NANOSTRUCTURED DEVICES FOR ULTRASENSITIVE AND HIGH THROUGHPUT VIBRATIONAL SPECTROSCOPIC IMAGING

BY

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DISSERTATION

Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Mechanical Engineering in the Graduate College of the University of Illinois at Urbana-Champaign, 2010

Urbana, Illinois

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ABSTRACT

An outstanding challenge in biomedical sciences is to devise systems that can enable rapid, simultaneous and quantitative imaging of tens to hundreds of species at ultra-low concentrations. Central to addressing this challenge is the availability of a modality capable of sensitive, rapid, cost-effective and multiplexed sensing. Further, the microscopic heterogeneity of intact biological systems necessitates that the sensing be in an imaging format. Vibrational spectroscopic imaging (both Raman and infrared) is an attractive tool due to its potential to obtain rich chemical and structural information using relatively accessible instrumentation. Its applicability in devising such modality however is limited by current detection limits, throughput and speed of acquisition. This dissertation discusses design of novel nanostructured devices for enhancing the sensitivity, acquisition rate and multiplexing capabilities in vibrational spectroscopic imaging.

First, Surface-enhanced Raman scattering (SERS)-based nanostructured particle probes are proposed. Their optical tunability with structure is discussed in detail and preliminary fabrication and validation are presented. Next, design and fabrication of a new class of filters for narrow-band optical reflection in mid-infrared spectral regions using guided mode resonances is demonstrated. The design principles and methodology presented in this dissertation are expected to provide a rational approach in development of sets of probes and filters to enable rapid, ultrasensitive acquisition of unlimited number of molecular targets.
To Reshma, the light of my life, for her love and endurance
ACKNOWLEDGMENTS

I would like to express my sincere gratitude to my thesis adviser, Dr. Rohit Bhargava, for his continual guidance, support and encouragement throughout the course of my doctoral research. I am very thankful to him for introducing me to exciting and interesting scientific horizons.

I would like to thank Professor Narayan Aluru for agreeing to serve as the chair of my committee, extending his support and offering valuable suggestions for my work. I would like to thank Professor Rashid Bashir for his support and suggestions. I owe my sincere gratitude for Professor Scott Carney, who pointed me towards the theoretical frameworks utilized in this dissertation.

My thanks are also due to current and past members in Dr. Bhargava group – Dr. Matthew Schulmerich, Laura Jane Elgass, Rohun Palekar, Pratik Randeria, Rohith Reddy, Jie Chou, Margie Mathewson, Kristina Govorovska, Megan Koop, Dr. Gokul Srinivasan, Dr. Brynmor Davis, Dr. Michael Walsh and Mr. Jason Ip. In addition, I would like to express my gratitude for Dr. Brian Cunningham, Mr. Gary Yen, Mr. Regis Menard, Mr. Laurent Aload, Dr. Adarsh Radadia, Dr. Xiaozhong Jin, Mr. Vishal Nandigana, and Dr. Minjun Yang for their suggestions and contributions.

My special thanks to my wife Reshma and my parents for their support.
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CHAPTER 1
INTRODUCTION

1.1 Motivation

The probability of successful treatment of diseases like carcinoma is highly dependent on their early detection. Through histologic assessments we can recognize disease, detect unusual cases and recognize diagnostic deficiencies. In current practice for pathology, a biopsy sample is fixed and embedded in a solid medium, following which it is microtomed and placed on a glass slide. Since tissue does not have useful contrast in optical brightfield microscopy, samples are stained prior to review by a pathologist. The hematoxylin and eosin (H&E) stain is commonly employed, staining protein-rich regions pink and nucleic acid-rich regions blue. A pathologist is trained to recognize the morphology of specific cell types and their alterations that indicate disease. In engineering terms, hence, recognizing disease can be a manual pattern recognition process that matches spatial patterns in the sample to the chart. An open question is the best method to use molecular and spatial patterns synergistically. A number of research groups have shown that a combination of spatial and biochemical information obtained through spectroscopic imaging (“chemical imaging”) may prove to be useful in pathology. Optical spectroscopic imaging techniques are especially attractive for this approach due to the richness of biochemical and structural information they provide. As opposed to stains, the biochemistry intrinsic in the spectral data is used for cell type recognition in these approaches.
The spatially-specific changes are however masked in ensemble averaged data obtained and specific molecular information cannot be derived. With the increasing realization that disease and development processes are dictated by simultaneous changes in levels of multiple molecules, assays today are no longer of a single chemical species. For example, molecular biology has provided specific protein targets that potentially relate to tumor aggressiveness. In this milieu, ultra-sensitive and high-throughput profiling of multiple biomolecular species in tissue samples becomes critical. Fluorescence imaging is typically employed for molecular visualization. Wherever fluorescence imaging is desirable and use of ultraviolet (UV)-excitation is feasible, quantum dots (QDs) provide an excellent choice for biomolecular labeling. From a technique perspective, the dynamic range of current methods limits the measurable concentration range, photobleaching limits sustained sensitivity while the intrinsically broad spectral signatures limit the ability to simultaneously monitor multiple targets.

Among spectroscopic techniques, Vibrational spectroscopy (IR and Raman) spectroscopy is especially attractive in that it can easily harness the power of optics and microscopy while providing richer molecular detail compared to the near-infrared (NIR), visible or ultraviolet regions. Imaging techniques based on vibrational spectroscopy offer stable signals and may be suitable for both in-vivo and ex-vivo use. Probing vibrational transitions can yield chemical and structural properties of a variety of molecular aggregate systems. Both Raman and infrared techniques have made considerable progress towards exceptionally high sensitivity, in recent years, with the availability of better components and computers. In particular, the advances in
their imaging instrumentation now allow for thousands of pixels of spectral data to be acquired in nearly the same time as single spectra required a few years ago. These emerging capabilities have led to their application in rapid acquisition of data from larger areas and/or smaller volumes at high resolution. Recently, automated approaches have been proposed for histologic differentiation within tissues by using statistical classification of vibrational spectra. For ultrasensitive molecular sensing, the inherent weak responses however limit the applicability of vibrational spectroscopy. In Raman spectroscopy, inelastic scattering of incident radiation due to its interaction with electron cloud of a functional group is detected. The inelasticity is a result of nuclei moving during the interaction of incident photons with the electron cloud. Such scattering events are significantly rarer compared to elastic (Rayleigh) scattering, resulting in extremely low cross sections (about $10^{-29}$ to $10^{-31}$ cm$^2$/molecule). The Raman response hence needs to be enhanced by about 14 to 15-orders of magnitude to achieve molecular sensitivity. On the other hand, Infrared (IR) spectroscopy detects the loss or absorption of incident IR frequencies that match those of vibrational transitions due to these interactions. Although the absorption cross sections are about 9 orders of magnitude higher than the Raman scattering cross sections, the IR signal is still not strong enough for monomolecular characterization, due to instrument limitations and simple thermal fluctuations at room temperature. Advanced instrumentation, such as multiple excitation wavelength sources in case of Raman spectroscopy, can be used to enhance signals. While increasing the expense and complexity, such techniques are often still limited in the highest sensitivity level attained.
Nanostructure-enhanced Raman spectroscopy has recently exceeded fluorescence detection limits and conventional immunoassays while being quantitative over a wide range of concentrations. The approach can also precisely quantify multiple analyte concentration in small volumes using imaging. The key principle behind this advance is the use of surface enhanced Raman scattering (SERS) and the recent emergence of nanotechnology, especially control over nanoscale structure in metals. The reliability of enhancement achieved in SERS is however an open question due to intractability of amplification phenomena. Indeed, this has led to the proposal of a “SERS-uncertainty principle” which states that the greater the enhancement, the less it is possible to know about the structural detail of the surface. Hence, the net result has been a choice between (a) an exceptionally large (up to single molecule level) but uncontrolled enhancement or (b) welldefined and controlled method probes due to the use of self-assembling monolayer reporters but of substantially lower enhancement and reduced label diversity. While the role and utility of controlled nanostructures on making the SERS effect practical via various architectures is now undisputed, a rational framework to design SERS probes for a desired enhancement level, spectral selectivity and size is lacking. In turn, this limits the design capability of assays in terms of multiplexing and sensitivity. Hence, new designs are needed to control the variability predict and manufacture a desired enhancement level and demonstrate reproducibility of the probes. In this thesis we have theoretically accomplished such a design for nanostructured SERS-based probes and investigate its effectiveness.
Fourier Transform (FT) - mode is mostly used in current practice for infrared imaging due its multiplex and throughput advantages. In FT-IR imaging, the field of view is illuminated by the output of a Michelson interferometer and imaged by a focal plane array (FPA) detector. The key enabling technology, mid-IR FPAs, were declassified ~10 years ago; FPAs with fast electronics and robust epitaxial fabrication are newer (~ 3 years) to enable robust detection of 16-65,000 pixels (spectra) in a single interferometer scan. Data analysis algorithms and statistical framework are also a mature resource in many groups. The available speed of scanning is however a barrier along with sensitivity that needs to be overcome for application of such automated spectroscopic approaches in clinical setting. For example, with current commercial IR imaging technology, acquiring data over a 1 cm x 1 cm area would require over 50 hrs. An acceptable speed would scan a glass slide in about a half hour or faster. In addition, the complexity of the instrument increases costs, with systems in the $150,000 to $250,000 range. Current setups are powerful general instruments but many features are not needed for histopathology. The cost of instrumentation can be reduced by reducing the complexity (generality). A reasonable target for pathologists seems to be in the $50,000 - $75,000 range which compares to optical coherence tomography (OCT) systems in ophthalmology that are sold for ~$65,000. To overcome the drawbacks above, here we propose a new approach in which spectral elements that are of interest in cancer research can be recorded directly using narrow line width filters. Here, we present design, fabrication, validation and application of narrow line width filters for this purpose.
1.2 Significance

1.2.1 Nanostructured probe-based modality for molecular pathology of cancers

The realization of nanostructured probes is expected to provide a general capability for ultrasensitive molecular measurements in malignancies and tissues. We propose that a vibrational assaying-modality can be devised as depicted in Fig 1.1 using reliable probes.

![Figure 1.1](image)

Fig 1.1 (A) Tailored nanoprobes with specific antibodies and tailored spectra (B) can be employed to visualize molecular expression levels and distribution in tissue (C) or serum assays (D). Quantitative expression levels (E) can be employed to provide a prognostic score for personalized medicine (F).

In step A, the focus is on designing the probe for controlled, giant responses. In particular, we concentrate on engineering rotationally invariant structures that have controlled
geometries to provide maximal sensitivity. The proposed probes are 3D aggregates composed of spherical gold nanoparticles aggregated with reporter molecules and dielectric spheres. The signal enhancement of reporter molecules arises from surface plasmon resonances of nanoparticles. At the same time, fluorescence may be quenched by nonradiative decay in the nanostructure, providing a secondary sensing advantage. Probes can be employed for two purposes: tissue analysis (B) and serum screening (C). Since these structures can be explicitly modeled, their structural and spectral reproducibility can be employed as inputs to a mathematical model to predict use in applications. The eventual goal for both is to identify individual prognosis by calculating expression levels (E) of biomolecules and mathematical models of risk scores (F). The probe design (step A) for use as imaging probes in a multiplexed sense is the key advance that is proposed here.

1.2.2 Narrow band filters for ultrafast acquisition in IR imaging

We propose to overcome the technological limitations of FT-mode by proposing a novel Discrete Frequency Infrared imaging (DF-IR) instrument to acquire discrete spectral elements. In an idealized measurement of spectra using FT-mode, the intensity of of the interference pattern \( I(\delta) \) arising from a source of output, \( B' \), is a continuous function of the optical path difference \( \delta \). The beam reflected back to the source and towards the detector are denoted by \( \pm \) (phase) of beams at zero path difference (ZPD). Hence, at each optical retardance, the full broadband image, albeit weighted, is measured. This has the following implications. First, at ZPD, a very
large signal (centerburst) due to the integrated bandwidth is seen; while at larger retardation (wings), the signal is much lower due to destructive interference. It is a major challenge to record both accurately with a given dynamic range of the detector.

Fig 1.2 Characteristic infrared absorbance spectra of ten histologic classes comprising prostate tissue (From bottom to top, normal epithelium, fibrous stroma, mixed stroma, muscle, nerve, lymphocytes, stone, ganglion, endothelium and blood.) Absorbance spectra are not uniformly informative.

Also, the measurement capability (full dynamic range) of the detector is only utilized once. Second, noise from the entire spectrum is distributed evenly and equally into informative spectral regions of interest. Last, the entire spectrum is always measured. Only small spectral regions or a small number of specific features are needed for classification. Hence, once the entire FT-IR spectrum has been used to determine the optimal spectral regions, the protocol is
fixed and the entire data set is not needed to be acquired. Typically we use 10-15% of spectral features, which is even less than the informative regions of typical spectra (Fig. 1.2). Hence, we collect more data than needed.

As opposed to interferometry, the DF-IR method records spectral elements directly using narrow linewidth filters. Mass production of the filters is enabled by recent advances in nanofabrication technology, optical modeling and use of unconventional materials for manufacture. In this approach recording spectral elements is decoupled from recording the entire bandwidth (the FT-IR case). The approach offers to:

1. Simplify instrumentation while providing performance at least comparable to an FT-IR spectrometer in terms of spectral coverage, resolution and data recording speed.
2. Make instrumentation considerably simpler and more robust by replacing the sensitive interferometer with a simple moving part. This will also result in lower cost.
3. Potentially use uncooled (significantly cheaper) detectors while providing acceptable performance.
4. The ability to selectively acquire data at few or all wavelengths and to do so in random access format.

Here we discuss design and development of filters with narrow band reflection characteristics to enable DF-IR imaging.
1.3 Background

1.3.1 Surface enhanced vibrational spectroscopy

Enhanced vibrational spectroscopy of molecules adsorbed on or in the vicinity of metal (inorganic) particles and/or surfaces is termed: surface enhanced infrared absorption (SEIRA) spectroscopy and surface enhanced Raman spectroscopy (SERS) for IR and Raman modalities, respectively. Together SERS and SEIRA can be broadly categorized under a common modality termed surface enhanced vibrational spectroscopy (SEVS).

SERS: SERS effect was first demonstrated for pyridine molecules adsorbed on roughened silver electrodes.\textsuperscript{1,2,3} The high Raman signal observed in 1974 was conceived to be due to increase in the number of pyridine molecules adsorbed on the increased surface area of roughened silver electrodes. Later experiments in 1977 however demonstrated that the surface enrichment by itself is not sufficient to explain the ~10\textsuperscript{5} enhancement and it was proposed that the Raman scattering efficiency was enhanced. Jeanmarie \textit{et. al}\textsuperscript{2} suggested that the enhanced intensity was a result of amplified electric fields in the vicinity of metal surfaces. In this line of thinking, Efrima \textit{et. al}\textsuperscript{4} considered absorbate molecule as a classical dipole at a smooth planar metal surface. The dependence of enhancement on excitation wavelength was explained using multiple scattering calculations of higher order harmonics between dipole and metal surface. The effect of roughness was taken into account by a study conducted later\textsuperscript{5} in which roughened metal substrates were modeled as a 2D colloid of metal spheres on top of a smooth metal surface. The
collective resonances due to excitations of conduction electron resonances were evaluated to
determine the effect of the degree of roughness on enhancement. Very soon, intense Raman
scattering of pyridine molecules adsorbed on gold and colloidal sols was reported. Apart from
paving the way for metal colloidal sols to be used as SERS substrates, this study corroborated the
explanations for enhancement based on EM calculations.

These first EM theory predictions of Raman enhancement for molecules near metal
nanoparticles were based on simple models, such as isolated spheres and spheroids. Gersten et
al. pointed out the curvature effects on the enhancement termed as ‘lightning-rod effects’. On a
parallel track to EM theory, Albrecht et. al. proposed that enhancement is due to resonance
Raman scattering which is a result of electronic interaction of adsorbate molecules with the metal
surface. Smardzewski et. al claimed that the giant enhancement is restricted to the spectra of
adsorbed species in the immediate vicinity (‘first-layer’) of the metal surface. In this study,
Raman spectrum of a thin film of deuterium-enriched pyridine on silver was examined. It was
observed that approximately 1000 times as much of pyridine had to be deposited on the top of this
thin film to yield Raman intensities similar to it. Based on these kinds of studies it was proposed
that adsorbate had to be bound on an atomic scale roughness for strong enhancements to be
possible. This view, termed chemical theory, proposed that SERS effect was a resonance Raman
phenomenon due to bonding of the adsorbate with the surface.
Zwemer et al.\textsuperscript{12} in 1980, considered a setup similar to that of Smardzewski et al.\textsuperscript{10} with a monolayer of pyridine on a iodine-roughened silver surface followed by deposition of deuterated pyridine on the top. They reported that both species contribute to the enhanced spectra and SERS certainly extends beyond the first-layer, as predicted by EM theories. This opinion was further popularized by other experimental findings in 1980 that large enhancements persist for molecule-metal separations as high as 100 nm.\textsuperscript{13,14} Such findings led to a further controversy and debate pertaining to the contribution of electromagnetic and chemical effects.

The contradicting theories and its immense potential made SERS an academically fascinating field during these early years. At the same time, a flurry of experiments were conducted and SERS effect was reported on several molecules and molecular groups like: cyanide complexes, 2,6-lutidine, methylpyridines, isonicotinic acid, hydrocarbons, cytochrome C, ruthenium red, pyrazine, nucleic acids, polystyrene, adenine mononucleotides, azides, benzene, acetonitrile, bile pigments, isoquinoline, cyclohexane etc. The first SERS substrates used in most of these experiments were metal (mostly silver) electrode surfaces subjected to alternating oxidation reduction cycles. Though the initial experiments were done using silver electrodes, by early eighties SERS was reported using substrates made of various coinage and alkali metals, including gold, copper, lithium, sodium, potassium, indium aluminum, platinum, cadmium, palladium and rhodium. In addition to different metals, SERS substrates were also fabricated using different approaches. An excellent review by Moskovits\textsuperscript{15} lists fabrication procedures of SERS-
active systems proposed by various groups. Table 1 summarizes some of these surface-based and nanoparticle-based SERS systems in no particular order.

<table>
<thead>
<tr>
<th>Substrates</th>
<th>Fabrication Procedure (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roughened Metal surfaces</td>
<td>Oxidation-reduction cycles of electrode surfaces, Ion bombardment in vacuo, Mechanical polishing, Acid etching, Metal halide films exposed to UV-radiation, Metal deposition on rough CaF2 films.</td>
</tr>
<tr>
<td>Island Films</td>
<td>Vapor deposition of silver on warm glass/quartz substrates</td>
</tr>
<tr>
<td>Cold-deposted films</td>
<td>Vapor deposition of silver on a cold substrate below 30 K</td>
</tr>
<tr>
<td>Spheroid assembly Gratings</td>
<td>Glancing angle evaporation of silver onto SiO2 posts produced by microlithography</td>
</tr>
<tr>
<td>Metal Gratings</td>
<td>Surface modulation of silver using lithography</td>
</tr>
<tr>
<td>Silver Colloidal sols</td>
<td>Reduction of dissolved metal salt in aqueous or non-aqueous medium, Quenching the metal vapor in an argon atmosphere and condensing on a low-temperature surface</td>
</tr>
<tr>
<td>Silver powders</td>
<td>AgNO3 reduced with glucose in aqueous KOH</td>
</tr>
<tr>
<td>Colloidal platinum</td>
<td>K₂PtCl₄ reduced by sodium citrate</td>
</tr>
<tr>
<td>Rhodium particles</td>
<td>Rhodium salt reduction</td>
</tr>
</tbody>
</table>

Table 1.1 Initial SERS Substrates

During 1980-85, several reports were published further validating the EM contribution to SERS enhancement. These early works of EM theory have been thoroughly reviewed. Liao
*et. al* measured Raman spectra of cyanide deposited on substrates with lithographically obtained silver ellipsoids. They validated the dependence of SERS enhancement on aspect ratio and dielectric constant of ambience as predicted by EM theory. The agreement of EM theory predictions was demonstrated on silver island films by *Weitz et. al*. Further some studies showed that SERS excitation spectra obtained for different molecules using rough films were similar. This led to further belief that resonance strongly depends on variation of localized plasmon on the surface geometry of the metal substrate. Some of these EM models were however based on calculations for isolated metallic spheroids; hence, they failed to account for important features of SERS spectra due to coupling effects in systems like cold-deposited films and aggregated colloids. The electrostatic EM calculations based on coupled particles also suggested that the interstices between metal features/particles are high-field locations or ‘hot-spots’. In case of spheres the fields in interstices were shown to exceed those near single spheres by an order of magnitude.

The EM models assume that the total enhancement is a product of enhancement in incident and emitted fields at their respective frequencies. Thus an order of magnitude increase in the field translates into four orders of magnitude enhancement in Raman scattering signal. Some of the earlier EM models were based on electrostatic calculations and hence did not include retardation effects due to higher order multipoles. The calculations made by Kerker *et. al.* on spherical particles were however electrodynamic in nature and accounted for these. Barber *et. al.* evaluated fields on surfaces of prolate spheroids using electrodynamic calculations. Despite the
fact that EM theory enjoyed success in explaining many effects, it couldn’t evolve into a comprehensive model to explain SERS effect. Moskovits\textsuperscript{15} reviewed a list of experimental SERS observations EM theory failed to explain. Some of these are: differences in SERS intensities of some molecules of equally strong ordinary Raman spectra, differences between relative intensities of many lines in SERS spectrum of a molecule, lack of correlation between SERS excitation spectra and absorption spectra in colloidal silver, absence of signal of electrolytes. Avouris \textit{et. al}\textsuperscript{24} reported a weak absorption in the electron-energy loss spectrum of several molecules adsorbed on silver consistent with their relative SERS enhancement. This absorption was interpreted as charge-transfer band leading to a charge-transfer mechanism. Based on other similar observations, a theory for chemical contribution to the enhancement was posed. This theory proposed that the weakness of the SERS effect in some adsorbates compared to other molecules was due to lack of a charge transfer band. The differences in the relative intensities of features of SERS spectra were attributed to the fact that the observed spectra are resonance spectra of a metal-molecule complex instead of the adsorbate itself. In 1984, Murray \textit{et. al}\textsuperscript{25} suggested that chemical effect can be included in EM theory models by using the polarizability of metal-molecule complex rather than that of the molecule in the pertinent equations. Zeman \textit{et. al}\textsuperscript{26} proposed corrections for electrodynamic and surface scattering effects in EM models based on electrostatics. Over the next few years the field of SERS continued to attract several studies on the theoretical front (in improving and extending on EM and chemical theories) and on the experimental front (in improving the reliability and extending the applications of SERS).
Surface enhanced resonance Raman spectroscopy (SERRS) of rhodamine 6G on colloidal silver particles was reported in 1984. The colloidal particles are anion activated to form closely spaced interstices with huge enhancements. Theoretically colloidal aggregates were modeled as fractal clusters and the role of scaling properties of the fractal on Raman scattering was examined.

