted for.

Experimentally, chemical detection on such substrates requires the molecules of interest to physisorb or chemisorb onto the metal surface, which is where these regions of intense plasmonic activity reside. This effectively demonstrates that the Nanowave substrate exhibits a “closely-packed array of hot spots”, thus making it an effective SERS substrate for sensitive, reproducible measurements. In this study, the EM enhancement is defined as $EME_x = |E_x|/|E_0| = |E_x|$ since the incident amplitude $|E_0| = 1$; for the 50nm Nanowave, a maximum enhancement of 15 was achieved at $\lambda = 580$nm. Since the SERS signal enhancement factor $SERS_{EF}$ is approximately proportional to the fourth power of the EM enhancement, $SERS_{EF} = |E_x|^4$, the 50nm Nanowave produces $SERS_{EF} = 5 \times 10^4$. Evidently, maximizing the $SERS_{EF}$ provided by a SERS-active substrate is important for obtaining the lowest detection limit, which is the key towards achieving ultra-sensitive detection.

Rather than map the three-dimensional E-field behavior at a single wavelength, which is useful for understanding the spatial distribution of the field, spectral information can be gleaned by calculating the absorption spectra of the Nanowaves as the metal thickness is varied, Figure 3.23.
Figure 3.21: Simulation data of unit cell of Nanowave structure consisting of 100 nm silica nanospheres coated with 50 nm gold; (a) geometric setup for FEM solving, detailing the 50 nm gold coating (red) and the 100 nm silica nanoparticles (clear). The PEC, PMC and PML boundaries are all denoted. A projection of the 3-D smoothing between adjacent particles can clearly be perceived on the PMC face in the x-z plane; (b) 3-D isosurfaces of the E-field, \( |E_x| \), in the vicinity of the Nanowave structure. The incident plane wave, \( E(\mathbf{r}, t) = E_x e^{i(\omega t + k_z z)} \), is polarized along the x-axis, propagates in the -z direction, has amplitude of 1 and a wavelength \( \lambda = 580 \text{nm} \). The strongest E-field is confined to the interstitial spaces aligned with the x-polarization direction, making the Nanowave an array of controllable hot-spots. The periodicity of the unit cell is evident by the presence of identical E-field patterns at the x-oriented interstitial spaces that are cut by PEC or PMC boundaries.

It is noteworthy that the thin-shelled Nanowaves exhibit two sharp absorption bands, a short-wavelength band and a longer-wavelength band, whereas the thicker-shelled Nanowaves have a single, short-wavelength absorption band and a hint of a longer-wavelength shoulder. As shell thickness increases, both absorption bands undergo a blue-shifting. Specifically, the short-wavelength bands blue-shift toward the typical absorption band positions of large 100 nm spherical nanoparticles: \( \sim 550 \text{nm} \) for gold and \( \sim 450 \text{nm} \) for silver. The
Figure 3.22: Further analysis of the 3D solution of $|E_x|$ in Figure 3.21(b): (a) Side view projection of the unit cell, depicting that $|E_x|$ is strongest in the junctions formed between adjacent nanoparticles, $|E_x|_{max} \sim 12V/m$, and that for this metal thickness, the field remains confined within the metal shell, not penetrating much into the silica nanoparticles; (b) Top view projection of the unit cell, with white dotted circles delineating the underlying metal-coated silica nanoparticles. For both (a) and (b), identical $|E_x|$ isosurface patterns are generated at the x-oriented interstitial spaces, confirming successful boundary periodicity in both the x- and y-direction. The dimensions of the structures are denoted by labeled axes in both figures.

longer wavelength bands also blue-shift, but disappear with increasing shell thickness at $\sim 650$nm for gold and $\sim 600$nm for silver. The nature of these peaks is addressed later on in the manuscript. It is noteworthy that the absorption peaks are of the same order of magnitude ($1 \times 10^{-14}$) across all thicknesses for both metals.

To understand the SERS potential of Nanowave substrates as a function of metal thickness, the E-field $|E|$ in the inter-particle space was probed at the positions reported in the last column of Figure 3.5. Again, the dual-peak nature of the thin-shelled Nanowave spectra is apparent, and the plasmon band blue-shift with increasing shell thickness, for both metals, points toward the possibility of plasmon tuning for optimizing the SERS detection
Figure 3.23: Spatial analysis of $|E|$ of the short-wavelength plasmon peak of gold Nanowaves of varying shell thicknesses. The diagrams are kept to scale relative to each other, clearly illustrating the thickening of the gold shell from 10nm to 50nm. The encircled letters A and B denote locations where E-field enhancement occurs and will be used to simplify the analysis.

Figure 3.24: Absorption spectra of the Nanowave structures for (a) gold and (b) silver metal shells. The discrete data points represent the calculated values from the simulation, and computed at a small enough $\lambda$-interval to capture the rapid variations of the plasmon bands. They are then interpolated using a spline function to smooth out the curves. The gold spectra convey two main absorption bands for the thinner shells 10–30nm, and only a hint of a shoulder for the 40nm thickness Nanowave. The 50nm shell has a single distinct absorption band. Silver Nanowaves have two, and sometimes three, absorption peaks for all metal thicknesses.
sensitivity of adhered analyte molecules. Interestingly, the maximal E-fields achievable for both silver and gold Nanowaves are quantitatively similar, which is unexpected because silver is renowned to be a stronger SERS material than gold. At the current probed position in 3-D space, gold Nanowaves exhibit a maximum $SERS_{EF} = 28^4 = 6 \times 10^5$ at $\lambda = 980$nm and silver’s $SERS_{EF} = 31^4 = 9 \times 10^5$ at $\lambda = 640$nm. It is noteworthy that the measurement point is located in the air medium in the inter-particle junction, exactly at equidistance from both particles, and is therefore not the location of the strongest E-field that would be experienced by surface-bound molecules.

The plasmonic behavior of both gold and silver Nanowaves is summarized in Figure 3.25. With increasing thickness, both gold and silver Nanowaves experience a decreasing peak until a plateau is reached at 50nm for both metals. For a given thickness, the peak of gold is red-shifted relative to that of silver, as expected given the permittivities of each metal. As the metal thickness increases for both gold and silver, the E-field enhancements at the peak wavelength also decreases, which suggests that thinner shells have the potential to provide the best SERS sensitivity, if excited at the appropriate wavelength.

**Figure 3.25:** E-field enhancement at positions defined in the last column of Table 3.5 as a function of excitation wavelength $\lambda$ for (a) gold and (b) silver Nanowaves of varying metal thicknesses.
Localization of the E-field hotspots

Understanding where the strongest fields occur on the Nanowave is critical in determining whether they would be efficient SERS substrates. Given the complex 3-D geometry of the structure, this is only achievable to analyzing the E-field isosurface plots, which capture the complexity of the E-field behavior in three-dimensions; the conventional slice plots are insufficient for this purpose and are therefore avoided. The spatial distribution of the $|E|$, probed at the low-wavelength peak for each metal-shell thickness, is presented in Figure 3.26. For conciseness, only gold Nanowaves are depicted since the silver Nanowaves exhibit similar characteristics in terms of their E-field distribution in 3-D space. The first row is the top view of the substrate and the bottom row represents the side view, to clearly depict the 100nm Silica nanoparticles and the overlaying metal shell. To simplify the discussion, the encircled letters A and B are used to denote two distinct locations of the E-field enhancement: A refers to the top of the interparticle junction and B refers to the bottom of the metal shell, on either side of the junction.

As a first observation, the E-field at A defocuses from a tightly confined focal spot for the 10nm shell to cover a wider area for the 50nm shell, most likely due to the smaller x- and y-dimensions of the junction for thinner shells relative to thicker ones, as depicted in Figure 3.20.

Secondly, it is noteworthy that over the entire 3-D Nanowave geometry and for all metal thicknesses, the E-field is strongest at locations A and B, but the relative extent to which the E-fields at A and B are enhanced by the local geometry is dependent on the metal thickness. The E-field $|E|$ is equally strong at A and B for a 10nm gold shell, but $|E|_B$ decreases with increasing shell thickness, such that the relative intensity $|E|_A/|E|_B$ also increases. For a gold thickness of 50nm, $|E|_B$ is no stronger than the background E-field. This is interesting because it can be geometrically inferred and measured that the shell thickness at B does not increase much as overall shell thickness increases, yet $|E|_B$
decreases, which suggests that the high $|E|_B$ for thin shells reflects the underlying 3-D geometry of the Nanowave.

Therefore, the short-wavelength plasmon band seems to be related to the E-field enhancement at A and could be due to the confinement in the x-direction of the Nanowave substrate (in the direction of incident E-field polarization). As the shell thickness increases, the plasmon is confined to the top junction of the metal shell, possibly because the incident wave is unable, at these incident wavelengths, to penetrate the thick shell to couple the two metal nanoparticles at junction B.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.26.png}
\caption{Analysis of the short-wavelength plasmon peak (a) and corresponding E-field enhancement (b) of gold and silver Nanowave substrates, as a function of metal thicknesses.}
\end{figure}

**Surface-averaged SERS enhancement**

Nanowave structures were first fabricated to be used as SERS substrates for trace analysis, so it is important to investigate the expected SERS enhancement, $SERS_{EF}$, that they are able to provide, and how this quantity varies with shell thickness. The $SERS_{EF}$ is calculated by evaluating the absolute electric field, $|E|$, and then raising it to the fourth power.\(^{139}\) However, rather than evaluate $SERS_{EF}$ at a single point in space (a popular method that presents the highest possible $SERS_{EF}$ at a hot spot but ignores the rest of the substrate),
a more meaningful measure of the theoretical $SERS_{EF}$ is obtained by integrating $|E|^4$ over the probed area of interest. Experimentally, the measured SERS signal is effectively proportional to the average $|E|^4$ signal emitted by the molecules over the entire probe area, normalized to the number of molecules (monolayer-coverage assumed) being excited by the incident source. Depending on the microscope objective magnification power and numerical aperture, the probe area typically ranges from a few of $\mu m^2$ to tens of $\mu m^2$; this implies that the probe area consists of both hot spots (regions of strong EM enhancement) and flat surfaces (regions of weak EM enhancement). Therefore, from a purely geometrical standpoint and for a given type of analyte molecules, it can be envisaged that the measured SERS intensity strongly depends on the spatial density of hot spots, the achievable EM enhancement in those hot spots, and the ratio of molecules adhered in hot spots to those adhered elsewhere.

Thus, the average $SERS_{EF}$ that Nanowaves can provide is estimated by first calculating the surface-area-averaged E-field, $|E_{avg}|$, and then raising it to the fourth power to obtain an approximate $SERS_{EF}$. This was performed by integrating the E-field over the 3-dimensional Nanowave top-surface, which is depicted in blue in Figure 3.27(a) and the areas of which are reported in Table 3.5. The surface-averaged E-field as a function of wavelength is reported in Figure 3.27(b,c), and the general trends observed in Figure 3.24 - the number of peaks and the shift of the peaks towards the respective plasmon bands of gold and silver - are also apparent here. Interestingly, however, the maximum E-fields of the gold and silver Nanowaves are comparable, for each shell thickness, even though silver showed consistently stronger $|E|$ relative to gold, Figure 3.24. This is due to the small inter-particle hotspot area relative to the total top-surface area, such that averaging diminishes the importance of the intense E-fields present in the hotspots compared to the low E-fields everywhere else.

The SA-average E-field is low, with a maximum calculated SERS EF of approximately $5 \times 10^4 \approx 625$ for gold or silver, at both $\lambda = 1000$nm and $\lambda = 940$nm, respectively.
FIGURE 3.27: Spectra of the surface-averaged $|E|$; (a) the top surface of the metal coating (blue) is where molecules would attach and experience the local E-field, and is the surface over which the E-field is integrated to obtain the average E-field over the entire structure for the wavelengths of interest. This is repeated for every shell thickness to yield the $|E|_{avg}$ spectra for gold and silver in (b) and (c); (b) $|E|_{avg}$ for the gold nanowaves; (c) $|E|_{avg}$ for the silver nanowaves.

though this finding does not validate the significantly higher experimental data reported by Vo-Dinh et al., a number of possible explanations could be offered to understand the difference. Firstly, capillary effect when drying that concentrates the analyte into the
crevices where it then dries and experiences the high local E-fields (hot spot concentration effect); secondly unaccounted surface roughness covering the entire metal surface, which would increase the local E-field such that the average E-field experienced by metal-bound analytes would be significantly higher; thirdly, the crevices are actually sharper in reality than they were simulated, which would translate to an increased average E-field, but more importantly, to much more significant E-field enhancement in the inter-particle hotspots. Assuming the analyte is concentrated in those junctions, this could explain the stronger measured signal. Nevertheless, all variables held constant, we propose that this methodology is a fairer comparison of the SERS EF potential of substrate, rather than merely presenting the highest achievable value at a single point in space.

It is noteworthy that a decrease in the diameter of the underlying silica nanoparticle, which would lead to higher density of hotspots, would not yield an increase of the SA-averaged E-field. For example, a 10nm-thick Nanowave constructed with 30nm silica nanoparticles (characterized by a $10 \times$ decrease in area to $3 \times 10^{-15} m^2$) did not yield any significant difference in SA-averaged E-field. This is possibly due to the relatively small contribution from the hotspot enhancements compared to the total averaged area, combined with an intrinsically complex dependence of E-field enhancement on nanoparticle size; in this case, the 30nm nanoparticles exhibited lower E-field enhancements than the 100nm nanoparticles.

The intriguing appearance of longer-wavelength (or lower-energy) peaks in the Figure 3.24 absorption spectra was investigated by probing the 3-D solutions at those wavelengths, which are depicted in Figure 3.28. It is noteworthy that the 40nm and 50nm Nanowaves of both gold and silver do not exhibit distinct long-wavelength peaks. As depicted in Figure 3.28, at these excitation wavelengths, the E-field enhancement is strongest at, and mostly confined to, the side junctions B.

As the shell thickness increases, the ratio $|E|_A/|E|_B$ decreases as $|E|_B$ remains high but $|E|_A$ weakens; this contrasts to the trend exhibited in Figure 3.26 for the short-wavelength
Figure 3.28: Spatial analysis of $|\mathbf{E}|$ of the long-wavelength plasmon peak of gold Nanowaves for shell thicknesses 10nm, 20nm and 30nm. The diagrams are to scale relative to each other. The encircled letters A and B denote locations where E-field enhancement occurs and will be used to simplify the analysis.

plasmon band. It is interesting that for 40nm and 50nm shells, the enhancement at B is not significant, possibly due to the incident field not being able to effectively penetrate into the shell to that depth.

When considered jointly, 3.26 and 3.28 suggest an explanation for the dual-plasmon peak nature exhibited by the Nanowave structure: the short-wavelength plasmon band is a manifestation of the E-field enhancement in the top crevice of the metal shell (junction A), and the long-wavelength plasmon band can be attributed to the E-field enhancement on either side of the interparticle gaps (junctions B). More generally, the short-wavelength plasmon band is a result of the spatial confinement in the x-direction, and the long-wavelength plasmon band is due to spatial confinement in the y-direction. This hypothesis was verified by relaxing the physical confinement along the y-direction such that the Nanowave structures became metal-coated cylindrical nanowires that extended to infinity in y, generically depicted in Figure 3.29. With an x-polarized incident excitation, the E-field enhancement
occurs along the entire nanowire ridge, unconfined in the y-direction, Figure 3.29(c).

**Figure 3.29**: Metal-coated cylindrical nanowires that extend to infinity in the x-direction, but are periodic in the y-direction: (a) as a generic model, 20nm of metal (red) is deposited onto the 100nm diameter cylindrical wires, and the finite rounding between adjacent wires is kept identical to its corresponding nanowave of equal metal coating, given in Table 3.5; (b) the top surface of the metal coating (blue) is where molecules would attach and experience the local E-field, and is the surface over which the E-field is integrated to obtain the average E-field, $|E|_{avg}$, over the entire structure; (c) a representative isosurface solution of $|E|$ for the 20nm silver-capped wire @ $\lambda = 510$nm (plasmon peak), conveys that the strongest E-field enhancement occurs in the groove and is invariable along the y-direction, as expected.

The absorption spectra of these nanowires are presented in Figure 3.30, for both gold and silver, and only exhibit a single plasmon band, which approximately co-registers with the corresponding short wavelength plasmon bands of the Nanowaves in Figure 3.24: for 10nm- and 20nm-thick gold shells, the peaks are located at 780nm and 600nm for Nanowires, respectively, compared to 710nm and 610nm for Nanowaves, respectively. For 10nm- and 20nm-thick silver shells, these peaks are positioned at 700nm and 520nm for the Nanowires, respectively, compared to 640nm and 520nm for the Nanowaves, respectively. The absorption spectra also possess similar magnitudes ($1 \times 10^{-14}$) and FHWM as those in Figure 3.24.

Figure 3.31 depicts the surface-averaged E-field, which is comparable magnitude to
FIGURE 3.30: Absorption spectra of the Nanowire structures for (a) gold and (b) silver metal shells. The discrete data points, representing calculated values from the simulation, are interpolated using a spline function to smooth out the curves. Compared to the Nanowave structure that exhibits two plasmon bands for both gold and silver, only one plasmon band is excited in the Nanowire structure, for both metals.

FIGURE 3.31: Spectra of the surface-averaged $|\mathbf{E}|$ of (a) gold and (b) silver Nanowires, integrated over the blue surface shown in Figure 3.29(b) for shell thicknesses 10nm to 50nm.

the Nanowave geometry, but yet again, only a single main plasmon peak is seen here for each metal thickness. To further investigate the hypothesis that increasing the spatial confinement of the structure leads to additional plasmon bands, the Nanowave structure was further confined in the $x - y$ plane by removing the periodic boundary condition in that same plane, such that the E-field of a cluster of only seven nanoparticles, schematically dis-
played in Figure 3.19, was evaluated. Indeed, this extra confinement led to the emergence of a longer wavelength plasmon peak in the 1100 – 1200 nm wavelength range, depicted in Figure 3.32 for silver; confined gold Nanowaves also introduced an extra, long-wavelength plasmon band.

The non-periodic Nanowave structure consistently reproduces the three short-wavelength plasmon bands, albeit sharper and of larger magnitude; this more confined structure, however, exhibits an additional, broad plasmon band appears at 1150 nm, whereas the unconfined, periodic Nanowave geometry does not. The dampening and broadening of the plasmon peaks for the unconfined Nanowave is probably due to the contribution from long-range, delocalized surface plasmons originating from the adjacent nanoparticles ad infinitum, which are absent in the confined structure; the investigation of this phenomenon is beyond the scope of this manuscript.
3.5.4 Conclusion

This study investigates the plasmon behavior in the vicinity of Nanowave substrates of various thicknesses, which has been sought after for over 25 years since this idea was first conceived. We report the first numerical study of the Nanowave structure in 3-D, taking into account infinite boundaries and interparticle rounding in order to reproduce the experimental behavior as faithfully as possible. To the best of our knowledge, this is also the first time a surface-area averaged E-field was evaluated to determine SERS, rather than single point measurement, making this a more realistic study.

For thin metal shells, the Nanowave structure exhibits two plasmons bands in the case of gold, and three in the case of silver. As the shell thickness increases, the long wavelength band vanishes possibly due to the incident field not being able to penetrate to the base of the shell to excite the long-wavelength plasmon. SERS was evaluated by surface-area averaging the E-field. The lower-than-expected average E-field resulted from low surface area ratio of hotspots to flat surfaces, implying that the highest SERS EF is obtainable by concentrating the molecules of interest into the periodic hotspots, where the local E-field is the strongest. Indeed, compared to a maximum E-field enhancement approximating 30 for both silver and gold, when evaluated at a single point in space, the maximum average E-field was merely 5.

Finally, this study provides insight into the appearance of multiple plasmon bands. Specifically for the Nanowave structure, the short-wavelength peak is attributed to the plasmonic coupling at the top of junction (site A), and the long-wavelength peak to the crescent-shaped base of the metal-dielectric function (sites B). By relaxing the periodic boundaries in the x-y plane, a third peak is exhibited in the NIR due to the even more confined structure under incident

SERS substrates can consist of colloidal nanoparticles, such as gold nanostars, or dry substrates, such as the Nanowave. Due to their differing nature, these two substrate types
are complementary to each other in that they each offer advantages over the other, yet also have their own limitations.

Colloidal nanoparticles are versatile, inexpensive to synthesize, offer plasmon tunability by geometric variations, but their largest drawback is their sensitivity to their chemical environment, which makes them prone to uncontrollable aggregation and irreproducible SERS signals. On the other hand, stabilizing them with large polymers or hydrophilic moieties to minimize this aggregation prevents them from being intentionally collapsed into a small volume to drastically increase their SERS signal via strong inter-particle plasmonic coupling.

Dry substrates can be reproducibly fabricated in batches and have the potential to provide strong, quantitative SERS signals as long as uniform drying is ensured on the surface. Unfortunately, the drying of solutions on surfaces is typically uncontrollable and it is unrealistic to assume uniform, reproducible drying of the analyte. Furthermore, dry substrates will most likely cost more in the long run, since they are one-time use surfaces, and they lack the versatility of their colloidal counterpart for in-vivo studies.

Rather than use a single type of substrate, however, the next two chapters investigate the result that can be achieved by combining the advantages of colloidal substrates with the agility and reproducibility of wafer fabrication. As such, the aim is to design and engineer a dual-mode device that combines the SERS capabilities of colloidal nanoparticles with spatial consistency of substrates. The vision is to be able to concentrate the SERS-active nanoparticles within a small volume, thereby achieving strong SERS signal, yet ensuring its reproducibility. Chapter 4 details the synthesis and functionalization of dual-functional SERS-active magnetic nanoparticles, and Chapter 5 discusses the design of micromagnetic structures for their manipulation, as well as the integration of these structures into a digital microfluidics system.
4

Design & Synthesis of Plasmonics-active Magnetic Nanoparticles

The research described in this chapter fulfills Aim 3

4.1 Introduction

Nanoparticles combine chemical accessibility in solution with physical properties of the bulk phase, representing intermediates between the molecular and solid states. They therefore represent ideal building blocks for the construction of nanostructure materials that can be physically and chemically adjusted.

Small iron oxide particles have been used for in-vitro diagnostics for the last several decades, but it is not until recently that the synthesis of nanosized, single domain magnetic particles, composed of maghemite $\gamma - Fe_2O_3$ or magnetite $Fe_3O_4$, has become important. Magnetite is particularly attractive because its biocompatibility has already been proven, eliminating that hurdle for future in-vivo studies. Iron oxide nanoparticles also exhibit superparamagnetic behavior, such that they do not retain their magnetism
after removal of the magnetic field.\textsuperscript{227} Ideally, they would display zero-loss hysteresis.

Although bare magnetic nanoparticles (MNPs) have recently gained increasing interest in the field of biomedical research for cellular therapy, tissue repair, drug delivery, magnetic resonance imaging (MRI), hyperthermia, magnetofection, etc.,\textsuperscript{228–231} one of the pursuits of this thesis is to employ plasmonic magnetic nanoparticles as efficient SERS substrates. In this case, plasmonic MNPs refer to gold/silver-coated MNPs - the metal shell provides the desired plasmonic behavior for SERS, while the magnetic core allows their manipulation.

SERS detection, to date, is undertaken with either free nanoparticles in solution or fixed nanostructured substrates. Free nanoparticles require robust stabilization in solution or will tend to aggregate which drastically increases any detected SERS signal, making quantification and reproducibility near impossible. They are, however, versatile and can be used for targeting and detection in cells, as our group has recently demonstrated.\textsuperscript{232} Fixed nanostructured substrates, if properly fabricated, have the advantage of providing a controlled 2-D map of hotspots, such as the Nanowave substrate, which leads to more reproducible, and therefore quantifiable, detected SERS signals, but are one-time use devices since the attached molecules cannot be easily washed or detached. Thus, combining the strengths of both techniques, while discarding the drawbacks, is the aim of this study - it is hypothesized that the controlled concentrating of plasmonic MNPs could enhance the SERS sensitivity and offer reproducible results, while maintaining the versatility associated with the small nanoparticle size.

The controllable concentrating of these nanoparticles would be induced by subjecting them to a strong magnetic field gradient that is confined in a small space; their superparamagnetic nature requires the field to be particularly strong since they have weak magnetic dipole moments. Such a strong field can be achieved by reducing the scale of the magnets,
to ‘micromagnets’, such that the local field gradient is extremely large. The effect of this high field has been demonstrated for concentrating both a high volume fraction solution of magnetic nanoparticles (ferrofluid)\textsuperscript{233–235} and nonmagnetic nanoparticles.\textsuperscript{236–240} The concentration of ferrofluid between the micromagnets intensifies as the applied magnetic field increases, demonstrating the aggregation capability of this technique.

With the hope of being able to controllably concentrate the synthesized MNPs@Au to yield a quantifiable and reproducible detected SERS signal, the MNPs@Au have to display critical properties: stability, monodispersity, sub-50nm in size, chemically functionalizable and be aqueous-based. Monodisperse particles are desired to ensure that their concentration leads to a predictable nanoparticle close-packed self-assembly, which in turn will lead to a more predictable SERS signal; the solution would also have a narrow plasmon band which helps determine the optimal excitation wavelength with which to probe the particles. A medium size is required since the E-field enhancement is known to be weak for very small or very large nanoparticles when exciting in the Vis/NIR region of the spectrum. Finally, the nanoparticles need to be stable in water, since most of the analytes and biomolecules of interest are water-based and the possibility of utilizing these particles in biological media is also desired.

Unfortunately, MNPs are significantly more complicated to synthesize, functionalize and stabilize than traditional metal nanoparticles, firstly because they are oxides and thus have limited available surface chemistry, and secondly, due to the inter-particle interaction of their intrinsic dipole moment, which makes them prone to aggregation in solution, especially during chemical reactions. Indeed, even though a wealth of protocols detailing the synthesis of gold-coated superparamagnetic nanoparticles exists in the literature,\textsuperscript{241–260} most of the resulting hybrid nanoparticles are polydisperse in distribution, are too small or too large in size, have multiple magnetic cores, suffer from aggregation, are synthesized
inorganic medium, or lack evidence to prove that the gold coating is actually present.

Synthesizing the ideal solution of MNPs@Au for the aforementioned SERS application unexpectedly became the biggest challenge of this thesis, and given the very limited background in synthetic chemistry among our group members, is unfortunately an effort that spanned between two to three years. Finally, after exhaustively trying the most promising protocols in the literature, in vain, the author discovered a new, simple method for generating MNPs@Au that were of optimal characteristics for the application at hand.

This chapter discusses the synthesis of magnetic nanoparticles (MNPs), and the obstacles in achieving MNP@Au with the desired aforementioned characteristics. The chapter concludes by presenting the author’s own novel synthesis protocol, which successfully yields MNPs@Au that are more suited for the intended application. These MNPs@Au were then further coated with a shell of silver to enhance the SERS signal, and functionalized with the resonant Raman dye, DTDC.

