LIGHT SCATTERING OF NANOSTRUCTURED MATERIALS

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LIGHT SCATTERING OF NANOSTRUCTURED MATERIALS

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Dissertation

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ABSTRACT

With the developments in technology, more and more materials and substances are being developed for different spheres of life, from construction works and computer industry to medicine. Many techniques and instruments are available to determine the macroscopic properties of bulk materials, such as tensiometric and rheological equipment, instruments to measure impact resistance and combustibility and many others. Currently, however, a big interest lies in nanotechnology, as different materials start to exhibit new and often very different properties with respect to the bulk state when the size of particles is much smaller than one micron. This, in turn, requires different instruments and techniques, which would be capable to reveal the properties of these nanomaterials.

Raman spectroscopy can be an effective tool for the study of nanostructures due to its high sensitivity to differences in structure, chemical functionalities and even to stresses on the nanoscale. However, there exist a number of drawbacks that hinder the use of Raman spectroscopy for nano-analysis. Among them are the low signal intensity, the diffraction-limited resolution of current instruments and difficulty in interpreting the acquired data.

The strategy for overcoming the low intensity is to induce an enhancement in the electric field in the sample by placing it next to noble metal particles. This phenomenon is called Surface Enhanced Raman Scattering (SERS). The key to successful application
of SERS is the correct choice of the particles and illuminating wavelengths, which determines the strength of the enhancement. In this Dissertation, we have studied SERS substrates prepared by different techniques and with different metals (Ag and Au) and characterized their SERS effectiveness.

To optimize the lateral resolution of SERS and to simultaneously access additional information such as surface topography, a Raman spectrometer and accompanying optics have been combined with a scanning probe microscope (SPM), a technique currently known as Tip-Enhanced Raman Scattering (TERS). However, there are still a few challenges for this technique, namely the efficiency of enhancing tips, their longevity and the potential damage they can induce in the sample through heating. We present a few promising designs of tips that could give high enhancement, suggest an effective method of their protection from environmental damage and show the increase in temperature in the analyte that will be caused by the enhancing tip.

Finally, we apply Raman scattering to the studies of carbon nanotubes separations and show how to interpret the data to get an understanding of the real changes in the sample after various treatments of the nanotubes.
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CHAPTER I.

INTRODUCTION

Recent advances in nanotechnology have shown that structures of different materials, from polymers and biological samples to metals, exhibit novel or improved material properties when the dimensions of a particle are comparable to some intrinsic length scale of the material. The enthusiasm for nanotechnology is inspired by the potential offered by these qualitatively unique properties.

Light scattering is a set of non-invasive techniques that can probe multiple interesting phenomena in different types of nanoscale materials, both organic and inorganic. Materials in solid, liquid and gaseous states can be effectively probed.

In particular, Raman spectroscopy is a powerful light scattering technique that is used by scientists for many different applications. Its ability to probe chemical functionalities of molecules is invaluable for the studies of organic molecules. In addition to that, Raman can be used for the determination of sizes of polymer crystals, can probe the structure and stress of different materials. The biggest advantage, however, lies in that fact that Raman spectroscopy can be utilized for studies of samples on the molecular and nanoscale levels.

The nanoscale dimensions of the studied materials impose several limitations on the technique. Raman cross-section of materials is typically much smaller than their
fluorescence cross-section, which makes small quantities of materials hard to detect and distinguish. At the same time, as with all other light scattering spectroscopies, Raman sample mapping is diffraction limited, i.e. its resolution is strongly dependent on the size of the laser spot. Some solutions have been recently developed, namely decreasing the size of the spot by small apertures and introduction of non-linear methods, such as superlenses. However, Surface-Enhanced Raman Scattering could be a much more promising technique with the ability to enhance the signal without sacrificing the resolution. In addition to that, Tip-Enhanced Raman Scattering is an emerging technique that can not only provide the necessary enhancement, but also introduce a confinement to the probed volume, effectively increasing the resolution of the scanning.

In this Dissertation, Raman spectroscopy, as well as other supporting spectroscopy techniques, has been employed as a powerful research tool for the characterization of properties of different nanoscale materials.

In the second Chapter, a brief historical background is presented on the development of light-scattering methods and the main principles of their operation are explained. The principles of SERS and most important advances in this field are discussed, together with the known experimental methods for the preparation of SERS substrates. The general idea of nearfield techniques is introduced and TERS is explained as one of the most prominent applications in the field. Similarly to SERS, recent advances in TERS and other nearfield optical scanning types are described and the implications of those findings on the pathways for the development of better TERS tips are discussed.
The third Chapter introduces the experimental techniques and samples used in this work. These include instrumentation and settings for SERS, TERS and regular Raman studies, as well as other supporting techniques.

Chapter 4 contains the studies on various SERS substrates, prepared by different means and with different metals. The overall enhancing properties of these metal particles are compared. The conclusions guide the further investigation on locating the exact positions of highly-enhancing spots within the metal clusters and their aggregates. The influence of introduction of dielectric layers to SERS clusters is also studied with respect to its applicability as a means of prolongation of shelf-life of SERS substrates.

In Chapter 5, the current problems of TERS are identified and solutions are developed. Issues of making of highly enhancing and durable tips are discussed, with the conclusions made from the protection of SERS samples being extended to TERS. Additionally, the heating of an organic film from TERS tips is studied, pointing to the existing risk for samples that could undergo a phase transition or get degraded.

Finally, Chapter 6 describes the use of Raman light scattering for the determination of characteristics of carbon nanotubes after different treatments. The findings are used as a basis for creating separation procedures that purify nanotubes of certain nature or chirality. The resulting nanotubes could be used for the growing number of applications, where specific nanotubes are required due to their particular radius or conductivity.
CHAPTER II

HISTORICAL PERSPECTIVE

Light scattering, as a scientific technique, originated from early studies of fluorescence and phosphorescence in medieval times. While fluorescence has been quite commonly observed and recorded by people in times of antiquity, both in the countries of the far east, like India and China, as well as by Greek and Roman authors, little effort was made to determine the origin of the observed phenomena.

The first reported observation of fluorescence in medieval Europe was made by N. Monardes in 1565 [1], who observed the emission of light by wood Lignim Nefriticum. That event was closely followed by the first recorded observation of phosphorescence from a Bolognese stone by V. Cascariolo in 1602 [2]. Later on, in 1680 [3], the phenomena have been described in more detail. The principles of refraction of light were discussed as well. Boyle also addressed the various colors of the observed fluorescence with respect to different conditions and wavelengths of illumination light, i.e. sunlight and candlelight. Additionally, the use of a triangular optical prism as an instrument for observing the refraction of light and, more importantly, for spectral analysis, has been discussed by the author and a correct schematic representation of the principle of a prism operation was presented in his work (Figure 1). Such a simple device can, however, find a use even for the most modern applications, as will be seen in later
chapters. In particular, one can use a phenomenon of total internal reflection to measure optical properties of objects on the surface of a prism.

![Optical prism schematic](image)

Fig. 1. Optical prism schematic taken from Reference 3.

Nearly two centuries later, in 1852, George Gabriel Stokes correctly used filters to suppress the incident light and establish the Stokes law. It said that the emitted light is always of wavelength longer than that of the excitation light. He also described in sufficient detail the use of optical absorption and fluorescence measurements as an analytical tool [4]. In 1859, it was postulated by Gustav R. Kirchhoff that each pure substance has its own characteristic spectrum [5]. This advance can be considered as making the dawn of modern optical spectroscopy.

Later on, in early 1928, an important addition to spectroscopic techniques was discovered—Raman spectroscopy, which was attributed to the works of C.V. Raman. It
was initially observed, however, by Landsberg and Mandelstam a week prior to that [6]. Raman was awarded the Nobel prize, while the other two were never fully acknowledged [7] for their discovery.

---

Raman spectroscopy is a technique to probe the characteristic relaxation of vibrational energy of molecules, when the emission energy is different from the incident energy. The type of emissions is denoted as either Stokes mode, when the emission energy is lower, or anti-Stokes mode, when the emission energy is higher. A corresponding Jablonski diagram is presented in Figure 2 [8]. The main difference between Raman scattering and fluorescence/phosphorescence lies in the fact that Raman scattering requires the excitation of an electron to a virtual electronic state, which is not equal to any ground state, from which it then relaxes. Fluorescence, on the other hand, involves a relaxation of an electron from a real electronic state.

Absorption, fluorescence and Raman spectroscopy can identify the chemical structure of a given substance and are sometimes able to differentiate between different structures of the same material [9]. In addition to the abovementioned techniques, there
exists a number of light scattering techniques which probe bulk properties of materials, such as molecular weight and modulus.

Of such optical techniques two are of particular interest: photon-correlation spectroscopy, established experimentally in 1970 [10], and Brillouin scattering, discovered in 1922 [11]. The latter measured the scattering of light from acoustic propagating wave modes in solids and liquids and therefore can probe the elasticity of the material. The former is used to monitor the motion of molecules or other small particles in a solvent and consequently could reveal the effective viscosity of the solvent and estimate some of the dimensions of the particles, e.g. the hydrodynamic radii of polymeric chains.

Light scattering, as a technique, is sensitive to fluctuations in the refractive index within the volume of interest. Thus, if any molecular motion results in some change in refractive index, such motion could be probed by a light scattering technique. If the particle is perfectly homogeneous and isotropic and its refractive index is the same as that of the solvent, its motion will not provide a change in refractive index and thus no light scattering signal.

With the development of light scattering methods, a very broad range of frequencies of motions can be probed. The combination of quasielastic (dynamic) and inelastic (Raman) scattering techniques allows covering a range of $10^{-3}$ to $10^{14}$ Hz in frequency. Light scattering is a non-invasive technique, unless very high power is used which can damage the sample. It possesses good frequency resolution, generally good statistics due to the ready availability of highly intense light sources and good spatial
resolution due to the possibility of focusing light into highly collimated, very narrow beams.

There are two approaches for the description of light scattering spectra. One approach is based on assigning each molecule its own polarizability, but its use is more specific. More commonly, a continuum approximation is used, which considers fluctuations of the dielectric constant, $\delta \varepsilon$, caused by some type of molecular motion.

The dependence of light scattering on $\delta \varepsilon$ can, therefore, be represented as:

$$I_{ab}(q,\omega) \sim \int_{-\infty}^{\infty} \exp(-i\omega t) dt \langle \delta\varepsilon_{ab}(q,t) \delta\varepsilon_{ab}(-q,0) \rangle$$

(2.1)

where $q = \frac{4\pi}{\lambda} \sin\left(\frac{\theta}{2}\right)$ is the scattering wave vector, and $\omega = \frac{2\pi v}{\lambda}$ is angular frequency.

Such an approach is perfectly applicable, as the wavelength of light is much larger than atomic size. To receive additional information from a molecule, it is possible to account as well for the polarization of the light. Such measurements help the user determine the type of motion. Polarized scattered light provides evidence of symmetric fluctuations, while de-polarized scattered light means that the fluctuation of the dielectric constant is asymmetric. Thus, the different types of fluctuation types can be measured by varying the polarization.

### 2.1 Photon-correlation spectroscopy for studies of nanostructured materials

Photon-correlation spectroscopy (PCS), or dynamic light scattering, is a technique which has found great popularity as a convenient method to determine the size of particles through the calculation of their diffusion coefficients. It is widely used
commercially due to highly reproducible results with a reasonable degree of accuracy [12-14], its simplicity and automatization for routine measurements. However, there are other ways in which this powerful technique, capable of spanning up to 10 orders in the time domain, can be utilized.

The underlying principle lies in correlating the current position of some particles with their position at some known fixed moment in the past. As an established technique, PCS can be used to investigate the influence of different factors on the motion of particles in a solvent, given that the structural relaxations arising from such factors can be distinguished from each other analytically.

PCS or dynamic light scattering is often used for more precise determinations of polymer radius of gyration than can be provided by static light scattering. The radius is extracted from the relaxation time. This can give useful information on polymer-solvent interactions.

More complicated applications for the PCS would require a deeper analysis of the shape of the relaxation curve. Such analysis makes it possible to determine the structural relaxation time and non-exponentiality of the relaxation, their temperature dependance and fragility, both for organic [15] and inorganic [16] materials. One can separate different relaxations by using polarized and depolarized scattering and by using different scattering wave vectors or, in other words, different scattering angles.

As signal is generally much weaker for depolarized scattering, more care is required for the set-up of such an experiment. In simple words, if there is any optical anisotropy in a particle, making it different from a uniform sphere, it would be seen in depolarized scattering. For an ellipsoid, PCS probes the rotational motion that is not
parallel to the long axis, as it is associated with displacement of mass. For diphenyl molecules, the rotation around the longer semi-axis can be possibly probed by another optical scattering technique – high resolution interferometric Raman bandwidth analysis [17]. However, the use of this technique is extremely limited, as it requires high concentrations of the molecules in the probed volume.

The biggest complication in the case of probing the rotational motion is the rather weak signal. In both cases of translational and rotational motion, the signal is proportional to the fluctuations of refractive index in a probed volume. The fluctuations become larger with the increase of optical contrast between the particle and the solvent. For rotational motion, the signal is often much weaker due to small deviation of the particle shape from spherical or small difference in refractive index for different areas of the particle. This implies that the signal would be smallest when the shape of the particle approaches a sphere and strongest when it approaches a rod and is highly anisotropic.

Consequently to this, it still remains a big challenge to reliably extract the rotational diffusion from depolarized dynamic light scattering and there are very limited literature data available on the topic. An effective experiment becomes harder to achieve for small particles on the order of several nanometers in diameter [18]. Later work [19, 20] has indicated that PCS can be reliably used at least for qualitative analysis of nanoscale particles, such as organic oligomers [19] and DNA [20]. It has also been shown that the possibilities of particle aggregation should be taken into account [20].

To conclude, PCS can characterize the motion behavior of small nanoparticles in different solutions. This was used to study the coupling of protein diffusion and rotation to solvent viscosity.
2.2 Raman spectroscopy for studies of nanostructured materials

Raman scattering is a technique which probes the vibrational relaxation of a molecule after the electrons of bonded atoms get excited to virtual energy states different from the ground state. Raman scattering identifies chemical structure in a manner, similar to that in infrared (IR) spectroscopy.

Raman signal is created when a modulation occurs in polarizability of the molecule, unlike the case for IR, which relies on the change of the dipole moment itself. Quite often, certain bands, which would appear at the same frequencies in Raman and IR, are restricted to only one of these techniques. Therefore, Raman and IR are used to complement each other [21] to give a more complete picture of vibrational motion of atoms.

To determine if a vibration is Raman-active it is often sufficient to determine whether a change in size, shape or orientation of a polarizability ellipsoid has occurred, given that the ellipsoid is not symmetric at two extreme configurations. A polarizability ellipsoid is visualized by simply plotting all $(\alpha_{ij})^{-1/2}$ components of the polarizability tensor, where $i$ and $j$ are coordinates, i.e. $x$, $y$ or $z$. Here, $\alpha_{ij}$ is the polarizability along the line joining a point $(i,j)$ on the ellipsoid with its center.

The principle can be described using the example of CO$_2$ and H$_2$O molecules, using Fig. 3. Note that only the first vibration is Raman-active for CO$_2$, while all three are Raman-active for H$_2$O.
Typically, Raman spectroscopy is used to distinguish the chemical composition of a substance or to monitor the change [21] in composition with a good degree of accuracy. Additionally, the difference in conformational structure can be probed.

It should be noted that Raman scattering has a lower signal than does IR. The Raman scattering cross-section is \( \sim 10^{-30} \, \text{cm}^2/\text{molecule} \), while for IR cross-sections are typically on the order of \( 10^{-20} \, \text{cm}^2/\text{molecule} \). This makes it significantly harder to extract useful data from beneath the fluorescence background, as the cross-section for fluorescence is as high as \( 10^{-16} \, \text{cm}^2/\text{molecule} \) [22].

Vibrational spectroscopy, including Raman spectroscopy, finds a great number of applications not only due to the reasons described above, but also due to the fact that it can be utilized both for solid and liquid samples, as well as gases, although Raman spectroscopy of the latter typically suffers from low concentration problems [21]. Raman
scattering has been shown to depend on different macroscopic parameters, such as pressure [23] and stress [24].

Symmetric vibrations are always active in Raman scattering [21]. Such vibrations as C=C and C≡C, as well as most C–H and C–C vibrations, which have high polarizability, give quite strong Raman signal [25, 26]. This makes Raman spectroscopy a very useful tool for the characterization of organic compounds with high enough accuracy to make even quantitative comparisons possible. It is also possible to measure Raman effectively from high-molecular weight molecules.

It should be noted that Raman is also sensitive to such important characteristics as conformations of polymer chains [27]. For more in-depth analysis, deuterium labeling can be applied in Raman [28], similar to other techniques such as neutron scattering. The high efficiency of simple Raman scattering at measuring all of these samples makes it an extremely versatile technique and a very important piece of equipment in a good characterization lab.

For example, one can calculate the thickness and thickness distribution of polymeric crystals through the most intense Raman band of folded chain polymeric crystals (Longitudinal Acoustic Mode, LAM) using a simple formula [29, 30], where the Raman frequency is inversely proportional to the crystal thickness. This technique is complementary to X-ray-based thickness determination. At the same time it requires simpler equipment.

Also, the extremely high resolving capability of Raman scattering has enabled scientists to distinguish the diameters and, consequently, chirality of different carbon
nanotubes (CNTs) through the frequency positions of their Radial Breathing Modes (RBM) [31, 32].

Additionally, it has been demonstrated [33] that a peak can be observed at <10 cm\(^{-1}\) in low frequency Raman for nucleated cordierite glass particles. Its frequency has been found to be inversely proportional to the particle radius.

The three examples above indicate high sensitivity of Raman to different vibrational modes in nanostructured materials and the possibility of probing different nanoscale properties without the use of much more sophisticated techniques. In Chapter 6 an effective use for Raman spectroscopy, as a scientific tool, the example of separation of different CNTs is described.

