Design and Fabrication of a Low-Cost, Portable, Battery-Operated Surface Enhanced Raman Scattering (SERS) Optical Device

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DESIGN AND FABRICATION OF A LOW-COST, PORTABLE, BATTERY-OPERATED SURFACE ENHANCED RAMAN SCATTERING (SERS) OPTICAL DEVICE

A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy

in

The Division of Electrical and Computer Engineering

by

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B.Eng., Osun State University, Nigeria, 2012
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May 2022
This dissertation is dedicated to the Almighty God and all black female USA minorities aspiring to earn a Doctorate in the field of Electrical Engineering.
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**Acronyms**

**SERS:** Surface Enhanced Raman Scattering

**PETE:** Polyethylene terephthalate

**R6G:** Rhodamine 6G

**nmol:** nanomole

**mmol:** millimole

**µm:** micrometer

**nm:** nanometer

**mm:** millimeter

**mW:** milliwatt

**GRIN:** Graded Index

**NA:** Numerical Aperture
Abstract

Raman Spectroscopy is a time-honored, non-invasive method for analyzing and identifying the molecular composition of materials. However, unenhanced Raman Spectroscopy has extremely low sensitivity which limits its sensing capability. SERS brings rough nano-metallic surfaces in contact with the material molecules to enormously enhance the Raman signals.

The sensitivity of SERS can be exploited in probe applications where the spectrometer needs to be brought near the specimen. For example, a long optical fiber coupled to a SERS device can be used to characterize and identify easy-to-reach cancerous tissues in organisms. Unfortunately, background signals in a long fiber can easily mask any signal returning from the end of the probe. A classical solution is to inject nanoparticles and use multiple optical fibers (one to deliver the excitation light and one or more to return the scattered Raman light). However, the coupling between the fibers is poor, reducing the signal strength, and reproducibility between locations, and removal of the injected nanoparticles present difficulties.

This work intends to address those challenges by designing and fabricating a low-cost handheld SERS device. The development of this SERS device is broadly split into (a) fabrication of a probe suitable low-cost SERS substrate (b) design and fabrication of the handheld SERS device optics.

The method used for the fabrication and characterization of the SERS layer low-cost substrate involved sandpaper imprint patterning of silver nanoparticles. This was accomplished at low cost with inexpensive equipment, readily available materials, and with no chemical or lithographic steps. The handheld SERS optics incorporated a solid-state laser, diffractive optics, the low-cost SERS substrate at one end of a GRIN lens, and a short-tube pathway to the Raman spectrometer.

The response of the optical system and imprinted SERS layer was tested to obtain Raman spectrum from 1nmol to 1mmol Rhodamine 6G suspension. This yielded good Raman scattering results.

The developed device was made with a SERS substrate fabrication method which is a low-cost alternative and with no lithography or chemical synthesis. This SERS portable design is also suitable for bio-probes or remote sampling without the disadvantages associated with injected clouds substrates and multiple collection fiber systems.
Chapter 1. Brief Description of Chapter Contents

This chapter briefly describes the contents of chapters 2 through 7. Chapter 2 presents the background to the general discourse of this dissertation. It discusses and reviews light scattering, Rayleigh and Raman scattering, Raman spectroscopy, Surface-Enhanced Raman Scattering (SERS), and various Surface-Enhanced Raman Scattering optical setups.

Chapter 3 justifies the need for this work. It follows chapter 2’s review, by stating the limitations of conventional SERS set-ups for probe applications, thereby outlining the research objectives of this work that are tailored at solving those limitations.

Chapter 4 opens with a brief introduction and literature review of SERS substrates and fabrication techniques. It presents a novel low-cost imprint SERS fabrication technique suitable for various SERS portable sampling applications. It also presents the materials, methods, characterization, and results, and a cost analysis of the imprint method.

Chapter 5 reviews SERS instrumentation for portable and remote samples. It discusses the need for a suitable SERS setup for remote sampling or probe applications. It describes how chapter 5’s low-cost imprinted SERS substrate can be transferred and securely bonded to other surfaces, most especially a GRIN lens for probe applications. It details the materials, methods, characterization, and results of the transferred SERS probe.

Chapter 6 reviews SERS instrumentation for table-top and portable applications. It outlines the need for a portable, low-cost SERS probe for remote sampling. It presents two portable SERS device designs: the first incorporates the compact collection optics with the portable sampling low-cost substrate described in chapter 4. The second design incorporates the compact collection optics with the remote sampling GRIN lens probe described in chapter 5. The SERS results of the first design are also presented.

Chapter 7 summarizes the results from chapters 4, 5, and 6. It states the general conclusion of this work and the recommendations for future work.
Chapter 2. Introduction

2.1. Light scattering

When light passes through or is incident on a material or target, the atoms or molecules of the material absorb the light and scatter it in different directions. This is referred to as light scattering. Light scattering is a common occurrence in our everyday world. A common example is a blue sky on a sunny day. Like white light, sunlight consists of various wavelengths corresponding to different colors. When sunlight passes through the atmosphere, the atmospheric particles scatter it in different directions. The blue color corresponds to the shortest visible wavelength. Since scattering is inversely proportional to the 4th power of the wavelength blue light is scattered the most. The human eyes, therefore, sees the dominant scattering of the short wavelength and concludes that the sky is blue [1, 2].

The intensity of scattered or radiated light depends on the structure of the molecules and the wavelength of the incident light. The scattered light could comprise wavelengths longer, shorter, and the same as the wavelength of the incident light as shown in Figure 2.1.

![Figure 2.1. A schematic diagram illustrating light scattering](image)

The scattered light whose wavelengths and energy are equal to the wavelength and energy of the incident light is referred to as Rayleigh or elastic scattering. This implies that Rayleigh scattering occurs when the molecules of the target neither absorb nor lose energy when photons collide with them.

Raman or inelastic scattering of light are those whose wavelength and energy aren’t the same as the wavelength and energy of the incident light. Raman scattering can be categorized further into Stokes and anti-Stokes Raman scattering. Stokes Raman scattering occurs when the molecules of the target absorb energy when photons collide with them. Their energy absorption in turn reduces the energy of the scattered photon resulting in a reduced energy, longer wavelength Raman shift. Anti-Stokes Raman scattering occurs when the molecules of the target lose energy when photons collide with them. Their energy loss in turn increases the energy of the scattered photon resulting in a higher energy, shorter wavelength Raman shift [3, 4].
If h = Planck’s constant, \( v \) = frequency of light, and \( e \) = vibrational energy level 0, 1, 2, 3..., \( v_i \) = frequency of incident light, \( v_s \) = frequency of scattered light, \( \lambda_i \) = wavelength of incident light, \( \lambda_s \) = wavelength of scattered light, and \( \Delta E \) = change in energy between the incident light and the scattered light. Then, the following applies differently to the scattering phenomena:

**Rayleigh scattering:**
\[
\lambda_i = \lambda_s \\
\Delta E = 0 \\
hv_i = hv_s
\]

**Stokes Raman scattering:**
\[
\lambda_i < \lambda_s \\
\Delta E = h(v_i - v_s) \\
E_i > E_S
\]

**Anti-stokes Raman scattering:**
\[
\lambda_i > \lambda_s \\
\Delta E = h(v_s - v_i) \\
E_i < E_S
\]

At room temperature, most of the atoms are at the lowest energy level ‘\( E=0 \)’ which is referred to as the ground state. In Rayleigh scattering, the vibrational energy level remains at ground state \( E=0 \) because the photon is excited from the ground state and returns to the ground state. In Raman Stokes scattering, the photon gets excited from the ground level and returns to a higher vibrational energy level. In Raman anti-Stokes scattering, the photon gets excited from a higher vibrational energy level and returns to the ground energy level [5]. Figure 2.2 shows the energy diagram for Rayleigh and Raman scattering.

![Energy Diagram Illustrating Stokes and Anti-Stokes Raman Scattering](image)
2.2. Raman spectroscopy

Raman spectroscopy is based on Raman scattering. It was first observed by C.V. Raman in 1928 [6]. He performed his initial series of tests with a single wavelength from a mercury lamp source and over time various incident light sources were used. However, the discovery of monochromatic laser sources in the early 1960s greatly revolutionized Raman spectroscopy studies [5]. Raman Spectroscopy has evolved as a non-invasive analytical tool for the identification of the biochemical composition of samples [7-10].

Its unique advantages are non-destructive sampling, minimal sample preparation, sample volume flexibility, robust suitability for most samples, simultaneous detection, and quantification of multiple molecules that generate fingerprints for the identification of unknown samples [11-16]. It has been applied in various fields such as biology [17, 18], physics [19], agriculture [20], chemistry [21-23], geology [24, 25], medicine [26-28], food science [29], and pharmacy [30, 31].

Raman Spectroscopy is based on the inelastic or Raman scattering of incident light interacting with sample molecules. This has an extremely low scattering cross-section; Raman signals have about $10^{-8}$ the intensity of the incident light [5]. This implies the filtering of emitted radiation at the laser wavelength. Using a high-intensity laser to amplify Raman signals could burn or bleach the sample [32].

