RAMAN AND FLUORESCENCE SPECTROSCOPY OF BIOMEDICAL NANOMATERIALS

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Abstract

Stabilized zirconia exhibits unsurpassed mechanical properties and biocompatibility, making it an indispensable ceramic material for biomedical implants. One of the most problematic features of stabilized zirconia has been its low-temperature degradation (LTD), which is associated to the observed transformation of its crystalline structure from tetragonal to monoclinic phase. The presence of monoclinic phases, therefore, is the red-flag for the impending catastrophic breakdown of mechanical properties. In this work, we establish characterization protocols to extend the sensitivity limit of conventional Raman spectroscopy for determination of extremely little amounts of monoclinic phase in zirconia implant prototypes. We accomplish this in two ways. First, we employ Raman spectroscopy and multivariate statistical analysis on a series of fully-dense and partially transformed Y-TZP zirconia prototypes. Incipient $t$-$m$ transformation is only revealed with high resolution spectral mapping and principal component analysis. The technique reveals the presence of islands of monoclinic phase that are otherwise not visible by simple observation and fitting of individual spectra. High resolution mapping likewise allows for probing homogenieties in the sample, which is a critical component in the development of implants.

The second protocol utilizes surface-enhanced Raman spectroscopy (SERS) with colloidal gold nanostars as substrate. The nanostars used have localized surface plasmon resonance (LSPR) at $\sim$690 nm. Two spectral maps, on clean and on nanostars-covered surface, were obtained exactly at the same position using confocal Raman spectroscopy. Comparison of the two maps shows that there are more monoclinic phases detected in the nanostars-covered surface possibly due to the “lightning rod” effect in the nanostar tips. We report an unprecedented attempt on SERS on solid zirconia, which provides early evidence of the effectiveness of the technique even on non-porous materials. With further improvement in sensitivity, SERS is a promising technique for the early detection of monoclinic phase in zirconia-based implants.
Sommario

La zirconia stabilizzata presenta una combinazione insuperabile di proprietà meccaniche e biocompatibilità, grazie alle quali diventa un materiale ceramico indispensabile per impianti biomedici. Una delle caratteristiche più problematiche della zirconia stabilizzata è la sua degradazione a bassa temperatura (LTD), che è associata ad una trasformazione di fase da tetragonale ($t$) a monoclinica ($m$). La presenza della fase monoclinica è il campanello d’allarme per l’imminente e catastrofica riduzione delle proprietà meccaniche. In questo lavoro, sono stati stabiliti dei protocolli di caratterizzazione con lo scopo di aumentare la sensibilità della spettroscopia Raman convenzionale per determinare quantità estremamente piccole di fase monoclinica in prototipi di impianti in zirconia. Questo obbiettivo è stato raggiunto in due modi. Nel primo è stata utilizzata la spettroscopia Raman e l’analisi statistica multivariata su una serie di prototipi in Zirconia Y-TZP completamente densi e parzialmente trasformati. È stato dimostrato che l’incipiente trasformazione da $t$ a $m$ può essere rivelata solo quando la mappatura spettrale è ad alta risoluzione e la matrice spettrale è valutata con l’analisi delle componenti principali. L’alta risoluzione della mappatura ha permesso di evidenziare piccole deformazioni, altrimenti non visibili con la tecnica convenzionale e l’analisi individuale degli spettri.

Il secondo protocollo utilizza la spettroscopia Raman amplificata da superfici (SERS) con nanostars di oro come substrato. Le nanostars utilizzate presentano la risonanza plasmonica di superficie localizzata (LSPR) a $\sim 690$ nm. Due mappe spettrali, una sul campione tal quale e una con la superficie ricoperta da nanostars, sono state acquisite esattamente nella stessa posizione utilizzando la spettroscopia Raman confocale. Il confronto delle due mappe mostra una percentuale di fase monoclinica maggiore sulla superficie ricoperta dalle nanostars, probabilmente a causa dell’effetto “lightning rod” dovuto alla forma a punta delle nanostar. È riportato il tentativo senza precedenti di misure SERS su zirconia allo stato solido, le quali potrebbero essere tra le prime prove dell’efficacia della tecnica anche su materiali non porosi. L’utilizzo del SERS per ottenere un ulteriore miglioramento della sensibilità
del Raman confocale è una tecnica promettente per la diagnosi precoce della fase monoclinica in impianti a base di zirconia.
To Malin Janna
and
Michael Isaac

The smallest ones,
indeed, bring about the
greatest enhancement.
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Lastly, to our resident tyrants, Janna and Isaac, who albeit their small size have greatly enhanced my life.
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<th>Full Form</th>
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<tr>
<td>AgNP</td>
<td>silver nanoparticle</td>
</tr>
<tr>
<td>Au</td>
<td>gold</td>
</tr>
<tr>
<td>AuNS</td>
<td>gold nanostar</td>
</tr>
<tr>
<td>AuNP</td>
<td>gold nanoparticle</td>
</tr>
<tr>
<td>c</td>
<td>cubic</td>
</tr>
<tr>
<td>CCD</td>
<td>charge-coupled device</td>
</tr>
<tr>
<td>Ce</td>
<td>Ceria</td>
</tr>
<tr>
<td>EF</td>
<td>enhancement factor</td>
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<tr>
<td>LSPR</td>
<td>localized surface plasmon resonance</td>
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<tr>
<td>LTD</td>
<td>low-temperature degradation</td>
</tr>
<tr>
<td>m</td>
<td>monoclinic</td>
</tr>
<tr>
<td>ME</td>
<td>2, mercaptoethanol</td>
</tr>
<tr>
<td>MPA</td>
<td>3, mercaptopropionic acid</td>
</tr>
<tr>
<td>NA</td>
<td>numerical aperture</td>
</tr>
<tr>
<td>PCA</td>
<td>principal component analysis</td>
</tr>
<tr>
<td>PS</td>
<td>piezospectroscopic</td>
</tr>
<tr>
<td>SERS</td>
<td>surface - enhanced Raman spectroscopy</td>
</tr>
<tr>
<td>t</td>
<td>tetragonal</td>
</tr>
<tr>
<td>TEM</td>
<td>transmission electron microscope</td>
</tr>
<tr>
<td>TTT</td>
<td>temperature - time to transformation</td>
</tr>
<tr>
<td>TZP</td>
<td>tetragonal zirconia polycrystal</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
<tr>
<td>VIS</td>
<td>visible</td>
</tr>
<tr>
<td>Y-TZP</td>
<td>yttria - tetragonal zirconia polycrystal</td>
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<tr>
<td>ZrO₂</td>
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Chapter 1

Introduction

1.1 Properties of Zirconia

Zirconia (ZrO$_2$) is a ceramic material known for its excellent mechanical properties. With hardness comparable to stainless steel and refractive index being a close second to that of diamond, it is often called a ceramic steel and fake diamond [1]. Pure or undoped zirconia is monoclinic (m) at room temperature up to 1170 °C. It begins to transform to a tetragonal (t) structure above this temperature and is stable up to 2370 °C. Further heating results to formation of cubic (c) phase. After sintering at high temperatures, depending on the cooling conditions, it transforms back to monoclinic structure. The transformation from tetragonal to monoclinic occurs at around 950 °C in the cooling direction. The main issue with this change in phase is the accompanying change in volume (about 3 % - 5 % increase), which induces fracture as temperature decreases, making pure zirconia ineffective for typical ceramic applications. These phase transformations can be restrained with the addition of oxide stabilizers such as calcia (CaO), yttria (Y$_2$O$_3$), magnesia (MgO) and ceria (CeO). By mixing with stabilizers, zirconia can retain a metastable cubic and/or tetragonal structure at room temperature [2]. This multiphase material is known as partially stabilized zirconia (PSZ). Addition of small amounts of stabilizer allows generation of ceramic containing only tetragonal phase, forming the so-called tetragonal zirconia polycrystal (TZP). The most successful and well-investigated composition is Y-TZP, which contains 3% of mol. yttria and more popularly known as 3Y-TZP. This ceramic possesses exceptional properties such as high flexural strength and fracture toughness, high hardness, excellent chemical resistance and high ion conductivity. Ce-doped zirconia, which has only recently been considered as an implant material, also exhibits superior
toughness [3, 4].

Once stabilized, zirconia can become technologically useful in applications that span a wide range of temperatures. ZrO$_2$-CaO contains conducting oxygen ions that are important for sensors and fuel cells while ZrO$_2$-Y$_2$O$_3$ finds applications in thermal barrier coatings in jet turbine engines [5].

As with all ceramics, stabilized zirconia is biocompatible, and hence, is suitable for manufacturing biomedical implants such as hip-joint and femoral head replacements. Apart from being utilized in orthopedic and industrial applications, zirconia is also gaining popularity in the field of dentistry [6]. It has been shown that zirconia-based dental ceramics are stronger materials compared to conventional glass-ceramics [7]. In addition, naturally occurring zirconia has color similar to tooth, which makes it more suitable for aesthetic dentistry. ZrO$_2$-Y$_2$O$_3$ has an excellent reputation for this particular purpose.

Zirconia, in its stabilized form has unsurpassed mechanical strength. However, it has been shown that 3Y-TZP exhibits low-temperature degradation (LTD) [8] or aging in the presence of water and other solvents. The different stages of LTD is depicted in Figure 1.1. The initial transformation is caused by one or a combination of these contributions: composition of stabilizers, presence of residual stress and the grain size. Aging occurs as slow transformation to the monoclinic phase beginning at the surface. With the increase in volume of individual grains, cracks form at the surface, making way for water to penetrate the material. As a result, fracture propagation accelerates in the material leading to loss of strength and reduced density. Now, the consequence of this transformation are not all bad. Ironically, the material undergoes phase transformation toughening when cracks emerge at the surface. Crack propagation is arrested as zirconia transforms its crystal structure.

1.2 Raman Spectroscopy on Zirconia

While metastable zirconia serves as a robust material for dental implants, its low temperature degradation still requires further study. Raman spectroscopy is a suitable detection technique for this purpose. There are some remarkable advantages in using Raman spectroscopy for detection of the $t$-$m$ transformation in zirconia. The technique is highly sensitive to polymorph, i.e., it is able to distinguish different crystal structures of the same composition. It provides high lateral resolution and produces the spectral signal in a matter of seconds. The observed Raman spectra
is information-rich. Several physical properties may be extracted and qualitatively obtained without extensive alternative testing. It is possible to study materials in the actual experimental environment and in bulk. One can also perform piezospectroscopy or measurement of stress-induced frequency shift in the Raman bands [9–12].

The extant literature is replete of reports on the use of Raman spectroscopy in determining the monoclinic content in zirconia and zirconia composites [9–16]. Most of these studies report the ability to detect monoclinic content higher than 5%, and rely on extrapolation for lower concentrations. This defines the lower limit of the sensitivity for monoclinic determination using regular Raman. As will be discussed in more detail in Chapter 4, the roadblock is due to the absence of large-enough monoclinic spectral peaks at 5% and below. There is a need for alternative routes to overcoming this limitation, which we hope to achieve with the aid of nanoparticles. This technique is introduced in the next section.

Figure 1.1. Different stages of low-temperature degradation. (A) Stabilized tetragonal zirconia, (B) Initial t-m transformation occur with accompanying volume change, (C) Water and moisture seep through the gaps and transformation accelerates inside the material. (D) Surface uplift due to increase in volume becomes more apparent.
1.3 Surface - Enhanced Raman Spectroscopy

One general limitation of conventional Raman spectroscopy has been the weakness of Raman scattering signals in materials. This limited the technique to those substances exhibiting strong Raman spectra or those that could withstand strong light sources that could be used to induce stronger Raman signals. A revolutionary technique to overcome this limitation was discovered in 1978 by Fleischmann [17], Van Duyne [18], Creighton [19], and their coworkers [20]. They observed that when pyridine was placed on top of a roughened silver electrode, the Raman scattering intensity of pyridine was enhanced by up to $10^5$ [21]. This observation has brought forth what is known as surface-enhanced Raman spectroscopy (or SERS), which has significantly extended the reach of Raman spectroscopy as an analytical tool.

For completeness, SERS mechanism will be described first in the context of detecting and enhancing the Raman signal of a molecule. As we will see later on, the description is adapted for the system considered in the current work, i.e. SERS on solid zirconia. Typically, there are three main components of a SERS set-up: the analyte molecule, the exciting radiation and the metallic substrate containing nanoparticles [22]. The chemical species are either adsorbed or located close (> 10 nm) to the metallic substrate, usually silver or gold. Monochromatic light is made incident onto a metal substrate and activates the surface plasmons, which are essentially electronic vibrations at the surface. In smooth surfaces, the plasmons are bound to the surface, but for appropriately rough surfaces the plasmons have a perpendicular component and are thus able to induce inelastic scattering on the molecule. Plasmonic oscillations generate a secondary EM field, which acts on the molecules. The scattered light then escapes from the surface as light with shifted wavelength and amplified intensity. The enhancement arises from electromagnetic effects, which can be achieved mainly through improving the design of the nanostructure substrates as well as ensuring adsorption of the molecules onto the substrate. A critical requirement in this process is the use of nanoparticles at the surface. As such, SERS is a truly nanoscience phenomenon as it relies on nanostructures for enhancement to occur [23].

SERS is now popular as a high-sensitivity analytic tool. Coupled with instrumental improvements brought about by developments in nanotechnology, its versatility in various applications has significantly increased. A wide variety of applications has been developed around the use of SERS as a quantitative tool, including single
molecule [24] and trace detection [25], detection of organic pollutants [26], microfluidics [27], separation science [28] and DNA analysis [29]. SERS technique is favored primarily for its high selectivity feature. It is able to discriminate between materials of the same composition but different structure. In addition, its high sensitivity enables detection of samples even in small volumes. The key step to achieving the giant optical amplification lies on finding a suitable nanoparticle system that supports the effect. Thus, preparation of SERS substrates remains a very active research area. The quality of the signal as well as its reproducibility is dictated by the size, shape and optical properties of the nanostructures. The size of the nanoparticles must be small with respect to the wavelength of the exciting light, with diameters between 1 and 100 nm. Platforms for SERS measurement can be in the form of colloids of metallic nanoparticles, nanoparticle islands on planar support or periodic arrays of nanoparticles grown on solid support [30]. While suspensions easily generate tiny regions of localized electromagnetic field, referred to as hotspots, very small enhancement is produced and the spectral signal is not reproducible. Despite this drawback, there are a number of reasons for choosing to utilize nanoparticle suspensions as substrates. They are easy and economical to synthesize through wet chemical preparation techniques. Other than mixing the analyte with the colloid, no further sample preparation is needed since most analytes come in solution form. Adsorption of analyte molecules by nanoparticles is very likely since nanoparticle suspensions have very large surface area. The presence of solvent prolongs the shelf-life of the nanoparticles, especially of silver because it is highly reactive. Apart from this, the suspension serves as buffer between the sample and the radiation, causing less damage to the sample during measurement. Reproducibility of the Raman spectra is addressed by using metallic nanoparticles grown on a planar support. Thin films or glass slides serve as support for immobilized nanoparticles. With the interplay of various growth parameters, better control of substrate morphology and thus reproducibility can be achieved. The most sophisticated type of SERS substrates is the fabrication of periodic arrays of nanoparticles directly on solid substrates in a top down fashion, which includes those prepared by nanolithography and template synthesis of nanostructures. Each of these classes of substrates has proved to be suitable for specific uses; there is no best SERS substrate available for universal use in applications.