In a series of papers, Xu et al developed and applied analytical expressions for calculating the primary Raman-Stokes moments using the amplitudes of scattered multipolar fields. They further made calculations for CO and benzene physisorbed on pairs and linear chains of particles made of silver, platinum, germanium and SiO. By this time, numerical methods like finite element method, discrete dipole approximation, finite difference time domain method and T-matrix method along with exact methods based on Mie scattering theory were all used to account for the enhancement based on EM fields. Kneipp et al proposed possibility of single molecule SERRS using colloids. Very shortly, Kneipp et al and Nie et al reported the first findings of single molecule surface enhanced Raman scattering (SM-SERS). The former group demonstrated large effective crosssections ($10^{-17}$ to $10^{-16}$ cm$^2$/molecule) of crystal violet molecule in aqueous colloidal silver solution while the latter group demonstrated a similar order of enhancement for rhodamine 6G molecules adsorbed on selected nanoparticles. These observations were further corroborated on Hemoglobin molecules using isolated silver nanoparticles and on rhodamine 6G molecules using silver nanocrystals. These reports accompanied by the potential widespread applicability of an SM-SERS modality refueled interest of several research groups in SERS theory of colloidal nanoparticle substrates. Based on the prospect of huge enhancements of SERS using colloidal nanoparticles, self-assembled nanoparticle aggregates and composite nanoparticles were of
interest with a hope to control the reliability of enhancement. Oldenburg et al. fabricated core-shell nanoparticles made of dielectric core and metal shell nanoparticles (called nanoshells) using colloidal growth chemistry. Nanoshells confirmed optical tunability of particles with a combination of dielectric and metal layers, an idea initially proposed by Neeves et al. Around the same time, programmed assemblies of gold nanoparticles using DNA linkers were proposed. The gold nanoparticle assemblies were later adapted as probes for genomic applications.

While these were seemingly bottom-up applications in which the enhancement was achieved using sequential building blocks, several advances were made in top-down nanofabrication procedures, which involved large scale patterning of surfaces. One significant approach was the use of lithography to produce nanoscale features. One of the most common ones is based on natural microscale lithography. In this technique, random and/or ordered submicron columnar structures were produced using colloidal particles as etching/or deposition masks. Based on extending this approach, nanosphere lithography to obtain nanometric periodic particle arrays was proposed, which was later improved to create arrays of structures of different geometries. During recent years, significant progress has been made in creating different types of SERS substrates. Some of lithography based substrates reported of late are by Baumberg et al., Perney et al., Qin et al. Some of the recent and popular particle self-assembly based substrates are nanoshells, dye-embedded nanoparticles, protein-functionalized nanoparticles and coupled nanowires. From the perspective of the discussion here, a very interesting idea was to
symmetrically structure materials on the nanoscale. H Xu\textsuperscript{54} proposed the idea of multilayered core-shell metal nanospheres to induce large optical fields at the core that could be used for very high Raman enhancements. No theoretical basis of designing optimal structures, however, based on this principle has been reported so far. In SM-SERS, understanding the quantitative specificity of Raman enhancement is incomplete unless the charge complexes and chemical mechanism are considered. In designing the optimal structures however one can hope that with a given set of materials and metal substrates the chemical enhancement factor remains constant and instead optimize the nano scale geometry of the substrates.

**SEIRA:** Soon after the first experiments of SERS, SEIRA was also observed in attenuated total reflection (ATR) geometry\textsuperscript{1} in thin films of p-nitrobenzoic acid deposited on silicon substrates with thin metal (either gold or silver) layer evaporated on the top.\textsuperscript{55} The enhanced bands reported were incorrectly assigned to C-H stretching modes of aromatic molecules in this report, while much later they were found to be due to contaminants.\textsuperscript{56} Also the limited transparency of the ATR prism used in the experiment allowed for only spectral features above 2800 cm\textsuperscript{-1} to be recorded. This led Hatta et. al\textsuperscript{57} to measure features below 2000 cm\textsuperscript{-1} for nitrobenzoate deposited on silver layer demonstrating the existence of enhancement. A multilayer Fresnel formula was used to calculate the fields at the metal-air interface in such a configuration theoretically\textsuperscript{58}

\textsuperscript{1} Though the term attenuate total reflectance finds common usage, we contend that the correct etiology should dictate that the ATR configuration be termed attenuated total reflection for the geometry and AT reflectance for the measurement.
suggesting that enhanced IR absorption is due to surface plasmon polariton of the metal surface. This was not adequate in explaining certain polarization dependent observations.\textsuperscript{59} Nakao \textit{et. al}\textsuperscript{60} reported enhanced IR spectra for polymer samples using silver, nickel, palladium and platinum films. The maximum enhancement in this experiment was observed at an angle different from the plasmon angle and the authors suggested that the effects of multiple reflections in the metal film, a decrease in penetration depth and chemical interactions at the sample-metal interface might contribute to the absorption enhancement. Hatta \textit{et. al}\textsuperscript{61} reported that the short-ranged enhancement and polarization effects could be explained by considering the collective electron resonance of small metal islands in the films based on EM theory. Later, it was shown that in-plane component of the electric-field in the metal film can enhance IR absorption of monolayer of stearic acid consistent with the island nature of the films and EM theory.\textsuperscript{62}

Wadayama \textit{et. al}\textsuperscript{63} showed that enhanced IR absorption of thiocynate (SCN\textsuperscript{-}) ions on Au film is restricted to the immediate vicinity and reaches a maximum at negative electrode potential. Based on this observation, a charge-transfer mechanism was suggested for IR enhancement. Hatta \textit{et. al}\textsuperscript{64} further tried to distinguish EM and chemical enhancement mechanisms and noticed that for films of polycyanoacrylate deposited on silver films, enhancement extends up to a thickness of 5 nm. Using Langmuir-Blodgett (LB) monolayers of a rhodamine derivative on silver island films, Muraki \textit{et. al}\textsuperscript{65} demonstrated the long-range EM enhancement characteristic of SEIRA. EM computational approaches proposed for SERS by Zeman \textit{et. al}\textsuperscript{66} were general for surface enhanced mechanism and were also applicable to SEIRA.
In normal configuration, SEIRA was demonstrated in transmission mode specifically for p-nitrobenzoic acid on top of silver island films evaporated on a CaF$_2$ substrate. This study suggested and examined three contributions to SEIRA: long ranged EM enhancement due to the metal island films, enhanced vibrational polarizability due to chemical interactions of metal surface and molecules (charge transfer mechanism) and orientation effects of vibrational dipoles of molecules. SEIRA in reflection configuration was reported for thin organic films on low reflective substrates by evaporating thin silver films either on the sample surface or on the substrates. Enhanced IR spectra of LB monolayers on metal island films were reported in both reflection and transmission configurations. These authors showed that a complete surface enhanced vibrational characterization (SERS and SEIRA) can be achieved using a single sample substrate. Also in reflection configuration using effective medium theory in which the island and LB film are modeled as a composite film, they showed effect of distance of the thin film from the metal and angle of incidence on enhancement.

Most of the thin metal films mentioned above were prepared by using a high vacuum evaporator. During the last ten years, different SEIRA-active systems have been reported. Kang et al. (1998) have demonstrated SEIRA using silver colloidal particles spread on a transparent substrate. Further metal particle layers were obtained on transparent substrates by techniques like silane induced immobilization of laser ablated silver colloids, sputtering of metals like gold, silver and platinum. Nanoparticle films of Ag, Au, Cu and Pt were also successfully prepared using
chemical deposition. Apart from coinage metals, SEIRA was reported on island films and other substrates of metals like Sn, Pb, Fe, Pt, Ni, Pd, Rh, Ir etc. The enhancement in transition metals was characterized by using phonon resonances. Periodic reviews on SEIRA\(^1\) provide an excellent listing of various theories used to model SEIRA and various SEIRA substrates fabricated. The progress made in recent years has been well reviewed.\(^1\)

**Biomolecular applications of SEVS:** Table 1.2 gives information about some recent reviews of biological fields of applications of SERS. This by no means, is a complete list of such reviews but only provides few pointer-reviews to important directions in the field. The applications of SEIRA for biomedical analyses are relatively rarer, due in part to the significantly lower enhancements observed. For current SEIRA applications, readers are referred to Aroca.\(^1\)

The experimental signal enhancement observed in SEVS, is a compounded contribution of several factors. The primary and dominant effects in determining the spectral enhancement characteristics of the analyte molecule are the surface plasmon resonance of the substrate and its variations due to surface roughness. In addition, resonance effects of analyte molecule, its charge-transfer coupling with the substrate and other chemical interactions play a contributing role. Such complex interactions have often resulted in case-specific results of enhancement and defied general design principles for experimentally reliable SERS and SEIRA since their discovery.
Current SEVS substrates can be divided into two major categories based on methodology for fabrication: substrates based on surfaces with nano-engineered features and substrates based on colloidal solutions of nanoparticles. Nanostructured surfaces, when carefully designed, offer controllability and reliability in enhancement. These applications represent the true promise of SEVS in that the enhancements can reasonably be predicted, the desired substrates fabricated and the results consistently validated. Patterned substrates, unfortunately, are limited in the achievable sensitivity levels and are especially not suitable for \textit{in-vivo} applications. Metal
nanoparticle aggregate (colloidal) probes, in contrast, are ideal for *in-vivo* applications. Further, the giant enhancements reported for molecules adsorbed on particle surfaces and in interstices provide an excellent opportunity for single molecule sensitivity. The detection reliability of target molecules is however affected by target adsorption variability on nanoparticle probe surface and the nature of the interstitial space in which it is localized. Hence, one avenue that has been suggested is to avoid direct measurement of the desired analyte and improve data reproducibility by detecting SERS probes associated with the target molecule(s). Surface enhanced Raman scattering (SERS)-based probes\textsuperscript{81} offer extensively multiplexed imaging,\textsuperscript{82} enhancement of signal with facile readout\textsuperscript{83} and ultrasensitive assays\textsuperscript{84}, but not all at the same time. The enhancement depends on the reporters’ molecular characteristics as well as nanoscale size, shape, geometry, local aggregation state and surface characteristics of the metal. These parameters can all possibly be optimized for maximal enhancement. Variability in SERS signal arises, however, due likely to the synergistic effects of atomic mobility,\textsuperscript{85} surface reorganization\textsuperscript{86} and molecular mobility. The net result is an unpredictable variation in enhancement, including blinking\textsuperscript{87} or “hot-spots”.\textsuperscript{88}
The current state of the art in vibrational spectroscopic labels is well-illustrated in Fig 1.3 given for nanostructures supporting surface enhanced Raman scattering. The trade-off between sensitivity and reproducibility is exemplified by the two extremes of generating enhanced signals. The two categories for nanoscale structures are based on methodology for fabrication: substrates based on surfaces with nano-engineered features and substrates based on colloidal solutions of nanoparticles. Nano-engineered features are realized on surfaces using top-down approaches like nano-lithography while probes based on nanoparticle aggregation are fabricated in a bottom-down approach using molecular self-assembly. Efforts to understand the enhancement effects and harness them using rationally designed substrates, where undertaken,
have proven to be very effective. Nano-patterned surfaces, when carefully designed, offer controllability and reproducibility in enhancement. Applications of these substrates represent the true promise of SEVS in that the enhancement can reasonably be predicted, the desired substrates fabricated and the results consistently validated. This achievement promises an exciting opportunity to extend such understanding to metal nanoparticle aggregate (colloidal) probes that are ideal for in-vivo applications. The giant enhancements reported for molecules adsorbed on particle surfaces and in interstices, in particular, provide excellent opportunity for single molecule sensitivity. Since it will be difficult to get the analyte of interest to reside in interstitial spaces, the alternate is to avoid the direct measurement of the desired analyte but detect SEVS reported on a probe that associates with the target molecule(s). The challenge then remains in designing multiplexed probes with immense signals reliably.

1.3.2 Narrow-band mid-IR reflection filters

Mid-infrared (IR) vibrational spectroscopy is one of the most common molecular analysis techniques in use. IR spectral data is almost exclusively acquired using Fourier transform infrared (FT-IR) spectrometers due to their multiplex advantage, ordinate precision, mature instrumentation and versatility. A major difference from when FT-IR spectroscopy was being developed and the present day, however, is that IR spectroscopy has been largely supplanted by nuclear magnetic resonance (NMR) spectroscopy to identify molecular structure. Molecular structure verifications today usually consist of comparisons of measured spectra to databases,
rather than to first principles elucidation – which is considerably less restrictive than acquiring high-resolution data for fundamental studies. At the same time, advances in instrumentation and their user-friendliness have resulted in the use of IR spectroscopy for fairly routine tasks in process monitoring and quality control. For many of these applications, only a portion of the spectrum is often useful, for example in reaction monitoring, and a low-resolution spectrum is often sufficient for database matching. The use of interferometers for these applications is contraindicated. IR interferometers are exceptionally useful, in contrast, for sophisticated experiments, including imaging, circular dichroism and perturbation-response experiments. Even in these cases, the expense and time required argues against full spectral acquisition as it is often minimally more informative than selected spectral regions and certain applications could be faster if smaller volumes of data were acquired, for example, for tissue identification in cancer pathology.

In FT-IR spectroscopy, large spectral regions are typically acquired. For large regions, the multiplexing advantage for signal-to-noise ratio (SNR) is also accompanied by a disadvantage for noise. The noise is spectrally uniform while the signal is not; hence, increasing SNR for even a single feature requires substantial effort over the entire spectrum. For characterization of many biological systems, optimal spectral regions and features have recently been identified. For example, in a recent application of IR spectral imaging to automated breast pathology, we were able to deduce the appropriate structure of breast tissue and the presence of cancer to high accuracy using only six spectral features. By limiting the acquisition to these smaller optimal
data sets, the SNR can be increased and acquisition time can be greatly decreased, especially for imaging applications.\textsuperscript{100} If small spectral regions were acquired, the dynamic range required to detect the signal for a given noise level will also be much lower, allowing for the use of simpler detectors.\textsuperscript{101} The advantages for acquiring smaller spectral regions and specific feature sets are the primary arguments driving discrete frequency IR (DFIR) spectroscopy,\textsuperscript{102} in which a small set of spectral features are acquired to characterize the entire spectrum. While it is possible to utilize the conventional grating/slit monochromator and select individual wavelengths for DFIR spectroscopy, light throughput is greatly reduced by the need to spatially restrict the beam and the throughput decreases sharply with aperture size, in turn decreasing the SNR. The recent use of a focal plane array detector for grating based systems\textsuperscript{103} is very promising but requires an expensive array detector and still measures a large bandwidth. Hence, it is advantageous to explore better and faster approaches to generate narrow-spectral band IR beams. Narrow linewidth, tunable IR quantum cascade lasers\textsuperscript{104} and nanoscale modified emitters\textsuperscript{105,106} have been proposed as narrow band sources and can be used for this purpose, but these are expensive and/or span a smaller wavelength range. Similarly, a combination of a fixed bandpass transmission filter and pyroelectric detectors can be used to acquire only one or two measurement wavelengths\textsuperscript{107}. The throughput in such cases is limited and the acquisition rate is suitable where only one or two wavelengths is of interest limiting their application for measuring different spectral bands over a large spectral region. Recently, tunable infrared detectors have been demonstrated using micromachined Fabry-Perot filters\textsuperscript{108} in 3-5 μm spectral range with bandwidths close to 100 nm, but such devices are limited in the spectral range that could be
spanned with a set and their fabrication can be cumbersome and expensive. Hence, technologies that are based on inexpensive components and are easy to deploy with common IR optical elements are still needed.

1.4 Outline

We present new classes of nano-structured devices for Raman and infrared spectroscopic imaging to enhance the detection limits, throughput and speed of acquisition. First, in chapter 2, the structures of probes using nano-spheres with alternating metal and reporter-filled dielectric layers and nanospherical aggregates are proposed. Theoretical models to evaluate the responses of probes are presented. In chapter 3 the optical tunability with structure of probes is discussed in detail and it is shown that by theoretical design of structure, they can be designed for sensing over a concentration range of over 20 orders of magnitude. Further tunability is demonstrated by presenting optimized structures for maximal response at a particular excitation laser or for optically flat response at multiple wavelengths. In chapter 4, a layer-by-layer approach is proposed for fabrication of the probes and results of validation against theory are shown for some preliminary structures. The design and a fabrication of a new class of filters for narrow-band optical reflection in mid-infrared spectral regions using guided mode resonances is demonstrated in chapter 5. A theoretical model based on rigorous coupled wave analysis for design and lithography-based fabrication of a set of filters will be discussed. The reflection spectra of filters obtained were shown to be well-predicted by the theory and rigorous
comparisons will be shown for validation. A discrete frequency infrared imaging instrument set
up based on the filters is presented and preliminary measurements made are given. Last, in
chapter 6, the results presented are summarized and future applications are noted.
CHAPTER 2
STRUCTURE AND THEORETICAL MODELS FOR TAILORABLE RAMAN PROBES

2.1 Philosophy of tailored Raman probes

“...although much remains to be discovered both theoretically and experimentally, we understand a great deal about origin of SERS, certainly for the knowledgeable experimentalist to design workable SERS systems and successful SERS experiments and otherwise make useful predictions. The notion that the basic physics behind SERS is either absent or wrong, is therefore a bewildering shibboleth that often creates doubt in the ability to design SERS-based applications, rationally.”

Kneipp et. al (2006)

“There is a consensus on the electromagnetic origin and fundamental properties of the signal enhancement of SERS, as assisted by surface plasmon excitation on certain nanostructures. Thus the presence of this component in the observed enhanced intensity will define the observed spectrum as SERS spectrum. Defining SERS in terms of one of the components of the observed enhanced intensity may, at first, seem limited and narrow. However, this definition provides the basis for a full discussion of the observations and also a guide for the experimentalist to tune experimental conditions according to the ultimate goal of their research project. The definition
does not necessarily imply that the plasmon assisted contribution ought to be the largest; other resonances may contribute and, in some cases, produce dominant contributions. However it is the presence of the plasmon resonance that will define the observed spectral intensities as a SERS spectrum”.

The two excerpts quoted above, brilliantly sum up our motivation and philosophy in devising nanostructured probes for SERS. We propose that the reliability of surface enhanced vibrational phenomena can be exceptionally controlled and sensitivity enhanced by a rational theoretical design and controlled fabrication of nanoscale-structured probes.

![Diagram](image)

**Fig 2.1.** Typical application of probes in immunohistochemistry. Bioconjugated SERS probes can be used in molecular staining applications.

The application of typical probes for immunohistochemistry (IHC) staining is as shown in Fig 2.1. The probes can be conjugated to specific analytes of interest utilizing biomolecular interactions like antigen-antibody, DNA-cDNA and hormone-receptors. Such interactions are highly specific in nature and have been utilized earlier in conjugating quantum dots and gold
nanoparticles to biomolecular analytes of interest. Two classes of rotationally symmetric nanoparticles are proposed for the design as shown in Fig 2.2.

2.2 Structure

![Diagram showing structures of Nano-layered Alternating Metal-dielectric Probes (Nano-LAMPs) and Structured NanoAggregate Probes (SNAPs).](image)

Fig 2.2 Structural characteristics of Nano-layered Alternating Metal-dielectric Probes (Nano-LAMPs) and Structured NanoAggregate Probes (SNAPs). (A) 3D view of Nano-LAMPs, (B) 2D representation of Mid-sectional view of SNAPs.
**Nano-LAMPs:** The first class is a multilayered nanosphere with alternating metal and silica layers. The configuration (Fig 2.2A) consists of dielectric layers with embedded reporter molecules alternating with metal layers. The dielectric embedment shields reporter molecules from direct contact with the metal surfaces. The probes are protected with an outer silica shell for biocompatibility as well as shielding molecules outside the probe from enhancement effects within the probe.\(^{109}\) In reference to the structural design of various layers, we term these constructs Nano-Layered Alternating Metal-dielectric Probes (Nano-LAMPs). Since the outer layer is fixed, resulting odd number-layered nano-LAMPs have a dielectric core while even number-layered probes have a metal core. The probe’s spherical symmetry ensures enhancement uniformity with respect to the direction of illuminating radiation and simplifies fabrication. Similar layered sphere systems with smaller number of layers and smaller sizes have previously for tunable far-field properties, but as probes their potential has not been fully investigated. In addition, although the possibility of achieving intense internal field resonances using multilayered metal-dielectric systems has been previously identified, here we intend to thoroughly investigate and demonstrate the benefits of such systems as probes.

**SNAPS:** The second class of probes is a 3D spherical aggregate composed of gold/silver nanospheres linked with organic molecules. We term these structured nano-aggregate probes (SNAPs) and a 2D representation of their mid-section is shown in Fig 2.2B. The organic linker molecules also act as reporters and their signal enhancement arises from surface plasmon resonances of nanoparticles. At the same time, fluorescence may be quenched by nonradiative
decay in the nanostructure, providing a secondary sensing advantage. Further, SNAPs are encapsulated in a protective silica layer to provide shielding in aqueous environments. Although core-shell DNA-linked gold nanoparticles have been investigated before, a rational approach has not been attempted in designing them. Here we propose systems with a large number of spheres and attempt to investigate the field enhancements obtained in them.