2.2.1. Raman Spectrum

The Raman spectrum of a material is a plot of the intensities of the Raman shift of the inelastically scattered photons. The Raman shift is the shift in the wavelengths of the scattered photons from the incident or excitation wavelength. It is usually expressed as wavenumbers with a unit of cm$^{-1}$ [33]. Figure 2.3 shows the Raman spectrum of 1mmol Rhodamine 6G solution obtained using Surface Enhanced Raman Scattering (SERS).

Figure 2.3. Raman spectrum of 1mmol Rhodamine 6G solution obtained using a silver SERS layer and a console Raman spectrometer equipped with a 30mW 632.81nm HeNe laser.
The Stokes Raman scattering is usually far more intense than anti-Stokes Raman scattering. Hence, the Stokes Raman spectrum is commonly used for Raman spectrum analysis [34, 35]. Since energy is lost in the Stokes Raman scattering, the shift in wavelength of the Stokes scattered photon can be derived using the equations below:

\[ E = h \nu = \frac{hc}{\lambda} \]
\[ \Delta E = h(v_i - v_e) \]
\[ \Delta E = hc \left( \frac{1}{\lambda_i} - \frac{1}{\lambda_s} \right) \]

where Planck’s constant, \( h = 6.626 \times 10^{-34} \text{ J/s} \) and velocity of light, \( c = 3 \times 10^{10} \text{ cm/s} \)

\[ \Delta E = 1.24 \ast \left( \frac{1}{\lambda_i} - \frac{1}{\lambda_s} \right) \]

In practical units, it’s 1.24 where energy is in eV and wavelength is in microns.

\( \Delta E \) is the Raman shift expressed in cm\(^{-1}\). Intensities of Raman bands are used to interpret Raman spectroscopic fingerprints of materials. Raman scattering depends on the wavelength of the excitation laser, but the resulting Raman shifts \( (\Delta E) \) are independent of the excitation wavelength [36, 37]. Hence, the Raman spectrum of a specimen remains constant irrespective of the excitation wavelength but the intensity of the Raman shifts or bands may vary [38]. The Raman spectrum of 1mmol Rhodamine 6G (R6G) solution was obtained using a 30mW 633nm laser with a SERS layer and console Raman spectroscope, while that of Figure 2.4 was obtained using a 4.5mW 650nm laser diode with a SERS layer and portable Raman spectroscopy optics. Rhodamine 6G Raman shifts (spectral peaks) were observed in both spectra at the characteristic wavelengths that have been widely reported [39-42]. As expected, the relative intensities of those shifts varied. This could arise from the different optics, laser sources, and varying power of the laser sources. However, the Raman shifts of a specimen remain the same. The Raman spectrum of a specimen is often referred to as the unique fingerprint of the material. Hence, one
of the unique advantages of Raman spectroscopy is its ability to identify unknown materials using the fingerprint of the material.

![Raman spectrum of 1mmol Rhodamine 6G solution](image)

**Figure 2.4.** Raman spectrum of 1mmol Rhodamine 6G solution obtained using a silver SERS layer and a portable Raman spectrometer equipped with a 4.5mW 650nm laser diode.

### 2.2.2. Basic Raman Spectroscopy Optical Setup

The basic instrumentation for Raman spectroscopy consists of an excitation laser source, light collection optics, filters, spectrometer, and the specimen [43, 44]. Figure 2.5 shows the arrangement of components in our Raman spectroscopy setup.

The excitation laser source is a monochromatic light source that illuminates the specimen resulting in scattered photons. The collection optics consists of optical components that channel the scattered photons to the spectrometer. This can include a fiber-optic probe, mirrors, lenses, etc. A Rayleigh rejection filter is essential to eliminate Rayleigh scattered photons which would mask the weaker Raman signals. Since the longer wavelength Stokes signals are more likely to occur than the anti-Stokes signals, a long-pass filter is suitable in rejecting the shorter wavelength photons (Rayleigh and Anti-Stokes photons) while allowing the longer wavelength photons to pass through to the detector.

The intensity of the excitation wavelength is usually much stronger when compared to the intensity of the Raman photons. The wide-range wavelength rejection of the long-pass filter has been found to be insufficient to completely attenuate the excitation wavelength since its bandpass extends very close to the desired Stokes wavelengths. Hence, the need for a more selective notch filter. Notch filters have very high attenuation at their specific wavelength. A 650nm notch filter effectively blocks 650nm photons. Notch filters are therefore an important component in Raman spectroscopy instrumentation [45].
The spectrometer detects the Stokes photons and converts them to a Raman spectrum. It contains an entrance slit, diffraction grating, and detector [46]. The beam of Stokes photons is focused to a small diameter by a lens and directed to the spectrometer slit. The narrow slit is necessary for good optical resolution. The diameter of the beam should be narrower than the slit opening. The beam afterward diverges on a diffraction grating which separates the beam into various wavelengths. The dispersed wavelengths are focused on a detector. The detector is typically an array of Charge-Coupled Devices (CCD) [43]. The CCD detects the dispersed wavelengths as electronic charges. The wavelength and intensity of the charges are converted into a spectrum. The wavelength with more photons builds up more charges and it’s seen in the spectrum as a Raman peak with higher intensity count.

![Schematic diagram showing the basic optical setup for Raman spectroscopy.](image)

**Figure 2.5.** Schematic diagram showing the basic optical setup for Raman spectroscopy.

### 2.3. Surface-Enhanced Raman Scattering (SERS)

Raman spectroscopy has a low scattering cross-section which limits its specificity and sensitivity [47]. Its weak signals could be easily suppressed by Rayleigh scattering and other background signals. Hence, the need for a signal amplifying technique such as Surface-Enhanced Raman Scattering (SERS).

SERS amplifies the intensity of Raman scattered photons using electromagnetic enhancement and chemical enhancement mechanisms [48, 49].

a. Electromagnetic enhancement occurs based on the principle that localized surface plasmons are excited when an electromagnetic wave interacts with a metal surface when there is an excitation of localized surface plasmon resonance of metallic substrates [50]. This can guide the electromagnetic field to a focus, or act as an antenna.

b. The chemical enhancement mechanism is attributed to the formation of a bond or charge transfer between specimen molecules absorbed on the metal surface and the metal
surface [49, 51]. It has been observed that molecules with lone pair electrons bond rapidly with the metallic surface thereby having the strongest enhancement [51, 52].

It has however been reported that the electromagnetic enhancement accounts for most of the SERS enhancement. The SERS enhancement factor (EF) is usually calculated based on the electromagnetic enhancement [49, 51].

As shown in Figure 2.6, the laser excitation beam interacts with the substrates to amplify the spectra of molecules adsorbed to the specimen surface. The underlining SERS principle is that the specimen must be in contact with metallic substrates and the amplification occurs at the contact point. If the SERS substrate layer is placed after the specimen as shown in Figure 2.7, the surface enhancement would only occur at the contact point between the specimen and the SERS substrates, not just somewhere in the specimen. This concentrates the electric field in the substrates nanostructure thereby producing “hot spots” which enhance the Raman Effect [48, 53, 54].

![Figure 2.6. Schematic diagram showing Raman scattering in SERS](image)

### 2.3.1. SERS substrates

For maximum enhancement, the choice of SERS substrate depends on both the substrate properties and the excitation source. Also, for electromagnetic enhancement purposes, SERS substrates can be roughened metallic substrates or can be shaped as tiny antennas.

The enhancement of various forms of SERS substrates are well known; some are Platinum electrodes [55], plasmonic metal colloids [56], and thin films [57]. The combination of metallic thin film and colloids has also been explored [58]. However, plasmonic metal colloids are more advantageous because they allow direct analysis with the sample's natural medium, they have a wide surface area for more interaction with samples, their colloid-solvent interaction minimizes sample damage under high laser excitation, and they can be converted to thin films for flexible on-site applications [57].
The commonly used plasmonic materials for SERS substrates are silver (Ag), Copper (Cu), and gold (Au). They are more suitable for SERS because they have their Localized Surface Plasmon Resonance (LSPR) within the visible and near-infrared laser excitation wavelength used in SERS. On one hand, silver has been proved to be more optically efficient than gold due to its higher enhancement [59] and excitation flexibility from ultraviolet (UV) to infrared (IR). Gold is mostly used in biological applications due to its structural flexibility and higher biocompatibility [60]. On the other hand, copper isn't frequently used because of its high reactivity [61].

2.3.2. SERS Optical Setup

As shown in Figure 2.7, the SERS optical setup is an extension of the basic Raman optical setup. The major difference is the addition of the SERS substrate.

![Figure 2.7. Schematic diagram showing the basic optical setup for SERS where the enhancement layer is on either part of the specimen.](image)

2.3.2.1. Bringing specimen to optics

The most common approach for SERS instrumentation involves bringing portable specimens to a stationary Raman spectrometer equipped with the SERS layer (Figure 2.8). Typically, a Raman spectrometer is equipped with a laser beam that is focused by a microscope objective on a small area on the substrate [48, 62, 63]. The microscope objective performs several important functions:

a) If the specimen is inhomogeneous, adjacent areas may be compared with high spatial resolution.

b) The large solid angle sustained by the microscope objective collects a substantial fraction of the isotropic Raman light.
c) Because the Raman light originates from a small area it is readily focused by the objective and transmitted back through the spectrometer slit.