The quality of detection is dictated by the composition, size, shape and optical properties of the nanostructures. In most applications, the typical choices of metals
are silver and gold. It has been shown that silver is generally more optically efficient while gold is favored for its biocompatibility [31]. Another way of controlling SERS intensity is varying the size of the nanoparticles. Larger particle sizes produce greater SERS intensity provided that the nanoparticle size is smaller than the wavelength of the exciting light. The sizes of nanostructures that yield successful enhancement typically ranges from 5 nm to 100 nm. However, recent studies show that high intensity signal can also be achieved for particle sizes up to 200 nm [32]. Another factor that affects SERS amplification is the shape of the nanoparticles. Spherical gold and silver colloids have long been used in SERS studies. While these generate significant enhancement in low concentrations of molecules, it typically requires aggregation in the nanospheres to achieve SERS enhancement. Reproducibility of the SERS signal is thus difficult to achieve because of the randomness in the aggregation mechanism in spherical nanoparticles. Aside from spherical nanoparticles, nanorods, triangular platelets and nanocubes have been explored. The main goal is to create several regions of high field concentrations over the nanoparticle surface without the need for aggregation. It has been observed that these focused electromagnetic fields occur at sharp apexes such as those present in gold nanostars [33] and thorn-shaped nanowires [34]. Among colloidal nanoparticles, nanostars favored for their superior SERS properties and flexibility. Nanostars are made of a spherical core with multiple sharp branches. Apart from having strongly enhanced electromagnetic (EM) field localized at its tips, nanostars have tunable plasmon resonance that can be easily matched to the excitation wavelength of the laser. Given the versatility and flexibility of the surface-enhanced Raman spectroscopy, it continues to be a very active research subject in many scientific fields. Its flexibility comes from its dependence on several factors that are readily controllable in the laboratory. The goal is then that of tuning the SERS method to suit the particular analyte of interest.

As mentioned above, SERS will be ultimately used on a solid zirconia. In this particular case, there are no molecules that will be adsorbed onto the nanoparticles. The single requirement then is very close proximity of the nanoparticles to the zirconia surface.

1.4 Objectives

At present, identifying the ideal composition for a zirconia implant possessing excellent mechanical properties and perfect reliability is yet to be realized. In
particular, developing an implant that can withstand the harsh conditions inside the body and can last more than 60 years is a huge endeavor. Fortunately, we are getting closer and closer to realizing this goal through the continued efforts of the LONGlife project (http://longlife-project.eu), to which this work is a major component. From the point of view of characterization, a key step to achieving lifelong implants is early detection of the formation of the monoclinic phase in the prototypes. In this work, detection of extremely little amounts of monoclinic at the onset of transformation is demonstrated on implant prototypes by combining Raman mapping and multivariate statistical analysis techniques. Moreover, to further improve the sensitivity of detection down to trace amounts of monoclinic zirconia, we propose to use gold nanostars as substrate for SERS. We exploit the two pieces of information that we know to be true: (1) the transformation to monoclinic commences at the surface and (2) the intrinsic confocality offered by SERS allows us to tap into only the surface modes of zirconia. The amplification of the solid surface is carried out by solely relying on the intrinsic property of very small metallic nanoparticles and sharp apexes to have high concentration of electromagnetic field at its surface - this is the so-called "lightning rod effect". Owing to the presence of numerous sharp tips, nanostars take full advantage of such effect and offer exactly the geometry needed for this approach. This work is an unprecedented attempt to apply SERS techniques through the addition of gold nanostars on the polished surface of fully-dense yttria-stabilized zirconia implants.

There is no doubt that nanostars and other nanoparticles/nanostructures are able to produce enhanced Raman signals even in low concentrations of molecules. The lowest concentration that has been reported in literature is down to attomolar levels \[35, 36\]. However, SERS has only been demonstrated in the detection of liquids, solutions as well as gaseous molecules. In contrast, very little work has been done to exhibit SERS in solid materials. Recently, SERS was performed on SnO\(_2\) pellets, pulsed-laser deposited SnO\(_2\) films, and SnO\(_2\) colloids in the presence of Ag \[37\]. For zirconia, there has only been a report on SERS effect in thin platinum electrodes deposited on yttria stabilized zirconia (YSZ) due to oxygen migration in closed circuits during catalytic activity \[38\]. However, YSZ has a porous surface similar to powders. It remains a challenge to demonstrate SERS on dense and polished solid, such as zirconia implants. The use of SERS in detecting trace concentrations of the monoclinic phase in zirconia implants shows great promise and is worth investigating further. The work described in this dissertation is a pioneering attempt to accomplish
1.5 Organization of this Thesis

The succeeding chapters are organized as follows: We first review in Chapter 2 the theoretical principles of Raman spectroscopy and surface enhanced Raman spectroscopy. In Chapter 4 we explain how Raman spectroscopy is generally implemented on zirconia samples. Our solution to the shortcomings of traditional Raman on zirconia is also explained here. Chapter 5 describes the work done towards the development of gold nanostars as substrate for SERS. We then describe in Chapter 6 the interaction of nanoparticles with zirconia samples, namely, partially stabilized Y-zirconia powder and fully stabilized zirconia disc. Finally, in Chapter 7, we present the conclusions and speculate on future avenues related to this topic.
Chapter 2

Theoretical Background

2.1 Scattering and Raman Spectroscopy

Scattering belongs to a family of optical processes, which involves simultaneous absorption of an incident photon and emission of another photon. When light illuminates a sample, it can be scattered in different directions. Most of the scattering process happen elastically, i.e., the incident and the scattered photon have the same energy but may differ in direction and/or polarization via Rayleigh scattering. However, a small portion of light may scatter inelastically, i.e., the scattered photon has a different energy than the incident photon. This is the Raman scattering and the energy difference corresponds to a transition in the vibrational energy level. An interesting feature of Raman scattering is that the scattered photon may have an energy either lower (Stokes) or higher (anti-Stokes) than the incident photon. These processes can be explained classically and quantum mechanically.

2.1.1 Classical Approach

In the classical approach [39], we can represent the incident electric field, $E_L$, of frequency $\nu_0$ and amplitude $E_0$ as

$$E_L = E_0 \cos(2\pi\nu_0 t) \quad (2.1)$$

This electric field interacts with the molecule and induces an electric dipole, $P$, given by

$$P = \alpha E_L \quad (2.2)$$
where $\alpha$ is the polarizability of the molecule.

The vibration of the molecule can be described as a simple harmonic motion with frequency, $\nu_{\text{vib}}$ and displacement given by

$$q = q_0 \cos(2\pi \nu_{\text{vib}} t) \quad (2.3)$$

The polarizability of the molecule is a linear function of $q$. For small amplitudes, $\alpha$ can be approximated by the first two terms of a Taylor series in $q$.

$$\alpha = a_0 + \left( \frac{\partial \alpha}{\partial q} \right)_0 q_{\text{vib}} + \ldots \quad (2.4)$$

$$\approx a_0 + \left( \frac{\partial \alpha}{\partial q} \right)_0 q \cos(2\pi \nu_{\text{vib}} t) \quad (2.5)$$

We can combine Eqs. (2.1), (2.3), (2.5) and (2.2).

$$P = \alpha E_0 \cos(2\pi \nu_0 t) \quad (2.6)$$

$$= a_0 E_0 \cos(2\pi \nu_0 t) + \left( \frac{\partial \alpha}{\partial q} \right)_0 q E_0 \cos(2\pi \nu_0 t) \quad (2.7)$$

$$= a_0 E_0 \cos(2\pi \nu_0 t) + \left( \frac{\partial \alpha}{\partial q} \right)_0 (q_0 \cos(2\pi \nu_{\text{vib}} t)) E_0 \cos(2\pi \nu_0 t) \quad (2.8)$$

$$P = a_0 E_0 \cos(2\pi \nu_0 t)$$

$$+ \frac{1}{2} \left( \frac{\partial \alpha}{\partial q} \right)_0 q_0 E_0 \cos 2\pi (\nu_0 + \nu_{\text{vib}}) t + \cos 2\pi (\nu_0 - \nu_{\text{vib}}) t$$

The first term corresponds to the Rayleigh scattering and take note that its frequency is equivalent to the incident frequency. The second term is the Stokes term with a shifted frequency given by $(\nu_0 - \nu_{\text{vib}})$. The third term is the anti-Stokes term with a shifted frequency given by $(\nu_0 + \nu_{\text{vib}})$. The partial derivative $\left( \frac{\partial \alpha}{\partial q} \right)$ serves as the coefficient of the Stokes and anti-Stokes terms. Raman bands arise when this coefficient is not zero, which physically means that there is a change in the polarizability. In other words, when $\left( \frac{\partial \alpha}{\partial q} \right) = 0$, the vibration is not Raman-active.

The intensity from an oscillating dipole is proportional to the second time derivative of the induced dipole moment.

$$I \propto \left( \frac{\partial^2 P}{\partial t^2} \right) \quad (2.10)$$
Performing the derivative on Eq. (2.9) gives us the expression for the intensity of the scattered light, $I_{\text{Rayleigh}}$, $I_{\text{Stokes}}$ and $I_{\text{anti-Stokes}}$.

\[
I_{\text{Rayleigh}} \propto \nu_0^4 \alpha_0^4 I_0 \tag{2.11}
\]

\[
I_{\text{Stokes}} \propto (\nu_0 - \nu_{\text{vib}})^4 \left( \frac{\partial \alpha}{\partial q} \right)_0^2 I_0 \tag{2.12}
\]

\[
I_{\text{anti-Stokes}} \propto (\nu_0 + \nu_{\text{vib}})^4 \left( \frac{\partial \alpha}{\partial q} \right)_0^2 I_0 \tag{2.13}
\]

$I_0$ is the intensity of the incident light. We can see here that the classical theory predicts a strong frequency dependence of $I_{\text{Stokes}}$ and $I_{\text{anti-Stokes}}$ and are almost the same magnitude. However, the experimentally observed intensities are different. Anti-Stokes scattering is generally weaker than that of Stokes and the quantum explanation will clarify this.

### 2.1.2 Quantum Mechanical Approach

Consider the simplified diagram of the vibrational states of a molecule in Figure 2.1. In a Stokes process, the molecule in the vibrational ground state, $\nu_0$, get excited by the incident photon, $E_L$, through interaction with a virtual state. At the same time another photon is emitted from the virtual state to the first excited vibrational state, $\nu = 1$, with energy $E_S = E_L - \hbar \nu_{\text{vib}}$. On the other hand, in an anti-Stokes process the scattered photon energy has more energy than the incident photon energy. The incident light excites a molecule originally at a higher vibrational state, $\nu = 1$, then interacts with a virtual state while simultaneously, a photon relaxes to the vibrational ground state, $\nu = 0$. The energy of the vibration is given by $E_S = E_L + \hbar \nu_{\text{vib}}$. An Anti-Stokes process generally occurs through thermal excitations.

The energy lost by the photons is the Raman shift and is given by $E_R = E_L - E_S$. A Stokes process will give a positive Raman shift and correspondingly, an anti-Stokes process will yield a negative shift.

At room temperature, more molecules reside in the lowest vibrational state than the upper states. The population between two states with energy difference, $\Delta E$,
2 – Theoretical Background

follow the Boltzmann distribution.

\[
\frac{N_0}{N_1} = e^{\frac{\Delta E}{kT}}
\]  

where \(N_0\) and \(N_1\) are the population of the lower and upper vibrational state, respectively. \(k\) is the Boltzmann’s constant and \(T\) is the absolute temperature measured in K. The ratio of the two intensities in Eqs. (2.12) and (2.13) can be expressed as:

\[
\frac{I_{Stokes}}{N_{anti-Stokes}} = \frac{\nu_0 - \nu_{vib}}{\nu_0 + \nu_{vib}} e^{\frac{\Delta E}{kT}}
\]  

At low \(T\), the ratio is a large value and Stokes scattering dominates. On the other hand, anti-Stokes becomes more intense at higher \(T\).

2.1.3 Fluorescence

Another inelastic scattering may occur depending on the energy of the incident light. This process is fluorescence and is very similar to the scattering described above. The only difference is that the molecule is excited to a physical electronic state rather than a virtual state. If the incident energy is high enough, it can excite a molecule to a higher electronic state by being absorbed. The molecule then almost instantaneously relaxes to the lowest level in the excited electronic state without emitting radiation. This process is caused by collision with other molecules and undergoes internal conversion. The molecule then relaxes to the ground electronic state by emitting fluorescence light.
2.1.4 Raman Spectroscopy

As an analysis tool, Raman spectroscopy is non-destructive and provides information about the composition and structure of the material. A typical Raman setup is depicted in the schematic diagram in Figure 2.2, the laser beam enters the spectrometer and then passes through the directing optics towards the microscope. The light source is then focused onto the sample by an objective lens. Any backscattered radiation from the sample is collected by the objective lens and directed to the collection optics. All the Rayleigh scattered light is blocked by the notch filter and the rest of the light is focused onto the CCD detector. An edge filter can also be used to filter out both the anti-Stokes and Rayleigh scattering leaving only the Stokes scattering to be detected. The result of the measurement is the Raman spectrum which contains the intensity of the Raman scattered light versus the energy difference (frequency shift), typically in units of cm\(^{-1}\). The intensity of the peak corresponds to the population of the molecule. The shift in frequency corresponds to the energy of the vibrational motion of the molecules in the sample. While not all vibrational modes of the molecule are Raman active as dictated by the selection rules, the vibrational motions and corresponding frequency shifts are unique for each molecular species. Each molecular species therefore has its own unique fingerprint. Raman spectroscopy can then be used for multi-species concentration measurements. Aside from the intensity and Raman shift, other features of the Raman spectrum contain different information about the sample. The Raman bandshape can broaden depending on the pressure. In gases, increase in pressure causes broadening in the lineshape [40–42]. In sufficiently dilute liquids, Raman lineshape has direct information on the temporal evolution of the solute-solvent interactions in the sample [43]. For solids, broadening of the linewidth increases with increasing temperature due to unharmonic vibrations [44, 45].

2.2 Confocal Raman Spectroscopy

Confocal Raman spectroscopy overcomes some of the limitations of conventional Raman. Pinhole apertures are introduced to the system to limit the light reaching the sample and to filter out unfocused light scattered by the sample from reaching the detector [46].

As in standard Raman the excitation light is focused on the specimen by the objective lens. The scattered light from the specimen is collected by the same
objective lens and then focused onto a pinhole aperture. With the pinhole, only the scattered light from the focal plane can pass through and reach the CCD. Light from out-of-focus region does not pass through the pinhole and will therefore be blocked from reaching the detector. The elimination of out-of-focus planes makes resolution in depth possible. Figure 2.3 shows the schematic diagram of the light rejection process of unfocused light. The sample receives light at the sample plane and the focus appears at point B. In an opaque medium, scattering diffuses the focus at points A and C. When an aperture is placed at a back focal plane of the objective lens, only those rays that originate near point C are transmitted, hence improving the depth resolution. Depth resolution is dependent on the volume of the laser focus, which is controlled by the objective’s numerical aperture (NA) according to the relation [47] given by

$$\text{depth resolution} = \frac{n\lambda}{(NA)^2} \quad (2.16)$$

where \( n \) is the refractive index of the immersion medium, \( \lambda \) is the laser wavelength. For example, a laser of 785 nm wavelength focused onto a solid sample in air using an objective with NA=0.75 will have a depth resolution of 1.4 \( \mu \)m. With confocal Raman spectroscopy, one measures the vibrational modes exclusively from the focal plane of the sample and isolates the contribution from out-of-focal plane regions. This is particularly advantageous when isolating surface contributions in a bulk sample from
sub-surface features. Since the detector only measures one point of the specimen at one time it is necessary to move the illuminated point across the specimen. This can be done more commonly by moving the stage holding the specimen. In contrast to a conventional microscope, a confocal microscope needs some form of image processing to produce an image.