2.3 Theoretical Models

In modeling and tailoring enhancement, two mechanisms - electromagnetic and chemical - are generally invoked. Electromagnetic enhancement (EE) is now well understood and is usually larger compared to the chemical enhancement (CE). CE mechanisms continue to be a subject of much research. Since the EE is larger, it is beneficial to focus on it in designing optimal structures. The dielectric embedment in Nano-LAMPs shields reporter molecules from metal surfaces and, in turn, reduces CE to negligible levels. To quantify signal enhancement from a single probe, we define a net enhancement factor (NEF) as ratio of a probes’ Raman signal to that of a single analyte molecule. Without loss of generality, we can assume that the analyte and probe reporter have equivalent spectra and do not consider resonance Raman enhancement (RRE). The NEF is the combined consequence of EE and numerical enhancement (NE) due to the bulk of reporter molecules within the probe. Assuming that the reporter is uniformly distributed in the dielectric layers at low concentration, the NEF is defined by the volumetric integral as:
\[
NEF = \iiint \left| E_{\text{Raman}}(\omega_S) \right|^2 \left| E_{\text{loc}}(\omega_o) \right|^2 c_r \, dV
\]  

(2.1)

where \( E_{\text{Raman}} \) is the electric field at the shifted frequency \( \omega_s \) and \( E_{\text{loc}} \) is the field at the incident laser frequency \( \omega_o \), \( c_r \) is the concentration of reporter and \( V \) the volume of the reporter embedment. Following the treatment considered earlier,\(^ {116} \) the enhancement in Raman signal of each reporter molecule scales as \( G = \left| E_{\text{Raman}}(\omega_S) \right|^2 \left| E_{\text{loc}}(\omega_o) \right|^2 \approx \left| E_{\text{loc}} \right|^4 \). The local fields (\( |E_{\text{loc}}| \)) are calculated using layered Mie theory for Nano-LAMPs and Generalized Mie theory formulation for SNAPs.

The theoretical modeling of enhancement obtained in SEVS using EM predictions offers explanations for crucial questions such as \(^ {38} \): dependence of the enhancement on 1) size of nanoparticles 2) dielectric properties of the metal 3) interstitial spacing 4) polarization of exciting and scattering radiation with respect to geometrical configuration illuminated. Further, it explains why nanoparticle aggregates offer much higher enhancement levels in comparison with isolated nanoparticles and why only appropriately structured nanoparticle clusters have higher enhancements and others do not.\(^ {117} \) For single molecule applications involving resonance Raman molecules,\(^ {118} \) however such understanding does not suffice and the charge complexes and the chemical mechanism of enhancement have to be duly considered.\(^ {119} \) Theoretically, larger colloidal aggregates have been considered as fractal clusters and scale-invariant theory\(^ {120} \) has been used.
In smaller aggregates, exact (Mie-based) and numerical methods have been applied to study the EM enhancement.\textsuperscript{121,122} Exact methods are particularly useful for a thorough mathematical understanding of the coupling of multipolar components. These methods are then extrapolated to larger aggregates to approximately model amplification per molecule in the cluster. Each of the interstices in large aggregates however has its own characteristics of polarization and field strength resulting in a smaller overall amplification per molecule. This has led to the most pertinent current question in the field of SEVS: \textit{Is it possible to design and fabricate a precise geometrical configuration that exploits the inter-particle coupling to the fullest for an arbitrary polarization?} Recent efforts have focused on designing spherical core-shell nanoaggregates to utilize the symmetry for tunable optical properties and Raman enhancement.\textsuperscript{123,124,125} The idea of multilayered core-shell metal nanospheres to induce large optical fields at the core that could be used for very high Raman enhancements has also been proposed.\textsuperscript{126} However, no theoretical basis exists for optimizing the structures to maximize Raman/infrared overall response. The effect on resonances and characteristic excitation spectra due to geometrical symmetry has not been investigated.

We first discuss the theoretical formulations necessary for evaluating the local fields in probes from which the Raman enhancement and overall optical responses of the probes are evaluated.
2.3.1 Layered Mie theory formulation

Nano-LAMPs are multilayered spheres (Fig 2.3) with layers of arbitrary sizes and refractive indices. Their optical responses and local electromagnetic fields are evaluated using layered Mie theory. The overall sizes of the spheres considered are typically much smaller than the wavelengths of excitation considered. Hence it would suffice to consider the incident excitation as a plane wave excitation and the optical response to a laser field can be approximated to be a linear superposition of responses due to plane wave excitation. Below we describe an analytical formulation based on Mie theory to evaluate the local fields within Nano-LAMPs and in their vicinity when illuminated by a plane EM wave based on a recursive approach. The parameters of significance in a model system are the wavelength ($\lambda$) of incident EM wave, the number of layers in the sphere (L), radii of each layer ($r_1$, $r_2$, …,$r_L$) and relative
refractive indices of each layer \( (m_1, m_2, \ldots, m_L) \). The embedding medium is referred to as \((L+1)\)th layer and the relative refractive index of an arbitrary \( j \)th layer is defined as \( m_j = N_j/N_{L+1} \), where \( N_j \) and \( N_{L+1} \) are the refractive indices of \( j \)th layer and the embedding medium respectively. Since we are interested in the electric fields only, the sphere and surrounding medium are considered to have a magnetic permeability of 1. Without loss of generality we consider the incident wave to be propagating along positive z-axis and polarized along positive x-axis of an arbitrarily chosen reference coordinate system. Such a field is expressed as:

\[
E_{inc} = E_0 e^{i \kappa_{L+1} z} e_x
\]

(2.2)

where \( \kappa_{L+1} = 2\pi N_{L+1}/\lambda \) is the propagation constant of the surrounding medium.

To satisfy the continuity of fields at the spherical surface boundaries, the electric and magnetic fields need to be expanded in terms of geometrically similar basis functions, specifically, vector spherical harmonics (VSH). The incident plane wave field can be expanded in terms of VSH as:

\[
\mathbf{E}_{inc} = E_0 \sum_{n=1}^{\infty} i^n \frac{2n+1}{n(n+1)} (M^{(1)}_{eln} - iN^{(1)}_{eln})
\]

\[
\mathbf{H}_{inc} = -\frac{\kappa}{\omega} E_0 \sum_{n=1}^{\infty} i^n \frac{2n+1}{n(n+1)} (M^{(1)}_{eln} + iN^{(1)}_{eln})
\]

(2.3)

The scattered fields outside the sphere can be expanded in VSH as:

\[
\mathbf{E}_{sc} = E_0 \sum_{n=1}^{\infty} i^n \frac{2n+1}{n(n+1)} (a^{(N+1)}_{eln} M^{(3)}_{eln} - ib^{(N+1)}_{eln} N^{(3)}_{eln})
\]

\[
\mathbf{H}_{sc} = -\frac{\kappa}{\omega} E_0 \sum_{n=1}^{\infty} i^n \frac{2n+1}{n(n+1)} (b^{(N+1)}_{eln} M^{(3)}_{eln} + ia^{(N+1)}_{eln} N^{(3)}_{eln})
\]

(2.4)
The fields in \( j \)th layer in the sphere can be expanded as:

\[
\begin{align*}
\mathbf{E}_j &= E_0 \sum_{n=1}^{\infty} \frac{2n+1}{n(n+1)} (A_n^j \mathbf{M}_{on}^{(1)} - iB_n^j \mathbf{N}_{on}^{(1)} + C_n^j \mathbf{M}_{on}^{(3)} - iF_n^j \mathbf{N}_{on}^{(3)}) \\
\mathbf{H}_j &= -\frac{\kappa_j}{\omega} E_0 \sum_{n=1}^{\infty} \frac{2n+1}{n(n+1)} (B_n^j \mathbf{M}_{on}^{(1)} + iA_n^j \mathbf{N}_{on}^{(1)} + F_n^j \mathbf{M}_{on}^{(3)} + iC_n^j \mathbf{N}_{on}^{(3)})
\end{align*}
\] (2.5)

In equations [(2.3) - (2.5)] \( A_n, B_n, C_n, F_n, a_n^{(L+1)} \), and \( b_n^{(L+1)} \) are the coefficients of expansion of VSH that need to be evaluated. The superscripts on 1 and 3 on VSH (\( \mathbf{M} \) and \( \mathbf{N} \)) denote the usage of spherical Bessel and Hankel functions respectively in their definition. The fields are expanded in appropriate VSH (Bessel or Hankel) based on the requirements of being mathematically well-defined at certain locations. Specifically, the incident field has to be well-defined and be zero at the origin. Similarly, the scattered field has to be finite at infinity. The internal fields on the other hand can be a combination of both inward and outward waves and accordingly both the Bessel functions of first kind and Hankel functions are chosen to define them. The propagation constant in \( j \)th layer appearing in equations (2.5) is given by \( \kappa_j = 2\pi m_j/\lambda \).

The analytical expressions for VSH are given by:

\[
\begin{align*}
\mathbf{M}_{on}^{(kr, \theta, \phi)} &= z_n^m(kr)\pi_n^m(\cos \theta) \cos \phi \ \hat{e}_\theta - z_n^m(kr)\pi_n^m(\cos \theta) \sin \phi \ \hat{e}_\phi, \\
\mathbf{M}_{on}^{(kr, \theta, \phi)} &= -z_n^m(kr)\pi_n^m(\cos \theta) \sin \phi \ \hat{e}_\theta - z_n^m(kr)\pi_n^m(\cos \theta) \cos \phi \ \hat{e}_\phi, \\
\mathbf{N}_{on}^{(kr, \theta, \phi)} &= n(n+1) \frac{z_n^m(kr)}{kr} \pi_n^m(\cos \theta) \cos \phi \ \hat{e}_r + \frac{d(krz_n^m(kr))}{d(kr)} \left[ \tau_n^m(\cos \theta) \sin \phi \ \hat{e}_\theta + \pi_n^m(\cos \theta) \cos \phi \ \hat{e}_\phi \right], \\
\mathbf{N}_{on}^{(kr, \theta, \phi)} &= n(n+1) \frac{z_n^m(kr)}{kr} \pi_n^m(\cos \theta) \cos \phi \ \hat{e}_r + \frac{d(krz_n^m(kr))}{d(kr)} \left[ \tau_n^m(\cos \theta) \cos \phi \ \hat{e}_\theta - \pi_n^m(\cos \theta) \sin \phi \ \hat{e}_\phi \right].
\end{align*}
\] (2.6)
where \( z_n \) are appropriate first order Bessel functions \((j_n)\) or Hankel functions \((h_n^{(1)})\). The angle dependent functions are functions of associated Legendre polynomials and are given as:

\[
\tau_n = \frac{P_n^{(1)}(\cos \theta)}{\sin \theta}, \quad \tau_n = \frac{dP_n^{(1)}(\cos \theta)}{d\theta}
\]  

(2.7)

The coefficients of expansion are obtained by enforcing the continuity conditions at the interfaces. To accommodate the discontinuity between \( j^{th} \) and \((j+1)^{th}\) layer we have:

\[
(E_{j+1} - E_j) \times \hat{e}_r = 0
\]

\[
(H_{j+1} - H_j) \times \hat{e}_r = 0
\]

(2.8)

Similarly the boundary conditions between the outermost layer and surrounding medium would lead to:

\[
(E_{se} + E_{inc} - E_L) \times e_r = 0,
\]

\[
(H_{se} + H_{inc} - H_L) \times e_r = 0
\]

(2.9)

The above equations can be reformulated using Ricatti-Bessel functions (defined as \( \psi_n(\rho) = \rho j_n(\rho), \xi(\rho) = \rho h_n^{(1)}(\rho) \)), logarithmic derivative functions \((D_n^{(1)}(\rho) = \psi_n'(\rho)/\psi_n(\rho), D_n^{(3)}(\rho) = \xi_n'(\rho)/\xi_n(\rho))\) and ratio function \((R_n(\rho) = \psi_n(\rho)/\xi_n(\rho))\). By defining: \( a_n^j = C_n^j/A_n^j, b_n^j = F_n^j/B_n^j \), a stable outward recursion could be worked out for coefficients \( a_n \) and \( b_n \). In this formulation we start with core, where \( a_n^1 = b_n^1 = 0 \) and evaluate the rest of the coefficients \( a_n^j \) and \( b_n^j \) from the values \( a_n^{j-1} \) and \( b_n^{j-1} \). Defining \( x \) as the size parameter given by \( x = 2\pi r/\lambda \), for \( j = 2 \ldots L \), we have the following equations.
The coefficients of the scattered field can be obtained as:

\[
U_n(m_{j-1}x_{j-1}) = m_{j-1} \frac{R_n(m_{j-1}x_{j-1})D_n^{(1)}(m_{j-1}x_{j-1}) + a_n^{L-1}D_n^{(3)}(m_{j-1}x_{j-1})}{R_n(m_{j-1}x_{j-1}) + a_n^{L-1}},
\]

\[
V_n(m_{j-1}x_{j-1}) = \frac{1}{m_{j-1}} \frac{R_n(m_{j-1}x_{j-1})D_n^{(1)}(m_{j-1}x_{j-1}) + b_n^{L-1}D_n^{(3)}(m_{j-1}x_{j-1})}{R_n(m_{j-1}x_{j-1}) + b_n^{L-1}}.
\]

(2.10)

\[a_n^L = -R_n(m_jx_{j-1}) \frac{U_n(m_{j-1}x_{j-1}) - mJD_n^{(3)}(m_jx_{j-1})}{U_n(m_{j-1}x_{j-1}) - mJD_n^{(3)}(m_jx_{j-1})},\]

\[b_n^L = -R_n(m_jx_{j-1}) \frac{mJV_n(m_{j-1}x_{j-1}) - D_n^{(3)}(m_jx_{j-1})}{mJV_n(m_{j-1}x_{j-1}) - D_n^{(3)}(m_jx_{j-1})}.\]

(2.11)

From this point, the remaining internal coefficients can be obtained by starting at the outer layer and using inward recursion. For the outer layer we have:

\[
A_n^L = m_L \frac{\xi_n(x_L)}{\xi_n(m_Lx_L)} \left( \frac{R_n(x_L) + a_n^{L-1}}{R_n(m_Lx_L) + a_n^{L-1}} \right),
\]

(2.12)

\[
B_n^L = \frac{\xi_n(x_L)}{\xi_n(m_Lx_L)} \left( \frac{R_n(x_L) + b_n^{L-1}}{R_n(m_Lx_L) + b_n^{L-1}} \right).
\]

For the inner layers \( j = L \ldots 2 \) we have:

\[
A_n^{j-1} = A_n^j \frac{m_{j-1}}{m_j} \frac{\xi_n(m_jx_{j-1})}{\xi_n(m_{j-1}x_{j-1})} \left( \frac{R_n(m_jx_{j-1}) + a_n^j}{R_n(m_{j-1}x_{j-1}) + a_n^{j-1}} \right),
\]

(2.13)

\[
B_n^{j-1} = B_n^j \frac{\xi_n(m_jx_{j-1})}{\xi_n(m_{j-1}x_{j-1})} \left( \frac{R_n(m_jx_{j-1}) + b_n^j}{R_n(m_{j-1}x_{j-1}) + b_n^{j-1}} \right).
\]
By using appropriate cutoff for the maximum order of VSH and stable recursive evaluations of logarithmic derivative and ratio functions involved, an accurate mathematical solution can be obtained for coefficients and fields in each layer. For metal layers of thickness greater than 10 nm it is typically acceptable to use the values obtained from bulk dielectric constants reported in literature. In case of metal shells thinner than 10 nm, we modify the imaginary part of bulk dielectric constant to account for restricted mean free path according to the previously reported methods for a $j$th layer as:

$$\varepsilon = \varepsilon_{\text{bulk}} + i r_{j+1} \frac{\lambda_p}{L_{\text{eff}}} \left( \frac{v_F}{2\pi c} \right) \left( \frac{\lambda}{\lambda_p} \right)^3$$

(2.14)

in which $\lambda_p$, $v_F$, $c$ and $L_{\text{eff}}$ are respectively plasma wavelength, Fermi velocity, light velocity in vacuum and effective mean free path for electrons. The reduced mean free path for electrons is obtained using:

$$L_{\text{eff}} = r_{j+1} \left\{ \frac{1}{1+t^2} - \frac{t}{2} \frac{(1-t)(1-t^2)}{4(1+t^2)} \ln \left( \frac{1-t}{1+t} \right) \right\}, \quad t = \frac{r_j}{r_{j+1}}$$

(2.15)

The far-fields are dependent on the coefficients of scattered field and can be evaluated using the asymptotic expansions of the scattered fields. The scattering and extinction efficiencies in the far-field are obtained using:

$$Q_{\text{ext}} = \frac{2}{\kappa r_{j+1}^2} \text{Re} \left\{ \sum_{n=1}^{\infty} (2n+1) \left( a_n^{L+1} + b_n^{L+1} \right) \right\},$$

$$Q_{\text{sca}} = \frac{2}{\kappa r_{j+1}^2} \left\{ \sum_{n=1}^{\infty} (2n+1) \left( |a_n^{L+1}|^2 + |b_n^{L+1}|^2 \right) \right\}$$

(2.16)
2.3.2 Generalized Multiparticle Mie theory formulation

Fig 2.4 SNAP configuration modeled as a multispherical cluster in the incident plane

The problem of EM scattering by a cluster of spheres is evaluated by using generalized multiparticle Mie theory formulation. Consider $L$ non-intersecting homogeneous spheres, with an arbitrary $j^{th}$ sphere having a radius $R_j$ and complex refractive index $N_j$, embedded in a non-absorbing medium of refractive index $N_{med}$. A reference coordinate system is chosen at the origin of the center sphere ($j = 1$) and an arbitrary $j^{th}$ sphere’s origin is located at $r^j$ with respect to this reference origin. The EM fields involved are then expanded in terms of vector spherical waves about this fixed reference system origin. The initial incident fields about the reference system can be expanded as:
\[ E_{inc,i}^j = -i \sum_{n=0}^{\infty} \sum_{m=-n}^{n} E_{nn}^j \left[ p_{mn}^j N_{mn}^{(1)} + q_{mn}^j M_{mn}^{(1)} \right] \]

\[ H_{inc,i}^j = -\frac{K}{\omega \mu} \sum_{n=0}^{\infty} \sum_{m=-n}^{n} E_{mn}^j \left[ q_{mn}^j N_{mn}^{(1)} + p_{mn}^j M_{mn}^{(1)} \right] \]  

(2.17)

The fields scattered by \( j^{th} \) sphere can be represented as:

\[ E_{sc}^j = i \sum_{n=0}^{\infty} \sum_{m=-n}^{n} E_{mn} \left[ a_{mn}^j N_{mn}^{(3)} + b_{mn}^j M_{mn}^{(3)} \right] \]

\[ H_{sc}^j = \frac{K}{\omega \mu} \sum_{n=0}^{\infty} \sum_{m=-n}^{n} E_{mn} \left[ b_{mn}^j N_{mn}^{(3)} + a_{mn}^j M_{mn}^{(3)} \right] \]  

(2.18)

The internal field of \( j^{th} \) sphere can be expanded as:

\[ E_{int}^j = -i \sum_{n=0}^{\infty} \sum_{m=-n}^{n} E_{mn} \left[ d_{mn}^j N_{mn}^{(1)} + c_{mn}^j M_{mn}^{(1)} \right] \]

\[ H_{int}^j = -\frac{K}{\omega \mu} \sum_{n=0}^{\infty} \sum_{m=-n}^{n} E_{mn} \left[ c_{mn}^j N_{mn}^{(1)} + d_{mn}^j M_{mn}^{(1)} \right] \]  

(2.19)

The total incident field on a \( j^{th} \) sphere is due to initial incident wave and the fields scattered due to all other \((L-1)\) spheres in the cluster.

\[ E_{inc,tot}^j = -i \sum_{n=0}^{\infty} \sum_{m=-n}^{n} E_{mn} \left[ p_{mn}^j N_{mn}^{(1)} + Q_{mn}^j M_{mn}^{(1)} \right] \]

\[ H_{inc,tot}^j = -\frac{K}{\omega \mu} \sum_{n=0}^{\infty} \sum_{m=-n}^{n} E_{mn} \left[ Q_{mn}^j N_{mn}^{(1)} + P_{mn}^j M_{mn}^{(1)} \right] \]  

(2.20)

The total incident field on a \( j^{th} \) sphere is scattered and the coefficients of expansion of the field scattered can then be given by:

\[ a_{mn}^j = a_n p_{mn}^j, \quad b_{mn}^j = b_n q_{mn}^j \]

\[ c_{mn}^j = c_n p_{mn}^j, \quad d_{mn}^j = d_n q_{mn}^j \]  

(2.21)
Where \(a_n\) and \(b_n\) are Mie scattering coefficients that were described in section 2.3.2. The coefficients \(P_{mn}^l\) and \(Q_{mn}^l\) can be expressed in terms of the individual coefficients as:

\[
P_{mn}^l = p_{mn}^l - \sum_{j=1}^{L} \sum_{\nu=1}^{\nu'} \sum_{\mu=-\nu}^{\nu} \left[ A_{mn\mu\nu}^l a_{\mu\nu}^l + B_{mn\mu\nu}^l b_{\mu\nu}^l \right]
\]

\[
Q_{mn}^l = q_{mn}^l - \sum_{j=1}^{L} \sum_{\nu=1}^{\nu'} \sum_{\mu=-\nu}^{\nu} \left[ B_{mn\mu\nu}^l a_{\mu\nu}^l + A_{mn\mu\nu}^l b_{\mu\nu}^l \right]
\]

The coefficients of expansion of all the scattered fields are translated from their initial reference frame with respect to the \(j^{th}\) sphere’s origin to origins of different spheres and such fields are summated over all the spheres in the cluster. The coefficients \(A_{mn}^l\) and \(B_{mn}^l\) facilitate this translation and can be obtained using the standard procedures.