Figure 2.8. Schematic diagram showing the most common SERS instrumentation where the specimen is brought to the SERS setup.

2.3.2.2. Bringing optics to specimen

For remote specimens, e.g., partially transparent tissue in animals or humans, the incident radiation must be brought to the specimen since it is awkward to bring large organisms to the optical bench. The most flexible way of doing this is through an optical fiber or probe (Figure 2.9). However, studies have shown that background signals generated within the long fiber mask the returning Raman signals [64-67].

Figure 2.9. Schematic diagram showing the SERS instrumentation where the instrumentation is brought to the specimen using a long optical fiber.
2.3.2.3. Classical multi-fiber SERS solution

To bring the SERS instrumentation to a remote sample and exclude contamination of the Raman light generated within a long fiber, usually light from the sample is returned to the spectrometer through a separate fiber or fibers [68] as shown in Figure 2.10. The SERS nano metallic structures are distributed as a ‘cloud’ within the specimen [69-71]. This can be referred to as the multi-fiber SERS solution.

![Figure 2.10. Schematic diagram showing the SERS instrumentation where the SERS setup is brought to the specimen using multiple fibers.](image)

Table 2.1. Raman intensity of different microscopes objectives and fiber probe with different numerical aperture. Extracted from Basu et. al.

<table>
<thead>
<tr>
<th>Objective</th>
<th>NA</th>
<th>Raman intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5X</td>
<td>0.075</td>
<td>200</td>
</tr>
<tr>
<td>4X</td>
<td>0.10</td>
<td>600</td>
</tr>
<tr>
<td>Probe</td>
<td>0.12</td>
<td>460</td>
</tr>
<tr>
<td>10X</td>
<td>0.25</td>
<td>2900</td>
</tr>
<tr>
<td>10X</td>
<td>0.30</td>
<td>3500</td>
</tr>
<tr>
<td>50X</td>
<td>0.55</td>
<td>14000</td>
</tr>
</tbody>
</table>
Chapter 3. Motivation and Research Objectives

Raman spectroscopy has been widely explored for various applications due to its non-destructive nature, material identification, and simultaneous detection of molecules capabilities. It is however limited by its weak signals which could be easily masked by Rayleigh scattering and background signals. The amplification of its signals has been made possible by various techniques such as Surface-Enhanced Raman Scattering (SERS). The unique advantages of SERS make uniquely suitable for the non-invasive characterisation of biological specimen.

As discussed in the previous chapter, the typical SERS setup cannot always be used to bring the optics to the sample because it’s impossible to bring some organisms to the optical bench and strong background signals would mask the Raman signals if a single long optical fiber is used. A probe is essential to bring the instrumentation to the specimen. The SERS multi-fiber system is the classical SERS solution that is employed in applications where the optics needs to be brought to the sample. However, the distributed cloud of nanoparticles in the classical multi-fiber SERS instrumentation has serious limitations. The extended geometry of the cloud and the lack a collimating lens seriously degrade the performance. In addition, each site requires a separate cloud making site to site comparisons difficult, and nanoparticles left in biological cells may lead to cancer metastasis [73].

Previous work provided a single pathway alternative to the multi-fiber system [74]. It incorporated a long-articulated arm and a SERS layer at the end of a short optical fiber as shown in figure 3.1. The GRIN lens was an effective substitute for the microscope objective.

Figure 3.1. SERS optical setup with a long-articulated arm and a short fiber [74]

This solution minimized background signals by using only a short fiber and was successfully used as an ex-vivo SERS diagnostic tool to differentiate between the healthy and cancerous colon (figure 3.2).
However, the SERS set-up was built for use with a large console Raman spectrometer. It is suitable for stationary sampling where the samples are brought to the fixed console instrumentation. To extend this application to remote sampling where the SERS instrumentation is brought to the sample, would ideally require a compact and affordable SERS device for remote and non-invasive in-vivo diagnosis. For example, it is impractical to bring a live horse to a tabletop spectrometer for skin-lesion SERS diagnosis. It is more feasible to bring a compact SERS bio-probe device to the horse. The small size and affordability of the SERS device also makes it more suitable for ships at sea and rural clinics. This can be critical concerns to laboratories faced with limited space or diminishing funding resources.

In summary, SERS bio-probes are needed for in-vivo applications. However, unlike SERS substrates, there are currently no commercial SERS probes.

The objectives of this research are therefore to:

- Fabricate an affordable, low-cost alternative for the SERS self-assembly fabrication method which does not involve any lithography or chemical synthesis.
- Design and fabricate the optics for a compact SERS device that can be used with a portable spectrometer for remote non-invasive characterization without the disadvantages associated with injected nanoparticles and multiple collection fiber systems.
- Fabricate a securely bonded SERS layer well-suited for bio-probes or remote sampling applications.
Chapter 4. Fabrication of Low-Cost Imprinted SERS Substrate

4.1. Introduction

4.1.1. SERS and Plasmonic Nanosubstrates

Surface-Enhanced Raman Scattering (SERS) amplifies Raman scattering by the excitation of localized surface plasmon resonances in metallic substrates. As previously shown in Figure 2.6, the laser excitation beam interacts with the substrates to amplify the spectra of molecules in contact with the target surface. The high substrate enhancement factor (EF) increases the signal intensity per molecule, leading to a wider scattering cross-section and higher sensitivity of the SERS process for single-molecule detection [58].

The SERS layer consists of nano-metallic particles that concentrate the electric field in the incident laser beam, producing “hot spots” where the Raman effect is enhanced. Plasmonic currents are induced in the electrons in these nano-metallic structures. These currents may

1) Be concentrated at a narrow portion of the nano structure, producing a high electric field there.
2) Oscillate around the nanostructure as a tuned circuit, tuned to the optical frequency of the laser beam and building up a high electric field.
3) Act as an antenna, collecting energy from an area whose width is comparable to the wavelength of the incident light, and generating a high electric field across a gap of just a few nanometers.

Arrays of nano-metallic particles may be fabricated lithographically, using an electron beam. This can produce large scale arrays of precisely controlled dimensions, but the method is expensive.

For maximum enhancement, the choice of SERS substrate depends on the substrate properties and the excitation source. Enhancement prospects of various forms of SERS substrates have been previously tested; some of which were Platinum electrodes [55], plasmonic metal colloids [56], and thin films [57]. The combination of metallic thin film and colloids has also been explored [58]. However, plasmonic metal colloids are more advantageous because they allow direct analysis with the sample's natural medium, they have a wide surface area for more interaction with samples, their colloid-solvent interaction minimizes sample damage under high laser excitation, and they can be converted to thin films for flexible on-site applications [57].

4.1.2. SERS Nanosubstrates Fabrication Techniques

The major limitation in the practical application of SERS for biosensing is the difficulty in the fabrication of practical SERS nanosubstrates. The basic requirements for practical SERS substrates are a high enhancement, uniform response, stable shelf-life, and ease of fabrication [75].
There are many SERS substrates fabrication techniques, most of which produce uniform large area substrates. Some of them are Electron Beam lithography (EBL), Nanosphere Lithography (NSL), Oblique Angle Deposition (OAD), and Template method.

A. Electron Beam Lithography

Electron Beam lithography (EBL) is an expensive fabrication technique that produces uniform SERS substrates with discretely controlled size and shape. An electron beam in the range of 50 to 150 KeV is focused on an electron resist on top of a metal layer that was previously deposited on a support such as silicon wafer. The exposed resist is developed, and the pattern is transferred to the metal by Reactive-Ion Etch (RIE). The resist is stripped off leaving a large area covered with discrete nanoparticles. Alternatively, the metal may be deposited after the resist is patterned. The resist is then stripped off leaving discretely patterned nanoparticles [76].

B. Nanosphere Lithography

Nanosphere Lithography (NSL) is an inexpensive fabrication technique used for making various shapes and sizes of SERS nanosubstrates in large quantities. The substrates are cut into desired sizes, polished, and assembled into two-dimensional colloids. A double-layer nanosphere mask is fabricated by spin-coating concentrated nanospheres (diluted in surfactant) to substrates [77]. Nanospheres crystallize by evaporation to form closely packed patterned structures. Physical Vapour Deposition is then used to deposit thin-film substrate on the nanosphere mask. The nanosphere mask is removed by lift-off leaving behind the colloid nanosubstrates [77]. The flexibility of NSL fabricated substrates (substrate thickness, mask dimensions, etc.) allows for the switching of LSPR frequencies in SERS applications [78].

C. Oblique Angle Deposition

OAD uses evaporation to deposit tilted Ag nanorod arrays on a large substrate area. The substrates are evaporated at an angle to the normal of the deposition surface. Random arrays of metallic nanorods are grown over time [75].