### 2.3 Surface-Enhanced Raman Spectroscopy

The two main ingredients for SERS process are: Raman spectroscopy and plasmon resonance, more particularly the localized surface plasmon resonance (LSPR) of the nanoparticles. The former was elucidated in the previous section. Now, we clarify the role of surface plasmon in the enhancement process. A surface plasmon is a traveling wave oscillation of electrons that can be excited in the surface of certain metals with the right properties [48]. For nanoparticles, a surface plasmon is more specifically called local surface plasmon due to the absence of a flat surface. We can describe the nanoparticles in terms of the resonance wavelength, which may be extracted from optical absorption and UV-visible spectroscopy. This measures absorption/transmission spectrum as a function of the incident energy. It probes the different excitations in the medium depending on the scattered energy. Absorption is indirectly measured from the transmitted intensity. When an incident light interacts with the medium, photons can be absorbed or scattered. Absorbed and scattered photons are transformed to heat or re-emitted in a different direction resulting in power reduction of the transmitted beam. As a result, \( P_{\text{trans}} < P_{\text{incident}} \). The
extinguished power therefore becomes

\[
P_{\text{ext}} = P_{\text{incident}} - P_{\text{trans}} = P_{\text{abs}} + P_{\text{scat}}
\]  

(2.17) (2.18)

For molecules, scattering is negligible giving \( P_{\text{ext}} \approx P_{\text{abs}} \). But for nanoparticles, the UV-vis spectrum measures both the absorbed and scattered power (\( P_{\text{ext}} = P_{\text{abs}} + P_{\text{scat}} \)), hence, it is more accurately called the extinction spectrum. The peak wavelength in the extinction spectrum corresponds to the localized surface plasmon resonance wavelength, LSPR \( \lambda_{\text{max}} \). SERS occurs as a result of interaction with the LSPR where the large enhancement is expected when the incident laser or the wavelength of the Stokes-shifted spectrum is at or close to the resonance wavelength. Moreover, as a general rule, nanoparticles which possess low intensity in the extinction spectrum gives a high enhancement effect. However, for nanoparticles giving high intensity in the extinction spectrum such as those in aggregated nanoparticles, extinction wavelength is not a good indicator for enhancement.

**Size and Shape Effects**

The size of the nanoparticle has consequences on the LSPR \( \lambda_{\text{max}} \). The larger the particle size, the more red-shifted the LSPR \( \lambda_{\text{max}} \) becomes. Moreover, broadening of the resonance wavelength is observed as size increases. LSPR also depends on the nanoparticle shape. Monodisperse spherical nanoparticle exhibit a single sharp LSPR peak. Elongated nanoparticles, such as nanorods with high aspect ratio, will register two well-separated peaks in the extinction spectrum. The peak at shorter wavelength corresponds to the transverse oscillations along the short axis and the peak at longer wavelength is due to the longitudinal oscillations. The more prominent peak at longer wavelength blue-shifts as the aspect ratio decreases. Another class of nanoparticle configuration is composed of spherical core covered with a shell made from a different material. The interplay between the size of the core and the thickness of the shell affects the extinction spectrum. Typically, higher intensity is achieved for increasing core size while red-shifting of the LSPR \( \lambda_{\text{max}} \) is observed as shell thickness increases [49]. Another morphology of particular interest for SERS are branched gold nanoparticles, or nanostars, because of their peculiar plasmonic properties [50–54]. The extinction spectrum of this class of nanoparticles features a sharp high intensity peak in the red up to near infrared wavelength and a broad shoulder at shorter wavelength. The prominent LSPR \( \lambda_{\text{max}} \) correspond to the oscillations
at the tips while the resonance at shorter wavelength is due to the oscillations of
the spherical core. As will be seen later, the LSPR $\lambda_{\text{max}}$ affects the SERS performance.

**Origin of Enhancement: Electromagnetic Effect**

SERS enhancement is largely an electromagnetic (EM) effect. When metal parti-
cles with dimensions less than the excitation wavelength are illuminated, localized
surface plasmon resonance can be induced in the nanoparticle. Plasmon resonance
or plasmonic oscillations, as defined above, are collective oscillations of the parti-
cle’s conduction band electrons. Excitation of these plasmon modes leads to the
generation of a secondary electromagnetic field hence, drastically increasing the local
electromagnetic fields at the particle’s surface. A molecule that is sufficiently close
to this particle’s surface is therefore affected by an enhanced incident field which
is equivalent to having a higher powered laser. Consequently, the scattered Raman
signal is amplified. In another instance, the Raman scattered light can also be
amplified if a molecule experiences enhanced emitted field. Recall that the Raman
process is directly correlated with the excitation field as well as the emission field.
Hence, any enhancement of these field sources can bring about enhanced Raman
scattered field. The theoretical *enhancement factor* (EF) associated with the EM
mechanism can be described in terms of the square of the average field intensity at
the plasmon frequency ($E$), the square of the field at the scattered frequency ($E'$)
and the incident field, $E_0$, given by [55]

$$EF = \frac{|E|^2 |E'|^2}{|E_0|^4}. \quad (2.19)$$

In the case where the incident frequency is equal or close to the plasmon frequency,
EF can be approximated as

$$EF = \frac{|E|^4}{|E_0|^4} \quad (2.20)$$

**Note on Chemical Effects**

A few works in the literature [56–58] argue that another source of enhancement is
from chemical effects. Its contribution to enhancement, however, does not exceed
more than a factor of 100 and is neglected in most cases. Le Ru *et al.* had defined
this chemical effect as any change on the Raman polarizability due to the adsorption
of molecule to the metal [59]. The change can be in the form of increase in intensity,
appearance of a new Raman band or even quenching. Recently, Moskovits asserted
in his critical article, that the enhancement observed over the last 40 years solely
originates from EM effects [60]. In fact, all nanoparticle substrates being developed are directed towards generating hotspots and not to promote more chemical reaction on the surface. Currently, the chemical effect hypothesis is highly disfavored, and it will remain so until such time that scientists are able to develop a substrate to promote and control the chemical reaction of individual molecules.
Chapter 3

Principal Component Analysis (PCA)

3.1 Introduction

A statistical tool frequently employed in this work is Principal Component Analysis (PCA). This is a standard technique in modern data analysis, and finds use in a wide variety of fields [61, 62]. To improve readability of this thesis, I shall provide a brief overview of this technique, and try to convey a conceptual understanding of what the analysis is all about and how it helps us for our purposes.

Like most data analysis methods, the main goal of PCA is the extraction of useful information from confusing data sets. Given a set of samples for which one has a large amount of data (which can be different types of measurements made on the samples), one of the most basic questions one can ask is: How is it 'best’ to characterize the samples? In particular, how can we best differentiate one sample from another using the data? If one is lucky then one particular data set (from the many) might already be enough to discriminate the samples. In many situations, this is not possible. The data sets might be very confusing and/or strongly overlapping and are likely to contain much redundancies. A goal is to determine a measure for which the overlapping data sets appear 'most different’ or show the greatest variance. This is precisely what PCA does.

Specifying this to our particular usage, since our goal is the ability to locate very low-concentrations of monoclinic zirconia within bone implants, we first coordinatize the implants into many pixels (of the order of a thousand), and we obtain Raman spectra for each pixel. Our “samples' then consist of the pixels into which we have
divided the implants, and the voluminous data characterizing each “sample” (each pixel) are their Raman spectra (i.e. intensities vs frequency over a given range). (See Figure 3.1). When a large concentration of monoclinic zirconia is present in a pixel, this will clearly be reflected by its Raman spectra – the characteristic peaks at 180 cm\(^{-1}\) and 190 cm\(^{-1}\). But for very small concentrations that are of interest to this work, the spectra of all samples/pixels look very similar.

![Example of measured Raman spectra](image)

**Figure 3.1.** Example of measured Raman spectra.

### 3.2 Spectra as vectors; vectors as spectra

Consider the Raman spectra for a given pixel, which is essentially an array of intensities at specified frequencies: \(\{I(\nu_1), I(\nu_2), \ldots, I(\nu_m)\} \equiv \{I_1, I_2, \ldots, I_M\}\), where \(M\) is the number of frequencies at which intensities are measured (in our case, \(M\) is typically >231). We can represent this as a column vector:

\[
|I^i\rangle = \begin{pmatrix}
I_1^i \\
\vdots \\
I_j^i \\
\vdots \\
I_M^i
\end{pmatrix},
\]

(3.1)

where, using the familiar Dirac “ket” in his bracket notation from quantum mechanics to represent column vectors [63]. The index \(i\) pertains to the \(i^{th}\) position on the surface of our sample, where \(i = \{1,2,\ldots,N\}\), with \(N\) being the total number. We shall refer to a position on the surface as a *pixel*. So, \(I_j^i\) pertains to the measured intensity at wavelength \(\nu_j\) of pixel \(i\). One can think of these as the “natural variables” in which
the data is originally represented. Each pixel will have its own \( M \)-dimensional column vector \( |I^i\rangle \) consisting of the measured intensities at its location at \( M \) frequencies, or in short, the Raman spectra measured at its location. Raman spectra at each pixel is thus represented by column vectors, and the entire data set for a given implant (say Implant 1) is therefore represented by the matrix

\[
\mathbf{I}(\text{Implant 1}) = \begin{pmatrix}
I_{11} & I_{12} & \cdots & I_{1M} \\
I_{21} & I_{22} & \cdots & I_{2M} \\
\vdots & \vdots & \ddots & \vdots \\
I_{N1} & I_{N2} & \cdots & I_{NM}
\end{pmatrix}_{N \times M}
\]

We shall refer to this from now on as "the entire data set".

Continuing to represent the spectra as vectors \( |I^i\rangle \), we see that the representation of Eq. (3.1) comes with a natural basis set of vectors

\[
|b^1\rangle = \begin{pmatrix}
1 \\
0 \\
0 \\
\vdots \\
0
\end{pmatrix},
|b^2\rangle = \begin{pmatrix}
0 \\
1 \\
0 \\
\vdots \\
0
\end{pmatrix}, \ldots, |b^M\rangle = \begin{pmatrix}
0 \\
0 \\
\vdots \\
0 \\
1
\end{pmatrix}
\]

such that we can write the spectra/column vectors as

\[
|I^i\rangle = \sum_{j=1}^{M} I^i_j |b^j\rangle.
\]

Interpreted as spectra, the basis vector \( |b^j\rangle \) would correspond to a measurement of unit intensity at frequency \( \nu_j \) and zero intensity at all other frequencies.

It is helpful to think of the spectra as (abstract) “arrows” in an \( M \)-dimensional space. The numerical values, \( I^i_j \), (which are the actual measured values) are then merely the components of the vector in the natural basis, i.e. \( I^i_j \) is the projection of the arrow \( |I^i\rangle \) onto the basis vector \( |b^j\rangle \), or “how much of the arrow \( |I^i\rangle \) points in the \( j \)-direction”.

From linear algebra, we know that the choice of basis vectors is not unique, and hence the “natural basis” which comes with the actual measurements may not be the
most useful one. This will be the case for instance when there are correlations between the measurements. For example, if the intensity at some frequency $\nu_i$ is correlated with the intensity at another frequency $\nu_j$, as is typically the case, then given $\nu_i$, the intensity measurement at $\nu_j$ is redundant, since it carries the same information as the intensity measurement at $\nu_i$. Thus, it can happen that the spectra of all the samples actually lie in an $\bar{M}$-dimensional hyperplane, where $\bar{M} < M$. In this case, it makes sense to use new basis vectors, say $\{|\bar{b}^i\rangle\}$, that better characterize this (lower-dimensional) hyperplane. These new basis vectors will be linear combinations of the original ones:

$$|\bar{b}^i\rangle = \sum_{j=1}^{M} P_{ij} |b^j\rangle,$$

and for $i > \bar{M}$, $|\bar{b}^i\rangle$ is orthogonal to the hyperplane. These latter basis vectors “point out” of the hyperplane in which the data lie, and correspond to “directions” which have nothing to do with the entire data set.

In changing to this new basis, the spectra $|I^i\rangle$ can then be expressed as

$$|I^i\rangle = \sum_{j=1}^{M} \bar{I}_{ij} |\bar{b}^j\rangle = \sum_{j=1}^{\bar{M}} \bar{I}_{ij} |\bar{b}^j\rangle,$$

where

$$|I^i\rangle = \begin{pmatrix} \bar{I}_{i1} \\ \vdots \\ \bar{I}_{i\bar{M}} \\ 0 \\ 0 \\ \vdots \\ 0 \\ 0 \end{pmatrix},$$

that is, the components after $\bar{M}$th one are all zero (i.e. $\bar{I}_{ij} = 0$ for $j > \bar{M}$). This means that we would need only $\bar{M}$ pieces of information, instead of all $M$ of the original intensity measurements, to describe the entire data set in Eq. (3.2) exactly. This change of basis then amounts to a dimensional reduction. Unlike the component $I_{ij}$ (i.e. the old variables describing the data), note that the barred component $\bar{I}_{ij}$ can no longer be interpreted as the intensity measurement at frequency $\nu_j$ in the pixel $i$. The new components are already linear combinations of the original spectral
measurements. These can be viewed as a (linear) change of variables. We can write

\[ |I^i⟩ = ∑_{j=1}^{M} I^i_j |b^j⟩ = ∑_{j=1}^{M} I^i_j \left( ∑_{k=1}^{M} P^j_k |b^k⟩ \right) = ∑_{j=1}^{M} \left( ∑_{k=1}^{M} I^i_j P^j_k \right) |b^k⟩. \] (3.8)

Now comparing this to Eq. (3.4), we see that

\[ I^i_k = ∑_{j=1}^{M} I^i_j P^j_k, \] (3.9)

or

\[ I^i_k = ∑_{j=1}^{M} \left( P^j_k \right)^{-1} I^i_j, \] (3.10)

where \( \left( P^j_k \right)^{-1} \) is the inverse of the matrix \( P^j_k \).

Even in the more realistic case where correlations between measurements are not perfect (so that there does not exist a hyperplane within which all spectra exactly lie), it often happens that the data set can still be “well-approximated” by a hyperplane. And by “well-approximated” we mean that deviations between the spectra and some hyperplane are minimal, or in other words, that all the spectra lie “close” to some hyperplane. In this case, changing to an appropriate basis is still worthwhile because fewer than \( M \) would be sufficient to approximately describe the entire data set. With the new basis, the spectra can be approximated by

\[ |I^i⟩ \approx ∑_{j=1}^{M} I^i_j |b^j⟩. \] (3.11)

Note that this is now just an approximation instead of the exact equality in Eq. (3.6). The components in this new basis do not have to exactly vanish for it to be useful. But in typical applications (such as ours), only a few of the components are needed to approximately represent the entire data set.