Finally the vector spherical wave functions are defined as follows using spherical Bessel functions and associated Legendre functions, and angular functions:

\[
M_{mn}^{(J)}(\kappa\tau, \theta, \phi) = \left\{ i e^{i m\phi} \pi_{mn}(\cos \theta) \frac{\kappa \tau z_n^{(J)}(\kappa\tau)}{\kappa \tau} e_\theta - e^{i m\phi} \tau_{mn}(\cos \theta) \frac{\kappa \tau z_n^{(J)}(\kappa\tau)}{\kappa \tau} e_\phi \right\}
\]

\[
N_{mn}^{(J)}(\kappa\tau, \theta, \phi) = \left\{ n(n+1) P_n^{(n)}(\cos \theta) \frac{z_n^{(J)}(\kappa\tau)}{\kappa\tau} e_\tau + e^{i m\phi} \tau_{mn}(\cos \theta) \frac{\kappa \tau z_n^{(J)}(\kappa\tau)}{\kappa \tau} e_\theta + i e^{i m\phi} \pi_{mn}(\cos \theta) \frac{\kappa \tau z_n^{(J)}(\kappa\tau)}{\kappa \tau} e_\phi \right\}
\]

The angular functions are functions of associated Legendre functions and are defined as:
\[ \pi^m_{mn}(\cos \theta) = \frac{m}{\sin \theta} P^m_n(\cos \theta), \tau^m_{mn}(\cos \theta) = \frac{d}{d\theta} P^m_n(\cos \theta) \]  

(2.24)

The Bessel functions, angular functions and associated Legendre functions can all be evaluated using stable recursive formulations. Substituting (2.22) in (2.21), a system of equations can be generated and the linear system can be solved. The solution can be obtained using an efficient numerical schemes and Bi-CG STAB iterative technique. The fields are then computed and the complete numerical formulation is referred to but not described here. The scattering and extinction cross sections can be evaluated as:

\[
C_{\text{ext}} = \frac{4\pi}{\kappa^2} \sum_{l=1}^{L} \sum_{n=1}^{\infty} \sum_{m=-n}^{n} \text{Re}\left[q^{l^*}_{mn} P^{l^*}_{mn} + b^{l^*}_{mn} q^{l^*}_{mn}\right], \\
C_{\text{scat}} = \frac{4\pi}{\kappa^2} \sum_{l=1}^{L} \sum_{n=1}^{\infty} \sum_{m=-n}^{n} \text{Re}\left[q^{l^*}_{mn} a^{(l)}_{mn} + b^{l^*}_{mn} b^{(l)}_{mn}\right] 
\]

(2.25)

2.3.3 Genetic Algorithm Selection

The important parameters that determine the local fields and far-field optical properties in case of Nano-LAMPs are the sizes of different layers and refractive index of each layer. In case of SNAPs the radii and refractive indices of different spheres are crucial. In this study, whenever required, we have employed an optimization of peak wavelength and height for far-field spectra and internal fields for Raman spectra of reporter molecules. A set of 1000 probe structures is
chosen at random and tournaments are conducted on sets of 4 probe structures to evaluate the best configuration (with maximal/minimal internal field in dielectric layers or minimal difference with requisite ratio of far-field spectral peak wavelength to peakwidth). These “fit” structures are entered into a mating pool and undergo a simulated binary cross over recombination with a probability of 0.9 and polynomial mutation recombination with a probability of 0.1. For each pair of structures undergoing recombination in cross over, on average half of the shell sizes are modified using either contracting or expanding crossover operations. The pairs of structures undergoing recombination in polynomial mutation the shell sizes expand or contract based on the closeness to upper or lower bounds. The population is then updated by replacing the “least fit” individuals with the recombinated structures. The updations are performed iteratively until the convergence of fitness of the best individual structure in the population is apparent. Typically about 30 to 50 iterations were required for the convergence in all cases.
CHAPTER 3

NANOSTRUCTURED PROBES:
OPTICAL TUNABILITY AND OPTIMIZATION

The far-field and near-field properties of Nano-LAMPs and SNAPs can be tuned with respect to the size, spacing and refractive index of the nanoparticles. Here we investigate the optical tunability of probes and provide optimal configurations devised using a genetic algorithm selection of configuration parameters.

3.1 Optical Tunability

3.1.1 Nano-LAMPs

Using calculations discussed in the previous chapter, we can evaluate far-field and near-field properties of nano-LAMPs. Their optical tunability is with respect to different parameters: size (and number of layers or a combination), refractive indices of metal and dielectric. Before considering their near-field properties that are directly relevant to SEVS enhancement, let us consider the optical tunability of their extinction and scattering efficiency. Such effects are relevant in their applicability for tissue imaging modalities based on elastic light scattering, low coherence tomography and light scattering spectroscopy. In these applications, the dielectric
layers within, are designed to tune peak positions of scattering while metal layers determine the scattering.

**Far-field tunability:**

We first consider the effects on scattering efficiencies for nano-LAMPs of given total size and of different numbers of layers, in visible and NIR excitation. Here, each layer of a nano-LAMP is considered to be synonymous with one metal layer and one dielectric layer together. For example $L = 1$ corresponds to a metal core-dielectric shell particle and $L = 2$ corresponds to this core-shell particle covered with a metal shell on the top of which a dielectric shell is coated. The metal layer is considered to be made of gold and the dielectric of silica, the refractive index of which is considered to be 1.45. The total size of nano-LAMPs is fixed to be 60 nm and the number of layers $N$ is varied from 1 to 4. Further each metal shell is of thickness 10 nm whereas the thickness of each dielectric layer is 5 nm. The size of the metal core is chosen according to the number of layers. In keeping with the theme of biomedical applications, these nano-LAMPs are assumed to be embedded in a water-like host medium whose refractive index is chosen to be 1.33. The normalized scattering efficiencies are plotted with excitation wavelength in Fig. 3.1.

In the range of excitation wavelengths considered, for $L = 1$, there are two dominant resonances in the spectral distribution of normalized scattering efficiency. The incorporation of an
additional metal shell and dielectric shell, when \( L \) is increased to 2, shifts the existing resonances
very slightly to the left.

\[ \text{Fig 3.1 Tunability of normalized scattering efficiency with number of layers when total size is constant} \]

Also the relative proportions of the resonances slightly change and a new smaller peak
appears on the right. With further addition of metal and dielectric shells (\( L = 3 \) and 4), new
resonances appear and the existing resonances shift. This is in accordance with the existing
understanding that, in alternating metal-dielectric multilayered spheres, the plasmonic coupling of
metal shells results in hybridized multiple extinction resonances. The coupling strength and in
turn resonances further depend on the extent and properties of the spacer dielectric (Sinzig et. al 1994, Prodan et. al 2004). Such effects are predominant in layered materials with metals showing narrow plasmon resonances and dielectrics with large refractive index in the chosen spectral region. In the systems considered here which start with gold core and shelled with alternate silica and gold layers, these shifting and splitting effects are perhaps not obvious as in multilayered spheres considered by Sinzig et. al (1994). Moreover lower-energy plasmon peaks might have superposed each other and are hence not spectrally resolved. Nevertheless, it is clear that changing the number of layers provides an opportunity to design layered probes for appropriate scattering resonance peaks.

Next, we consider a similar calculation on nano-LAMPs made of silica and silver, but with core size kept constant at 20 nm. The number of layers L is varied from 1 to 3; the sizes of silver layers are fixed at 20 nm, the size of dielectric layer is fixed at 5 nm and the embedding medium is chosen to be aqueous. The normalized scattering efficiency spectra obtained are plotted in Fig. 3.2. The effects due to increasing number of layers as well as size as observed in Fig. 3.2 are physically similar to those in Fig 3.1. The peaks are much sharper for these nano-LAMPs, due to sharper plasmon resonances in case of silver. Upon adding silver and silica shells to the initial L = 1 nano-LAMP configuration, the sharp resonance peak broadens and a very slight splitting effect can be noticed. Also, a new resonance peak occurs on the right. Adding another set of silver and silica shells (L = 3), further splits the left peak, shifts the right peak and adds a new peak on the
far-right. Also here, in contrast to the previous example, the new plasmon peaks and shifting of the existing ones are demonstrated better.

![Graph showing tunability of normalized scattering efficiency](image)

Fig 3.2 Tunability of normalized scattering efficiency with number of layers when core size is constant

The total sizes and number of layers that could be accommodated are both dependent on experimental feasibility and size criteria. Based on such fabrication constraints, the geometrical configuration of nano-LAMPs can then be optimized for given excitation frequency.

The other important parameter that determines the location, extent and height of far-field resonance peaks in nano-LAMPs, is the chemical composition. To demonstrate this, consider the
scattering efficiency spectral distribution of nano-LAMPs of varying dielectric spacer in a fixed geometrical configuration of \( L = 2 \) (with Cu core 20 nm, Dielectric 5 nm, Cu shell 20 nm, Dielectric 5 nm).

![Graph showing scattering efficiency spectral distribution](image)

**Fig. 3.3** Tunability of scattering efficiency in a fixed 4-layered LAMP with changing outer dielectric

The dielectrics chosen are of refractive indices 1.1, 1.45, 2 and 3. An index of 1.1 represents a layer similar to biomolecules (but a separation of 5 nm is perhaps not practically achievable with biomolecular linkers along with producing a continuous shell of nanoparticles), 1.45 represents silica while high refractive indices 2 and 3 are chosen as hypothetical values for
comparison. The embedding host medium is again assumed to be water. From the scattering efficiency spectra plotted in Fig 3.3, it is clear that the relative dominance of the resonances in the given excitation region vary with the dielectric.

![Graph showing scattering efficiency](image)

**Fig. 3.4** Tunability of scattering efficiency of bimetallic 4-layered LAMPs with same core, but different outer metal shell

More importantly, the resonance peaks for $n = 1.1$ and $1.45$ are of interest because most bio-polymers have refractive index values between 1 and 1.5. Also, the clear distinction between some of the peaks is relevant in creating multicolor probes. Next, we consider the same geometry with $N = 2$, but change the chemical composition of metal shell. The core of the particle is fixed
to be made of Au and the metal shell is varied between Au, Cu and Ag. The dielectric layers are fixed to be made of silica and the embedding host medium is water. The scattering efficiencies for this case are presented in Fig 3.4.

From Fig. 3.4, it can be observed that the scattering characteristics for a Cu shell are closer to that of Au shell. The greater differences between plasmon characteristics of Ag and Au than those between Au and Cu might result in such effects. The above discussed tunability can be further used to design multicolor labels in visible and NIR ranges of excitation. The controllability of spectral positions of extinction resonances can be used in designing dielectric core metal shell particles (nanoshells) with different core/shell ratios (Jackson et. al 2001). Three layered particles with metal core-dielectric shell-metal shell, consisting of thin metal shells have been proposed to provide ultra sharp resonances (Chen et. al 2005). In this study the size effects on dielectric functions of metal layers thinner than bulk electron mean free path were not considered. In actuality, this dependence on size results in much wider resonances (Khlebstov et. al 2006).

A representative example of the scattering efficiencies calculated using corrected refractive indices for probes proposed by these authors is provided in Fig. 3.5. The three configurations in figure can be used as multicolor labels in Vis range. The caption for Fig. 3.5 provides the details of layer thicknesses in each configuration. The total size for these probes is kept constant to avoid diffusion disparities and hence ability to bind to receptor sites.
Fig 3.5. Multicolor labels in Vis range. Size 10 nm; Geometry: Ag core, Silica shell, Ag shell. 1: 2.5 nm, 4 nm, 3.5 nm; 2: 4.25 nm, 2.75 nm, 3 nm; 3: 5 nm, 3 nm, 2 nm

Such probes of multiplexing capability can be obtained but the number of distinct probes that can be created is going to be limited by the widths of resonance peaks; this is also the case for nanoshells. Nano-LAMPs with higher number of layers however can provide rich absorption spectral response. Their full distribution responses in the excitation regions might be used along with multivariate curve analysis for multiplexed detection of greater number of nano-LAMPs. Although an unlimited multiplexing might not be possible detecting elastic scattering of nano-LAMPs, the results above show that once they have been designed for optimum near-fields, their far-field properties can be used in multimodal techniques. The specificity of detecting Raman/IR
responses of the dielectric layer is much higher due to the characteristics of vibrational transitions. Such a sensing mechanism for nano-LAMPs would, in theory, provide an opportunity for multiplexing unlimited number of targets.

Fig. 3.6 5-layered gold-silica sphere (a) effect of changing protective silica layer thickness between 0, 6, and 10 nm (b) effect of changing embedding medium.

Below, we demonstrate the effect of these parameters on the optical characteristics through some more examples.

**Layer Size:**

First, the effect of changing an individual layer size in LAMPs is illustrated using an example 5-layered gold silica LAMP of radius 50 nm. In Fig 3.6A, the extinction spectra are
plotted for three configurations with different thicknesses for 4th (gold) and 5th (silica) layers. The spectra show that in the absence of outer silica layer (R5-R4 = 20 nm; R6-R5 = 0 nm), the LAMP configuration possesses a broader NIR extinction peak. As the thickness of outer silica layer increases (and the outer gold layer decreases) the NIR extinction peak shifts towards longer wavelengths, however the narrowest achievable peak is at an optimum combination of outer gold layer size and outer silica layer size.

**Embedding Medium:**

To show that the LAMPS can be devised for a particular application, we considered a configuration designed for aqueous medium and show its extinction spectra in comparison to its spectra when used in a biological medium (N = 1.1) and air (N = 1.0). In both cases as shown in Fig 3.6B, the proposed configuration has narrowest peak only when used in aqueous medium.

**Composition:**

LAMPS of different compositions can also be devised for a desired response. This facilitates using different synthesis techniques to fabricate these particles as well as allow creating designs for various applications. We considered one of the coinage metals Au, Ag and Cu for metal shells and silica as the dielectric for all the results shown in this article. To show the tunability with composition, we show the tunability of extinction characteristics in LAMPS when the composition of metal and dielectric are changed. In Fig. 3.7A, for the structural configuration of a 5-layered
sphere, the extinction characteristics are shown for the three different metals. This illustrates the
dependence of peak positions and widths on dielectric properties of the metal shells. In Fig. 3.7B,
we consider the same configuration with copper as metal and change the refractive index of
dielectric material. As the refractive index of dielectric increases, the resonances can be tuned
towards infra red.

Fig. 3.7 5-layered metal dielectric sphere (a) effect of changing metal composition, (b) effect of
changing dielectric composition
Near-field tunability:

The optical near-fields within nano-LAMPs play a very crucial part in designing optimized Raman/IR enhancement.

Fig 3.8 SEIRA enhancement on nanospheres of Au, Ag and Cu

Let us, for example, consider SEIRA for the molecules in the reporting layers of probes. For high sensitivity applications the maximum size allowed for probe is restricted to be less than 100 nm. In case of mid-IR excitation, the nanoscale probes usually are under small particle limit and any size effects are negligible. Using the small particle limit the surface enhancement in SEIRA for a molecule on a nanosphere is given by:
where \( g = (\varepsilon - \varepsilon_o)/(\varepsilon + 2\varepsilon_o) \), in which \( \varepsilon \) and \( \varepsilon_o \) are the dielectric constants of the metal particle and surrounding medium respectively. SEIRA enhancement factors on single nanospheres of Au, Ag and Cu evaluated using equation (3.1) are depicted in Fig. 3.8. From the figure, it can be observed that the differences in enhancement factors of SEIRA for the three different metals are minor. Also the enhancement is not going to be benefited much by a careful design of layer thicknesses and size. So we focus on optimizing Raman enhancement by studying near fields with nano-LAMPs made of different chemical compositions and topology. Nevertheless the principles remain same for SEIRA and these calculations can be extended to SEIRA, if necessary.

The electromagnetic contribution to Raman enhancement factor given in equation (2.1) depends on enhanced intensities at both laser and stokes frequencies. However it is a common practice to evaluate one of the intensities and approximate the EM contribution to be of the order of its square (Schatz et. al 2002). If we consider only the order of magnitude of the signal from the probe then it suffices to evaluate the maximum value of the field intensity within the probe. However, it is impossible to generate multilayered structures with uniform surface coverage for each shell. So sometimes, a more conservative approach would be to evaluate the order of surface average of intensities. Such a design criterion would ensure the maximization of the extent of intense fields called ‘hot-spots’. Here, we use both these approaches and mention the approach used when referring to a particular result. Another important aspect in the applicability of probes
based on enhancement in interstices of metal features is the dependence on polarization of incident beam due to orientation variability. Due to the spherical configuration of nano-LAMPs, such dependence on polarization is absent.

Fig 3.9 depicts the Raman enhancement calculated from maximum intensity on the surface of Au, Ag and Cu nanospheres of different sizes at an excitation wavelength of 532 nm. It can be observed that along with depending on plasmon characteristics of the metal, the enhancement is dependent on size. We will use this tunability further, to design Raman probes for a given excitation.
By plotting the near-fields of different configurations, the effect of geometrical parameters like relative sizes of shells can be observed. Probing into near-fields of nano-LAMPs also provides an opportunity to understand the extent of ‘hot-spots’ in their reporter dielectric layers. In such multilayered configurations, the outer layers seem to focus the field onto the surface of the core (H Xu 2005).

Fig. 3.10 Enhanced electromagnetic fields of a multilayered sphere in different planes

Fig. 3.10 provides a plot of the logarithm of magnitudes of electric fields within a representative nano-LAMP made of gold layers and a dielectric of refractive index 1.1. The radii
of the layers starting from the metal core are 20 nm, 21 nm, 26 nm, 27 nm, 32 nm, 33 nm, 53 nm, 54 nm. The excitation wavelength is taken to be 532 nm and the nano-LAMPs are considered to be embedded in vacuum. The polarization of the incident field and its propagation with respect to each plane are represented at the bottom of each subfigure. The subfigure at bottom right represents the field at a spherical surface 0.5 nm above the core. E-field magnitudes of \( \sim 10^2 - 10^3 \) are observed at the surface of the core as shown in the figure and this translates into a Raman enhancement factor of order \( \sim 10^8 - 10^{12} \). Moreover, it can be observed that all dielectric layers are bright, representing enhanced E-fields. Along with these ‘hot-spots’, dark spots or regions of no enhancement are also observed mostly in the metal regions. These regions of no enhancement also exist in dielectric layers; although they are not obvious in the representations chosen here. Mathematically, these can be interpreted to be due to cancellation of higher order spherical harmonics and physically this is due to the retardation effects of higher order multipoles. Hence we needed to consider such higher order effects in order to completely define enhancement and EM scattering solutions based on electrostatics would not be sufficient to design hot-spots.

The enhancement predicted for nano-LAMPs under appropriate configurations are huge compared to what is achievable by single nanoparticles or nanoshells. Their near-field tunability can be demonstrated by considering the E-field magnitude near the core of particles with different number of layers and sizes but with core size kept constant. For the results here, a gold core of 10 nm is used and the refractive index of dielectric shells is considered to be 1.1. The geometrical configurations considered are given by: \( L = 1 : 20 \text{ nm Au core} + 5 \text{ nm dielectric shell}; L = 2 : 10 \)
nm Au core + 5 nm dielectric shell + 10 nm Au shell + 5 nm dielectric shell; N = 3: 10 nm Au core + 5 nm dielectric shell + 10 nm Au shell + 5 nm dielectric shell + 2.5 nm Au + 5 nm dielectric. Fig. 3.11 represents the spectral distribution of E-field magnitude at a point 2 nm above the core in the direction of incident field polarization. From the figure, it can be observed that as the number of layers increases the maximum E-field magnitude increases. This amplification is due to the additional metal layers interacting with the plasmon resonances of the core. The peak positions are located at 537, 542 and 552 nm respectively.

Fig. 3.11 Near-field tunability of E-field magnitude of particles with constant core and increasing metal and dielectric layers
The shift towards lower energies is due to additional layers and spacing between the layers. This shows that by adding more layers of appropriate size one can keep the shift in the near-field resonances low while increasing the maximum E-field possible. Fig 3.12 presents the log values of E-fields in a plane at the symmetric section of these configurations. The propagation of E-field in this case is into the paper and it is polarized in upward direction. It can be noticed from Fig. 3.12 that the E-field at surface of the core increases with adding layers on the top of the initial nano-LAMP configuration. Further it can be observed that, some of the enhanced regions extend into metal layers and with proper selection of sizes and properties of different layers the probes can be designed to restrict to and maximize enhancement in dielectric layers.

Fig. 3.12 E-field distribution in the incident plane for nanoLAMPS considered in Fig. 3.11 at resonance illumination
Figure 3.13 shows the maximum intensities within the six Nano-LAMPs. The structures considered are:

A – 20 nm Au core/ 5 nm silica shell/10 nm Au shell/5 nm silica shell/ 15 nm Au shell/ 5 nm silica shell

B – 15 nm Au core/ 5 nm silica shell/20 nm Au shell/5 nm silica shell/ 10 nm Au shell/ 5 nm silica shell.

C – 25 nm Au core/ 5 nm silica shell/10 nm Au shell/5 nm silica shell/ 10 nm Au shell/ 5 nm silica shell.

D – 10 nm Au core/ 5 nm silica shell/15 nm Au shell/5 nm silica shell/ 20 nm Au shell/ 5 nm silica shell.

E – 20 nm Au core/ 5 nm silica shell/10 nm Au shell/5 nm silica shell/ 15 nm Au shell/ 5 nm silica shell.

F – 20 nm Au core/ 5 nm dielectric shell/10 nm Au shell/5 nm dielectric shell/ 15 nm Au shell/ 5 nm dielectric shell.

G – 20 nm Ag core/ 5 nm silica shell/10 nm Ag shell/5 nm silica shell/ 15 nm Ag shell/ 5 nm silica shell.

H – 20 nm Ag core/ 5 nm dielectric shell/10 nm Ag shell/5 nm dielectric shell/ 15 nm Ag shell/ 5 nm dielectric shell.

The local field intensity enhancements are dependent on the plasmon coupling and thus on the configuration of the six layered spheres. It can be observed in cases A-D, that the enhancement can be tuned using the thickness of the layers.
In addition, some of the resonances are broadened or sharpened and are dampened or amplified due to the choice of the relative shell sizes. Using a dielectric of lower refractive index shifted peaks towards higher energies as shown comparing case E with F and G with H. Also, the dielectric spacer of higher refractive index yields slightly higher maximum intensities. In case of silver spheres the resonances are sharper and higher in number compared to those of gold spheres in the given excitation range.

**Design Example:**

Using the properties discussed above, nano-LAMPs can be designed for maximum signals at given excitation laser characteristics. Further, the enhancement of the fields in each dielectric layer can be specifically designed for specific excitation frequencies. To demonstrate this, in Fig.
3.14, the enhancement of each dielectric layer for a probe with $N = 2$ is presented. The metal layers considered are of silver, the dielectric is assumed to be of silica and the particle is assumed to be in vacuum. In this case, the enhancement at Stokes frequency is considered by using a Raman shift of about $1010 \text{ cm}^{-1}$. The geometry of configuration is defined as: 10 nm Au core+ 2 nm silica shell + 10 nm Au shell + 5 nm silica shell + 5 nm Au shell. The probes are assumed to be embedded in vacuum.

![Graph](image)

Fig. 3.14 Multiple excitation capability of nano-LAMPs; $G_1$ and $G_2$ correspond to enhancement factors in dielectric layers 1 and 2.
Here, enhancement factor is evaluated as a volume integral of the dielectric shell and represents that the contribution of each dielectric layer to total signal of the probe. Both layers exhibit resonant Raman responses at an excitation of about 470 nm, but layer 1 has a higher order of enhancement at an excitation of 664 nm. Due to contribution of the signal from each layer being an integral of the values over the entire volume, $G_2$ is usually higher than $G_1$. The spectral variation of the layers is slightly different but the total signal of the probe is high at multiple excitations throughout the distribution.