D. Template Method

The template method is an inexpensive substrate fabrication technique. It involves the electrochemical deposition of metallic substrates on a fabricated nanopore template. The template is formed by dissolving alumina in acid solution under high voltage to form hexagonally arranged nanopores of anodic alumina oxide (AAO). Desired pore sizes are achieved by varying voltage, acid solution, temperature, and process time. Metals are deposited in the AAO nanopores at a voltage of about 10-15V. The AAO template is then dissolved in phosphoric acid solution, leaving the metallic nanorods. It was discovered that the metallic nanorods have strong polarization due to their closely packed pattern; this, in turn, increases their SERS enhancement [76].
While the template method described by Fan et al [76] might be considered inexpensive, the complexity of the fabrication method could pose a challenge. Hence, the need for an inexpensive but straightforward fabrication technique. The imprint fabrication method presented in this chapter is inexpensive, easy, and can be achieved with commonly available materials.

Large scale mechanical process can fracture or deform bulk metal leaving surfaces which contain rough structures, some of which are useful for Raman surface enhancement. Small metallic nano particles can self-assemble into larger structures suitable for Raman surface enhancement. A variation of this technique is reported below.

4.2. SERS Imprint method
4.2.1. Materials and methods
4.2.1.1. Nanoparticles

Silver nanoparticles were obtained from ink sold for ink-jet printers [79]. The specification for the ink states that the nanoparticles are 10 nm spheres in a 10% by weight suspension of hexane [80]. If the hexane is allowed to evaporate in bulk, the silver nanoparticles congregate and fuse into a solid three-dimensional structure. In normal printer operation, the droplets overlap each other but evaporate so quickly that there is minimal mixing of the nanoparticles. The nanoparticles then fuse to a planar structure of nearly constant thickness. In this work additional hexane was added to dilute the suspension 1000 X, resulting in an average separation between the nanoparticles of 42 nm.

4.2.1.2. Imprinting

Substrates were prepared from 15 mm squares of 0.5 mm thick PETE-1 (Polyethylene terephthalate) thermoplastic [81]. An imprinting step was used to form microscopic pits on the surface of the PETE-1. Costly lithographic processing was avoided by using fine-grit sandpapers as the template. The sandpapers used were 6µm [82] and 1µm [83] grit sandpapers. The imprint fixture had two blocks joined by two 1/4-20 screws, and a rubber pad to distribute the pressure (Figure 4.1). The screws were tightened by a torque wrench to 50 inch-pounds. This produced a force of 12,566 pounds or pressure of 250MPa on the 15mm substrates. The assembly was placed in a 35°C oven [84] for 12 minutes. The low oven temperature was used to minimize the depth of the imprint.

4.2.1.3. Self-Assembly

Drops of diluted nanoparticle suspension were cast from a pipette onto the PETE-1 substrates (Figure 4.1, step 4). Surface tension drove the hexane suspension into circles. On smooth PETE-1 surfaces, the nanoparticles followed the hexane as it evaporated, with majority congregated in a narrow ring along the circumference (Figure 4.2a).
However, more uniform nanoparticle distributions were obtained with the imprinted substrates. As the hexane suspension spread towards the circumference of a circle, most of it became trapped in pits in the surface along the way. The volume of these pits depended on the oven temperature and the roughness, or grit, of the sandpaper. Figures 4.2b and 4.2c show that finer sandpaper isolated smaller volumes of the suspension and therefore smaller numbers of nanoparticles.

Figure 4.2. Optical micrographs of deposited nanoparticle suspension. a. Ag nanoparticles on non-imprinted PETE congregate along the rim of a circle. b. Isolated islands of Ag nanoparticles form on 6µm grit imprinted PETE. c. Much smaller islands of Ag nanoparticles form on 1µm grit imprinted PETE.
Figure 4.3a is an SEM image of the 1 µm grit sandpaper. Figure 4.3b is an SEM of the imprinted PETE-1 containing drop-cast silver nano-spheres. As the hexane evaporated, the nano-spheres were formed by nearby nanoparticles adhering to each other and self-assembling into spheres. The size of the spheres depended on the number of nanoparticles available. Casting multiple drops provided fresh nanoparticles and increased the size of the spheres. Three consecutive drops of the diluted suspension of Ag in hexane on PETE imprinted with 1 µm grit sandpaper grew spheres of about 80nm diameter and produced optimum SERS result.

In contrast, no structure can be seen in an SEM of non-imprinted PETE-1 (Figure 4.3c), and few if any nanoparticles can be found after the nanoparticle suspension is cast on non-imprinted PETE-1 (Figure 4.3d). The comparison between Figures 4.3b and 4.3d shows the critical role of the pits in the imprinted PETE-1 to trap small volumes of the suspension.

Figure 4.3. Scanning electron micrographs of a. 1µm grit sandpaper template. b. Silver nanoparticles assembled in the pits of PETE-1 thermoplastic imprinted with 1µm sandpaper. c. non-imprinted and featureless PETE-1. d. Non-imprinted PETE-1 with few if any nanoparticles after drop-casting of the nanoparticle suspension.
4.2.1.4. SERS Measurement

SERS measurements were obtained with a table-top HORIBA confocal Labram Raman spectrometer equipped with a 50X, 0.55NA microscope objective and a 30mW, 633nm HeNe excitation laser. Rhodamine 6G solution was prepared from powdered rhodamine dye using the method described by Basu et. al [39].

4.2.2. Results

A solution of R6G was placed on a SERS substrate made by nano-sphere deposition on an imprinted PETE-1 substrate. Rhodamine 6G Raman spectral peaks were observed at the characteristic wavelengths that have been widely reported [39-42]. With no SERS layer present, the Raman spectrometer detected no Raman spectra from a 1mmol solution of R6G.

The Raman signal obtained from a 1 mmol solution of R6G is shown in Figure 4.4 (top trace). The background spectrum from the PETE-1 SERS substrate was also obtained by taking a run with no R6G present (Figure 4.4, bottom trace). The small background is readily separated from the R6G spectrum. Spectra were also taken in which the PETE-1 substrate was not imprinted (Figure 4.5). In this case, only a background signal, largely from the PETE-1, was obtained. This demonstrates the important role imprinting plays in generating the R6G Raman signal.

![Figure 4.4](image-url)  
Figure 4.4. Top trace, Raman spectrum of 1mmol R6G after drop-casting silver nano-sphere suspension on imprinted PETE-1. Bottom trace, Background spectra obtained after drop-casting silver nano-sphere suspension on an imprinted PETE-1 SERS with no R6G present.
Spectra of R6G solutions on PETE-1 substrates with concentrations ranging from 1nmol to 1mmol are shown in Figure 4.6. Although the background is more significant at the lower concentrations, individual R6G peaks can be identified down to the lowest concentration of 1nmol. The relative strength of the enhanced spectra as a function of the concentration of the solution is shown in Figure 4.7. This behavior is characteristic of SERS enhancement.

Figure 4.5. Top trace, 1mmol R6G spectra after drop-casting nano-sphere suspension on imprinted PETE-1. Bottom trace, 1mmol R6G spectra after drop-casting nano-sphere suspension on PETE-1 that was not imprinted.
Figure 4.6. Raman spectrum of 1nmol to 1mmol concentrations of R6G obtained after drop-casting silver nano-sphere suspension on imprinted PETE-1. For clarity the spectra have been offset vertically.

![Raman spectrum](image)

**Figure 4.7.** Raman signal strength vs. Rhodamine 6G concentration on PETE-1 SERS

### 4.2.3. Cost estimate

The components used to fabricate the PETE SERS substrate are moderately priced in quantities so large that the cost of a single substrate is virtually negligible.
PETE

PETE was taken from a $6 pastry box containing about 60 square inches of usable surface. Assuming most of the cost was for the pastry the cost of a 1 square cm PETE is less than 1 cent.

Silver (Ag) Ink

The ink containing silver nanoparticles cost $154 for 25ml [79]. After diluting 1000X in hexane about 0.15ml (3 drops) were used on each substrate, at a cost less than 1 cent.

Hexane

4 liters of hexane were purchased for $12. The 0.15ml (3 drops)[85] used on each substrate also cost less than 1 cent.

Sandpaper

The cost of the sandpaper is hard to estimate as a single 1.5cm² piece was used repeatedly without apparent degradation.

It’s apparent that the cost of materials required to fabricate this SERS substrate is negligible. In addition, it presents a robust SERS option applicable to a variety of applications.

4.3. Conclusion

This chapter described a straightforward method of producing a SERS layer on an imprinted thermoplastic. Surfaces of thermoplastic were imprinted using sandpapers of known grit size. SERS layers were made by self-assembly of silver nanoparticles on the imprinted thermoplastic. Using this SERS substrate, Raman spectra from R6G solution were detected at concentrations as low as 1 nmol. This SERS substrate has potential usage in a wide range of portable Raman sensing applications. For potential bio-probe or remote applications, a transferable SERS substrate is described in the next chapter. The fabrication of these SERS substrates does not require expensive equipment or processes. Consequently, the developed SERS substrates are useful for applications needing low-cost SERS substrates.
Chapter 5. Design and Fabrication of Portable Probe SERS Device

5.1. Portable Spectroscopy

Portable spectroscopy is important in bringing the spectroscopic device to the specimen [86]. It has become necessary in different areas where immediate characterization of materials is required to make real-time decisions and emergency responses [87]. Portable spectrometers are useful for the characterization of explosives, narcotics, impurities, lesions, etc. [88-90].