4.2 Synthesizing MNPs@Au

Given the recent exponential growth of manuscripts detailing nanoparticle syntheses in the literature, it was surprising to find only a limited number of manuscripts that convincingly described the coating of gold on magnetic nanoparticles. In contrast to synthesizing nanoparticle shapes of a single material, such as our gold nanostars\(^{90,188}\) or other rods,\(^{176}\) cubes,\(^{261}\) octahedra,\(^{262}\) nanoprisms,\(^{263}\) or even compared to the growth of a silver shell on gold nanoparticles,\(^{264}\) the growth of gold (metal) on iron-oxide (dielectric) is incredibly challenging for a number of reasons: crystal lattice mismatch, surface energy mismatch, surfactants that are incompatible to either metal or dielectric, intrinsic magnetic moments of the iron-oxide that induce aggregation and require further stabilization.

Scouting manuscripts in the literature, it is fairly difficult to determine how complex
the synthesis really is, although it is fairly obvious when a study reports questionable data, such as, for example, badly focused TEM images,\textsuperscript{251} evident by the fact that the density of iron oxide is significantly less than that of gold, and not vice versa. Those anomalous manuscripts aside, however, there are a number of interesting chemical syntheses that are worth scrutinizing.

A number of research groups have demonstrated the gold coating of both small and large MNPs that unfortunately generate large MNPs@Au of diameter ranging between 100 and 400nm, making them intrinsically unstable and also beyond the optimal size for SERS, which lies in the range of 30 – 80nm for gold and silver.\textsuperscript{247,252,257}

When attempting to properly coat small MNPs, maintaining sufficient repulsion, either steric or ionic, to ensure MNPs can be individually coated with gold with minimal aggregation becomes challenging. Xu et al. addressed this problem by performing the metal coating at room temperature in the original organic solvent, in which the MNPs were extremely well stabilized by a mixed monolayer of oleic acid and oleylamine.\textsuperscript{250} The resulting MNPs@Au were small, stable individually coated with gold or metal and in very high yield, but the issue now became the efficient solubilization of these MNPs@Au so that they could be easily functionalized with biomolecules. Although solubilization was achieved by CTAB ligand exchange, the CTAB bilayer that forms on each MNP@Au and renders them stable in water also makes functionalizing them a challenge.

Another research group developed two methods to coat organically-stabilized MNPs in organic solvent, which employed elevated temperatures to ensure the removal and recapping of stabilizing oleic acid and oleylamine molecules during elemental gold deposition on the MNPs’ surface.\textsuperscript{242,249} These two procedures enabled the gold-coating of 5nm and 10nm MNPs, respectively, but did not solve the issue of solubilization and surface functionalization.

With the synthesis of MNPs and their subsequent gold-coating performed in aqueous medium, the methods proposed by Xie et al.\textsuperscript{259} and Goon et al.\textsuperscript{256} both generated monodis-
perse MNPs@Au in the 50nm-range and with no need to further solubilize the particles. However, the thick coating of ‘sticky’ polymer poly-ethyleneimine (PEI), comprising a long web of imine groups, that acts both as a stabilizing surfactant and a reducing agent, renders subsequent functionalization via ligand exchange extremely hard, if not impossible. The only other means would be to chemically conjugate the molecule or biomolecule of interest to the PEI coating, but their separation from the metallic surface by the 5nm PEI thickness, in turn, diminishes their potential as SERS substrates. This also significantly complicates the functionalization process and hinders further use of the nanoparticles in biological systems: if attempting to attach a biomolecule such as DNA, the mere charge of the PEI molecule would probably seriously interfere with any DNA manipulation or hybridization that would be of interest.

The following groups successfully developed protocols to yield monodisperse MNPs@Au in aqueous solution that were stabilized by weak repulsive charges or a citrate surfactant.

Ji et al. first coated a thick shell of silica onto small, 10–15nm Fe_3O_4 NPs, followed by functionalization with APTES, and subsequent attachment and growth of tiny 2nm gold nanoparticles. This resulted in the final MNP@Au diameter exceeding 80nm, with the magnetic core being very small with respect to the overall size, which compromises their magnetic response. Moreover, the TEM images suggested that the MNPs@Au were clustered together as aggregates and were thus unstable, which was indirectly confirmed by their very broad and nearly peakless UV-Vis spectra and deep blue solution color. The speed at which the MNPs@Au were attracted to a nearby rare-earth magnet is also secondary confirmation of their instability.

Levin et al. modified this method by directly coating larger, 50nm Fe_3O_4 NPs with APTES, followed by attachment and growth of tiny 2nm gold nanoparticles; however, as indicated by their murky solution (highly scattering) and corresponding TEM images, the
resulting nanoparticles were large (> 100nm) and thus also unlikely to be stable for more than a few hours.

Lim et al. synthesized Fe₃O₄ NPs organically, transferred them to aqueous solution with TMAOH, then functionalized them with MUA, which attached to the Fe₃O₄ surface via the -COOH group, leaving the thiol -SH group available for covalent binding of tiny 2nm gold nanoparticles, onto which gold growth ensued.²⁵³ The length of the MUA relative to that of APTES aided in the stabilization of the 2nm-gold encrusted Fe₃O₄ NPs during subsequent gold coating, allowing for the successful synthesis of non-aggregated MNPs@Au. It is noteworthy that although monodisperse, these particles were not spherical and consisted of a coating gold protrusions from uneven gold growth that influenced their plasmonic properties by shifting their resonance towards 600nm. The author was unsuccessful at obtaining the MNPs@Au claimed by Lim et al., even with direct input from JitKang Lim himself.

Lyon et al.’s method employed an iterative method to achieve the gold coating of aggregates of Fe₂O₃ NPs to yield fairly large MNPs@Au (60nm) with a high polydispersity. By adding progressively more gold ion and hydroxylamine reducing agent, the initially rough-surfaced MNPs@Au, that exhibited a deep blue color characteristic of surface protrusions, became successively redder indicating smoothening and growth of the gold shell. Although the MNPs@Au surface could be functionalized, they were highly polydisperse and would cause stability issues.

The most elegant method is probably that of Jin et al., which was also employed to coat quantum dots²⁶⁵ and lipid particles²⁶⁶ with gold. This method uses organically synthesized, oleic acid-capped Fe₃O₄ MNPs, but solubilization is achieved via the amphiphilic polymer PL-PEG-COOH, whose organic end interdigitated into the oleic acid monolayer, leaving the hydrophilic PEG-COOH end for both stabilization in aqueous media and further functionalization with the positive PLH polymer. The PLH chelates Au³⁺ ions in high density to allow the nanometer-controlled growth of a gold shell. The largest obstacle that
pertains to this method is the expense of the chemicals, as well as its intrinsic complexity of the chemistry involved. Moreover, once synthesized, the MNPs@Au are claimed to require instant stabilization by SH-PEG molecules to prevent their aggregation.

A number of protocols were attempted and their results are presented in the following section.

4.2.1 Synthesizing MNPs

MNPs can be synthesized either organically or inorganically, depending on the desired outcome.

The inorganic method is by far the simplest of the two, since it usually occurs at a low reaction temperature, makes use of inexpensive, abundant chemicals and simple equipment, and most importantly, produces hydrophilic MNPs that are water-soluble. The most significant drawback is the MNPs’ lower crystallinity and higher polydispersity relative to its organic counterparts. It is also relatively hard to control the size of the produced nanoparticles. These nanoparticles also tend to be less stable in solution, flocculating and settling down over time.

The organic synthesis of MNPs, developed independently by Colvin and Sun, employs high reaction temperatures to slowly thermally decompose the reagents into hydrophobic MNPs. This ensures a high monodispersity, good lattice crystallinity, stability and size control. For use in biological systems, however, the hydrophobic MNPs need to be solubilized to make their surfaces hydrophilic. This process, although considered a drawback, in fact allows to enhance MNP stability by choosing the appropriate solubilization chemicals, making the organic pathway the most attractive of the two and the one used for this study.

The high temperature nature of the reaction requires an inert atmosphere, which is supplied via a Schlenk line setup, Figure 4.1. The synthesis requires solution degassing
followed by ramping up to a temperature of $320^\circ$C, at which it is maintained for a length of time depending on the desired MNP size. After cooling to room temperature, the MNPs were thoroughly washed via repeated flocculation and redispersion with acetone and chloroform. A typical TEM image of monodisperse 12-nm MNPs is shown in Figure 4.2. Their monodispersity is confirmed by the fact that they produce a self-assemble monolayer as the toluene solvent evaporates on the TEM grid. These MNPs are extremely stable in organic solvent (chloroform or toluene), remaining dispersed in solution without flocculation for months.

**Figure 4.1:** Inert atmosphere Schlenk line setup for the organic, high-temperature synthesis of monodisperse superparamagnetic nanoparticles. Here, the reaction had finished ($T_{\text{set}} = 20^\circ$C and $T_{\text{actual}} = 260^\circ$C) and flask was cooling to room temperature from $320^\circ$C.

An important feature with nanoparticle synthesis is size control. For gold or silver nanoparticles, there is an intricate relationship between size and SERS efficiency. For MNPs, an increase in size is accompanied by an increase in the particle’s magnetic moment which allows MNP concentration at lower fields strengths, but also increases the likelihood of MNP aggregation in solution because the volume to surface area ratio increases such
that the stabilizing surfactant becomes less effective. Figure 4.3 conveys MNP sizes of 20nm, 25nm, 30nm and 60nm, which were achieved by increasing the oleic acid reagent concentration and increasing the growth time. As observed, the monodispersity is still very high.

4.2.2 Solubilizing MNPs

The organically synthesized MNPs have a hydrophobic coating of oleic acid and therefore require solubilization into aqueous solution to be useful with biological molecules of interest, such as DNA or antibodies. Several methods for solubilizing hydrophobic MNPs are ligand exchange, encapsulation or silica coating.

Ligand exchange involves replacing the oleic acid on the MNP by a chemical that has
Figure 4.3: TEM images demonstrating the size-control achieved by adjusting the oleic acid concentration and the reaction time. Each sample conveys high particle monodispersity and the average particles sizes are (a) 20nm (b) 25nm (c) 30nm (d) 60nm.

A stronger affinity for the underlying iron oxide material and that is also soluble in water. Some ligand exchange chemicals include TMAOH, silanes, Carboxylic acids, PEI or dopamine. Depending on the ligand used, these MNPs remain stable in water from days to months.
Encapsulation, however, is the technique by which amphiphilic molecules, usually polymers, ‘encapsulate’ the MNPs such that their hydrophobic moieties intercalate into the oleic acid monolayer and their hydrophilic parts extend into the surrounding water. This can be achieved using CTAB\(^{275}\), carboxylic-backbone polymers\(^{276,277}\) or lipid-PEGs.\(^{278}\) The successful phase-transfer of MNP from organic solvent to water is confirmed by a clear solution that is stable over days to weeks or months.

Finally, silica coating of the MNPs can be achieved by reverse-microemulsion such that the negative -OH surface of the silica shell enables the MNPs to be sterically stabilized in aqueous medium.\(^{275,279}\)

4.2.3 MNP@Au

**MNPs@Au on Fe\(_3\)O\(_4\) via SiO\(_2\) shell growth and 2nm-Au seeds attachment**

This idea of attaching small gold seeds onto a dielectric core to act as nucleation sites for the further growth of gold, to ultimately form a shell, stemmed from Halas’ well-known gold nanoshell protocol.\(^{280}\) This protocol for synthesizing large, gold-coated silica nanoparticles was reproduced and its results are depicted in Figure 4.4.

![Image of TEM images](image)

**Figure 4.4:** TEM images detailing the growth sequence of the coating of 100nm silica nanoparticles with a gold shell. The silica nanoparticles are functionalized with APTES, leaving amine groups on their surface; these capture small 2nm THPC-capped gold seeds (a) which then serve as growth sites for further gold deposition (b-d).
The 100nm silica nanoparticles were synthesized via the Stober process, following which their surface was functionalized with APTES to populate the surface with positively charged amine moieties. Small THPC-capped gold nanoparticles of between 2 – 3nm were synthesized in tandem and kept in the dark at 4°C until further use. An excess of THPC-capped gold was added to the silica nanoparticles, and left overnight to electrostatically attach to the silica surface. Aliquots of these THPC-capped silica nanoparticles are then injected into a growth solution containing partially reduced gold ions. Reduction to elementary gold is then induced by addition of the reducing agent formaldehyde. As gold is progressively reduced onto the silica core, the solution color changes from reddish, to purple to blue as a complete shell is formed on the silica nanoparticles. This is observed under TEM by an increasingly dense shell coating that thickens with further gold reduction. Thus, the standard gold-coating of silica nanoparticles proved successful with little complications.

Silica-coating of MNPs

After significant research, the concepts from 4 different procedures were combined to obtain the best possible synthesis scheme that would allow for the synthesis of small, monodisperse, stable MNPs@Au. This synthesis consists of silica-coating MNPs via a reverse-microemulsion procedure, functionalizing the silica surface with amine groups, attaching small gold seeds, and growing a gold shell via reduction of gold ions in solution (See MNPs@Au: Silica coating, attachment of Au seeds & shell growth in Appendix 2).

Rather than adhere to the report by Ji et al. and silica-coat the MNPs using the standard ‘TEOS method’, whereby TEOS (Tetraethyl orthosilicate) is non-specifically condensed in basic solution onto the surface of any available NP, a more elegant “reverse microemulsion” method based on the formation of isolated ‘nanoreactors’ as proposed by Jun et al. was employed. This was done for two reasons: firstly, by using a reverse mi-
croemulsion, the MNPs could be silica-coated in their original organic medium; secondly, it enabled tight control of the silica coating thickness, allowing the synthesis of smaller and more monodisperse MNP@SiO$_2$ compared to the TEOS method, thus improving their stability and therefore likelihood of a successful gold shell coating.

**Figure 4.5**: TEM images detailing the growth of a silica shell on MNPs at (a) 5 hours (6nm), (b) 10 hours (10nm), (c) 20 hours (15nm) and (d) 36 hours (25 nm).

Figure 4.5 depicts TEM images of silica-coated MNPs as the silica shell was grown
progressively larger simply by letting the reaction proceed. Since TEOS hydrolysis is a slow process, the silica shell could be grown controllably and stopped at a given time by quenching the reaction with ethanol. It is noteworthy that reverse-microemulsion is a simple process that affords every high yield (~100% MNPs are coated with silica).

Au-seed attachment to silica-coated MNPs

Utilizing a similar approach to the Halas method, these synthesized silica-coated MNPs were then functionalized with APTES and incubated overnight with THPC-capped Au nanoparticles. Since MNPs are intrinsically light brown (at low concentrations), and the THPC-capped Au nanoparticles are dark brown, the washed Au-encrusted silica-coated MNPs appeared only slightly darker than their bare counterpart. Only the TEM imaging could confirm their good coverage of THPC-capped Au nanoparticles, both for thin silica shells and thick silica shells alike, Figure 4.6.

An increase in this surface coverage was attempted by adding a low concentration of sodium chloride salt, so as to increase the ionic strength of the solution and reduce the inter-particle repulsion between THPC-capped Au particles. This procedure did not seem to affect the final particles, and if anything, had a negative effect on their size distribution, as observed by the larger THPC-capped Au nanoparticles in Figure 4.7.

![Figure 4.6](image.png)

**Figure 4.6:** TEM displaying the coverage of silica-coated MNPs by THPC-capped Au NPs. Regardless of the silica shell thickness, the coverage appears to be identical. The red dotted line delineates the silica shell boundary for visualization purposes.
Figure 4.7: TEM displaying the coverage of silica-coated MNPs by THPC-capped Au NPs that were slightly aggregated with a low concentration of NaCl. The Au NPs appear slightly larger than those in Figure 4.6.

In an attempt to further increase the surface coverage, small citrate-capped Au nanoparticles were synthesized and incubated in excess with APTES-functionalized silica-coated MNPs, Figure 4.8, but the results were worse than the THPC-coated batch.

**Gold-coating of Au-encrusted silica-coated MNPs**

Once the thin-silica shelled nanoparticles from Figure 4.6(a) were washed and ready for coating with gold, they were injected in the similar aforementioned growth solution, followed by formaldehyde. Unfortunately, the growth of the gold shell resulted in significant aggregation among the nanoparticles, such that the resulting solution was unstable and flocculated within one hour. Even though the typical color transitions were observed with thickening shell, TEM revealed large intertwined particle clusters, Figure 4.9.

The gas-phase reducing agent, carbon monoxide, was recently employed to more controllably coat nanoparticles with gold, but yielded similar results to the formaldehyde reducing agent, Figure 4.10.

The result was similar regardless of injected seed volume, growth solution concentra-
Figure 4.8: TEM depicting the electrostatic attachment of small citrate-capped Au nanoparticles. Compared to the THPC-capped Au NPs, these are significantly larger and the surface coverage is relatively poor.

It was concluded that this unforeseeable obstacle was most likely due to the small size of the silica-coated MNPs. Compared to the significantly larger 100 nm silica nanoparticles used by Halas, these were about 30 nm. Simplistically, stability of nanoparticles in solution ensured by the zeta potential at their surface. The higher the zeta potential magnitude, the stronger the inter-particle repulsion. However, since it is a surface phenomenon, the repulsive forces are most likely proportional to the nanoparticle surface area. Even though they might have the same zeta potential, small silica nanoparticles are significantly more prone to aggregation than larger ones. Since surface area is proportional to the radius squared, the 100 nm silica nanoparticles have a surface area that is approximately $11 \times$ larger than the 30 nm nanoparticles; this large surface charge build up is like to be the reason why the gold shell can be successfully grown on large silica nanoparticles, and not on small ones. After exhaustively trying the synthesis in vain, a new route was sought after.
FIGURE 4.9: TEM showing the formation of a gold shell on Au-encrusted silica-capped MNPs, for increasing growth solution volumes from $500\mu$L to $4mL$ (a-d), using formaldehyde as the reducing agent.

*MNPs@Au on Fe$_3$O$_4$ using PL-PEG-COOH and PLH*

Recently, Jin and Gao proposed a protocol, which, through a complex functionalization scheme, allows the growth of extremely thin gold shells onto small, organically-capped nanoparticles of different sizes ranging from $4\text{–}5\text{nm}$ to $50\text{nm}$.$^{260,265,266}$

Due to the protocol’s complexity, however, this exciting methodology was exhaustively tried for over two months, but in vain, even with several corresponding discussions with the paper’s author. There were a number of challenges that made this procedure particularly...
Figure 4.10: TEM showing the formation of a gold shell on Au-encrusted silica-capped MNPs using carbon monoxide as the reducing agent, with growth solution volumes of (a) 500 µL, (b) 1 mL and (c) 2 mL.

difficult to replicate:

1. The unconventional solubilization method

2. The high speed washing steps requiring an ultracentrifuge

3. The pH-sensitive attachment of PLH to PL-PEG-COOH, monitored only via DLS

4. The inability to directly observe intermediate results by eye or TEM

5. The small volume of MNPs@Au synthesized in each batch

6. The monetary expense of the required equipment and chemicals

7. The time expense of the entire procedure

Indeed, although the protocol claimed to generate very nice particles, these 7 items prevented them to be replicated. Item 1. comprises the interdigitation of the lipid tails of the PL-PEG-COOH molecule into a monolayer of OA at the MNP surface. If an excess of OA is present at the MNP surface (due to insufficient washing), the PL-PEG-COOH molecules are unable to completely envelope each MNP and these MNPs become prone to aggregation during high speed washes (item 2.) and prevent the efficient binding of the
positive PLH molecule to the negative COOH tail group. Conversely, the insufficiency of OA on the MNP surface (due to excessive washing) also yield the same ultimate problems. Thus, because the method of growing a gold shell hinges on having a full coverage of PLH molecules on the MNPs’ outer surface to chelate added Au$^{3+}$ ions, the state of the initial coverage of OA on the MNP surface can lead to failed experiments. This is very problematic especially when considering that an excellent MNP solubilization can occur even with partial coverage of the PL-PEG-COOH, because of the extremely hydrophilic PEG segment; this partial coverage, however, implies a low coverage of the negative COOH tails, which in turn lead to severe aggregation once the PLH molecules attach (due to insufficient charge repulsion) and also failed gold coating. Moreover, initial monitoring of the coverage at any step in the functionalization process is unfeasible, since even zeta potential measurements provide no baseline for the charge of fully covered MNPs.

Assuming that the PL-PEG-COOH is performed perfectly, the next step involved directly coating the negative lipid-PEG-COOH-functionalized MNPs with positive PLH-polymer via a pH-sensitive layer-by-layer deposition procedure. Theoretically, the surface-bound PLH-polymer chelates gold ions that are then reduced by a weak reducing agent, completing the gold shell. The challenge, however, was to obtain the right pH and stirring conditions to ensure the full coverage of PLH atop the PL-PEG-COOH. The pH needed to be adjusted because there exists only a small range (pH=5-6) for which the PLH and COOH molecules are positive and negative, respectively; it was thus crucial to obtain the correct pH to achieve full coverage of PLH. Because polymers are not observable under TEM unless stained a priori, the successful polymer layer-by-layer deposition could only be confirmed via $\zeta$-potential measurements for which a sign reversal was to be expected. Moreover, although a zeta potential switch from negative to positive indicates a resultant positive surface, it neither qualifies nor quantifies the state of the PLH coverage.

Finally, the combination of items 5 – 7. made this entire protocol sub-optimal for the intended application, which was to propose a novel, inexpensive SERS platform for the de-
tection of biomolecules. With 10mg of PLH costing $130 and 100mg of PL-PEG-COOH costing $400, together with the need of a DLS machine to verify PLH attachment and an ultracentrifuge to wash the particles, this procedure is by far one of the most costly in the literature. Given the fact that the claimed MNPs@Au were not achievable, alternative syntheses were sought after.

4.2.4 MNPs@Au on Fe₃O₄ using Au(acac)₃

MNPs@Au were successfully synthesized by the method proposed by Wang et al.²⁴² and later employed by other groups.²⁴⁹,²⁵⁸ This two-step process, undertaken completely under inert atmosphere of argon, firstly involved the high-temperature, organic synthesis of OA/OAm-capped 4 – 5nm iron oxide NPs, followed by the gold-coating at elevated temperature. The gold coating process is based on the de-capping and re-capping of the OA and OAm molecules that stabilize the MNPs, which occurs at elevated temperatures of 180 – 190°C. At these high temperatures, the amine-groups of the OAm act as weak reducing agents such that the gold salt, Au(acac)₃, is reduced unto the MNP in between surfactant de-capping and re-capping.

As is expected, uncoated MNPs have a featureless absorption spectrum since iron oxide does not plasmonically interact with light. The MNP@Au absorption spectrum, however, exhibits a distinct peak at around 540nm, which is red-shifted relative to the typical 520nm peak normally attributed to Au NPs. Gold nanoshells that have a dielectric core, as is the case here, have a plasmon band that is red-shifted relative to their Au NPs counterpart, with the degree of red-shifting depending on the ratio of shell thickness to overall diameter: the smaller this ratio, the more-red shifting is observed. Since the MNPs are 4 – 5nm and the overall diameter of the MNPs@Au is 7 – 8nm, the ratio is around 0.5, which explains the only slight shift to 540nm. This shift is enough to make the MNPs@Au solution appear deep purple in color, which, given their overall size, can only be explained by their structure having a core-shell formation.
There are several limitations with this procedure, however, as described below. Although MNPs@Au were finally synthesized, many trials were required to successfully obtain the particles. This stemmed from the very nature of the complex synthesis, which critically required the reaction to be performed and kept under argon, during both the synthesis of MNPs and coating of MNPs with gold. As such, several failed trials resulted in the devising of a comprehensive procedure to ensure a successful synthesis (see A2.2). In brief, the process involved separating 3 reagents solutions so that each one could be separately degassed at an elevated temperature without initiating the reduction of gold acetate by 1, 2-Hexadecylamine, which starts around $50 - 60^\circ C$. Thus, the procedure is complex and requires significant skill in the general synthesis of NPs.

Interestingly, the gold-coating is not very homogeneous: the final solution consists of uncoated MNPs, gold-coated single MNPs and gold-coated MNP aggregates. Nevertheless, as originally suggested by Wang et al., centrifuging could be used to efficiently separate those three types of NPs.

Another limitation of this synthesis is that the synthesized MNP@Au NPs are capped by OA/OAm surfactants and thus only dispersible in organic solvents. This was addressed by adapting the solubilization method proposed by Tamang et al., which employed a two-phase solution and the ligand MUA to efficiently transfer OA-capped QDs to aqueous medium (see A2.2).

To improve the SERS signal of these MNPs@Au, a thin silver shell was further coated onto the MUA layer via reduction of AgNO$_3$ by sodium citrate at reflux (see A2.2). The MUA-capped MNPs@Au solution is heated to reflux to activate the reducing potential of the -COOH end of the MUA. Silver nitrate is then added and the solution is refluxed for 5 min to initiate the reduction of silver atoms onto the MUA layer, which serve as nucleation sites for subsequent silver deposition. Sodium citrate is then added while heating at reflux is continued for another 20 min, which allowed citrate to reduce the remaining AgNO$_3$ in solution onto the previously formed nucleation sites, to form a thin silver shell.
It is noteworthy that this silver coating method was found to be successful only if the MUA-capped MNPs@Au are first perfectly stable in water. Although Park et al. suggested the ligand exchange mechanism in which MUA replaces the OA/OAm surfactant via extensive sonication, this method did not yield stable MUA-capped MNPs@Au, as seen by a significant red-shift of their absorption spectrum relative to the original OA/OAm-capped MNPs@Au in hexane. When growth of a silver shell was subsequently attempted, the addition of AgNO$_3$ further precipitated the MNPs@Au, which is the reason Tamang et al.’s much more robust solubilization method was employed.

Finally, after much excitement about synthesizing these stable, small, aqueous MNPs@Au@Ag, it was discovered that MNP core was in fact too small to have an adequate magnetic response to an externally applied field, even if the solution was concentrated to enhance the collective magnetic response of the MNPs@Au@Ag. The search continued to discover the optimal MNPs@Au for the application in mind.

### 4.2.5 MNPs@Au on Fe$_2$O$_3$ using an iterative process

The iterative gold shell growth method proposed by Lyon et al. was an important milestone toward obtaining gold-coated iron oxide nanoparticles. The synthesis employed inexpensive reagents, was relatively simple and yielded MNPs@Au of size 50 – 100nm. One of the key findings of this study was the unexplained observation that gold could only be reduced onto MNPs if these were Fe$_2$O$_3$ MNPs, and not Fe$_3$O$_4$ MNPs. Although this discovery could not be explained from a crystal lattice mismatch standpoint (since both Fe$_2$O$_3$ and Fe$_3$O$_4$ had very similar lattices), it provided insightful information that enabled other groups, including ours, to customize their syntheses. Instead of using inorganically synthesized Fe$_3$O$_4$ that are subsequently oxidized to Fe$_2$O$_3$, monodisperse and organically-synthesized Fe$_2$O$_3$ were directly generated by following Hyeon et al.’s method (see A1.2), a TEM of which is shown in Figure 4.11. As observed, these magnetic nanoparticles are monodisperse and small, which are therefore optimal for subsequent gold coating.
These OA-capped MNPs were solubilized with TMAOH, prior to iterative gold reduction, and the iteration procedure (Lyon’s method in A2.3) produced the results depicted in Figures 4.12 and 4.13.