At its core, PCA is a prescription for finding the “best” set of basis vectors with which to represent the entire data set in Eq. (3.2). In this specific context, the basis vectors \( \{ |b^j⟩ \} \) (which are linear combinations of the natural basis vectors) are called the principal components or the loading vectors (or just loadings). A given data set (in our context, corresponding to a given implant) will have a unique set of loading vectors according to the PCA prescription. The new components \( \bar{I}^i_j \), which are projections of the spectra onto the new basis are often called scores.
3.3 Principal components are eigenvectors of the covariance matrix

Up to now, we have been loose about what we might mean by the “best” set of basis vectors for a given data set. Intuitively, we imagine the spectra at each pixel to correspond to higher-dimensional arrows and we imagine the arrows to “lie close” to some hyperplane of lower dimension than the original dimensionality of the data set (specifically, the number of frequencies at which intensities are measured, which in the previous section was \( M \)). We thus want the hyperplane to capture as much of the variation in our data set as possible.

To make this more precise, we shall need to review a few concepts. But first, we reprocess our data set by centering our data about its mean. Consider the \( j \)th row vector of the data set represented by the matrix of Eq. (3.2) (i.e. all the intensity measurements for each pixel at frequency \( \nu_j \)):

\[
\langle I_j \rangle = (I^1_j, I^2_j, I^3_j, \ldots, I^N_j),
\]

where again we use the familiar Dirac “bra” in his bracket notation to denote rows. Note that in this notation, \( |I_j\rangle \) is a column vector, given by the transpose of the row vector \( \langle I_j \rangle \), and we can represent the matrix product between row and column succinctly as

\[
(I^1_j, I^2_j, I^3_j, \ldots, I^N_j) \times (I^1_k, \ldots, I^N_k) = \sum_{i=1}^{N} I^i_j I^i_k \equiv \langle I_j | I_k \rangle.
\]

We shall then subtract from each of these the average (over all pixels)

\[
\langle I_j \rangle = \frac{1}{N} \sum_{k=1}^{N} I^k_j,
\]

that is

\[
(I^1_j, I^2_j, I^3_j, \ldots, I^N_j) \mapsto (I^1_j - \langle I_j \rangle, I^2_j - \langle I_j \rangle, I^3_j - \langle I_j \rangle, \ldots, I^N_j - \langle I_j \rangle).
\]
Henceforth, when we talk about data sets, we shall refer to mean-centered ones.

The variance over all pixels (at a particular frequency) is

$$\sigma^2_{(I_j)} \equiv \sigma_j^2 = \frac{1}{N} \sum_{i=1}^{N} (I^i_j - \langle I_j \rangle)^2. \quad (3.16)$$

But since we have already centered to the mean, this is simply

$$\sigma_j^2 = \frac{1}{N} \sum_{i=1}^{N} (I^i_j)^2 = \frac{1}{N} \langle I_j | I_j \rangle \quad (3.17)$$

The covariance between measurements at two different frequencies, $\nu_p$ and $\nu_q$, is

$$\sigma^2_{I_p I_q} \equiv \sigma^2_{pq} = \frac{1}{N - 1} \sum_{i=1}^{N} I^i_p I^i_q = \frac{1}{N - 1} \langle I_p | I_q \rangle. \quad (3.18)$$

We note first that if $p = q$, $\sigma^2_{pq} = \sigma^2_p$ (i.e. the covariance of the $\nu_p$-measurements with itself is just its variance). More importantly, $\sigma^2_{pq} = 0$ if and only if the $\nu_p$-measurements are completely uncorrelated with the $\nu_q$-measurements.

It helps to recall now the original basis vectors, $\{|b^i\}\$, and what they physically mean. A basis vector $|b^1\rangle$ corresponds to an unphysical spectrum of unit intensity at frequency $\nu_1$ and of zero intensity at all other frequencies. The intensity measurements at $\nu_1$ over all pixels characterize the data set in the direction of $|b^1\rangle$. Typically though, the variation in the intensity at $\nu_1$ is correlated with the variation at some other frequency, say $\nu_q$. Again, this means that the measurement at $\nu_q$ is somewhat redundant, since some (or all) of the information it can convey about our set of pixels is already contained in the $\nu_1$-measurements. Correlations between $\nu_j$-measurements are quantified by the covariances between the row vectors (i.e. such as Eq. (3.12)) of our mean-centered data set in Eq. (3.2). These covariances represent informational redundancy.

To eliminate redundancy of measurements, the PCA prescription is to find a new basis such that the covariances of the row vectors of

$$\begin{pmatrix} \bar{I}_1^1 & \bar{I}_1^2 & \ldots & \bar{I}_1^N \\ \bar{I}_2^1 & \bar{I}_2^2 & \ldots & \bar{I}_2^N \\ \vdots & \vdots & \ddots & \vdots \\ \bar{I}_M^1 & \bar{I}_M^2 & \ldots & \bar{I}_M^N \end{pmatrix}$$

are zero. (The matrix above corresponds to the same data set as in Eq. (3.2) expressed
in the new basis). Again the rows in this new matrix no longer represent intensity measurements at a fixed frequency over all pixels, but rather linear combinations of these measurements at different frequencies. The variances of the new rows do not necessarily vanish. And in fact, the row with greatest variance becomes the most informative measure for discriminating the pixels – “variance is information”.

Another important part of PCA is that it ranks its new basis vectors according to these variances, so that row of greatest variance is the first row, and so on.

The mathematical object that succinctly captures these ideas is the so-called covariance matrix. Given the original data set (in the natural basis), this is the matrix \( S_{ij} \) with matrix elements given precisely by the covariances between the row vectors of Eq. (3.2):

\[
S_{ij} = S_{ji} = \frac{1}{N-1} \langle I_i | I_j \rangle = \sigma^2_{ij}.
\]

The covariance matrix is a very useful object in data analysis. We first note that it is a square \( M \times M \) matrix, where the dimensionality \( M \) is given by the number of “measurement types” in the data set. In our case, this is the number of frequencies in the measured spectrum. Also, the diagonal terms of the covariance matrix are just the variances over all pixels.

Having defined all of this, the mathematical PCA problem of finding the “best” basis that gets rid of all covariances is that of diagonalizing the covariance matrix, a standard problem in linear algebra. This amounts to computing the eigenvalues and eigenvectors of \( S_{ij} \), or solving the eigenvalue equation

\[
([S] - \bar{\sigma}^2_j |I|) |\bar{b}^j\rangle = 0,
\]

where \( \bar{\sigma}^2_j \) is the eigenvalue corresponding the eigenvector \( |\bar{b}^j\rangle \). The eigenvector is purposely written as the “new” basis vector that best represents the data set. Moreover, the corresponding eigenvalues automatically give the variances of the data set in each of the new variables. Detailing the methods for solving Eq. (3.21), like the singular value decomposition in particular, would take us beyond the scope of this thesis. Suffice it to say that for the large data sets we deal with (\( M \geq 231 \) frequencies), statistical software such as R takes care of solving this eigenvalue equation for us.

In this new basis the covariance matrix \( \bar{S}_{ij} \) takes the diagonal form

\[
[\bar{S}_{ij}] = diag(\bar{\sigma}^2_1, \bar{\sigma}^2_2, \ldots, \bar{\sigma}^2_M),
\]

where
where the variances are arranged such that $\bar{\sigma}_1^2 > \bar{\sigma}_2^2 > \ldots > \bar{\sigma}_M^2$. The basis vectors that give us this diagonal representation for the covariance matrix are the one in Eq. (3.5) and are called loading vectors or loadings.

As already mentioned, the new components $\bar{I}_i^j$, which are projections of the original spectra (the column vectors) onto this new basis,

$$|I^i⟩ = \sum_{j=1}^{M} \bar{I}_i^j |\bar{b}^j⟩,$$

(3.23)

are called scores. In R, this matrix of scores is the output of a PCA calculation:

$$\begin{bmatrix}
\bar{I}_1^1 & \bar{I}_1^2 & \ldots & \bar{I}_1^N
\bar{I}_2^1 & \bar{I}_2^2 & \ldots & \bar{I}_2^N
\vdots & \vdots & \ddots & \vdots
\bar{I}_M^1 & \bar{I}_M^2 & \ldots & \bar{I}_M^N
\end{bmatrix} \text{ } M \text{ scores at each pixel}$$

(3.24)

The real benefit of all this machinery comes from the fact that because we have ordered the new basis vectors according to their information content (or the variance of the data along the basis directions), it typically happens that we can write,

$$|I^i⟩ \approx \bar{I}_1^1 |\bar{b}^1⟩ + \bar{I}_2^2 |\bar{b}^2⟩ + \bar{I}_3^3 |\bar{b}^3⟩.$$

(3.25)

Thus it is enough to capture most of the information of the data set with only the first few scores of each pixel (sometimes even just the first score):

$$\begin{bmatrix}
\bar{I}_1^1 & \bar{I}_1^2 & \ldots & \bar{I}_1^N
\bar{I}_2^1 & \bar{I}_2^2 & \ldots & \bar{I}_2^N
\bar{I}_3^1 & \bar{I}_3^2 & \ldots & \bar{I}_3^N
\end{bmatrix} \text{ } \text{just 3 scores at each pixel, instead of } M = 231 \text{ intensities}$$

(3.26)

The reduction from $M = 231$ intensities to just 3 scores for each pixel constitutes a significant dimensional reduction, thus illustrating the power of PCA.

The number of loadings (or scores) one needs depends of course on the intended application, but for the purpose of monoclinic determination described below at most only three loadings have been needed. By construction, the set of first scores $(\bar{I}_1^1, \bar{I}_2^2, \ldots \bar{I}_N^N)$ has the largest variance and is the most useful quantitative discriminant between the pixels.
To understand this less abstractly, a representation of Equation (3.25) in actual data is shown in Figures 3.2 and 3.3. We take a single spectrum at a given pixel and center it at the mean. The resulting mean-centered data is equal to the sum of the first two loadings multiplied by their corresponding scores plus a small residuals.

Figure 3.2. Mean-centering of the spectrum.
3.4 PCA for monoclinic zirconia determination

When given high-quality (mostly tetragonal) zirconia samples such as ours, which is almost spatially homogeneous except for tiny concentrations of monoclinic phase, it is impossible to ascertain the presence of monoclinic ZrO$_2$ at a given pixel/point on the surface just from the Raman spectra. Plotting the spectra from all pixels together
shows that they are highly degenerate (see Figure 3.1 above), making it impossible to differentiate monoclinic-contaminated pixels from purely-tetragonal pixels. PCA allows us to tease out one class of pixels from the other by using coordinates (i.e. the first few scores) that give the greatest variation between pixels.

Our guiding expectation was that a significant variation between the spectra taken at different points on the surface (different pixels) could be attributed to differences in monoclinic content. In which case, monoclinic content should be picked up by one of the first few loading vectors. From the Raman maps of each ZrO$_2$ sample (these maps are essentially the set of spectra at each pixel), we compute up to about seven loading vectors and check if any of them peak around the monoclinic excitation band between 175 cm$^{-1}$ and 200 cm$^{-1}$. (Typically, beyond the 7th, the loading vectors have very little discernible structure that we can associate with monoclinic content.) If none of the loading vectors peak around the frequency band of interest, then we consider the monoclinic concentrations within the sample too small to be detected. In most samples, however, we are able to identify a monoclinic-like loading vector. Thus, the score associated with this monoclinic-like loading vector indicates the presence (or absence) of monoclinic phase. We subsequently produce a map of the scores to determine the spatial distribution of the monoclinic concentrations within our sample.

To summarize, PCA provides a useful coordinate with which to indicate the presence of monoclinic phase on the surfaces of implants. This method can be used to produce spatial maps of monoclinic distribution, which is useful in the characterization of zirconia implants.
Chapter 4

Fine determination of monoclinic content and hydrostatic stress in zirconia-based implants

4.1 Introduction

Stabilized zirconia is a ceramic material known for its excellent mechanical properties and biocompatibility, and has been widely employed as a biomaterial [64] - most recently including dental and spinal implants [8]. However, Low-Temperature Degradation (LTD) [8], associated to a phase transformation from tetragonal to monoclinic. This transformation leads to a loss of mechanical properties [8, 65] - makes detection of low levels of monoclinic a critical aspect in developing and engineering zirconia-based biomaterials.

4.1.1 Quantitative Determination of Monoclinic Phase in Zirconia by Raman Spectroscopy

Raman spectroscopy is an effective analytical tool for detecting the presence of monoclinic phase in samples. The basis of this technique relies on the fact that the intensities of light scattered from monoclinic and tetragonal phases are proportional to their concentration. To illustrate this, let us examine the Raman spectra of various phases zirconia depicted in Figure 4.1 [66]. The bottommost curve represents a purely monolinic phase, where the characteristic doublet at 180 cm$^{-1}$ and 190 cm$^{-1}$ is observed. The topmost curve, which is a purely tetragonal phase zirconia, has
prominent peaks at 147 cm\(^{-1}\) and 265 cm\(^{-1}\) that are visible while the monoclinic doublet is suppressed. The two intermediate curves are the spectra of zirconia containing mixed concentrations of monoclinic and tetragonal phases, where all four peaks are visible simultaneously. Clearly, the intensities of light scattered from monoclinic and tetragonal phases are proportional to their concentration. For such materials which only contain monoclinic and tetragonal phases, the individual volume fractions, \(V_m\) and \(V_t\), can be linearly expressed as \(V_m + V_t = 1\). These are the conditions which make the current quantitative detection methods possible. When detectable concentrations of monoclinic phase are present, isolated peaks can be used to calculate the actual monoclinic content.

![Figure 4.1. Raman spectra of yttrium-stabilized zirconia under heating showing the characteristic peaks as the structure transforms from monoclinic phase to tetragonal phase.](image)

We will follow the method that is described in Ref. [16]. In practice, a single Raman spectrum of the sample is taken, the data is fitted to standard curves from which the integrated intensities at the appropriate peaks are extracted. The proposed linear model for the calculating the volume fraction of monoclinic, \(V_m\), is given by the expression,

\[
V_m = \frac{I_{180}^m + I_{190}^t}{k(I_{147}^t + \delta I_{265}^t) + I_{180}^m + I_{190}^m}
\]

(4.1)
where \( I \)'s are the integrated intensities and \( k \) and \( \delta \) are calibration constants. The integrated intensities are calculated by fitting the prominent peaks of the spectra with mixed Lorentzian and Gaussian distributions as opposed to the one described in literature where Lorentzian distributions with a linear background. \( k \) and \( \delta \) were determined from the linear fitting procedure performed by Katagiri et al \cite{67}. In their work, \( k \) is found to be \( 2.2 \pm 0.2 \) and \( \delta = 0 \). Setting \( \delta \) to 0 means that the peak intensity at 265 cm\(^{-1}\) is not included in the fitting.