Although, the performance of portable spectrometers might not be at par with their console laboratory alternatives in terms of their versatility, signal-to-noise ratio, and resolution their specific adaptability, ease of use, and portability are cherished for specific and timely applications [86].

Portable spectroscopy has been adapted across various fields of spectroscopy. Jakub et al. described the application of portable near-infrared spectroscopy in the forest sector [91]. Mach et. al. and Malcolm et. al. used portable mass spectroscopy in the detection of environmental pollutants and liquid chromatography applications respectively [92, 93]. Rakovsky et. al. reviewed the various applications of portable laser-induced breakdown spectroscopy [94]. Yang et. al. presented preliminary results using portable bioimpedance spectroscopy in pathology [95]. Tadeu Costa Junior et. al. used portable x-ray fluorescence in determining the mineral contents of food [96]. Portable Raman spectroscopy was used by Fujihara et. al. to differentiate between human and non-human blood [97]. Tondepu et. al. demonstrated the screening of unapproved drugs using portable Raman spectroscopy [98].

5.2. Portable Raman Spectroscopy

Portable Raman spectrometers have made possible the diversification of Raman spectroscopy applications. The dual advantages of portability and affordability have strengthened their markets. Laser collimating dimensions, dispersion, CCD pixel, spectrometer focal lengths, and aperture determine the resolution of a Raman spectrometer. There is therefore a resolution compromise in miniaturizing the spectrometer. However, some portable Raman spectrometers have achieved good resolution in the range of 8-12 cm$^{-1}$ [99]. This improvement has also been possible in some Raman spectrometers using transmission grating designs [100, 101].

The cost, weight, size, excitation source, resolution, data system, specimen coupling, etc., are factors usually considered in the design and choice of portable Raman spectrometers [102]. The small size, long lifetime, low fluorescence interference, low cost, and low heat generation of laser diodes make them the preferred excitation laser sources for portable Raman spectrometers [103]. The development of CCD and CMOS detectors, laser diodes, Rayleigh rejection filters, battery technology, and mobile computing accelerated the miniaturization of Raman spectrometers [86, 104].
5.3. Portable SERS devices

The difference between Raman spectroscopy and Surface Enhanced Raman Spectroscopy (SERS) instrumentation is the presence of metallic nanoparticles in contact with the specimen in the SERS setup. This greatly amplifies the Raman signal making SERS a worthwhile characterization method for good specificity and sensitivity in Raman spectroscopy.

As discussed in chapter 2, the most common approach for SERS instrumentation involves bringing portable specimens to a stationary Raman spectrometer equipped with the SERS layer. Typically, a Raman spectrometer is equipped with a laser beam that is focused by a microscope objective on a small area on the substrate [48, 62, 63]. Since it’s impossible to bring large organisms or remote specimens to the optical bench, the instrumentation must be brought to the specimen.

The most flexible way of bringing the instrumentation to the specimen is incorporating surface enhancement with an optical fiber or probe. However, it’s been shown that background signals generated within the long fiber mask the returning Raman signals [64-67]. To exclude the contamination of the Raman light generated within a long fiber, usually, multi-fiber SERS instrumentation is used. This involves distributing the SERS metallic structures within the specimen [69-71] and returning the Raman light from the sample to the spectrometer through a separate fiber or fibers [68]. However, the extended geometry of the cloud and the lack of the advantages provided by a collimating lens seriously degrade the sensitivity. In addition, each site requires a separate cloud making it hard to compare to other sites, and nanoparticles left in biological cells may lead to cancer metastasis [73].

As discussed in chapter 3, for portable sampling, there is a need for SERS instrumentation without the disadvantages of the long optical fiber or multi-fiber instrumentation. Previous work incorporated a long articulated arm and an epoxied SERS layer at the end of a short optical fiber while using a GRIN lens as an effective substitute for the collimating lens [74]. This eliminated the background signals and the disadvantages of the multi-fiber instrumentation.

However, that solution was built for use with a large console Raman spectrometer where the samples were brought adjacent to the fixed console instrumentation. This application needs to be adapted for remote characterization where the SERS instrumentation is brought to the sample. This would ideally use a compact and affordable SERS device for remote sampling and non-invasive in-vivo diagnosis. The small size and affordability of the SERS device would also make it more suitable for ships at sea and rural clinics.

5.4. Portable SERS Device Designs

This work presents two portable SERS device designs. The first is a compact design while the second is a handheld design. Many of the factors influencing the choices of the key optical components are the same. However, some components such as lenses varied based on the dimensions suitable for each design.
5.4.1. Portable Compact design

A portable compact SERS device is presented in Figure 5.1. It shrinks the bench-top collection optics to compact and low-cost alternatives while retaining the unique SERS functionality. This compact design fit into a 21" X 16" X 9" enclosure used to eliminate background light. This design includes the excitation laser source, 45° mirror, 45° prism, long-pass filter, two notch filters, specimen focusing lens (lens 1), low-cost PETE SERS layer, specimen stage, pre-spectrometer lens (lens 2), and portable Raman spectrometer.

Figure 5.1. Schematic diagram of the designed portable compact SERS device. All dimensions are in inches.

Figure 5.2. Side view photograph of the fabricated portable compact SERS device.
5.4.1.1. Optical configuration

a. Excitation light source: The choice of the excitation laser source was based on its monochromatic wavelength, stability in its laser frequency, and casing dimensions. A monochromatic light source is important because the Raman spectrum was to be calculated based on the shift in that wavelength [105].

A Thorlabs 4.5mW 650nm laser module was used [106]. It generates an elliptical-shaped collimated beam. Its beam profile measured at 2 inches from the front of the laser is 5mm X 2.4mm. The laser module is packaged in an 11mm diameter x 54mm long casing which makes it very portable.

The laser module was powered by a Thorlabs 5VDC battery pack [107]. This module can also be powered from the 110 volts line. The battery back was used due to its portability and suitability for mobility in a handheld optical configuration.

b. Collection optics

The collection optics included 45° mirror, 45° prism, long-pass filter, notch filters, one specimen focusing lens (lens 1), and one pre-spectrometer lens (lens 2).

The 45° mirror was a 25mm X 25mm Edmund Optics aluminum-coated optically flat mirror [108]. It was aligned to 45° using an Edmund Optics mirror mount [109] adjustable in angle by two 64-pitch screws.

The long-pass filter was selected based on its 45° angle of incidence, and ability to reflect photons with shorter than or equal to the 650nm excitation wavelength while transmitting wavelength longer than the excitation wavelength. The long-pass filter used in this design is the Edmund optics 660nm, 12.5 X 17.6mm high-performance fluorescence dichroic filter [110]. It reflects 98% of the wavelength between 580 and 651nm and transmits 90% of the wavelengths between 670 and 800nm.

The specimen focusing lens is a 50X, 0.55NA Nikon microscope objective with a working distance of about 0.5inch. This working distance was sufficient to have both the SERS layer and sample on the sample stage.

Due to the high intensity of the Rayleigh scattering, and the fact that 650nm is at the boundary of the reflection range of the long-pass filter, many Rayleigh photons still filter through the long-pass filter. It is therefore necessary to incorporate additional filters with a narrow ranges and sharp cut-offs at that wavelength. The two notch filters used for this design are Iridian Spectral Technologies 650nm narrow notch filters [111]. They
have a $0^\circ$ angle of incidence excitation and 90% transmittance except at the 650nm wavelength. Combining two filters completely blocks the 650nm Rayleigh photons.

The choice of the pre-spectrometer lens 2 was determined by the numerical aperture and width of the spectrometer slit. This lens had a working distance of about 2 inches. The spectrometer slit was placed after a fiber-optic connector which was about 0.5cm thick. The portable spectrometer's fiber-optic connector and cable connectors are situated at the entrance plane of the spectrometer. The entrance plane is parallel to the slit; the body of the spectrometer is at 45 degrees. The working distance of the lens allowed an appreciable focusing distance between the lens and the spectrometer slit, gave room for the $45^\circ$ angle of the portable spectrometer with respect to the entrance plane, and some space for the computer and power cable connections.

c. Low-cost SERS layer
The low-cost SERS layer incorporated into this compact design is the PETE SERS layer described in chapter 4. SERS layers were made by self-assembly of silver nanoparticles on a sandpaper imprinted thermoplastic.

d. Sample stage
This sample stage was a flat 1.5-inch square aluminum block with a flat horizontal surface for a liquid sample holder. This was supported by a Newport xyz stage [112] with three precision screws for adjustments in the x, y, and z directions.

e. Raman spectrometer
The spectrometer incorporated into this design was a portable Raman spectrometer. It’s a custom-made QE Pro Raman Series spectrometer by Ocean Optics [113]. The custom specifications are 0.22 NA, 25 $\mu$m slit, H4_600 lines blazed at 750nm grating, DET-QE Nonelens detector, and 529-908nm bandwidth.