**Figure 4.11:** TEM of the Fe$_2$O$_3$ NPs resulting from Hyeon et al.’s method.

**Figure 4.12:** Growth progression of MNPs@Au monitored via absorption spectra. The initial synthesis conditions were tweaked to yield varying MNPs@Au, but the iterations were identical in each case: (a) 250µL MNPs with 500µL 0.1M citrate, (b) 500µL MNPs with 500µL 0.1M citrate.
The obtained spectra in Figure 4.12 illustrate the blue-shifting of the solution’s absorption spectrum, with iterative addition of gold salt and reducing agent. This trend is in agreement with Lyon et al.’s original report. With confirmation from TEM images, this shifting translates the increasing gold deposition on MNP nanoparticles, which have a jagged surface after the first iteration, but become rounder as more gold is deposited.

Figure 4.13: MNP@Au obtained by coating Fe_{2}O_{3} nanoparticles using Lyon’s iterative method. This solution of nanoparticles has an absorption peak at 540 nm, and depict the (a) original sample that contains MNP@Au and uncoated MNPs and (b) centrifuged sampled, demonstrating the size-based separation that is possible.

It is noteworthy that this iterative procedure does not coat individual MNP, but rather aggregates them during the coating process to generate significantly larger particles. Indeed, after five iterations, the gold-coated nanoparticles are relatively large, Figure 4.13. The advantage of obtaining such large nanoparticles is that they can easily be separated from the uncoated MNPs by simple centrifuging.

Nevertheless, although this method has been employed by a number of groups for their own application, it quickly became evident that it would not suit the application at hand for a number of reasons. Firstly, although they were relatively stable, these large MNP@Au settled down fairly quickly, even with a thick coating of SH-PEG_{5000} that increases stability in aqueous medium. Secondly, and more importantly, the thickness of
gold deposition on single or even multiple MNP core was significant such that the magnetic response of the MNPs@Au was significantly reduced. Finally, the inhomogeneity of their size distribution, ranging from 30nm to 100nm, would reduce the predictability of their formation when concentrated by the external magnetic field.

4.2.6 MNP@Au using a one-step, sodium citrate method

The methods explored thus far were unsuccessful at producing small and stable gold-coated magnetic nanoparticles via a simplistic route. Among the aforementioned syntheses that were attempted, the most attractive in terms of its coating simplicity was Lyon et al.’s, but it was accompanied with the most notable limitation being their large size and inhomogeneity.

After synthesizing MNPs@Au using Lyon’s iterative procedure, it became evident that each addition of the strong reducing agent hydroxylamine resulted in the immediate reduction of gold ions in solution onto the MNPs, chaotic. This hypothesis was supported by analyzing TEM images of the resulting NPs following each iteration step, which appeared similar to those provided in Lyon’s paper. The first addition of hydroxylamine results in a rapid color change of the solution to dark blue, which is associated with a jagged gold-coating on the MNPs. With subsequent steps, the reduction of gold adds to the crevices to effectively smoothen the MNPs@Au, which translates to a blue-shifting of their plasmon band towards 540nm. The main reason for the initial blue solution is not due to a thin gold shell on individual MNPs, but rather to the growth of gold protrusions on MNP aggregates. This also elucidated the reason for having multiple iteration steps, to attempt to control the reduction of gold onto the MNPs.

Rather than employ the strong reducing agent hydroxylamine which rapidly and uncontrollably reduced gold onto the MNPs, it was hypothesized that the weaker, much slower reducing agent sodium citrate, which was originally employed by Turkevich et al. to synthesize gold colloids, would allow for the more controllable synthesis of MNPs@Au
possessing a narrower size distribution, a smaller average size and thus better stability in solution.

To minimize uncontrolled MNP aggregation during gold-coating, a small MNP size was selected onto which to grow the gold shell. Thus, this protocol successfully employed either oxidized Fe₃O₄ MNPs (Colvin method) or directly synthesized Fe₂O₃ MNPs (Hyeon method).

*MNP@Au Synthesis and Analysis*

Before diving into the details of the actual synthesis, it is important to first discuss the challenges with synthesizing core-shell NPs. Apart from the actual details pertaining to the synthesis of MNP@Au, the greatest difficulty lies in properly characterizing such core-shell structures, especially when the electron density of the core is significantly lower than that of the shell.

Both the AFM and SEM yield surface topologies at the nanoscale, but they are unable to provide information of the interior of a NP. Furthermore, the sample preparation for AFM imaging is very demanding since the concentration of NPs needs to be dilute enough to obtain single, isolated NPs. Then comes the time investment required to image a sufficient number of NPs to enable confident characterization of the whole solution. Finally, the resolution of the AFM is limited by the tip resolution and movement along the z-axis, which poses problems for NPs that are too small or too large. The SEM is based on imaging the signal reflected off nanostructured surfaces, which requires the surface to be conductive. However, the poor resolution for NPs < 50nm and inability to image dielectric NPs such as Fe₃O₄ severely limit its use for characterizing both MNPs and MNP@Au.

For the characterization of individual NPs, the TEM is by far and large the easiest, fastest and most accurate imaging method. A beam of electrons is focused onto the sample specimen and a 2D image of shadows, whose contrast is based on the electron densities of the nanostructures in the sample, is acquired in transmission mode below the focal plane.
plane; this therefore allows both metallic and dielectric NPs to be imaged with nanometer resolution. Similarly to the AFM, a monolayer of individually separated NPs is best to obtain clear images; however, in contrast to the line-scanning tip of the AFM, the resolution is theoretically limited by the wavelength of an electron and 2D imaging and magnification occurs in real-time allowing rapid acquisition and sample scanning. Importantly, the transmission nature of this imaging method enables the internal structure of core-shell nanoparticles to be visualized, but only under two specific conditions: firstly, if the core is denser than the shell (such as for silica-coated iron oxide NPs previously presented, or silica-coated gold NPs), or secondly, if the core is less dense than the shell and the shell is extremely thin. Due to the high electron density of gold, a gold shell thicker than $2 - 3\text{nm}$ completely blankets the interior of MNPs@Au such that these MNPs@Au appear to be whole Au NPs under TEM.$^{260}$ As such, although the TEM provides accurate information on the size and shape of MNPs@Au, it is unable to confirm the coating of MNPs by a gold shell.

With regards to specifically characterizing magnetic nanoparticles, such as $\text{Fe}_3\text{O}_4$ and MNPs@Au, magnetic AFM (MFM) might provide confirmation about the magnetic properties of the MNPs@Au in response to an external field. Nevertheless, in terms of resolution and imaging capabilities, the MFM is as limited as the AFM. Superconducting Quantum Interference Devices (SQUIDs) are another instrument that allow the measurement of extremely small magnetic fields down to the attoTesla regime. The gold coating of iron oxide nanoparticles would reduce their effective magnetic dipole relative to uncoated MNPs, which would help confirm the presence of a magnetic core, however, the unavailability and expense associated with this device prevented its use in this research.

One of the most useful characterization methods of NPs in solution is obviously the UV-Vis spectrometer. However, it is important to also realize the limitations of the UV-Vis analysis: although core-shell structures typically exhibit a red-shift of their plasmon peak relative to their whole counterparts, with the shift extent being inversely proportional to
the shell thickness, such a red-shift does not only necessarily imply core-shell NPs. Indeed, as any expert in the field of nanoparticle synthesis would acknowledge, an increase in diameter of whole NPs is accompanied by slight red-shifting, surface protrusions on the surface of spherical, whole NP also translates to a red-shift, different NP geometries (rods, cubes, stars) significantly red-shift the NP plasmon peak even as far as the NIR, and finally, because surface plasmon is also directly related to the difference in dielectric between the NP and surrounding environment, its surface chemistry and solvent in which they are dispersed also produce a red-shift in the plasmon peak. Baring these characteristics in mind, however, the absorption spectrum of a nanoparticle solution gives a wealth of information that can be used to infer the structure of NPs in solution. In addition to the plasmon peak position, the width of the plasmon band, the tail-end of the plasmon band, the magnitude of the plasmon peak, and finally the evolution of the plasmon band over time can be employed together to shed light on the type of NP that is present in solution. Moreover, compared to the aforementioned imaging techniques that characterize individual NPs, the UV-Vis provides an ensemble average of all the NPs in solution, which therefore offers both important and complementary information.

Considering the advantages and limitations of the available techniques, the synthesized MNPs@Au were characterized via a combination of UV-Vis absorption and TEM to understand their geometry, and the use of a simple rare-earth magnet to confirm their magnetic behavior.

**Synthesis protocol**

The MNP seeds were synthesized organically, either via Colin’s method to produce 12nm monodisperse Fe₃O₄ NPs, or via Hyeon’s method to generate γ-Fe₂O₃ NPs of about 10 – 12nm in diameter. Their syntheses were conducted under inert atmosphere as discussed at the start of this chapter and detailed in Appendix 2.

Following an involved washing procedure, solubilization to aqueous medium was achieved via ligand exchange with TMAOH. These two crucial steps are intricately de-
tailed in Appendix 2 under Washing and Solubilization of OA-capped MNPs. As observed by Lyon2004, gold seems to preferentially deposit onto oxidized Fe₃O₄ NPs, which is not explained by their nearly identical lattice parameters of \( a = 0.840 \) and 0.835, respectively. Oxidation of Fe₃O₄ in air results in Fe₂O₃ via Fe cation diffusion, but the oxidation reaction can also be accelerated by heating the solution of Fe₃O₄ at a low pH, as detailed in Appendix 2 under Oxidation of Fe₃O₄ MNPs.

The following results were obtained using the \( \gamma - \text{Fe}_2\text{O}_3 \) NPs that were directly synthesized via Hyeon’s method and left to aerate in a centrifuge tube, dispersed in 1% TMAOH for 3 months - the solution color had turned brown from its original black color, indicating its oxidative state.

The solution is then stirred for 10 minutes to replace some of the surface-bound TMAOH with citrate ions and facilitate gold reduction at the MNP surface. During the 20 min reaction, the solution underwent a series of colors that were atypical of standard gold colloid synthesis.

The reagents employed for this synthesis were sodium citrate (99%), silver nitrate (AgNO₃ 99.999%), HAuCl₄ 99.99%, TMAOH (1% in DI) and Ultrapure DI water. The required equipment was a 25mL conical flask with fitting condenser, a rectangular medium stirbar, a stir/hot plate and 20mL scintillation vials.

The reagent volumes were selected according to Table 4.1.

**MNPs@Au protocol**

1. Set the hot plate to 200°C and let equilibrate

2. Add 10mL of DI to a 20mL scintillation vial

3. Inject a volume \( V_{TMAOH} \) and \( V_{MNP} \)

4. Add a volume \( V_{Cit} \) to the solution and cap vial

5. Bath sonicate for 10min to disperse MNPs
Table 4.1: Experimental conditions investigated to analyze MNPs@Au

<table>
<thead>
<tr>
<th>Exp #</th>
<th>MNP</th>
<th>TMAOH (1%)</th>
<th>Citrate (0.1M)</th>
<th>HAuCl₄ (1%)</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50µL</td>
<td>0µL</td>
<td>100µL</td>
<td>150µL</td>
<td>dark red</td>
</tr>
<tr>
<td>2</td>
<td>50µL</td>
<td>0µL</td>
<td>50µL</td>
<td>150µL</td>
<td>dark purplish</td>
</tr>
<tr>
<td>3</td>
<td>50µL</td>
<td>0µL</td>
<td>25µL</td>
<td>150µL</td>
<td>cloudy pinkish-red</td>
</tr>
<tr>
<td>4</td>
<td>50µL</td>
<td>0µL</td>
<td>50µL</td>
<td>100µL</td>
<td>dark red</td>
</tr>
<tr>
<td>5</td>
<td>25µL</td>
<td>25µL</td>
<td>50µL</td>
<td>150µL</td>
<td>dark purplish</td>
</tr>
<tr>
<td>6</td>
<td>10µL</td>
<td>40µL</td>
<td>50µL</td>
<td>150µL</td>
<td>dark purplish</td>
</tr>
<tr>
<td>7</td>
<td>10µL</td>
<td>40µL</td>
<td>50µL</td>
<td>100µL</td>
<td>pinkish-red</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Exp #</th>
<th>MNP</th>
<th>TMAOH (1%)</th>
<th>Citrate (0.1M)</th>
<th>HAuCl₄ (1%)</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>0µL</td>
<td>50µL</td>
<td>100µL</td>
<td>150µL</td>
<td>light red</td>
</tr>
<tr>
<td>9</td>
<td>0µL</td>
<td>50µL</td>
<td>50µL</td>
<td>100µL</td>
<td>light red</td>
</tr>
</tbody>
</table>

6. Stir vigorously for 10 min to replace surface-bound TMAOH ions with citrate

7. Transfer contents to conical flask

8. Inject volume $V_{HAuCl_4}$ and place on heater with vigorous stirring

9. Insert condenser into vial and heat to reflux over 12 – 20 min, depending on reaction conditions

10. As the reaction proceeds, the initial clear yellowish solution slowly transitions from brownish/blue to deep purple and finally pinkish-red/red, depending on the reaction conditions

11. For MNPs@Au, remove from heat and let cool to RT, or continue with MNPs@Au@Ag protocol to add a silver shell

Compared to Lyon et al.’s method which iteratively reduces gold onto oxidized $\text{Fe}_3\text{O}_4$ to yield a wide distribution of relatively large nanoparticles, the method developed here produces a narrow distribution of smaller MNPs@Au with a single, initial injection of gold chloride. Although this protocol is simple, there are several variables that can be
tweaked to yield very different results, namely the concentrations of citrate, HAuCl₄ and MNP seed. These will be discussed next.

**Effect of sodium citrate concentration**

Citrate acts both as the reducing agent and as the surfactant that stabilized the nanoparticles as they are coated. The effect of citrate concentration on the final MNPs@Au can draw parallels from its effect in the synthesis of gold colloid, originally published by Turkevich et al.: a low citrate concentration results in larger nanoparticles than a high citrate concentration. As nanoparticles grow in size, their surface area to volume ratio decreases; thus, for the same concentration of gold in solution, the total volume remains the same but the total surface area decreases. For a low citrate concentration, the initially poorly stabilized nanoparticles will keep growing until their total surface area in solution is low enough to be completely stabilized by the available citrate molecules in solution; this results in large nanoparticles. For a high citrate concentration, the nanoparticles grow to a much smaller extent since the citrate is able to stabilize them at a much higher total surface area, such that the nanoparticles are unable to grow further.

The same observation is seen when synthesizing MNPs@Au using citrate, Figure 4.14. A blue-shifting of the solution similar to Lyon’s method is observed, but unlike that method, the blue-shifting here is not due to large jaggered nanoparticle aggregates that are immediately formed as soon as the strong reducing agent formaldehyde is injected into the solution. Rather, it is due to the nucleation of very small gold nanoparticle seeds, similar to the Turkevich method, which, as they grow, begin displaying the typical plasmon resonance that is expected of small gold nanoparticles. Indeed, the time scale over which this blue shifting takes place is much longer, indicating a much more controlled gold deposition in solution, which is desired. It is also noteworthy that the reaction time to completion increased substantially with increasing citrate concentration - a higher surfactant concentration is expected to reduce the nanoparticle growth speed since they are better protected and thus more stable. As the citrate concentration is decreased, the solu-
Figure 4.14: Absorption spectra depicting the effect of citrate concentration on the formation of MNPs@Au, keeping other reagents constant: (a) Comparison of obtained MNPs@Au for citrate volumes 100µL, 50µL and 25µL after the reaction time of 12 minutes. These conditions correspond to experiments #1, 2 & 3 in Table 4.1, respectively. Growth dynamics of MNPs@Au analyzed by icing 100µL aliquots to halt the reaction at times $t = 2, 4, 5, 6, 7, 8 & 12$ minutes, for (b) 50µL and (c) 100µL citrate.

Plasmon red-shifts as the resulting MNPs@Au increase in size. This size increase is also accompanied by a faster flocculation of the nanoparticles to the bottom of the vial.

Interestingly, the 50µL citrate volume produced a solution that was slightly purple, due to elongation of the nanoparticles, as inferred by its more prominent absorption tail. This is confirmed via TEM imaging, which shows the interesting formation of elongated
nanoparticles compared to a sample with twice the citrate volume, Figure 4.15. A close look at these elongated particles in (b) would suggest that they are formed by the joining of two or more MNPs that were in the process of being coated. Further investigation showed that this occurred not as a function of citrate concentration alone, but rather as a function of \([\text{HAuCl}_4]:[\text{Citrate}]\) ratio. Tweaking this ratio allows for the MNP@Au shape to be adjusted.

**Figure 4.15**: TEM images of MNPs@Au synthesized from experimental conditions in Table 4.1, illustrating the effect of changing the \([\text{HAuCl}_4]:[\text{citrate}]\) concentration ratio - (a) Exp #1 and (b) Exp #2.

It is crucial to observe that although elongated, these nanoparticles are still small and the solution remained stable for extended periods of time. This discovery also served as an important validation result that confirmed the successful coating of the MNPs by gold. Because the most energetically favorable shape is a sphere, this interesting shape is one that is most likely to occur by the merging of two or more core particles as they are continually coated with gold. This provided further confidence in the validity of the protocol.

As a final note, it is worth mentioning that the presence and concentration of the origi-
nal TMAOH surfactant used to solubilize and stabilize the MNPs also affects the reaction. TMAOH is a surfactant that keeps the MNPs stable during the slow deposition of gold at elevated temperature, and attempting to rid the original MNP solution of TMAOH results in large aggregated MNPs@Au similar to those generated by Lyon’s method.

**Effect of HAuCl$_4$ concentration**

The concentration of HAuCl$_4$ was not a crucial factor in the synthesis, except that a threshold did exist, below which no apparent gold growth could be visually observed ($\sim 75\mu$L of added HAuCl$_4$, data not shown). Figure 4.16 illustrates the reaction dynamics with varying HAuCl$_4$ concentrations. The slight increase in absorption intensity is likely a result of the slight increase in nanoparticle size due the greater availability of Au ions, which was also accompanied by slight plasmon red-shifting. Interestingly, the lower the concentration of HAuCl$_4$, the longer the reaction time, as observed by the rate of change in the absorption spectra.

With regards to MNP concentration, the volume initially injected into the solution did not show any remarkable effect on the resulting MNPs@Au. Similarly to Lyon’s method, the yield of coated MNPs is no where close to 100% and many uncoated MNPs remain in solution. In comparison, however, this protocol generates significantly smaller and more stable MNPs@Au that are more suitable for the application at hand.

**Validating MNPs@Au and not a mixture of MNPs and Au NPs**

Since the reaction solution was deep red and not purple, as is typically expected of a core-shell structure of gold, the reaction was performed with identical conditions, but without the initial injection of MNPs as seed. If gold was truly coated on the MNPs, it is expected that their corresponding reaction dynamics, and final absorption spectrum, would convey appreciable differences. The results are depicted in Figure 4.17.

It is noteworthy that because the MNPs were stabilized by TMAOH, a meaningful comparison was ensured by adding TMAOH to the seedless reaction vials to the same final concentration as their corresponding seeded-solutions.
Figure 4.16: Absorption spectra depicting the effect of (a) MNP volume and (b-c) HAuCl$_4$ volume on the formation of MNPs@Au, keeping other reagents constant, and ensuring reaction comes to a completion prior to measurements. Figure (a) presents a comparison of obtained MNPs@Au for initial MNP volumes of 50$\mu$L, 25$\mu$L and 10$\mu$L, which correspond to experiments #2, 5 & 6 in Table 4.1, respectively. Figures (b) and (c) display the effect of HAuCl$_4$ volume, for two different MNP concentrations, for which (b) corresponds to experimental conditions #2 & #4 and (c) corresponds to conditions #6 & #7 in Table 4.1, respectively.

The first row of figure 4.17 compares the final absorption spectra of Au NPs and MNPs@Au. From this side-by-side superposition, a number of key observations can be made. Firstly, the peak position of the MNPs@Au is in fact red-shifted relative to its Au NP counterpart. This is observed for both pairs of reactions, but is more prominent between experiments #1 and #9 with a red-shift of 8nm. Although this may not seem like much, it confirms that, if anything, the two solutions are dissimilar. Secondly, the larger width of the absorption spectra of MNPs@Au compared to Au NPs is also another confirmation that core-shell structures are present in the solution. The plasmonic behavior of a core-shell structure, where the plasmon oscillation is somewhat dampened by the dielectric core, typically yields a wider plasmon band.

A comparison of the dynamics of the reaction provides more evidence that the nanopar-
FIGURE 4.17: Comparison of the absorption spectra dynamics between Au NPs and MNPs@Au to validate the gold coating of MNPs. Two pairs of experiments from Table 4.1 are compared: Exp #8 (no MNP) v.s. Exp #1 (w/ MNP) and Exp #9 (no MNP) v.s. Exp #4 (w/ MNP), while keeping all other reaction conditions constant.

The most important observation here is that the reaction of MNPs@Au is significantly slower than pure Au NPs - this is true for both sets of pairs. Without being...
able to visually confirm (under TEM or by other means) that there really is a core under the dense gold shell, it can be inferred by the evidence thus far, and more so by the fact that the MNPs@Au could be separated from the solution by application of an external magnetic field (rare earth magnet).

Thus, it can be inferred that the main reason that the MNPs@Au appear deep red is simply because the gold shell is relatively thick compared to the core size. The previously conducted simulation of gold-coated silica nanoparticles showed that if the shell thickness is above $40 - 50\%$ of the overall particle radius, the resulting plasmon shift would be unremarkable compared to a Au NP of the same size. This can be intuitively understood by realizing that as the gold shell thickness tends to infinity, the core-shell nanoparticles behaves increasingly more as if its dielectric core were absent. Here, the MNPs@Au are at least 30nm in diameter and the initially injected MNPs were approximately 10nm in diameter, implying a Au shell thickness of around 10nm. This would make the gold shell thickness greater than 50\% of the nanoparticle radius of 15nm and would explain the solution’s reddish color.

A final note on the organic v.s. inorganic synthesis of MNPs is worth mentioning. Although organically synthesized MNPs were employed in this synthesis, it is evidently easier to inorganically synthesize Fe$_3$O$_4$ NPs and subsequently oxidize them to Fe$_2$O$_3$, since this does not require an inert atmosphere. Based on the fact that Lyon et al. were able to coat Fe$_2$O$_3$ synthesized in such a manner, this novel citrate-based gold coating method is also expected to yield similar results. This was not confirmed due to lack of time, but will be done at a later date. The only drawback using this method is the lower crystallinity of the generated MNPs, whose smaller size is less controllable than organically synthesized MNPs.

**Purification of MNPs@Au**

Similarly to Wang et al. and Lyon et al., the synthesis yield of MNPs@Au was not 100\%, and the final solution comprised of both bare MNPs, MNPs@Au and most probably
Au NPs. Due to their stability and small size, efficient magnetic separation by an external magnet, even 6000G, takes several days for a few milliliters. Although this method was employed to confirm the coating of gold onto the MNPs, the unseparated MNPs@Au were used for further studies to demonstrate the feasibility of the micromagnets for concentrating the particles, with or without purification. With simplicity of the final device in mind, this would be an important attribute of the MNPs@Au synthesis. Also challenging to purify MNPs@Au from uncoated MNPs, since they were both similar in size and both stable in solution. Two possible ways were considered: dissolving the iron oxide with concentrated HCl, a method which also dissolved the MNPs@Au core as witnessed by the disappearance of their magnetic response, and gently tweaking the centrifugation speed and time to selectively collect the heavier MNPs@Au from the mixture of MNPs@Au and bare MNPs, a method proposed by Wang et al. Not too much time spent on optimizing this method and is something that an interested reader would be encouraged to attempt. However, to minimize the bare MNPs in the final solution, caution was taken to use only the minimum MNPs as seed onto which to grow the gold shell.

4.3 Synthesis and optimization of silver coated MNPs@Au

Gold NPs are typically weaker SERS substrates than silver NPs by about two orders of magnitude, owing to the stronger plasmon resonance of silver metal. Obtaining a strong SERS substrate is advantageous because it increases the detection sensitivity of the nanoparticle system. The synthesized MNPs@Au were thus coated with a silver shell, immediately subsequent to their synthesis.

A uniform, reproducible silver coating of the MNPs@Au was achieved by iteratively adding silver nitrate (AgNO$_3$) to the MNPs@Au solution under reflux, and allowing the citrate to both reduce the silver onto the MNPs@Au and stabilize the final MNPs@Au@Ag. The protocol is detailed below.
MNPs@Au@Ag protocol

1. Set the hot plate to 200°C and let equilibrate

2. Inject 5 – 25µL volumes of AgNO₃ into vigorously stirring solution of MNPs@Au under reflux

3. Let reaction proceed for 1 min and iterate through AgNO₃ additions until desired silver shell thickness is achieved (monitored via UV-Vis)

4. As the reaction proceeds and with each iteration, the initial deep purple or red solution slowly transitions to red, orange, brown and increasingly deep brownish-yellow as the silver shell thickens, which increases the scattering coefficient of the MNPs@Au@Ag

5. After reaction completion, remove from heat and let cool to RT

6. Centrifuge at 500g for 10 min to pellet out the oxide byproduct & repeat if necessary

7. Filter solution through 0.22µm cellulose membrane filters and store for further use

This iterative procedure, whereby small aliquots of AgNO₃ are incrementally added to the refluxing solution, allows the slow and controlled growth of silver on the MNPs@Au, while minimizing the unwanted nucleation of silver NPs. The growth of the silver shell was monitored via the solution’s absorption spectrum over time, Figure 4.18(a), as well as via TEM.

Figure 4.18(a) depicts the transitioning absorption spectrum peak of the MNPs@Au, from around $\lambda = 520\text{nm}$ to $380\text{nm}$, for MNPs@Au (corresponding to Exps 1 in Table 4.1), as they are successively coated with an increasing thickness of silver. In addition to its blue-shifting, the plasmon peak also increases in amplitude, characteristic of increasing absorption and scattering; as the NPs become larger and more silver-like in their plasmonic behavior. The increasing silver shell thickness was confirmed under TEM, depicted in
Figure 4.18: Synthesis and Raman characterization of MNPs@Au@Ag of increasing Ag shell thickness: (a) absorption spectra of the iterative growth of a silver shell on MNPs@Au, via the reduction of AgNO$_3$ by sodium citrate and (b) averaged Raman intensity ($\pm$SD) of the 1132 cm$^{-1}$ Raman peak of DTDC of 3 spectral acquisitions, as a function of Ag shell thickness, when 200 nM of DTDC was mixed into solution. The inset presents a baseline-corrected Raman spectrum of one of the 100 µL AgNO$_3$ acquisitions, denoting the 1132 cm$^{-1}$ Raman peak of interest.

Figure 4.18(c). Similar trends to those observed here were also reported in the literature for Au NPs that had been coated with silver shells of increasing thickness, but with ascorbic acid as the reducing agent.$^{288}$

The versatile silver-coating scheme herein presented allows the MNPs@Au to be coated with silver either directly subsequent to their synthesis (as detailed in the protocol above), or by reheating to reflux a previously synthesized stock solution of MNPs@Au solution that has since been stored at RT.