2.3 Surface Enhanced Raman Spectroscopy, “Towards a better substrate”

2.3.1 Theoretical predictions and simulations

With the advent of nanotechnology and the necessity to probe smaller and smaller volumes of materials, for which structural parameters begin to deviate from their bulk values, severe restraints have been imposed on the Raman scattering technique. It has been already noted above that Raman analysis is often hindered by the inherently low scattering cross section of molecules in comparison with IR and fluorescence. This problem becomes even more severe when the Raman signal from nanoscale features of samples is to be collected. Here, accumulation times beyond reasonable are often
required. Fairly recently, however, an approach has been developed which has overcome this challenge.

Surface Enhanced Raman Spectroscopy (SERS) is generally considered as a phenomenon of an increase in the intensity of the signal, collected from an analyte molecule, when it is in proximity to a metal particle. Such an increase in intensity should be many orders of magnitude, i.e. more than ten thousand times typically, and enables the researcher to see signal from nanometer-scale amounts of analyte molecules, which would be next to impossible in normal experimental conditions. This ability to enhance signal from small volumes of very weakly scattering molecules is considered the characteristic feature of SERS and has been the topic of considerable interest since its very discovery thirty years ago.

It is hard to overestimate the importance of SERS for scientists and industry. In addition to studies of nanomaterials, more people are trying to address such critical issues as cancer cell detection [34] and their destruction via photothermal heating [35]. It is possible not only to use silver or gold particles as SERS fillers for different organic cells and cell parts, but also to use these SERS particles with dye labels to do imaging of live cells by monitoring the positions of the particles [36].

There are attempts to use Raman scattering for poisonous gas detection [37], as well as for bacteria detection, since all of them would have their specific chemical fingerprints. Needless to say, such use of Raman spectroscopy would require significant enhancement of the incoming signal, regardless of the initial cross-section of the probed molecule, as the number of such molecules in air would be extremely small. With the
advances in SERS substrates preparation, there is no doubt that such detection will be achieved in the near future.

SERS studies the increase of the Raman signal, when analyte molecules are in a close proximity to metal particles. The free electron cloud of the metal creates the possibility of such enhancement. The frequency position of the collective electron vibration, or plasmon resonance, with respect to the absorption and emission frequency of analyte molecule is very important. Also, enhancement is stronger for highly conducting metals and lower for lower conducting ones.

SERS is known to originate from two main mechanisms – electromagnetic enhancement (EE) and chemical enhancement (CE). It is generally considered that the EE mechanism is the dominant one, contributing up to twelve or more orders of enhancement [38], while the CE mechanism can give only up to two orders.

CE can originate from formation of charge transfer states of the metal-absorbed molecule complex. Such charge transfer states, however, are effective at fairly close ranges, which suggests that they could not be the main contributor to the overall enhancement effect. As was calculated in Ref. 39, CE extends only five or less Ångstroms away from the surface of the metal particles, since the very close proximity of the molecules to the surface of a metal is required for wavefunction overlap, responsible for the “charge-transfer” mechanism.

The most common theoretical representation of the EE mechanism is coupling of the molecules to the surface plasmons, created by the metal surface. The enhancement from the electromagnetic mechanism falls off significantly slower with distance than in
the case of CE. The long-range and the short-range enhancement from CE and EE, respectively, has also been proven experimentally [40].

It has also been noted by several authors [41, 42, 43], that the most effective enhancement happens at sharp small structures, namely bumps, pits and other metal roughness features. The features provide “localized” resonance for the analyte molecules, which is strongly coupled to the surface electromagnetic modes of the metal itself.

As has been further noted [42], not only the size of the metal particles of roughness features has an impact on the level of enhancement, but also the shape of the particle. There is a significant difference in enhancement with respect to the position of the absorbed molecules at the metal particle. Highest enhancement is expected to be at the apex of the longer semi-axis of an elliptical particle. The signal from molecules absorbed near high curvature edges of surface irregularities is enhanced to a greater extent than is that from one absorbed along a flat metal feature. Calculated values of enhancement factor for different aspect ratios of metal ellipsoids versus molecule-surface distance are shown in Fig. 4. It is clear that higher aspect ratio particles provide higher enhancement, but only for the molecules in the direct vicinity of the particle, i.e. less than 10 Ångstroms away.
Fig. 4. Enhancement factor R as function of molecule-surface distance H for four sets of (a,b) values (particle dimensions): (1) (500; 200) Å; (2) (500; 100) Å; (3) (500; 50) Å; (4) (500; 500) Å. Molecular polarizability is assumed $10\,\text{Å}^3$ and $\hbar\omega = 2.5\, \text{eV}$ (496 nm). Reprinted with permission from [42]. Copyright 1980, American Institute of Physics.

2.3.1.1. Overlapping of absorption and emission bands

Resonance Raman (RR) scattering occurs when the sample is irradiated with light whose energy corresponds to that of the electronic transition of a particular chromophoric group in a molecule (pp17,53,54 in [21]). The intensities of Raman bands of such chromophores will be selectively enhanced by a factor of $10^3$ to $10^5$. 
Therefore the level of SERS enhancement is be significantly different if scattering occurs from a molecule in its resonant condition. The collected signal is proportional to the squares of the incident and emitted fields at the molecule:

\[ I \sim E_i^2 \cdot E_s^2 \quad E_i^2 = f(\lambda_1) \quad E_s^2 = f(\lambda_2) \quad \lambda_1 \neq \lambda_2 \quad (2.2) \]

where \( E_i, E_s, \lambda_1, \lambda_2 \) are incident field, scattered field, incident wavelength and scattered wavelength of the absorption and emission bands of the molecule, respectively. In simple terms, the plasmon resonance should overlap not only with the laser line wavelength, but also with the absorption and emission bands of the analyte molecule.

The simplest case is for very small spherical particles. Both the incident electric field and the field scattered by the analyte molecule are enhanced by plasmon peaks located at the same wavelength [44]. The Raman emitted field is enhanced by a different amount than is the incident field, since the frequency is different due to the inelastic nature of Raman scattering. However, the enhancement factors of the incident and the emitted fields are comparable as frequency shifts are usually rather small. For non-spherical and large spherical particles, the case is more complicated. This is due to the fact that the scattered field is spatially different from the incident one, as it arises from an oscillating dipole located at the position of the emitting molecule.

2.3.1.2. Shape and size factors for crystalline nanoparticles

If spherical particles with different sizes are considered, studies based on theoretical simulations have shown that highest enhancement is achieved for the smallest particles [45]. The enhancement was calculated to be decaying similarly for both small (5
nm) and medium-sized (50 nm) particles, so the statement should be true regardless of the proximity of the analyte molecule (see Fig. 5). Further calculations have been done by other authors showing a similar result, but they were all based on a quasielectrostatic treatment of isolated spheres.

In short [44], a sphere with a dielectric constant of $\varepsilon_i$, independent of the size of the sphere, is embedded in a medium with a dielectric constant $\varepsilon_o$. The incident electromagnetic field is represented by $\vec{E}_0$, a vector pointing along the z-axis which is assumed to be independent of its location for distances on the order of the sphere size. The resulting field outside the sphere can then be written as:

$$
\vec{E}_{out} = \vec{E}_0 \hat{z} - \alpha \varepsilon_0 \left[ \frac{\vec{z}}{r^3} - \frac{3\vec{z}}{r^5} (\vec{z} \cdot \vec{x}) \vec{x} + \vec{y} \cdot \vec{y} \right]
$$

(2.3)

where the first term is the applied field, and the second is the induced dipole that results from polarization of the sphere’s electron density. $\alpha$ is the metal polarizability, $x, y, z, r, x, y, z$ are coordinates, radial distance and unit vectors in the Cartesian directions, respectively. $E_o$ is the magnitude of $\vec{E}_0$. For a metal sphere with the dielectric constant indicated above, the polarizability is:

$$
\alpha = ga^3 = \frac{\varepsilon_i - \varepsilon_o}{\varepsilon_i + 2\varepsilon_o} a^3, \text{ where } a \text{ – is the particle radius.}
$$

(2.4)
Fig. 5. Enhancement versus distance of dipole from origin (in units of particle radius) for silver particles with $a = 5$ nm ($\lambda = 382$ nm), $a = 50$ nm ($\lambda = 511$ nm) and $a = 500$ nm ($\lambda = 528$ nm). Reprinted with permission from [45]. Copyright 1980, Optical Society of America.

So whenever the real part of $\varepsilon_i$ equals $-2\varepsilon_o$ and the imaginary part is small, $\alpha$ becomes very large, and as a result the induced field becomes very large. It is this induced field that is responsible for the electromagnetic enhancement. For vacuum (or air) $\varepsilon_o$ is equal (or close) to one. In such a case, the polarizability and, therefore, Raman signal, is maximized when $\varepsilon \sim 2$, based on (2.4). Theoretically from the same formula, the highest polarizability can be achieved in water ($\sim 80$).

The expression for the Raman intensity for a field at the surface of the sphere ($r = a$) can be written as:

$$ I \sim E_{out}^2 = E_0^2 \left[ |l-g|^2 + 3\cos^2 \theta \left( 2 \Re(g) + |g|^2 \right) \right] $$

(2.5)
In the above expression, $\theta$ is the angle between the applied field direction and the vector $\mathbf{r}$ that locates positions on the sphere surface ($Re$ is the resonance condition). If we consider molecules located at the position of maximum enhancement ($\theta = 0$ or $180^\circ$) and the limit of $g \gg 1$, then the overall enhancement arising from incident and scattered fields is approximately:

$$Enhancement = R(\omega)R'(\omega') = \frac{E_{out}^2 E_{out}'^2}{E_o^4} = 16|g|^2|g'|^2$$  \hspace{1cm} (2.6)

where the primed symbols refer to fields evaluated at the scattered frequency and $R(\omega)$ denotes field enhancement. The corresponding angle-averaged (particle-averaged) enhancement factor would be smaller than this by a factor of 4. If we use the simplified expression for the metal dielectric constant from the free-electron Drude model [46]:

$$\varepsilon_i = 1 - \frac{\omega_p^2}{\omega(\omega + i\gamma)},$$  \hspace{1cm} (2.7)

the value of $g$ would be equal to 10 when the ratio of the plasmon frequency $\omega_p$ to plasmon width $\gamma$ is roughly 10. If substituted into the above equation, this would result in $\sim 10^5$ enhancement. Several materials, especially silver, have $|g| \gtrsim 10$. However, the free electron model for dielectric constant is rarely sufficiently accurate for this prediction to be quantitative.

A somewhat more complicated expression [47] for Raman enhancement is written for a spheroid whose major axis is of length $2b$ and minor axis $2a$, with a constant field $E_o$ applied along the major axis and random distribution of molecules on the surface:

$$Enhancement = R(\omega)R'(\omega'),$$  \hspace{1cm} (2.8)
where \( R(\omega) = |g| + \left[ \frac{2 \text{Re}(1 - g)g^*}{Q_1(\xi_0)} + \frac{|g|^2}{Q_1(\xi_0)^2(\xi_0^2 - 1)} \right] \left[ -\left( \frac{\xi_0^2 - 1}{\xi_0^2} \right)^{1/2} + \xi_0^2 \sin^{-1}\left( 1/\xi_0 \right) \right] \) \\
\[ \xi_0 = \left( 1 - \frac{a^2}{b^2} \right)^{-1/2} \]

\[ Q_1(\xi_0) = \frac{1}{2} \frac{\xi_0}{\xi_0 - 1} \ln \left( \frac{\xi_0 + 1}{\xi_0 - 1} \right) - 1 \] \quad (2.9) \\

\[ g = \frac{\varepsilon_i - \varepsilon_0}{\varepsilon_i + \chi\varepsilon_0} \] and \[ \chi = -1 + \left[ \frac{\xi_0}{2} \ln \left( \frac{\xi_0 + 1}{\xi_0} - 1 \right) \right]^{-1} \] \\

From simple math, \( \chi \) is more than 2 for prolate spheroids and less than 2 for oblate ones. Similarly, if there is an excitation perpendicular to the symmetry axis, or, in other words, along the minor axis, the last expression would be:

\[ \chi = -1 - \left[ \frac{\xi_0}{2} \ln \left( \frac{\xi_0 + 1}{\xi_0} - 1 \right) \right]^{-1} \] \quad (2.10)

with all other formulas left the same. Regardless of the case, when \( \chi > 2 \), the plasmon resonance condition \( \text{Re}(\varepsilon_i + \chi\varepsilon_0) = 0 \) is satisfied for a red-shifted wavelength, when compared to a sphere, according to the expression for the dielectric constant in the free electron model. Thus both oblate and prolate spheroids have plasmons that are red-shifted from those for the sphere case. An electric field distribution for an elliptical silver particle is shown in Fig. 6a. The field intensity distribution was calculated with respect to the main resonance peak of any particular orientation, as their positions differ \[48\] (see Fig. 6b). This will be shown to be important in the Results chapter for SERS. Unfortunately, such simple models generally cannot account for extremely large enhancements (i.e. far above the mentioned \( 10^5 \)), which makes their validity for SERS doubtful.
There is some debate about the overall efficiency of the high aspect ratio metal particles over more spherical ones. The work of F. J. Adrian [49] suggested the superiority of prolate spheroids with the two shorter semi-axes being approximately two times less than the longer one versus all other spheroids. The works of Kerker et al. [50], in which a clear increase of the magnitude of the enhancement has been reported for silver, gold and even copper prolate spheroids with increasing aspect ratio, and Barber [51], who made a similar conclusion, present a contrary picture. However the latter work didn’t study particles with aspect ratio of more than 2. When particles with clearly discernible edges are considered, a theoretical study [52] has revealed that enhancement is highest with increasing sharpness of the feature, assuming zero radius of curvature. The enhancement on the apexes of a prolate ellipsoid is highest when the polarization is along the longer axis of the particle [53], which is in agreement with a simpler model described in detail above.
Yet, there have been hardly any experimental works, which report direct evidence of such overall superiority of extended particles over spherical ones due to complications in sample preparation and accurate measurements of those. One could refer, for example, to the work of McLellan et al. [54], where, in fact, silver nanocubes were found to produce lower enhancement than did silver spheres, with the exception of edges, which produce similar enhancement to that of a sphere. Such a finding is opposite to the earlier theoretical works discussed above. However, the discrepancy could be explained by the smaller thickness of the square particle.

For a sample prepared by either roughening of a metal surface or evaporation/sputtering of metal particles onto a substrate, there exists a huge number of plasmons, located at different wavelengths, since the sizes and shapes of the particles are different from one another. Consider the particular case in which they are characterized by a Gaussian distribution. The distribution of the plasmons over the different wavelengths would be broader than Gaussian (i.e. two overlapping Gaussians), since particles with higher aspect ratios would contribute two plasmon bands at wavelengths lower (short semi-axis) and higher (long semi-axis) than the wavelength of the plasmon from a spherical particle of the same overall volume.

Enhancement by elongated particles has been studied in considerable detail. There exist a number of simulations on the position of a plasmon resonance for silver [55] and gold rods [56, 57] and its change with respect to the size distribution [58, 59].

Multi-particle enhancements were first studied by P. K. Aravind and co-workers [60] and a significant increase of the local field in the space between two spheres due to their electromagnetic interaction was reported, although at a cost of field decrease outside
of the “gap”. Multiple further studies have been made by several different groups [61, 62, 63], which considered cases where molecules were trapped in enclosed cavities between spherical particles, as in the first case, or in spherical cavities in the surface of a metal film, as in the other two papers. All of these studies were aimed at finding a theoretical proof of a possibility of extremely high enhancement, observed in experimental works, since the enhancement, calculated to arise from single metal particles was not enough to justify such enhancement levels. Such theoretical confirmation was indeed found in all three papers. Later, more sophisticated theoretical modeling was performed [64], which dealt with molecules trapped between metallic rods, partially embedded into the surface of the same material. In that work, it has been reported that the best enhancement is found when the rods are barely touching each other, creating a very narrow gap for the analyte molecules (too loose, optimal case and too tight, as seen in Fig. 7).

Fig. 7. A chain of silver semicylinders placed upon a silver slab with thickness $l = 2R$, where R is the radius of the semicylinders. Reprinted with permission from [64]. Copyright 1996 by the American Physical Society (http://prl.aps.org/abstract/PRL/v77/i6/p1163_1).
More recently, an important principle has been proposed – cascade amplification, which justified the possibility of the highest enhancements observed in experiments [65]. This principle stated that the highest enhancement is achieved between the ultimate and penultimate particles in a “train” of particles of decreasing size. Spherical particles of different selected radii have been placed in a sequence with smaller spheres placed consecutively further away from the larger ones (see inset in Fig. 8a). Moreover, when two such structures are combined together with their tops facing each other (Fig. 8b), the enhancement is even greater. It should be emphasized that the effect requires the small particles be located in the enhanced field of the larger ones. This cascade enhancement is possible only when the emission bands of bigger spheres exactly correspond to the absorption bands of the smaller spheres located in their region of enhancement.

Fig. 8. Field enhancement distribution for a three sphere assembly (a) and for two combined three sphere assemblies (b). Reprinted with permission from [65]. Copyright 2003 by the American Physical Society (http://prl.aps.org/abstract/PRL/v91/i22/e227402)
2.3.2. Preparation procedures and known experimental data

2.3.2.1. Electrode surfaces, vacuum evaporation and sputtering techniques

Starting from the discovery of SERS, which was done on a roughened silver electrode, multiple groups accepted this approach as the main approach to reliably manufacture an effective SERS substrate. This could be typically the approach undertaken by groups who felt themselves new to the technique.

More recently, vacuum deposition, as well as sputtering, the latter also known as “cold-deposition” technique, became known. Shortly after the first publications, where this technique has been utilized [66, 67], more groups started using it and vacuum deposition became much more widespread. The advantages of this technique over the former one are obvious: higher availability of equipment, which is used for other purposes, and low cost of expendables.