5.4.1.2. Working principle
The 650nm monochromatic laser beam is reflected by the flat $45^\circ$ mirror (Figure 5.1). The mirror reflects it towards the long pass filter which is also at $45^\circ$. The two fine adjustment screws on the mirror mount direct the beam to the long pass filter. The long-pass filter reflects the incoming beam to the prism. The beam is reflected by the prism to the microscope objective used here as the specimen focusing lens (lens 1 in Figure 5.1). It focuses the incoming laser beam on the specimen, collects the Raman light from the specimen, and redirects it back as a parallel beam through the prism to the long-pass filter. The long-pass filter retro-reflects any Rayleigh and anti-Stokes photons back towards the laser and transmits the Stokes Raman photons to the notch filters. The notch filters block any Rayleigh photons that are transmitted through the long-pass filter. Two notch filters were incorporated to completely eliminate the Rayleigh photons.
The Stokes Raman photons pass through the notch filters to the pre-spectrometer lens (lens 2). This lens focuses the Raman light through the spectrometer slit. The spectrometer generates the Raman spectrum and displays it on the laptop computer screen.

5.4.1.3. SERS characterization results

As described in chapter 4, the SERS capability of this compact SERS device was tested using 1mmol Rhodamine 6G (R6G) solution. The solution was prepared from powdered R6G dye using the method described by Basu et. al [39].

R6G Raman spectra were obtained both with the laser beam going through the PETE SERS layer to the R6G solution (Figure 5.3a) and through the R6G solution to the PETE SERS layer (Figure 5.3b). R6G peaks were identified at known R6G Raman bands.

![Figure 5.3](image)

Figure 5.3. Detail in figure 5.2. (a) beam incident through the PETE SERS layer to the specimen (b) beam incident through the specimen to the PETE SERS layer.

The almost identical spectra of R6G (Figure 5.4) obtained with beam incident through the PETE SERS layer to the R6G and with beam incident through the R6G to the PETE SERS layer demonstrates the versatility and transparency of the layer. The versatility shows that the device is suitable for both portable and remote SERS characterization as described in chapter 4. The transparency of the layer further demonstrates its suitability for remote or probe applications since the SERS layer would be placed before the specimen in probe applications.
5.4.2. Portable Probe design

While the compact design had the shrinking capability of the bench-top optics as its major influencing factor, the major factor considered here was the addition of a SERS GRIN lens probe that could be stuck into a biological sample. The laser, portable probe, collection optics, and the specimen holder were designed to fit into smaller dimensions that could be handheld.

Figure 5.5 shows the schematic diagram of the SERS device portable probe design. This design features a unique probe mounting stage housing some part of the collection optics, specimen holder, and specimen. Its dimension is about 15cm X 5cm mounted at a height of about 13cm for the initial experimental analysis.

This design includes the excitation laser source, 45° mirror, 45° prism, long-pass filter, notch filter, two-post laser lenses (lens 1 and lens 2), one pre-spectrometer lens (lens 3), portable probe, specimen holder, and a portable Raman spectrometer.
Figure 5.5. Schematic diagram of the SERS device probe design. Dimensions are in cm.

Figure 5.6. Top-view photograph of the fabricated portable probe design.

Figure 5.6. shows the photograph of the SERS device probe design. The top left picture in Figure 5.7 shows a close-up view of the portable laser diode and its battery pack. The top right picture shows the probe mounting stage holding the portable probe, long pass filter, notch filter and specimen holder. The diverging laser beam after the SERS layer can be seen in the sample.
The bottom left picture shows a fabricated 90° external alignment fixture positioned on the stage. This alignment fixture is a combination of a flat mirror and a flat sheet of glass at right angles to each other. The bottom right picture in Figure 5.7 shows the portable Raman spectrometer with a protruding fiber-optic connector at 45° to the body of the spectrometer.

Figure 5.7. Top left, close-up view of the laser diode module and battery pack. Top right, close-up view of the probe mounting stage. Bottom left, close-up view of alignment fixture and long pass filter. Bottom right, close-up view of the portable Raman spectrometer.
5.4.2.1. Optical configuration

a. Excitation light source
   The 650nm excitation light source was previously used in the portable compact design. As before, it was powered by a portable Thorlabs 5VDC battery pack.

b. Collection optics
   The collection optics included two post-laser lenses, a 45° mirror, a long-pass filter, a notch filter, and one pre-spectrometer lens. The optical configuration of these components is similar to the compact design.

   The post-laser lenses lens 1 and lens 2 have focal lengths of 200cm and 100cm respectively. The first lens was placed close to the laser and separated about 55cm from lens 2 to form a combined focus at about 50cm from lens 2 at the long-pass filter. Lenses 1 and 2 converged the laser’s 5mm X 2.4mm elliptical beam to a spot small enough to be channeled through the 2mm hollow tube.

   The 45° mirror was the same 25mm X 25mm Edmund Optics aluminum-coated optical flat mirror as before. It was aligned to an angle within the range of 45° using a 1.5-inch square Edmund optics mirror mount [109]. The two 64-pitch fine adjustment screws on the mirror mount center the beam on the long pass filter and into the hollow tube, focusing on the SERS layer at the back focal plane of the GRIN lens.

   The long pass filter and the notch filter are the same as used in the compact design. The pre-spectrometer lens (L3) has a focal length and diameter of about 3.5cm and 1.5cm respectively.

c. Portable probe
The portable probe in this design consists of a hollow tube and a SERS GRIN lens.

i. GRIN lens and SERS layer
   The SERS layer incorporated into this compact design is the low-cost SERS layer transferred to the back focal plane of a GRIN lens as described in chapter 6.

ii. Hollow tube
   The hollow tube was 10 cm long with an inside diameter of 2 mm. This length corresponds to the length of the probe and can of course be made shorter. The diameter of the tube matched the 1.8mm SERS GRIN lens held parallel to the tube by a thin layer of epoxy. This minimizes reflections from the tube walls by light traveling in either direction.

d. Specimen holder

   The bathtub-shaped liquid specimen holder was fabricated from transparent plastic with about 0.5cm thickness. Two rectangular pieces of plastic with dimensions of 2cm X 1.5cm made
up the holder. An oval opening of about 1.5cm x 1cm was drilled through the thickness of the first piece. Another round opening of about 3mm was drilled through the side of the rectangular block into the oval opening. The first piece was then glued to the second piece in such a way that the second rectangular block serves as a leak-proof base for the first piece.

The oval opening in the first piece holds the sample while the round opening at the side holds the probe in place. The probe was glued into the side opening to avoid leakage of a liquid specimen.

e. Raman Spectrometer

The Raman spectrometer incorporated into this design is the same portable Raman spectrometer used in the compact design.

5.4.2.2. Working principle

The 650nm laser beam goes through the post-laser lenses 1 and 2 to the flat mirror. The post-laser lenses are placed at distances such that their combined focus is at the long-pass filter.

The flat mirror positioned at 45° to the beam reflects the incoming beam towards the long-pass filter. The alignment fixture was used to align the long pass filter such that the incoming laser beam retro-reflect back through its original path. This also ensures that the returning Raman light goes through the long pass filter without missing the notch filter.

The two 64-pitch fine adjustment screws on the mirror mount are used to tilt the beam through the long pass filter, into the hollow tube, and down to the SERS layer at the end of the portable probe where it comes in contact with the specimen in the specimen holder.

The incoming beam is focused on the end of the portable probe where the back focal plane of the GRIN lens is in contact with the SERS layer and the SERS layer is in contact with the specimen. The scattered photons from that interface are collected by the GRIN lens and directed back through the hollow tube to the long pass filter. The long-pass filter reflects the Rayleigh and anti-Stokes photons and transmits the Stokes photons to the notch filter. The notch filter filters out any Rayleigh photons that pass through the long-pass filter.

The Stokes Raman photons pass through the notch filter to the pre-spectrometer lens (lens 3). This lens focuses the Stokes photons to a width smaller than the width of the spectrometer slit and it is placed at a focal length from the spectrometer slit. Going through the slit, the photons afterward diverge on the spectrometer diffraction grating which separates them into various wavelengths. The dispersed wavelengths are focused on the spectrometer detector. The spectrometer detector converts the detected photons to a spectrum. The spectrum is then displayed on the laptop screen that controls the spectrometer.
5.4.2.3. Ideal handheld configuration

To make the optics in Figure 5.5 entirely handheld, the excitation laser source should fit on the probe mounting stage. However, due to the elliptical beam profile (5mm X 2.4mm) of the laser source in the current portable probe design, post-laser focusing lenses (Lens 1 and Lens 2) were needed. The ideal handheld configuration in Figure 5.8 is possible with a rounded small diameter laser diode and a shorter tube or optical fiber. The laser beam could be placed close to and focused directly on the long pass filter. This eliminates the need for the post-laser focusing lenses.

Figure 5.8. Schematic design of the ideal handheld design without the excitation laser elliptical beam constraints. All dimensions are in cm.

5.5. Conclusion

This work presents two newly fabricated low-cost portable SERS device designs. The portable compact design was characterized with Rhodamine 6G spectra. The result showed good R6G Raman spectra when the low-cost SERS layer was both before and after the sample. This demonstrates the transparency and suitability of the low-cost SERS layer for probe application.