It is noteworthy, however, that the solution becomes cloudy upon addition of AgNO$_3$ due to this chemical’s reaction with the basic TMAOH that was used to stabilize the original MNPs in aqueous medium. The reaction of AgNO$_3$ with a base produces a dark-grey, insoluble silver oxide, Ag$_2$O, which renders the solution cloudy and also explains the high
concentration of AgNO₃ required to grow even a thin silver shell. The majority of this Ag₂O byproduct, as well as any large NP aggregates that form during the synthesis, is successfully removed from the solution via low speed centrifugation followed by simple filtration.

Moreover, the filtered MNPs@Au@Ag solution appears more stable than the solution of MNPs@Au that serve as seed and onto which the silver shell is grown. This was noted by the very minimal settling of MNPs@Au@Ag at the bottom of their storage flask after several days, compared to the solution of MNPs@Au, which displayed nanoparticle depositions after 24 hours. This is most likely due to a number of reasons, including an increased concentration of the stabilizing citrate moiety in solution, an increased MNPs@Au@Ag surface area that effectively hosts a larger number of molecules and thus stronger stabilizing, repelling surface charge and finally the filtration of all large aggregates from the solution that were formed during synthesis. Combined with the fact that the final MNPs@Au@Ag are still not very large, as per their TEM, they are able to display very good stability in solution.

A final remark before moving on to the next section is that the entire reaction process, from coating of MNPs with Au to further coating of MNPs@Au with Ag, is scalable. The reaction was 10× scaled up in volume and yielded very similar nanoparticle solutions. This is an important feature because it allows the preparation of large batches, which taps into economies of scale in terms of manufacturing time and resources.

While it may be desirable to keep increasing the silver shell thickness deposited on the MNPs@Au to maximize their potential as SERS substrates, there is most probably a compromise that exists between NP shell thickness, and thus overall diameter, and magnetic maneuverability of the NPs. Similarly to how a gold shell coating on MNPs decreases their magnetization moments, it can be envisaged that a thickening silver shell further decreases these moments, effectively reducing the MNPs@Au@Ag’s responsiveness to an
externally applied field. In order to retain as much of their magnetic response as possible while exploiting the plasmonic response of silver for SERS, the MNPs@Au@Ag should be designed to have a silver shell thickness that gives strong SERS while keeping the overall MNP@Au@Ag diameter as small as possible; this also has the added advantages of maximizing their stability in solution, as well as minimizing their optical absorption and scattering coefficients, which increase with silver shell thickness.

With the vision of integrating these MNPs@Au@Ag with optical and Raman microscopies, together with their manipulation at high concentration and ultimate desire of SERS quantification, the last two characteristics (high stability and low optical density) are particularly important: firstly, unstable NPs flocculate out of solution such that their controllable manipulation becomes challenging, secondly, their SERS signal would not be quantifiable since the uncontrollable formation of hotspots would prevent any reliable prediction to be made, thirdly, since optical and Raman microscopies are to be used, both of which rely on the optical penetration of light with minimal absorption and scattering, the scattering and absorption coefficients of MNPs@Au@Ag must be minimized to allow their concentration to a high volume fraction in solution, which greatly facilitates their manipulation.\textsuperscript{233} Thus, the aforementioned desired characteristics impose the limitations by which the synthesized MNPs@Au@Ag need to adhere.

The compromise between overall size of the MNPs@Au@Ag and their potential as SERS substrates was investigated by functionalizing MNPs@Au@Ag of increasing silver shell thickness with the Raman molecule 3’-diethylthiadicarbocyanine iodine (DTDC) and detecting the generated SERS signal from their surface. This allowed the optimal silver shell thickness to be determined and selected for the subsequent studies. This result of this study is presented in Figure 4.18(b).

The Raman molecule DTDC was selected for two specific reasons. First, it has strong electronic transitions in the visible spectrum, exhibited by a broad absorption peak at $\lambda = 655\text{nm}$. Using a He-Ne laser centered at $\lambda = 633\text{nm}$ as the excitation source allows access
to resonance Raman enhancement, which greatly amplifies the Raman signal and thus increases the nanoparticle system sensitivity. Second, it contains two sulfur groups in its ring structures and therefore offers an “affinity tag” for binding to gold or silver surfaces, yielding a strong metal-sulfur bond that is stable against washing and detaching over time.

In order to determine the relationship between silver shell thickness and generated SERS signal, the DTDC concentration in solution was ensured to be low enough to prevent nanoparticle aggregation, which would invalidate any comparison. A 200nM final concentration of DTDC in solution was found to allow the stable suspension of MNPs@Au@Ag while producing a strong enough Raman signal for detection by the spectrometer. The measurements were performed on 100µm samples in a 96-well plate, via excitation with a 20mW laser through a 20× objective. Ten second acquisitions were repeated three times. The results are presented in Figure 4.18(b).

The inset depicts the baseline corrected Raman spectrum detected using MNPs@Au@Ag with the addition of 100µL AgNO₃, and highlights the peak at 1132 cm⁻¹ that is isolated to subsequently produce the bar graph.

The results suggest that an optimal silver-shell thickness exists at which the SERS is maximized. Although this is counterintuitive, this trend is in accordance with the report published by Liu et al. Analyzing the data in more detail reveals several important insights. The uncoated MNPs@Au exhibit undetectable SERS for their size and at this DTDC concentration. As soon as a very small amount of silver is grown onto the MNPs@Au, denoted by a slight blue shift of the absorption spectrum, the Raman signal becomes prominent, confirming the strength of silver as a stronger SERS substrate. The strongest SERS was obtained for a 100µL addition and will thus be the volume used for future experiments. Interestingly, the sudden drop of the SERS signal between AgNO₃ additions of 100µL and 125µL is intriguing and worthy of discussion. This can be understood by simultaneously analyzing their corresponding absorption spectra. As aforementioned, the basic solution of nanoparticles turns cloudy with the addition of AgNO₃ because of
the formation of particulate Ag$_2$O. The small changes in the absorption spectra following the initial incremental additions of AgNO$_3$ up to 100µL suggest that substantial amounts of this volume are used up by the reaction with the TMAOH base, with only the remaining small amount contributing to shell growth. The 25µL AgNO$_3$ increment from 100µL to 125µL is accompanied by a jump in absorption intensity. It is hypothesized that at this point, most of the TMAOH base had been used up, such that the added AgNO$_3$ volume would contribute nearly entirely toward growing the silver shell. This sudden growth in shell thickness would have masked the incrementally decreasing SERS intensities expected by a slowly increasing shell thickness, which is reported by Liu et al.. However, since the SERS intensity remains at a low plateau from that point onward, the optimal silver thickness for SERS detection can be assigned by a volume addition of 100µL.

Finally, it is important to bear in mind that in contrast to gold, silver is oxidized over time by oxygen in solution. Although the oxide layer is relatively thin such that significant silver may still lie beneath to ensure a strong SERS effect, the problem with the oxide is that it alters the nanoparticle surface chemistry such that silver-binding entities (thiol, amines, etc) are no longer able to bind. Since SERS is a surface-confined effect, this effectively renders the MNPs@Au@Ag obsolete after an extended period of time. Simple solutions to this would be to functionalize them as soon as they are made, or make a new batch prior to each functionalization.

4.4 Development of a stable Raman probe using MNPs@Au@Ag

Although a dilute solution of MNPs@Au@Ag can remain well-dispersed even post-addition of the Raman dye, the efficient concentration of MNPs@Au@Ag by an applied magnetic field requires a high volume fraction of particles; as per the MNP theory later discussed in Chapter 5, the higher the concentration of particles, the stronger the collective force experienced by the MNPs@Au@Ag in solution. Practically, this is achieved via strong
centrifugation of the NPs into a pellet and redispersion into a small volume. Unfortunately, the MNPs@Au@Ag collapse and cannot be redispersed, unless they are first further functionalized by stabilizing ligands.

The thiolated-PEG ligand family is often used to stabilize gold and silver nanoparticles in solution, because the affinity of the sulfur moiety these metals. Compared to bare PEG, which binds non-specifically and is easily displayed from the nanoparticle surface by stronger chemical bonds, the presence of a thiol group ensures the PEG is properly anchored to the surface, making further functionalization and washing possible.

To a series of 100µL samples of MNPs@Au@Ag placed in a transparent 96-well tray, an increasing concentration of SH-PEG7 was injected, mixed and left to incubate for 10 minutes to ensure good thiolate attachment. To each well, the same volume of 2µM DTDC (10µM) was added and mixed. The final concentration of DTDC in solution was 200nM, chosen because it displayed minimal NP aggregation (assumed sub-monolayer coverage). After two-minute incubations, the SERS spectra were measured with a 20× objective, 100% power, and 10 second accumulation. Three accumulations of each well were measured for consistency.

The effect of increasing the MNPs@Au@Ag surface coverage with PEG is depicted by Figure 4.19. The schematic in (a) illustrates the degree of surface coverage by PEG as its concentration is increased prior to DTDC addition. With no addition of PEG, the nanoparticle surfaces are bare and all the DTDC molecules in solution adhere to the MNP@Au@Ag surface. Although DTDC displaces a fraction of the stabilizing citrate, the absence of PEG at the surface results in the maximum detected SERS signal. At the other extreme, full surface coverage of PEG leaves no room for attachment of DTDC, such that the injected dye remains floating in solution. Now the nanoparticle are very stable, but do not exhibit any SERS signal. The solution, however, has also become fluorescent due to the unattached dye that is no longer quenched. A compromise therefore exists between excessive and insufficient coverage by PEG, such that the nanoparticles are stable yet also
Figure 4.19: Study investigating the stabilization of MNPs@Au@Ag with increasing surface coverage of SH-PEG7: (a) schematic illustrating the process of increasing surface coverage by PEG; (b) SERS spectra of increasing PEG coverage with 0.2 µM DTDC dye.

The background fluorescence in this set of data was intentionally not subtracted be-
cause it provides insight into the relative concentration of DTDC dye that is suspended in solution. The trend depicted in (b) is as expected: the strongest detected SERS is attributed to an absence of PEG, and as PEG is incrementally increased, the measured SERS signal decreases. This occurs until a point where SERS signal becomes undetectable by the system and instead a fluorescent background appears, which keeps increasing with decreasing PEG coverage. Thus, to obtain SERS-active MNPs@Au@Ag with a strong enough SERS signal while ensuring their stability, a final concentration of 0.5\( \mu \)M PEG was selected. These SERS-active MNPs@Au@Ag were stored in the dark for their use described in Chapter 5.

4.5 Conclusion

The controllable manipulation of SERS-active, gold-coated magnetic nanoparticles in aqueous solution requires them to be stable (and therefore small by default), as well as easily functionalizable for labeling with SERS dye, antibodies or DNA capture probes. Although there is wealth of protocols in the literature that claim to produce functionalizable, gold-coated magnetic nanoparticles that abide by such stringent conditions, it was surprising to discover that these claims were in fact mostly subjective. For example, the term “stability”, when referring to colloidal nanoparticles, seems to have a subjective definition: to some, no flocculation of the nanoparticle after 10 minutes, or a couple of hours, is “stable” but to the author of this thesis, stable is defined as insignificant flocculation even after 24 hours, if not longer. Anything less typically implies that the nanoparticles are too large, and therefore settle down under gravity, or insufficiently stabilized in solution by their surfactant. In this definition of the word, stable metal-coated nanoparticles are essential for their controllable manipulation, and in that sense, there was a definite lack of protocols that detailed the successful aqueous synthesis of such particles.

After an exhaustive period of trying to produce nanoparticles with ideal characteristics
for their manipulation by an external magnetic field, the author developed a simple, novel synthesis that involved coating Fe$_2$O$_3$ nanoparticles with gold using citrate as the both the reducing agent and surfactant. These nanoparticles were extensively characterized by UV-Vis and TEM to ensure that the injected magnetic seeds were indeed coated with gold. The gold-coated magnetic nanoparticles were stable in solution and easily functionalizable. In order to improve the SERS potential of these substrate, they were further coated with a layer of silver, whose thickness was optimized to maximize the generated SERS signal originating from the surface-bound resonant Raman dye DTDC.

The final chapter details a method that allows for the controllable manipulation of the aforementioned nanoparticles to yield an enhanced and reproducible SERS signal. The nanoparticles are concentrated by the intense magnetic field gradient that is produced by custom-designed micromagnets with the application of an externally applied magnetic field. These micromagnet structures are then integrated into a digital microfluidic chip to demonstrate a proof-of-concept SERS-based diagnostic device, which provides a strong, reproducible SERS signal and which is portable, reusable and inexpensive to fabricate.
A Unique Concept for Sensitive & Reproducible SERS Detection

The research described in this chapter fulfills Aim 4

5.1 Introduction

This final chapter details the implementation of the authors vision, which was formed early on but took several years to achieve. As a reminder, the main aim of this thesis was to contribute a versatile platform that employs SERS as the transducer to sensitively and quantitatively detect cancer biomarkers from homogeneous samples. Chapter 4 presented the development of novel plasmonic magnetic nanoparticles, MNP@Au@Ag, which were synthesized to maintain strict properties that enabled their controllable manipulation while enhancing their emitted SERS signal. Chapter 5 focuses on utilizing these nanoprobe in conjunction with micromagnets and microfluidics to produce a novel platform that achieves the aforementioned versatility.

The distinct problem that needs to be addressed to leverage the SERS technique for
use in disease diagnostics is devising a platform that can yield sensitive and quantitative SERS data.

The first challenge is that of enhancing the detected SERS signal in a controllable manner such that the signal can be quantified within the same sample, as well as across different samples. This can be divided into two types of SERS substrates: colloidal nanoparticles and dry substrates. As previously discussed in Chapter 1, enhancing SERS via the controlled aggregation of nanoparticles has been attempted by several research groups. These systems that are employed to control NPs can be further subdivided into two categories, as defined by the author: passive systems, that do not employ external factors to induce aggregation, and active systems, that manipulate and engineer the surrounding environment to trigger nanoparticle aggregation. Passive systems comprise solution-based, colloidal substrates and dry, wafer-type substrates. In the case of colloidal substrates (nanoparticles), the SERS signal enhancement is achieved by collapsing the nanoparticles in solution with NaCl, or by adding specific molecules that trigger the formation of nanoparticle networks, such as complementary single strand DNA probes. The former represents a primitive, yet somewhat effective manner, to enhance the SERS signal but remains uncontrollable, producing nanoparticle clusters of hugely varying sizes and thus unquantifiable SERS. Although the latter systems are inexpensive and yield reproducible, quantitative SERS signals, their biggest limitation is the added complexity of the nanoparticle system that required to induce aggregation. The dry SERS substrates are an alternative to colloidal systems, and they achieve a high SERS enhancement factor by designing their nanostructured surface to exhibit a high density of electromagnetic hotspots, where the local E-field is strongly enhanced. Although this may seem as a flawless solution, there are also drawbacks with wafer-type substrates. Firstly, these substrates are one-time use devices that cannot be reused since SERS is obtained from target molecules by their adhesion to the substrates metallic surface. Secondly, their design and fabrication can be extremely complex, and therefore very expensive to manufacture, especially considering
their one-time use. Thirdly, the SERS signal is still not completely reproducible unless a single monolayer of target molecules is deposited onto the surface. This condition rarely happens unless the molecule comprises a metal-binding moiety (such as an amine or a thiolate group), and even with this very limiting feature, the substrate would still necessitate excessive surface washing to discard excess, unbound molecules. With the vision of integrating SERS into microfluidic chips, it is evident that there are unsolvable limitations with dry SERS substrates.

As such, the development of novel, active systems to induce nanoparticle aggregation has become an attractive field of SERS research. Active systems employ external structures or forces, such as tapering channels, capillary forces or magnetic forces, to induce aggregation in a more controllable fashion. Two notable strategies that have shown promise are the use of customized microfluidic channels and magnetic forces. Wang et al. demonstrated a single channel microfluidic device that used a 20nm channel pinch-off to trap silver nanoparticles and free molecules in solution, such that they collect and aggregate at this narrow junction, thereby increasing the measured SERS signal. Although this concept is elegant in that its use is simple, the largest drawbacks of this microfluidic device is its delicate fabrication and one-time use property: the nanoparticles and molecules in solution, once aggregated, cannot be washed out of the device. This means that each test requires a new device, rendering this method expensive in the long run. Additionally, since SERS is so sensitive to changes in aggregation state, this one-time nature also suggests foreseeable problems in device reproducibility, which will require high product quality control given the very strict pinch off dimensions of 20nm needed to pinch off the channel. Lee et al. synthesized novel SERS-nanoparticles that comprised of silica-coated superparamagnetic nanoparticle clusters that were encrusted with small silver nanoparticles. This allowed for their aggregation, and thus enhanced SERS, via application of an externally applied magnetic field supplied by a rare-earth magnet.

Although passive systems have demonstrated the capability to quantify SERS to a
greater extent that active systems, most of the published techniques in the literature experience similar limitations of SERS reproducibility and reusability, which unravels a strong need for new techniques that would address these problems.

The second challenge is integrating the SERS technique for cancer biomarker detection into a microfluidic chip, to tap into the features that make point-of-care devices attractive, such as high-throughput detection, automated sample manipulation, minimal sample usage and wastage, reusability, overall simplicity. Until now, this integration step has not been attempted by many groups, as detailed in the comprehensive review by Myers and Lee. This is most probably due to the complexity involved in successfully achieving all steps required to make it a truly SERS-based diagnostic chip:

1. Design of a sensitive SERS substrate (colloidal or wafer-based)
2. Functionalization of the SERS substrate to target disease biomarker of interest
3. Integration of SERS substrate onto microfluidic device
4. Reproducible and quantitative detection of SERS
5. Reusability of integrated system to reduce cost and encourage its widespread use

A number of research groups have been investigating the integration of SERS with microfluidics. These techniques all employ colloidal solutions with continuous flow microfluidics. For example, Choi et al. developed the “CD microfluidics” approach, but which a spinning disk comprising of radially extending microfluidic channels achieves the maneuverability required for sample manipulation and SERS detection. Strehle et al. employed a continuous flow microfluidic system that segments the sample flow into discrete sample packets, allowing for the high-throughput, reproducible detection of SERS in each sample packet. Such methods have successfully demonstrated that
the SERS technique can be integrated into microfluidic systems, similar to fluorescence-based techniques, however, they still lack the reusability feature that is required of versatile point-of-care devices and do not amplify the SERS signal via nanoparticle aggregation.

These challenges have yet to be satisfactorily addressed by the research community, and the author’s attempt to combine all 5 critical steps into a single device is the focus of this last chapter. It should be clarified, however, that the majority of this chapter is devoted to investigating and developing a novel SERS platform that facilitates the magnetic manipulation of plasmonics-active magnetic nanoparticles such as to yield an enhanced and reproducible SERS signal; only the last part of this chapter will address integration of this SERS platform with digital microfluidic technology. These novel concepts are explained in the next section.

Novel magneto-controlled SERS platform and integration with digital microfluidics

The system will consists of 3 independent, yet integrated, sub-systems that are designed to be modular by nature: each sub-system is able to function without the other two, yet they can also be assembled into a single system to achieve one purpose, whence stems the versatility of this platform. The concept is conveyed in Figure 5.1: the novel SERS platform is depicted in (a) and its integration onto a digital microfluidic platform is presented in (b).

The first system consists of the plasmonic magnetic nanoparticles, MNPs@Au@Ag, that were developed in Chapter 4. These are stabilized by citrate molecules, and thus can easily be functionalized with the capture probe of interest (such as thiolated-DNA strands that are complementary to cancer gene sequences). As a proof of concept, the MNPs@Au@Ag were functionalized with the Raman dye DTDC and SH-PEG for stabilization purposes. It is important to stress that the parameters required of these nanoparticles were stringent to ensure that they could be integrated into, and used with, the main system. This system deals with steps #1 – 3 above.
The second system consists of thin cobalt micromagnets, which, with the application of an external magnetic field, create intense local magnetic field gradients that are able to concentrate even superparamagnetic nanoparticles into a small volume. Their very small height of 70 nm make these micromagnets ideals for use in conjunction with aqueous droplets since their minimal z-profile prevents obstruction with fluidic movement. Finally, their simple fabrication allows these micromagnets to be conveniently grown on any smooth surface, and in this case, deposition on the top plate of a dual-plate microfluidic system. This top plate was made of quartz to allow the penetration of excitation sources and minimize autofluorescence, which is prominent in acrylics or glass. This system al-
allows for the enhanced, quantifiable and reproducible detection of SERS, and therefore covers step #4 in the above list.

The third system comprises of the overarching digital microfluidic technology that is based on electrowetting; the system is able to manipulate single nanoliter-droplets by controllably actuating the voltage between a grounded top plate and a bottom plate onto which tracks of electrodes are designed. In this case, the bottom plate comprised of the electrode tracks and the top plate was customized with the addition of micromagnets. The dual hydrophobic coating on the top and bottom plates, in conjunction with the separating oil medium, ensures that sample droplet can be completely dispensed once detection occurs, such that the channel remains contamination-free. This deals with the final step #5 in the above list.

It is noteworthy that each system spans a different scale: the nanoparticle system lies in the nanometer regime, the micromagnets span micrometers in length and width, and the microfluidic system manipulates droplets over millimeter distances.

Prior to diving into the details, however, it is important to briefly summarize some of the extensive work that has already been done in the fields of ferrofluid manipulation via externally-actuated micromagnets and digital microfluidics; both these concepts will be applied toward fabrication of the novel substrate demonstrated in this chapter.

Yellen and Friedman were pioneers in the field of ferrofluid manipulation by cobalt micromagnets\textsuperscript{234–236,239,240} and they extended their understanding of such structures by correlating the relationship between induced concentration gradients and externally applied magnetic field.\textsuperscript{233} They also demonstrated the successful manipulation of non-magnetic fluorescence beads by intricately controlling the applied magnetic field in three-dimensions such that the locally- induced magnetic field experienced by these micron sized particles shifted in the plane of the micromagnets.\textsuperscript{237,240} The proximity of the Yellen research group
to the Vo-Dinh lab, as well as the extensive interaction with its group members, fostered a good working environment from which was born the novel idea of integration magnetic SERS nanoparticles with micromagnet technology to achieve SERS enhancement and reproducibility.

Digital microfluidics is also a technology that was pioneered at Duke University by Fair et al., and is based on electrowetting, based on charge-control manipulation at the solution/insulator interface of discrete droplets by applying voltage to a control electrode. Compared to continuous flow microfluidics, there are distinct advantages with digital microfluidics:

- software-driven electronic control eliminates mechanical tubes/pumps/valves
- complexity of diverse set of biomedical applications can be reduced to a manageable set of fluidic operations, making the device programmable, reconfigurable, reusable
- top plate can be a disposable and processed independently of the microfluidic elements on the bottom surface
- highly specialized to a particular application
- high degree of automation and significantly smaller sample sizes
- programmable electrodes can merge/split/transport/mix/incubate droplets by programming electrodes to carry out specific tasks

Digital microfluidics is extremely versatile since it can perform all fluidic operations needed for sample manipulation, such as transport, mixing, dispensing, splitting/merging, filtering, analysis, storage of droplets. Since its recent development, it has seen widespread use in fields such as electrophoretic separations, DNA analysis, protein/enzyme analysis, immune- and bio-assays, pathogen detection. Optical sensors have also been successfully
integrated into the digital microfluidics platform,\textsuperscript{305} demonstrating its versatility and robustness. The interested reader is encouraged to scrutinize the excellent review by Fair.\textsuperscript{306} Digital microfluidics therefore shows the potential to be used as an optimal platform for manipulating SERS nanoparticles in an automated and reusable fashion.

This final chapter presents a novel concept that leverages the ability of cobalt micromagnets to concentrate magnetic nanoparticles and their reproducible fabrication, together with the versatility of plasmonics-active magnetic nanoplatforms for SERS. The integration of both systems boasts an enhanced and reproducible SERS signal, and its simplicity offers new possibilities for its integration with microfluidics technology, which is also touched upon at the very end.

5.2 Concentrating MNPs with micromagnets - Theory

The ability to concentrate MNPs in a solution can be understood by first considering the force induced on a single MNP, and then extending the formulation to include the surrounding fluid’s magnetization.

The force induced by an externally applied H-field, $\mathbf{H}$, on a single MNP of magnetic dipole vector $\mathbf{m}_p$, through the equation:

$$ F = \mathbf{m}_p \cdot \nabla \mathbf{B} = \mu_0 \mathbf{m}_p \cdot \nabla \mathbf{H} \quad (5.1) $$

where $\mu_0$ is the permeability of free space, $\mu_r$ of water is unity and the subscript $p$ denotes ‘particle’.

In general, a sample comprising $N$ magnetic dipole moments of volume $V$ has a magnetization $\mathbf{M}_p$ given by:

$$ \mathbf{M}_p = \frac{N}{V} \mathbf{m}_p \quad (5.2) $$
Substituting $m_p$ into 5.1 for a single MNP ($N = 1$) yields

$$F = \mu_0 V M_p \cdot \nabla H$$

The basic equation 5.3 is then extended to include the surrounding fluid’s magnetization, which is an important variable that affects the force on the MNP.

$$F = \mu_0 V M_p \cdot \nabla H$$

$$= \mu_0 V [(M_p - \langle M_f \rangle) \cdot \nabla] H$$

where $\langle M_f \rangle$ is the local fluid magnetization and $(M_p - \langle M_f \rangle)$ represents the net magnetization of the individual nanoparticle inside a concentrated suspension. This formula suggests that the generation of a large force is achievable by using particles with large volumes, maximizing the contrast between the magnetizations of the particle and surrounding fluid, and employing manipulation systems that are capable of delivering high magnetic field gradients.

The two magnetizations can then be further expanded. The magnetization $M_p$ of an individual isolated superparamagnetic nanoparticle exposed to an external magnetic field $H$ is typically described by the Langevin behavior.

$$M_p = M_s L(\xi) = M_s [\coth(\xi) - \xi^{-1}]$$

The Langevin function $L(\xi)$ explains the temperature dependence of the susceptibility of paramagnetic materials by assuming that the atomic magnetic moments react independently to applied magnetic fields and to thermal energy. At any temperature above absolute zero, thermal energy vibrates the crystal lattice, causing the atomic magnetic moments to oscillate rapidly and randomly in direction. In the absence of a magnetic field, the atomic moments are equally distributed in all directions such that the net magnetization equals zero. When a magnetic field is applied, the atomic moments shift slightly toward the field direction. It should be reiterated that each MNP is considered a single magnetic dipole $m$.
since they comprise a single magnetic domain in the superparamagnetic regime. Further derivation via Boltzmann statistics\textsuperscript{308} leads to

\[
M_p = M_s L(\xi) = M_s [\coth(\xi) - \xi^{-1}]
\]  

(5.7)

The local fluid magnetization \(\langle M_f \rangle\) in equation (5.5) depends linearly on the local magnetic nanoparticle concentration via the linear constitutive relation:

\[
\langle M_f \rangle = C(r) M_p
\]  

(5.8)

where \(C(r)\) is the local volume fraction of particle material, ranging from 0 for infinitely dilute to 1 for close-packed particles. This linear relationship is reasonable since the magnetization of any material is proportional to the local density of magnetic dipoles.