Two other groups, namely Clarke and Adar \cite{68} and Lim et al \cite{69} have done a similar calculation to extract the value of \( k \) and \( \delta \). While the calibration done by Clarke and Adar (\( k = 0.97 \) and \( \delta = 1 \)) is the most widely used to quantify the monoclinic phase fraction, recent work \cite{16} showed that their proposed equation underestimates the monoclinic content. Lim et al \cite{69} has also retrieved a more accurate monoclinic concentration with \( \delta = 1 \) and \( k = 3 \pm 0.3 \). Based on Figure 4.2, which plots the calculated versus the predicted \( V_m \), both Katagiri and Lim were able to recover the \( V_m \) concentrations that are consistent with the actual value. However, an advantage of Katagiri’s procedure is that only the tetragonal peak at 147 cm\(^{-1}\) is used, this eliminates having to fit the overlapping peak at 224 cm\(^{-1}\).

![Figure 4.2](image.png)

**Figure 4.2.** Plot of the predicted vs actual volume of monoclinic concentration as performed by various groups.

Once the integrated intensities are determined and a choice of the appropriate calibration constants is made to be that obtained by Katagiri, Eqn. (4.1) is then
easily evaluated as
\[ V_m = \frac{I_{m180} + I_{m190}}{2.2 \cdot I_{t147} + I_{m180} + I_{m190}} \] (4.2)

It is important to emphasize, however, that the procedure just described only works when large concentrations of monoclinic phase are present in the sample. Volume concentrations greater than 10% can be conveniently fitted using this technique. However, distinct and detectable peaks are required to be able to perform the fitting.

4.1.2 Zirconia with very small monoclinic concentrations

When the monoclinic content is extremely small, especially near the onset of the t-m transformation, a single Raman spectrum will not show the characteristic monoclinic peaks. For such cases, employing the previous method can lead to the wrong conclusion that no monoclinic phase is present. Instead, an array of Raman spectra has to be collected in the regions of interest.

Principal Component Analysis (PCA) can then be applied to the array of spectra. PCA is a very useful data processing tool, which has been shown to be sensitive to small scale changes in particular spectral features. In using PCA, several new bases that are linear combinations of the original ones are generated. Then data points are re-expressed as projections onto the new bases. Each new basis vector is called the principal component (PC) or loading vector, which represents an axis rotated from the original. The data in the rotated axis are assigned as scores. As discussed in Chapter 3, among these loading vectors, we are able to choose the most relevant one. In some cases, since the first component is always in the direction that contains the greatest variance, one loading is sufficient. If one is looking for finer features, more loadings are needed. For the application to zirconia, the most important component is the one that most emphasizes the presence of monoclinic phase. If there is even a little amount of monoclinic in the sample, the first few scores will show monoclinic peaks at around 181 cm\(^{-1}\) and 190 cm\(^{-1}\) in the new axis. Combining the 2D coordinates in the map with this data, we will be able to plot a map showing the spatial distribution of the monoclinic phase.

4.1.3 Stress Measurements using Raman Spectroscopy

Aside from the presence of peaks in particular bands, another indispensable feature found in spectroscopic data is the shift of the characteristic bands. Because of this,
it is possible to perform piezospectroscopic measurements and quantify the amount of stress in the sample. An illustration of this is found in Figure 4.3. Very little to stress-free zirconia have $\nu_{265}$ slightly higher frequency compared to that containing residual stress.

Figure 4.3. Illustration of stress-induced frequency shift in Raman bands. The amount of stress is proportional to the shift.

Tomazewski, et. al. [9], indirectly determined the piezospectroscopic coefficient of Ce-TZP. It was described that for a free-standing piece containing random distribution of grains, the relationship between the observable shift in spectral peaks and stress can be described by the equation:

$$\Delta \nu = \Pi \langle \sigma \rangle$$  \hspace{1cm} (4.3)

where $\Pi$ is the piezospectroscopic (PS) coefficient, $\langle \sigma \rangle$ the spatial average of the trace of the stress tensor. An assumption in this work is that the equilibrium condition

$$f_1 \langle \sigma \rangle_1 + f_2 \langle \sigma \rangle_2 = 0$$  \hspace{1cm} (4.4)

holds true. $f_1$ and $f_2$ are volume fractions which are determined during sample preparation. Rearranging Eqn. (4.3) and Eqn. (4.4), we have

$$\Pi = -\frac{f_2 \Delta \nu_2}{f_1 \langle \sigma \rangle_1}$$  \hspace{1cm} (4.5)

With the parameters of material 1 is determined, $\Pi$ will also be known. The advantage of Eqn. (4.5) is that it is valid for any shift in the Raman band of material 2. There is no instrumental restriction in using this equation. Frequency shifts can
be obtained either by spectroscopic or diffractive means. The origin of stress is also irrelevant in the calculation. It can be used for randomly combined ceramics that are immiscible.

As an example, Tomazewski, et. al. [9] performed the stress measurements on zirconia/alumina composite. The hydrostatic stress is induced by varying the alumina content and sintering at 1600 °C. The stress-free sample was a pure alumina sample processed in the same manner. Once the stress in alumina was determined, it is now possible to determine the stress on zirconia through systematic implementation of Eqn. (4.5). The important results for us are the calculated values for PS coefficient of the Raman bands of zirconia summarized in Table 4.1, which are the slopes of the lines in Figure 4.4.

<table>
<thead>
<tr>
<th>Raman Band cm⁻¹</th>
<th>PS Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>141</td>
<td>-1.8 ± 0.1</td>
</tr>
<tr>
<td>256</td>
<td>4.9 ± 0.2</td>
</tr>
<tr>
<td>316</td>
<td>-5.3 ± 0.3</td>
</tr>
<tr>
<td>457</td>
<td>-5.9 ± 0.3</td>
</tr>
<tr>
<td>634</td>
<td>-1.8 ± 0.1</td>
</tr>
</tbody>
</table>

Table 4.1. Piezospectroscopic Coefficients of tetragonal Ce-TZP Raman bands.

Figure 4.4. Plot of frequency shift of the Raman bands versus the average hydrostatic stress. Ce-TZP Raman bands are located on the tension side while alumina bands are on the compression side.
4.2 Materials and Methods

Materials
All the samples were provided by the partners in the LONGlife consortium. Four fully dense 3Y-TZP samples were subjected to pin-on-disk tribological tests, leaving distinct wear marks on the samples’ surface. Two of the four samples had been treated in autoclave before the tribological test. All samples present an evident circular mark. Rough portions are distinguishable from the smooth parts.

The second type of sample is Ce-TZP that have undergone biflexure testing. Branch-like surface deformations on this sample are visible under a microscope. All the samples were measured in their as-received state.

Raman Instrumentation and Mapping
Spectroscopic measurements were done using an inVia Raman system (Renishaw, Wotton-under-Edge, UK) shown in Figure 4.5. The Raman system is equipped with a combination of optics for directing incoming and collecting scattered light, an optical microscope, grating monochromator and a CCD camera. Collection of the spectra is done in a 180° scattering configuration. The microscope objective used 50x with numerical aperture (NA) of 0.75. The corresponding penetration depths in polycrystalline zirconia is 10-15 µm. Laser used was a Toptica laser with excitation wavelength of 785 nm and the grating used was 1200 l/mm. Standard spectral map were obtained using the 576 pixel CCD. The accompanying Wire software for spectral acquisition was also utilized.

An array of Raman spectra was first collected from a radial sector areas across the pristine and the worn surface of each sample’s surface in order to identify possible large-scale inhomogeneities. Map sizes vary depending on the area of interest, but typically ranges from 300 µm by 600 µm and 200 µm by 2000 µm consisting of about 1300 spectra or higher. High-resolution mapping on Ce-TZP is of the size 7 mm x 7 mm and increment of 40 microns. The number of spectra was kept around 30 000 spectra.
4 – Fine determination of monoclinic content and stress

Data Analysis using Principal Component analysis
All data pre-processing and analysis was performed using several packages of the R software for statistical analysis [70]. Raman and SERS spectra were analyzed with hyperSpec [71], an R package to handle hyperspectral data sets. The spectral array was treated by means of standard univariate analysis and by multivariate analysis based on Principal Component Analysis (PCA), also an R package.

4.3 Results and Discussion

4.3.1 Monoclinic Phase Detection of 3Y-TZP
The amount of monoclinic is generally extremely small, below the confidence limit of any quantitative detection method currently known. Figure 4.6 is a single spectrum on the rough surface of the disc and it shows only six peaks belonging to the tetragonal structure. Simple observation and fitting of individual Raman spectra would suggest that the sample is purely tetragonal.

We analyze now the collected spectral array. First, we performed background correction using a linear baseline and normalization. After this, we plot the intensity map. The intensity map shown in Figure 4.7 corresponds to the integrated area under the curve within the Raman shift range of 124 cm$^{-1}$ to 733 cm$^{-1}$. We can observe that the distinct line in the middle that separates the figure into two marks the interface between the pristine and the worn side of the sample. The top is is the worn side with small red spots (indicated by the circles). From this map, we can say that there are no large-scale inhomogeneities. There are no other striking features observed except for the tiny red spots at the upper half.
4.3 – Results and Discussion

Figure 4.6. Single Raman spectrum obtained from a spot on the roughened portion of the disc.

Figure 4.7. (A) Diagram of the disc with circular wear marks (shaded ring) indicating the area of measurement (red rectangle), which includes rough and pristine portions. (B) Intensity map of the spectral array which is equivalent to the integrated area under the curve within the frequency range of 124 cm\(^{-1}\) to 733 cm\(^{-1}\). Red circles on the map highlight the striking features. The faint line in the middle denotes the rough-pristine interface.
To be able to interpret the meaning of the red spots, we have to perform PCA. The results of PCA are presented through scores and loadings. The loading that we are interested in is the one that will contain an information about the monoclinic phase, i.e., the presence of the characteristic doublet peak at $\sim 180 \text{ cm}^{-1}$ and $190 \text{ cm}^{-1}$. The scores can be plotted in the same manner as the intensity map. Figure 4.8 summarizes the results for on Sample 1. Shown in this figure is the loading containing the monoclinic feature. Also visible are islands of approximate size of 50-100 $\mu$m and containing 1-2 vol.% of monoclinic emerging from areas where monoclinic is barely detectable. We can now confirm that the red spots found in the intensity map accounts for the presence of monoclinic phase zirconia.

The succeeding figures present a gallery of representative results that can be obtained in PCA. Figure 4.9A is the plot of the mean and standard deviation of all the spectra in Sample 2. It is fully tetragonal phase as confirmed by the three loadings. Maps are not shown since they do not represent any physical meaning. This is an example of the case where there is no monoclinic phase present.

Sample 3, similar to Sample 1, contains islands of monoclinic phase as shown in Figure 4.10. This sample has a more shallow marking, thus the interface is only slightly visible.

Figure 4.11 shows the results for Sample 4. We can see that the first PC represents the monoclinic content. This sample contains a smaller island of monoclinic phase of about 50 $\mu$m compared to Sample 1. We also observed other PCs (Figure 4.12) and found that the third PC feature a sharp change in sign from the negative to positive in the vertical axis. This indicates that the Raman band at $\sim 255 \text{ cm}^{-1}$ is shifting due to residual stress.

### 4.3.2 Monoclinic Content Determination and Piezospectroscopy of Ce-TZP

**Monoclinic Content**

The mechanical testing on the Ce-TZP sample produced a branch-like surface deformation propagated radially outward from the center. Surface uplift is visible in Figure 4.13. The distribution of the monoclinic content in the sample is shown in Figure 4.14. The highest monoclinic content is concentrated mostly at the center. With high density spectral mapping, we are also able to resolve finer defects found in between the major branches.

The area of analysis can be selected to look into a particular branch. In Figure
Figure 4.8. Sample 1: (A) Plot of the first loading vector, which contains monoclinic information. (B) Map showing the scores corresponding to the plot in (A) where the bright red spots indicate the area of incipient $t$-$m$ transformation while the blue regions consist of purely tetragonal phases.
Figure 4.9. Sample 2: (A) Mean and standard deviation of the spectral array composed of 1300 spectra. (B) Plot of the first three loading vectors or principal components showing that monoclinic phase feature between 180 cm\(^{-1}\) and 190 cm\(^{-1}\) is absent.

Figure 4.10. Sample 3: (A) Plot of the first loading vector or principal component (B) Map of the corresponding scores indicating the areas of large monoclinic content.

4.15, we calculated the monoclinic content along a branch (indicated by the dashed line) again using the technique described in Sec. 4.1.1 with Eq. 4.1. The amount of monoclinic phase is about 75 vol. % at the center and amount tapers to 57 vol. % towards the end of the branch.

The monoclinic profile across a branch is shown in Figure 4.16. We find that the monoclinic is highest in the middle of the branch and decreases sharply towards the sides.
4.3 – Results and Discussion

Figure 4.11. Sample 4: (A) Plot of the first loading vector, which contains the feature for monoclinic phase. (B) Map of the corresponding scores indicating the areas of larger monoclinic content.

Figure 4.12. Sample 4: (A) Plot of the third loading vector, which contains a feature about shifting of band at $\sim 250$ cm$^{-1}$. (B) Map of the corresponding scores indicating the areas of large bandshift due to residual stress.

Piezospectroscopy

For the stress calculation, we considered the difference between the tetragonal peak positions at $\nu_1 \sim 140$ cm$^{-1}$ and $\nu_2 \sim 260$ cm$^{-1}$ as derived from spectral fitting of
Figure 4.13. Micrograph of surface deformation on Ce-TZP sample as a result of biflexure testing. Surface uplift is visible due to transformation from tetragonal to monoclinic structure.

Figure 4.14. Overall monoclinic phase distribution across the 7 mm × 7 mm area on the sample surface. Color scale indicates the presence of high monoclinic content (red) at the center, lower monoclinic content (yellow to green) are located at the branches and tips, and little to none (blue to black) are in between the branches.

the equation

$$\sigma = \frac{\Delta \nu - \Delta \nu_0}{\pi_2 - \pi_1}. \quad (4.6)$$
4.3 – Results and Discussion

Figure 4.15. Monoclinic map (left) with dashed line indicating the selected region along a single branch and plot (right) of monoclinic content along a branch as a function of distance from the center of the map.

Figure 4.16. Monoclinic map (left) with dashed line indicating the selected region across a single branch and plot (right) of monoclinic content as a function of edge-to-edge distance across the branch.

\[ \Delta \nu = \nu_2 - \nu_1 \] denotes difference in peak positions in the stressed state and \( \Delta \nu_o = \nu_{2,o} - \nu_{1,o} \) denotes the equilibrium state. Here, we utilize the derived PS coefficients in Table 4.1, \( \pi_{2,260} = +4.9 \) and \( \pi_{1,140} = -1.8 \).

Shown in Figure 4.17 is the stress distribution on the sample surface. The maximum stress is located at the center, which was the point of contact during testing. It is also the location of high monoclinic content. It can be matched with the corresponding monoclinic map in Figure 4.14.

Similarly, we can also examine the stress at certain points of interest in the map. Figures 4.18 and 4.19 show the stress distribution along a branch and across a branch, respectively.
Figure 4.17. Overall stress distribution across the 7 mm × 7 mm area on the sample surface. Color scale indicates the maximum stress (yellow) are located at the center and lower stress (red) at the branches and tips. Region of little or no stress (purple) are located at the edges and in between the branches respectively. As expected, the position with high monoclinic content experience higher stress.