This work also presents a portable probe design incorporating a portable probe with the SERS layer at the back focal plane of a GRIN lens. This could be inserted into biological specimen. This design prompted the transfer of the low-cost SERS layer to a GRIN lens as presented in Chapter 6.
Chapter 6. Robust Transfer of SERS Surfaces for Probe Applications

6.1. Introduction

Raman spectroscopy has been extensively explored for various applications including in-vivo molecular diagnosis. Eberhardt et al. suggested that Raman spectroscopy has a major advantage for the in-vivo diagnosis of plagues due to its ability to provide information about the biochemical composition of the plague [47]. They further stated that diagnosing the biochemistry of the plague is important for more personalized medication.

Raman spectroscopy is usually used for in-vivo diagnosis using probes. A probe is essential in bringing the instrumentation to the specimen. Zhao et al demonstrated the in-vivo detection of skin cancer using a fiber-based Raman probe in conjunction with an automated internal algorithm analysis of the spectrum [114]. Taketani et al used a Raman endoscope for the in-situ diagnosis of the response of colorectal tumors to anti-cancer drugs [64].

The signal strength in Raman spectroscopy is weak, but in Surface-Enhanced Raman Scattering (SERS) it is strengthened up to millions of times by close contact between the molecules and nanoscale metallic structures [58-60, 115, 116]. SERS also improves the specificity of Raman spectroscopy[47]. This implies that SERS could be used for the early diagnosis of diseases and physiological disorders.

6.1.1. SERS Instrumentation

As discussed in chapter 2, the instrumentation for SERS may be configured in two ways:

1) Portable samples may be brought to a stationary instrument equipped with the SERS layer (Figure 6.1a). The layer may consist of a substrate on which pairs of conductors are separated by gaps as small as 2 to 4 nanometers. The conductors act as antennae for the incident light. They concentrate the electric field in the gaps and produce “hot spots” which enhance the Raman Effect [48, 53, 54]. Typically, the incident light is a laser beam that is focused by a microscope objective on a small area on the plate. This is the most common approach for SERS [48, 62, 63].

2) For remote samples, e.g., partially transparent tissue in animals or humans, the incident radiation must be brought to the sample (Figures 6.1b and 6.1c). The most flexible way of doing this is through an optical fiber or probe. However, studies have shown that background signals generated within the fiber mask the returning Raman signals [64]. To exclude contamination of the Raman light generated within the fiber, light from the sample is returned to the spectrometer through a separate fiber or fibers [68] as shown in Figure 6.1b. The nano metallic structures are distributed as a ‘cloud’ within the sample [69-71]. These structures may be spherical since spheres of the right diameter also concentrate the electric field of the incident radiation. However, the extended geometry of the cloud and the lack of the advantages provided by a lens seriously degrade the performance. In addition, each site studied requires a separate cloud, and nanoparticles left in the tissue may lead to cancer metastasis [73].
Figure 6.1. a. Portable sample configuration. The specimen is brought to the laser/spectrometer combination. b. Remote sample configuration. The SERS nanoparticles are injected as a cloud into the specimen. c. biological probe. The SERS layer on the GRIN lens is brought to the surface or the interior of the specimen.

The SERS layer in Figures 6.1a and 6.1c may be achieved by lithographically patterning a flat metallic layer, or by chemically treating a suitable substrate so that metallic nanoparticles self-assemble on its surface [117, 118]. The exact dimensions are critical for optimal SERS performance [119-122]. The frictional forces inserting a SERS probe in biological applications require strong adhesion of the metallic nanoparticles so as not to dislodge the nanoparticles from the probe. This work describes an inexpensive method for the self-assembly and collection of nano-spheres as an alternative method to produce a SERS layer on a flat substrate. It also shows how their growth is controlled to the optimum diameter for Raman enhancement. Most importantly, it shows how they may be transferred and securely bonded to secondary surfaces,
e.g., the rear surfaces of GRIN lenses. This is accomplished at low cost with inexpensive equipment, readily available materials, and with no chemical or lithographic steps. These may be critical concerns to laboratories faced with diminishing funding resources.

The choice of the SERS substrate, type, material, and fabrication method depends on the specific application[118]. Although, this work shows the suitability of the fabricated SERS substrates for both portable and bio-probe applications; the substrates are targeted towards remote sampling or SERS bio-probe applications. In comparison to existing SERS bio-probe applications, this work presents:

(a) SERS substrates suitable for bio-probes or remote sampling without the disadvantages associated with injected clouds substrates and multiple collection fiber systems.

(b) A low-cost alternative for the SERS self-assembly fabrication method which does not involve any lithography or chemical synthesis.

6.2. Transfer to a different substrate

The PETE SERS layer described in chapter 4 might need to be transferred to a new substrate, for example, as part of the SERS portable probe device described in chapter 5. This is necessary in a biological probe for the following reasons:

a) The nanoparticles must be securely attached so they do not fall off and remain in the tissue.

b) Transmission to the spectrometer is poor without a lens to collect and collimate the Raman light.

6.2.1. Materials and methods

The transfer process used a widely available jell [123] as a sacrificial layer. The jell protruded slightly from a syringe which was gently tapped against the thermoplastic (Figure 6.2). Next, a thin layer of quick setting epoxy [124], was spread on a coverslip, pressed against a flat sheet of “non-stick” silicone rubber, and allowed to partially harden. This procedure was necessary to prevent the epoxy from flowing around and encapsulating the nanoparticles. Then the syringe was pressed against the surface of the epoxy and the jell was forced out against it. The fixture was left for 24 hours to fully cure. The syringe[125] was removed, taking along the Jell and leaving the nanospheres securely epoxied to the coverslip[126]. Multiple taps transferred more nanoparticles, which then self-assembled into larger spheres. Two taps collected spheres of about 80nm diameter (Figure 6.3a) which were optimum for SERS applications as shown in the results section. Ten taps produced spheres as large as 175nm diameter (Figure 6.3b) that weren’t suitable for SERS applications.
Step 1. Load Jell into syringe. Position PETE-1.

Step 2. Press Jell on imprinted PETE-1.


Figure 6.2. Schematic flowchart of the transfer process. Portions enlarged for clarity.

Figure 6.3. Scanning electron micrographs of silver nanospheres transferred to epoxy on a glass coverslip. a. Two taps. b. Ten taps.
6.2.2. Measurements and characterization

SERS measurements were obtained with a HORIBA confocal Labram Raman spectrometer equipped with a 50X, 0.55NA microscope objective, and a 30mW, 633nm HeNe excitation laser. Rhodamine 6G solution was prepared from powdered rhodamine dye using the method described by Basu et. al [39].

6.2.3. Results

As with the PETE substrate, solutions of Rhodamine 6G (R6G) were used to test the enhancement of the transferred SERS layer. Raman spectra of R6G were obtained with the SERS layer after bonding to epoxy on a coverslip as needed for SERS bio-probe. A small background from the epoxy was observed with no R6G present but it was readily separated from the R6G spectrum (Figure 6.4). R6G Raman spectrum peaks were observed at the characteristic wavelengths that had been widely reported [39-42]. Spectra were obtained using R6G concentrations from 1nmol to 1mmol (Figure 6.5).

As with the PETE substrates described in Chapter 4, individual R6G peaks can be identified down to the lowest concentration of 1nmol. The relative strength of the enhanced spectra as a function of the concentration of the solution is shown in Figure 6.6, again as expected for SERS enhancement.

![Figure 6.4. Top trace, Raman spectrum of 1mmol R6G obtained after transferring nanospheres to epoxy on glass. Bottom trace, Background spectra from the transferred nanospheres to epoxy on glass with no R6G present.](image-url)
Figure 6.5. Raman spectrum of 1nmol to 1mmol concentrations of R6G obtained after transferring silver nano-sphere suspension to epoxy on glass. For clarity, the spectra have been offset vertically.

Figure 6.6. Raman signal strength vs. Rhodamine 6G concentration on epoxy SERS
6.3. Transfer to GRIN lens

A particularly interesting location for a SERS layer is the back focal plane of a GRIN lens (Figure 6.1c). Parallel light, typically from a laser, enters the lens and is brought to a focus on the SERS layer. A large fraction of the generated Raman light is collimated by the lens and returned as a nearly parallel beam. It is separated from the laser beam, for example by a wavelength filter, and focused on the entrance slit of a spectrometer. If the GRIN lens is part of a probe far from the spectrometer portions of the light path may be in optical fibers, but care must be taken to avoid contamination of the signal by Raman background generated in the fibers.

Previous work retained the advantages of the microscope objective by placing it with a GRIN lens at the end of a remote probe [74]. The SERS layer was in the focal plane at the rear of the Graded Index (GRIN) lens as shown in Figure 6.1c. This is an ideal configuration for a SERS biological probe since the GRIN lens is narrow enough to be inserted into the specimen. In the arrangement in Figure 6.1c, the incident laser light is reflected into the GRIN lens by a “long pass” interference filter. The longer wavelength Raman light is then transmitted through the filter to the spectrometer.