In Fig. 3.15, the logarithm values of intensity ($M = |E_{loc}|^2$) distribution of this probe at laser frequencies is shown in the incident plane. It can be observed that the ‘hot-spot’ at core in this

Fig. 3.15 E-field distribution for the probe considered in Fig. 3.14 at an excitation of 664 nm; M values are plotted in log scale.
case fully includes its surface except for the points in equator. The outer dielectric is also completely enhanced at this excitation frequency. In this figure, the regions with retarded fields can be observed to a small extent in the outer dielectric layer.

Using the above properties of layered particles one can optimize nano-LAMP structures subject to experimental constraints. An optimization algorithm can be used to devise a configuration with either maximum enhanced intensity or volume integral of enhanced intensity at given laser properties. The constraints that will be incorporated into such a design problem would be the fabrication and experimental constraints. The fabrication constraints limit the size of layer and chemical composition, while the experimental constraints would include the applicability and diffusion characteristics in-vivo for nano-LAMPs of different sizes. In addition to designing the metal and dielectric layers, one need to consider the effect of the protective layer and antibody layer (both act as dielectrics) over the Raman response of nano-LAMPs. The protective glass layer should be as thin as possible but with optimal shielding characteristics; whereas the antibody layer should be as thin as possible but with optimal binding efficiencies.

3.1.2 SNAPs

The far-field and near-field properties of SNAPs can be tuned with respect to the size, spacing and refractive index of the nanoparticles. Using a nano-LAMP-template, we have theoretically demonstrated that the local field intensities (and, consequently, the vibrational
responses) are dependent on the plasmonic coupling which is in turn dependent on overall probe configuration and individual layer thicknesses for a given set of materials. Plasmonic interactions between the thin metal shells results in multiple extinction peaks, which can be maximized and/or tuned by rational design of the nano-LAMP. The resulting resonances can be further tailored by specifying the size and composition of the dielectric layers between metal shells.

Fig. 3.16 Tunability in extinction spectra (A) and local field at midpoint of separation (B) of dimers of gold nanoparticles of size 40 nm with separation d.

Here we investigate the optical tunability in SNAPs. First we consider simple configurations of nanoparticle aggregates and then present results pertinent to SNAPs.
Nanoparticle aggregates:

Effect of Size:

In Fig. 3.16 we consider dimers of gold nanoparticles of same size with changing separation. The illuminating field is considered to be polarized along the axis of dimer while its propagation is perpendicular to the dimer axis.

![Fig. 3.16](image)

Fig. 3.17 Tunability in extinction spectra (A) and local field at midpoint of separation (B) of dimers of gold nanoparticles at 2 nm separation with one nanoparticle’s size varying.
As the separation decreases the extinction efficiency peak shifts towards infrared. Separations as low as 0.5 nm are considered here, but it must be noted that EM predictions might not be accurate at metal surfaces placed closer than 2 nm. For separations, 1 nm and 0.5 nm EM theory calculations predict a split in the peaks. In the EM theory calculations, the bulk refractive indices of gold are considered and when electronic scattering effects are considered, the broadening of peak is expected to be observed instead of the split. When the amplitude of local field is considered at the midpoint of separation, as the separation decreases the amplitude increases and the wavelength at which the maximal field amplitude is obtained shifts towards right.

**Effect of Separation:**

In Fig. 3.17, a dimer of gold nanoparticles of 80 nm and a nanoparticle of varying size are considered when separated by 2 nm. The illumination field is again considered to be propagating perpendicular to the dimer axis and polarized along the dimer axis. As the second nanoparticle’s radius increases from 5 to 40 nm, the split in extinction peak becomes prominent and the resonances shift towards right. This shift in resonance towards longer wavelengths can also be observed in case of local field amplitudes at midpoint of separation.

**Effect of Composition:**

The effect of composition on the near-field and far-field optical properties is illustrated in Fig. 3.18. The extinction efficiencies are plotted against wavelength and compared for dimers of gold, copper and silver nanoparticles of similar size and separation. The illumination field properties are similar to the cases considered earlier. The silver dimers at this separation and size
posses two peaks and locations of resonances are dependent on the plasmon properties of gold, copper and silver. Similarly the resonances in E-field amplitude are strongest for silver dimers and least for copper in comparison.

Fig. 3.18 Tunability in extinction spectra (A) and local field at midpoint of separation (B) of dimers of 50 nm nanoparticles at 2 nm separation with metal composition varying.

It must be noted that the amplitudes in the interstice of nanoparticles are enhanced upto 50 times which will translate up to 7 orders of magnitude enhancement in Raman spectra of the molecules embedded.
Orientation Effects:

In smaller aggregates of nanoparticles however, the enhancement in field amplitude is dependent on the orientation resulting in the variability and strength of hot-spots created. To illustrate this we consider the field distribution in incident plane for dimers of silver nanoparticles of size 45 nm separated by 5 nm when excited by plane EM wave of wavelength 514.5 nm, in Fig. 3.19.

![Diagram](image)

Fig. 3.19 Effects of orientation on the hot-spots created in dimers. The illumination field is polarized at angles (A) 90°, (B) 30°, (C) 0° and (D) 60° to the dimer axis.

The strength and extent of hot-spot in the interstice of dimers is maximal for field polarized along the dimer axis and field polarized at 30° to the dimer axis. As this angle increases
to $60^0$, the strength and extent decrease and the interaction between the scattered fields of nanoparticles, is minimal when illuminating field is polarized perpendicular to the dimer axis.

**Increase in number of nanoparticles in the aggregate:**

![Field distribution in aggregates with increasing number of spheres](image)

Fig. 3.20 Field distribution in aggregates with increasing number of spheres (A – 1, B – 2, C – 3, D – 4, E – 6 and F – 9) but of same effective volume

In a fixed volume available for a probe, the extent and strength of enhanced spots available can be increased by considering higher number of nanoparticles. This can be related to increasing the number of layers in case of Nano-LAMPs. In Fig. 3.20, we consider the field distribution in silver nanoparticle aggregates with increasing number of nanoparticles while
keeping the effective volume constant. The nanoparticles are considered to be separated by 2 nm and the illuminating plane EM wave is of wavelength 514.5 nm. The strength of hot-spots increase as the number of spheres increase; at the same time the extent of hot-spots available also increases.

**SNAPS:**

By considering symmetric nanoaggregates the effects on hot spots due to orientation of nanoparticles in the aggregate can be eliminated and symmetric hot-spots can be achieved. For example let us consider circular aggregates of silver nanoparticles and the enhanced field distributions in the incident plane when excited by a 514.5 nm laser. In Fig. 3.21A, the spheres are separated by 2 nm, the core sphere is of radius 40 nm and the surrounding spheres are of radius 5 nm.

![Fig. 3.21 The field distribution in incident plane for symmetrical nanoparticle aggregates](image)

Fig. 3.21 The field distribution in incident plane for symmetrical nanoparticle aggregates
In Fig. 3.21B, the spheres are separated by 2 nm, the core sphere is of radius 40 nm, the surrounding spheres in second layer are of radius 10 nm and the surrounding spheres are of radius 5 nm. From the figure it can be observed that the achieved enhanced regions are mostly symmetric in nature and as the number of particles increase the extent of hot spots increase. In addition their strength increases as well, and the Raman spectra of reporter molecules can be enhanced up to a million fold.

**Tunability in SNAPs:**

**Effect of Size**

![Figure 3.22 Near-field distributions in incident plane for gold SNAPs with size of spheres in the first shell varying from 5 nm, 10 nm, 15 nm and 25 nm (left to right), when excited by a plane EM wave of wavelength 532 nm.](image)

Here we focus on the optical tunability of near-fields in SNAPs. We first consider gold nanoparticle SNAPs of two layers with core particles of radius 25 nm in Fig. 3.22. The radius of first shell spheres are varied between 5 nm, 10 nm, 15 nm and 25 nm. The core sphere and the
first shell sphere are separated by 2 nm. The projections of all spheres in the incident plane are taken and the fields inside the sphere are not calculated due to the lack of interest in them. The illumination EM wave is considered to be of wavelength 532 nm and is polarized vertically while propagating horizontally from left to right. It can be observed from Fig. 3.22 that as the size of the first shell spheres increases the extent of hot-spots and their strengths increase which results in higher NEFs.

Fig. 3.23 Near-field distributions in incident plane for gold SNAPs with 25 nm and 15 nm sized particles with separations 15 nm and 25 nm, when excited by a plane EM wave of wavelength 532 nm.

Effect of Separation: 

Next we consider gold SNAPs with 25 nm and 15 nm sized particles with separations 15 nm and 25 nm, when excited by a plane EM wave of wavelength 532 nm. Again, the fields
inside the projections of spheres on the incident plane are not calculated due to the lack of interest in them. The illumination EM wave is considered to be of wavelength 532 nm and is polarized vertically while propagating horizontally from left to right. The field distributions plotted in the incident plane are shown in Fig. 3.23. The results indicate that the extent and strength of hot-spots are higher as one decreases the separation.

Such tunability in SNAPs is observed to closely follow other observations in LAMPs. The metal layer sizes can be associated with the metal nanoparticle sizes while the separations can be associated with the dielectric layer sizes. In addition, the extent of hot-spots will be much higher in SNAPs when compared to Nano-LAMPs, but the complex interaction might decrease the strength as well as symmetry in the hot-spot. However, Nano-LAMP configuration provides an easy template and mathematical formulation to evaluate and optimize the Raman responses of SNAPs. Hence, below we focus on the design of Nano-LAMPs using a genetic algorithm selection of layer sizes for requisite responses.
3.2 Optimization of Nano-LAMPs

3.2.1 Optical characteristics of LAMPS:

The inter-plasmon coupling of the metal shells in LAMPs governs their optical properties. The mixing of dipolar contributions due to the metal shells typically result in stronger resonances at longer wavelengths while the higher order multipole contributions and their interaction result in weaker resonances at shorter wavelengths.

Fig. 3.24 (A) Extinction efficiencies of gold-silica Nano-LAMPS of 5 layers \( \{r_1, r_2, r_3, r_4, r_5\} = \{5, 15, 30, 44, 50 \text{ nm}\} \) and 6 layers \( \{r_1, r_2, r_3, r_4, r_5, r_6\} = \{5, 10, 26, 34, 44, 50 \text{ nm}\} \). (d) Internal intensity distribution in the incident plane for LAMPS in (c) when illuminated by plane waves at resonant wavelengths. The boundaries of different layers are indicated using black circles.
However the relative strength of interaction and their manifestation in far-field characteristics is strongly dependent on the overall size of the particle, the relative thicknesses and compositions of each layer. For example, extinction spectra are shown in Fig. 3.24A for a 5-layered gold-silica LAMP ($r_1 = 5$, $r_2 = 15$, $r_3 = 30$, $r_4 = 44$ and $r_5 = 50$ nm) and a 6-layered gold-silica LAMP ($r_1 = 5$, $r_2 = 10$, $r_3 = 26$, $r_4 = 34$, $r_4 = 44$ and $r_6 = 50$ nm) LAMP. In the spectra shown, there exist two distinct peaks at wavelengths of 531 and 676 nm for 5-layered LAMP and at 543 and 837 nm for the six-layered structure. The resonance peaks at shorter wavelengths can be observed to be a combination of resonances, which are due to higher order multipole modes. In addition, the contributions from different metal shells can be understood by examining the local reorganization of electric field within and in the vicinity of LAMPs when illuminated at the resonant wavelengths. In Fig. 3.24B, this reorganization is depicted in the intensity distributions in the incident plane. For the 5-layered structure, a relatively stronger resonance at 676 nm illumination is manifested in the fields being enhanced up to 3 orders of magnitude in the dielectric layer between the two metal layers. At 531 nm, the field in the dielectric layer is enhanced only up to 1-2 orders of magnitude indicating a weaker resonance. For the 6-layered structure, the contributions due to plasmonic interaction of different metal layers are more apparent. At 543 nm illumination, the coupling between core and first outer metal layer is dominant resulting in enhanced fields of higher magnitudes in the innermost dielectric layer between them. At 837 nm illumination, the coupling of the first and second outer metal layers is stronger resulting in enhanced fields of higher magnitudes for the second dielectric layer between them. The ability to reorganize the enhanced and depleted regions into different
dielectric layers can further be employed to design probe structures with different enhancement characteristics for dye molecules filled in different layers.

3.2.2 Design of structures with hot-spots localized in various layers:

Fig. 3.25 Localizing hotspots internally to various silica layers in silver-silica nano-LAMPs of 5 layers (top) and 6 layers (bottom) through internal field distributions in incident plane.

For example, in Fig. 3.25 various configurations optimized for localizing internal fields into various dielectric layers are shown. For the 5-layered LAMPs the structure, that maximizes the field to the silica layer between the first and second silver layers, possesses maximal NEF.
This structure has comparatively thicker metal shells, however by increasing the dielectric core size and/or by minimizing the thicknesses of metal layers it is possible to achieve structures that possess maximal NEF’s within core silica layer or outermost silica layer. Similar results can be observed for 6-layered silver-silica LAMPs. When bigger core silver is chosen for the structure the maximal NEF spot is located in the silica layer surrounding the core. However it is possible to achieve greater NEF’s in the outer layers by choosing a smaller core and thicker outer metal shells. However such NEF’s are lower in comparison to what could be achieved in the silica layer surrounding the silver core.

The spectra of LAMPs can precisely be understood by utilizing a plasmon hybridization model [36,37] and evaluating the coupling of various plasmon modes with the optical fields. Using the models, insights can be gained into engineering the thicknesses of various layers for simple structures. However having to consider plasmon modes of higher number of metal layers can prove to be cumbersome and such a complex study is often not necessary for designing probes of requisite responses. We can alternately engineer the thicknesses of various layers using optimization procedures in combination with Mie theory calculations. As examples of such approach, below we propose some designed LAMP structures.
3.2.3 Design of optical probes for multiplexing

With different layer thicknesses in a LAMP, different higher order multipole modes can be excited resulting in resonances at different wavelengths. The tunability can be employed to design LAMPs as probes in a multiplexing application. While the tunability is greater with probes of different compositions and total sizes, here we restrict ourselves to designing probes of a specific size and composition. The constant total size is relevant in designing probes of similar diffusion characteristics and hence similar availability to the labeled sites while a single composition is beneficial towards simplicity of fabrication. For biological imaging applications, probes of a size below 100 nm and below are relevant. Keeping the above in mind, as a specific example here, we demonstrate the design of silver-silica LAMPs of 100 nm size that have distinct spectral characteristics in the 200-1200 nm range of excitation.

For smaller layer sizes, it is often difficult to obtain a continuous uniform layer. Hence we impose a constraint that the designed LAMPs should have metal layers exceeding or equal to 10 nm and dielectric layers exceeding or equal to 5nm. For achieving the peak in a desired wavelength region, a simple contrast ratio (=Cumulative extinction in the desired illumination region/Cumulative extinction in rest of the range) is chosen as the cost function to be maximized. Figure 3.26a shows the radii and distinct extinction spectra of 3-layered silver-silica LAMPs designed using a genetic algorithm selection of layer thicknesses. In this structure, the interaction and mixing of the plasmons of outer and inner surfaces of the metal layer can result in tunability
with layer thicknesses. In structures with thinner metal layer \(\{(35,45,50), (33,44,50), (28,38,50), (22,32,50), \text{ and } (9,19,50)\}\), the stronger interaction of plasmons can result in stronger and narrower resonances.

Fig. 3.26 Odd-layered Silver-Silica LAMPs of 100 nm size with distinct extinction spectra in 200-1200 nm excitation range. (a) 3-layered LAMPs (b) 5-layered LAMPs.

The thicker outer silica layer however lowers the strength of this resonance. In contrast, the structures with thicker metal layers \(\{(6,45,50), (20,44,50)\}\) possess broader peaks. While the thicker layer can effectively isolate the plasmons of two surfaces, the total size considered here is still smaller in comparison to result in two well separated peaks. Figure 3.26b depicts the radii and distinct extinction spectra of 5-layered silver-silica LAMPs designed. The
presence of two metal layers and the interaction of multipole contributions due to these layers can be utilized to achieve multiple resonances. In the provided structures most possess thinnest metal layers possible. The presence of a thin outer metal layer ensures the existence of a resonance at longer wavelengths. Using the thinnest outer metal layer further maximizes this resonance. This is due to the fact that the cost function chosen for optimization is biased towards maximizing the strength of resonances. A different cost function could be chosen to optimize for focusing on narrowing the peak and/or achieving higher number of peaks. It can also be observed that the resonances at longer wavelengths can be shifted further into near-infrared for the 5-layered LAMPs. Moreover, the resonances in the near-infrared for 5-layered LAMPs are well separated in comparison to those for 3-layered LAMPs.

In Figure 3.27, the radii of the designed 4-layered and 6-layered Silver-Silica LAMPs and their distinct extinction spectra shown indicate that similar results could be achieved with even-layered structures. For the designed 4-layered Silver-Silica LAMPs with near-infrared resonances, the thickness chosen for the silica layer between the metal layers is minimal, in consistence with the scaling law for plasmon resonance shift proposed in case of gold-silica-gold spheres. The presence of the extra (protective) silica layer here, further red-shifts the resonances. As in the case of 3-layered and 5-layered LAMPs, the outermost metal layer is thinnest possible for achieving resonances in the near-infrared wavelengths, but the core is bigger. As the core size becomes smaller and the outer metal layer becomes thicker the resonances at shorter wavelengths become stronger and broader. The presence of additional metal and silica layers in
the designed six-layered LAMPs result in distinguishable peaks at the shorter wavelengths as shown in Figure 3b. As in the case of odd layered LAMPs, the additional layers add to the tunability and structures with resonances further into near-infrared can be achieved. In both odd-layered and even-layered LAMPs cases, the multiple peaks achieved are distinguishable in characteristics and along with a multivariate curve resolution analyses, the probes could be easily employed for multiplexing analyses.

Fig. 3.27 Even-layered Silver-Silica LAMPs of 100 nm size with distinct extinction spectra in 200-1200 nm excitation range. (a) 4-layered LAMPs (b) 6-layered LAMPs.

The interaction between metal layers in smaller particles (sizes below 100 nm) can be relatively weaker and all the plasmon modes might not be resonant. Although in some cases
shown, extinction spectra can possibly be attained by designing simple two-layer and three-layer structures, for a given total size the LAMPs with higher layers can be designed to possess resonances at longer wavelengths. The extinction characteristics of LAMPs are due to contributions from scattering and absorption. The scattering intensity has a much higher order dependence on the sizes in comparison to the absorption intensity. The scattering and absorption characteristics of LAMPs can similarly be designed and multiplexing probes could be realized for various spectroscopy modalities with different detection.

3.2.4 Designing probes with scalable responses

The LAMPs can be designed such that their signal arises not directly from the overall scattering and absorption of the probe but from the emission or inelastic scattering characteristics of inorganic molecules doped into the dielectric layers. Such signals can be greatly amplified at a particular wavelength by maximizing the electric fields in the dielectric layers. In addition, the interaction with the metal for the molecules could be reduced through the separation offered by the dielectric material. This ensures the reliability of enhancement in the signal by basing it purely on the composition and structure of the probe. The stronger enhancements achieved with multilayer particles like LAMPs is very beneficial, but at the same time their tunability can be employed to achieve probes with scalable responses. For example, such scalability at 785 nm excitation is demonstrated in the internal field distributions provided for 6-layered Gold-Silica LAMPs in Figure 3.28. The structures can be tuned such that the maximal field achieved within
can be tuned from 1-5 orders of magnitude. It can be observed that by choosing a very thick outermost metal shell the fields inside can be damped in comparison to outside the probe.

Fig. 3.28 Scalability of enhancement in the internal fields in dielectric layers using 100 nm sized six-layered gold-silica LAMPs of various layer thicknesses when illuminated by a plane wave of wavelength 785 nm. The distributions are shown in the incident plane with incident field polarized horizontally. The structures shown have radii (a) 5,14,16,17,48,50 nm, (b) 7,17,20,21,44,50 nm, (c) 8,23,25,26,35,50 nm (d) 6,18,23,26,31,50 nm (e) 15,25,32,36,40,50 nm (f) 13,20,25,35,40,50 nm (g) 10,14,22,32,41,50 nm (h) 18,22,33,38,41,50 nm (i) 14,16,28,34,46,50 nm (j) 7,8,26,38,48,50 nm. The boundaries of different layers are depicted using black circles.

When the inner metal and silica layers and the outer most silica layers are thicker in comparison to this layer, the enhancement within the dielectric layers can be amplified to higher orders of magnitude. For the structures with greater enhancements the metal layers are separated by thin dielectric layers and one of the inner metal layers (core or the first outer metal layer) is
bigger. It can be observed that the stronger enhancement regions achieved within LAMPs extend symmetrically over the entire dielectric layer.

Fig. 3.29 Internal field distributions in 60 nm sized six-layered Gold-Silica LAMPs ((a) and (b)) and Copper-Silica LAMPs((c) and (d)) designed for minimal and maximal enhancements at excitation wavelengths 532 nm ((a) and (c)) and 785 nm ((b) and (d)). The distributions are shown in the incident plane with incident field polarized horizontally. The structures shown have radii (a) minimal-left: \( \{5,7,9,10,12,30 \text{ nm}\} \), maximal-right: \( \{5,9,13,17,28,30 \text{ nm}\} \) (b) minimal-left: \( \{5,11,13,14,26,30 \text{ nm}\} \), maximal-right: \( \{7,8,18,24,29,30 \text{ nm}\} \) (c) minimal-left: \( \{5,6,8,10,12,30 \text{ nm}\} \), maximal-right: \( \{6,7,13,19,29,30 \text{ nm}\} \) (d) minimal-left: \( \{6,12,14,15,27,30 \text{ nm}\} \), maximal-right: \( \{7,8,18,24,29,30 \text{ nm}\} \). The boundaries of different layers are depicted using black circles.

This is also significant in ensuring that enough reporter molecules exist in the enhanced regions. The strongest enhancements are much higher than what could be achieved with the single nanoparticles and the extent of enhanced regions is much higher in comparison to the simple nanoparticle aggregates.
LAMPs of same size can also be designed for different wavelengths of excitation using both Copper-Silica and Gold-Silica LAMPs. Figure 3.29 shows the internal field distributions in LAMPs designed that possess maximal and minimal enhancements in the innermost dielectric layer for wavelengths of excitation 532 nm and 785 nm. It can be observed that at 785 nm excitation, the structures designed for maximal enhancement utilizing Copper-Silica and Gold-Silica are the same. The structures for minimal enhancements derived are also similar. Moreover, the achieved maximal and minimal enhancements are of similar magnitude and at longer wavelengths of excitation Copper or Gold could be employed to design probes with equal effectiveness. At 532 nm excitation however, the maximal and minimal enhancements are of higher order magnitude for Gold-Silica LAMPs in comparison to Copper-Silica LAMPs. This is due to the plasmon modes for Gold layer sizes considered being resonant at wavelengths closer to 532 nm in comparison to the Copper layers. In all the cases shown the structures for minimal enhancements possess a thicker outermost metal or silica layer and the structures for maximal enhancements possess thinner dielectric layers in consistence with observations made in Figure 3.28.