4.4 Conclusion

In this chapter, we described how we detected very low concentrations of monoclinic phase, which are indicative of the incipient $t \to m$ transformation. Principal component analysis was applied to achieve monoclinic phase detection and determination of residual stress through band shifting. With the aid of high-resolution mapping, the spatial distributions of both the monoclinic phases and stress were visualized. Our technique has advantages over diffraction methods because it is able to locate the presence of monoclinic content lower than 1 - 2 volume %. Another invaluable advantage of our mapping is that it probes the over-all homogeniety of the sample. The ability to assess sample uniformity over large scales is a critical aspect in the design and development of zirconia-based implants.
4.4 – Conclusion

Figure 4.18. Stress distribution (left) with dashed line indicating the selected region along a single branch and plot (right) of residual stress along a branch as a function of distance from the center of the map.

Figure 4.19. Stress distribution (left) with dashed line indicating the selected region across a single branch and plot (right) of residual stress as a function of edge-to-edge distance across the branch.
4 – Fine determination of monoclinic content and stress
Chapter 5

Synthesis and Characterization of Nanostars as substrate for SERS

5.1 Introduction

Nanoparticles of various shapes, sizes and compositions have been studied for a wide variety of applications, a non-comprehensive listing of which includes drug delivery [72, 73], cancer diagnosis [74] and therapy [75], contrast enhancers for imaging [76], as well as surface-enhanced Raman spectroscopy (SERS) [77–80]. Nanoparticles are relatively straightforward and inexpensive to synthesize through wet chemical preparation techniques. Well-known routes are available for almost any desired morphology. Of particular interest for SERS are branched gold nanoparticles, or nanostars, because of their peculiar plasmonic properties [50–54].

At present, many sophisticated synthetic routes ranging from seed-mediated [51, 54, 81–83] to one-pot methods [84, 85] are widely available for nanostars. The most common seed-mediated synthesis requires use of cetyltrimethylammonium bromide (CTAB) [86–88], polyvinylpyrrolidone (PVP), N, N-dimethylformamide (DMF) solution [89, 90] and hydrazine [91]. Unfortunately, these surfactants are generally difficult to replace and challenging to functionalize for further applications [85]. Notable exceptions are the works of Casu, et al. [51] and Pallavicini, et al. [92], who have addressed this problem by using zwitterionic lauryl sulfobetaine (LSB) surfactant, which is significantly easier to remove than CTAB. The straightforward synthesis of stable gold nanostars with no impeding outer layer is attractive from the viewpoint of applications. Yuan et al. [83], recently reported a protocol for producing surfactant-free nanostars with very little amount of citrate, a well-known
biocompatible electrostatic stabilizer for gold nanoparticles. This protocol proves to be cost- and time-effective, in spite of being a seed-mediated route. However, in using this protocol, we have observed the occurrence of rapid aging (within 5 to 10 hours) in all our suspensions of surfactant-free gold nanostars; hereafter, by aging we mean a morphological change of the nanoparticles that is reflected in the UV-visible absorbance spectrum. To our best knowledge, long term stability and aging studies on surfactant-free nanostars have not been previously reported. A few papers on the stability of surfactant-stabilized nanoparticles exist. The stability studies performed by Trigari et al. [85] were done solely on nanostars fabricated with CTAB capping and they found that even in presence of surfactant, the nanostars can degrade after aging in suspension for some hours. Nanostars generally need to be coated to maintain stability. The use of thiolated compounds to maintain stability of nanostars is now becoming a standard. Examples of thiol-coated gold nanostars that gained excellent stability after surface coating with polyethyleneglycol-thiol (PEG-SH) was demonstrated in the works of Sironi et al. [93] and Cavallaro et al. [94]. Another stability study was done by Gao et al. [95], who used tandem coating of PEG-SH and MPA on spherical gold nanoparticles. Unlike these previous works, we focus on surfactant-free nanostars. We study the morphology and optical properties of surfactant-free nanostars upon aging at 4 °C, i.e. the common conditions of conservation for such kind of particles. We then propose to improve the existing protocol by forming a monolayer of MPA via Au-S bonding. One of the advantages of this approach is the solubility of MPA in water, which allows carrying out surface modification in an entirely aqueous environment.

5.2 Materials and Methods

5.2.1 Materials

Sodium tetrachloroaurate (III) dehydrate (AuCl\textsubscript{4}Na · 2H\textsubscript{2}O), sodium citrate tribasic dihydrate (Na\textsubscript{3}C\textsubscript{6}H\textsubscript{5}O\textsubscript{7} · 2H\textsubscript{2}O), hydrochloric acid (HCl), silver nitrate (AgNO\textsubscript{3}), L-ascorbic acid (C\textsubscript{6}H\textsubscript{8}O\textsubscript{6}), 3, mercaptopropionic acid (C\textsubscript{3}H\textsubscript{6}O\textsubscript{2}S), 2 mercaptoethanol (C\textsubscript{2}H\textsubscript{6}OS) were purchased from Sigma-Aldrich and used without further purification. Ultra pure Milli-Q water (Merck Millipore, Billerica, MA, USA) was used for the preparation of all solutions.
5.2.2 Synthesis

In accordance to the protocol proposed by Yuan et al. [83], we first synthesized spherical gold seeds. In a typical synthesis, aqueous solution of \( \text{AuCl}_4\text{Na} \cdot 2\text{H}_2\text{O} \) (100 ml, 1 mM) is brought to boiling point followed by rapid addition of sodium citrate (15 ml, 1% wt.). The solution was allowed to reflux for 15 minutes under vigorous stirring. After cooling down, the solution is kept at 4 °C. We then produced the nanostars by preparing a growth solution consisting of \( \text{AuCl}_4\text{Na} \cdot 2\text{H}_2\text{O} \) (10 ml, 0.25 mM), \( \text{HCl} \) (10 µl, 1 M) and 100 µl of gold seeds. Under moderate stirring, ascorbic acid (50 µl, 100 mM) and \( \text{AgNO}_3 \) (100 µl, 1.5 mM) were simultaneously added. Instantly, a bluish-black color is achieved. We performed centrifugal washing at 3500 rpm for 30 minutes. The extracted nanostars were redispersed in MilliQ water and filtered with 0.22 µm nitrocellulose membrane. The final product was kept at 4 °C. Surface modification of nanostars with MPA was done immediately after filtration by adding MPA (10 µl, 20 mM) to 10 ml of nanostars under vigorous stirring. The mixture was continuously stirred for 10 minutes at room temperature. No further centrifugal washing was done.

5.2.3 Characterization

Absorption spectra in the wavelength range of 400 - 800 nm were measured with UV-vis spectrophotometer (Perkin-Elmer, Lambda 20 Bio). The morphological features of the particles were characterized by Transmission Electron Microscope (TEM, Philips EM 208). The nanostar colloids were monitored for several days after the synthesis. On the day of the measurement, suspensions were sonicated for 10 minutes before recording the UV-vis spectrum. An aliquot was further diluted to 10 % of the original stock concentration and 10 µl were drop cast onto a carbon TEM grid. The grid was dried at ambient temperature and TEM images were obtained on the same day of deposition. We used Image J software (ver. 1.46r) to examine the TEM images.

5.2.4 Raman and SERS Measurements

Spectroscopic measurements were done using an inVia Raman system (Renishaw, Wotton-under-Edge, UK). 10x objective is used for measuring liquid drops while 50x objective is used when measuring drops dried on CaF\(_2\) slide. Drop typically consist of 1:4 analyte to nanoparticle volume ratio to create 50 µl drop. All drops are kept
at constant volume of 50 µl.

## 5.3 Results and Discussion

### 5.3.1 Morphological and Optical Characterization

Figure 5.1 shows a TEM image of the typical nanostars and gold spherical particles used as seeds, together with their characteristic UV-vis extinction spectra. The gold spheres are highly monodisperse and have a mean diameter of 12.0 nm ± 0.4 nm. A suspension of such seeds exhibits a spectrum with a sharp absorption maximum at 520 nm. The nanostars, on the other hand, have a broader size distribution with an average diameter of 78 ± 13 nm (measured from tip to tip across the nanostar). TEM images reveal that a single nanostar can contain sharp as well as blunt tips. The UV-vis absorption spectrum of Au nanostars exhibits a broad, intense band centered at 680 nm, along with a less intense shoulder at 520 nm.

We measured the absorption spectrum again after coating the nanostars with MPA. The primary absorption peak red-shifts by 10 nm, which is associated with the formation of a layer of MPA on the surface of the nanostars.

![Figure 5.1](image)

Figure 5.1. UV-vis absorption spectra of spherical Au seed (red), nanostars (blue) and MPA-coated nanostars (black). Insets: TEM images of gold seeds and nanostars. The scale bars shown correspond to 100 nm.

The tunability of the LSPR of the nanostars lies on the characteristics of the tips, which are controlled by the concentration of the silver nitrate. To demonstrate this, we also synthesized nanostars with different silver nitrate concentration (0.5 mM and 3.0 mM). Since morphology is strongly coupled with the optical properties, results
such as solution color (Figures 5.2), size (5.3) and UV-Vis absorption spectrum (5.4) can be inspected alongside each other. All data labeled as (A) have 0.5 mM, (B) have 1.5 mM and (C) have 3.0 mM silver nitrate. One can already visually observe the solution color differences that resulted from that simple change. Nanostar A is blue-violet in solution, Nanostar B solution is greenish blue and Nanostar C is bluish black when in solution. Their corresponding TEM micrographs (refer to Figure 5.3) show that low silver nitrate concentration produces nanostars (A) with less spikes and more blunt tips. When silver nitrate concentration is slightly increased, nanostars (B) have more spikes, longer and sharper tips. Further increase results to nanostars (C) that have even more number of spikes, not necessarily longer in length but with the most narrow tips compared to the other two. These different morphological properties affect the way the interact with light. In the UV-Vis absorption spectrum, blunt tips of nanostar (A) have maximum absorption, $\lambda_{max} = 640$ nm, sharper tips of nanostar (B), have $\lambda_{max} = 689$ nm and lastly, the sharpest nanostar (C) have $\lambda_{max} = 770$ nm.

![Figure 5.2](image1.png)  
(A) (B) (C)  
Figure 5.2. Photo of as-prepared nanostar suspensions showing the different shades of blue that are produced when (A) 0.5 mM, (B) 1.5 mM, and (C) 3 mM AgNO$_3$ concentration were used.

![Figure 5.3](image2.png)  
(A) (B) (C)  
Figure 5.3. TEM images of nanostars produced using (A) 0.5 mM, (B) 1.5 mM, and (C) 3 mM AgNO$_3$ concentration. Scale bars shown are 100 nm.
Figure 5.4. Extinction spectra of the nanostar colloids produced using (A) 0.5 mM, (B) 1.5 mM, and (C) 3 mM AgNO$_3$ concentration.

### 5.3.2 Long-Term Stability of Surfactant-free Gold Nanostars

We examined six batches of nanostars for the stability studies. All nanostars were produced with 1.5 mM of AgNO$_3$ (categorized as Nanostar B in Sec. 5.3.1). Three batches were surfactant-free while the rest were coated with MPA. Figure 5.5 summarizes the changes in absorption wavelength maximum ($\lambda_{\text{abs}}$) of different batches of nanostars. For surfactant-free nanostars denoted by SF1, SF2 and SF3, $\lambda_{\text{abs}}$ decreases with time indicating changes in the nanostar tip radius. Centrifugation and filtration do not effectively halt the aging process. $\lambda_{\text{abs}}$ continues to decrease in the following days of observation and the fall-off rate varies for different batches. SF1 has an initial absorbance at 689 nm and has the fastest decay rate, decreasing by as much as 67 nm within 9 days of aging. SF2 has an initial $\lambda_{\text{abs}}$ at 672 nm and it only decreased by 37 nm in a span of 9 days. The slowest decay rate was observed for SF3 with an initial $\lambda_{\text{abs}}$ at 693 nm and only decreased by 11 nm in 9 days. To show the long-term behavior of surfactant-free nanostars, we measured the UV-vis
spectra of SF1 after 11 months. The suspension continues to decay, as evident from the further decrease of $\lambda_{abs}$ and change in color. SF1 turned to purple with $\lambda_{abs}$ of 585 nm. As we have noted, the different batches of surfactant-free nanostars have different aging kinetics. We are currently investigating whether the starting primary absorbance peak can possibly be the determinant of this decay rate. However, from the examples we presented in this paper, no conclusive correlation can be made. More tests should be done on this.

Figure 5.5. Shift of the wavelength at maximum absorption upon aging at 4°C of surfactant-free (SF1, SF2 and SF3) and MPA-capped nanostars (M1, M2 and M3). The confidence on the wavelength is of the order of the dimensions of the data points.

To support our hypothesis that such changes in the extinction spectra can be explained in terms of morphological variations, we measured the radius of curvature of the nanostar tips. In Figure 5.6, the radius of curvature of the nanostar tips after aging in solution at 4 °C is reported. Each column represents the average of about 200 tips measured from the TEM images and the bar represents the standard error. For the three batches of surfactant-free nanostars (SF1, SF2 and SF3), we report the
average radius of curvature of the tip at day 0 (right after synthesis and filtration), and after one week of aging. For the three batches of MPA-capped nanostars (M1, M2 and M3), we report the radius at day 0 and after 30 days. The radius of the surfactant-free nanostars aged in solution for more than one week is consistently higher than the ones measured on the day of synthesis. On the other hand, no significant change is evident for the MPA nanostars even after 30 days. In Figure 5.7, we show the extinction spectra of M1 for day 0, after 7 days and after 6 months. The fact that the spectra remain unchanged confirms that there are no changes in the nanostar tips radius.

An example of blunting of the tips in surfactant-free nanostars is presented in Figure 5.8, which shows the nanostar SF1 at day 0, after 7 days and after 11 months. At the bottom panel of Figure 5.8, it is worth noting that the extinction spectrum after 11 months almost resembles that of a spherical particle with a larger diameter. This means that the contribution of the longitudinal mode to the plasmon resonance has decreased due to the loss of sharp protrusions. This is consistent with the observed decrease in the plasmon resonance wavelength of nanorods, which
are comparable to the spikes of the nanostars, with decreasing aspect ratio [96, 97]. We are led to conclude that the gold atoms from the tips are migrating towards the core and filling the valleys, in a mechanism analogous to a typical sintering process whereby atoms move from convex to concave regions driven by the chemical potential difference associated to the respective curvatures [98]. We hypothesize that upon addition of MPA on the surface of the nanostars, the Au - S bond limits the mobility of Au atoms via surface diffusion and kinetically hinders the diffusion of the Au atoms from tips toward the core. We noted earlier in Fig. 5.5 that the different batches of surfactant-free nanostars have different aging kinetics. We are currently investigating However, from the examples we presented here, no conclusive correlation can be made. More tests should be done on this.

Figure 5.7. Extinction spectra of MPA-coated nanostars, M1, recorded on day 0, after 7 days and after 6 months.