Currently, this method of preparation has evolved into more complex deposition techniques such as the so-called Nano-Sphere Lithography (NSL) [68], which enables the production of patches of gold with some flexibility in their size and shape during the deposition process. Such flexibility is achieved through a technique called silver (or gold) shadow evaporation, where a substrate with a monolayer of spheres is tilted at an angle and some areas become shadowed by the spheres from the evaporating metal (see, for eg. [69]).

Studies of a number of etched and or otherwise roughened films resulted in some very important conclusions [70, 71] – it was confirmed, that active SERS sites with the
size below 100 nm should exist on the surface of a silver film in order to produce high enhancement for Raman signal. On the contrary, smooth silver surfaces produce very little enhancement.

2.3.2.2. Chemical synthesis

While extensive works on the preparation of gold [72] and silver [73] colloids have been done as early as the XIX century, they were of little practical value to the scientific community. Although silver clusters were used as anti-microbial surfaces [74], there is little available scientific literature on this topic.

The discovery of SERS effect has spurred additional interest to all types of gold and silver cluster preparation. The very first chemical syntheses, used for the preparation of SERS-active samples, have involved the use of NaBH₄ [75], an extremely powerful reducing agent, which resulted in almost instantaneous reduction of all available silver or gold ions and formation of fairly monodisperse colloids. In such colloids, diameters of particles were typically around 5 nm. Reliable formation of high fractions of particles with higher diameters is very difficult to achieve.

Later articles [76, 77, 78] have shown the effectiveness of organic reducing agents, in synthesis of bigger silver nanoparticles. For example, such agents as sodium citrate [76] and ethylenediaminetetraacetic sodium [77] have been utilized. A recent paper [79] has demonstrated a significant difference in the nature of the resulting particles depending on the introduction of other reducing agents as reactants. For
example, simple sodium citrate method results in higher crystallinity of silver particles [80].

All of the above authors have observed the main optical absorption peaks in the area of 400-405 nm for small silver particles and 520 nm for the gold ones. The peak tended to shift to longer wavelengths by a ten to twenty nanometers with the increase of the particle diameter. In another paper, it has been demonstrated that the optical absorption shows a separate peak at 700 nm and higher for silver if a formation of strings of particles occurs [81], which makes it potentially possible to fine-tune the plasmon resonance for a specific laser wavelength. More recently, with spreading availability of TEM, detailed characterization of metal nanoparticles became easier, which, in turn, led to the resurge in the development of colloidal solutions of gold and silver.

As an example, the synthesis of fairly monodisperse elongated silver [82, 83] and gold [84, 85, 86, 87] nanorods has been achieved. Some work was done in the development of a synthesis of cubic [88] and triangular silver particles [89] and bimetal core-shell particles [90, 91] with gold being typically the core due to ease of making monodisperse gold particle solutions, although the Ag-core and Au-shell particles have also been synthesized [92]. The principle is generally based on sequential reduction of two metals, silver and gold, so that the initial core particles serve as nucleating agents for the shell growth which significantly reduces the possibility of single-metal particle formation.

Even more complex core-shell structures, where silver particles reside at tunable spacings on the surface of silica cores, have been synthesized recently [93]. One could
also refer to a review article by Wiley et al. [94] to find pathways to synthesize such unusual shapes as hollow core particles, as well as more conventional ones.

Several detailed optical studies have been done on the gold particles with the intent of determining the position of the optical absorption resonance with respect to their aspect ratio [95, 96]. The results from the two different works were in good agreement.

All of these could be potentially used for surface-enhanced Raman spectroscopy, as has been done in a work by Orendorff et al. [97]. However, most research has been done on SERS in solutions rather than on flat substrates. Only a few studies on the latter were conducted due to complications in the characterization of surface morphology of the SERS substrates and its general non-uniformity.

2.3.2.3. The possibility of strong enhancement (nanogaps, nanoroughness)

The discovery that the strongest enhancement seen in most experimental works [22, 98] could not be justified by the existing theoretical modellings of the possible enhancement on the surface of gold or silver particles led scientists to the notion that it must be interference of the plasmons in the multi-particle assemblies (two or more). Such a possibility has been studied in some of the articles shown above.

Some insight into the strong enhancement from conventional rough silver surfaces has been given in a paper by Tsang and coworkers [99], where they have established a correlation between surface roughness and higher enhancement. Although the authors failed to get ultrahigh enhancement from their samples, reporting only a factor of 20 of signal enhancement for a monolayer of analyte molecules, correctly accounting for the
higher amount of analyte-silver interaction for rougher substrates, the main conclusion that higher roughness of a silver film is increasing the level of enhancement is nevertheless quite significant.

Important observations were made in an article, dealing with porous silver films, deposited at temperatures below 180 K [100]. It has been found, that highest enhancement can be achieved from deep pores, where analyte molecules have been trapped.

Rather interesting conclusions were made in a paper by T. H. Wood [101], where strong SERS is achieved only for rough silver layer of at least 15 nm thickness. Very low enhancement was observed for layers of 5 nm or less. The author stated that this ruled out the possibility of atomic-scale roughness creating extreme enhancement. The exact surface roughness was not studied, since AFM spectroscopy was not yet developed at the time. Given that the experimental results were quite reliable, such a conclusion comes in contradiction with many theoretical studies.

In a recent paper by Funston et al. [102] it has been shown that relative positions of two gold rods influence not only the scattering intensity of such an assembly, but also the position of the plasmon resonance, which can easily differ by 200 nm or more for two rods placed end-to-end and two parallel rods with side-to-side placing (see Fig. 9)
2.3.2.4. Intermittent enhancement (blinking effect)

The very first observation of intermittent signal from an analyte in SERS has been reported more than a decade ago [44; 98]. In those papers, the authors have observed the appearance and disappearance of discernible peaks over a weak or zero background. The peaks were characteristic to the analyte molecules under investigation. Moreover, in the
second case, the authors have calculated statistically that they have in fact observed the appearance of one, two and three molecules under the objective. Additionally, in the first paper, the effect of peak shifting has been observed and this has been attributed to molecules being adsorbed to different sites of an enhancing particle.

Further research has proved that enhancing particle aggregation is a necessary condition for “blinking” to occur [103]. The enhancement was estimated to be around $10^{10}$. It has been suggested that temporal fluctuations of the spectra appear to be characteristic of single-molecule detection limit. In another paper by the same authors, an observation has been made that “blinking” does not appear if relatively small (30 nm) silver particles were used [104]. Another paper [105] confirmed the necessity for metal particle aggregates to achieve effective SERS hotspots and provided AFM images of such aggregates.

“Blinking” is typically associated with the motion of the analyte molecules on the surface of SERS particles [106], confirmed by an abrupt loss of a large fraction of Raman signal for most of the observed molecules. The unpredictability of spectral fluctuation has been further confirmed [107].

An important observation has been reported in a paper by Futamata et al. [108] that a molecule without electron transitions in bulk state in visible light was still giving “blinking” spectra. This supported the assumption that molecule diffusion is the reason of blinking, rather than some electron transitions. A suggestion of thermal activation of molecules has been proposed, which assumes that molecules need to diffuse into a hot spot, based on measurements at 77 K and room temperature. This suggestion has been supported in a paper by Emory and co-workers [109]. The authors made an overall
conclusion that blinking SERS arises from thermally activated diffusion of individual molecules on the particle surface, perhaps also coupled with photo-induced electron transfer and structural relaxation of surface active sites. In a different paper [110] the reason for the activation was argued to be different, namely aggregation of particles or Ostwald ripening, confirmed by AFM images, when molecules get gradually squeezed in between the particles. There is still considerable debate over the exact nature of blinking or whether there could be several simultaneous mechanisms for its appearance.

An effective pathway to confirm single molecule detection has been proposed [111, 112], which involves the so-called “bianalyte approach”. In this approach, the molecules are effectively competing for a single spot (given that the probability of finding several hot spots under the illuminated spot is very low), thus creating the observed dynamic behavior. This pathway allows one to infer single-molecule detection without the necessity to maintain a monolayer of molecules over the SERS cluster.

It is apparent, that only blinking suggests high enhancement, while SERS samples, which do not exhibit this phenomenon, most likely possess only very moderate enhancement or, as has been suggested in a recent paper [113], no enhancement at all, as there could be different sources of the signal increase.

The literature review above suggests that SERS metal particles, capable of strong Raman enhancement, can be synthesized by different methods, physical or chemical. Theory also suggests that extreme enhancement can be obtained from aggregated or touching particles, which apparently is not possible for isolated ones. The aim of our study was to determine how to tailor a SERS substrate in such a way, so that the
efficiency of the enhancement becomes highest and it becomes a substrate of choice for single-molecule detection. The results of this study are presented in Chapter 4.

2.3. Tip Enhanced Raman Spectroscopy, possibilities and challenges

2.4.1. The definition of TERS and its importance

Scanning near-field optical microscopy (SNOM) is a technique for optical and spectroscopic imaging of different materials with spatial resolution far better than the diffraction limit [114-117]. With the advent of commercialized near-field optics, SNOM became a powerful optical tool to study nano-structures. A general SNOM system can be depicted as in Figure 10 [118].

Fig. 10. Schematic of a near-field spectrometer with an inverted microscope. The illumination beam is supplied through an aperture probe with a proper feedback loop to control the distance between the aperture probe and the sample. The signal from the near-field region is collected with an inverted probe and a spectrometer after filtering out the incident beam. Based on ref. [118].
It has been proposed [119] that a SNOM can be used for data recording by inducing photochemical reactions in a highly localized area. Infra-red optical signal amplitude from equal in size gold and polystyrene 12 nm-high particles was shown to be different, suggesting the possibility for material differentiation even without the use of Raman signal [120]. Optical transmission images with appertured SNOM set-ups have shown fair resolving capability of at least 300 nm for imaging of interiors of stained cells [121] (Fig. 11), according to the image.

Two different approaches exist for implementing SNOM: aperture-limited [121-126] which has laid the foundations of the technique, and apertureless [121, 127, 128] optics.

A probe with an aperture smaller than the wavelength of visible light has been developed to improve the lateral resolution of the aperture-limited system. The general probe uses a tapered fiber optic waveguide coated with a reflective metal coating. The
lateral resolution is directly dependent on the aperture size of the probe. The aperture size must, therefore, be comparable to the desired resolution. The transmission ratio decreases an order of magnitude with each 50% decrease in aperture size. This means that to achieve resolution below 50 nm, tremendous intensity must be sacrificed in any optical spectroscopy. When compared to a focal spot of the size of 1.5 μm in diameter in apertureless Raman, a 50 nm aperture will let through 900 times less light.

In the second case of an apertureless system, a sharp tip is used in order to create a local source of light, which greatly increases resolution [129-131]. One of the widely used apertureless techniques is the so-called tip-enhanced spectroscopy (TES). It utilizes a sharp metal or metal-coated tip, which provides high enhancements of the optical field in the vicinity of the tip. One particular type of SNOM of interest here is tip-enhanced Raman spectroscopy (TERS) [116, 117, 121, 125-128, 132, 133]. It is essentially a SERS probe of very small dimensions, which is scanned over a surface, collecting both AFM and Raman signal at the same time. If this enhancement is large, in the first approximation the resolution is limited to the size of the tip, which is much smaller than the laser spot. TERS has received significant attention in recent years [134, 135] and optical (Raman and fluorescence) imaging with lateral resolution of 10-20 nm has been achieved [136, 137].

The key element of the apertureless optics is the metalized (or metallic) tip. Development of tips that can provide enhancements of the optical signal of the order of ~10^6 remains a challenge [138, 139], although it is feasible. There are many other complications in TERS technology, including mechanical and thermal damage as well as chemical alteration of the analyte, substrate and even the tip itself. While mechanical
damage can be significantly reduced by the use of non-contact scanning probe (e.g. tuning-fork assembly), thermal damage and chemical alteration are still issues to be reckoned with.

For such a useful tool as TERS, one could find an even bigger range of practical applications, than just for regular surface-nearfield optical absorption or transmission. This technique, combining high resolution chemical and topographical imaging, as has been proved in an important work of Hartschuh et al. [136], where optical resolution of about 10 nm for single carbon nanotubes has been achieved, can be used for characterization of different nanostructured materials. A good example would be the works of Lee et al. and Zhu et al. [137; 140], where images of strained silicon/silicon oxide stripes with high resolution have been presented, making TERS potentially suitable for mapping strain in developing strained silicon computer processor circuits.

It has been shown recently that TERS can also resolve short-ranged chemical or mechanical interactions between the metal on the tip apex and the molecules of an adenine sample, which are observable only within the subnanometric vicinity of the tip [140]. This suggests that TERS can probe interactions on scales, unreachable by most characterization techniques.
2.4.2. Theoretical predictions for tips efficiency

Numerous theoretical modellings have been made, which tried to address the important question of how to produce a tip with high enhancement, which would also provide high resolution.

As one of the first factors to be considered is the issue of light polarization with respect to the tip. It has been best summarized in a research article by A. Bouhelier [141]. In Figure 12 one can see the comparison of enhancement for horizontal (left) and vertical (right) polarization of incident light with respect to the tip. It clearly shows that higher enhancement should be achieved in the latter case.

![Figure 12](image)

Fig. 12. Calculated induced surface charge density of a gold tip illuminated by a laser for different polarizations. Reprinted with permission from [141]. Copyright John Wiley & Sons 2006.

The changes of the opening angle of metal/metallized tips were studied, however the theoretical predictions are not conclusive, as the findings were quite controversial. Martin and co-workers [142] concluded that an increased opening angle increases enhancement, while Kawata et al. [143] has reported an opposite finding.
Another factor to be considered was the radius of a tip. Demming et al. [144] has come to the expected conclusion that a bigger tip radius decreases the level of enhancement. Such a result is not likely to be challenged, since a big radius means lower resolution, as has been also noted in the article, so such tips will not be used for scanning anyway.

An important parameter to be addressed was the material of the tip. It is now generally accepted that tips made of or coated with silver or gold provide the most enhancement, especially in the case of silver. Silver also has the highest reflectivity, which can be also contributing to signal enhancement, although this “enhancement” will not improve resolution. To achieve highest enhancement, one should use illumination wavelengths in resonance with the plasmon wavelength of the manufactured tips. Resonance wavelengths can be ~150 nm apart for tips made of silver and gold and having the same radius [145], as can be seen in Fig. 13.

Unlike AFM, tuning-fork assemblies give the user the ability to control tip to sample distance by monitoring the magnitude of the frequency shift. For these assemblies, enhancement dependence on tip-to-sample distance was studied. The abovementioned paper [144] reported a sizeable drop-off in enhancement for separations of 10 nm and more. A study on small tip-to-sample distances [146] has revealed that there is little to no change in enhancement up to 5 nm, at least in air environment.

Finally, the enhancement produced by metallic “self-similar” nano-antennae on the apex of a tip has been investigated theoretically by Kapeller et al. [147]. This approach follows the proposal of Bergman et al. discussed above to employ the principle
of cascade amplification. In TERS, as in SERS, this has been proved to be feasible, but so far only theoretically.

2.4.3. Manufacturing a TERS tip with good enhancement

In AFM-based TERS, where a change in the reflection of a laser beam from a cantilever arm on a diode is monitored, commercially manufactured tips are commonly used. Such tips are somehow altered to have a metal coating or a metal structure on its surface. Tuning-fork (TF) microscopy is using frequency and phase control of said TF to raster the probe across the surface of the substrate and can use any type of probe, which can be later attached to a tuning fork.

Although most apertured scanning is performed without enhancing the field, two manufacturing methods for aperture-limited TERS have been proposed as well. Such
methods are perfectly applicable for normal apertureless TERS and therefore deserve to be mentioned. In the following section, ways to manufacture TERS probes for apertureless scanning will be reviewed.

2.4.3.1. Wire etching in electrolyte solution

The method is based on etching a tip made of conducting metal in an electrolyte solution, where one electrode is placed in the solution and one is attached to the tip itself. A simple and inexpensive way to manufacture tips for tuning-fork TERS would be to use a typical procedure of tungsten tip etching with subsequent silver or gold coating [148]. More commonly, however, wire tips, etched directly from noble metal wires are used [148-151]. Such tips are rather robust, since they are made completely out of one material and their good resolving capability as well as durability has been unambiguously shown in the works mentioned. Moreover, even sharper tips have been reported in a more recent work [152]. Unfortunately, these tips do not have rough enough surfaces, which are often considered as detrimental for high enhancement.

2.4.3.2. Vacuum evaporation and sputtering

Vacuum evaporation or sputtering is, perhaps, the most common method for TERS tips preparation for AFM-based apertureless scanning. For the vacuum evaporation, tips are coated in vacuum with a layer of silver or gold by heating a boat with the chosen metal to temperatures above its evaporation temperature. The boat is
heated by applying high voltage to it. Both gold [153] and silver tips [154], prepared by the evaporation method, are regularly used. Plasma cleaning of evaporated metal surfaces is employed sometimes [127] as a way to get rid of contaminations. However, this can possibly alter the roughness of tips too.

Tips for TERS, prepared by sputtering a metal layer on the surface of the probe, are not very common [139]. The preparation procedure is also quite simple and is based on the ejection of atoms from a silver or gold flat substrate in vacuum due to bombardment of the target by highly energetic particles of argon plasma. The noble metal atoms then adhere to the tip, which is placed above the metal facing down, similar to the vacuum evaporation set-up.

2.4.3.3. Focused ion beam milling

A rather interesting approach has been taken by Taminiau et al. [123], where a metallic protrusion has been placed near the opening of the scanning fiber used for surface-nearfield Raman scanning. The problem with such devices is that the enhancing particle position is offset to the side of the incoming beam. This considerably diminishes enhancement and questions the very essence of an experiment involving such a tip. Although the probe was manufactured for an aperture-limited set-up, which has a number of drawbacks, such method could be perfectly viable for AFM or TF-based TERS.
2.4.3.4. Chemical deposition

It is no surprise that some research was done on the possibility of making a TERS tip using a method of direct reduction of a noble metal on its surface [155]. Apparently, the method is not yet suited for TERS, since the quality of the data in the paper collected using such tips is highly questionable.