3.2.5 Optimal LAMPs

Optimal enhancements depend critically on both the total probe size (R) and internal structure manifest nominally in the number of layers (L) for odd or even layered LAMPs (Figure 3.30).
Fig. 3.30 The maximum NEF for even (a) and odd (b) layered silver-silica LAMPs of different sizes for 785 nm excitation. Purely numerical enhancement is significantly smaller, hence, the net amplification in signal arises from a couple of the NE and EE. The color bar code for (a), (b), (c) and (d) is given below (c) and (d).

NEFs upto $10^{12}$ can be achieved for ordinary, non-resonant organic reporters. This availability is substantially higher than that encountered in using SAMs for consistent CE or resonant dyes$^{127}$ in simple geometries. The elimination of CE here is counter-intuitive but structuring the probe balances the loss by enabling high EE. Further, as opposed to dyes or SAMs, the proposed approach allows the use of almost any reporter, considerably enhancing the multiplexing capability of SERS probes. A distribution of the enhancement within LAMPs (Figures 3.31 and 3.32) indicates that the optimal structure reorganize the surface plasmons to
create local hot spots with a high NEF density at the core and appreciable contributions from elsewhere. While there is some contribution from the increased loading of molecules (Fig. 3.30c and Fig. 3.30d), it is the interplay of molecular and Plasmon density that determines the NEF.

Fig. 3.31 Enhancement distributions in the incident plane of illumination for even-layered Silver-Silica LAMPs of various configurations that are designed for maximal enhancements at an excitation wavelength of 785 nm. From top to bottom, the number of layers increases from 2 to 10. From left to right the total size increases as shown.
Fig. 3.32 Enhancement distributions in the incident plane of illumination for odd-layered Silver-Silica LAMPs of various configurations that are designed for maximal enhancements at an excitation wavelength of 785 nm. From top to bottom, the number of layers increases from 3 to 9. From left to right the total size increases as shown.

Similar results can be obtained and maximal NEF’s can be obtained for other wavelengths of excitation as shown in Fig. 3.33.
Fig. 3.33 Maximal net enhancement in Silver-Silica probes of different number of layers for laser excitations: A) 532 nm B) 633 nm C) 830 nm and D) 1064 nm.
3.2.6 Dynamic range available with LAMPS

![Diagram showing enhancement distributions in the incident plane of illumination for even-layered Silver-Silica LAMPS of various configurations that are designed for minimal internal enhancements at an excitation wavelength of 785 nm. From top to bottom, the number of layers increases from 2 to 10, while from left to right the total size increases as shown.](image)

Fig. 3.34 Enhancement distributions in the incident plane of illumination for even-layered Silver-Silica LAMPS of various configurations that are designed for minimal internal enhancements at an excitation wavelength of 785 nm. From top to bottom, the number of layers increases from 2 to 10, while from left to right the total size increases as shown.
Fig. 3.35 Enhancement distributions in the incident plane of illumination for even-layered Silver-Silica LAMPs of various configurations that are designed for minimal internal enhancements at an excitation wavelength of 785 nm. From top to bottom, the number of layers increases from 3 to 9, while from left to right the total size increases as shown.

While single molecule assays and large multiplexing will be possible with LAMPs, biochemical assays often involve a large concentration range that must be measured. Hence, tunable and large NEFs must be co-addressed with the challenge of devising a large dynamic range. Just as we enhanced the signal, we optimize Raman signal for quenching. Quenching is enabled by optimizing the electric field distribution such that the total enhancement of signal
from the probe is measurable but minimized. Quenching is illustrated in Figure 3.34 and 3.35, where distributions in minimized configurations are depicted in the plane of incident illumination.

The enhanced and quenched probe pair for a given size and excitation wavelength provides the allowable dynamic range as shown in Figure 3.36a.

Fig. 3.36 Allowable probe signal and multiple excitation capability. (a) Range of NEF attainable via Silver-Silica nano-LAMP configurations for 785 nm excitation as a function of probe size. (b) LAMPs can be made capable of excitation at multiple frequencies by tailoring the internal structure. Here, a six layer LAMP provides similar signal at multiple frequencies for the same size (50 nm) as a 3-layered LAMP, while maintaining high enhancement.

It must be noted that using a lower concentration or weaker Raman scattering reporter, the total signal could be even lower. The shaded regions represents the viable tuning space of probe
signals for any common organic used as a reporter. With resonant enhancement and optimized quenching, we estimate that analytes over 20 orders of magnitude in concentration would be measurable in a single assay.

Last, the structuring of a particle into the LAMP configuration allows us to tailor the response of probes to be spectrally flat (Figure 3.36b). The spectral uniformity indicates that LAMPs can be widely excitable and enhancements at shifted frequencies do not experience a drop-off from the excitation laser frequency that is often selected to coincide with the spectral plasmon resonance peak of SERS probes. Hence, the LAMP configuration promises sensitive, tailored, reporter-independent and spectrally consistent responses while being amenable to fabrication.\textsuperscript{129}
CHAPTER 4

PROPOSALS FOR REALIZATION AND VALIDATION OF NANOSTRUCTURED PROBES

We propose that Nano-LAMPs can be fabricated by using a layer-by-layer seeding method using colloidal gold and colloidal silica solutions while SNAPs can be fabricated via layer-by-layer aggregation of gold nanoparticles using synthetic oligonucleotide linkage. In this chapter, we propose some of the approaches that could be used to fabricate Nano-LAMPs and SNAPs and some techniques to validate their responses. While we acknowledge that the discussion in this dissertation is not a complete account for fabrication and validation of probes, it provides a first step in making such structures.

4.1 Fabrication

4.1.1 Nano-LAMPs

The structure of Nano-LAMPs proposes that the reporter molecules are embedded in the silica spheres. The techniques to embed organic molecules in silica spheres are limited in the smallest sizes that could be created. Hence an easier variant of Nano-LAMP that could be created is using surface assembled monolayer on the metal surfaces while fabricating each layer. Accordingly we
propose that the first generation LAMPs can be created using a layer-by-layer seeding method as illustrated in Fig 4.1.

Fig 4.1 Layer-by-layer fabrication of a 4-layered gold-silica LAMP

The method followed can be described step-by-step as follows:

1. **Formation of Silica-Coated Gold Nanoparticles**

   5 ul of 1 mM (3-aminopropyl)trimethoxysilane (APS) was added to 1 ml of gold nanoparticle solution under magnetic stirring. The mixture was allowed to stand for 15 minutes after which 40 ul of active silica (0.54 wt %, pH 10) was added to it under magnetic stirring. The resultant solution was allowed to age for 24 hours in the dark to allow for proper binding of active silica to the gold particles.

2. **Growth of Silica Layer by Stober Method**

   To allow further growth of the silica shell, 500 ul of the silica-coated gold nanoparticle solution was treated with 2 ml of ethanol, 6 ul of tetraethoxysilane (TES), and 10 ul of ammonia.
The mixture was then allowed to stir overnight. The mixture was then centrifuged at 2500 RPM for 6 hours and re-dispersed in 1.5 ml of ethanol.

3. Seeding of 2 nm Gold Nanoparticles

To prepare the silica coated gold nanoshells for the addition of outer gold layer; 1 ml of the solution of particles dispersed in ethanol was treated with 50 ul of a 1 mM solution of APS, added under magnetic stirring. The mixture was then allowed to age overnight in the dark to allow for the reaction. To enhance the binding of APS to the outer silica shell, the solution was refluxed for 1 hour. It was then centrifuged for 1 hour at 2000 RPM; re-dispersed in 1 ml of water and then was sonicated for 15 minutes to prevent aggregation. 2 ml of a 2 nm gold colloid suspension was added to the solution under gentle magnetic stirring and the solution was allowed to age in the dark overnight.

4. Formation of Outer Gold Layer

The formation of a uniform outer gold layer was accomplished by reduction of a gold hydroxide on the surface of the multilayered gold-silica particle. The gold hydroxide was prepared by adding 60 ul of 1.0 wt % HAuCl₄ to 4 ml of 1.8 mM potassium carbonate solution. The solution was allowed to react in the dark overnight. The solution of gold hydroxide was then added to 1 ml of the gold-primed silica-coated gold nanoparticles. The reduction was completed by the addition of 10 ul of 37 wt % formaldehyde solution under magnetic stirring. The mixture was allowed to react in the dark overnight.
We propose that the steps illustrated above can be repeated to obtain LAMPs of any number of layers.

The TEM images and UV-Vis spectra along with theoretical comparisons are shown for the spheres in steps 2-4 described above are shown in Fig. 4.2.

![TEM images and UV-Vis spectra](image)

Fig 4.2 The TEM images and Uv-Vis spectra at various steps of proposed fabrication (2) gold nanoparticles with reporter (3) silica coated gold and (4) gold coated over gold core silica shell particle. The subfigure shown between (3) and (4) is the phase when silica coated gold particles are seeded with 2 nm colloidal gold particles.

The yield of specific structures obtained using this method is a problem and separation procedures might lead to non-specific aggregation in the colloidal nanoparticle solution.
4.1.2 SNAPs-Fabrication

Fig. 4.3 Aggregation of gold nanoparticles using DNA molecules. The Au NP surfaces are functionalized with thiolated DNA molecules of different amino acid sequences. The nanoparticles are then brought together by hybridizing with linker molecules partly complementary to each of the functionalized molecules.

Self-assembly approaches using oligonucleotides and nanoparticles offer an opportunity to form extensive constructs of multiple particles by facile temperature and/or pH control. Most importantly, it is possible to form such constructs with generally variable nanoparticle sizes and, hence, controllable optical properties. The basic principle in use by various groups has been to derivatize two batches of nanoparticle solutions with non-complementary oligonucleotides through 5'-, 3'-thiol groups. A linker oligonucleotide with half of it designed to bind with one of the derivatized oligonucleotides and the other half designed to bind with the other, is used to form aggregates in the solution. The linking is achieved by
Watson-Crick base pairing of oligonucleotides. We started with the same idea and Fig. 4.3 depicts this approach for DNA-mediated gold nanoparticle assembly for a set of oligonucleotides designed for our use.

The derivatized oligonucleotides with thiol group can include two parts: a spacer sequence and a linking sequence. The spacer can be used to optimize the number of oligonucleotides that are functionalized on the gold nanoparticle surface,\textsuperscript{136} to control the separation between nanoparticle networks\textsuperscript{69} and to avoid steric effects on hybridization of functionalized oligonucleotides due to nanoparticles.\textsuperscript{137} The oligonucleotides, further, are designed to minimize non-specific binding. Aggregation mediated by DNA leads to a characteristic change in optical response of the solution, which can be monitored using Vis-NIR spectroscopy.\textsuperscript{69} Direct evidence of aggregation comes typically from transmission electron microscopy (TEM) images\textsuperscript{138} to reveal the well-defined order, and three dimensional natures of the nanoparticle aggregates. Due to the specificity of Watson-Crick base pairing only aggregates of particles with hetero-dimeric composites with alternating sizes can be formed. Alternatively, one can design the derivatized oligonucleotides such that a certain base pair sequence in each is directly complementary, thus not requiring a linker molecule for binding. By using different stoichiometric variations of colloidal solutions and the number of linker DNA molecules added the resultant aggregates can be controlled to a certain extent.
By using a higher number of one of the particles, it is possible to generate structures similar to the multispherical aggregates we aspire to create. The oligonucleotides on the nanoparticle surface are not all bound to the specific linker molecule in the satellite-like structure and may form cross-links with the oligonucleotides in other satellite structures. Hence the resultant solution is usually a mixture of networks of particles and some isolated satellite-like structures with smaller nanoparticles. Again by stoichiometric variations, one can improve the yield of desired structures (the number of satellite-like structures in the sample). One obvious way to improve the yield is to cap away some of the sites on the gold nanoparticle surface by using agents such as 6-mercaptop-1-Hexanol. These agents reduce the number of oligonucleotides on the gold nanoparticle surface and are helpful in improving the yield by reducing cross-linking. It is apparent from these studies that the principle of self-organization may be facile but control over structures is not trivial. Solution phase approaches to controlled nanoparticle aggregation, hence, need some constraints to control this stochastic aggregation. We have used the above-mentioned ideas, to create solutions of isolated core-shell aggregate (satellite-like) structures using some of the colloidal gold nanoparticle solutions commercially available.

Further linkage of higher number of nanoparticles can be achieved. For example using the oligonuclotide linkage depicted in Fig. 4.4, another layer of nanoparticles can be linked to the core-shell particles. By stoichiometric variations, SNAPs can be generated in such solutions.
A step-by-step procedure can be used to fabricate isolated structures in the form of desired SNAP-configurations. Due to non-specific binding between the linkers it will be difficult to create solutions with only desired SNAP structures. Instead, the resultant solution will be a mixture of several random aggregates due to the combinations of linking possible. Nevertheless, the method provides a baseline for comparison with the controlled fabrication case proposed (task 3). A brief outline of the step-by-step by procedure is depicted in Fig 4.5. Using these principles we have fabricated SNAPs of two and three layers.
The procedural steps followed to make core-shell SNAPs with 40 nm gold core surrounded by 5 nm gold spheres are as listed below:

The gold nanoparticles are purchased from Tedpella, while the oligonucleotides were purchased from IGB at University of Illinois and IDT-DNA. The available quantities of gold nanoparticles and oligonucleotides are:

1) A solution of gold particle colloid of average diameter 40 nm at a concentration of $9 \times 10^{10}$ particles/mL.

2) A solution of gold particle colloid of average diameter 5 nm at a concentration of $5 \times 10^{13}$ particles/mL.
3) Thiol modified 3’ oligonucleotide of 30 bp aliquoted in samples of 20 μL at a concentration of 375 μM.

4) Dye labeled- thiol modified 5’ oligonucleotide of 30 bp aliquoted in samples of 20 μL at a concentration of 375 μM.

5) 1 M NaCl and 10 mM phosphate to be used as buffers.

Calculations summary:

1) No of oligos per 40 nm Au particle = \(4 \cdot \frac{\pi \cdot 40^2}{(\pi \cdot 2^2)} = 1600\).

2) No of oligos per 5 nm Au particle = \(4 \cdot \frac{\pi \cdot 5^2}{(\pi \cdot 2^2)} = 25\).

3) Ratio of oligo bound 40 nm particles to oligo bound 5 nm particles is
\[= 4 \cdot \frac{\pi \cdot 50.3^2}{(\pi \cdot (5+2 \cdot 10.3)^2)} \approx 16.\]

Based on the above, the quantities and method chosen are:

- Use 200 μL of 40 nm Au solution, it has \(1.8 \times 10^{10}\) particles.
- Choose a molar ratio of 120:1 between 5nm Au and 20 nm Au.
- Amount of 5 nm Au solution taken should have \(120 \times 1.8 \times 10^{10}\) particles i.e. \(2.16 \times 10^{12}\) particles. So the volume required is \(2.16 \times 10^{12} / 5 \times 10^{13} = 43.2\) μL.
- For 200 μL of 40 nm Au solution, no. of oligos required = \(1600 \times 1.8 \times 10^{10}\) particles = \(1600 \times 1.8 \times 10^{10} / (6.023 \times 10^{23}) = 47.8\) pmol. Choose to use 20 μl of oligo, then concentration needed = \(47.8 \times 10^{-12} / (20 \times 10^{-6}) = 2.39\) μM.
• For 43.2 μL of 5 nm Au solution, no. of oligos required = 25 x 2.16 x 10^{12} particles = 25 x 2.16 x 10^{12} / (6.023 x 10^{23}) = 89.66 pmol. Choose to use 16 μl of oligo, then concentration needed = 89.66 x 10^{12} / (16 x 10^6) = 5.6 μM.

• To create oligo at 2.39 μM to 10 μl of oligo at 375 μM add 1.56 mL of DI water.

• To create oligo at 5.6 μM to 10 μl of oligo at 375 μM add 659 μl of DI water.

**Procedural Steps for example 2-layered SNAPs:**

1) Take 10 μL of ‘375 μM thiol modified dye labeled oligo at the 3’ end’ in a sterilized micro centrifuge tube and add 1560 μL of DI water to get 1570 μL oligo at 2.39 μM.

2) Mix this solution from 1 well by creating vortices using pipette (by sucking in and releasing the solution for a number of times).

3) Take 10 μL of ‘375 μM thiol modified dye labeled oligo at the 5’ end’ and add 659 μL of DI water to get 669 μL of oligo at 5.97 μM.

4) Mix this solution from 3 well by creating vortices using pipette (by sucking in and releasing the solution for a number of times).

5) Take 200 μL of ‘40 nm Au solution’ in a small plastic/glass vial and add 20 μL of ‘solution obtained from 2’ to it.

6) Mix the solution from 5 well by vortex formation using pipette. Also gently shake the vial for a number of times. Place the vial on the shaker horizontally and shake for around 10 min at a tilt of 10.
7) Store the vial from 6 in a dark place and let it age for around 24 hrs.

8) Take 43.2 μL of ‘5 nm Au solution’ in another small plastic/glass vial and add 16 μL of ‘solution obtained from 3’ to it.

9) Mix the ‘solution from 8’ well by vortex formation using pipette. Also gently shake the vial for a number of times. Place the vial on the shaker horizontally and shake for around 10 min at a tilt of 10.

10) Store the ‘vial from 9’ in a dark place and let it age for around 24 hrs.

11) After 24 hrs centrifuge the vial from 7 at 1000 rpm for 30 s.

12) Add ‘solution from 7’ to ‘solution from 9’ in a small vial. Add 75 μL of 1 M NaCl, 75 μM of 10 mM Phosphate to it.

13) Mix the ‘solution from 11’ well by vortex formation using pipette. Also gently shake the vial for a number of times. Place the vial on the shaker horizontally and shake for around 10 min at a tilt of 10.

14) Store the ‘vial from 12’ in a dark place and let it age for around 6 hrs.

15) Transfer this solution with nanoparticle constructs into a micro centrifuge tube and centrifuge it for around 4 min at 6000 rpm to separate unbound particles from particle constructs.

16) Draw the sample from the bottom of the micro centrifuge tube to get a solution of constructs and redisperse them in the salt buffer.
The TEM images of the fabricated 2-layered SNAPs are as shown in Figs. 4.6 and 4.7.

Fig. 4.6 TEM images of two-layered SNAPs of various sizes with core and shell particles respectively of (A) 60 nm and 15 nm (B) 50 nm and 10 nm (C) 50 nm and 15 nm.

Fig. 4.7 TEM images of two-layered SNAPs of various sizes with core and shell particles respectively of (A) 30 nm and 15 nm (B) 15 nm and 5 nm.

Similarly this can be extended to there-layered SNAPs and the procedureal steps for an example 3-layered SNAPs are listed below.
**Procedural Steps for 3-layered SNAPs:**

1) Take 20 μL of ‘375 μM thiol modified dye labeled oligo at the 3’ end’ in a sterilized micro centrifuge tube and add 882.6 μL of DI water to get 902.6 μL oligo at 8.3 μM.

2) Mix this solution from 1 well by creating vortices using pipette (by sucking in and releasing the solution for a number of times).

3) Take 10 μL of ‘375 μM thiol modified dye labeled oligo at the 5’ end’ and add 1.5 mL of DI water to get 1.51 mL of oligo at 2.48 μM.

4) Mix this solution from 3 well by creating vortices using pipette (by sucking in and releasing the solution for a number of times).

5) Take 4.13 μL of ‘20 nm Au solution’ in a small plastic/glass vial and add 2 μL of ‘solution obtained from 4’ to it.

6) Mix the solution from 5 well by vortex formation using pipette. Also gently shake the vial for a number of times. Place the vial on the shaker horizontally and shake for around 10 min at a tilt of 10.

7) Store the vial from 6 in a dark place and let it age for around 24 hrs.

8) Take 2 mL of ‘5 nm Au solution’ in another small plastic/glass vial and add 500 μL of ‘solution obtained from 2’ to it.

9) Mix the ‘solution from 8’ well by vortex formation using pipette. Also gently shake the vial for a number of times. Place the vial on the shaker horizontally and shake for around 10 min at a tilt of 10.
10) Take 202 μL of ‘10 nm Au solution’ in another small plastic/glass vial and add 100 μL of ‘solution obtained from 4’ to it.

11) Mix the ‘solution from 10’ well by vortex formation using pipette. Also gently shake the vial for a number of times. Place the vial on the shaker horizontally and shake for around 10 min at a tilt of 10.

12) Store the ‘vials from 9 and 11’ in a dark place and let it age for around 24 hrs

13) Add ‘solution from 7’ to ‘solution from 9’ in a small vial. Add 75 μL of 1 M NaCl, 75 μM of 10 mM Phosphate to it.

14) Mix the ‘solution from 13’ well by vortex formation using pipette. Also gently shake the vial for a number of times. Place the vial on the shaker horizontally and shake for around 10 min at a tilt of 10.

15) Store the ‘vial from 12’ in a dark place and let it age for around 6 hrs.

16) To ‘Solution from 15’ add ‘solution from 11’ and store them in a dark place

The TEM images of an example fabricated three-layered SNAPs is shown in Fig. 4.8.
Fig. 4.8 Three-layered SNAPs of core particle, 1\textsuperscript{st} and 2\textsuperscript{nd} shell respectively of 60 nm, 15 nm and 5 nm (A), (B) and (C) show different positions in the grid indicating the presence of a mixture of two-layered and three-layered SNAPs in the solution.

4.2 Validation

Fig. 4.9 Raman spectra obtained from the solutions whose TEM images are shown in Fig. 4.8.

Fig. 4.9 shows the Raman spectra obtained from the mixture of two-layered and three-layered SNAPs shown in Fig. 4.8. The spectra were acquired using a laser of wavelength 785 nm
and for 10 seconds. A probe channel is used for measurements with Senterra 70 of Bruker Instruments. The concentration of oligos that are in this solution is around 1 μM and accounting for the concentration differences a net enhancement of $10^6$ is predicted in this solution which agrees with the EM theory predictions.