Thus, combining equations (5.7) and (5.8) into (5.5) results in the force on a MNP or small aggregate of MNPs equal to

\[
F_m = \mu_0 V (1 - C(r)) L(\xi)(M_s \cdot \nabla)H
\]  

(5.9)

Erb et al.\textsuperscript{233} then solved the equilibrium concentration profile \(C(r)\) by evaluating the balance of fluxes due to magnetic drift and Brownian diffusion at steady state. Briefly, the thermal diffusive flux \(J_{\text{diff}}(r)\) and magnetic drift flux \(J_{\text{drift}}(r)\) are given by:

\[
J_{\text{diff}}(r) = -D \nabla C(r)
\]  

(5.10)

\[
J_{\text{drift}}(r) = \eta C(r) F_m
\]  

(5.11)

where \(\eta\) is the mobility of the particle acted upon by a locally applied magnetic force \(F_m\), and \(D\) is the diffusion constant that is assumed independent of particle concentration \(C(r)\).

At steady state, both fluxes must exactly balance each other, leading to:

\[
\frac{\partial C(r)}{\partial t} = \nabla \cdot [-D \nabla C(r) + \eta C(r) F_m] = 0
\]  

(5.12)

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Combining expressions 5.9 and 5.12 and incorporating the Einstein relation \((\eta/D) = (k_B T)^{-1}\) generates the following differential equation

\[
\frac{\nabla C(r)}{C(r)[1 - C(r)]} = \left[ \coth(\xi) - \frac{1}{\xi} \right] \nabla \xi
\]  

(5.13)

Integration followed by enforcing the boundary condition that the nanoparticle concentration far from any local sources of field equals the bulk concentration \(C_b\) provides the final solution

\[
C(r) = \left[ 1 + \left( \frac{1 - C_b}{C_b} \right) \frac{\sinh(\xi_0)}{\sinh(\xi)} \frac{\xi}{\xi_0} \right]^{-1}
\]  

(5.14)

where \(\xi\) is the ratio of local magnetostatic energy and \(\xi_0\) is the ratio for a MNP in the bulk fluid far from any sources of magnetic field.

This equation describes the concentrating of MNPs in regions of strong magnetic field gradient: when \(\xi >> \xi_0\) the volume fraction \(C(r) \to 1\) (closed packed), whereas when \(\xi = \xi_0\), the volume fraction \(C(r) \to C_b\). Thus, MNPs are concentrated in regions where the local field is greater than the external uniform field, \(\xi > \xi_0\), and the greater this difference, the stronger the concentrating effect. This is schematically illustrated in 5.2.

**Figure 5.2:** Schematic of cobalt micromagnets illustrating the manipulation of the local field gradient with an applied magnetic field \(H_{ext}\). Depending on the direction of \(H_{ext}\), the local field will either be amplified (large green arrow), diminished (small green arrow) or unchanged, which results in the formation of H-field minima and maxima in space. The MNPs will concentrate in areas of H-field maxima, where \(\nabla H\) will also be strongest.
5.2.1 Discussion on MNP volume for controlled concentration and SERS

It is noteworthy that the direct correlation between force $F$ and volume $V$ implies that the force experienced by a MNP is very sensitive to its diameter ($V = \frac{4}{3}\pi r^3$), which effectively dictates how easily it can be manipulated by an externally applied H-field, all other variables held constant. On the other hand, as the size of the MNP exceeds $20 - 30\text{nm}$, it loses its superparamagnetic status (single domain magnetic dipole), begins growing multiple magnetic dipoles and its effective magnetic dipole increases; the increased magnetic interaction among neighboring MNPs renders the whole solution of MNPs more prone to aggregation, which makes its controllable manipulation a significant challenge. Thus, there exists an optimum MNP size that constitutes a compromise between being too small and unresponsive to an externally applied H-field, and too large and uncontrollable.

It is also important to recall that for the MNPs to be SERS-active, they must be coated with a shell of either gold or silver metal. This added weight, increased distance between MNPs and decreased mobility of each particle due to stabilizing surfactants that need to be added to ensure stability (such as SH-PEG, or long polymer chains) effectively decreases the magnetization of each particle and its responsiveness to surrounding MNPs, which further constrains its ability to be moved. Therefore, controlling these coated MNPs to the same extent as their non-coated counterparts will require a higher applied H-field and/or larger MNP cores to counteract the negative impact of adding a non-magnetic metal shell. In addition, another design parameter is for the metal shell thickness to be large enough such that its plasmonic band resides in the mid-visible range for excitation by Renishaw’s 633nm laser line, and such that the total MNP@Au volume is large enough for the particle to exhibit a strong plasmonic response, which is critical to obtaining a strong SERS signal. Typically, the optimal size is $60 - 80\text{nm}$ for gold and $30 - 50\text{nm}$ for silver.

Since the MNPs employed by Erb et al. in their experiments were uncoated and high
volume fractions could be used, small micromagnets \((3 \times 8 \mu m^2)\) were sufficient to exert the necessary force to obtain strong MNP concentration at magnetic ‘hotspots’. The much weaker moment of the synthesized MNPs@Au@Ag, relative to bare MNPs, due to the increased weight, couple with the increased drag due to the larger overall volume, suggest that the same micromagnet dimensions would provide an insufficiently large H-field gradient to manipulate these particles. Moreover, the high optical density of these plasmonic MNPs@Au@Ag, relative to bare MNPs, imposes another practical constraint on the possible volume fraction that can be used: to maintain visibility through the solution of MNPs@Au@Ag as they are manipulated, the volume fraction cannot be as high as those used by Erb \textit{et al.}. These two constraints point toward the necessity of higher magnetic field gradients, which translate to the design of novel, more powerful micromagnets.

5.3 Design of novel micromagnets via numerical simulation

The optimal design of novel micromagnets had to take into account a number of variables that pertained to their use for the controlled concentrating of SERS-active MNPs. To maximize SERS sensitivity while ensuring a reproducible and therefore quantifiable signal, the largest number of SERS-active MNPs should be controllably concentrated into the focal spot. Thus, the micromagnets should:

1. generate the largest possible \(\nabla H\) to ensure maximum concentrating power

2. produce a concentrating region where \(C(\mathbf{r})\) consistently varies with the \(H_{ext}\) and that is large enough to accommodate the focal spot of a laser focused by \(10 - 100\times\) microscope objectives for probing the SERS signal

3. have size and geometry that allows them to be fabricated by standard photolithography, rather than by the more expensive and time consuming electron beam lithography
4. influence the greatest possible volume of MNPs to concentrate the largest number of MNPs, which would increase detection sensitivity

5. have a size commensurate with the size of the intended droplet to be analyzed in the microfluidic chip (150 µm diameter × 20 µm height)

Items (1) and (2) impose minimum and maximum constraints on the gap separating adjacent micromagnets: item (1) intuitively requires the gap to be as small as possible, but because the tightest laser focal spot realistically achievable with a 50 – 100× microscope objective is ~ 3 µm and since MNPs will tend to concentrate on the extremities of magnetized micromagnets (even in the absence of an applied H-field), item (2) suggests that the separation needs to be at least 3 – 5 µm (allowing for 1 µm of MNP “steady-state” aggregation at the extremities). This lower limit is also imposed by item (3). To concentrate the largest number of SERS-active MNPs into the focal spot, item (4), the micromagnet should span as large an area of MNPs as possible such that the generated local H-field becomes ‘local’ to as large a population of MNPs as possible, while also ensuring a strong concentrating ability. Finally, item (5) suggests that the micromagnets should not exceed much more than 150 µm to remain integratable with the digital microfluidic chip technology.

Prior to their fabrication, numerical simulation was employed to design novel micromagnet shapes that would maximize the local field gradient, thereby increasing the concentration efficiency of MNPs@Au. The simulations were performed using the electrostatics and magnetostatics AC/DC module in Comsol Multiphysics. Rather than trying to obtain an accurate quantitative solution to the workings of the micromagnets, more qualitative understanding of their induced magnetic field with respect to their geometrical properties (shape, size, orientation) was desired; this would enable the fair comparison among different geometries to determine the ideal shape and size for the application at hand.
The response of a material to an applied magnetic field is determined by the material’s relative permeability, $\mu_r$, which, for a ferromagnetic material, is proportional to the slope of the B-H curve and thus varies with the magnetic field strength. To simplify the numerical simulation, the function detailing the variation of $B$ with respect to $H$ for cobalt was not incorporated into the simulation, rather a linear relationship between the two was assumed. Since the initial and maximum $\mu_r$ of cobalt are 70 and 250, respectively, the $\mu_r$ used in the simulation was set to 150 as a reasonable estimate.

### 5.3.1 Initial validation of the numerical model

The micromagnet model was initially validated via intuition by analyzing the induced local magnetic field in the junction of rectangular $3 \times 8 \mu m$ cobalt micromagnets. The micromagnets were set to a thickness of 70nm and assumed to uniformly magnetized along the x-direction. An infinite 2D array of micromagnets was simulated by defining the computational domain as a unit cell with periodic boundaries in the x-y plane, such that the field behavior was repeated in the x-y plane at infinitum.

The conditions simulated here assume that the micromagnets have previously been magnetized by a large externally applied field, which was then removed ($B_{ext} = 0$). As with all ferromagnetic material, such as cobalt, this magnetizing action aligns the magnetic dipoles within the material along the same direction. When the applied field is then removed, the magnetized material exhibits a remnant field vector pointing in the same direction that it was magnetized.

As depicted in the 3-D schematic, the magnetization field, $B_M$, was set to an arbitrary magnetization vector of value of 60G along the x-axis, because this is typically the approximate value of the coercive field of cobalt micromagnets. This magnetization vector produces a magnetic field that is strongest at the micromagnets longitudinal extremities, and decreases as $1/d^3$, where $d$ is the distance from the extremity. As such, each micromagnet behaves like a large-sized bar magnet that is magnetized along its length.
The pairing of two micromagnets to form a junction results in magnetic coupling between them, leading to an increased magnetic field in the junction than over the micromagnet. This is more clearly visualized by the $|H|$ and $H_x$ line profiles, which were taken through $y = 0$ and at a height of 70nm. It is noteworthy that the uneven sharp peaks conveyed by
the line profiles should in fact be of same magnitude, but the limited meshing resolution prevents accurate interpolation at this sharp discontinuity.

The $|\mathbf{H}|$ profile confirms that the minimum magnetic field occurs in the center above the micromagnet, and the maximum occurs at the extremities. The $H_x$ profile depicts that a sign reversal of the magnetic field occurs at each longitudinal extremity, such that the magnetic field direction above the magnet is in the -ve $x$-direction (opposite to the magnet’s magnetization vector) while that in the junction is in the +ve $x$-direction (with the magnet’s magnetization vector). This is also illustrated by the 3-D map of red arrows in the 3-D solutions that convey the magnetic field direction throughout the computational domain. These observations confirm the intuitive understanding of micromagnet arrays conveyed in Figure 5.2.

The top and side views of the 3D solution illustrate the extent of the magnetic field beyond the micromagnet surface in the $z$-direction and in the $x$-$y$ plane, respectively. Finally, a 3D plot of the magnetic field gradient confirms that very high gradients can be obtained with micromagnets, reaching $1 \times 10^{11}$ in this case, and that their location in space is correlated with the location of the strongest magnetic field, as expected. All these observations have been validated by experimental research performed by the Yellen group and instills confidence that the numerical simulation can be employed to design and understand novel micromagnet shapes for their optimized integration into the desired application.

It is noteworthy that the attractive feature of micromagnets, and the premise of using them to concentrate stable MNPs, is based on the fact that even though the local magnetic field might be relatively low, or in the same range as the externally applied field, the magnetic field gradient, which is ultimately the quantity that defines the force on magnetic nanoparticles, is greatly enhanced because of the microscopic scale over which the fields are produced: as demonstrated numerically, the sharp change in magnetic field over a junction gap of $3\mu$m generates magnetic field gradients in the order of $10^{10} - 10^{11}$ A/m², which would be unobtainable with large solenoids or rare-earth magnets.
5.3.2 Design of new micromagnet shapes via numerical modeling

FIGURE 5.4: Simulation data analyzing the local magnetic field induced by magnetized rectangular micromagnets ($3 \times 8 \mu m$), in the absence of an applied magnetic field.

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Figure 5.4 demonstrates that increasing the externally applied magnetic field, $B_{ext}$, is accompanied by an increase in the local magnetic field gradient between the micromagnets. Importantly, as $B_{ext}$ increases, the extent in space over which the locally generated magnetic field has influence also increases, both in the x-y plain and in the z-direction. This is particularly important for this application since the aim of using such micromagnets is to concentrate as many nanoparticles into a small spot to increase the detected SERS signal.

\[ \text{Figure 5.5: Numerical solutions conveying the effect of micromagnet separation on generated magnetic field.} \]

Intuitively, reducing the gap between adjacent micromagnets will also increase the magnetic field gradient they generate, and this is numerically confirmed in Figure 5.5. It is noteworthy that the finite focal spot of a laser, that is the excitation source of choice for Raman, will impose a lower limit to the micromagnet separation, especially if attempting to excited beneath the substrate through the quartz. For example, a $50 \times$ objective typically has a focal spot diameter of $3 - 5 \mu m$, therefore reducing the gap any further would have negative consequences on the detected SERS.

Interestingly, increasing the length of the micromagnets only slightly increased the local magnetic field in the junction, until a saturation point was reached beyond which further increase had no effect (data not shown). The saturation length was found to be $\sim 45 \mu m$, so a micromagnet length of at least $60 \mu m$ was used throughout the tests to
maximize the concentrating power of the micromagnets.

\textbf{FIGURE 5.6:} Numerical solutions conveying the effect of micromagnet shape on generated magnetic field. The bow-tie shaped micromagnets generated the strongest $|H_{\text{norm}}|$, particularly visible for the externally applied field $\mathcal{B} = 500\text{G}$.

The shape of the designed micromagnet is ultimately one of the defining parameters that can be adjusted, Figure 5.6. Since the magnetic field gradient generated by the micromagnets dies off exponentially with distance, the ideal micromagnet design will spread out as much as possible in the x-y plane to influence the greatest volume of nanoparticle solution, yet still maintain a single focal spot. The bow-tie and cross designs offer a greater area of influence than the traditional rectangular magnets, as well as a significantly higher generated field in their junctions. These were thus chosen as the designs of choice, and were designed, fabricated and tested alongside rectangular micromagnets in the next section.
5.4 Cobalt Micromagnets Fabrication

With the hope of integrating the micromagnets as the top-plate of a digital microfluidic through which excitation would occur, there was a necessity for the underlying substrate material to be thin, optically transparent and possess minimal intrinsic fluorescence. The silicon wafers that are typically employed by the Yellen lab to deposit the cobalt micromagnets would not be feasible, and instead, 300µm quartz wafers were selected and processed. Importantly, since the micromagnets will be designed to be very thin (≈ 70nm), it is crucial for the surface roughness of the substrate onto which they are grown to be as low as possible, such that adjacent micromagnets are properly aligned in the z-direction to allow for magnetic coupling between their junctions. Compared to silicon wafers which are atomically flat, quartz surface roughness varies substantially across suppliers. The flattest wafers that were found had a claimed surface roughness of ≈ 6nm, which is less than 10% of the desired micromagnet thickness and therefore adequate for their intended purpose.

The reason for making the micromagnets so thin are two-fold. Firstly, such a thin magnetic material ensures that the majority of the magnetization by an externally applied field orients itself along the longitudinal direction in the plane of the substrate. This probably enables greater manipulation of the magnetic fields in their vicinity by a three-dimensional solenoidal system, as often employed by the Yellen group. Secondly, and perhaps more importantly given the intended desire to integrate them within a digital microfluidic channel, the nanometer thickness of the micromagnets would allow droplets that are several micrometers thick to flow over them essentially unimpeded as if the surface was flat. This would ensure proper digital microfluidic manipulation of the droplets, which could involve the shuttling, splitting, merging and mixing of droplets at high speeds. Other micromagnet designs that involve thicker micromagnets would, in contrast, act as protruding physical obstacles to the droplet manipulation and would be incompatible with the digital microfluidic chip. Moreover, the combination of the teflon coating to eliminate surface contamina-
tion/sticking together with the micromagnets nanometer profile would undoubtedly minimize any unwanted bio-fouling or dirt deposition around the micromagnet edges, where waste deposits are normally prone to accumulate; this would assure substrate reusability, which is a key feature of the intended prototype.

The fabrication of micromagnets on substrate was achieved by photolithography, which is a technique that employs patterned masks, photoresist and UV-illumination to produce the desired micro-scale features of interest. The limited resolution of the industry-fabricated mask allows for features down to a radius of curvature of approximately $3\mu\text{m}$. Two different masks were designed in Autocad to explore several interesting micromagnet designs and geometrical parameters, and their corresponding chrome masks were implemented by the company Photosciences.

**Photolithography protocol**

The detailed photolithography protocol is presented in Appendix A.3, but its process is illustrated in Figure 5.7. The fabrication was performed in a clean room to minimize surface contamination and ensure a reproducible, clean process.

![Micromagnets on Quartz](image)

**Figure 5.7:** Schematic detailing the procedure involved in the fabrication of cobalt micromagnets on a quartz wafer.
Several adjustments to the standard silicon photolithography had to be implemented to successfully fabricate the micromagnets on quartz substrate. Compared to Silicon wafers that require simple cleaning with acetone and IPA, the quartz substrates requires the additional use of Piranha solution to chemically etch the surface-bound organic material and hydroxylate the quartz surface, making it hydrophilic by populating with -OH groups. Unfortunately, even with this extra step, the NFR-016 photoresist still failed to properly adhere to the quartz surface, which in turn prevented proper Cr/Co deposition. As a final solution, the adhesion promoter AP3000 which is silane-based, was spun onto piranha-cleaned wafers. The silane bound covalently to the quartz surface, leaving an organic monolayer exposed to air and to which the photoresist was able to properly bind.

The exposure duration was also an important variable that was tweaked to prevent under- or over-development of the photoresist. After iterations spanning times of 12 seconds to 20 seconds, the optimal time of 17.5 seconds @ 13mW/cm$^2$ was selected, which produced sharp, vertical walls in the photoresist.

Combined with longer 3-min oven-bake times, the NFR-016 negative resist remained adhered during the MF-319 developing stage, and the micromagnets patterns were successfully incrusted and ready for metal deposition. An adhesion layer of 5nm chromium, followed by a 70nm thickness of cobalt were deposited using an E-beam evaporator, vacuumed down to $< 1 \times 10^{-6}$ Torr. After liftoff of the negative photoresist via thorough dissolving with acetone and rinsing with IPA, the substrate was dried with a stream of N$_2$. A typical substrate is presented in Figure 5.8.

In order to render the surface hydrophobic and allow it to undergo multiple uses, a 50nm layer of Cytop (Teflon) was spun onto the substrate, followed by two baking steps to evaporate the solvent and uniformly spread the teflon throughout the substrate. For another set of experiments, 3 more quartz substrates were fabricated and sputtered with 200nm, 500nm and 1000nm thicknesses of PECVD prior to the teflon coating, in order to investigate the magnetic field response of ferrofluid that is located progressively further.
Finally, the substrate had to be diced into manageable pieces. It was discovered that the simpler “scribe and break” method failed to dice the quartz, possibly since this quartz had been hardened or tampered by the manufacturer, so dicing was the only other option. Since dicing uses a diamond tip blade to cut through the quartz, producing flying shards of quartz, the microstructures are prone to damage without appropriate surface protection. Although this is normally achieved by a simple spin coating and subsequent baking of photoresist, the presence of the teflon coating that prevented proper adhesion of any liquid to the surface complicated things quite substantially. Rather than spin coating, the most practical solution was found to be a very gentle, repeated glazing of S1813 photoresist on the substrate surface with a spongy swab. As the photoresist began drying out, it began sticking to the teflon and oven baking at $90^\circ C$ was then achievable.
5.5 Analysis of the Cobalt micromagnet structures

5.5.1 Customized microscope setup for simultaneous magnetization and excitation of MNPs@Au@Ag for controllable SERS

Thus far, it has been shown that the magnetic field, and therefore magnetic field gradient, generated by the micromagnets strongly depends on the magnitude and direction of the externally applied magnetic field \( B_{\text{ext}} \). For a uniform field along the micromagnets longitudinal direction, the periodic geometry of the micromagnet array would ensure quantitatively identical (assuming a perfectly made substrate) magnetic field ‘hotspots’ at each of their junction throughout the substrate, and is therefore the most desirable condition when performing experimental measurements that attempt to demonstrate system reproducibility.

Unfortunately, obtaining both a uniform and controllable magnetic field across a substrate width of about 2cm is not an easy feat with simple rare-earth magnets: the magnetic field profile between two such magnets separated by a distance is ‘U’-shaped and a uniform field is only obtainable over a micrometer scale at a position that is equidistant from both magnets. Although the simplicity of this setup is ideal for a simple portable diagnostic device, which is the goal of this thesis, the initial system tests require finer control of the applied magnetic field such as to properly characterize the system parameters and limitations.

In order to obtain a uniform and controllable magnet field across the micromagnet sample, a solenoid system with attached current source and labview setup was borrowed a multiple occasions from the Yellen group at Duke University. The solenoids were each about 1 foot long and comprised of an iron core to increase the relative permeability of the medium through which the field was produced, thereby also increasing the generated magnetic field. The uniform field is achieved by having two very large solenoids, whose extremity areas are significantly larger than both the sample size and their inter-magnet
Figure 5.9: Customized integration of electromagnets onto a Raman microscope for the study of magnetic field modulated SERS. The top schematic details the dual setup while the photo on the bottom presents the actual setup that required assembly of a customized sample holder as well as solenoid holders.

separation distance. This ensures that the fringing effects of the magnetic field are kept far from the sample and that the field is also uniform along the substrate plane between
the magnets. With a distance of $\sim 2$cm between the solenoids and a current generator spanning $\pm 5$A, uniform magnetic fields up to $\pm 500 G$ were obtainable.

The customized system, involving the integration of an inverted Raman microscope and the two solenoids that were controlled by Labview, is presented in Figure 5.9. Although the figure is self-explanatory, it is worth mentioning that one of the biggest challenges for the intended experiments was the need for co-registration between the position of the micromagnet hotspots, where the nanoparticles would concentrate, and the laser focal spot, which has an approximate x-y diameter of $5 - 8 \mu m$ and a z-depth of also few micrometers. Proper x-y alignment and focus required a very robust sample holder that was connected directly to the microscope build, to avoid random vibrations from the optical table.

5.5.2 Experimental investigation with Ferrofluid

The novel teflon-coated micromagnet substrates were first tested by using a nanopartic- ular system of known magnetic behavior, namely ferrofluid EMG705 by Ferrotec. This solution consisted of magnetic nanoparticles of nominal size 10nm and of volume fraction $\sim 4\%$, and a similar solution was employed by Erb et al. to investigate the optical absorption of the concentrated ferrofluid as a function of applied magnetic field.\(^{233}\)

In addition to the desire of understanding how micromagnet shape and size affected their ability to concentrate the MNPs in their junction, an even more fundamental question was whether the 50nm teflon coating significantly impair the micromagnets ability to do just that. Since the teflon coating actually increases the distance between the micromagnet and the MNP solution, it is expected to have some degrading effect, yet it was hypothe-
sized that the coating would be thin enough to allow the local magnetic field gradient to sufficiently influence the MNPs into concentrating.

Thus, a 5$\mu L$ volume of 0.4% ferrofluid solution ($10 \times$ diluted stock solution) was sand-
wiched and sealed between a coverslip and the micromagnet substrate. The electromagnet
setup in Figure 5.9 was employed to generate a uniform magnetic field ranging from $0G$ to $200G$ across the substrate, and bright field microscopy images were captured to document the observed behavior, Figure 5.10.

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**Figure 5.10:** Investigation of micromagnet shape and size using 0.4% volume fraction ferrofluid solution EMG 705 from Ferrotec. The micromagnets appear black because transmission mode bright-field imaging was employed. The bulk solution appeared transparent even though a high volume fraction of MNPs were used because MNPs are not plasmonic and do not interact much with light.

The first promising result was that the MNPs were observed to concentrate between
micromagnet junction that were aligned with the direction of the applied magnetic field. This meant that a 50nm coating was thin enough for the magnetic field gradient produced in the MNP solution to generate sufficient force on the MNP to induce their concentration. Interestingly, however, a 200nm coating of PECVD (glass) sandwiched between the Cobalt and teflon layers did prevent MNP concentration under the exact same experimental conditions (data not shown). With the PECVD, the distance between the micromagnet and the MNP solution was 250nm, and thus it is noteworthy that an upper limit separation between Cobalt and MNP solution does exist beyond which the field gradient experienced by the MNPs is too weak to induce a sufficient magnetic response to overcome the chaotic Brownian motion. Secondly, the fact that they hydrophobic teflon coating, which prevents sticking and contamination, is compatible with this technique also suggests that the surface protected micromagnet substrate achieves the goal of being multiply reusable. Indeed, the MNP solution is easily washed off the substrate by a rinsing with few drops of DI water, and no MNP residue is observed after drying.

Figure 5.10 analyzes three distinct micromagnet shapes - circles, rectangles, and the newly designed bow-tie - for four increasing magnetic field strengths. It is noteworthy that prior to application of the $B_{ext}$ field, the micromagnets had already been magnetized along the axis passing through their junction. The circles and rectangles behave as expected since they generate an increasingly strong magnetic field gradient in their junction with increasing $B_{ext}$, which is visually demonstrated by an increasing ferrofluid density in their junction. The bow ties, however, behave surprisingly at first glance: the MNPs are well focused in the junction for $B_{ext} = 0 \text{G}$ but as $B_{ext}$ increases, they spread out away from the junction in the direction normal to $B_{ext}$ such that their density in the junction effectively decreases. This trend was also observed for bow tie micromagnets of different size and varying junction distances. Nonetheless, although surprising, this experimental observation is in accordance with the numerical simulation of bowtie micromagnet structures, which portrayed increasing magnetic field coupling between the micromagnet pair, on
both sides of the junction, with increasing $B_{ext}$. This provides insight into why the MNPs are spread over an increasingly large area about the junction as $B_{ext}$ increases. Although this result was unexpected, it could in fact be a beneficiary feature because it implies that even with no external field, the remnant magnetization of bow tie micromagnets is strong enough to sustain a strong magnetic field gradient in the junction and strongly concentrate the MNPs. With a portable diagnostic device in mind, this becomes an additional advantage of this micromagnet shape, and thus significant effort will be placed on using this particular shape in the integrated chip design.

5.6 Magnetic hotspots as concentrators of SERS nanoplatforms: potential for magnetic field modulated SERS

This section deals with the micromagnet manipulation of the previously synthesized and functionalized MNPs@Au@Ag to obtain reproducible and more intense SERS signals, that would then offer a novel platform to be integrated into a digital microfluidic system. Specifically, the two concepts of SERS signal reproducibility/quantification and increased SERS signal sensitivity, which pertain to optimizing the microfluidic system reliability and limit of detection, will be investigated and discussed.