2.4.3.5. Attaching a metal particle

Some attempts have been done to attach pre-made noble metal particles to the AFM tip. Placement of colloid silver particles has been reported [156], however, the description of the method of particle preparation, as well as the method of their attachment, were missing in the article.

In a different paper [122], authors glued a gold particle of approximately 100 nm diameter next to the waveguide opening. The resolution of scans performed with this SNOM probe was at or slightly larger than 100 nm. Such an approach could be potentially applied for the case of apertureless TERS. However, smaller spheres should be used to improve at least the topography resolution, if not Raman.

2.4.4. Issues which compromise the effectiveness of TERS tips

There exists a number of requirements for metal or metal-coated tips to be effective as TERS probes. The first and foremost is the requirement for a tip to provide
high enhancement. This is important, as higher enhancement results in higher signal, making better chemical species differentiation and higher resolution possible.

However, TERS tips should also be quite durable and withstand mechanical, chemical and heat degradation, as very minor nanoscale changes at the very apex of a tip can alter its enhancing ability. This will make an experiment unrepeatable, regardless whether the enhancement is decreased or increased. Severe changes in morphology of a tip can drastically decrease its efficiency for topographic scanning in the case of AFM-based TERS or impose additional challenges with the feedback control in the case of tuning-fork TERS.

2.4.4.1. Chemical instability

It has been reported by several authors [157, 129], that enhancement from a typical vacuum evaporation-coated silver tip fades after just half of a day in ambient conditions when running experiments. This has been generally attributed to chemical degradation of a tip.

Certain things were identified incorrectly in prior research. The erroneous assumption that oxygen quenches the enhancing ability of silver and copper metal layers through the formation of submonolayer coatings on the surface [158] was not challenged for some time. Currently most researchers believe that the effect of oxygen is in oxidation of the noble metal. It is generally considered that oxidation is the primary type of chemical degradation of Raman enhancing surfaces [159, 160]. The possibility of silver surface inhibition through oxidation has been perfectly illustrated by Erol et al. [161],...
who showed that oxidation of SERS substrates can severely decrease their efficiency even in water, which has rather low levels of oxygen, in comparison with ambient air.

However, sulfidation has been identified as a much more important factor in a number of publications [162, 163], where it was shown that it is tarnishing of silver substrates rather than oxidation, especially at lower temperatures. Franey et al. have pointed out that H₂S and COS result in ten times higher rates of sulfidation than CS₂ and SO₂ [163]. Sulfidation has been shown to have three different effects, which prohibit chemical enhancement to occur for tarnished SERS silver structures. First, chemical enhancement was not possible due to the newly formed separation layer. Second, a significant portion of silver was converted to silver sulfide that does not possess the free electrons. Finally, optical properties of silver plasmonic structures were altered as the Plasmon peak shifted in position and changed in shape [164]. The effect was measured for silver sulfide films from 1.3 to 400 nm. Silver nanoparticles were shown to be much more prone to atmospheric contamination as the growth of tarnish was measured to be 7.5 times higher than for bulk silver [165]. Estimates for tarnish grows are in the range of 0.3 [164] to 3 nm [165] per day in normal laboratory air conditions and up to 10 nm/h for humid conditions with 10% H₂S in the air [164].

2.4.4.2. Annealing of metal clusters by heating

It has been shown experimentally [151], that nanoscale roughness, similar to the case of SERS substrates, is very important for high enhancement in TERS. In the work of
Lee [9] a direct dependence has been shown between the degree of enhancement and roughness of the metal layer deposited on the TERS tip (Fig. 14).

![Graph showing enhancement dependence on RMS film roughness.](image)

Fig. 14. Enhancement dependence on the RMS film roughness.

It has been also shown, that $300^\circ$ C is more than enough to completely smooth out roughness features for 40 nm triangular polycrystalline silver particles on a substrate [166] and convert them into spheres. One would expect that nanoscale roughness features would disappear at significantly lower temperatures.

The very first attempts to determine the exact degree of laser-induced heating for SERS substrates were done quite some time ago [167] and the degree of this heating was determined to be very small for even high laser powers. However the experiment was done for a thick silver electrode, where the heat could easily dissipate into the bulk. This is quite unlikely to be the case for nanometer-scale gold or silver features. We are not aware of other works done to measure the raise of temperature in SERS.
Zhang et al. [168] have shown in simulations that the degree of heating for a TERS can go up to about 50° C for the tip apex for rather low powers of 1 mW. Moreover, their experiments has showed that hot-spot features can be irreversibly annealed with such temperatures. Simulations by Downes et al. [169] have resulted in quite comparable levels of heating. However, no attempts for direct temperature measurements for either small SERS nanoparticles, or tips for TERS have been done experimentally so far.

As can be seen, various methods have been developed for the preparation of enhancing tips. Many of the approaches were based on conclusions made from theory, however the available theory itself can guide to opposite conclusions. Also, very few systematic studies of the tip efficiency have been done so far. In addition to the complication of producing a highly-enhancing TERS tip, there are multiple issues, which can compromise the tip efficiency. Attempts at solving these issues and ways to create more effective tips are described in Chapter 5 of this Thesis. Apart from SERS and TERS studies, applications of regular Raman spectroscopy will be shown in the field of carbon nanotube purification.
CHAPTER III

EXPERIMENTAL

The sample preparation, materials and equipment used were grouped by projects. Each section gives complete information about the experiments pertinent to a particular project or a scientific challenge.

3.1. Preparation of SERS substrates

3.1.1. Procedures of preparation

3.1.1.1. Vacuum chamber deposition

Metallized substrates were prepared by physical vapor deposition of silver at low pressures ($10^{-7}$ Torr). Depositions were performed using a conventional vacuum chamber designed for evaporation of metal and deposition onto a flat substrate. The thickness of the deposited layer was controlled by manipulating deposition rate and time of exposure to the evaporated metal. The amount deposited was monitored with a Sycon (STM-100) quartz-crystal microbalance. Silver and silicon oxide ($\text{SiO}_x$) were evaporated from separate tungsten boats. A deposition rate of 0.2 Å/s was used for the deposition of silver. Silicon oxide was deposited at a typical rate of 0.6 Å/s to minimize the exposure of the substrate to the high temperatures required for $\text{SiO}_x$ evaporation. However, the typical
thickness of a SiO₂ layer was sufficient to ensure complete coverage of the substrate. Protected and unprotected substrates were removed from the deposition chamber and placed inside a desiccator to minimize their exposure to the environment. Others were stored under ambient conditions to evaluate the effect of commonly used storage conditions on the optical properties and enhancement capabilities.

3.1.1.2. Preparation of polymer films

Prior to polymer film deposition, the silicon wafers were treated by Piranha solution (mixture of H₂SO₄ and H₂O₂) to remove all organic contamination from the surface. The native oxide had been etched away using a 5% hydrofluoric acid (HF) solution. Etching improves the spreading of the polymer film on the substrate. Polymer films were used as substrates for both vacuum deposition of a plasmonic silver layer and drop-casting of solutions containing silver or gold metal particles. In both cases, the polymer films were created first and were an inherent part of all SERS substrates which are discussed in the following chapters of this dissertation. The polymers used for film preparation were poly(3,4-ethylenedioxythiophene)/poly(styrene-sulfonate) (PEDOT-PSS) from HC Starck (in a stabilized solution) and linear polystyrene (PS) primary GPC standards (MW = 15k, 110k, 470k) from Waters Corporation.

Below is the description of film preparation for the “protection” experiments. The polymer films were deposited by spin coating a 2.5% wt. polystyrene solution in toluene at 2000 RPM for 2 minutes on a silicon wafer. After annealing the polystyrene film in a high vacuum oven, at approximately 10⁻⁷ Torr at 180°C for 2 days, to remove residual
solvent and any stress from the coating step, two different types of SERS substrates were fabricated. In one case, the plasmonic structure was deposited directly on the polystyrene film. In the other, an ultrathin silica layer was deposited on the polymer film first, and then the plasmonic structure was deposited to produce a SiOx/Ag/SiOx/polymer multilayer structure. In both cases, the surface of the metal structure in contact with air was protected by a 5 nm thick SiOx layer. All of these layers were deposited by thermal evaporation PVD as described above and in all cases the plasmon-active structure was deposited as a silver layer of nominal 4.5 nm thickness. All silicon oxide coatings (SiOx) were deposited by evaporation of a silicon monoxide (SiO) precursor.

For the development of better SERS substrates, a few changes were done to the above procedure. The silicon wafers were washed carefully with boiling chromic solution (100% H$_2$SO$_4$ and K$_2$Cr$_2$O$_7$) with no HF etching. The cleaned wafers were rinsed with deionized water and dried with blowing nitrogen gas. Additionally, no annealing was done for these substrates.

PEDOT-PSS films were prepared according to the following procedure: 50% or 10% vol. solutions of PEDOT-PSS in ethanol were used for spin casting, after which the sample was left to dry for no less than 12 hours in roughing vacuum.

3.1.1.3. Chemical synthesis
3.1.1.3.1. Synthesis of silver structures

Elongated silver particles have been synthesized by the reduction of AgNO$_3$ (2.5*10$^{-5}$ M aqueous solution) by 1% wt. trisodium citrate aqueous solution in a 1:1
molar ratio. The volumes used were 1 and 0.2 mL respectively. Sodium citrate also served as a capping agent for the resulting particles. The silver nitrate was added gradually during the course of the reaction to facilitate the growth of longer particles (0.2 mL five times with 10 min intervals for 1 mL total). The reaction mixture in a 250 mL flask was brought to boil prior to the addition of the nitrate and was stirred during the course of the synthesis (70 min).

The procedure described above was performed with the following variations: 1:1, 1:2 and 1:5 molar ratio of silver nitrate and trisodium citrate; five times higher concentration of reagents. The color of the resulting mixture was grayish-green.

Square-shaped silver structures have been synthesized by the reduction of AgNO₃ (5*10⁻³ M aqueous solution) by 1% wt. trisodium citrate aqueous solution in the presence of polyvinylpyrrolidone (5*10⁻³ M aqueous solution) (PVP) in a 1:2:3 molar ratio. PVP served as a templating agent for the growing particles. The volumes used were 3.5, 1 and 10.5 mL respectively. The reaction mixture of 100 mL in a 250 mL flask was brought to boil prior to the addition of the nitrate and was stirred during the course of the synthesis (80 min). In a different reaction, a molar ratio of 1:2:1 was used with the other parameters kept constant. The color of the resulting mixture was grayish-green.

Raspberry-like structures of silver were synthesized at the surface of silica nanoparticles. These 100 nm “nanoraspberries” were made by the Stöber method from tetraethyl orthosilicate (TEOS) precursor in ethanol, with NH₄OH as basic catalyst, at 40°C for 1 hour and followed by the ammonia reduction of AgNO₃ at the surface (60°C, 20 hours). These particles were centrifuged and sonicated with fresh ethanol three times before drying [170].
3.1.1.3.2. Synthesis of gold structures

Spherical gold nanoparticles have been synthesized according to the Zhigmondi method. A water solution of 100 mL HAuCl₄ (0.001 M) was treated with 10 mL trisodium citrate (1% wt. in water) as both a reducing and a capping agent. The reaction mixture in a 250 mL flask was brought to boil prior to the addition of the citrate and was stirred during the course of the synthesis (15 min). The color of the resulting mixture was brick-red.

Rod-like gold nanoparticles have been synthesized according to the method, described by Wu et al. [87], which is itself a modification of the seed-mediated Murphy method [84]. A volume of 0.2 mL of 0.025 M trisodium citrate solution was added to 19.8 mL of aqueous solution containing 2.5*10⁻⁴ M HAuCl₄ and stirred for 3 min. Concurrently, 10 mL of 0.01 M NaBH₄ solution was prepared by adding NaBH₄ to 10 mL of ice-cold 0.025 M sodium citrate solution. When 0.6 mL of the NaBH₄ solution was added to the HAuCl₄ solution, the resulting solution immediately turned orange-red. 100 mL of growth solution was made by dissolving 0.01 mol of cetyltrimethylammonium bromide (CTAB) surfactant in 100 mL of 2.5*10⁻⁴ M HAuCl₄ aqueous solution. In two flasks, labeled “A” and “B”, 25 μL of 0.1 M ascorbic acid was added to 4.5 mL of growth solution. In flask “C”, 250 μL of 0.1 M ascorbic acid and 200 μL of nitric acid were added to 45 mL of growth solution. Next, 400 μL of the gold seed solution was added to the solution in flask A and stirred slightly for 3 s. Then 400 μL of the solution in flask A was added to flask B and stirred for 3 s. Finally, 4 mL of the solution in flask B
was transferred to flask C and stirred for 3 s. Flask C was left undisturbed for 12 h for the reaction to go to completion.

3.1.2. Characterization of substrates
3.1.2.1. Transmission electron microscopy of separate particles

The particles were deposited from their water solution on commercially available carbon-coated copper grids (SPI, 62 micron pitch and 37 micron hole width). The deposition was done by drop casting small volumes of the sols on a grid with an insulin disposable needle and letting them evaporate freely. The grids were imaged using a FEI Technai 12 Scanning Transmission Electron Microscope operating at 120 kV.

3.1.2.2. Ellipsometric thickness measurements

The hydrophobic layers thicknesses were measured using a Gaertner L116C ellipsometer. The instrument was equipped with a He-Ne laser (=632.8 nm) fixed at an incident angle of 70° with respect to surface normal. All thicknesses reported were obtained by subtracting the thickness of the silicon oxide (SiO$_x$) from that of the hydrophobic layer with the oxide using a single layer model with refractive index value of 1.456 for SiO$_x$. A minimum of three measurements of at least three different locations at the surface was acquired.
3.1.2.3. Atomic-force microscopy of evaporated substrates

A Park Scientific Autoprobe® CP atomic force microscope operated in a tapping mode with rectangular silicon cantilevers (Veeco, $R_{\text{curvature}} < 10 \text{ nm}$) was used to characterize the surface morphology and thickness of unprotected and protected evaporated silver structures on flat substrates.

Samples with gold particles deposited on them were measured using a Park Scientific Autoprobe® CP AFM, as well as a Quesant Qscope 250 AFM (Quesant) with an nPoint XY100A XY piezo sample stage. Imaging was done in tapping mode using rectangular silicon cantilevers (MikroMasch, $R_{\text{curvature}} < 10 \text{ nm}$).

3.1.2.4. Optical absorption of hydrosols

Optical absorption of gold and silver particle solutions was performed in a Agilent 8453 UV-Visible Spectroscopy System optical spectrophotometer equipped with deuterium and Hg lamps. The signal was collected in the UV, visual and near-infrared ranges. The samples were loaded in quartz or plastic couvettes and placed in the path of the beam. Additionally, gold nanorod particles were drop-coated (15 minutes) onto ~0.1 µm thick PS films on Corning 30x50 mm glass slides. Optical absorption measurements were done on the same instrument for this slides.
3.1.2.5. Determination of Raman enhancement

Scheme 1 shows a schematic of the Nano-Raman instrument with side illumination. The Nano-Raman system consists of a Horiba Jobin Yvon Labram HR-800 Raman spectrometer coupled with a Quesant (QScope 250) AFM with a XY piezo sample stage (nPoint XY100A) and side illumination optics. A confocal microscope using a long-working distance Mitutoyo (APO SL50) objective (50X, 0.42 NA) was used for measuring optical properties and signal enhancement. The objective is fixed on a XY-stage controlled by step motors with an accuracy of ~ 40 nm to position the objective with respect to the probe. The 514.5 nm line of a Coherent Innova 70C argon ion laser and the 647 nm line of a Lexel Raman-Ion krypton laser were used to obtain the Raman signals with an incident power < 2 mW on the sample. The objective was placed at a 60° angle with respect to the surface normal.

The same instrumentation, but without the Quesant AFM, was used to measure the enhancements from flat SERS substrates. The measurements were performed in 60° and 0° angle geometries. The signal was measured at three different locations on the surface for TERS tips and at least five different locations for all SERS substrates.
3.1.2.6. MicroRaman and NanoRaman scanning

NanoRaman (nearfield) and MicroRaman (farfield) scanning was performed using the system described above. The piezo crystal was moved in a 2D pattern under the confocal objective, which was focused on the surface, with or without the tip, respectively. The Raman signal and topography data (for NanoRaman) for every point were recorded with a home-made Labview software. Another home-made Labview program was used to process and analyze the data and then plot it in 3D (base on [9] with several improvements).
3.2 Preparation of tips for TERS

3.2.1 Procedures of tip preparation

The protected probes were prepared by sequential physical vapor deposition of silver and silicon monoxide (SiO$_x$) or silver and aluminum (Al) at low pressures ($10^{-7}$ Torr) using the same vacuum chamber used for flat substrates. A deposition rate of 0.2 Å/s was used for deposition of the silver to minimize distortion of the cantilever. Silicon monoxide was deposited at a typical rate of 0.6 Å/s, higher than that of silver, to minimize exposure of the cantilever and the silver structure to the high temperatures required for this evaporation. Annealing of unprotected or protected probes was avoided in order to reduce any chemical degradation and any bending of the cantilever caused by the difference in thermal expansion coefficients of the different layers.

In the case of aluminum, a deposition rate slower than 0.2 Ångstroms/s was used. Silver and aluminum were evaporated from separate tungsten boats. The adhered Al layer easily generates a passivating Al$_2$O$_3$ coating due to the high reactivity of Al with environmental O$_2$ under normal condition [170]. One duplicate of each protected and unprotected metallized probe was removed from the deposition chamber, placed inside a plastic desiccator containing fresh desiccant, and kept under vacuum to minimize contact with humidity or other possible contaminants. This storage condition is very close to a completely dry atmosphere (relative humidity ~0). Others were stored under ambient conditions to evaluate the effect of commonly used storage conditions on signal enhancement and optical properties.
3.2.2 Characterization of obtained tips

3.2.2.1 Transmission electron microscopy

A FEI Technai 12 Transmission Electron Microscope operating at 120 kV was used to measure thickness and to observe the morphology of the protective coatings using a sample holder specially constructed for SPM probes. The sample stage was tilted at an angle of 55° with respect to the surface plane to image the probes and several images were taken at various magnifications. A low beam intensity during focusing and a short acquisition time of 1 s were used to reduce cantilever bending caused by heating of the different layers covering the probe.