**Microchannel-based validation:**

We propose that the oligonucleotide-nanoparticle mixtures can be monitored in a micro-capillary while they aggregate in each step of fabrication. To illustrate this the Raman spectra of two-layered SNAPs with 50 nm core and 10 nm shell are monitored while they are aggregated using the oligonucleotide linker. The microcapillaries chosen are light-guiding silica capillaries with a polyimide cladding on their surface. These were purchased from Poly Micro Tech. The polyimide coating is burnt off to create a window for Raman measurements. The solutions with the nanoparticles and oligos are pumped into a small capillary which has a small measuring window at the center. The ends are sealed and the spectra are acquired at regular intervals. Laser excitation of 785 nm is used and a microscopic objective of 100X is used. The field of view of this objective is 0.22 mm and the depth of focus is 0.73 μm. The spectra were acquired for 120 seconds and the instrument is left idle for 180 sec between the measurements. The schematic of the microchannel used, the spectra acquired for 10 hrs, TEM image of the solution recovered are shown in Fig. 4.10.
Fig. 4.10 (A) Light-guiding silica capillary used for measurements of kinetics of Raman spectra (B) TEM image of sample recovered (C) Raman spectra acquired over time for 10 hours.

The intensity in peaks of Raman spectra acquired increase with time indicating the aggregation of nanoparticles and formation of SNAPs with time. As an example the intensity of the peak at 1050 cm$^{-1}$ is plotted over time through which the kinetics is illustrated in Fig. 4.11.
A fit shown for the data points indicate that the enhancement in the intensity stabilizes after about 6 hrs which is the time followed for aggregation during fabrication methods. The methods proposed here can be further extended to different steps of fabrication and a validation with the theoretical predictions can be obtained.
CHAPTER 5
DESIGN AND FABRICATION OF MID-IR REFLECTION FILTERS USING GUIDED MODE RESONANCES

Here, we propose optical reflection filters based on guided mode resonances (GMR) to provide an inexpensive alternative. GMRs are typically observed in substrates with geometrically tailored multilayer thin films of grating and waveguide layers and are a result of coupling diffraction and wave-guiding modes. While narrow bandwidth resonant reflection was first experimentally observed as early as 1902, recent advances in theory, simulation and microfabrication have made large-scale realization of the needed structures feasible. The geometric parameters required to support GMR in the mid-IR spectral region, further, are well within the dimensions of routine contact lithography, making GMR filters (GMRF) feasible for inexpensive, mass production. Several groups have experimentally demonstrated filters based on GMR devices for microwave, visible and near-IR regimes. However, GMR optical filters have not been extended to mid-IR as the region represents a unique challenge in terms of fabrication due to strict limits on materials and the extended spectral range. We demonstrate here the first mid-IR GMRFs. In the following sections, we first describe the theoretical framework for GMRF design based on rigorous coupled wave analysis (RCWA). Second, we report a lithography-based fabrication and successful realization of narrow-band reflectance filters.
Finally, we compare predicted and recorded responses of filters to validate the production of filters for spectroscopy.

5.1 Theory and Modeling

The typical design we propose is depicted in Fig. 5.1 and consists of a soda lime substrate with a thin film of silicon nitride (Si$_3$N$_4$) on its surface. The top part of the silicon nitride film is periodically etched to provide for a grating layer, while the bottom part acts as a wave-guide layer. The introduced periodic modulation allows for phase-matching of an external incident beam into modes that can be reradiated into free space. These modes possess finite lifetimes and are often referred to as ‘leaky modes’. An incident beam of particular wavelength and orientation undergoes a resonance on coupling to these leaky modes, resulting in high reflection efficiencies. The filter structure critically affects the coupling of incident light. The bandwidth and peak position of the reflected beam spectra can be tuned with parameters of the grating structure, including the grating period (Λ), grating depth ($d^g$), duty cycle (f) and thickness of the waveguide layer ($d^{wg}$) depicted in Fig. 5.1. While grating period can be changed relatively easily for a set of GMRFs, depth and thickness of layers are more difficult to change due to the nature of the fabrication process. Hence, we designed structures with different periods while keeping all other parameters constant to allow for simultaneous fabrication of all structures in our validation set.
The incident plane EM wave on the GMR filter structure is diffracted into waves of different orders. The diffraction efficiencies for each order can be exactly evaluated using RCWA. In brief, this method finds solutions to electromagnetic (EM) fields in each of the regions (cover, grating, waveguide and substrate as depicted in Fig. 5.1 that satisfy Maxwell’s equations and invokes the continuity of the tangential components at various interfaces to find their complete description. An efficient and stable implementation of this approach for binary\textsuperscript{147} and multi-level\textsuperscript{148} surface relief gratings has already been reported. This method is now well implemented and available in existing commercial software. However, implementation of dispersion of the material in such software is often not straightforward and hence we have developed an implementation specific to the proposed filter structure. A brief description of our implementation is described below (and in the supplementary information provided).

Fig. 5.1 : Geometrical configuration and illustration of light incident at a generalized angle to the GMRF.
**Coupled Wave Analysis Model**

The cover region and substrate region are considered to be semi-infinite and existing in the regimes $z < 0$ and $z > (d_{gr} + d_{wg})$ respectively, while the grating layer and wave-guiding layer exist in regimes $0 < z < d_{gr}$ and $d_{gr} < z < (d_{gr} + d_{wg})$ respectively. All regions, except for the grating layer, are homogenous in the transverse plane and the grating layer is modulated in the $x$-direction with a period, $\Lambda$, and fill factor, $f$ (fraction filled with a higher refractive index material). The normalized EM field vector of a complex EM wave can be represented in terms of position vector ($\mathbf{r}$), wavenumber ($\nu$) in free space, propagation vector ($\mathbf{s}$) and normalized amplitude vector ($\mathbf{A}_0$) as

$$\mathbf{E} = \mathbf{A}_0 \exp \{i2\pi \mathbf{s} \cdot \mathbf{r}\} \quad (5.1)$$

For an EM wave incident on the GMRF as shown in Fig., the propagation direction is at an angle $\theta$ relative to the $z$-axis, $\varphi$ relative to the $x$-axis and $\psi$ with respect to the plane of incidence. Hence, the incident field can be represented by equation (5.1) with amplitude function $\mathbf{A}_0$ and propagation vector $\mathbf{s}$ given by

$$A_0 = (\cos \psi \cos \theta \cos \varphi - \sin \psi \sin \varphi)\mathbf{x} + (\cos \psi \cos \theta \cos \varphi - \sin \psi \cos \varphi)\mathbf{y} - (\cos \psi \sin \theta)\mathbf{z} \quad (5.2)$$

$$s = s_x \mathbf{x} + s_y \mathbf{y} + s_z \mathbf{z}$$

In the homogeneous cover region with dielectric constant ($\varepsilon'(\nu) = [n'(\nu) + ik'(\nu)]^2$), the propagation components in transverse plane (XY plane) are defined as:
\( s_x = \sqrt{\varepsilon(\nu)} \sin \theta \cos \phi, s_y = \sqrt{\varepsilon(\nu)} \sin \theta \sin \phi. \) (5.3)

These relations explicitly describe the coupling of the electrical/optical properties of the material with the electric field of the incident beam. In the mid-IR spectral region, most materials undergo large and, often, anomalous dispersion. Hence, the choice of materials to use is limited both by the desired refractive index and the need to avoid anomalous optical effects. The propagation in z-direction in general can be obtained from the dispersion relation,

\[ s_z = \sqrt{\varepsilon(\nu) - s_x^2 - s_y^2} \] (5.4)

The incident field can be expanded in terms of space-harmonic components in each layer. Each mode of the incident field is diffracted by the presence of modulation in x-direction (in the grating layer). Accordingly the propagation of \( p^{th} \) mode in x-direction in the cover region is altered and can be obtained as:

\[ s'_{x,p} = \sqrt{\varepsilon(\nu)} \sin \theta \cos \phi - \frac{p}{v\Lambda} \] (5.5)

Finally, the diffracted waves in a homogeneous layer \( \mathcal{L} \) (cover, waveguide and substrate) can be expressed as:
To describe the diffracted field in the grating layer, we first need to define its dielectric function $\varepsilon^{gr}$. The grating layer has alternate regions of different dielectric properties (ridges and grooves). For simple rectangular ridges and grooves, the periodic relative permittivity (dielectric function) can be conveniently expressed in a Fourier series as:

$$
\varepsilon^{gr}(x) = \sum_h \left[ (\varepsilon_1 - \varepsilon_2) \sin \frac{\pi hf}{\Lambda} \right] \exp \left\{ -\frac{i2\pi h}{\Lambda} \right\} 
$$

where $\varepsilon_1$ and $\varepsilon_2$ are the refractive indices of the ridges and grooves. Note that tailoring the optical properties of either the ridges or grooves or the difference of both, hence, can also be used to modulate the effective response of the GMRF. The tangential electric and magnetic
fields are of particular interest in the grating layer and they can be expanded in harmonic series as:

\[
E^{gr} = \sum_p (M_{xp}(z)x + M_{yp}(z)y + M_{zp}(z)z)\exp\left\{i2\pi\nu(s_{xp}x + s_{yp}y)\right\}
\]

\[
H^{gr} = \sum_p (N_{xp}(z)x + N_{yp}(z)y + N_{zp}(z)z)\exp\left\{i2\pi\nu(s'_{xp}x + s'_{yp}y)\right\}.
\]  (5.9)

The normalized vector amplitude functions \(M\) and \(N\) can be expressed as functions in position and uncoupled coefficients by relating electric and magnetic fields using Maxwell’s equations, as described in the supplementary information. The expansions given by equation (5.9) for the grating layer, equations (5.6), (5.7) for all the other layers and equations [(5.1)-(5.4)] for the incident field can be related. Thus the problem simply reduces to solving for the unknown amplitude coefficients \((A_p, B_p\) in equation (5.6) and \(\alpha_p\) and \(\beta_p\) defined in following discussion). Equations governing these quantities can be obtained by applying continuity conditions.

In the substrate region, there is only a forward-wave component. Accordingly the quantities \(A^c\) and \(B^s\) vanish. The field in the cover region is due to both \(E^c\) and the incident field \(E^{inc}\) and the field in the substrate region is only due to \(E^s\). Other specific relationships are:

At the cover(c)-grating(gr) layer interface \((z=0)\):
where the inclination angle of the diffracted mode is given by: \( \phi_p = \tan^{-1}(s_y/s'_{x_p}) \).

At the grating (gr)-waveguide (wg) layer interface (z=d^gr):

\[
-\sin \phi_p M_{x_p}(0) + \cos \phi_p M_{y_p}(0) = \sin \psi \delta_{p0} + \left\{ -\sin \phi_p B^c_{x_p} + \cos \phi_p B^c_{y_p} \right\}
\]

\[
\begin{align*}
\cos \phi_p M_{x_p}(0) + \sin \phi_p M_{y_p}(0) &= \cos \psi \cos \theta - \left( \frac{s'_{x_p}}{s'(v)} \right) \left( \begin{array}{c}
\left( p \cos \phi_p s'_z \right) B^c_{x_p} \\
- \left( \sin \phi_p s'_z \right) \left( \cos \phi_p s'_{x_p} + \sin \phi_p s'_{y_p} \right) B^c_{x_p}
\end{array} \right) \\
\cos \phi_p N_{x_p}(0) + \sin \phi_p N_{y_p}(0) &= i \left\{ \sqrt{s'(v)} \sin \psi \cos \theta - \left( \begin{array}{c}
\left( p \cos \phi_p s'_z \right) B^c_{x_p} \\
- \sin \phi_p B^c_{x_p} + \cos \phi_p B^c_{y_p}
\end{array} \right) \right\}
\end{align*}
\]

\[
\begin{align*}
\sin \phi_p N_{x_p}(0) - \cos \phi_p N_{y_p}(0) &= i \left\{ \sqrt{s'(v)} + \left( \begin{array}{c}
\left( p \cos \phi_p s'_z \right) B^c_{x_p} \\
- \left( \sin \phi_p s'_z \right) \left( \cos \phi_p s'_{x_p} + \sin \phi_p s'_{y_p} \right) B^c_{x_p}
\end{array} \right) \right\}
\end{align*}
\]

where the inclination angle of the diffracted mode is given by: \( \phi_p = \tan^{-1}(s_y/s'_{x_p}) \).
At the waveguide (wg)-substrate (s) interface ($z = d^s + d^{wg}$):

\[
\begin{align*}
\cos \phi_p N_{x,p}(d^s) + \sin \phi_p N_{y,p}(d^s) &= i \left( -s_z^{wg} \left( -\sin \phi_p A_{x,p}^{wg} + \cos \phi_p A_{y,p}^{wg} \right) - s_z^{wg} \left( -\sin \phi_p B_{x,p}^{wg} + \cos \phi_p B_{y,p}^{wg} \right) \right) \\
\sin \phi_s N_{x,p}(d^s) - \cos \phi_s N_{y,p}(d^s) &= i \left( \left( p \cos \phi_p \right) s_z^{wg} A_{x,p}^{wg} - \left( \sin \phi_p s_z^{wg} \right) A_{y,p}^{wg} - \left( \cos \phi_p s_z^{wg} + \sin \phi_p s_y \right) A_{y,p}^{wg} \right) \\
&+ \left( \left( p \cos \phi_p \right) s_z^{wg} B_{x,p}^{wg} - \left( \sin \phi_p s_z^{wg} \right) B_{y,p}^{wg} - \left( \cos \phi_p s_z^{wg} + \sin \phi_p s_y \right) B_{y,p}^{wg} \right) \\
\end{align*}
\tag{5.11}
\]

At the waveguide (wg)-substrate (s) interface ($z = d^s + d^{wg}$):

\[
\begin{align*}
A_{x,p}^s &= A_{x,p}^{wg} \exp \{i2\pi v s_z^{wg} d^{wg}\} + B_{x,p}^{wg} \\
A_{y,p}^s &= A_{y,p}^{wg} \exp \{i2\pi v s_z^{wg} d^{wg}\} + B_{y,p}^{wg} \\
\left( s_y A_{x,p}^{wg} - s_z^{wg} A_{y,p}^{wg} \right) \exp \{i2\pi v s_z^{wg} d^{wg}\} + s_x B_{x,p}^{wg} + s_z^{wg} B_{y,p}^{wg} &= s_y B_{y,p}^s - s_z^{wg} B_{y,p}^s \\
\left( s_z^{wg} A_{x,p}^{wg} - s_x A_{x,p}^{wg} \right) \exp \{i2\pi v s_z^{wg} d^{wg}\} - s_z^{wg} B_{x,p}^{wg} + s_x B_{z,p}^{wg} &= s_z^{wg} B_{x,p}^s - s_x B_{z,p}^s
\end{align*}
\tag{5.12}
\]

For a system which considers $N_F$ coefficients for series expansions, hence, there are $24N_F$ coefficients. $6N_F$ of these coefficients are assigned a value of zero (all $A^c$ and $B^c$). Consequently, $12N_F$ independent equations are obtained from [(5.10)-(5.12)] and $6N_F$ equations are obtained from transversality conditions.

**Transversality Conditions:**

In the grating region, the electric and magnetic fields can be relating using Maxwell’s equations:
Using (5.13) in the expression for field description in grating layer provided in the manuscript and eliminating \(M_d(z)\) and \(N_d(z)\), a finite system of differential equations can be obtained as follows for all modes:

\[
\begin{bmatrix}
\frac{\partial M_x}{\partial z} \\
\frac{\partial M_y}{\partial z} \\
\frac{\partial N_x}{\partial z} \\
\frac{\partial N_y}{\partial z}
\end{bmatrix} = i2\pi \nu \begin{bmatrix}
0 & 0 & S_y \varepsilon^{-1} S_x & I - S_y \varepsilon^{-1} S_y \\
0 & 0 & S_x \varepsilon^{-1} S_x - I & -S_x \varepsilon^{-1} S_y \\
S_x S_y & \varepsilon - S_y^2 & 0 & 0 \\
S_x^2 - \varepsilon & -S_x S_y & 0 & 0
\end{bmatrix} \begin{bmatrix}
M_x \\
M_y \\
N_x \\
N_y
\end{bmatrix}
\]  

(5.14)

where \(S_x\) and \(S_y\) are diagonal matrices with diagonal elements \(s_{x,p}^{gr}\) and \(s_{y}^{gr}\) respectively and \(\varepsilon\) is a matrix in which \(\varepsilon_{jh} = \varepsilon_{j-h}^{gr}\). This set of equations is solved using an Eigen decomposition of the matrix involved in equation (5.14) in the form of \(G \Gamma G^{-1}\), where \(\Gamma\) is a diagonal matrix with Eigen values in the diagonal and columns of matrix \(G\) are the corresponding Eigen vectors. With this decomposition, a solution set can be constructed as:

\[
M_{xp} = \sum_{j=1}^{N_F} \{ \alpha_j g_{j,p} \exp[i2\pi \nu \gamma_j z] + \beta_j \tilde{g}_{j,p} \exp[i2\pi \nu \gamma_j (d_{gr} - z)] \},
\]  

(5.15)
where $g_{j,p}$ is element of $j^{th}$ Eigen vector column that corresponds to the positive Eigen value $\gamma_j$ and similarly $\tilde{g}_{j,p}$ is Eigen vector value corresponding to Eigen value $-\gamma_j$. $M_z(z)$ and $N_z(z)$ can be obtained using equation (5.15), in the following expression eliminated to obtain (5.14):

$$M_{z,p} = -\frac{1}{2\pi V} \left\{ \sum_{p'} \eta_{p'-p''} \left\{ s_{x,p'}^{gr} M_{y,p''} \right\} \right\},$$

$$N_{z,p} = \frac{1}{2\pi V} \left[s_{x,p}^{gr} M_{y,p''} \right]$$

where $\eta_h$ are the Fourier coefficients of inverse of the dielectric function $\varepsilon^{gr}$. Substituting $M$ and $N$ into equations (5.10) and (5.11), they can be expressed in terms of unknown coefficients $\alpha_j$ and $\beta_j$.

In addition, in the homogeneous layers, Gauss’s equation results in conditions:
Equations \((5.15)-(5.17)\) thus provide for the required \(6N_F\) equations.

This set of equations is then solved using a standard matrix decomposition (LU or QR) method. The fields can then be explicitly calculated using the obtained coefficients.

The diffraction efficiency, \(DE\), for each \(p^{th}\) propagating diffracted order is the diffracted power divided by the incident power. With unit incident amplitude, the diffraction efficiency for reflection \(DE_{\text{ref},p}\) can thus be obtained by using the spatially averaged Poynting vector as follows:

\[
DE_{\text{ref},p} = \left\{ \frac{s'_{x,y}B_{x,y}^c + s_{x,y}A_{x,y}^c + s_{x}A_{x}^c}{\sqrt{\varepsilon'(\nu)\cos\theta}} \right\}^2 \left\{ \frac{s_{x,y}B_{x,y}^c + s_{x}B_{x}^c - s_{x}B_{x}^c}{\sqrt{\varepsilon'(\nu)\cos\theta}} \right\} + \left\{ \frac{s_{x,y}B_{x,y}^c + s_{x}A_{x,y}^c + s_{x}A_{x}^c}{\sqrt{\varepsilon'(\nu)\cos\theta}} \right\}^2 \left\{ \frac{s_{x,y}B_{x,y}^c + s_{x}B_{x}^c - s_{x}B_{x}^c}{\sqrt{\varepsilon'(\nu)\cos\theta}} \right\}
\]

The diffraction efficiency for transmission, \(DE_{\text{tran},p}\) can be obtained by substituting coefficients \(B^c\) with \(A^c\) in equation \((5.18)\).
Filter Design

The efficiency of each diffracted order can be calculated using RCWA. However, it is an informative first step to analyze the filter structure as a bi-layer waveguide to find a range of parameters for the eventual GMRF structure. Accordingly, the effective average refractive index of the grating layer to a first order approximation can be written as

$$\varepsilon^{gr} = \varepsilon^{SN} f + \varepsilon^c (1 - f) \quad \text{(TE case), and}$$

$$\frac{1}{\varepsilon^{gr}} = \frac{f}{\varepsilon^{SN}} + \frac{1 - f}{\varepsilon^c} \quad \text{(TM case)} \tag{5.19}$$

For the surface-relief structure to resemble a waveguide, the refractive indices of grating and waveguide layers have to be such that ($n_{wg}, n_{gr}^r > n_s^r > n_c^r$). The materials are chosen to be silicon nitride and soda lime, and a duty cycle $f$ is chosen to be 0.5 for easier fabrication. For the same structure, the reflection resonances are dependent on the polarization state of the incident wave and the angle at which it is incident. With a TE polarized incident wave at angle $\theta$, for a particular order diffracted wave to be a leaky Eigen mode, the introduced modulation parameter should satisfy the inequality:  

$$\text{Max}\{n^r, n^s\} \leq n^r \sin \theta - \frac{D}{vA} < \text{Max}\{n^{gr}, n^{wg}\} \tag{5.20}$$
For maximal efficiency, we propose designing filters such that only zero-order propagating waves contribute to the reflected wave while all the higher orders become guided modes (evanescent).

Here, our focus was on fundamental studies involved in validating a framework for GMRFs, rather than a fabrication and use for spectroscopy. Hence, we focused on validations with a small number of filters in the spectral region around the C-H stretching vibrational mode (2600-3000 cm$^{-1}$). Fig. 5.2A represents the inequality in equation (5.20) for the first diffraction orders to be evanescent in this range for different incident angles. The refractive indices are assumed to be...
real constants for the spectral range listed. At normal incidence, the parameter range overlaps for $p = 1$ and $p = -1$ and approximately obeys $1.7 < \Lambda < 2.7 \mu m$. Larger dimensions are preferred for simpler fabrication; accordingly, we chose 2.2, 2.3, 2.4 and 2.5 μm as Λ values for proposed filter set. The thicknesses of the waveguide and grating layers, subsequently, have to be appropriately chosen to maximize the chosen resonances. Hence, for the selected Λ, we examined different sets of values for $d^{wg}$ and $d^{gr}$ using RCWA to maximize zero order diffraction reflection efficiency. Fig. 5.2B shows the results obtained for normally incident TE polarized wave when $d^{wg} = d^{gr}$. In these calculations the refractive indices are assumed to be constant and are assigned values listed in the figure. It can be observed that a minimum thickness of 0.3 μm is needed to achieve a reflectance peak in the desired spectral range. We choose the minimum thickness possible to minimize losses due to absorption.