5.6.1 Micromagnet dimensions and region of capture (ROC)

The increased intensity of the SERS signal depends on the ability of the micromagnets to concentrate the nanoparticles in their predefined magnetic hotspots, or junctions. It can be envisaged that for the same nanoparticle volume fraction and applied field, the shape of the micromagnet is likely to play a role in its ability to concentrate NPs, as was previously theoretically illustrated. For a given micromagnet shape, however, it would be interesting to investigate whether its dimensions also affect this concentrating ability. This was achieved by designing rectangular micromagnets of different x-y dimensions, as shown in Figure 5.11. Specifically, the effect of width and length of rectangular micromagnets
on the density of MNPs@Au@Ag in their 3µm junction was investigated for the same MNPs@Au@Ag volume fraction and applied field, $B_{\text{ext}}$.

**Figure 5.11**: Effect of micromagnet width and length on its ability to concentrate MNPs@Au@Ag (100× conc.), assessed by visually estimating changes in optical absorption. An external magnetic field ($B = 500G$) is applied to all structures. The first row depicts an increasing micromagnet width, while the second row presents micromagnets of increasing length; both rows maintained a fixed inter-magnet separation of 3µm. For clarity, the micromagnet boundaries are delineated with dotted white lines. The red rectangles overlaid on the images delineate the “region of capture” (ROC) of each micromagnet array. The cross geometry, with a 5µm gap, is included to illustrate the significantly larger ROC afforded by this exotic geometry.

In order to discuss and better qualify the ability of micromagnets to concentrate nanoparticles, a new term “region of capture” (ROC), is introduced here. The ROC is schematically illustrated in the figure inset and is defined as the volume of nanoparticle solution that is under the influence of the local magnetic field gradient generated by each micromagnet.
pair, or in other words, the magnetic “reach” of each micromagnet hotspot. The ROCs for each micromagnet design are overlaid onto the bright field images Figure 5.11.

The first row depicts an increasing micromagnet width, with its length fixed at 30µm, which is also accompanied by an increasing ROC width, i.e. along the y-direction. The increasing micromagnet width effectively increases the edge length of the micromagnet junction, and therefore increases the nanoparticle population in its proximity. Interestingly, however, the density of nanoparticles does not appear to increase much. This is speculated to be due to the counterbalancing of the incremental increase of the ROC width (and thus additional volume of nanoparticles accessible by the micromagnet junction) by the same incremental increase in the micromagnet width; therefore, although a greater number of nanoparticles are concentrated by the wider micromagnet junction, their density remains unchanged.

It is noteworthy that the density of MNPs@Au@Ag is the crucial factor of interest, since the Raman signal is measured by probing a focused laser source at the region of interest, and the detected signal will be proportional to the number of excited molecules in the focal volume, which in this case is equivalent to the density of MNPs@Au@Ag in the focal volume (since the Raman molecules are physisorbed to the particles surface). Ignoring Raman signal loss due to absorption, scattering and other competing processes, As this density tends to the close packing of nanoparticles, the SERS signal would continue to increase linearly until plasmonic coupling takes over and increases the signal exponentially.

The second row, however, demonstrates that an increase in the micromagnet length for a fixed width is accompanied by a proportional increase of the ROC along the x-direction, e.g. when doubling from 15µm to 30µm, the ROC also doubles in volume. In contrast to an increasing width, an increasing length actually increases the density of nanoparticles in the micromagnet junction; because numerical simulation showed that lengthening of the micromagnets does not correlate with an increased local magnetic field generated by the
micromagnets (a result that is intuitive and not shown here), it can be inferred that this density increase stems from the increasingly large ROCs for the same micromagnet width. As a matter of comparison, a $70 \times 75 \mu m$ cross-shaped micromagnet is presented as the last image in the figure, to show that by altering the shape of the micromagnet to exert a magnetic field over as large an area as possible in both the x- and y-directions, the corresponding ROC can be maximized to cover the entire magnet, such that the nanoparticle density is also maximized for a given nanoparticle volume fraction and $B_{ext}$.

From the given analysis and with the integration of micromagnets with microfluidics in mind, the best micromagnet size and shape in terms concentrating a high density of nanoparticles in its junction is a large bow tie or cross. These two shapes were designed to maximize their ROC and both experimental and theoretical analyses have confirmed that, compared to rectangular micromagnets of equal gap separation and with all other variables constant, they can achieve a higher density of nanoparticles and thus a higher SERS signal sensitivity.

In concluding this section, it is important to mention that the analyses undertaken here not only produced insights pertaining to optimization of the micromagnet system, but also provided very promising results with regards to the nanoparticulate system of MNPs@Au@Ag suspended in solution. Indeed, following high speed centrifugation that was required to concentrate the nanoparticles to the desired volume fraction for subsequent magnetic manipulation, the MNPs@Au@Ag were easily redispersed for dilution without sonication, and that bright field microscopy at $40 \times$ did not reveal large micrometer clusters or aggregates of NPs that are typically found in unstable solutions. Although the presence of SH-PEG7 could contribute toward their stability, this extremely short PEG (7 repeats) is unlikely to be the main reason, rather the small size of the MNPs@Au@Ag prior to functionalization with PEG would explain this good stability. This simple, yet crucial, observation further strengthens the validity of the synthesis method developed in Chapter 4 to produce stable, magnetic and plasmonic nanoparticles for their manipulation by the
extremely high field gradients as those produced by the micromagnets.

5.6.2 SERS signal dependence on MNP@Au@Ag concentration

Similarly to bare MNPs, or ferrofluid, the ability of the micromagnets to concentrate the MNP@Au@Ag strongly depends on their concentration in solution: the higher their concentration, or volume fraction, the greater the force they collectively experience and the easier they can be concentrated. It is quite typical to use a very large volume fraction of MNPs in the range $0.4 - 4\%$ to manipulate them at will, and due to their low absorption and scattering coefficients, light is still able to penetrate to some extent into a thin layer of such highly concentrate fluid.$^{237}$ Unlike bare MNPs that do not exhibit any plasmonic response when excited by visible light, however, MNP@Au@Ag are coated with Au and Ag metals that are both strongly plasmonic and thus exhibit large absorption and scattering cross sections. As such, significantly lower volume fractions of MNP@Au@Ag have to be used to allow light penetration into the thin layer of fluid. It is hypothesized that although an increased volume fraction of MNP@Au@Ag would allow for them to be concentrated to a higher degree, the penetration of light through the micromagnet junction would decrease and will in turn negatively influence the detected SERS.

To investigate this concept, the term signal increase (SI) is introduced here. SI is defined as ratio of the SERS signal detected in the micromagnet junction to that detected in bulk solution away from the junction.

The bow tie micromagnets were employed since their concentrating ability was determined to be higher than rectangular micromagnets. Five concentrations of MNP@Au@Ag were prepared via centrifugation for 5 min at 12,000 rcf and dilution with DI, after which 5 µL of the solution was sandwiched between the micromagnet substrate and coverslip, followed by sealing with oil. This volume of NPs spread over an $2 \times 2$ cm area effectively translates to a solution layer thickness of approximately $16 \mu$m. The Raman detection specifications were a laser power of 0.2 mW, a $40 \times$ objective, and 10 s acquisition time.
Each measurement point was repeated at a different micromagnet junction and their variation was plotted with error bars representing the signal standard deviation. The results are presented in Figure 5.12.

**Figure 5.12:** Investigating the signal increase (SI) of $70 \times 75 \mu m$ bowtie micromagnets as a function of MNPs@Au@Ag concentration, with the application of a $500G$ uniform magnetic field. The SI is the ratio of SERS signal obtained by NPs in the magnet junction, SERS$_{junction}$ (red cross), to that detected in bulk solution far from the junction, SERS$_{bulk}$ (blue cross): $SI = I_{red} / I_{blue}$. $SI_{1x} = 177/10$ (assuming $I_{blue}=10$), $SI_{10x} = 4300/240 = 17.9$, $SI_{20x} = 5825/700$, $SI_{50x} = 8640/1400 = 6.2$, $SI_{100x} = 1100/2170 = 5.1$. The standard deviation error bars indicate the variation in detected SERS at 3 adjacent micromagnet junctions along the y-direction. The two curves on the right document the relationship between SERS intensity and MNPs@Au@Ag concentration in the junction and in bulk solution.
Figure 5.12 presents several important results.

Firstly, the SERS signal detected in the micromagnet junction, SERS_{junction} is several times higher than that in bulk, SERS_{bulk}, for all MNPs@Au@Ag concentrations, which in itself is an important result for two reasons: it provides another validation that the MNPs were indeed coated with Au and then Ag, and also confirms that these multi-shell NPs were still able to magnetically respond to an externally applied field. Moreover, the magnetic force did not seem to collapse the MNPs@Au@Ag (indicated by a reasonable, non-exponential SERS signal), which would be expected if these are unstable in solution.

Secondly, the stock solution of 1× conc., despite having a very low volume fraction of MNPs@Au@Ag, also experiences a concentrating effect between the micromagnets. This was surprising because the low volume fraction of NPs prevented any visual confirmation of their concentrating between the micromagnets, and only a large SI ~ 18 was confirmation of their presence. The detected SERS signal for the 1× conc. could not be resolved above the background noise, which fluctuated around 10 counts.

Thirdly, the error bars being < 10% implies a robust reproducibility across three different micromagnets, and also most likely extends throughout the entire substrate, which in turn suggests the possibility of reliably quantifying the SERS signal at different points on the substrate. Although the error bars should ideally be smaller, this variability is also likely due to human error associated with attempting to properly focus onto the substrate and acquire data despite using a customized and somewhat unstable Raman/magnetic system. This will be further analyzed in the next section, but it is still noteworthy that the highly stable MNPs@Au@Ag are the main reason for this spatial consistency.

Fourthly, the highest SI of approximately 18 was discovered to belong to the low volume fraction solutions of MNPs@Au@Ag, and this value decreased monotonically with increasing volume fraction, reaching a value of 5 for 100× NP concentration. This interesting decrease in SI is the expected result that was postulated by the earlier hypothesis and can be explained as occurring for two possible reasons, both which stem from the
increasing volume fraction, and therefore density, of MNPs@Au@Ag in the micromagnet junction: firstly, the higher the density of MNPs@Au@Ag in the micromagnet junction, the harder it is for the laser source to penetrate such that an increasingly smaller fraction of the MNPs@Au@Ag would be probed; secondly, the increasing density is also accompanied by an increasing likelihood of re-absorption and re-scattering of the emitted Raman photons by the plasmonic MNPs@Au@Ag, before they can escape the high NP density and be detected and accounted for by the spectrometer. This decreasing trend in the SI is further emphasized by the lines of best fit presented in the two graph insets to the right. The top graph exhibits a linear relationship between the detected SERS signal and the nanoparticle concentration in bulk solution. This is expected since for stable NPs in solution, a linear increase in the NP concentration should be accompanied by a similarly linear increase in measured SERS signal - this relationship breaks down when the NPs are unstable and experience aggregation, which exponentially increases the measured SERS signal. The bottom graph depicts a relationship between the detected SERS signal in the micromagnet junction and the nanoparticle concentration that is typical of a saturation mechanism: the initially sharp gradient eventually flattens out with increasing nanoparticle concentration, which is indicative of the challenge that exists of obtaining high SERS signals from an increasingly dense volume of MNPs@Au@Ag. Indeed, further increasing of the MNP volume fraction would result in a incrementally decreasing SI value, as $SERS_{bulk} \rightarrow SERS_{junction}$, at which point no benefit would result from having a micromagnet substrate.

Thus, although the highest overall SERS intensity is obtained with the highest volume fraction of MNPs@Au@Ag, the highest SI of around 18 is achieved by using a MNPs@Au@Ag concentration between 1 and $10 \times$. In this low range, the density of MNPs@Au@Ag that is concentrated between two adjacent micromagnets is still low enough such that the laser excitation penetrates the solution and the Raman signal exits with minimal competing absorption and scattering from surround MNPs@Au@Ag.
For this reason, it is worth contemplating whether the addition of uncoated MNPs (that are strongly magnetic yet not plasmonic) to the solution of MNPs@Au@Ag would in fact be beneficial in assisting their concentrating between micromagnets, especially for low volume fractions of MNPs@Au@Ag that do not suffer from the aforementioned two drawbacks. This might increase their SI and could therefore be worth further investigation.

5.6.3 Spatial reproducibility of SERS signal

The two main purposes of using micromagnets to manipulate magnetic plasmonic nanoparticles was to increase signal detection sensitivity and enable reliable quantification of the detected SERS signals. SERS signal reproducibility can be defined in the spatial context (identical detected SERS signal at different positions on the substrate), as well as in the temporal one (identical detected SERS signal at the same point, over time). This section addresses the former, and the next section will investigate the latter.

The idea of the micromagnet substrate being able to provide spatially reproducible SERS signals in the micromagnet junction stems from the intrinsic fabrication process of the micromagnets, which is a definite and reproducible one. Coupled with homogeneously small and stable MNPs@Au@Ag, two qualities that would also make this nanoparticulate system equally reproducible, it is hypothesized that the application of an external magnetic field could rapidly and predictably concentrate these plasmonic nanoprobes into predefined locations in space, or magnetic junctions, so as to yield a quantifiable SERS signal.

This was investigated by probing a number of different locations in space, 15 micromagnet junctions and 15 bulk solution positions, both in a $5 \times 3$ array arrangement as demonstrated in part by Figure 5.13(a). A $5\mu L$ volume of high volume-fraction MNPs@Au@Ag ($100\times$) was used in conjunction with a substrate of $3 \times 8 \mu m$ micromagnets separated by $3\mu m$, under the influence of a uniform $500G$ magnetic field. Once again, the optical components consisted of a $0.2mW$ laser and a $40\times$ objective, with a $10s$ acquisition time.
Figure 5.13: Spatial reproducibility of the detected SERS signals for 15 adjacent positions in the micromagnet junction and 15 adjacent positions in the bulk solution (far from the junction) for an array of $10 \times 30\mu m$ rectangular micromagnets, using $100\times$ concentration of MNPs@Au@Ag and $B_{ext} = 500G$; (a) illustration of some of the laser probe positions in junctions (red cross) and in bulk solution (blue cross), (b) DTDC SERS spectra measured at the junction positions ($SERS_{bulk}$), with their $5 \times 3$ arrangement indicating their position on the array relative to one another, (c) average and standard deviation error bars of the $1128\text{cm}^{-1}$ DTDC Raman peak for 5 row measurements in each of the 3 columns, both in the junction and in bulk solution.

The SERS spectra measured between the 15 different junctions are displayed in Figure 5.13(b) as a function of position at which they were obtained. These spectra were processed to remove the fluorescent baseline and allow for a reliable comparison of their SERS peaks. This layout evidently conveys that the SERS spectra are consistent throughout space, and this was further confirmed by spatially averaging the $1128\text{cm}^{-1}$ DTDC Raman peak across each of the 3-columns investigated. These results are conveyed by Figure 235...
5.13(c), where the averaged 1128 cm$^{-1}$ peak of the bulk solution measurements are also presented. The peak averages and error bars together confirm that the micromagnet substrate concentrates the nanoparticles in a spatially reproducible manner in their junctions. Compared to the small error bars of the SERS$_{bulk}$ measurements, the larger error bars of the SERS$_{junction}$ may be of concern, but it is noteworthy that this may be simply a measurement inaccuracy when acquiring the SERS spectra from the micromagnet junctions. Although not evident by the bright-field image in Figure 5.13(a) (since it is a top-view of the micromagnet substrate), the focusing requirements are much stricter when probing the nanoparticles concentrated in the junctions, compared to the nanoparticles in bulk. This is illustrated in the schematic inset to the right of Figure 5.13(a), which presents a cross-sectional view of the system under consideration. Assuming a nanoparticle solution layer thickness of $\sim 20 \mu$m (previously estimated), the focal volume of the 40× objective can probe anywhere in that $\sim 20 \mu$m thickness to yield a consistent SERS$_{bulk}$ signal. On the other hand, the micromagnets are only 70nm thick, and since the field gradient is strongest in the vicinity of the magnets and weakens with increasing distance away from the magnets, it can be inferred from simulation results that the MNPs@Au@Ag will be concentrated in the junction very close to the micromagnets, such that the “pellet” thickness would not exceed a few hundred nanometers - the remainder of the solution thickness above the junction would in fact have a bulk concentration of nanoparticles. Therefore, unless the focal spot is consistently positioned at the same height above the micromagnet substrate, there is a chance that different regions of the nanoparticle concentrate would be probed, leading to slightly different detected SERS$_{junction}$ signals (this was indeed observed while performing the experiment, but the data is not reported here). The same idea applies to the laser position in the x-y plane, which, due to the donut shaped concentrate of NPs by the micromagnets, also requires consistent positioning at each micromagnet junction for reproducible SERS measurements. Thus, by using a properly built stage that can accommodate the magnetic solenoids and Raman microscopy, the error bars are ex-
pected to diminish and show that high spatial reproducibility can indeed be obtained with this dual nanoparticulate/micromagnet system. Moreover, due to the identical fabrication process for micromagnets of different shapes, the predictable spatial behavior of rectangular micromagnets can be interpolated to other micromagnet shapes with a good degree of confidence.

5.6.4 Temporal reproducibility: magnetic field modulation of SERS

With the goal of yielding quantitative SERS signals, it is important to evaluate whether the micromagnets reproducibly concentrate the magnetic nanoparticles to the same degree for each on/off switch of the B-field. This was achieved by applying a cyclical magnetic field and continually interrogating the same micromagnet junction where the nanoparticles were being concentrated. It is hypothesized that a reliable system should generate the same SERS signal under the same magnetic field, keeping all other variables constant (such as NP concentration, dye concentration, and optical system parameters). In this case, the system is in fact a two-state system: it comprises of a substrate of in-plane micromagnets (dry-state), onto which is coupled a solution of MNPs@Au@Ag (liquid-state). Importantly, these two sub-systems are physically separated from one another by a teflon coating. The only form of interaction between the two is magnetic, that is, the magnetic response of the MNPs@Au@Ag to the local B-field generated by the micromagnets.

This study aims to address a number of questions to confirm whether this dual-state system can be integrated into a microfluidic platform to confidently yield reproducible and thus quantifiable results. Specifically:

1. Is the liquid system of MNP@Au@Ag stable enough to be manipulated by such high and frequently changing B-fields?

2. Do the micromagnets reproducibly concentrate the MNPs@Au@Ag in the same location and to the same extent?
3. What is the response time of the MNPs@Au@Ag to the applied field? Is it immediate or does it long as the MNPs@Au@Ag progressively concentrate?

4. Is the detected SERS quantitatively consistent as the MNPs@Au@Ag are cyclically concentrated and released in the micromagnet junctions?

The optical/Raman/electromagnetic experimental setup shown in Figure 5.9 was employed to simultaneously control the applied magnetic field while probing the MNPs@Au@Ag. Large bowtie micromagnets (70 × 75µm) were used in conjunction with a solution of MNPs@Au@Ag of 100× concentration. A 5µL of NP solution was sandwiched and carefully sealed between the substrate and a thin coverslip, through which the 633nm He-Ne laser was focused. Again, this volume of NPs translated to a solution thickness of approximately 16µm. Sealing was achieved via a combination of vacuum grease and immersion oil, and, given the duration of the experiment, was of utmost important to ensure a controlled environment with minimal solution evaporation, which would drastically alter the experimental conditions. Coupled with a 40× objective, a 1% laser power (0.2mW) was employed to minimize dye photobleaching and solution heating, which would decrease the measured SERS and accelerate evaporation, respectively. Indeed, DTDC is strongly fluorescent with an absorption peak at 650nm and is thus prone to some photobleaching, especially with a laser source centered at 633nm; this is true despite the dye being surface-bound to the MNPs@Au@Ag which quenches the fluorescence and substantially decreases photobleaching.

The customized merging of a Raman microscope with an independent, Labview controlled solenoid setup meant that the experiment had to be performed manually. For each new B-field, B_{ext}, applied across the micromagnets, proper focus on the sample was ensured, followed by switching of the filter wheel to allow laser passage through the system and finally careful probing of the sample solution with the laser to record the generated Raman signal. The laser shutter was automatically engaged after the 10s acquisition, the
B-field was varied, and the process was repeated. A total of three B-field cycles (18 sampling points in total), that varied from −50G to 500G, were applied to the system and their result is displayed in Figure 5.14.

Figure 5.14(a) depicts the correlation between detected SERS intensity between the bow-tie micromagnets and a sinusoidally varying applied magnetic field, $B_{ext}$.

The first row of bright-field images depicts a single cycle of $B_{ext}$, varying from a minimum of -50G to a maximum of 500G. This -50G offset was required to demagnetize the micromagnets after they had been magnetized by the large positive fields. It was surprising to discover how well the magnetized, bow-tie micromagnets kept their magnetization, even after removal setting $B_{ext}$ to zero; this was ostensibly due to their small intrinsic thickness of 70nm which ensured the magnetization remained strong and in-plain. This system “memory” resulted in a distinct difference between $B_{ext} = 0$G on the rising edge of the sine wave, prior to micromagnet magnetization nanoparticles, and $B_{ext} = 0$G on the falling edge of the sine wave; these were differentiated by assigning the notation $+0$G to the former, and $−0$G to the latter, for explanation purposes. The bright-field images also conveyed the extent to which the SERS-active magnetic nanoparticles were concentrated, for different values of $B_{ext}$.

The two superimposed cycles below the bright-field images convey the measured SERS signal intensity as a function of applied field $B_{ext}$. The SERS signal intensities were evaluated by processing the 1132 cm$^{-1}$ DTDC peak from the DTDC Raman spectra that were measured at each $B_{ext}$ value, conveyed in Figure 5.14(b). At first glance, there is an evident positive correlation between $B_{ext}$ and the detected SERS, as expected and demonstrated in the previous figures: a strong $B_{ext}$ concentrated the SERS-active nanoparticles and therefore increased the detected SERS intensity. Interestingly, there was no discernible difference between the detected SERS at $B_{ext} = 250$G and $B_{ext} = 500$G, suggesting that for the given concentration of nanoparticles in solution, a saturation point had already
Figure 5.14: Magnetic field modulation of SERS signal between two micromagnets: (a) SERS data as a function of a periodic magnetic field $B_{\text{ext}}$. The first image depicts bright-field microscope snapshots of $70 \times 75 \mu\text{m}$ bowtie micromagnets showing one complete magnetic field modulation cycle and the spatial concentration and relaxation of MNPs@Au@Ag. The vectorized $B_{\text{ext}}$ is depicted as green arrows above the images, and the induced magnetization in the micromagnets is illustrated with red arrows. The laser cross-hair is overlaid on each picture to convey where the laser excitation occurred. The left graph presents the measured SERS signal as a function of $B_{\text{ext}}$ and the right graph superimposes $B_{\text{ext}}$ and a hysteresis loop schematic to explain the magnetic response of the micromagnets; (b) SERS spectra sequence as a function of $B_{\text{ext}}$; (c) SERS intensity averaged at each $B_{\text{ext}}$, over 3 cycles, with corresponding standard deviation error bars.

been reached at 250G and beyond which increasing $B_{\text{ext}}$ would not further concentrated the nanoparticles in solution. When the external field $B_{\text{ext}}$ was cycled three times between $-50G$ and $500G$ in a sinusoidal manner, the SERS signal cycle did not respond in like, but
rather followed an on/off behavior to a first approximation. This is explained bearing in mind the schematic of the micromagnet hysteresis loop presented to the right of the cycle. When the cobalt micromagnets are magnetized by an external magnetic field $B_{\text{ext}}$, they retain a magnetization which depends on the magnitude of $B_{\text{ext}}$ as well as their physical geometry. Due to the magnetic memory of the micromagnet system, the removal of $B_{\text{ext}}$ was not accompanied by a drop in the detected SERS. This was visually confirmed in the corresponding bright-field image at $B_{\text{ext}} = -0G$, for which the magnetic nanoparticles were still strongly concentrated between the micromagnets. In order to deplete this region of nanoparticles, $B_{\text{ext}}$ had to be reversed to a value corresponding to the coercive field of cobalt micromagnets, which was estimated to be approximately $50G$. This sign reversal was then faithfully accompanied by a depletion of nanoparticles in the laser focal spot and a drop in the SERS signal to a value slightly higher than the background. When $B_{\text{ext}}$ was increased back to $+0G$ to start a new cycle, the SERS signal actually decreased by a small amount, that was statistically significant as confirmed by the error-bars in 5.14(c). This was most probably due to a slight overestimation of the coercive field of cobalt micromagnets by a few Gauss units, which would result in a slight magnetization in the opposite direction. The presence of this slight $B_{\text{ext}}$ would concentrate the nanoparticles to just a slight extent, insufficient to be perceived by eye, but enough to yield a stronger SERS reading. This very slight $B_{\text{ext}}$ would not sufficiently magnetize the micromagnets for them to retain memory, such that an increase of $B_{\text{ext}}$ to $+0G$ reduce the locally experienced field to $0G$, resulting in a lowered measured SERS intensity corresponding to the background counts.

It is noteworthy that a slight decreasing trend is observed in the SERS cycle peak as time progressed, most probably due to progressive photobleaching incurred by repeated laser probing in the same spot and on the same subset of nanoparticles. This degrading SERS signal also contribute to the larger error bars conveyed in Figure 5.14(c). By using a non-resonance Raman dye, the SERS quantification ability of the system would be more
It is also worth pointing out that the SERS signal intensity difference between the peaks (∼ 12,000 counts) and troughs (∼ 1800 counts) of the cycle yields an SI of ∼ 6.5, which is corresponds to the evaluated SI for this nanoparticle concentration of (100×). This concentration was simply used to enable a clear visual confirmation of the correct working of the system.

Finally, it should be re-emphasized here that obtaining this cycle is only possible with small and stable MNPs@Au@Ag, since thermal energy needs to be significantly stronger than inter-particle attractive energy in order to redisperse them with simple removal of the applied magnetic force. For larger, unstable NPs, simple removal of the field would not completely redisperse the NPs in a reproducible or controlled fashion and would not provide such a reliable cycle - this was tested with the larger, unstable MNPs@Au that were synthesized via Lyon’s method previously described.

5.7 Integration of the novel SERS platform with digital microfluidics

This last section briefly describes the integration of SERS with microfluidics to produce an advanced platform that has the potential for reproducible, reusable and automated SERS detection. The simplicity of the novel, hybrid SERS platform comprising the plasmonics-active magnetic colloids and the micromagnet-based substrate allowed for easy integration into a digital microfluidic device, Figure 5.15.