3.2.2.2 Scanning electron microscopy

Scanning electron microscopy images were captured using a JEOL JSM 7501 Scanning Electron Microscope (SEM). The tips were placed face up on aluminum cylinders covered with conductive tape to minimize charging by electrons. Qualitative elemental analyses of the probes were obtained using the energy dispersive X-ray spectrometer (EDX) coupled to the SEM, to check for degradation and contamination of the probes. An accelerating voltage of 5 or 10kV was used with an acquisition time of 10 s. The SEM images of gold particles were obtained under the same conditions.
3.2.2.3 Optical properties of tips

The principle idea for the measurements of optical properties of AFM tips is illustrated in Scheme 2. A white light beam is directed through one of the sides of a quartz prism and is reflected onto the other surface at an angle above the critical angle. Due to the fact that the refractive index of air is smaller than that of the prism material, the beam is internally reflected from the prism surface. At the same time, a short-range evanescent field is created at the surface, which effectively illuminates only the particles in the very close vicinity of the surface. The spectrum of the subsequent reflection from these particles can be collected and analyzed. The prism was illuminated with white light from a ACMI Xenon lamp with both UV and IR internal filters removed. The light was guided through a waveguide to a Mitutoyo Plan APO 20 (20X, 0.42 NA) long working distance objective to a side of the quartz prism and focused on the top surface of the prism. The AFM tips were carefully approached to the illuminated spot on the prism surface (the AFM approach procedure has been modified for this purpose). After that, another objective (Mitutoyo APO SL50, 50X, 0.42 NA) was focused at the very apex of the AFM tip. The optical signal in the range from 400 nm to 800 nm was collected both for the case of the tip in contact and the tip withdrawn. The collection was done using the same monochromator as described above, but without any notch filters.
3.2.2.4 Determination of Raman tip enhancement

The nano-Raman instrument described above was used to characterize signal enhancement and optical spectra of unprotected and protected probes. TERS contrasts provided by the probes were estimated using measurements on a 50 nm thick PEDOT/PSS film spin coated on an aluminum mirror. The error in the determination of the contrast was estimated to be on the order of 10%. Enhancement from all probes was measured at three different locations on a sample.

3.3 Separation of carbon nanotubes and Raman characterization

3.3.1 Centrifugation separation of nanotubes

3.3.1.1 Separation of “raw” SWCNTs.

Separation of low degree of treated and untreated SWCNTs (produced via the high-pressure carbon monoxide method, HiPCO™) with poly[(m-phenylenevinylene)-
alt-(p-phenylenevinylene)] (trans-PmPV) was performed as follows: 0.3 mg of SWNTs was added to a 20 mL solution of trans-PmPV (Mₙ=25,000, concentration of 0.1 mg/mL), in tetrahydrofuran (THF), and the mixture was sonicated for 3 h in an ice-water bath. The obtained suspension (Sus1) was subjected to centrifugation (7000 g, 6 h) to remove the nondispersible SWNTs. The resulting supernatant solution and sediment of SWNTs were designated as Sup-1 and Sed-1, respectively (Scheme 3). Sed-1 was collected and redispersed in trans-PmPV solution. When the sonication-centrifugation process was repeated, the second suspension, supernatant, and sediment were designated as Sus-2, Sup-2 and Sed-2, respectively. The sonication-centrifugation process was repeated 7 times to disperse the tubes sufficiently, thereby allowing the tubes to have an optimum interaction with the wrapping polymer chains. The same separation procedure as above was done using cis-PmPV as a dispersing/separating agent.

Separation of low degree of treated and untreated SWCNTs (produced via the high-pressure carbon monoxide method, HiPCO™) with [(p-phenylene-ethynylene)-alt-poly(p-phenylene-vinylene)] copolymer (cis-PPE-PPV) was performed as follows: “raw” nanotubes are dispersed in THF solution under sonication for 10 hours. Then cis-PPE-PPV in THF was added (~1 part per 20 parts of the solution). The solution of SWNTs and polymer was sonicated for another 2 hours. After centrifuging at 7000 rpm for 3 hours, the mixed solution was separated as supernatant and sediment. After that the sediment was mixed with polymer and sonicated for 2 hours and then centrifuged. The separation cycle was repeated several times.
3.3.1.2 Separation of “pure” SWCNTs.

SWCNTs, produced via the HiPCO™ method and purified using a nitric acid reflux process, were dispersed in THF solution under sonication for 10 hours. Then cis-PPE-PPV or PMPV in THF was added. The solution of SWCNTs and polymer was sonicated for another 2 hours. After centrifuging at 7000 rpm for 3 hours, the mixed solution was separated as supernatant and sediment. The sediment was mixed with polymer and sonicated for 2 hours and then centrifuged. The separation cycle was repeated until no (12, 6) tube was detected in the supernatant solution.
3.3.2 Atomic-force microscopy imaging of nanotubes.

3.3.2.1 AFM imaging of PmPV-separated nanotubes.

The microstructure of a PmPV/SWNTs film, obtained by spin-casting from Sup5 of a respective separation process, was examined using the Quesant AFM operated both in contact and tapping mode. Standard cantilevers from MikroMasch (NSC15/AIBS, manufacturer spring constant of shortest probe \( k = 14 \text{ N/m}, R_{\text{curvature}} < 10 \text{ nm} \)) and Park Scientific (silicon nitride tip, \( k \sim 0.01 \text{ N/m}, R_{\text{curvature}} \sim 20 \text{ nm} \)) were used for the tapping and contact mode, respectively. Scan size was 10x10 microns².

3.3.2.2 AFM imaging of PPE-PPV-separated nanotubes.

The microstructure of a PmPV/SWNTs film, obtained by spin-casting from a supernatant or suspension of a respective separation process, was examined using the Quesant AFM, Autoprobe CP and Veeco Nanoscope IIIe all operated in tapping mode. Standard cantilevers from MikroMasch (NSC15/AIBS, manufacturer spring constant of shortest probe \( k = 14 \text{ N/m}, R_{\text{curvature}} < 10 \text{ nm} \)) were used for the tapping and contact mode, respectively. Scan size was 10x10, 5x5 and 2x2 microns.

3.3.3 Raman characterization of nanotubes

Raman spectra of the initial and wrapped SWCNTs, both in THF and solid were measured using the Horiba Jobin Yvon Labram HR-800 Raman spectrometer and 647 nm
laser line of a Krypton Lexel Raman-Ion or 514.5 nm laser line of an Argon Coherent Innova 70C laser lines. The laser power was kept at around 1.0 mW at the sample. Solid samples were measured with a 50X long-range Mitutoyo objective, while solution samples were measured with a 10X Melles Griot objective (0.25 NA).
CHAPTER IV

SURFACE-ENHANCED RAMAN SCATTERING

Depending on a preparation method of a SERS substrate (evaporation-sputtering-roughening, chemical synthesis) and the material used (Ag, Au), the morphology of places which give highest enhancement is expected to vary. Such places, commonly referred to as “hot spots” [171], are expected on the basis of electromagnetic enhancement theory [172] to be located at the narrow junctions of metal clusters [103; 105]. It is conjectured that such junctions are occasionally formed when semi-crystalline chunks of silver are deposited on the surface or when particles form aggregates due to capillary forces upon drying of a liquid suspension. Schematics of perceived hot-spots in these two cases are presented in Fig.15.

![Schematic representation of field enhancement distribution](image)

Fig. 15. Schematic representation of field enhancement distribution anticipated for a) evaporated rough noble metal and b) chemically synthesized noble metal particles.
The purpose of this research was to determine the best procedures for the reproducible formation of “hot spots” in SERS substrates, identify the factors increasing or diminishing the enhancement, and develop the methodology for the extension of the effective lifetime of such materials.

4.1. Vacuum deposited silver

4.1.1. General description of silver-island films

As the most commonly used SERS substrate, films of silver islands created by evaporation were investigated for their effectiveness. Silver is less expensive than gold and can be easily obtained in a very pure form (99.999+). Additionally, it is known to have a very low damping of free electrons due to high conductivity, which is very important for strong plasmon resonance. A 100 nm film of polystyrene (PS) (~30k MW) is first spun on an etched silicon wafer. After 12h annealing of the film at around $T_g$ (~100\(^\circ\)), a thin silver layer of nominal 4.5 nm thickness was vacuum-deposited on top of PS. The silver layer of this thickness possesses the highest level of roughness [173] when a silver-island film is formed through the process of dewetting. The PS layer is created first to ensure the uniformity of its thickness.

As can be seen from Fig. 16, such a substrate can be quite effective for the enhancement of Raman signal. Measurement of a 100 nm PS film gives only several counts per second for the most intense peak at ~1000 cm\(^{-1}\), when illuminated with a laser beam of moderate intensity (1 mW) with 514.5 nm laser. Typical measurements of silver-coated PS substrates with this laser show 200-fold enhancements of the collected signal.
Assuming that only the signal from the top 20 nm of the PS film is, in fact, enhanced [174], the enhancement factor is found to be $10^3$ or similar. This level of enhancement is typically enough to investigate the Raman signal of the near surface region of layers of

Fig. 16. a) SERS spectra (10s each) of a 100 nm thick PS film spun cast on an etched Si wafer and then stored in a desiccator for 5, 10, 100 and 250h. The surface enhancement mechanism has been enabled by deposition of a 4.5 nm Ag film atop the PS layer; b) Raman spectrum of a bulk PS sample for the range of wavenumbers marked by a red square in “a”.
most materials. However, Figure 17 shows that the level of enhancement severely degrades over the first several days. This makes the Ag SERS substrates useless very quickly, as well as making the measured signal dependent on the time of the measurement. Figure 17 shows how the intensity of the main PS peak in the Raman spectrum varies for two different SERS substrates with different initial levels of enhancement (in other words, a “good” substrate and a “bad” substrate). The enhancements for the substrates decay to the same level, with the “good” sample degrading faster. The reasons for the wide variation in behaviors are not known, but could include varying degrees to which a particular degradation mechanism (thermal, chemical), or their combination is operative for a given substrate. Moreover, the initial level of enhancement could be different due to a number of factors: variation in the evaporation rate, air pressure inside the CVD chamber (i.e. quality of the vacuum) and even traces of materials that were used for earlier evaporations in the chamber. One thing could be noted with confidence – all unprotected SERS samples showed decreases of enhancement over time.

Another problem is immediately recognized from Figure 16. The spectrum from bulk polystyrene does not exhibit any other peaks as strong as that for the benzene ring breathing mode (~1000 cm\(^{-1}\)). However, the SERS spectrum shows some strong broad peaks at wavenumbers <300 cm\(^{-1}\) and at 1100 – 1700 cm\(^{-1}\). The former could come from silver sulfidation on the surface, while the latter are commonly identified as arising from amorphous carbon.

Amorphous carbon (a-C) on the sample can come from the deposition of semi-volatile organic substances from the ambient air or it could be formed by the degradation
of the polymeric material itself, presumably through thermal decomposition. It should be noted that the intensities of the a-C peaks are proportional to the intensity of the main PS band (see Fig. 16a).

![Graph](image)

**Fig. 17.** Change in intensity of the PS peak at 1000 cm⁻¹ as a function of time for two SERS samples (silver on PS on etched Si) prepared in the same way.

4.1.2. Protection of silver films with SiOₓ

In an attempt to protect the silver island film both from tarnishing and from buildup of organic molecules deposited from the ambient air, a 5 nm SiOₓ film was deposited on top of the silver, ensuring complete surface coverage. In Fig. 18 one can see a sequence of spectra, measured over a period of time during which the protected sample was constantly illuminated by the laser beam. The beam was focused at an adjacent spot 50 microns away, then moved to the acquisition location and signal acquisition started immediately. The peaks at 1100 to 1700 cm⁻¹ increase in intensity during the 5 minutes of laser exposure, indicating the formation of a-C at the interface between PS and silver in
the region in which the highly enhanced field is located. On the other hand, the intensity of these peaks did not increase after several days of storage, suggesting that a-C buildup from the air is not the primary cause. This behavior was similar to that of the control (unprotected) sample. Continuous 1s measurements at different spots have shown that the a-C peaks gradually grow and then decrease in intensity in the span of 5 minutes. The decrease could arise for the following reasons: subsequent evaporation of decomposed a-C, annealing of the SERS structure and decrease of a-C enhancement, or a combination of the two. The intensity of the main PS peak at 1000 cm\(^{-1}\) decreased at various speeds depending on the spot illuminated, but fluctuated less than the intensities of the a-C peaks. All these observations suggest that the appearance of the a-C peaks arises from thermal decomposition of the PS during illumination.

![Graph showing SERS spectra](image)

**Fig. 18.** SERS spectra (10s each) after 0, 30, 120 and 300 seconds of laser exposure for a sample stored for 123 hours in desiccator (4.5 nm of silver evaporated on top of 100 nm PS, protected by 5 nm of SiO\(_x\) above the silver). The substrate is an etched Si wafer.

Unfortunately, thermal degradation of the analyte molecules is inherent with strongly-enhancing SERS substrates and can only be dealt with by lowering power,
decreasing exposure time or even by forced heat dissipation from the substrate. Tarnishing of silver samples can be stopped or decreased by blocking the interaction of silver with air by some insulating material. Fig. 19 shows that the rate of signal decay can be decreased by the deposition of a 5 nm layers of SiO$_x$ on top of the silver film. While retaining a level of enhancement (see the intensity of the measured peak at time zero) comparable to that of the non-protected samples, the protected sample degraded significantly more slowly, with the resulting preservation of signal being most pronounced at longer times. After nearly three months of storage, the protected sample still retained ~16% of the initial enhancement, while the unprotected samples gave nearly no SERS signal. Note that the exponential functions fit using all the data points and those fit using the data for times up to 400 hours overlap well within experimental error.

![Graph a)
Intensity of the main PS peak over a period of 400 hours for three unprotected samples (red triangles, green circles, blue inverted triangles) and one protected with 5 nm SiO$_x$ (black squares) samples. The dashed lines are single-exponential fits to the data points over the entire time range. The solid line is a single-exponential fit to the data points of the protected sample up to 400 hours.

![Graph b)
Intensity of the main PS peak over a period of ~2000 hours for three unprotected samples (red triangles, green circles, blue inverted triangles) and one protected with 5 nm SiO$_x$ (black squares) samples. The dashed lines are single-exponential fits to the data points over the entire time range. The solid line is a single-exponential fit to the data points of the protected sample up to 400 hours.
Finally, an experiment was carried out to determine the required level of proximity of the metal structure to the analyte for strong signal enhancement. The same type of 4.5 nm silver island films on top of 100 nm PS, protected by 5 nm of SiO$_x$ as investigated. However, two samples had a SiO$_x$ spacer film of thickness 2.5 nm or 5 nm between silver and PS layers. These were called “sandwich” samples. A corresponding control sample without the spacer film was also prepared and measured. All samples were stored in a desiccator for no more than one day before the measurement.

![SERS spectra](image)

**Fig. 20.** SERS spectra of “sandwich” samples with a spacer layer of 2.5 or 5 nm SiO$_x$ between PS and the silver island film, as well as a control (no spacer) sample. Inset shows relative intensity of the peak at 1000 cm$^{-1}$ versus spacer thickness.

As shown in Fig. 20, the signal is a factor of seven lower for a sandwich with a 2.5 nm spacer than for the control sample. Addition of another 2.5 nm of SiO$_x$ results in only a factor 1.5 additional decrease in enhancement. Thus one can infer that very close positioning of the analyte molecule to the SERS structure is required. The behavior of the enhancement factor (EF) closely follows an exponential dependence, i.e.
\[ EF \sim I \sim e^{(-l/l_0)x}, \quad l_0 \approx 1 \text{nm}, \] when plotted in a graph of EF as a function of \( x \), where \( x \) is the spacer thickness (see inset). This result may be compared with the characteristic decay length found for another dielectric protective material, Al\(_2\)O\(_3\), by van Duyne and collaborators [p. 28 ref 135]. They characterized the decay using the distance over which the signal falls to 0.1 times its value in the absence of the dielectric layer, which was 2.8 nm. In the present case this distance is 3.5 nm for the SiO\(_x\) coating, so the two values are quite similar.

4.2. Chemically grown particles

In addition to evaporation and sputtering, so-called wet chemical synthesis methods can be employed for the preparation of SERS substrates. This method can be viewed as a more versatile one, since the user can decide on how the particles will interact with the analyte molecules of interest by fabricating structures of particular morphologies. As with the former methods, the particles can be deposited on top or beneath the analyte. They can also be added directly to analyte solutions, from which the substances adsorb on their surface. Their main advantage is that they provide more freedom in the way of their placement and control over their aggregation, when deposited on the surface of substrates. As will be seen from the following Chapter, aggregation and orientation of particles can be essential for very strong enhancement.
4.2.1. Gold particles

The use of SERS substrates made of gold particles is a more reliable approach, as the choice of the material negates the probability of its chemical change after heat treatment or laser illumination, or simple exposure to the environment. The solutions of gold particles used in creating the SERS substrates are generally transparent and vary in color. This enables some simple primary analysis of the solution using visual examination.

4.2.1.1. Spherical gold particles

Nearly monodisperse spherical particles were prepared using the citrate-reduction synthesis at elevated temperatures. The procedure was repeated twice and the resulting particles were identical in all properties. Thus, the procedure was quite reproducible. The average size of the particles was ~17 nm, as determined by visual counting of a total of

![TEM image of 17nm gold spheres and optical absorption spectrum of a solution.](image)

Fig. 21. a) TEM image of 17nm gold spheres and b) optical absorption spectrum of a solution.
100 particles on several electron microscopy images. A typical TEM image of the particles is presented in Fig. 21.