### 5.2 Experimental Methods: GMRF fabrication, microscopy and spectroscopic measurements

**Filter Fabrication**

The filters are fabricated on a 4 inch diameter soda-lime glass wafer using contact photolithography. The sequence of steps involved in fabrication is depicted in Fig. 5.3. Briefly, a 600 nm layer of Si₃N₄ is deposited onto the substrate by plasma enhanced chemical vapor deposition (PECVD) from a mixture of N₂, SiH₄ and NH₃ flowing at rates of 1960, 80 and 55 cm³/min respectively, all at a pressure of 900 mTorr and temperature of 300 °C. The wafer is
then baked at 125 °C for 2 min as a preparatory step to remove any moisture. A photoresist (AZ5214E) is spun coat on the wafer using an adhesion promoter (Hexamethyldisilizane). The adhesive is spun at 4000 rpm for 30 seconds and the photoresist is spun at 5000 rpm for 45 seconds. The wafer is then baked at 110 °C for 50 seconds. The thickness of the photoresist layer obtained is estimated to be 1.25 μm. The wafer surface is then exposed to ultraviolet radiation at 7.5 mW/cm² for 10 seconds through a chrome mask with the four grating patterns printed on its surface.

Fig. 5.3: Sequence of steps involved in fabrication of surface-relieved film on soda lime wafer
To reveal the grating pattern, the wafer is then immersed in a developing agent (AZ400 in a 1:4 dilution with water) for 9 seconds. The wafer is then hard baked at 125 °C and then etched by reactive ion etching (RIE) using Freon-14 at 35 mTorr using 90 W. Approximately 490 Å are estimated to be etched in a minute and a grating depth of 300 nm is targeted to be achieved. The wafer is finally cleaned using oxygen plasma treatment, this final de-scum step ensures that the grating layer obtained is smooth.

*Analytical Measurements*

*Microscopy measurements:*

Atomic force microscopy (AFM) measurements were performed with the probe in AC mode at a frequency of 0.1 Hz with voltage set at 2V. Data were acquired over an area of 5μm x 10μm on 512 pixels over 256 scan lines at a 5 μm/s scan rate with no proportional gain and integral gain of 10. The scanning electron microscopy (SEM) images are acquired using a field-emission environmental scanning electron microscope. The structural profiles were obtained from the AFM data by averaging over the 256 scan lines.

*Spectroscopic Measurements:*

The filter responses to a broadband beam from a silicon carbide element were recorded. An off-axis parabolic mirror is used to collimate the radiation and a wire-grid polarizer was used for
polarization. Two parallel circular apertures that restrict the beam to around 10 mm have been employed for filtering out any divergent rays. A beam splitter was employed for measurements of normal incidence case. The beam splitter and all other optics have been assumed to be of 100 % efficiency. The filter wheel was mounted on a stage with precise control on translation and rotation. The responses were recorded such that there was a single noticeable resonance. The data was acquired at 4 cm\(^{-1}\) resolution and averaged 16 times. The reflection spectra were evaluated by comparing the filter responses with that of a silver coated substrate. Bruker Vertex 70 spectrometer is used as a detector and the spectra were acquired in the 7000-400 cm\(^{-1}\) spectral region.

5.3 Results and discussion

Simulations

Using the parameters for the filter set above in the developed theoretical framework, we simulated reflectance spectra for broadband IR illumination for each of the selected structures as shown in Fig. 5.4A. The four structures of \(\Lambda=2.2, 2.3, 2.4\) and 2.5 \(\mu\)m possess a maximum reflection efficiency of ~1 at 2987, 2869, 2760 and 2659 cm\(^{-1}\) respectively and distinct bandshapes in the characteristic reflection peak with full width at half-maximum (FWHM) of 50, 42, 36 and 33 cm-1 respectively. Along with these resonance peaks, there exists a much weaker reflection peak due to a higher order guided mode at 5456, 5250, 5063 and 4892 cm\(^{-1}\) respectively.
Fig 5.4 A) Reflection spectra (zero-order diffraction efficiency) of the designed filters. The parameters chosen are: $\Lambda = 2.2, 2.3, 2.4$ and $2.5 \mu m$, $f = 0.5$, $d^{gr} = d^{wg} = 0.3 \mu m$, $n_{Si_3N_4} = 2.0$, $n^S = 1.48$, $n^c = 1.0$. B) E-field distribution in the vicinity of structure with $\Lambda = 2.5 \mu m$ at a resonant wavenumber, $\nu = 2659$ cm$^{-1}$ (top) and off-resonance wavenumber, $\nu = 3400$ cm$^{-1}$. The surface relief of the film to be fabricated is depicted using white lines.

While the higher order modes cannot be avoided, we note that the reflections are substantially weaker and the peak positions are shifted towards the near-IR. While one strategy may be to optimize filter structures such that these peaks are minimized, the $\sim 2500$ cm$^{-1}$ shift from the major reflectance peak implies that bandpass or longpass filters may be used to eliminate their effects. For example, a longpass filter commonly used for undersampling in FT-IR spectroscopy (transmittance of $\sim 0.95\%$ for $\nu < 3950$ cm$^{-1}$) can be used to eliminate the effects of the same. The bandwidths for the structures can potentially be changed (especially, reduced) by considering different values for thicknesses of grating and waveguide layers. Our intention here, however,
was to achieve the fabrication of the entire filter set on a single wafer for purposes of comparison with theory. Hence, we choose the same thickness values for grating and waveguide layers.

Finally, the out-of-bandpass or side-band reflections in these filters reach up to 17%. The ratio of the peak reflectivity to the maximum side-band reflectivity can be termed the contrast ratio for the filter. The contrast ratio can be improved, for example, by using additional layers.\textsuperscript{142} The approach here was to compare theory and fabrication and such methods have not been considered here to keep the fabrication and optical setup simple. To understand the behavior of GMRFs further, we examine one specific case. At peak reflection a lateral standing wave is created inside the filter\textsuperscript{150}, due to the coupling of the two counter-propagating diffracted waves (+1 and -1 orders) which are phase matched to the leaky waveguide mode. The strength of the local electric field in the surface-relieved film can depict the strength of the lateral standing wave setup and the extent to which coupled waves propagate resulting in the multiple interferences of the energy coupled to the waveguide from every grating period. The higher the electric field enhancement inside the structure is\textsuperscript{151}. In Fig 5.4B, this standing wave is visualized by the electric field amplitude distribution at a resonant frequency (2659 cm\textsuperscript{-1}) for a filter (\(\Lambda = 2.5 \mu\text{m}\)), which is significantly smaller at an off-resonance frequency (3400 cm\textsuperscript{-1}). Due to the strong modulation for the GMRFs considered here, the maximum electric field enhancement inside the structure is smaller in comparison with enhancements reported in other structures.\textsuperscript{151}
Prior to a comparison with measurements, it is instructive to consider experimental factors that can possibly influence the recorded data and to understand their potential effects using the developed theoretical framework. First, we recognize that spectral reflectance of filters can be significantly altered by differences in the values of refractive indices used in simulation and those that are realized during fabrication. Such differences may arise from spectral dispersion, from film structure at the nano-microscale as well as due to confinement effects. Second, light in experimental conditions is unlikely to be highly collimated. Hence, the effect of small variations in angles on incidence must be studied. These are two of the largest effects and we examine them next.

The refractive index of silicon nitride in visible region decreases gradually with increasing wavelength. It has a significant dispersion, however, in mid-IR spectral region with intense absorption peaks in the 1400-600 cm\(^{-1}\) spectral range. Fig 5.5A shows the effect on reflectance spectra for one of the structures when dispersion characteristics are considered. When dispersion of the underlying substrate, soda lime, is considered, the peak is slightly shifted towards higher wavenumber (from 2869 cm\(^{-1}\) to 2875 cm\(^{-1}\)). When dispersion of Si\(_3\)N\(_4\) is considered, the peak shifts towards a lower wavenumber (from 2869 cm\(^{-1}\) to 2853 cm\(^{-1}\)) and is of lower efficiency (0.96 in comparison to 1.0). The combined effect of the absorption of Si\(_3\)N\(_4\) layer and surface scattering is a lower peak efficiency (0.95 in comparison to 1.0) and a slight broadening of the reflectance peak (150 cm\(^{-1}\) in comparison to 138 cm\(^{-1}\)).
Fig. 5.5 A) Effect on resonances of dispersion characteristics of Si$_3$N$_4$ and soda lime, for structure of $\Lambda = 2.3$ $\mu$m, $d^{\text{gr}} = 0.3$ $\mu$m, and $d^{\text{wg}} = 0.3$ $\mu$m, when a TE-polarized wave is normally incident. B) E-Field amplitude distribution at resonance wavenumber ($\bar{\nu} = 2869$ cm$^{-1}$) without considering the dispersion properties of both Si$_3$N$_4$ and soda lime. C) E-field amplitude distribution at resonance wavenumber ($\bar{\nu} = 2858$ cm$^{-1}$) considering the dispersion properties of Si$_3$N$_4$ and soda lime. The surface relief of the film to be fabricated is depicted using white lines. D) Comparison of E-field amplitude distributions shown in (B) and (C) at the interface of grating and waveguide layers.

The effects due to anomalous dispersion are also apparent in the region below 1600 cm$^{-1}$. Fig. 5.5B depicts the E-field amplitude distributions considering a constant real refractive index while FigC depicts the E-field distribution when the dispersion characteristics are incorporated into the model. In both cases, a standing wave field is observed indicating the presence of resonances. The local E-field as shown in FigD is only slightly lower for the latter case indicating decreased the slightly broader reflectance peak$^{155}$. In summary, we note that the refractive index values can
influence the position, peak reflectivity and bandshape of the filter. Hence, it is critical to control this parameter during fabrication and operation.

Figure 5.6: Spectral variability with changes in incidence angle $\theta$ for structure of $\Lambda = 2.3$ μm, $d^{gr} = 0.3$ μm, and $d^{wg} = 0.3$ μm, when a TE-polarized wave is incident with azimuthal angle $\phi = 0^\circ$. Only regions around the two resonances observed in Fi are plotted and significant alterations are observed within both regions. Other spectral regions are insignificantly impacted and hence not shown here.

The peaks obtained are also strongly coupled to the angle of incidence of the light to be filtered due to the strong modulation in the filter structures. For example, Fig 5.6Figure shows the effect of varying incidence angle, $\theta$, on the two reflectance peaks compared to those obtained using normal incidence. Even a 1$^\circ$ variation in $\theta$, results in splitting of the peak at 2858 cm$^{-1}$ into two peaks at centered at 2880 and 2822 cm$^{-1}$. The two peaks are slightly different and one of the peaks is broader and shorter. A 5$^\circ$ variation could result in splitting and shifting of peaks by as
much as of 140 cm$^{-1}$. Hence, the optical setup needs to be as precise as possible in measurements and consistently automated when utilizing the filters. At the same time, we note that the peak splitting and shifts can be a facile means to achieve modulation in spectral position. As opposed to amplitude modulation, which may be achieved by a chopper for example, this type of spectral modulation is not possible in interferometry. Hence, the filters present an additional modulation mechanism that remains to be utilized in a smart manner. A trivial application is, of course, in filter alignment such that tilting the filter would bring the peaks closer, eventually resulting in a single reflection peak.

*Measurements*

![Image](image.png)

Fig 5.7. (A) Scanning electron microscopy (SEM) image of one of the fabricated GMRFs, and (B) atomic force microscopy (AFM) images of a 1D GMRF, demonstrating the consistency in depth and the periodicity in surface relief.
Filters were fabricated as described in the experimental section. We subsequently characterized the obtained structures using SEM and AFM. While SEM provides a global view of the consistency of fabricated microstructures, AFM provides a quantitative measure of the achieved thickness. From these measurements, we can iteratively refine experimental conditions such that filters with consistently similar structures are obtained. We refined our experimental protocol using several runs and validated that the results are consistent in implementation of the protocol for fabrication. Once routinely consistent filters were obtained, we characterized the measured geometrical parameters with a goal of using them as inputs to the theoretical framework to predict filter response. Results from both modalities of such consistent filters are shown in Fig. 5.7. By averaging over 256 scan lines the values for $d^{gr}$ and $\Lambda$ are estimated. The standard deviation in the values obtained for $d^{gr}$ is observed to be less than 5 nm while that for $\Lambda$ is observed to be less than 15 nm.

**Structural Parameters**

The response of the filter is also very sensitive to the actual geometrical configuration obtained\textsuperscript{149}. The filters fabricated show a good homogeneity of the grating layers as characterized by the structural profile of the grating layers obtained using AFM measurements. Fig. 5.8A shows the average structural profile of the grating layer in the four filter structures offset from each other. The fill fractions can be approximated to be 0.5 from these profiles. The filter responses are relatively insensitive to slight variations in fill fractions and hence an
approximate value will suffice. The rectangular profile of the grating grooves cannot be verified from these results, but for convenience we assume straight edges for the grooves in calculations. The pitch values derived are close to the targeted values within a ~1% variability. Although the targeted $d^{gr}$ is 0.3 μm, the actual depths obtained were somewhat lower (~0.25 μm).

Fig. 5.8 : A) Structure of the grating layer obtained for the four structures using AFM, (depicted offset from each other here). The AFM results are averaged over 256 scan lines. The structure of the grating layer is used to evaluate $\Lambda$ and $d^{gr}$, the standard deviation of $d^{gr}$ is ≤ 0.005 μm and the standard deviation in evaluating $\Lambda$ is ≤0.015 μm. B) The reflectance efficiencies of the four filters with TM-polarized normal incidence, an appropriate $d^{wg}$ is found with which results predicted by RCWA match those obtained from experiments. C) Structural parameters for the four filters fabricated.

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<tr>
<td>$\Lambda$ (μm)</td>
<td>2.201</td>
<td>2.28</td>
<td>2.39</td>
<td>2.49</td>
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<tr>
<td>$d^{gr}$ (μm)</td>
<td>0.247</td>
<td>0.243</td>
<td>0.257</td>
<td>0.249</td>
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<tr>
<td>$d^{wg}$ (μm)</td>
<td>0.4</td>
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This can be attributed to the etch rate being smaller than that estimated due to a higher SiH₄/NH₃ flow ratio used in our fabrication process to achieve a Si₃N₄ film of higher refractive index.¹⁵⁶ The thickness of the waveguide layer is obtained using the reflectance measurements at TM-polarized normal incidence. As depicted in Fig. 5.8B, the theoretical predictions and experimental measurements match very well for all the filter structures when a value of 0.4 μm is chosen for \( d_{wg} \). The peaks are relatively broader in the measured data, which is likely due to small angular distribution in the incident beam. The relatively higher base reflectance in the experimental data could be attributed to the background radiation in the optical setup and the scattering at Si₃N₄-soda lime interface and a finitely thick actual soda lime substrate while the RCWA simulation assumes a semi-infinite medium substrate.¹⁵⁷

**Validation: comparing predicted and measured spectra**

The reflectance spectra of the fabricated filters are measured for TE incidence and are compared with the theoretical predictions. The acquired spectra show distinct peaks in the spectral region of interest as shown in Fig. 5.9. The four structures provide maximal reflection efficiencies of 0.79, 0.74, 0.73 and 0.69 respectively with side bands of peak reflection efficiency of 0.2 – 0.25. The bandwidths are approximately the same as the design values. The H₂O and CO₂ present in air that permeates the optical setup results in small differences from predicted values around 3800 and 2350 cm⁻¹. The higher baseline for the reflectance exists in TE polarization, as seen for the TM measurements, and can be attributed to the effects due to non-
specific reflection at the interface, substrate properties and background response due to optics in
the experimental setup.

Fig. 5.9: Comparison of experimentally measured and theoretically calculated reflectance spectra
of fabricated GMR filters for a TE-polarized incident wave. The structural parameters are
obtained from AFM measurements and reflectance measurements from TM-polarized incidence.
These values are listed in Fig. 5.8C.

The splitting of the peaks can be modeled better when the responses are considered as an
integral average over slight variation in angles of incidence and this has been illustrated in
supplementary information. The angular dependence arises from the imperfect collimation of the
beam as well as small alignment differences that may be improved further.

Due to the unavailability of exact refractive index values, the losses due to scattering (at Si₃N₄
and soda lime interface) and absorption (of bulk material) cannot accurately be accounted for in
the simulations. The imperfections in the structure and the deviations from straight edges in grooves and edges have also been excluded from the theoretical model. This, along with the slight variability in the incident beam could be the cause for deviations from ideal predictions. The base reflectance can be eliminated by multiply reflecting off the filters. The experimental measurements at other angles of incidence have also been verified against theoretical predictions and the results have been presented in supplementary information. The unavailability of a collimated IR source is a significant hurdle in rigorously and exactly validating the filter responses. Similarly, the optical setup can be further optimized to yield better efficiencies and lower side-bands. An accurate validation can however only be done by a proper characterization of the incident beam and inclusion of all optical effects in the model. Nevertheless, the two major conclusions of the observations and modeling are that, first, fabricated filters possess distinct reflection characteristics with reasonably high efficiencies. Second, reflectance spectra can be understood theoretically. Combined, the two results form a significant first step towards creating appropriate GMRFs for mid-IR measurements.
CHAPTER 6

CONCLUSIONS AND FUTURE WORK

In summary, we have theoretically demonstrated the possibility of designing SERS probes that possess designed enhancement, wavelength-tuning capability and potential for multiwavelength excitation. The layered structure and aggregate structures permit both the reduction of variance by shielding reporters from direct interaction with metal surfaces as well as being spherically symmetric. In principle, the configuration offers unlimited multiplexing using simple reporters, whose enhancements do not depend on their scattering cross section but can be independently tuned by the probe. The use of any reporter, tailored enhancement and reproducible response will open the possibility of measuring multiple molecular species in complex samples. A practical translation of this technology also requires the development of a large dynamic range and suppression of the signal from the surrounding media, which are both often a limiting factor. While the concept of enhancements in signal has been previously examined, the simultaneous incorporation of quenching in the optimization makes this work especially practical. The response of the probes may be tuned to reject out of probe enhancement to any desirable level. Similarly, an enhancement of the region outside the probe can be imagined. In this case, the use of LAMPs will be more powerful and generally applicable than the use of solid substrates. While we have focused on utilizing the reporter in the probe in this manuscript, we believe that the tailoring can be extended to a sample volume outside the probe
using the same methods. The proposed structures are amenable to modern fabrication methods\textsuperscript{159} and a number of biocompatibility and molecular targeting strategies are available for nanoparticles\textsuperscript{160} that can be employed for the proposed structures. At the same time, strategies to use spectroscopic signatures for quantitative analyses via efficient computer algorithms\textsuperscript{161} are available to analyze the data. Hence, the realization and use of nano-LAMP probes, as designed in this thesis, is poised to enable progress in a wide variety of fields ranging from clinical diagnostics, environmental sensing to basic biomedical research.

We have also demonstrated GMR reflectance filter structures for the first time. The design is based on a surface-relief film of silicon nitride and four structures with distinct characteristics have been fabricated on the same wafer using a lithography approach. The simpler design and fabrication demonstrated will allow for mass production and this study is a first step in creating a filter wheel with regions of distinct reflectance bands. The strong modulation conditions resulted in devices with responses that are sensitive to the variability in geometrical structure and incident beam properties. The fabricated filters exhibited slightly lower reflectance and broader bands than those expected due to incidence angle variability and scattering/absorption losses. Although the reflection characteristics realized are not the optimal achievable, the structures can be improved by adding more layers or changing the refractive index properties of the film by changing deposition/etching parameters.
The principles proposed here for design of structures can be extended to the design of substrates and surfaces for surface enhanced Raman spectroscopy. We propose nanoslits similar to Nano-LAMPs which have nanoscale disks fabricated using e-beam lithography. The structure, surface electron micrographs and SERS spectra of Rhodamine Isothiocyanate obtained using nanoslits made of gold and silver are as shown in Fig. 6.1.

Fig. 6.1 A) Side view of the structure of nanoslits made of gold or silver. The thin Cr layer on the Si substrate is to facilitate the lift off in E-beam lithography. B) SERS spectra obtained of 0.2 mM Rhodamine Isothiocyanate adsorbed onto these structures C), D) SEM images of nanoslits made of gold and silver.
The fabrication and capability of such structures is shown here and the design principles in this thesis can be further extended to optimize these structures.

**DFIR Imaging**

A DFIR imaging instrument proposed here in comparison to an FT-IR setup is as shown in Fig. 6.2.

Fig. 6.2 A) Typical FT-IR optical instrumentation in comparison with B) a typical DF-IR instrumental setup.

The interferometer has been replaced with GMR reflection filter wheel. The filter wheel has to possess regions that can reflect narrow regions in mid-IR while a set of such region should cover an entire broad band range. To achieve this filter wheel has been designed with more than
40 regions of distinct reflection characteristics. A schematic of the filter wheel and the experimental reflection characteristics are shown in Fig. 6.3.

Fig. 6.3 A) The GMR reflection filter wheel and B) their distinct reflection characteristics

Their reflection spectra shown in Fig. 6.3B as shown are distinct, narrow and extent over broadband range. The reflection efficiencies achieved here are less than what has been achieved in the case of four-region filter described in Chapter 4. This is due to the fact that the incident IR beam in characterizing this beam is of considerably lower area (2 mm as opposed to 10 mm) resulting in larger and significant deviations in the intensity and divergence of the incident beam reaching the filter. In the work shown here, there is also a limit to the spectral resolution achieved in the reflectance peaks and superstate layers can be deposited onto further optimize the parameters in the GMR filters. By incorporating such advances with the principles proposed here it is possible to match the resolutions achieved in case of FTIR. Last Fig. 6.4 demonstrates some
of the spectral data acquired and IR images acquired using the DFIR setup.

![Spectra comparison with FTIR for polycarbonate and polystyrene. IR images acquired for USAF target at different spatial resolutions. IR image of a prostate tissue sample.](image)

Fig. 6.4 Data acquired with preliminary DFIR setup A) Spectra comparison with FTIR for polycarbonate and polystyrene. B) IR images acquired for USAF target at different spatial resolutions. C) IR image of a prostate tissue sample.

The data depicted here show the applicability of DFIR, but the spectral resolutions needs to be enhanced. Further optimization of the optical setup and filters are a work in progress.

In conclusion, this dissertation proposes application of electromagnetic theory and principles for rational approaches to design devices at nanoscale. The physical principles can be extended to several modalities and can enhance the throughput, detection limits and sensitivity in molecular imaging applications.
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