6.3.1. Materials and Methods

Placing the SERS layer precisely at the back focal plane of a GRIN lens requires additional steps. The nanoparticles are first transferred to a thin mylar film, mechanically backed by a flat surface such as a microscope cover glass. The GRIN lens used here is an Edmund optics uncoated GRIN lens with a 1.8mm diameter, 4.31mm length and ±5% length tolerance[127]. The GRIN lens is placed in a parallel laser beam (Figure 6.7). Freshly mixed epoxy is placed between the GRIN lens and the mylar film. The mylar film[128] is brought to the GRIN lens until the light reflected from the SERS layer forms a parallel beam directed back to the laser. After the epoxy is cured the SERS layer is permanently bonded at the back focal plane of the GRIN lens.

![Diagram of SERS layer at back focal plane](image)

Figure 6.7. Positioning the SERS layer at the back focal plane of a GRIN lens. NOTE: Some dimensions have been exaggerated for clarity.
6.3.2. Measurements and Characterization

The SERS measurement was obtained with a HORIBA confocal Labram Raman spectrometer equipped with a 30mW, 633nm HeNe excitation laser. Unlike the measurements for the PETE and epoxy-on-c over slip substrates, the microscope objective of the spectrometer was removed. Hence, the GRIN lens replaced the objectives for remote sampling as emphasized earlier in the chapter.

6.3.3. Results

Figure 6.8 bottom trace shows the Raman spectrum of 1mmol concentration of R6G obtained from the GRIN lens, mylar, and SERS layer combination in Figure 6.7. As with the PETE and epoxy-on-glass SERS substrates, strong R6G Raman peaks were identified. It was compared to the spectrum obtained with SERS layer on epoxy-on-glass (Figure 6.8 top trace). The intensities of these peaks were comparable to the epoxy-on-glass substrates (Figure 6.9) proving that the transfer process was robust.

![Figure 6.8](image-url)

Figure 6.8. Top trace, Raman spectrum of 1mmol R6G obtained after transferring nanospheres to epoxy on glass. Bottom trace, Raman spectrum of 1mmol R6G obtained after transferring nanospheres to mylar which was epoxied to the back of a GRIN lens.
6.4. Conclusion

The SERS substrate based on silver nanoparticles assembled over imprinted plastic can be used for portable and in-vitro analysis schemes. The transferable nature of the SERS substrate also makes it a candidate for remote sampling or in-vivo SERS applications. A comparative review of similar SERS substrates that were developed for bio-probe or re-applications is summarized in Table 6.1.

Table 6.1. Summary of different SERS substrates reported for bio-probe applications.

<table>
<thead>
<tr>
<th>SERS substrate</th>
<th>Fabrication</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silver (Ag) film on nanosphere surfaces</td>
<td>Nanosphere lithography: Drop-coating nanospheres on copper substrates and depositing 200-nm thick Ag film on the nanospheres; used in-vitro</td>
<td>[129]</td>
</tr>
<tr>
<td>Gold nanostars</td>
<td>Chemical synthesis: Seed mediated route using buffer; injected in-vivo</td>
<td>[130]</td>
</tr>
<tr>
<td>Gold film over nanospheres</td>
<td>Nanosphere lithography: Silica microsphere are self-assembled on a silicon wafer, followed by evaporation of gold layer (200-nm thick); used in-vitro</td>
<td>[131]</td>
</tr>
<tr>
<td>Gold nanowires with nanogaps</td>
<td>Gold nanowire made by vapor transport method on sapphire substrate, followed by sputtering of gold; used in-vitro</td>
<td>[132]</td>
</tr>
<tr>
<td>Gold nano-biopyramids</td>
<td>Chemical synthesis: seed-mediated method; injected in-vivo</td>
<td>[133]</td>
</tr>
<tr>
<td>Gold nanoparticles</td>
<td>Self-assembled from 20 nm thick sputtered gold film on aluminum foil; used in-vitro</td>
<td>[74]</td>
</tr>
<tr>
<td>Gold nanoparticles</td>
<td>Gold nanoparticles formed by sputtering on carbon nanotubes; used in-vitro</td>
<td>[39]</td>
</tr>
<tr>
<td>Silver nanoparticles on polymer microneedle array</td>
<td>Chemical synthesis to grow silver nanoparticles on micromolded polymethyl methacrylate needles; used in-vivo but no probe for large specimen sampling</td>
<td>[134]</td>
</tr>
<tr>
<td>Silver nanoparticles on imprinted plastic</td>
<td>Dispersion and self-assembly of silver nanoparticles on an imprinted plastic surface; intended for in-vivo and probe use</td>
<td>This work</td>
</tr>
</tbody>
</table>

It can be seen that the use of nanoparticles of gold or silver is a common approach. Planar SERS substrates based on silver or gold layers over nanospheres [129, 131] and silver-coated microneedles have also been employed in implantation-based bio-probe schemes [134]. In the
case of nanoparticle-based approaches, the nanoparticles are either combined with the analyte in-vitro [132] or injected into analyte tissue in-vivo [130, 133]. The limitations inherent to using free nanoparticle clouds have been discussed earlier in this work. When designing nanoparticle-based SERS sensors for potential in-vivo applications, the whole sensor must stay intact in-vivo to produce the desired signal, after which the nanoparticles must be expelled from the body [135]. Nanoparticle clouds injected into tissue are well separated from the collecting optics and consequently, only a small fraction of the Raman light is collected. The advantage of a defined nanoparticle location is that the incident light from a laser can be focused to a small spot, and a large fraction of the Raman light generated can be collected by the same or a confocal lens. It is well known that the strength of the Raman signal is proportional to the square of the numerical aperture (NA) of the collecting lens. In this context, the transferable SERS substrate described here can potentially address the limitations of randomly dispersed nanoparticle clouds.

It is often easier to fabricate a SERS layer on the surface of a wafer than on where it is to be deployed. In particular, the back focal plane of a GRIN lens is ideally suited for collecting the Raman light and transmitting it as a collimated beam that can be focused on the slit of a spectrometer. This chapter describes a straightforward method for transferring a SERS layer to a GRIN lens with readily available materials.
Chapter 7. Summary and Recommendation

7.1. Summary of results

Chapter 4 presented a low-cost imprinted SERS layer. Silver nanoparticles were imprinted on PETE using a sandpaper template. The imprint method was straightforward, and the materials used were inexpensive and readily available. A console Raman spectrometer and the imprinted PETE SERS layer were used to characterize the R6G solution. There were no Raman peaks without the imprinted SERS layer as seen in the blue trace in figure 7.1. Figure 7.2 shows that Raman peaks were identified at concentrations as low as 1nmol.

![Figure 7.1](image1.png)

**Figure 7.1.** Top trace, 1mmol R6G spectra after drop-casting nano-sphere suspension on imprinted PETE-1. Bottom trace, 1mmol R6G spectra after drop-casting nano-sphere suspension on PETE-1 that was not imprinted. (Repeated from figure 4.8)

![Figure 7.2](image2.png)

**Figure 7.2.** Raman spectrum of 1nmol to 1mmol concentrations of R6G obtained after drop-casting silver nano-sphere suspension on imprinted PETE-1. For clarity, the spectra have been offset vertically. (Repeated from Figure 4.9)
Portable compact and probe SERS device designs were fabricated in Chapter 5. The compact design incorporated a portable Raman spectrometer and the low-cost PETE SERS layer while the handheld design had a portable Raman spectrometer and the GRIN lens SERS probe. The performance of the compact design was evaluated with a 1mmol concentration of R6G solution. Raman peaks were identified looking through the specimen to the SERS layer and vice versa (figure 7.3) proving the transparency and suitability of the SERS for probe application.

![Figure 7.3](image-url)

Figure 7.3. Blue trace, Raman spectrum of 1mmol R6G obtained by looking through the PETE SERS layer to the Rhodamine solution. Green trace, Raman spectrum of 1mmol R6G obtained by looking through the Rhodamine solution to the PETE SERS layer. (Repeated from figure 5.4)

Chapter 6 described a simple method of transferring the low-cost SERS layer to other surfaces suitable for probe applications. A console Raman spectrometer and different concentrations of R6G solution were used to evaluate the performance of the SERS layer transferred to a thin layer of epoxy. R6G Raman peaks were identified down to 1nmol (figure 7.4). R6G Raman peaks were also identified when the layer was transferred to the back focal plane of a GRIN lens (figure 7.5).
Figure 7.4. Raman spectrum of 1nmol to 1mmol concentrations of R6G obtained after transferring silver nano-sphere suspension to epoxy on glass. For clarity, the spectra have been offset vertically. (Repeated from Figure 6.5)

Figure 7.5. Raman spectrum of 1mmol R6G obtained after transferring nanospheres to mylar which was epoxied to the back of a GRIN lens. (Repeated from Figure 6.8)
7.2. Recommendation for future work

As concluded in chapter 6, the SERS portable probe device design should be optimized and characterized to evaluate its SERS performance. Preliminary results in chapter 5 have been shown with R6G solutions but ultimately, the device should be characterized with biological specimens.
References


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81. Polyethylene Terephthalate, widely available for transparent containers and embossed with the PETE-1 logo.

82. Widely available as ‘2500 P-scale’ sandpaper.


84. 3499M-3 Imperial IV Ultra Clean 100 Microprocessor Oven, Lab-line Instruments, Inc., USA.


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