Upon coating the nanostars, we tested its sensitivity to detect the MPA bands. Figure 5.9 shows that the Raman bands of MPA are enhanced when AuNS are
5.3.3 SERS Results

Let us recall that the development of nanostars in this research was initially intended for application to solid zirconia. The SERS performance of the nanostars were done on liquid analytes to verify the effects of the different parameters on the SERS signal. In doing so, we hope to identify which type of nanostars would give the best enhancement. First, we used the freshly prepared bare nanostars in diluted solution of mercaptoethanol (ME). In Figure 5.10, the Raman bands are not visible in pure ME. Upon addition of nanostars, Raman bands between 600 cm$^{-1}$ to 1100 cm$^{-1}$ become visible, with intensity significantly higher for nanostars with $\lambda_{\text{max}}$ at 689 nm. It is common practice in SERS measurement that the LSPR of nanoparticles should match or very close to the excitation wavelength. However, we observed that the nanostar with LSPR at 689 nm gave a greater enhancement than that with 772 nm. Other groups [99, 100] have also recently reported greater enhancement at when the absorption maximum of the nanoparticle substrate is blue shifted from the excitation wavelength.
5.4 Conclusion

This chapter was devoted to developing a protocol for establishing long-term stability of nanostars. Caution should be exercised when using surfactant-free suspensions of nanostars as they undergo rapid morphological evolution especially during the first few days after synthesis. The rate of decrease in the absorption maximum wavelength, as a consequence of the decrease in the aspect ratio of the nanostar spikes, slows down but does not plateau even after several months of aging. The surfactant-free nanostars, after almost one year of aging in suspension, become more spherical in shape with larger diameter compared to the seeds used in producing them. Aging is the result of migration of atoms from the convex tips to concave valleys toward the core of the nanostars. We have shown that such aging can be prevented by capping the nanostar surface with a thiolated compound such as MPA, without greatly affecting the optical property of the nanostars. From our study, we find that MPA-coated nanostars exhibit much longer stability than surfactant-free nanostars.

Figure 5.9. Raman spectrum of 20 mM MPA (bottom, black) and SERS spectrum of 20 mM MPA with gold nanostars substrate (top, blue)
Figure 5.10. Raman spectrum of 1 mM ME, (bottom, black), SERS spectrum of AuNS($\lambda_{max} = 772$ nm) + ME (middle, red) and SERS spectrum of AuNS($\lambda_{max} = 689$ nm) + ME (top, blue)
Chapter 6

SERS on Solid Zirconia

With a protocol established for stabilizing nanostars, we are now in a better position to use them for SERS on solids. First, a simple experiment was performed to test the efficiency of the nanostars in enhancing the monoclinic signal in partially stabilized zirconia powders. Quite interestingly, an off-shoot result of our experiment reveals that gold nanostars can be utilized as nanoheaters, rendering gold nanostars-zirconia powder system a potential platform for studying transformation kinetics in ceramic materials. Details of this off-shoot study are relegated to Appendix A, as they seem to be outside the main narrative of this thesis. The rest of the chapter discusses the work done in using SERS techniques to enhance the monoclinic peaks in solid zirconia.

6.1 Introduction

Aging transformation has been the most problematic feature in stabilized zirconia, especially when utilized as implants or prostheses. A key step to preventing the detrimental failure in zirconia-based implants is early detection of \( t-m \) transformation in prototypes. At the time of incipient transformation, the monoclinic phases are concentrated at the surface. Early detection of the presence of monoclinic phases therefore entails detection of extremely little amounts of monoclinic phase (less than 1 - 2 vol %) at a thin layer on the sample surface. Efforts to identify a procedure to detect such small amounts of \( t-m \) transformation were described in Sec. 4.3.1. While the current procedure offers high reliability and sensitivity, advances in nanotechnology provide avenues for further improvement of the technique.

This work employs gold nanostars as substrate for SERS to amplify the monoclinic
peak in zirconia. SERS is now a well-established detection technique. While it is extensively used especially for the detection of trace amounts and even single-molecule of chemical species \([24, 101]\), it has not been widely applied to detection in solids. Very little work has been done to exhibit SERS in solid materials. Recently, SERS was performed on \(\text{SnO}_2\) hot-pressed pellet, pulsed-laser deposited \(\text{SnO}_2\) films, and \(\text{SnO}_2\) colloids in the presence of Ag \([37]\). For zirconia, in particular, to the best of our knowledge, there has only been one report on SERS in thin platinum electrodes deposited on yttria stabilized zirconia (YSZ) due to oxygen migration in closed circuits during catalytic activity \([38]\). However, YSZ has a porous surface similar to powders. It remains a challenge to achieve SERS enhancement on dense and polished solids, such as zirconia implants. Owing to the fact that the transformation to monoclinic commences at the surface, our guiding insight is that we would be able to tap into the surface modes of zirconia by enhancing the near field intensity. This amplification relies on small metallic nanoparticles whose sharp apexes can concentrate strong electromagnetic fields at its surface. This is the so-called 'lightning rod effect' . Due to its numerous sharp tips, nanostars take full advantage of this effect and offer exactly the geometry needed for this approach. To our knowledge, this work is an unprecedented attempt to apply SERS techniques through the addition of gold nanostars on the polished surface of fully-dense yttria-stabilized zirconia implants.

### 6.2 Materials and Methods

Typically, SERS on molecules using colloidal substrates is done by mixing the analyte with the nanoparticles first. Then, a small volume of the solution is dropped on a glass slide. The drop can immediately be measured even when wet. However, in doing SERS where the 'analyte' is a solid material, the solid disc replaces the glass slide and the nanoparticle 'substrate' is simply dropped or sprayed on top. The setup for performing SERS on solid is shown in 6.1. The drop has to be dried before measurement is done.

We used the Raman in confocal mode because we are interested in measuring surface monoclinic phase. Most monoclinic transformations originate and are concentrated at the surface (2 \(\mu\text{m} - 4 \mu\text{m}\)). The spectral map size is 900 \(\mu\text{m}\) by 160 \(\mu\text{m}\) with 5 \(\mu\text{m}\) increment composed of 5973 spectra. The origin centered at the interface of the smooth and worn parts (see Figure 6.2). First, the clean surface was measured.
6.2 – Materials and Methods

Figure 6.1. Setup for SERS measurement on zirconia disc. Zirconia disc serves as the base and the nanoparticle SERS substrate is dried on the surface.

Each spectrum was taken with 3s exposure time and single accumulation using 50x objective. Without moving the sample, we put 10 µl of nanostars making sure that the whole mapping area is covered and does not contain the edge of the drop. The nanostars used are MPA-stabilized and have LSPR at 690 nm. We let the drop dry before taking the next map with exactly the same parameters. For comparison, the whole process was repeated and Raman spectra were obtained in nonconfocal mode.

Figure 6.2. Mapping region on the zirconia disc. The map is centered at the interface between the worn and pristine interface.

All spectra were corrected with a linear baseline and normalized on all the spectra. Figure 6.3 shows the mean and standard deviation of all the spectra. We see that the area measured appears to be fully tetragonal both in the clean and with-nanostars state. Quantitative determination of monoclinic by spectral fitting is not possible since no detectable monoclinic peaks are present. We analyze the data further by essentially looking at the standard deviation. The region of interest is the position of the monoclinic peaks between the 170 cm\(^{-1}\) and 195 cm\(^{-1}\) Raman shift. Below we discuss our results.
6.3 Results and Discussion

6.3.1 SERS using AuNS with LSPR $\lambda_{max} = 690$ nm

Confocal mode
Figure 6.4 shows the standard deviation of all the spectra. The dashed lines indicate the region where monoclinic peaks appear. We see that there is more variation in the monoclinic peaks for the surface with nanostars. We then cut the spectra to include only the region between 170 cm$^{-1}$ and 195 cm$^{-1}$ and create a level plot. We can correlate the result of the level plot to the amount of monoclinic content because it only takes into account the integrated area under the curve between the frequencies for monoclinic peaks. From hereon, level plot pertains to the plot obtained from the integrated area under the curve segment between the frequencies, 170 cm$^{-1}$ and 195 cm$^{-1}$. The level plots for the clean surface and with nanostars are shown in Figure 6.5. Both level plots have uniform color scale. For the clean surface, the most noticeable feature is a small spot on the upper lefthand side. There is also a faint line at the -200 mark on the x-axis.

For the surface with nanostars (see Figure 6.5(B)), both the spot and the faint line became more clearly visible. The intensity was also higher as seen from the appearance of the red mark. The defect at the -200 mark formed a solid line all the way to the top of the map.

To uncouple the signal contributions from the high monoclinic from the low monoclinic regions, we analyze the two regions separately. It is possible to cut the map to include only the spectra of the region containing the strip of low monoclinic. Figure 6.6 shows the standard deviation of the selected spectra. We see that in the clean case, there are no large variations in the spectra in the region of the monoclinic peaks. In the presence of nanostars, the peaks emerge in the frequencies between 170 cm$^{-1}$ and 195 cm$^{-1}$.

We can appreciate the difference better in the level plot of the two cases in Figure 6.7. A clear line is visible when there are nanostars. We also see a red spot in the middle, indicating a higher monoclinic content in that area. Recall that it does not appear in the previous level plot in Figure 6.5 because it gets saturated by the intensity of the other spot with an even higher monoclinic concentration. Notice that the intensity scale here is only up to 0.8 units. Thus, we can more confidently conclude that monoclinic phases are present in this region.

Similarly, we get the portion of the map containing high monoclinic phase. In
Figure 6.3. Mean and standard deviation of normalized spectra of (A) clean surface and (B) surface with nanostars (AuNS$_{690\text{nm}}$). Spectra were taken in confocal mode.

Figure 6.8, we can see that there is still a noticeable increase in the variation in the nanostars case. The corresponding level plot (Figure 6.9) will show that it is due
Figure 6.4. Standard deviation of the all the spectra of (A) clean and (B) with nanostars (AuNS$_{690\,nm}$) surface. Spectra were taken in confocal mode. The region between the red dashed lines indicate the frequency range that is considered in constructing the level plot.

to the additional monoclinic detected in that area. The spot of monoclinic is larger when the map was obtained with nanostars on the surface.

Overall, the presence of nanostars offers a detectable enhancement of the monoclinic signal due to the intrinsic high concentration of electric field or hotspots located at the tips of the nanostars. The tips exhibit the lightning rod effect in efficiently capturing the incoming light, which results to the increased Raman scattering. The nanostar tips act as large antennae for electromagnetic field. In effect, it is similar to using a more powerful laser. The elevated near-field intensity compensates for the reduced scattering cross-section when operating in confocal mode.
6.3 – Results and Discussion

Figure 6.5. Level plot of the monoclinic region of the spectra, i.e. the integrated area under the curve between frequencies 170 cm$^{-1}$ and 195 cm$^{-1}$. Color scales shown are uniform for both plots. Spectra were taken in confocal mode.

**Nonconfocal mode**

We consider now the maps obtained in nonconfocal mode. All the maps were taken with exactly the same data collection settings and on the same area as before. The only difference is that the Raman signals were taken in nonconfocal mode. For brevity, the figures shown here are partial areas containing the strip with monoclinic phase. Figures 6.10 and 6.11 are the plots of standard deviation and the level plot, respectively.

The two cases, clean and with nanostars, exhibit almost similar standard deviation and the level plot shows even slightly higher monoclinic on the clean surface. The reason why the enhancement effect is visible in the confocal mode while absent in nonconfocal mode could be explained by the operating mechanism of the two configurations. Illustrated in Figure 6.12 is the difference in penetration depth of the laser focus in the sample when noncofocal and confocal modes are employed. In the confocal mode, the signal accounts only for the small section of the surface layer of the sample, where more monoclinic phase resides. In nonconfocal mode, the volume of laser focus is scattered well within the subsurface level and the signal
6.3.2 SERS using AuNS with LSPR $\lambda_{\text{max}} = 630$ nm

Confocal mode
The experiment was also performed using nanostars with plasmon resonance at 630 nm. First, we looked at the results of the confocal Raman. The plots of mean and originating from these regions containing tetragonal phase are detected. In other words, enhancement is not observed in nonconfocal mode because of the competition between suban intrinsically small enhancement effect and the large subsurface volume which is still tetragonal.

Figure 6.6. Plot of standard deviation of the spectra taken from a portion of the map from -300 $\mu$m to 100 $\mu$m along the x-axis. Plot (A) represents the clean surface and (B) represents the surface with nanostars (AuNS$_{690nm}$). Spectra were taken in confocal mode.
Figure 6.7. Level plot of the partial region of the map from -300 µm to -100 µm along the x axis. Color scales shown are uniform for both plots. Plot (A) represents the clean surface and (B) represents the surface with nanostars (AuNS$_{690nm}$). Spectra were taken in confocal mode.

The standard deviation of the spectral array for the clean and nanostar-covered surface are depicted in Figure 6.13. The monoclinic peaks are not visible in both plots, however, slightly more fluctuations can be seen in the case with nanostars. Similar to the analysis in the previous section, we plot the standard deviation of the normalized spectra as shown in Figure 6.14. From this plot, a larger fluctuation can be seen in the case with nanostars. The spectra is then restricted to the region inside the indicated by the dashed lines to account for the monoclinic peaks. The integrated area within this region is shown in the level plot in Figure 6.15. A similar trend as the results in section 6.3.1 was observed. There is considerable enhancement detected when Raman signal is obtained in confocal mode.
Figure 6.8. Plot of standard deviation of the spectra taken from a portion of the map from -450 µm to -300 µm along the x axis. Plot (A) represents the clean surface and (B) represents the surface with nanostars (AuNS$_{690nm}$). Spectra were taken in confocal mode.

**Nonconfocal mode**

In the nonconfocal mode, no significant difference is observed between the clean and covered surfaces. Figures 6.16 and 6.17 correspond to the standard deviation and level plots, respectively. Again, we see that the enhancement effect is absent when in nonconfocal mode. The signal detected accounts for the subsurface volume which is mostly tetragonal and this dominates over the weak monoclinic enhancement effect.
6.3 – Results and Discussion

6.3.3 SERS using AgNP with LSPR $\lambda_{\text{max}} = 410$ nm

In order to investigate the effect of substrate composition on the enhancement, we then used silver nanoparticles instead of gold nanostars. The silver nanoparticles (AgNP), which were provided for us by the group of Muniz-Miranda, were produced through laser ablation process. The procedure and characterization of the Ag colloids
are well-documented in Ref. [102]. Extinction spectrum of such colloids shows a wavelength maximum, $\lambda_{\text{max}}$ at 410 nm. For conciseness, only the relevant plots on the partial area of the maps are presented here. Figure 6.18 corresponds to the results of the confocal Raman acquisition while Figure 6.19 is that for nonconfocal mode. With AgNP, a different result is observed.

In the confocal mode, plots of the standard deviation (Figure 6.18 A and B) show almost similar magnitude. While there is no significant enhancement observed, the level plots (Figure 6.18 C and D) show an improvement in contrast when AgNPs
6.3 – Results and Discussion

Figure 6.11. Level plot (A) represents the clean surface and (B) represents the surface with nanostars (AuNS$_{690\text{nm}}$). Taken in nonconfocal mode.

Figure 6.12. Schematic diagram showing the operation of an objective lens in focusing the laser onto the sample. The volume of laser focus within the sample is different when operating in nonconfocal and confocal mode.
Figure 6.13. Mean and standard deviation of normalized spectra of (A) clean and (B) with nanostars, AuNS$_{630\text{nm}}$ surface. Taken in confocal mode.