The particles have been deposited from a solution droplet on a thin layer of poly-2-vinilpyridine (P2VP) used to immobilize them on a pre-cleaned Si wafer. The analyte, a film of PEDOT/PSS blend, where PSS is acting as a dopant for PEDOT, providing very strong Raman activity, was spun on the substrate either beneath or on top of the particles. A tapping mode AFM image of the particles deposited on a polymer film is shown in Fig. 22a. The size of the particles may be estimated at around 35-40 nm which, considering the specifications of the tip ($R_{\text{curvature}} \leq 10$ nm), is in good agreement with the TEM images, once one accounts for apparent increase in sphere size caused by convolution of the tip size with the particle size. The slight distortion of the particle shape in the vertical direction should be attributed to the AFM piezo drift. The image shows that there is a wide distribution of interparticle separation distances, so that at least some groupings of particles could be good “hot-spots”. For comparison, a scan of a 4.5 nm thick silver layer deposited on a Si slide is presented in Fig. 22b. The images were brought to the same scale in X and Y axes. The preceding section presenting results from SERS samples created by evaporation of Ag onto films documented good enhancement from such silver samples. The surface coverage is estimated to be a factor of five less in the case of solution deposited particles.
Fig. 22. Tapping mode AFM images of a) 5x5 micron\(^2\) image (Quesant) of 17 nm Au spheres on a thin (~2 nm) P2VP layer on top of a precleaned Si wafer and b) 1x1 micron\(^2\) image (Park Scientific) the rough film formed by the dewetting of a grainy 4.5 nm thick film of Ag.

According to the optical absorption spectrum of such particles (Fig. 21b), the main plasmon peak is located at 520 nm, which is very close to the 514.5 nm line of an Argon laser. However, if this line is used as an excitation source, no enhancement is observed, as seen in Fig. 23a. On the contrary, the deposition of 100 nm gold particles [175] with less regular shapes (Fig. 23c) provided an increase in the intensity of the peak at 1450cm\(^{-1}\) of approximately 1.8 as measured by a ratio of peak areas (Fig. 23b).
Thus, from the analysis of the experimental data, it has been shown that there are factors beyond the interparticle spacing that should be taken into account in order to achieve any enhancement. The next section will show that consistent enhancement can be achieved for particles with shapes different from that of a sphere.

4.2.1.2. Irregularly-shaped and rod-like gold particles

Irregularly-shaped gold particles have been obtained as a by-product of the synthesis of high-aspect ratio nanorods. The particles were found in the supernatant of the solution. A low magnification TEM image of the particles is presented in Fig. 24.
Such particles had only one absorption peak at ~530 nm (Fig. 24c). However, they provided enhancement when deposited on PEDOT/PSS films both at green (514.5 nm) and red (647 nm) illumination wavelengths (Figs. 25a and 25b, respectively). It was observed that for every illuminated spot the enhancement increased over time and then decreased slightly (checked for at least 10 spots). Moreover, the enhancement was not uniform across the whole range of wavenumbers for the red laser illumination. The peaks at ~1150 and 1550 cm\(^{-1}\), which likely correspond to CH\(_2\) and CH\(_3\) vibrations, were preferentially enhanced. Such behavior was never observed with plain PEDOT/PSS samples on the same time scales. This peculiarity was attributed to the possible annealing
of the thin particles and their possible subsequent shrinking. This suggested some strong heating in the area of the gold particles and was the topic of further investigation, the results of which are reported in another chapter of this dissertation.

Fig. 25. Raman spectra (each spectrum acquired for 10s) from samples on which were deposited irregularly-shaped gold particles at a) 514.5 nm (after 0, 30, 90, 180 and 300 seconds of exposure) and b) 647 nm (after 0, 600, and 1200 seconds of exposure) illumination wavelengths.

In order to understand whether simply elongating the particles would help in achieving good enhancement, short Au rods have been studied. The optical absorption peak was nearly identical to that of the 17 nm spheres, although one would expect it to be located at a higher wavelength [47] due to red-shifting of the longitudinal optical bands, and thus again 514.5 nm laser excitation was considered to be the most suitable. Fig. 26. shows a TEM image of the low aspect ration gold rods on a carbon-coated copper TEM grid. Also shown are two spectra from PEDOT/PSS films without and with rods prepared in the same manner as those for spherical Au particles (PEDOT/PSS film on the particles on a thin P2VP film on top of a precleaned Si wafer). As in the case of 17 nm uniform spheres, no noticeable enhancement has been found when the short gold rods were
deposited atop the PEDOT/PSS. The absence of enhancement has been attributed to the total absence of facets and sharp edges on the particles.

As the next step, a study was carried out to determine whether strong enhancement can be seen from faceted particles, especially long rods. It is apparent that to obtain faceted particles one should increase the number of highly favorable nucleating spots on a nucleated particle. This is achieved using two methods. First, milder reducing agents and conditions should be used. Such conditions would include lowering the temperature and decreasing the concentrations. Second, and most important, templating agents should be introduced, which would create a preferential direction for particle growth. Generally, this is realized through the formation of micelles of surfactant. The different shapes of the micelles will be a governing factor in the shapes of the resulting particles. For extended particles, namely rods, surfactant molecules such as CTAB [82] with long straight chains in trans conformation are used. Additionally, the concentration of the surfactant is maximized, while that of the metal ion precursor is minimized.

Fig. 26. a) TEM image of short gold rods on a commercial TEM copper grid and b) Raman spectra from PEDOT/PSS films without and with short gold rods, illuminated with the 514.5 nm laser line.
Moreover, an even more complicated approach would involve several steps of growing of the particles.

Work has been done to identify the structures that provide the very large enhancement needed to make high resolution imaging and extremely sensitive detection practical. There is ample evidence in the literature [103, 105, 108, 176-178] that clusters of particles, rather than individual particles, provide extremely high enhancements. Nevertheless, we have analyzed whether either faceted particles or high aspect ratio particles can form highly enhancing plasmonic structures.

Collections of particles including nanorods have been synthesized according to a well-known seed-mediated procedure [84], which has recently been significantly improved by Wu et al. [87]. The surfactant, present in a very high concentration, was removed by several redispersion steps with deionized 18 MΩ water, in which the upper supernatant fraction was removed and the sediment was retained for further purification. To understand what a typical assortment of particles from a purified solution is, one can refer to Fig. 27a. The particles formed include fairly well-defined rods with a narrow distribution in diameters (~20 nm) and lengths of hundreds of nanometers, which therefore have aspect ratios above 20. In addition to rods, the solution contained a number of smaller particles of various shapes. According to TEM data from three different batches of rods, the diameters of ~90% of the rods are 18-22 nm.

According to the literature, the transverse plasmon band of such a rod is expected to be observed around 530 nm [179]. With increasing aspect ratio, the transverse resonance shows a small shift to shorter wavelengths [180]. Our optical absorption measurements from a solution of particles show a peak at 530 nm (Fig. 28). The peak at
630 nm corresponds to absorption of small faceted particles, particularly triangles, which agrees with Kottmann et al. [52]. The shoulder of a broader peak above 800 nm, well-separated from the one at 630 nm, corresponds to the longitudinal band of the long rods. This is in excellent agreement with previous results published by Eustis and El-Sayed [58]. Simulations have shown that the scattering spectra red-shift slightly when two gold rods are getting close to one another [102, 181]. Experimental results show similar trends [102, 181]. The information from both experiment and theory would result in the position of the transverse band in the range of 520-560 nm. This would correspond to the left shoulder of the optical absorption peak for the particles deposited on a glass slide with a thin polymer coating (to simulate the substrate for SERS). The peak for dry particles is centered at ~610 nm and therefore could only correspond to aggregates of small particles. Moreover, Hao and Schatz [182] have shown that for peak prisms with sizes of 30-60 nm, similar to particles in our samples, most typical geometries of aggregates would yield extinction spectra with maximum at ~650 nm. Illuminating of the particles at this

Fig.27. a) TEM image of the particles of various geometries formed in one solution and b) Raman spectra from PEDOT/PSS films with deposited long gold rods with some other faceted particles, illuminated with green and red lasers. Spectra from blank samples (PEDOT/PSS only), represented by the red and green curves, were normalized to match maximum peak height.
maximum with 647 nm laser wavelength should result in the very high Raman enhancement in such “hot spots” in the particle aggregates.

In Fig. 27b one can see the comparison of the Raman signal from a PEDOT/PSS film with and without the particles. Clearly, enhancement is observed with both 514.5 nm and 647 nm illumination, although it is about a factor of two weaker in the latter case.

![Optical absorption graph]

Fig. 28. Optical absorption of a solution of the irregularly shaped particles (blue curve) and of a layer of particles deposited on a glass slide on top of a PS film (black curve).

The most important property of the synthesized elongated particles, however, was their ability to reproducibly create consistent Raman “blinking” of various peaks in the spectrum of a sample. The blinking manifests itself as both changes in intensities and positions of peaks. The changes were considerable, with complete appearance and disappearance of peaks and confirmed shifts in position of up to 45 cm\(^{-1}\) for a given peak during an acquisition of one second. This phenomenon was described to some extent in
a) 

b)
Fig. 29. Three sequences of Raman blinking observed (only 5 curves chosen randomly) for a hot-spot; the patterns in “a” and “b” can be identified as belonging to PEDOT, while “c” can correspond to a PSS molecule. Each curve was measured using 1s acquisition. The sequences were ≥40 s apart.

the Historical Perspective section. It is generally agreed that such blinking is an indication of very strong enhancement. The presence of very strong enhancement was evidenced by the common observance of a-C background peaks, which were identified in the previous section as resulting from polymer decomposition.

Fig. 29 shows sets of spectra for a sample of PEDOT/PSS, drop coated with the particle collections containing a predominance of nanorods. These spectra show a richly complex behavior, including blinking. Individual spectra correspond to 1 s acquisition time with 0.5s delay to the next acquisition. It is interesting to note that blinking appears intermittently. It was strong during the first 40 seconds of illumination (Fig. 29a), then no blinking was observed for the next 40 seconds, then intense blinking appeared for 30 seconds (Fig. 29b), and then no blinking was observed for the next 70 seconds, and after
that another set of blinking was observed for 15 seconds (Fig. 29c). Peak intensity and position fluctuations are clearly visible in all cases.

Based on literature databases of Raman and IR peaks [25, 26], the peaks in our experiments could be best assigned in the following way: symmetric and asymmetric deformations of CH$_3$ (1300-1500 cm$^{-1}$); aromatic C-C groups, sulfur compounds and even C=C double bonds (1000-1100 cm$^{-1}$); CH$_2$ rocking (~800 cm$^{-1}$). It should be noted that the peaks at ~1400 and 800 cm$^{-1}$ consistently appeared for both PEDOT/PSS and PS samples, which speaks in favor of the assignment. Moreover, in some cases the shift in position of one peak was correlated to the shift in position of another peak, as demonstrated in Fig. 30, which suggests that the natures of the corresponding peak vibrations should be closely related, as would be the case of symmetric and asymmetric deformations of CH$_3$. In contrast, the CH$_2$ rocking vibrations should not be correlated with the abovementioned ones, as is indeed the case.

![Fig. 30. Peak positions for symmetric and asymmetric CH$_3$ vibrations (a) and CH$_2$ rocking (b). Open circles and crosses show positions of extra peaks next to the main ones.](image)

The drop cast samples of gold rod particles invariably produced blinking spectra on different types of polymer substrates: PS (30k, 110k, 470k MW), P2VP (41.5k MW)
and PEDOT/PSS blend. However, such blinking occurred only in a small percentage of illuminated spots on a sample. Thus, it was considered that a particular conformation of a hot spot is required in order to produce blinking spectra. Such spots were located using the following technique. A fine razor scratch was made in the polymer film as a noticeable mark. The optical image of the illuminated area was recorded after each blinking spot was found (a cut-out is presented in Fig. 31a). Then, this picture was correlated to an optical image from an optical microscope with higher resolution and a better digital camera (seen in Fig. 31c). That, in turn, was correlated to a low-magnification SEM image (Fig. 31b). When the area was identified, the close-up high magnification images of individual hot spots were recorded.

From such SEM images, it was possible to identify characteristics showed by “hot-spots”, i.e. locations producing blinking spectra. Fig. 32 shows images of separate particles and aggregates that did not give blinking Raman spectra. Separate spheres, as well as single and parallel rods did not produce good hot-spots. In fact, about 10% of all spherical particle clusters did not produce effective hot spots with fast enough induction times (i.e. the time between the illumination by the laser and the onset of blinking). On the contrary, aggregates of small faceted particles of various shapes appeared to produce blinking spectra (Fig. 33). This is in excellent agreement with the optical properties data, which show the absorption peak of the aggregates to be situated at the wavelength of the illuminating laser (647 nm). At the same time, the absorption peaks of separate particles, as well as separate rods and rod assemblies lie either above or below the wavelength of illumination and are not able to provide extreme enhancement.
Fig. 31. A representative set of a correlation of a) Raman microscope, b) SEM and c) high-resolution optical microscope images. Red squares represent areas that “blinked” in Raman. Note that “b” and “c” are offset for clarity. The principle of the experiments is shown in d).
Fig. 32. SEM images of a) single and adjacent spherical particles, b) separate rods, c) adjacent rods that did not produce blinking spectra.
Fig. 33. SEM images of aggregates composed of multiple small round or faceted particles that gave rise to blinking spectra.
Finally, polarization effects have been tested on a particle aggregate. Blinking spectra for three different polarizations with respect to the cluster have been accumulated and compared with each other. The SEM image of the aggregate with indication of polarization directions is shown in Fig. 34. The laser intensity was kept same.

![SEM image with polarization directions shown in the inset (a) and Raman blinking spectra for different 647 nm laser polarization directions acquired in this sequence: 0° (b), 45° (c), 90° (d), 0° (e). Each spectrum was acquired in 1s.](image)

The spectra were collected for 15 s for each polarization. The laser was blocked while the polarization direction was switched. While the decay of blinking over time (i.e. when “b” and “e” are compared) is certainly evident, it is still present in “e”, which was collected last. Thus, the complete absence of blinking in “d” (90°) indicates that this direction is not favorable for the creation of a strong plasmon. It should be noted that blinking was seen only for the small particles and not for the rod aggregates, regardless of polarization direction. This result has been confirmed for four rod aggregates.

The analysis of samples coated with spherical, irregularly-shaped and faceted gold particles, short and long rods has shown that, within the constraints of the
experiments, only faceted particles appear to be able to produce good enhancement and their aggregates produce hot spots, capable of extreme enhancement, manifested as blinking. Both our experimental results and literature theoretical results suggest the very high enhancement in the hot spot requires coincidence of the wavelength of the optical resonance with the excitation wavelength. Considering the size of the hot spot to be on the order of 1-2 nm based on SEM images of blinking SERS clusters, a mechanism of chain diffusion seems to be the most plausible explanation for the blinking phenomenon. In this case the changes in the spectra may be associated with diffusion of polymer molecules (PEDOT, PSS, PS) into and out of a hot spot, as has been suggested previously [22, 171, 183]. However, it is less possible that molecules, or portions of molecules, diffuse into hot spots and then thermally decompose or otherwise react [184]. While the observed sharpness of the blinking peaks does speak in favor of thermal decomposition and can be further justified by our recent experimental and theoretical work showing significant heating from TERS tips, it seems unlikely that such high temperatures are attained in all of the clusters that produced blinking, as suggested by the level of temperatures attained in our TERS experiments, discussed in the following chapter.

4.2.2. Silver particles

The major advantage of SERS substrates based on silver particles is the low damping of free electrons which stems from the high conductivity of silver. The price of the precursor, silver nitrate of high purity, is quite low and is also a notable factor, especially when such particles are considered for use in industrial applications.
4.2.2.1. Spherical and rounded silver particles

One can routinely create spherical particles of silver using strong reducing agents. The spherical nature of the particles can be explained by the fact that there is little preference for silver ions for a site to attach themselves to. Such hydrosols tend to be relatively polydisperse (more than an order of difference in size of smallest and largest particles). The reason is that the power of the reducing agent greatly diminishes the energetical favorability of particle growth over particle formation. Unfortunately, this is also the case when different capping or templating agents, such as surfactant molecules, are used. Thus the yield of non-spherical particles is extremely small. See in Fig. 35 the TEM image of particles from a typical solution of NaBH₄-reduced silver particles, deposited on a carbon-coated TEM copper grid. Such rounded silver particles were not expected to provide good enhancement for the reasons explained in the section discussing the spherical gold particles.

Fig.35. TEM image of NaBH₄-reduced silver particles on a carbon-coated grid.
4.2.2.2.  Faceted and rod-like silver particles

As has been noted in the section for the gold rods, the use of surfactants is required to achieve non-rounded particles. As with the gold particles, very high surfactant concentrations and multiple growth steps are required for precise shape control of the resulting particles. However, unlike the gold particles, the silver particles are easily damaged by the environment. Thus, synthesis procedures with as few preparation steps as possible should be considered for silver SERS particle preparation.

The first procedure involved using trisodium citrate both as the templating and reducing agent and concentrations of $6.25 \times 10^{-6}$ M for citrate and silver nitrate in the reaction mixture. The reaction mixture was kept boiling to compensate for the very low reducing power of trisodium citrate. TEM images of the resulting particles are shown in Fig. 36. The Raman enhancement spectra from PEDOT/PSS films with these particles for 1 and 5 minutes particle deposition from a droplet (the droplet was removed from the polymer surface in 1 and 5 minutes, respectively) is shown in Fig. 37. The spectrum for the 5 min deposition shows approximately a factor 1.6 enhancement, but also shows an

![Fig. 36. TEM images of silver particles formed after the reaction of silver nitrate with trisodium citrate in a 1:1 ratio.](image-url)
increase of the baseline with particle deposition and the appearance of a broad band from 1200 to 1650 cm$^{-1}$, hinting at the possible formation of a-C, as has been identified in the first section of this chapter.