In order to integrate the SERS micromagnet platform with microfluidics, its fabrication had to be altered slightly. Since the electrowetting phenomenon that is the basis of digital microfluidics requires a voltage drop between the electrodes and a grounded bottom plate, a conducting layer of indium tin oxide (ITO) has to be sputtered onto the quartz wafer, prior to the chromium and cobalt deposition that form the micromagnets. Another procedure that required investigation was the boring of < 1mm holes using a sand blaster, which
FIGURE 5.15: Photos showing the integration of the SERS platform with digital microfluidics, in the absence of colloidal nanoparticles: (a) combined device is about $2 \times 3$ cm in size, and the rare-earth magnet provides the external magnetic field that concentrates the NPs; (b) zoom-in of the digital microfluidic electrodes that transport the droplets in the PDMS channel, also showing the triple-reservoir configuration of this particular device; (c) alignment of three different micromagnet shapes such that their foci are located above the center of the underlying electrode.

was deemed the best way to obtain such intricate features. The top-plate surface was protected with photoresist and tape to prevent surface damaging from the coarse grain. The bottom plate that comprises the electrodes was fabricated on quartz using the standard protocols employed in the Fair group.
A typical protocol for the operation of the device would be as follows: about 10µL of MNPs@Au@Ag solution would then be inserted into the reservoir and dispensed by the electrodes such that each droplet covers approximately the area of a single electrode. It is important to realize that for droplet dispensing and transport to be successful, the surface tension of the nanoparticle solution needs to be matched to that of the surrounding oil immersion; this procedure requires tweaking of the surfactant concentration in the oil or the aqueous phase and is time consuming and dependent on the aqueous content (surfactant, nanoparticle concentration, etc.). Once the droplet containing MNPs@Au@Ag is positioned at the electrode over which a micromagnet junction exists, the externally applied magnetic field would concentrate the particles in the junction and an incident laser beam would then probe the same junction to detect an enhanced and reproducible SERS signal.

It is therefore evident that only a few more steps were required to obtain a working device, but the lack of time during the author’s Ph.D. experience is the sole reason why it was not completed. The author expects to achieve a working device in the few months post-graduation.

5.8 Conclusion

This chapter strung together many concepts and experimental results that have been presented and discussed thus far in this thesis, and which were each independently developed as a unique piece of a big puzzle that painted a picture of a proof-of-concept diagnostic system for cancer detection.

Building on the research Yellen et al. had conducted with respect to cobalt micromagnet fabrication, a novel bow-tie shaped micromagnet was designed and numerically simulated to enhance its concentrating capabilities for the manipulation of low volume fraction of gold-coated magnetic nanoparticles. This was achieved by increasing the extent of the micromagnets’ in-plane magnetization, such that its region of capture (ROC) was maximized,
while still ensuring a single magnetic focal spot between a pair of adjacent micromagnets. Bearing in mind the intended integration of these micromagnets into a digital microfluidic device, which required a hydrophobic coating, as well as its desired use for SERS, a new protocol was devised to fabricate the micromagnets onto polished quartz wafers, which were then protected with a thin, 50-nm layer of teflon.

The capture efficiency of various micromagnet shapes were tested with standard ferrofluid, confirmed that the bow-tie micromagnets produced both the strongest and most far-reaching magnetic field gradient, thus making them the optimal shape for future use. Another important result was the confirmation that the thin teflon layer did not detrimentally increase the separation between the micromagnets and the ferrofluid solution, such that strong ferrofluid concentration in micromagnet junctions was still observed. Further separation incurred by sputtering thicker layers of silica did have drastic effects on the micromagnets’ ability to concentrate ferrofluid.

The concept of magnetic-field modulated SERS was finally demonstrated, after having been conceived by the author over 3 years ago. The SERS-active magnetic nanoparticles from Chapter 4 were controllably concentrated between micromagnets by the application of an externally applied magnetic field to yield reproducible and enhanced SERS signals. The generated SERS signals was quantitatively similar at different micromagnet junction, for the same sample, confirming that the controlled fabrication of the micromagnets translated to reproducible SERS data. The robustness of this system for generating quantitative SERS signals, thereby tackling one of the most notorious challenges in SERS, was showcased by obtaining a magnetic-field modulated SERS cycle, depicted the cycling of the detected SERS signal as the externally applied magnetic field was sinusoidally varied. This study confirmed that this system provided novel control on the concentration state of SERS-active nanoparticles, which could open doors for the more reliable use of SERS in diagnostic devices.

Finally, the plasmonics-active magnetic nanoparticles and micromagnet technique were
incorporated into a digital microfluidics device and tests were initiated. This required further modification of the micromagnet fabrication to ensure that a conductive layer of ITO was sandwiched between the quartz wafer and the cobalt micromagnets - this ensured that the top plate could be grounded for droplet manipulation by voltage actuation. Although the tests were not complete, preliminary data would suggest that the concept is realistically achievable.

The proof-of-concept diagnostic device that integrates plasmonics-active magnetic nanoparticles as versatile SERS substrates, custom designed micromagnet structures as concentration inducers, and finally a digital microfluidic platform for the automated manipulation of sample droplets with minimal cross-contamination. This device boasts key attributes that are crucial to make the SERS modality competitive for diagnosing diseases: signal sensitivity and reproducibility, sample automation and manipulation, and culminating in a low-cost and reusable device.
The four aims that were formulated at the start of this thesis were completed in full.

The finite element method (FEM), upon which the numerical simulation package COMSOL Multiphysics is based, was confirmed to be a versatile tool that can confidently solve nanoscale electromagnetics of 3-D geometries. When compared to the multipole expansion as well as the Mie theory, which are both established analytical methods for modeling 3D nanostructures in the optical regime, the FEM exhibited excellent correlation. Although the algorithm is memory-intensive, the obtained solution is accurate, as a function of both wavelength and space, to within a few percent of its theoretical counterparts. This was demonstrated when solving near-field electromagnetics in the vicinity of 3-D single and dimer nanoshells, when excited by a plane wave incident or a radiating dipole. It is particularly important to stress that the tetrahedral mesh elements enable curved boundaries to be discretized such that potential field discontinuities across boundaries are reliably accounted for. The comparative studies performed in this work indicate that FEM is a robust algorithm for solving more complex, interesting and realistic geometrical models for further advancing the field of plasmonics.
These results suggest FEM to be a more promising computational electrodynamics modeling algorithm than FDTD for use in the plasmonics arena.

The contribution of these studies to the field of nanoscale simulation, and to the broader audience of nanotechnology in general, was recognized when they were both accepted to visible journals in the field - ACS Nano & Journal of Nanotechnology. Prior to these studies, the author discovered that the consensus among the research groups that are intricately involved with nanoscale modeling was that the FEM algorithm employed by Comsol to model 3D nanostructures in the optical regime was inaccurate to the point of erroneous. Evidently, the author acknowledges that Comsol has been improving their algorithm by releasing a newer version approximately every two years. Nevertheless, by demonstrating that the accuracy of the FEM algorithm was typically within 5% of the analytical solutions in both spacial and spectral resolutions, compared to 10-30% for the more established FDTD, the author’s hope is that established research groups will start endorsing the more accurate FEM to produce more realistic modeling of nanostructures.

Once the FEM algorithm was properly characterized for the modeling of three-dimensional nanostructured plasmonics, it was utilized as a complementary tool to study more exotic SERS substrates, such as the gold nanostar. The author’s contributions in the domain of SERS substrate development were exhibited in three related domains.

Firstly, the synthesis of high-yield gold nanostars of varying sizes, the growth mechanism, their physical modeling, optical response and use as SERS substrates were investigated and reported for the first time. The results indicated that it is possible to vary the size and morphology of the nanostar structures in a controlled fashion, by carefully adjusting the volume of preformed Au seed added to the growth solution. The homogeneously-sized star samples conveyed interesting absorption spectra comprising a short and long plasmon band, with the latter becoming increasingly broad and red-shifted with enlarging nanos-
tars, indicating a heterogeneous distribution of morphologies within a given sample. The gold nanostars’ potential as a promising SERS substrate was demonstrated with the Raman dye pMBA. This study comprised the author’s first publication, dating back to 2008, in the Journal of Physical Chemistry C.

Secondly, the gold nanostar’s plasmonic behavior were modeled both as a function of space and excitation wavelength. This study provided important results that contribute to the advancement of nanoparticle characterization, as well as to the more complete understanding of the plasmonic behavior of gold nanostars. Other than the challenge of modeling such a complex nanostructure in three-dimensions, which exceeds any published result thus far, the nanostar branch tips were confirmed to generate the strongest E-field enhancement, as long as the branch was aligned by a component of the excitation polarization vector. The nanostar’s plasmon band position was shown to be dependent on the branch aspect ratio more than any other geometrical parameter, and thus draws parallels to the plasmonic response of gold nanorods.

Moreover, the broad absorption spectrum of gold nanostars was determined to be the result of a combination of effects of key geometrical parameters: branch AR, that is determined either by varying the branch width for a given length, or by varying the branch length for a given width, and hyperbranching, a term that coins the appearance of small protrusions from the main branches. Thus, although nanostars are homogeneous on a particle-basis, each nanostar comprises of randomly formed branches of differing parameters, and whose combination produces large plasmon broadening both for single nanostars and nanostar ensembles. This suggests that each nanostar can be excited by a range of wavelengths, which makes this geometry particularly attractive for SERS, PDT and PTT.

The first study was recently published via co-authorship with Scott Yuan in the journal Nanotechnology in 2012.

Finally, the first numerical study of the Nanowave structure in 3-D was reported since
its experimental demonstration over 25 years ago, taking into account infinite boundaries and interparticle rounding in order to reproduce the experimental behavior as faithfully as possible. For thin metal shells, the Nanowave structure exhibited two plasmons bands in the case of gold, and three in the case of silver. As the shell thickness increased, the long wavelength band vanishes possibly due to the incident field not being able to penetrate to the base of the shell to excite the long-wavelength plasmon. SERS was evaluated by surface-area averaging the E-field. The lower-than-expected average E-field resulted from low surface area ratio of hotspots to flat surfaces, implying that the highest SERS EF is obtainable by concentrating the molecules of interest into the periodic hotspots, where the local E-field is the strongest. This detailed study was published in the Journal of Physical Chemistry C in 2012.

With a good understanding of both colloidal and wafer-based SERS substrates, a hybrid system combining dual-functional plasmonics-active magnetic nanoparticles and micromagnet structures, to exploit the versatility of the former and the reproducibility of the latter, was designed and developed.

The controllable manipulation of SERS-active, gold-coated magnetic nanoparticles in aqueous solution requires them to be stable (and therefore small by default), as well as easily functionalizable for labeling with SERS dye, antibodies or DNA capture probes. Although there is wealth of protocols in the literature that claim to produce functionalizable, gold-coated magnetic nanoparticles that abide by such stringent conditions, it was surprising to discover that these claims were mostly subjective. After an exhaustive period trying to reproduce, in vain, published protocols of gold-coated magnetic nanoparticles, the author developed a simple, novel synthesis that exhibited the ideal characteristics for manipulation by an external magnetic field. The synthesis involved the organic synthesis of Fe$_2$O$_3$ nanoparticles, followed by their solubilization in water, and finally gold-coating using citrate as the both the reducing agent and surfactant.
These nanoparticles were extensively characterized by UV-Vis and TEM to ensure that the injected magnetic seeds were indeed coated with gold. The gold-coated magnetic nanoparticles were stable in solution and easily functionalizable. In order to improve the SERS potential of these substrate, they were further coated with a shell of silver, whose thickness was optimized to maximize the generated SERS signal originating from the surface-bound resonant Raman dye DTDC.

Although this research has not yet been published, it provides the most crucial component required for obtaining the envisioned diagnostic chip: an aqueous, stable, easily functionalizable, plasmonics-active, magnetic nanoparticles. Without this nanoparticle system, the data detailed in Chapter 5 would not have been possible, which is the reason the author spent excruciating time obtaining the “ideal” colloidal system.

The final chapter strung together many concepts and experimental results that have been presented and discussed thus far in this thesis, and which were each independently developed and finally combined into a hybrid system consisting of a micromagnet-based substrate and versatile plasmonics-active magnetic nanoplatforms.

The major contributions details in this chapter are the demonstration of the concept of magnetic-field modulated SERS.

The first study involves controllably concentrating SERS-active magnetic nanoparticles between micromagnets by the application of an externally applied magnetic field to yield reproducible and enhanced SERS signals. The generated SERS signals was quantitatively similar at different micromagnet junction, for the same sample, confirming that the controlled fabrication of the micromagnets translated to reproducible SERS data. The robustness of this system for generating quantitative SERS signals, thereby tackling one of the most notorious challenges in SERS, was showcased by obtaining a magnetic-field modulated SERS cycle that varied as a function of an externally-applied, sinusoidal magnetic field. This study confirmed that this system provided novel control on the concentration
state of SERS-active nanoparticles, which could open doors for the more reliable use of SERS in diagnostic devices.

The design and development of this hybrid system concludes this Ph.D. thesis, although there is still much to be done that was unfortunately not achievable by the author in the available time frame. The author foresees many tasks that require attention to further this device as a promising platform for cancer biomarker detection; these are discussed in the Proposed Future Research section below.

Proposed Future Research

Although it is always tempting to formulate increasing complex ideas to be added to extant projects to make them the next big medtech device in the healthcare realm, the author will try to keep this final section to a practical minimum.

Future work can be divided into two parts: 1) immediately possible additions that would keep the device simple, yet workable, and 2) the more long-term goals to could make the device competitive in the field:

The first item follows smoothly from the where the thesis left off, and would involve testing the integrated SERS-microfluidic prototype platform. Specifically, a proper holder for the system would need to be designed and the dispensing of an aliquot of MNPs@Au@Ag into the fluidic channel would need to be tested. A reproducible SERS signal from several droplets manipulated on the chip would then have to be detected and quantified to validate this proof of concept.

The second item on the list would be the functionalization of the plasmonics-active magnetic nanoparticles with the appropriate capture probes that are specific to antigens or cancer genes, such as antibodies or DNA capture probes for ErbB2 or Ki-67 breast can-
cer antigens or genes, respectively. The nanoparticles could be functionalized using the in-house developed Molecular Sentinel, or using a simpler DNA-sandwich assay.

Although this appears simple to perform, the key to this procedure is first characterizing the nanoparticle binding/SERS detection process and determining the system’s limit of detection. Integration into the chip is then only a matter of pipetting.

The third, more involved, task would follow from the first, and would comprise of utilizing real human sample biomarkers, and compare the results of this system to systems currently available in the market. In addition to the pros and cons that would need to be established from a technical perspective, a cost-benefit analysis would be in order. Further development of the system might be worth pursuing if the analysis is financially sound.

In terms of projects that enhance the functionality and portability of the microfluidic system, several ideas are proposed: Since the ultimate goal is the detection of disease biomarkers in patient blood, the purification of blood prior to detection is of great desire, and implementing a system that can purify blood to excrete the biomarkers of interest would be indispensable. The simultaneous detection of multiple samples via parallel processing is a feature that the digital microfluidic platform can offer, due to the versatility of the electrode programming. In the simplest case, increasing the sample throughput by N would require the addition of N tracks. Evidently, the upper limit of N is determined by the practical feasibility of being able to simultaneously control these tracks, as well as the detection setup used to collect the emitted SERS signal. It is envisaged that significant system customization and optimization, both on the microfluidic front as well as on the optical system train, would be needed to accomplish this task. Finally, miniaturizing the optical system would allow the
device to be more portable and use less expensive optics, although a spectrometer would most likely still be required. The laser excitation source, however, could be directly integrated into the microfluidic device, as has recently been demonstrated by the Jokerst group. Again, once integrated, extensive testing would be needed to properly calibrate the system and ensure a sensible limit of detection is maintained.

The author realizes that each proposed task by itself is likely to span a whole Ph.D. career or more, but they were presented as potential directions stemming from his own passion to positively contributing to the diagnosis and eradication of deadly diseases.
Author’s Publications

1. **Christopher G. Khoury** and Tuan Vo-Dinh, "Plasmonic ‘Nanowave’ Substrates for SERS: Fabrication and Numerical Analysis”, just accepted to Journal of Physical Chemistry C

2. **Christopher G Khoury**, Hsiang-Kuo Yuan (co-first authors), Hanjun Hwang, Christy M Wilson, Gerald A Grant and Tuan Vo-Dinh, "Gold nanostars: surfactant-free synthesis, 3-D modelling, and two-photon photoluminescence imaging”, Nanotechnology, 2012, pp 075102


6. Hsiang-Kuo Yuan, **Christopher G. Khoury**, Christy M. Wilson, Gerald A. Grant, Adam J. Bennett, Tuan Vo-Dinh, "In Vivo Particle Tracking and Photothermal Ablation using Plasmon Resonant Gold Nanostars”, just accepted to Nanomedicine

7. Pae C Wu, **Christopher G. Khoury**, Tong-Ho Kim, Yang Yang, Maria Losurdo, Giusepe V. Bianco, Tuan Vo-Dinh, April S. Brown and Henry O. Everitt, "Demonstration of Surface-Enhanced Raman Scattering by Tunable Plasmonic Gallium Nanopar-
icles,” Journal of the American Chemical Society (JACS), 2009, 131 (34), pp 12032-12033

Appendix A

Nanoparticle Synthesis Protocols

This appendix details the synthesis protocols employed by the author for the research on the gold-coated magnetic nanoparticles.

A.1 Synthesis of MNPs

A.1.1 Fe₃O₄ via Colvin method

This section details the organic synthesis of Fe₃O₄ first introduced by the Colvin group.²⁶⁷

Reagents: 1-Octadecene (90%), FeO(OH) crystals, Oleic acid (99%) all from Sigma-aldrich, Argon gas

Equipment: Schlenk line setup, vacuum pump, digital vacuum gauge, digital temperature controller and thermocouple (7-inches long, 2mm diameter wide), heating mantle for 50mL flask, thin NMR tube to fit a thermocouple (6-inches long, 3mm diameter opening), glass thermocouple insert with rubber sealings (19/22), 50mL 3-neck round-bottom flask glass flask (19/22 neck), condenser tube (19/22 neck), thick-walled vacuum tubing, medium egg-shaped stirbar
Reagent Preparation: To ensure proper dissolution and reaction of the FeO(OH) chemical, it needs to be crushed to a fine powder with a pestle and mortar prior to addition into the reaction flask. As such, prepare a stock powder of FeO(OH) by crushing finely until no more shine is observable in powder (the original black shiny granules becomes fine brown matt powder). Store in a foiled 20mL glass vial at room temperature.

Schlenk line initialization:
1. Connect Argon line and test flow throw Schlenk line and bubbler
2. Turn on digital vacuum gauge
3. Close Schlenk line valves and turn on vacuum pump and let system reach ∼ 60mT
4. System is ready for degassing of reaction flask

1. Remove clean glassware from oven
2. Assemble reaction setup as illustrated in figure above, with PTFE coating on each neck insert
3. Add stir bar
4. Close Ar inlet and open vacuum inlet to test system sealing
5. Vacuum gauge should read ∼ 200 – 300mT
6. Backfill with Ar and system is ready to proceed

Reagent addition
1. Place clean 20mL glass vial on balance and tare
2. Measure 5g 1-Octadecene (∼ 6.4mL) & transfer to 3-neck flask
3. Add 0.178g of FeO(OH) powder
4. Stir gently at # 2
5. Add 2.5mL Oleic Acid
6. Pipette in and out to wash FeO(OH) deposited on side wall
7. Seal the flask with the glass stopper

Temperature controller profile
1. Insert thermocouple into NMR tube
2. Program temperature controller as follows:

   Degas
1. Increase stir speed to #5
2. Gently open reaction flask to line vacuum → vigorous bubbling
3. Initiate temperature profile program
4. Initiate gentle water flow into condenser
5. Supervise bubbling until temperature reaches 100°C
6. At $t = 45$ min, switch vacuum degas for Ar flow and backfill with Ar
7. Insulate the reaction vessel with foil (or $320°C$ will not be reached)

**Reaction**

1. Ramp to $200°C$ and let FeO(OH) dissolve
2. Ramp to $320°C$ to nucleate and grow $Fe_2O_3$ NPs
3. Once reaction complete, remove foil and gently lift assembly to cool
4. Let cool to RT
5. Execute glassware wash protocol
6. Execute particle wash protocol
7. TEM verification

Finally, follow the *Glass wash* and *Particle wash* protocols.

**A.1.2 Fe$_2$O$_3$ via Hyeon method**

The following synthesis yields $Fe_2O_3$ nanoparticles of 10nm in diameter, as per Hyeon et al.\textsuperscript{285}

*Reagents:* Fe(CO)$_5$ (481718 – 25mL 99%), Octyl Ether (245599 – 25G 99%), Oleic acid
(OA 99%), dehydrated \((\text{CH}_3)_3\text{NO}\) \((T0514 – 100\text{G})\), pure argon gas, PTFE tape. NB: Keep \(\text{Fe(CO)}_5\) in original packaging (moisture absorbing towel, in the dark and at 4°C)

**Equipment:** For reaction: 50mL 3-neck flask \((19/22 \text{ neck})\), thermocouple probe input \((19/22)\), rubber bung \((19/22)\), glass stopper \((19/22)\), condenser, medium new stir-bar \((\text{egg-shaped})\), Schlenk-line setup, 500µL tuberculin syringes with attached needle, 20mL glass scintillation vial.

**Step 1**
1. Place 20mL vial on balance and tare
2. Weigh 1.28g of OA
3. Add 10mL of Octyl Ether, mix and transfer to 3-neck flask
4. Add stirbar
5. Insert thermocouple, condenser and rubber bung
6. Initiate water flow into condenser
7. Stir speed #5 (vigorous stirring)
8. Program *Temp Profile #1* in Fig. A.3
9. Start degassing and initiate *Temp Profile #1* (let vacuum decrease to ~ 200mT)
10. Once temperature hits 100°C, backfill reaction flask with Ar and let stabilize for 5min
11. Inject 200µL of Fe(CO)$_5$ using a 0.5mL syringe
12. Insulate reaction flask with multiple layers of foil
13. When reaction is over, remove foil and let cool down to RT under Ar
14. Black solution is very viscous as it cools

**Step 2**
1. Program **Temp Profile 2** in Fig. A.3
2. Weigh 0.34g of (CH$_3$)$_3$NO
3. Stop stirring
4. Close air flow to bubbler but maintain Ar flow into reaction flask (keep positive Ar pressure inside reaction flask)
5. Remove bung, quickly add (CH$_3$)$_3$NO and cap with PTFE coated glass stopper
6. Ensure thermocouple inlet is well sealed subsequent to initial MNP synthesis
7. No degassing required (since trying to oxidize particles, presence of some O$_2$ should be insignificant)
8. Open air flow to bubbler and let Ar flow throughout
9. Initiate Temp Profile #2 and stir vigorously
10. As temperature ramps to 130°C, some bubbling occurs but quickly disappears
11. Solution color starts turning brownish-black as MNPs are oxidized
12. Insulate reaction flask with multiple layers of foil
13. When reaction is over, remove foil and let cool down to RT under Ar

Finally, follow the Glass wash and Particle wash protocols.

Two important considerations should be taken into account when synthesizing Fe$_2$O$_3$ nanoparticles with Fe(CO)$_5$: when extracting Fe(CO)$_5$, ensure the container is backfilled with Ar or it reacts with air and decomposes. This can be achieved by the use of a tube with one needle at each end, with one end inserted into an Ar source, and the other end into Fe(CO)$_5$ bottle, such that extraction of reagent replaced the lost volume with an equal volume of Ar gas). The 200µL Fe(CO)$_5$ solution should be injected at once into the reaction flask to ensure spontaneous nucleation of the entire reagent volume.

A.1.3 Particle wash protocol

Reagents: Hexane (100%), Toluene (100%), Ethanol (200 proof)

Equipment: 4 × 50mL centrifuge tubes, centrifuge, organic waste container

Once the black MNP solution has cooled to RT, it becomes viscous so an organic solvent is required to efficiently transfer solution to centrifuge tubes for washing.

Transfer to centrifuge tubes
1. Remove 3-neck flask from assembly
2. Add 20mL of hexane into the 3-neck flask containing ∼10mL MNP & stir to mix
3. Distribute ∼30mL MNP solution into 4 × 50mL centrifuge tubes
4. To each tube, add 15mL EtOH (excess) to flocculate the particles
5. Bath sonicate for 1min to mix well

Centrifuge
1. Centrifuge @15,000g for 30min
2. Decant deep yellow supernatant
3. Add 3mL Hexane & sonicate briefly
4. Top up to 15mL mark with EtOH & sonicate to mix
5. Centrifuge @15,000g for 30min
6. Decant clear yellow supernatant
7. Repeat steps 3 – 6 and see Solubilization protocol to solubilize, or continue below
8. Add 2.5mL Toluene to each tube to redisperse black MNP ppt
9. Combine contents of 4 tubes into 20mL glass vial → ~ 10mL stock solution

A.1.4 Glass wash protocol

*Reagents:* Hexanes (100% not required), Toluene (100%), DI water, Alconox detergent, concentrated HCl, Aqua Regia (3 : 1 ratio of concentrated HCl and HNO₃)

*Equipment:* scrubbing brush, organic waste container

The organic nature of this synthesis means that the glassware becomes particularly challenging to clean and requires several washing steps to ensure complete removal of particles and inorganic matter. Once the MNP solution is transferred from 3-neck flask:

1. Add hexanes and swirl / stir & dump into waste container
2. Wash again with hexanes until clear
3. Rinse 3× with EtOH to remove hexanes
4. Rinse 3× with DI to remove EtOH
5. Wash with Alconox and DI
6. Incubate in conc. HCl to dissolve remaining Fe₃O₄
7. Rinse 3× with DI
8. Incubate in aqua regia to remove inorganics
9. Rinse 3× with DI
10. Dry in oven

A.1.5 Washing and Solubilization of OA-capped MNPs

The synthesized OA-capped MNPs (either Fe₂O₃ or Fe₃O₄) were solubilized using the method employed by Lim et al., which ligand-exchanged the OA monolayer for TMAOH, with some modification.

*Reagents:* Hexane, Chloroform, Ethanol, Acetone, TMAOH (25%) were purchased from Sigma Aldrich

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Equipment: 50mL centrifuge tube, centrifuge, bath sonicator, tip sonicator, rare-earth magnet (∼ 3000 – 5000G)

Wash
1. Transfer 10mL MNP solution to 50mL centrifuge tube
2. Add 20mL EtOH to flocculate the MNPs
3. Shake vigorously, bath sonicate for 1min & separate MNPs with magnet
4. Decant clear yellowish / brown supernatant
5. Wash MNPs with solution of Chloroform:Acetone (v:v = 1:1)
   a. Add 20mL CHCl₃:Acetone and bath sonicate for 2min
   b. Centrifuge at 6000g for 10min
   c. Decant supernatant
   d. Repeat cycle (a-c) four more times
6. After last decant, add 20mL of EtOH and bath sonicate 5min (washes with EtOH)
7. Flocculate MNPs with magnet
8. Solubilize in aqueous medium by following Solubilize protocol below
   OR
   Redisperse in 10mL Toluene to store in organic medium

Solubilize
1. Add 10mL TMAOH followed by 10mL DI water
2. Bath sonicated for 1min then transfer to tip sonicator
3. Sonicate at amplitude 10 for 20min
4. Place magnet on the tube wall and collect MNPs OR centrifuge 6000g for 10min
5. Decant supernatant
6. Repeat steps #1 – 5 twice more
7. Disperse final ppt into 20mL of Ultrapure DI
8. Add 0.8mL TMAOH to give final conc ∼ 1% or adjust to pH 11 with TMAOH
9. Bath sonicate for 2 min → stock aqueous MNP
A.1.6 Oxidation of Fe₃O₄ MNPs

The 12nm Fe₃O₄ nanoparticles synthesized via Colvin’s method above were subsequently oxidized prior to gold coating via gentle heating with nitric acid.