Figure 6.14. Standard deviation of the spectra. Plot (A) represents the clean surface and (B) represents the surface with nanostars, AuNS$_{630\text{nm}}$. Taken in confocal mode.

are present. The strip of monoclinic phase is more visible in level plot D. In the nonconfocal mode, there is a SERS enhancement observed in the plot of standard deviation (Figure 6.19 A and B). It is evident in the level plots (Figure 6.19 C and D) that higher intensity of monoclinic signal are present in plot D. This demonstrates that the morphology and composition of the nanoparticle substrate has a significant effect in the SERS enhancement. We are currently performing more tests to better understand this dependence.

Several factors can contribute to the observed results that were presented for both gold nanostars and silver nanoparticles substrates. It is expected that the enhancement effect in the zirconia disc is small due to the inherently low concentration of monoclinic phase present in the sample. Moreover, there could also be an issue
6.3 – Results and Discussion

Figure 6.15. Level plot of the integrated area under the curve segment between the frequencies 170 cm\(^{-1}\) and 195 cm\(^{-1}\). Plot (A) represents the clean surface and (B) represents the surface with nanostars, AuNS\(_{630\text{nm}}\). Taken in confocal mode.

Figure 6.16. Standard deviation of all the spectra. Plot (A) represents the clean surface and (B) represents the surface with nanostars, AuNS\(_{630\text{nm}}\). Taken in nonconfocal mode.

with depositing the nanoparticles onto the polished surface. In contrast to porous samples, the nanoparticles can very well adhere verywell to the surface. Uniformity in the distribution of the nanoparticles on the surface is also an important factor. Large steric forces are typical in aqueous suspensions and could affect the spatial distribution of the nanoparticles during drying. It is commonly observed that nanoparticles are pulled to the edges of the drop during the drying process. In effect,
Figure 6.17. Level plot of (A) clean surface and (B) surface with nanostars, AuNS$_{630\text{nm}}$. Taken in nonconfocal mode.

less nanoparticles end up at the central spot. It is important to determine the right concentration of nanoparticles needed to achieve the desired effect.

Several parameters could further be investigated. It is well-known that there is an intricate relationship among the laser excitation wavelength, LSPR wavelength and wavelength of the Raman band being detected in producing maximum enhancement. Variations in one or a combination of these parameters have nonlinear effects on Raman enhancement. The group of Van Duyne [103] has systematically determined the effects the three parameters on the enhancement factor (EF) through wavelength scanning techniques. Their system consists of benzenethiol adsorbed on Ag nanoparticle arrays. Their results show that the maximum SERS EF occurs for excitation wavelengths that are blue-shifted with respect to the LSPR $\lambda_{\text{max}}$ wavelength and that not all Raman bands are enhanced. Among the characteristic vibrational modes of benzenethiol, the smaller Raman shifted peak shows a maximum enhancement closer to the $\lambda_{\text{max}}$. Another example that demonstrate the complicated relationship of these parameters was recently reported by Ming et al. [100] through SERS of 4-nitrobenzenethiol (4-NTP) with colloidal nanostars. They showed that nanostars have an optimum concentration, which has a direct consequence on the optical extinction (and therefore, on the LSPR wavelength) to maximize the enhancement factor (EF). They clarified that the maximum EF occurs when the LSPR band is
appropriately blue-shifted from the excitation wavelength rather than at the on-resonance position. Moreover, it is not enough that $\lambda_{\text{max}}$ is blue-shifted from $\lambda_{\text{excite}}$ but it also has to differ by the right amount based on the EF profile. Le Ru, et al., [104] also clarified in their work that the connection between extinction wavelength and SERS enhancement is not direct and the highest enhancement factors can be achieved at off-resonance excitations. The determining factor is the spatial localization of collective resonances. The SERS enhancement cannot be obtained directly from the extinction spectra in general.

These examples affirm that there is no universal substrate for SERS application. Each class of samples require its own prescription in terms of the laser excitation wavelength, LSPR wavelength and Raman shifted wavelength.

It is important to note, however, that the prescriptions typically available in
Figure 6.19. (A) Plot of standard deviation of the normalized spectra of clean surface and (B) surface with silver nanoparticles, (Ag$_{410\text{nm}}$). (C) Level plot showing the monoclinic phase of a clean surface and (D) nanoparticle-covered surface (Ag$_{410\text{nm}}$). Taken in nonconfocal mode.

literature are tailored mainly for detection of molecules in solution and not for bound surfaces. We have demonstrated that the SERS effect can be observed in the nanostars-zirconia system. We presented the initial parameters on the nanostars and Raman operation for which enhancement could be achieved. Optimization of the EF is outside the scope of the current work and is reserved for future studies.

### 6.4 Conclusion

SERS was performed on solid zirconia disc using gold nanostars and silver nanoparticles as substrate. SERS on solid zirconia using gold nanostars as substrate shows a promising improvement to the confocal Raman spectroscopy technique for detection
of very low monoclinic content in zirconia-based implants. In the presence of nanostars, more spots of monoclinic were located on the surface of the implant. In this chapter, we presented early promising results that could serve as a starting point for succeeding investigations on the SERS of solid ceramics.
Chapter 7

Conclusions and Outlook

We summarize the major achievements of this thesis and cite possible avenues for future work.

7.1 Fine Determination of Monoclinic Phase in Zirconia Implants

First, we have established a characterization protocol for the fine determination of monoclinic phase in stabilized zirconia, which consists of a combination of high resolution mapping with multivariate statistical analysis. I have laid out the concepts of Principal Component Analysis and applied them in the context of monoclinic phase detection and the determination of residual stress through band shifting. With the aid of high-resolution mapping, I have determined and visualized the monoclinic phases and stress. This technique is superior to diffraction methods that it is able to detect and locate the presence of monoclinic phases lower than 1 - 2 volume %, while at the same time providing a direct probe of the over-all homogeneity of the sample. The methods we have developed will be most useful in the design and development of zirconia-based implants, for which large-scale uniformity is a critical aspect.

7.2 Synthesis of Gold Nanostars

Second, we have established a protocol for stabilizing surfactant-free nanostars. In the synthesis of gold nanostars, long-term stability is an important but underappreciated property. Long-term stability of suspensions of surfactant-free gold nanostars was investigated through observing the changes in the UV-visible absorption spectra and
mean radius of curvature of the nanostar tips. Results showed that nanostars whose surfaces are unprotected by any surfactant exhibit rapid morphological evolution. An aging process can be observed, evident in the blunting of the nanostar tips, leading to a blue shift in the absorption maximum. Such changes, which greatly impact their optical properties, consequently deter their utility as SERS substrates. Stability is greatly improved by depositing on the nanostars a monolayer of mercaptopropionic acid (MPA), possibly because of the formation of the gold-sulfur (Au-S) bond that limits the mobility of the Au atoms. Capping the nanostars with MPA is an easy additional step for extending the stability of the nanostars in suspension for several months without significantly affecting the original plasmonic resonance band.

7.3 SERS on Solid Zirconia

Finally, as a pioneering first attempt, SERS was performed on solid zirconia disc using gold nanostars and silver nanoparticles as substrate. Our results show that SERS using gold nanostars is a promising improvement to the confocal Raman spectroscopy technique for detection of very low monoclinic content in zirconia-based implants. In the presence of nanostars, more spots of monoclinic were located on the surface of the implant. Coating the surface with nanostars enable us to tap into the surface modes of zirconia. The enhancement effect is more visible when confocal mode is employed and very little enhancement is observed when operating in nonconfocal mode due to the competition between the contribution from the large volume of the subsurface level and an intrinsically small enhancement effect.

7.4 Outlook and Future Work

Several avenues can be explored for extending this thesis. We have not yet fully exploited the potential of Principal Component Analysis in various parts of this work. Further studies can be made to understand and interpret the results of PCA. In principle, by closely investigating more loading vectors, the technique could be used, not only in monoclinic phase detection, but also in finding finer features on the sample. One really exciting possibility for the future is the conjunction of PCA methods with enhancement by nanostars.

For the work on nanostars, we can further demonstrate various degrees of stability
in nanostar suspensions, including the ones coated with CTAB and PEG-SH. It would be beneficial to have information regarding the shelf-life of various nanostars suspensions. Furthermore, since nanostars exhibit a mechanism analogous to a typical sintering process whereby atoms move from convex to concave regions driven by the chemical potential difference associated to the respective curvatures, future work can be done on deriving an exact model to describe the diffusion kinetics. The current work can also be extended to determine the correlation between the initial LSPR $\lambda_{\text{max}}$ or the average radius of curvature of the tips and the decay rate.

Since this is a pioneering work on monoclinic phase detection through SERS, there is obviously a lot more that can be done to promote the technique and bring it to the level of a standard characterization tool. We have presented the promising results that could serve as a starting point for the succeeding investigations. Future work might include obtaining SERS enhancements from a larger area of the zirconia surface. Effort can also go into improving the depth resolution in nonconfocal mode and into optimizing the nanostar substrates. The dependence on the parameters, such as size and concentration of nanostars, can be studied to better understand their role in SERS enhancement. Other composition for nanostars can also be explored. In addition, it has been shown that stronger hotspots are generated at the interface of bimetallic nanostars in core-shell configuration [105]. With this, one can imagine taking the full advantage of the lightning rod effect from the presence of the tips, while at the same time benefiting from the additional enhancement provided offered by the radial anisotropy at the metal-metal interface. Aside from this, we can also make use of metal-semiconductor core-shell nanostars. In fact, our group presently has an existing protocol for coating the nanoparticles with a semiconductor layer by chemical bath deposition, which could be adapted for gold nanostars as well. Similarly, we could expect additional enhancement from the anisotropy at the core-shell boundary. The ultimate goal is to strengthen the ability of the nanostars to impart high confocality in the system, i.e. restricting the excitation to a very thin surface layer on the sample. This will enable us to observe the enhancement effect even when operating in nonconfocal mode.
Appendix A

Effect of Gold Nanostars on Yttria-Stabilized Zirconia Powders

In this side experiment, we investigated colloids of fine zirconia powders, which are partially stabilized with yttria. Samples were prepared such that the powder will have increasing coverage of nanostars as shown in the schematic diagram in Figure A.1. The goal was to be able to detect increasing monoclinic content with increasing nanostar coverage. Moreover, the critical concentration at which the detection plateaus was also investigated.

Suspension of zirconia powders with average grain size of 20 µm was prepared by mixing 1.5 mg with 6 ml MilliQ water in a vial. While under sonication, 4 equal volumes of zirconia colloid were drawn out and placed in separate Eppendorf vials. To three of these, increasing amounts of nanostars (AuNS@MPA, LSPR $\lambda_{max} = 690$ nm) were added to form mixtures with 0%, 29%, 44% and 50% by volume. Intermediate concentrations were also prepared, however only representative results are reported here. The solutions were dropped onto a CaF$_2$ slide using a pipette and then dried under the hood. Raman measurements done using a 785 nm Toptica diode laser with 50x focusing objective lens. Each spectrum was obtained with exposure time of 10s for single accumulation. Laser power at the laser shutter is 456 mW.

Initial measurement of the pure powder shows that the powder is poorly stabilized or may have undergone aging in time. Since no prior annealing was done, it contains 89% monoclinic. However, measurement of the powders with nanostars revealed interesting results. When using nanostars, measurements are typically done initially at 10% laser power to avoid oversaturation. Then gradually, the laser power is increased until optimum signal is achieved without saturation. The succeeding drops
containing powders with nanostars were then measured. Initially, at low power, the result showed that it is in monoclinic phase with large a background signal. And then when the laser power was increased, the spectrum showed that the same powder is tetragonal. A couple more trials were made at different spots and different concentration. Some spots were becoming more tetragonal than others. Upon observing this, all the samples were then measured as follows:

Step 1: Initial illumination at 10% laser power
Step 2: Second illumination 100% laser power
Step 3: Third illumination at 10 % laser power

The results are summarized in Figure A.2. Sample (A) consists of pure powder and it remained in monoclinic phase all through the three steps. Sample (B) contains 29% nanostars and it is in monoclinic phase in Step 1. Some monoclinic diminished upon illuminating with 100 % laser power (Step 2) and did not change back to monoclinic phase even when the power was lowered to 10 % (Step 3). The same trend was observed in the remaining 2 drops. Sample (C) contains 44 % nanostars. It is monoclinic in Step 1 and when it was illuminated with 100 % power, the powder transformed to tetragonal phase completely. Lastly, Sample (D) contains with 50% nanostars. In Step 2, the monoclinic phase transformed to tetragonal but not as completely as in Sample (C).

The plot of the amount of monoclinic as a function of nanostar concentration for all three illumination steps is found in Figure A.3. As seen in the figure, at 10 % laser power, all samples are monoclinic (black curve). At 100 % laser power (red curve), Sample (B) transformed from 100 % monoclinic phase down to only 58 %. Sample (C) transformed from 100 % monoclinic phase to 100 % tetragonal phase.
Sample (D) transformed from 100 % monoclinic phase down to only 18 % phase. We can see that with appropriate amounts of nanostars the heat energy generated at the sample surface is high enough to induce transformation on the monoclinic zirconia.

To be able to illustrate more about the transformations kinetics in the powders, Sample (B) is investigated at various exposure times in all the three illumination
A – Effect of Gold Nanostars on Yttria - Stabilized Zirconia Powders

Figure A.3. Monoclinic phase present in the samples at initial 10%, 100% and final 10% laser power. The vertical lines are guides to the eye for the nanostar concentrations, 0, 29, 44 and 50% on the x-axis.

steps. On a single grain, a spectrum is first obtained with exposure time of 10 s at 10% laser power and then the time is increased at 10 s increment until intensity saturation occurs. Figure A.4 shows the result of this. From 100% monoclinic phase, it is then slowly reduced to 88% monoclinic after 80 s of exposure time.

Similarly, at 100% laser power, we obtain a spectra for increasing exposure times. The result is depicted in Figure A.5. In this case, with the increased laser power, the sample was transformed to tetragonal after the first exposure (only 58% monoclinic). But the opposite happens when exposed to the laser at longer times. In contrast to the result at low power, the sample transformed back to monoclinic after being exposed to the laser for only 30 s.

Lastly, the laser power is lowered again to 10% and then we performed the same procedure. In Figure A.6, we see that the sample was initially at 64% monoclinic then with increasing accumulation time in increments of 10 s, the sample continues to transform to monoclinic phase at 70 s exposure time.

These preliminary results seem to indicate that nanostars can be utilized as nanoheaters, which can induce transformation to zirconia powders. In the presence of nanostars, the heat energy at on the sample is elevated. Through a more systematic study, one might hope to identify an appropriate concentration and exposure times that will enable transformation in both directions. We can also use this system
Figure A.4. Raman spectra obtained at increasing accumulation time at initial illumination of 10% laser power. Bottommost spectrum was taken with 10 s exposure time and the topmost spectra with 80 s.

Figure A.5. Raman spectra obtained at increasing accumulation time at illumination of 100% laser power. Bottommost spectrum was taken with 10 s exposure time and the topmost spectrum was with 30 s.

to study transformation kinetics in zirconia. Further investigation on this topic is reserved for future work. This is particularly interesting from the materials science point of view. As a possible application, the localized transformation induced by
Figure A.6. Raman spectra obtained at increasing accumulation time at illumination of 100 % laser power. Bottommost spectrum was taken with 10 s exposure time and the topmost spectrum was with 70 s

nanostars can be exploited for patterning surfaces of materials.
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