As the next step in the understanding of the factors that influence the level of Raman enhancement, forced aggregation of the particles has been introduced. The particles have been mixed with different concentrations of PEDOT/PSS blend with/without added glue molecules of polyethyleneimine (PEI). The PEDOT/PPS solution was used as is or in a diluted form of 1:10 in ethanol. Fig. 38. shows the additional gain in the enhancement when PEI was added to particles for the thick (factor of ~1.5) (a) and thin (factor of ~3.6) (b) analyte layers, spun on the surface of precleaned Si wafers. The gain, most pronounced for a thin film, is most likely originating from particle aggregation, facilitated by the adhesive (PEI).
Fig. 38. Influence of PEI on the overall enhancement from random crystalline Ag particles for a) thick and b) thin PEDOT/PSS layers. Spectra from PEDOT/PSS mixed with particles (red lines), mixed with particles and PEI (green lines) are compared to reference samples (black lines).

In the second and third procedures, the trisodium citrate was also used as the templating and the reducing agent. The concentrations were kept the same, however the ratio of the components has been changed. The second and third batches had an excess of 2:1 (marked “(2:1)”) and 5:1 (marked “(5:1)”) of the reducing agent, respectively. In addition to that, the particles were centrifugated in a water-ethanol mixture and only the lower fraction has been retained. TEM and Raman data are presented in Fig. 39 “a” and “b”, respectively.

Fig. 39. a) TEM images of silver particles (2:1) placed on carbon-coated TEM grids and b) Raman spectra of PEDOT/PSS films with drop-coated “(2:1)” and “(5:1)” silver particles compared to spectra from reference samples.
Not only there was less variety in shapes of the particles, but also the enhanced spectra do not show the a-C buildup and allow for a clear comparison of the data. Surprisingly, the 2:1 ratio of reactants was slightly more effective than 5:1 (enhancement of a factor of ~1.24 compared to a factor of ~1.12), at least judging from the overall enhancement level.

Based on the above results, it can be concluded that faceted particles with sharper features are more likely to produce higher enhancement than the ones with features that are less distinguished. Also, aggregation of particles seems to be important for good enhancement.

4.2.2.3. “Nano-raspberries”

Silica-silver core-shell particles have been synthesized according to the Stöber sol-gel method [185]. The resulting particles were called “nanoraspberries” due to the rough nature of the surface covered with closely spaced smaller silver particles. The diameter of such particles was on the order of several nanometers as seen in Fig. 40a.

Fig. 40. a) TEM image of ~80 nm nanoraspberries on a TEM grid and b) optical absorption spectrum of the particles in solution.
The optical absorption spectrum (Fig. 40b) exhibited a rather broad peak with a pronounced shoulder up to the maximum probed wavelength. According to theoretical electrostatic picture, highest enhancement is proportional to the position of the plasmon peak divided by its width. This factor was not particularly favorable for the SERS use of these particles. However, as has been shown in the case with the aggregated gold particles, described in the previous section, the close proximity of the small silver particles was expected to give rise to higher enhancement. For the “nanoraspberries”, this is achieved for every big 80 nm particle regardless of the employed casting method.

![Graph showing Raman enhancement spectra](image)

**Fig. 41.** Spectra of Raman enhancement of PEDOT/PSS films with “nanoraspberry” SERS particles as measured with 514.5 nm laser line compared to a reference sample (PEDOT/PSS) without particles.

As seen from Fig. 41, the particles posses above average enhancement (a factor of ~1.23) without the a-C contribution. Moreover, the enhancement would not be affected by the exact relative positions of the bigger particles in space, since high enhancement is already achieved on the surface of a single particle. The particles were considered most
effective for SERS among the reviewed silver ones, however they were still prone to environmental degradation (sulfidation), as their enhancing capability was diminishing with time. Nearfield Raman mapping of a single particle and TERS tips made with such particles are reported in further sections of this dissertation.

As a conclusion, several important behavior features, characteristic for SERS substrates, have been identified: high enhancement with even moderate laser powers often results in degradation both of the polymer layer and the metalized substrate. Also, high enhancement might facilitate adsorption of chemical species onto the metal particles, drastically decreasing their effectiveness. This can be overcome by the introduction of a “protection” layer, which apparently decreases the extent of oxidation and sulfidation of silver substrates thus significantly increasing their lifetime. The exact mechanism of the protection, however, is still not very clear. Experiment has shown an exponential decay in enhancement with increase in separation of metal and polymer analyte layers, which limits the range of thicknesses viable for such protection layers. A study was performed to identify the factors increasing or diminishing the enhancement in order to create more effective SERS substrates. Highly crystalline particles with sharp features or rough surfaces were found to give higher enhancement. Some procedures for reproducible formation of “hot spots” in SERS substrates have been suggested and the actual morphologies of the hot spots have been visualized.
CHAPTER V

TIP-ENHANCED RAMAN SCATTERING

As has been noted in the Introduction section of this Dissertation, a TERS tip is essentially a SERS probe of very small dimensions, which is scanned over a surface, collecting both AFM and Raman signal at the same time. Let us consider the case when the analyte of interest is scanned by a typical Raman confocal system. Here, a signal is detected from a feature on the surface if the feature is located anywhere within the focused laser spot. The image collected by scanning the surface while collecting this signal is often called the far-field image. The observed image of features will be distorted, due to the optical diffraction limit, by the size of the focal spot. In short, every part of a Raman-active object will be enlarged to the size of this spot. There are many applications that require better resolution that currently exists with confocal microscopes. One of the ways to answer this need for better resolution is to perform Tip-Enhanced Raman Spectroscopy. When a plasmonic tip is in close proximity to a sample feature, the Raman system collects a so called near-field signal in addition to the far-field signal, which is collected regardless of the tip presence. Since the area in which the signal is enhanced is quite small (approximately 20 nm in radius [186], defined by the radius of the tip), the features will be enlarged much less than in the case of simple confocal Raman scanning. Unfortunately, the very nature of TERS makes it impossible to acquire
the near-field mapping without the far-field signal. Thus, a TERS map is essentially an overlay of a near-field map over a far-field map. Only when the contrast between such a combined map and a far-field map is significant will TERS scanning be useful.

It is possible to perform an additional far-field scan and subtract its results from the TERS scan to obtain a map with higher resolution. However, one will encounter multiple problems when doing this. First is the extra time spent on the experiment. Second is the possible drift of optical and AFM components of the system or even the movement of the sample itself. Finally, the extra illumination time can significantly contribute to the thermal degradation of the sample. Here it becomes obvious that to be able to perform a good TERS imaging in one scan, one needs the near-field signal to be on the order of the far-field signal or higher.

In Fig. 42 three different types of samples for TERS with corresponding tip enhancement requirements are shown: quasi-1D sample (left), quasi-2D sample (right) and 3D sample (bottom).

![Fig. 42. Schematics of three different types of samples for TERS with corresponding tip enhancement requirements.](image)
For a quasi-1D object, e.g. a carbon nanotube, the far-field Raman-active volume would be around 1000 nm$^3$ ($1 \times 1 \times 1000$ nm$^3$). The near-field Raman-active volume would be $\sim 1 \times 1 \times 40 = 40$ nm$^3$, or 25 times smaller. So a tip should give a ~25x enhancement for a good TERS mapping. Similar assumptions give tip enhancement requirements of $10^3$-$10^4$ for quasi-2D objects and $10^5$-$10^6$ for 3D objects. Currently, enhancements of a few (1-3) orders of magnitude are considered to be typical.

The purpose of the studies presented in this Chapter is to find out how to maximize the enhancement obtained from common vacuum-evaporated tips as well as develop promising designs for even better levels of enhancement. Additionally, different tip-enhancement degradation mechanisms will be discussed and the ways developed to minimize their effects will be shown. Finally, TERS mapping of different nanoscale samples will be presented and discussed.

5.1. Tip morphologies and corresponding enhancement factors

This section will discuss our developments in the field of TERS on tips that would provide higher enhancement. Experiments with tips prepared with varying deposition rates will be shown. Two promising designs with tips that have different plasmonic particles formed on their apex will be outlined and the major properties of such tips, i.e. morphology, optical properties and enhancement, will be described.
5.1.1 Simple metal-deposited tips: effects of varying deposition rate.

Metal vacuum evaporation on a tip is a common method [153, 154] to obtain usable TERS tips in a fairly fast and easy procedure. There are a few parameters that can be changed in the procedure. However, only thickness and deposition rate can be reliably controlled using an appropriate quartz-crystal microbalance.

It appears that a metal island film with multiple separate clusters creating plasmons in the gaps could be the most effective coating for a TERS tip, based on the results of the previous Chapter. However, metal deposition by evaporation has been found to be a much more convenient and simple method, while still creating a plasmonic coating capable of producing usable enhancement. From experiment, a nominal thickness of ~50 nm [9] can be considered optimal (as detected by a microbalance placed directly above the metal source; the real thickness on a sloped tip might be different). It should be noted here that thicker tips do not exhibit better enhancement, while the tip radius becomes bigger, which is to be avoided [9].

The variation in enhancement with deposition rate can be quantified using "contrast", which relates the far-field and near-field intensities for a tip and is essentially a measure of a gain in signal from the presence of a tip [174]:

\[
\text{Contrast} = \frac{I_{\text{nearfield}} - I_{\text{farfield}}}{I_{\text{farfield}}} \quad (5.1)
\]

In Fig. 43 it is seen that contrast decreases with increasing deposition rate in the range tested. At deposition rates of below 0.1 Å/s, the deposition system used was highly unstable and good tips could not be made.
Fig. 43. Effect of deposition rate of silver on tip contrast for a PEDOT/PSS sample with 514.5 nm laser illumination.

From the data it is possible to say that the deposition rate is to be kept at the lowest possible level for highest enhancement. However, the exact influence of this factor on the actual enhancement is yet to be explained.

5.1.2 Simple metal-deposited tips: Different shapes and morphologies.

Several promising tip morphologies have been studied to understand the principal ideas in constructing a better TERS tip, which would be both sharp and give high enhancement. The first design tested was a “pillar” tip with a thin cylindrical gold structure protruding from the very apex of a regular gold-coated tip. The “pillar” has been created using the electron beam stimulated deposition process at the Center for Nanophase Materials Science (CNMS) at Oak Ridge National Laboratory. The vapor of hydrocarbon compounds containing gold ions has been burned out at the selected spot with a directed electron beam of high power. This resulted in decomposition of the
organic compound and formation of a long, sharp, needle-like structure of about 100 nm in length and ~20 nm in diameter (see Fig. 44a). This structure was expected to consist mostly of gold.

![Image](image.png)

**Fig. 44.** (a) SEM image of a “pillar” tip and (b) optical contrast spectra for two “pillar” tips that showed no enhancement.

Unfortunately, most of the tips have shown absolutely no enhancement. (All tips have been tested with the 514.5 nm laser, while some have also been tested with the 647 nm laser). Two tips did show contrasts below 0.3 (see below in text). Notably, their optical properties (optical contrast) were also markedly different from those of the “pillar” tips that showed no enhancement. In Figure 44b are shown two optical contrast spectra typical for two the “pillar” tips that showed no enhancement. The spectra show a peak centered at ~620 nm, which is not too far from the theoretical value for a 20 nm gold particle. If the optical contrast from one of the two tips that does show enhancement is compared to the optical contrast of a simple gold-coated tip (similar tip, but without a pillar), the positions of the absorption peaks are closely matching at ~700 nm. From theory, this is an indication of the presence of a particle or aggregate which is much larger than 20 nm in diameter. At the same time, the peak for the “pillar tip” is somewhat
wider (Fig. 45) than the one for a simple gold tip created by gold evaporation. This could be evidence for some pillars collapsing during initial contact [170]. If a pillar would collapse, the tip would resemble the original gold tip with the same trend in optical properties and enhancement.

Fig. 45. Optical contrast spectra for a “pillar tip” that shows enhancement (black) and a simple gold-coated tip (red).

The enhancement for one pillar tips that did show enhancement is compared with the enhancement of a simple gold-coated tip in Fig. 46. The contrast values for a PEDOT/PSS sample on an Al mirror measured with the 514.5 nm laser line were 0.30±0.2 and 0.13±0.1 for the pillar tips and 1.29±0.03 and 0.93±0.02 for two gold-coated tips. The contrast values with the 647 nm laser line were 0.17 for the first pillar tip and 1.44 and 1.13 for the two gold tips, respectively.
Fig. 46. Raman spectra of PEDOT/PSS for a gold tip with a pillar (a, c) and a typical gold tip (b, d). The laser wavelengths were 514.5 (a, b) and 647 nm (c, d).

The experiment shows that pillar tips, when intact, do not give any noticeable enhancement. They also appear to be prone to breakage during approach to the sample if regular contact-mode AFM is used. One possible explanation of no enhancement is the fact that the deposited Au structure can contain up to 80% impurities [187], including organic contamination, which can damp all plasmonic excitations.

The next type of tip considered was not obtained through deliberate preparation. During vacuum evaporation of gold, an aggregate formed on the very apex. As a result, the tip with the aggregate showed a significant increase in enhancement when compared to another tip from the same batch. The enhancement was measured on PEDOT/PSS on
an Al mirror with the 514.5 laser. The Raman spectra and the corresponding SEM images are shown in Fig. 47. The contrast values are 0.6 and 2.5. While the increase could come partially from the bigger size of the actual tip apex in contact with the analyte surface (see (a) and (c) in Fig. 47, note the slight difference in scale), the remarkable increase could not come from this fact alone. This is consistent with the idea of a particle in the field of an even bigger particle, which in this case is represented by the metalized tip.

![SEM images and Raman enhancement spectra](image)

**Fig. 47.** SEM images (a, c) and Raman enhancement spectra on PEDOT/PSS with 514.5 nm laser (b, d) for a gold-coated tip with (c, d) and without (a, b) an aggregate at the apex.

From all of the above experiments it is possible to say that placing a metal or metallized particle on the apex of a tip may give an increase in enhancement, however, it
should consist of high purity metal. It also appears that a grainy structure might be of high importance for a highly-enhancing tip, which is in agreement with literature [151].

5.2 Important factors affecting tip enhancement.

There are multiple factors which can affect tip enhancement. Among them is tip degradation from environment and prolonged use. The degradation from environment will be discussed in detail in the later part of the Chapter. In the following section the effect of signal reflection from the tip will be demonstrated. Unlike real enhancement at the tip apex, this effect does not provide a gain in resolution.

5.2.1 Reflection from tip.

When octagonal (Micromasch), rather than rectangular, tips with evaporated silver have been tested for their TERS effectiveness, it has been found that the contrast they provide is very low (0.1-0.2). This resulted in a consideration that some of the contrast can come from a reflection of the analyte signal from the tip surface itself, rather than from the enhancement of the electromagnetic field by the tip apex. In the literature, the problem of signal reflection from one of the sides of a tip has been discussed in detail as far as ten years ago by several authors [113, 188, 189]. However, quite surprisingly, this phenomenon has attracted little attention from other groups. Fig. 48 shows a bottom-view schematic representation of how extra signal can be generated when a TERS tip is in close proximity with the surface of an analyte. The tip cantilever and tip are shown in
gray, the green arrow represents the illuminating laser, and dim green represents the extra signal reflected from the tip surface.

![Diagram of laser illumination](image)

Fig. 48. Bottom view schematics for two different ways of rectangular tip laser illumination for side-illumination TERS geometry: illumination of the front side of a tip (left) and illumination of the tip corner (right).

When a beam strikes the tip apex, it also strikes the neighboring substrate. The signal from the substrate is scattered in all directions, including the direction of the collecting optics (same path as the incident beam). This gives the farfield signal. In addition to that, some scattered photons get reflected from the side of the tip pyramid and propagate in the direction of the collecting optics. These photons may be electromagnetically enhanced by the metal layer. However, they come from different spots on the illuminated area and thus the spatial resolution associated with this signal is approximately the same as that of the farfield signal, i.e. the size of the focused laser spot. While they do contribute to the collected signal, they do nothing to improve the Raman scanning resolution.

It order to understand better the “reflection from the tip” phenomenon, an experiment has been developed. It involved measuring gold and silver tips in 90° (front-illumination) and 45° (corner-illumination) to the laser beam. The results are shown in Fig. 49. Both tips have shown a significant decrease in contrast in 45° geometry: gold-
coated tip contrast went from 1.45 to 0.28, silver-coated tip contrast went from 1.09 to 0.124. This corresponds to a nearly one order drop off in the calculated level of enhancement. Several other gold-coated tips have been tested and have shown contrasts of 0.1-0.2 for the 45° geometry.

![Graph](image)

Fig. 49. TERS signals of PEDOT/PSS for 90° and 45° illumination of tip pyramids, vacuum-evaporated with a) silver and b) gold. The laser line was 647 nm. The top blue curves were measured in the 90° geometry, the lower ones – in the 45° geometry. The withdrawn signals shown as red curves are overlaid.

### 5.2.2 Tip degradation and ways to prevent it

As has been noted in the previous section, tips for TERS measurements should give high enhancement, which would allow scanning of more complex substrates. At the same time, however, these tips should retain this enhancement for a long period of time when stored. Ideally, the tips should withstand mechanical degradation from interactions with the substrate/analyte, which could scratch the tip and remove the metal layer. Moreover, the tip should be resistant to thermal and chemical degradation, which anneal, decompose or transform the enhancement layer.
The following section describes attempts to deal with these effects by introducing “protected” tips that withstand the degradation much better. In addition, detailed studies of temperature increases of TERS tips under illumination are discussed. They give an idea about the reasonable levels of laser power for such experiments to minimize the tip and sample degradations from heating.