Oxidation of Fe₃O₄ MNPs
1. Start with 20mL of MNPs in 0.1M TMAOH in 50mL centrifuge tube
2. Add 20mL absolute EtOH to flocculate MNPs
3. Magnetically separate flocculated MNPs for 5min
4. Redisperse in 20mL 10mM HNO₃ and bath sonicate for 2min to disperse
5. Add into 50mL round-bottom flask with medium egg-shaped stir bar
6. Place in oil bath at 90°C and stir vigorously for 6 hours
7. Solution color slowly transitions from dark black to reddish-brown
8. Transfer to 50mL centrifuge tube and let cool to RT
9. Flocculate by adding 10mL of 25% TMAOH and centrifuge @ 15,000g for 60min
10. Decant and redisperse in 20mL of 10% TMAOH to functionalize MNP surface with TMAOH, bath sonicate for 5min then magnetically separate for 10min
11. Redisperse in 20mL of 0.1M TMAOH and bath sonicate for 10min
12. MNPs are ready for gold coating via citrate method

A.2 Synthesis of MNPs@Au

A.2.1 MNPs@Au: Silica coating, attachment of Au seeds & shell growth

This method of synthesizing MNPs@Au was based on the method for coating large silica nanoparticles with a gold shell, but with several modifications. Basically, it consisted of first

Silica shell via reverse microemulsion

This required the organic stock MNP in toluene to be redisperse in Cyclohexane, which is done by, for example, flocculating a small aliquot (200µL) with an excess of acetone and redispersing the ppt into the same volume of Cyclohexane. The silica shell growth was performed as reported by Jun et al..

Reagents: Igepal CO-520, Cyclohexane (100%), stock MNPs in cyclohexane, ammonium hydroxide (30%), Ethanol (200 proof), Tetraethyl Orthosilicate (TEOS)
**Equipment:** 50mL round bottom glass flask, medium egg-shaped stir bar, stir plate, 50mL centrifuge tubes and centrifuge.

**Synthesis Protocol**
1. Place round bottom flask on balance and measure 0.23g of Igepal into flask
2. Add 4.8mL into round bottom flask & bath sonicate for 2min to disperse
3. Add 50µL of MNP in cyclohexane & cap with glass stopper
4. Stir vigorously (#5) for 2min
5. While stirring, add 50µL of ammonium hydroxide solution
6. Stir 1 hour to obtain reverse microemulsion
7. Add 50µL TEOS
8. Stir for 5hours

The ratio of Igepal to ammonium hydroxide determines the size of the formed microemulsion droplets. The thickness of silica grown on the MNPs can be controlled by varying the volume V of MNP added to reaction or by the altering the reaction time: increasing V increases the surface area for silica deposition, and thus decreases the silica thickness, keeping all other variables constant; increasing the reaction time allows for more silica condensation and a thicker shell, until the reagents are completely consumed.

**MNPs@SiO₂ Wash Protocol**
1. Transfer MNP@SiO₂ to 50mL centrifuge tube
2. Add 10mL EtOH to flocculate particles
3. Centrifuge @6000g for 10min
4. Decant supernatant
5. Repeat steps #2 – 4 twice more
6. Redisperse in 5mL EtOH → stock MNPs@SiO₂ in EtOH

**Functionalization of MNPs@SiO₂ with APTMS**

**Reagents:** stock MNPs@SiO₂ in EtOH, APTES, Ethanol (200 proof)

**Equipment:** 50mL round bottom glass flask, medium egg-shaped stir bar, stir plate, 50mL centrifuge tubes and centrifuge.

1. Transfer MNP@SiO₂ to 50mL round bottom flask with egg-shaped medium stir bar
2. Add 5 – 10μL of pure APTES while vigorously stirring
3. Reduce stirring to medium and let stir for 24 hours
4. Centrifuge @10,000g for 30min to collect all particles
5. Decant supernatant and add 5mL EtOH
6. Bath sonicate for 1min to redisperse well
7. Repeat steps 4 – 6 twice more
8. Redisperse in 5mL EtOH → stock MNP@SiO$_2$ in EtOH

Attachment of Au seeds to MNPs@SiO$_2$@APTES

This step consisted of first synthesizing very small 2 – 4nm Au seeds that would then be attached to the APTES-functionalized MNPs@SiO$_2$. The small seeds were synthesized via two different method using two different surfactants, THPC and Sodium citrate.

Reagents: APTES-functionalized MNPs@SiO$_2$ in EtOH, Ultrapure DI, HAuCl$_4$ 0.5M stock solution, NaOH, THPC, Sodium Citrate, NaBH$_4$

Equipment: 150mL conical flask, long stir bar, stir plate, 50mL centrifuge tubes and centrifuge.

Synthesis of THPC-capped Au seed: Au@THPC

1. Prepare 2mL of 1% HAuCl$_4$ and put aside in dark to let age for 15min
2. Pipette 45mL Ultrapure DI into conical flask
3. Insert stir bar and stir vigorously (strong vortex)
4. Inject 1mL of 1M NaOH
5. Gently add 12μL of THPC (precision is essential, one extra drop fail reaction)
6. Stir reaction for 5min vigorously
7. Rapidly inject (in one movement) 2mL of HAuCl$_4$
8. Instant color change from clear to dark brown (takes 2 – 3sec)
9. Keep stirring for 10min to react all the reagent
10. Store in dark @ 4°C → stock Au@THPC seed

Synthesis of Citrate-capped Au seed: Au@Citrate

1. Prepare 15mL of 38.8mM Citrate in 15mL centrifuge tube
2. Separate citrate into 2 tubes - tube #1: 5mL and tube#2: 10mL
3. Into tube #2 add 7.5mg NaBH$_4$ and shake
4. Add 1mL of 1% HAuCl$_4$ into 90mL Ultrapure DI in conical flask
5. Stir vigorously for 1min
6. Add 2mL of citrate from tube #1
7. Stir vigorously for 1min
8. Add 1mL of citrate/NaBH$_4$ from tube #2
9. Instant color change to light red is observed
10. Continue stirring for 10min and store @ 4°C → stock Au@Citrate seed

Attaching Au seed to MNPs@SiO$_2$@APTES

1. Add 10mL of Au seed (either Au@THPC or Au@Citrate) to 50mL centrifuge tube
2. While vortexing, add dropwise 1mL of MNPs@SiO$_2$@APTES in EtOH
3. Gently shake by hand for 1 – 2min
4. Place in dark @ 4°C overnight
5. Centrifuge @6000g for 30min
6. Decant supernatant and redisperse pellet in 5mL Ultrapure DI
7. Repeat steps #5 – 6 once → stock MNPs@SiO$_2$@Au$_{seed}$
8. The solution of MNPs@SiO$_2$ that are capped with Au@citrate appears reddish
   The solution of MNPs@SiO$_2$ that are capped with Au@TPHC appears slightly brownish than the original MNPs@SiO$_2$ solution

Gold shell growth on MNPs@SiO$_2$@Au$_{seed}$

Two method were employed to form a gold shell on MNPs@SiO$_2$@Au$_{seed}$ via reduction of Au ions to elemental Au ions. For each method, the ‘growth solution’ containing the Au ions was identical, different reducing agents: formaldehyde, as employed by Pham et al.\textsuperscript{280} and Brinson et al.,\textsuperscript{282} and carbon monoxide.\textsuperscript{282,283}

\textbf{Reagents}: MNPs@SiO$_2$@Au$_{seed}$, Ultrapure DI, HAuCl$_4$ 0.5M stock solution, formaldehyde, Carbon monoxide

\textbf{Equipment}: 150mL conical flask, large stir bar, 20mL glass vial, medium stir bar, stir plate

\textbf{Preparation of the gold growth solution}

1. Dissolve 25mg of K$_2$CO$_3$ in 100mL Ultrapure DI
2. After 10min of stirring, inject 1.5mL of 1% HAuCl$_4$
3. Initially clear yellow solution slowly turns colorless over 30min (Au$^{3+}$ → Au$^+$)

\textbf{Gold shell growth}

1. Pipette 4mL of growth solution into 20mL glass vial with medium stir bar
2. Add 200uL of MNP@SiO$_2$@Au$_{seed}$ while stirring
3. Inject 10µL of formaldehyde
   OR
   Bubble CO gas into solution via glass micropipette
4. Solution changes from colorless to blue over the next 2 – 4min
5. Once no further color change is observed, the MNPs@SiO$_2$@Au are washed via centrifugation and redispersed in Ultrapure DI for further use

The gold shell thickness is varied by controlling the volume of growth solution or the volume of added MNP@SiO$_2$@Au$_{seed}$. Once a gold shell is obtained (post-merging of gold island growth on the particle surface), decreasing the former decreases the gold shell thickness while decreasing the latter increases the shell thickness.

It is noteworthy that this method is scalable: one can increase the volume of each procedure without affecting nanoparticle or gold shell quality.

A.2.2 Wang’s Method combined with Korean method

- Reagents & Materials - Setup used, Pictures from phone

Synthesis of MNPs in organic medium

The success of this reaction depends critically on ensuring AIRLESS conditions, in other words, fully degassed solutions and proper Ar purging, proper solution transfers. To this end, a very involved 3-step process was devised to ensure an airless atmosphere throughout: separation of the reducing agent 1,2-Hexadecanediol from other reagents and degassing both batches separately prior to recombining.

The following two temperature profiles need to be programmed into the temperature controller prior to each corresponding section.

**First step: Degassing Reagents bar 1,2-Hexadecanediol**
1. Place a water bath on a hot plate/stirrer
2. Add 10mL Phenyl Ether, 2mL OA, 2mL OAm into 50mL 2–neck flask
3. Insert egg-shaped stir bar and stir gently
4. Add in 0.71g Fe(acac) & insert rubber bung to side-neck
5. Insert flask into condenser assembly
6. Initiate degassing & increase stirring to #4
7. Slowly increase bath temperature to 100°C by setting hot plate to 250°C
8. Degas for ~ 20 min at 90 – 100°C (a few minutes after bubbling stops / reflux begins)
9. Perform 3 degas/Ar-purge cycles at 90 – 100°C (1 min purge, 1 min degas)
10. Turn off hotplate, remove water bath and let flask cool slowly to RT under Ar (place foil between plate and flask to minimize heat to flask)
11. Once at RT, move setup to neighboring stir plate & keep stirring under Ar for 30min-1hr

Figure A.4: Temperature profiles for the synthesis of MNPs@Au
12. Denote as *flask 1*

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**Second step: Degassing 1,2-Hexadecanediol**

1. Place a water bath on a hot plate/stirrer
2. Into a 100mL 3-neck flask add a stir bar and 10mL of Phenyl Ether
3. Insert a plastic funnel into the middle neck & add 2.58g of 1,2-Hexadecanediol
4. Connect thermocouple-inlet & bung on side-necks, and middle neck to condenser
5. Add thermocouple and connect heating element to read off temperature
6. Initiate degassing & heat water bath to 100°C by setting hot plate to 250°C
7. 1,2-Hexa melts around 60°−70°C and refluxes around 70°−80°C
8. Keep degassing for 2min beyond when bubbling stops and refluxing begins
9. Purge with Ar for 1min & repeat vac/purge twice
10. Turn off hotplate, remove water bath and let cool to RT slowly under Ar (around 40°C, solution solidifies quite a bit)
11. Denote as *flask 2*

---

At this point, both flasks 1 & 2 are stirring under Argon.

---

**Third step: Combine flasks 1 & 2 under Argon atm**

1. Place heating mantle over stir plate
2. Place *flask 2* into heating mantle
3. Stop stirring in both flasks
4. Attach long, small gauge metal needle onto a 30mL syringe
5. Insert syringe into *flask 1* & purge syringe with Ar three times
6. Extract totality of solution from *flask 1*, invert flask/syringe assembly, suck out more Ar and remove syringe
7. Rapidly inject solution into *flask 2* & remove syringe
8. Start stirring again → ready for reaction

---

**Fourth step: Synthesis of MNPs**

1. First, ensure Ar flow into flask, vigorous stirring (#5), water flow into condenser
2. Program temperature profile #1
3. Initiate temperature profile #1
4. No foil is needed to thermally insulate flask (temperature not too high)
5. Once complete, remove heat source & let cool down slowly to RT under Ar
6. Denote as *flask 2*
7. **DO NOT REMOVE *flask 2* FROM ARGON** while next section is prepared
synthesis of MNP@Au

This step involves separating 3 solutions so that each one can be degassed separately at elevated temperature without initiating the gold acetate reduction by 1,2-Hexadecanediol, which occurring at 50 – 60°C.

First step: Degassing Reagents bar 1, 2-Hexadecanediol
1. Place a water bath on a hot plate/stirrer
2. Add 10mL Phenyl Ether to a 50mL 2-neck flask & egg-shaped stir bar
3. Add 0.5mL OA, 3mL OAm and 0.83g Au(acac) & stir vigorously
4. Place in water bath at 100°C & degas until bubbling ceases
5. Backfill with Ar
6. Perform 3 degas/Ar-purge cycles at 90 – 100°C (1 min purge, 1 min degas)
7. Turn off hotplate, remove water bath and let flask cool slowly to RT under Ar
8. Denote as flask A

At this time, both flasks A above and flask 2 containing the synthesized MNPs previously synthesized are sealed and under Ar.

Second step: Transfer of MNPs from flask 2 to flask A under Ar
1. Purge a 30mL syringe (long needle attached) with Ar
2. Syringe out 10mL of MNP from flask 2 and quickly inject into flask A
3. Flask A now contains 20mL Phenyl Ether, MNPs, Oam, OA and Au(acac) under Ar

Third step: Degassing of 1,2-hexadecylamine
1. Add 20mL phenyl ether into 100mL 3-neck flask & egg-shaped stir bar
2. Insert plastic funnel into middle neck & add 3.1g of 1,2-Hexadecanediol
3. Connect thermocouple inlet & bung on side-necks, and middle neck to condenser
4. Add thermocouple and connect heating element to read off temperature
5. Initiate degassing & heat water bath to 100°C by setting hot plate to 250°C
6. Keep degassing for 2min beyond when bubbling stops and refluxing begins
7. Purge with Ar for 1min & repeat vac/purge twice
8. Turn off hotplate, remove water bath and let cool to RT slowly under Ar
9. Denote as flask B

Fourth step: Transfer total contents of flask A to flask B under Ar
1. Purge a 30mL syringe (long needle attached) with Ar
2. Syringe out totality of flask A and quickly inject into flask B
3. Flask A now contains 40mL Phenyl Ether, MNPs, Oam, OA. Au(acac) and 1,2-Hexadecanediol under Ar

Fifth step: Synthesis of MNPs@Au

1. First, ensure Ar flow into flask, vigorous stirring (#5), water flow into condenser
2. Program temperature profile #2
3. Initiate temperature profile #2
4. No foil is needed to thermally insulate flask (temperature not too high)
6. As heat increases, Au(acac) is reduced and solution gains a hint of purple
7. Once complete, remove heat source & let cool down slowly to RT under Ar
8. Follow Wash Protocol

Fifth step: Washing of MNPs@Au nanoparticles

1. Collect dark black solution (+ hint of purple) in 50mL centrifuge tube
2. Divide solution into two by pouring half into another 50mL tube
3. Add EtOH until 45mL line mark and shake to mix and flocculate particles
4. Divide 2 tubes into 4, yielding 25mL of solution in each one
6. Centrifuge washing:
   a. Centrifuge @1000g for 10min
   b. Decant supernatant (deep yellow)
   c. Add 1:1 mix of Hexane:EtOH to 25mL line mark for each of 4 tubes
   d. Bath sonicate for 1 – 2 minutes
   e. Repeat cycle (a-d) again
   a. Finally, centrifuge @1000g for 10min & decant colorless supernatant

7. Redisperse in final hexane solvent that contains OA and OAm:
   a. Pour 100mL Hexane into 100mL glass container
   b. Add 1.5mL of OA and OAm (∼ 0.75mM final conc)
   c. From this flask, pipette 20mL hexane to each of the 4 centrifuge tubes
   d. Bath sonicate to redisperse well
   e. Pipette dark purple solution back into 100mL glass flask
   a. Bath sonicate glass flask for 2min to redisperse whole solution

8. Denote as MNPs@Au Organic

These MNPs@Au are stable for months, but it is worth bath sonicating prior to their use.

synthesis of MNPs@Au@Ag
To increase the sensitivity of these nanoparticles for SERS detection, a shell of silver was grown onto the gold shell. Shell growth with silver in aqueous solution was achieved via a method proposed by [249], but rather than solubilize particles via sonication, which was found to be ineffective and uncontrollable, another more efficient method used by [284] for quantum dot solubilization was applied here and yield extremely stable aqueous MNPs@Au.

First step: Prepare aqueous solution of MUA
1. Add 5mL DI into 15mL centrifuge tube
2. Inject 500µL TMAOH 25% & mix well
3. Add 220mg of MUA and bath sonicate (~ 0.2M solution @ pH 11.5)

Second step: Transfer stock MNPs@Au in Hexane to Chloroform
1. Pipette 1mL MNPs@Au in hexane into 15mL centrifuge tube
2. Flocculate with excess EtOH (3 – 5mL)
3. Centrifuge @6000g for 10min to obtain pellet
4. Redisperse in 1mL Chloroform and transfer to 20mL glass vial

Third step: Solubilization of MNPs@Au with MUA
1. To glass vial, add flat medium stir bar
2. Stir vigorously @ #5
3. Inject 1mL of aqueous MUA solution
4. Seal by screwing on cap and stir vigorously for 2hours (biphasic solution becomes milky purple)
5. Bath sonicate briefly for 1min (helps to separate phases)
6. Centrifuge at 6000g for 10min to separate two phases

   The bottom layer consists of the mikly organic CHCl₃ phase still containing unsolubilized MNPs@Au, and the clear deep purple top layer comprises MNPs@Au in DI
7. Extract top layer and place into 1.5mL Ultracentrifuge tubes
8. Perform 3 cycles of Ultracentrifuging @ 25,000rpm for 30min (decant and redisperse in DI each time)
9. Final solution of MNPs@Au are dispersed in 1mL DI and are deep purple in color
The solubilized MNPs@Au are very stable in DI, and no aggregation is observed after several months. Properly solubilized MNPs@Au are the same color in DI as they are in CHCl$_3$ - if they were improperly solubilized, they would display a bluer color (indicating partial aggregation) and would settle down over time.

Ag growth based on method proposed by Park et al..$^{249}$

**Final step: Growth of silver shell onto MNPs@Au**

1. Prepare oil bath at 100°C
2. Inject 200µL MNPs@Au into 5mL DI at pH 10 (adjusted with NaOH) in round bottom flask
3. Add medium egg-shaped stir bar & stir vigorously
4. Add volume ($V = 3, 5, 10, 15$µL) of 0.1M AgNO$_3$ & lower into oil bath
5. Let react for 5min
6. Add 50µL of 10mM Citrate and let react over 10 – 20min
7. Gradual color change from deep purple to red, to orange to brownish, with increasing volume $V$ of added AgNO$_3$

Both the volume of added MNPs@Au and the amount of added AgNO$_3$ control the shell thickness and can be tuned as desired.

### A.2.3 Lyon’s Method (modified)

Instead of using Fe$_3$O$_4$ MNPs that are then oxidized to Fe$_2$O$_3$ as undertaken by Lyon et al.,$^{241}$ Fe$_2$O$_3$ MNPs were directly synthesized as described in the aforementioned protocol. Gold was then deposited onto these MNPs via an iterative growth process using hydroxylamine hydrochloride as a reducing agent.

**Reagents:** HAuCl$_4$ (1%), Hydroxylamine hydrochloride (0.2M), Sodium Citrate (0.1M), Ultrapure DI

**Equipment:** 50mL round bottom flask, medium egg-shaped stir bar, timer

**Iterative growth of Au shell onto MNPs**

1. Place 10mL DI into flask & add stir bar
2. Inject 200µL of stock MNPs in TMAOH (solution is dark yellowish brown) 
3. Add 250µL 0.1M Citrate 
4. Bath sonicate for 1min to disperse well then stir vigorously 
5. Begin iteration in table below, with 15min between iteration # 
6. Monitor gold coating via solution color and UV-Vis measurements 
7. Once last iteration is performed, let stir for another 30min to react well 
8. Wash solution via 3 centrifuge washes, redispersing in citrate solution

<table>
<thead>
<tr>
<th>Iteration scheme</th>
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<tbody>
<tr>
<td><strong>Iteration #</strong></td>
</tr>
<tr>
<td>1</td>
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<tr>
<td>2</td>
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<tr>
<td>3</td>
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<tr>
<td>4</td>
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</tbody>
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It is noteworthy that the hydroxylamine is added prior to the HAuCl₄ during each iteration.

### A.3 Micromagnet Fabrication method

This protocol details the fabrication of Micromagnet substrates for use as magnetic field concentrators. The fabrication is performed in the cleanroom on nanometer smoothness 3-inch quartz wafers.

*Reagents:* Piranha solution (4:1 conc. H₂SO₄: H₂O₂ (30%)), Negative photoresist NFR-16, Photoresist developer MF-319, Cromium & Cobalt metals, Cytop (PTFE solution), Acetone, Isopropanol, DI

*Equipment:* wafer tweezers, amber-colored film (antireflection), deep glass dishes (4-inch wide), spin-coater, 2 hot plates, Mask-aligner/photo-developer "Photo1"(MJB3 350W), E-beam Evaporator

**CAUTION:** Piranha is extremely oxidizing and full-body protection must be worn when handling this solution.

Wafer surface cleaning
1. Set one hot plate to 90°C and the second to 180°C and let equilibrate
2. In the acid hood, prepare Piranha solution (gently pour H₂O₂ into H₂SO₄)
3. Place the quartz wafer into the bubbling solution for 2min with slight agitation
4. Rinse abundantly with DI and dry with N₂ gun
4. Place on 180°C for 5min to dry and let cool to RT → ready for deposition

Photoresist deposition and exposure

**Spincoater setup:**

Recipe #1: "Speed/Ramp Time": 3000/50030s
Recipe #2: "Speed/Ramp Time": 1000/500 5sec, 3000/500 30sec, 0/500 0sec

**Photo1 setup:**

Set Photo1 lamp to 10.5mW/cm² at 405nm and let warm up

Wash and dry micromagnet mask with Acetone/IPA/N₂ gun, then insert into Photo1 mask holder

**Photoresist developer setup:**

Pour MF-319 developer in glass dish and place in base hood

**Procedure:**

1. Spread AP-3000 (adhesion promoter) onto surface until fully covered
2. Spin Recipe #1
3. Place wafer on 180°C for 5min to evaporate solvent
4. Place on spincoater & let cool to RT
5. Spread NFR-16D2 onto wafer until surface is fully covered
6. Spin Recipe #2
7. Place wafer on hotplate 90°C for 180sec & cover with an inverted glass dish
8. Remove wafer and let cool to RT
9. Place wafer atop amber-colored film in Photo1 & ensure proper contact with mask
10. Irradiate for 15.5sec (optimum time to ensure properly beveled walls for lift-off)
11. Place on hotplate 90°C for 180sec & cover with an inverted glass dish
12. Micromagnet patterns are observable by naked eye; let cool to RT
13. Place wafer in MF-319 dish for 90sec with intermittent, gentle agitation
14. Rinse abundantly with DI and dry with N₂ gun
15. Confirm correct mask deposition via light microscope → ready for metal deposition
Cobalt evaporation
1. Gently clean the wafers with N₂ gun
2. Secure on E-beam evaporator turning mount & place into evaporator
3. Pump down to 5 × 10⁻⁶ (∼ 45min)
4. Deposit 5nm of Cromium and 70nm of Cobalt
5. Vent chamber and remove wafers
6. Pour acetone into a glass dish & soak wafers to dissolve NFR-16
7. Rinse abundantly with IPA and dry with N₂ gun

Cytop (PTFE) coating
1. Clean wafer with Acetone and IPA, then dry with N₂ gun
2. Spread Cytop solution onto wafer
3. Spincoat (Speed/Ramp Time): 1000/100 10s, 3500/300 45s, 0/500 0s
4. Place wafer on hotplate for 10min @110°C to evaporate solvent
5. Place wafer on hotplate 20min @190°C to ensure uniform thickness

Wafer dicing
1. Because of hydrophobic Cytop coating, use sponge-tipped stick to repeatedly spread
   S1813 resist onto wafer
   OR
   Plasma etch for 10sec @ 30W and spin
2. Bake for 100°C in oven for 1min to dry
3. Dice with dicing machine
4. Place each piece into acetone bath to dissolve S1813
5. Rinse with IPA and dry with N₂ gun
Bibliography


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[222] Siegel, R. Nanostructure science and technology. A worldwide study; Loyola College in Maryland, 1999.


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Christopher G. Khoury was born on January 22, 1983 in Manchester, United Kingdom to a loving family. Raised in England and France, Chris is fluent in both English and French. After finishing primary school in France, he enrolled in the private boarding school Epsom College in Surrey and served as Deputy Head of House under his house master (just like the ones you see in Harry Potter) in his senior year. Upon high school graduation, Chris matriculated at Imperial College, London for the Masters of Engineering course in Electrical Engineering. He graduated with First Class Honours after four years and decided it was time to experience a different part of the world - the United States of America. First destination: Durham, North Carolina.

With the Duke BME MS Fellowship, Chris started in the Masters of Biomedical Engineering program in the Pratt School of Engineering of Duke University. During the summer of 2007, he worked as a Research Assistant in the Trahey Ultrasound Lab. After meeting Professor Tuan Vo-Dinh, he pursued the Ph.D. degree in the Vo-Dinh Lab. His research involved the advanced synthesis of gold/silver/magnetic nanoparticles for SERS, FEM electromagnetic modeling of 3D nanostructures, and the design and fabrication of a proof-of-concept SERS-based microfluidic platform that combined multifunctional nanoparticles, magnetism and digital microfluidics.

During his time as a Ph.D. student, Chris was a TA for the BME 171 course “Signals and Systems” for two semesters and won the Outstanding Teaching Assistant Award of 2007. He also received the John T. Chambers Photonics Fellowship and the 1st Place
Poster Award at the Fitzpatrick Institute of Photonics Symposium in 2008 and 2011. Chris also filed an Invention Disclosure on “Nanoplatforms for diagnostics and therapy using optical modalities”. His dissertation is titled “Advanced SERS Sensing System With Magneto-Controlled Manipulation Of Plasmonic Nanoprobes.”

While shadowing Dr. David Tanaka in Dukes NICU and Dr. Lopez in Dukes Emergency Department in 2010, Chris conducted on an open heart surgery as a team of onlooking doctors gasped in awe!