The local loss of the metal layer through scratching would result in lost contrast and/or unstable feedback of the tip if used with a non tuning-fork AFM. Thermal and chemical degradation are also important factors threatening the TERS tip efficiency. Regular tips made by evaporation of gold or silver intrinsically are easily worsened by these degradations. Both metals can be annealed by moderate (couple hundreds of degrees) temperatures and are soft. Silver is also very easily tarnished in the air. In order to overcome these setbacks, a protection layer can be introduced on top of the tip. In Fig. 50, a change in silver evaporated tip contrast over time

![Graph showing contrast over time](image)

Fig. 50. Change in tip contrast over time. The silver evaporated (0.3 Å/s) tip was tested with 514.5 nm laser at a power fluctuating around ~0.7 mW.
is plotted. The tip was illuminated continuously at rather low power (0.7 mW) and approached every 15 minutes for 60s to a different spot on a PEDOT/PSS film to obtain the signal. The farfield signal for every spot was the same, indicating the same thickness.

It is apparent from the figure, despite the error bars, that the contrast falls by ~ 5% over the period of just 14 minutes. This is comparable for a time spent on a TERS scan of one line. For just one line this 5% decrease might not be very significant. However for larger scans, this might introduce noticeable errors. This decrease could come both from thermal degradation and chemical alteration of the surface.

5.2.2.1 Silicon oxide protection

Silicon oxide (SiO$_x$) has been chosen as a promising coating for the purposes of TERS tips’ protection due to its good adhesion to the metal, high rigidity and low porosity. SiO$_x$ has been evaporated sequentially with the plasmonic layer (silver) in the same chamber. At high temperatures, the oxide increases its oxygen content on the surface of the metal with the formation of SiO$_y$, where y = 1.1 – 1.7 [190]. Y is greater than X (see above in text), but is not equal to 2 due to low amount of oxygen present.

Mechanical tests (scanning of protected tips on silicon wafers) have been performed and it was found that SiO$_x$-protected tips have a higher probability of survival than non-protected ones. In other words, the AFM feedback has been stable for longer periods of time for similar imaging conditions, in comparison to non-protected tips.

Fig. 51 shows the Raman contrast trends for unprotected tips and tips with 1 and 3 nm thick protective coatings. The tip with 3 nm of SiO$_y$ starts with contrasts being 35-
40% lower, as the protection film appears to block some of the enhancement. However, after 10 days of tests the protected tip has contrast better than that of the non-protected one, as it degrades more slowly. The behavior of one tip with a nominally same protection layer of SiO$_x$ is similar to that of the non-protected tip with the decay rate being quite significant, while the starting contrast is the same. This could be likely due to the instability of the deposition due to silica’s very high melting and sublimation temperatures (1285 and ~1200 K, respectively), which resulted in a non-homogeneous coating.

The measurements of the optical properties of the tips indicate that the introduction of a SiO$_x$ layer significantly changes their optical absorption (Fig. 52). It should be noted that the positions of the two peaks vary from tip to tip and could be offset by up to 50 nm in each direction.

![Raman contrasts for an unprotected silver-coated Si$_3$N$_4$ tip (black squares) and two silver-coated tips protected by 3 nm of SiO$_x$ (red hollow and full squares) on a 20 nm thick CdS film for a period of 30 days. Relative humidity < 40%.](image)

Fig. 51. Raman contrasts for an unprotected silver-coated Si$_3$N$_4$ tip (black squares) and two silver-coated tips protected by 3 nm of SiO$_x$ (red hollow and full squares) on a 20 nm thick CdS film for a period of 30 days. Relative humidity < 40%.
The difference in Raman contrast between protected and non-protected tips, for which the silver coating was evaporated at 0.3 Å/s has been noticeably lower (the protected tip had its contrast at approx. 75% of that of the unprotected tip). This, most likely, is the consequence of a much rougher surface of tips. Tips prepared at higher evaporation rates have larger silver grains on the surface, which can be only partially coated by a 5.5 nm SiO$_x$ layer. Therefore, they remain partially “unprotected”, which gives a gain in contrast in comparison with fully protected tips.

Fig.52. Optical spectra for an unprotected silver-coated Si$_3$N$_4$ tip (black line) and a typical silver-coated tip with a 3 nm layer of SiO$_x$ (red line).

5.2.2.2 Aluminum oxide protection

Aluminum oxide (Al$_2$O$_3$) has been chosen as a promising coating due to its good adhesion to the metal, high rigidity and very low porosity. Alumina coatings have been created by direct evaporation of aluminum in a vacuum chamber, although the evaporation temperature has been lower than in the case of SiO$_x$. Unlike the evaporated silicon oxide, aluminum gets completely oxidized with the formation of aluminum oxide.
Mechanical (scratching) tests have been performed in a similar fashion as with other tips tested. However, the scanned areas of a silicon wafer were larger and the rate was lower. Tips were scratched over a 5 μm by 5 μm area on a silicon wafer at a rate of 0.5 Hz. Values of Raman contrast factors for two protected (hollow symbols) and two non-protected tips (full symbols) are plotted in Fig. 53.

![Graph showing Raman contrasts for scratched tips.](image)

Fig. 53. Raman contrasts for unprotected silver-coated Si₃N₄ tip (full squares) and silver-coated tips protected with 3 nm of Al₂O₃ (hollow spheres) tested on a 50 nm thick PEDOT/PSS film after scratching tests on Si wafers.

From the figure it can be noted that the enhancement fluctuates significantly for all the tips, which comes from the fact that such a severe treatment of tips results in the destruction of the plasmonic layer for both protected and unprotected tips. The difference, however, is in the manner in which the tips get degraded. Significant portions of silver get chipped off unprotected tips changing the area of the metal layer in contact with the surface. This results in increased or decreased effective contrast and thus, higher fluctuation of the values of contrast. Protected tips, on the other hand, can be contaminated by substances on the surface, but the metal layer on the tip apex stays intact. This conclusion is supported by TEM images of the tips after the experiment (Fig. 54). Note that in “b” the whole silver layer was removed from the tip due to high
stress; however, the apex and its protection layer retained their original shape. In “a” even the Si$_3$N$_4$ tip beneath the silver layer was flattened. These experiments suggest that for less severe and demanding experiments, the protection layer can be the determining factor of whether the plasmonic layer is destroyed or not.

Fig.54. TEM images of a non-protected (a) and protected by 3 nm of Al$_2$O$_3$ tip (b). Scale bar is equal to 50 nm [170].

Tips, protected by alumina, have been tested for their Raman contrast (plotted in Fig.14) for a period of 40 days. Tips were stored in dry conditions inside a desiccator or in ambient conditions. However, little difference was observed between the two types despite increasing overall humidity over the period of the experiment. On the contrary, there is some noticeable difference for unprotected tips, as tips used in more humid conditions degraded faster and even more so for tips stored in such conditions.

Tips have been coated with alumina layers of different thickness: one, two and three nanometers for three sets of tips. According to the trends of data points, the thicker was the protection layer, the slower was the drop off in Raman contrast. Tips with 3 nm of Al$_2$O$_3$ showed no decrease even after 40 days. While it is possible that such tips also lost some of the initial contrast as appears from the figure, in reality this lower contrast most likely resulted from fluctuating quality of plasmonic silver layers for different batches of tips.
Fig. 55. Raman contrasts for tips protected by 3 nm (a), 1 nm (b), 2 nm of Al₂O₃ (c) and unprotected tips (d) on a 50 nm thick PEDOT/PSS film for a period of 40 days. Black symbols correspond to tips stored in desiccators, red symbols – to tips stored in ambient conditions. Relative humidity was slightly increasing from left to right for different protected sets. Hollow markers for un-protected tips indicate experiments performed at higher humidity.

The measurements of the optical properties of the tips indicate that the introduction of a Al₂O₃ layer has not significantly changed their optical absorption (Fig. 56). This agrees well with the fact that Raman contrast values for protected tips are similar to those of unprotected tips.

Fig.56. Optical contrasts for an unprotected silver-coated Si₃N₄ tip (black line) and a typical silver-coated tip with a 3 nm layer of Al₂O₃
5.2.2.3 Tip-induced heating

In addition to the problems of mechanical and chemical degradation, partially addressed above, there exists a problem of tip thermal stability. It is obvious that the enhancement of the electrical field of the light, which, in the case of TERS, can be one order of magnitude or even higher (so the overall enhancement is \( \sim E^4 \) and is four orders and higher), generates heating in addition to heating produced in the case of regular micro-Raman. Earlier, the heating arising from the field enhancement has been partially addressed in recent experimental works both for surface enhanced Raman spectroscopy (SERS) [167] and for TERS [168], as well as in a theoretical work [169]. The level of heating has been estimated either theoretically [169] or indirectly through analysis of the light-induced damage [168]. In both works sample temperature, caused by Joule heating, was found to increase by amounts reaching tens of K. However, no attempts at direct temperature measurements have been presented so far.

In this study, a different experimental approach has been taken. The temperature was measured directly through Stokes and Antistokes Raman signals. As with other experiments, a spin-cast (200 rpm, 2 min) PEDOT/PSS film was used as the substrate. A polymer sample for optical absorption measurements was prepared in a similar way on a cover glass slide from Dow Corning. Optical absorption measurements were performed using a HP 8452A Spectrophotometer. For precise calculations, the thickness of the PEDOT/PSS film on each type of substrate was characterized using profilometry. A fine scratch was made on the surface of the sample in several places, while an AFM instrument was utilized in tapping mode to measure the depth of the scratch.
AFM was used in contact mode. Rectangular-based tips (referred to as Tip1 and Tip2 in the text), prepared by vacuum evaporation of gold, were illuminated at a 45° angle (with respect to one of the faces of the tip) to avoid collecting light directly reflected from the surface of the tip. Light reflection from the tip has been identified as an important effect, which can distort the value of calculated enhancement and has been partially addressed in a previous section of the chapter. While such a measure may result in a less favorable polarization with respect to the tip and, consequently, lower enhancement, it is less prone to result in a false value of real enhancement.

Two sets of experiments have been performed, each with a different laser line (Ar++ 514 nm and Kr++ 647 nm) with an incident power of 0.4 – 1.1 mW on the sample in either case. The measurements were performed at room temperature, T=298±1 K, and relative humidity of 38-39%. Temperature and humidity were monitored by a Traceable Hydrometer™ (Control Company™) during all the measurements. A different, randomly selected spot on the sample was illuminated for every experimental data point to ensure there were no effects due to irreversible changes in a particular spot on a sample as a result of exposure. The structure of two tips was imaged with SEM using a JEOL PC-SEM 7401 scanning electron microscope after all other experiments with them had been completed. Optical absorption spectra have been measured according to a previously published procedure [139, 191].

Light absorption in the polymer film was determined by measurements of the intensity of a laser beam reflected at incident angle of 60° with respect to the substrate normal (i.e. the same as in the Raman setup), assuming the value of the aluminum reflectance to be 92%. The absorption of light in the polymer layer was ~23% for red
light and \sim 13\% for green light. The values obtained agree with the values supplied by the polymer manufacturer and optical absorption measurements on a glass slide, since the absorption at the green wavelength was approximately 43\% weaker.

Fig. 57 Schematic diagram of the heated two-layer system. \(D_2=1\ \mu\text{m}\) is the diameter of the laser spot, \(D_1=40\ \text{nm}\) and \(h_3=20\ \text{nm}\) are the diameter and the depth of the tip-enhanced spot, respectively, \(h_1=50\ \text{nm}\) is the polymer film thickness and \(h_2=1\ \mu\text{m}\) is the Al-layer thickness.

The simulation was performed using an available program with the code written in Fortran for solution of the equation of heat conduction in solids. The numerical solution of the problem was done using the finite-difference method. The following model has been employed in the simulations: Heat transfer in a two-layer system has been considered (see Fig. 57). The upper layer with thickness \(h_1=50\ \text{nm}\) was the polymer; the lower layer with the thickness \(h_2=1\ \mu\text{m}\) was the aluminum substrate. The specific heat of the polymer was assumed to be 1500 J/(kg\times K), a value typical for this class of polymers. Thermal conductivity, \(\lambda\), was taken to be 0.17 W/(m\times K) \cite{192} and density, \(\rho_1\), was 1003 kg/m\(^3\), as reported by the supplier. The value of the aluminum reflectance was assumed to be 92\% and the absorption of aluminum assumed to be 8\%. The thermal conductivity, density and specific heat for aluminum were taken to be \(\lambda = 237\ \text{W/(m\times K)}\),
\( \rho = 2689 \text{ kg/m}^3, \ c = 1300 \text{ J/(kg} \times \text{K}) \) [193]. We assumed heating of the polymer film by the laser beam to be uniform within the horizontal plane of the round spot with the diameter \( D_2 = 1 \mu\text{m} \) (Fig. 57). Absorption of the laser radiation in the polymer was taken from the optical measurements (see above). This model presents heating of the polymer film without a tip.

In order to simulate heating by the tip, an additional small circle with the diameter \( D_1 = 40 \text{ nm} \) (estimated localization of the near-field signal [174]) was introduced in the middle of the main spot (Fig. 57). The radiant flux into the polymer within this small circle was also assumed to be uniform in the horizontal plane. The light absorption in the polymer at different wavelengths was taken from the measurements described above. The light enhancement from the tip was calculated from the contrast factors measured experimentally, assuming the enhanced volume had a shape of a cylinder with a radius and height both equal 20 nm [186], as estimated from tip radius and enhancement penetration depth, respectively.

The radiant flux was decreasing along its path towards the aluminum substrate, as well as on the way towards the surface of polymer after being reflected by the aluminum substrate. The radiant flux within the polymer decreased with the distance from the surface \( z \) in accordance with Buger’s law

\[
I(r, z) = I_0(r) \exp(-\alpha z) \quad (5.2)
\]

Heat transfer in the system is described by the equation [194]:

\[
\rho c \frac{\partial T}{\partial t} = \frac{1}{r} \frac{\partial}{\partial r} \left( r \lambda \frac{\partial T}{\partial r} \right) + \frac{\partial}{\partial z} \left( \lambda \frac{\partial T}{\partial z} \right) + \omega \quad (5.3)
\]
where $T$ is the temperature, $t$ is the time, and $\omega$ is the heat generation rate due to absorption of radiant energy in the polymer layer and at the boundary between the aluminum substrate and the polymer. The heat generation rate is caused by both the incident flux and the flux reflected from the boundary between the polymer and aluminum substrate. In addition to heat generation due to absorption of light in the polymer layer, some heat was generated by absorption in the aluminum substrate. Both the upper and lower boundaries, i.e. air/polymer and aluminum/glass interfaces, were considered adiabatic, since the heat conductivities of air and glass are much smaller than the conductivities of the polymer and aluminum. The heat dissipation was fairly fast, i.e. the experimentally measured signal per second was identical for a 10s and a 60s measurement, but the simulation did take the prolonged exposure into account for the sake of compatibility with the real experimental parameters.

5.2.2.3.1 Heating in a simple Raman set-up

First, regular micro-Raman measurements (without tips) were used to calculate the temperature of the sample via the Stokes and Antistokes intensities ratio:

$$\frac{I_s}{I_{AS}} = \exp(h\nu/kT)$$

(5.4).

The feasibility of such an approach for SERS has been demonstrated previously in Reference 167. In that paper, the authors have also confirmed that the temperature in a two-layered polymer-metal SERS system equilibrates in milliseconds and becomes independent of the exposure time. We used the main Raman mode at $\nu \sim 1430$ cm$^{-1}$ for the temperature estimate. Fig. 58 presents an example of the Stokes and anti-Stokes spectra
(the latter is multiplied by a factor 100 for better visibility) used to estimate the temperature. The spectra in the frequency range of interest (1290 – 1465 cm\(^{-1}\)) were fit

![Raman Spectra](image)

**Fig. 58** The comparison of Stokes and anti-Stokes signals measured with \(\lambda=647\) nm for one selected temperature for Tip1. Signal in the anti-Stokes side is multiplied by a factor of 100 (is marked “x100”) for better visibility.

with two Lorentzian distribution functions (the second one represents a weaker mode at \(\nu\sim1370\) cm\(^{-1}\)) and the corresponding temperature was estimated from the ratio of intensities (eq.3). For accurate temperature estimates a correction parameter \(A\) should be introduced into the Equation 5.4 in order to compensate for such distortions as monochromator transmission and CCD sensitivity. This factor also takes into account distortions of the signal due to possible resonance Raman scattering and other optical properties of the samples. Therefore, the eq.5.4 has been modified:

\[
\frac{I_S}{I_{AS}} = A \exp\left(\frac{h\nu}{kT}\right).
\]

The correction parameter \(A\) has been calculated for both illumination wavelengths using an extrapolation of the estimated temperature to laser power \(P=0\) mW (Fig. 59). Linear extrapolation of the data measured with a wavelength of 647 nm results in \(T=295\) K at zero power, which is very close to the ambient temperature (298 K). So, the
correction parameter, \( A = 1.07 \), is close to unity for 647 nm. The linear extrapolation of the data for 514 nm, however, results in a much lower \( T = 274 \) K, and leads to a much higher value of the parameter \( A = 1.81 \). Fig. 59 presents the corrected experimental data for all far-field (without a tip) measurements. The heat generation rate is caused by both the incident flux and the flux reflected from the boundary between the polymer and aluminum substrate. In addition to heat generation due to absorption of light in the polymer layer, some heat was generated by absorption in the aluminum substrate.

Fig. 59 Temperature as a function of the incident laser power in a micro-Raman setup (no tip) estimated using Stokes/Antistokes Raman signal ratio measured with illumination wavelength 647 nm (a) and 514 nm (b). Symbols present two independent series of the measurements. The thin lines present linear regressions of the data. The thick lines present the corresponding simulation results.

5.2.2.3.2 Heating from a TERS set-up

After the initial experiments, two series of experiments with two different gold-coated tips were performed. The near-field Raman signal was calculated by subtracting the far-field signal (tip withdrawn) \( I_W \) from the signal measured when the tip is in contact, \( I_C \): \( I_{NF} = I_C - I_W \). The resulting Stokes and Antistokes component of \( I_{NF} \) were used in the same eq.5.4. The tip enhancement can be different for the Stokes and Antistokes signals.
So, the value of the correction factor $A$ could be different as well. The temperature estimated from $I_{NF}$ for the two tips, using measurements at $\lambda=647$ nm extrapolated to zero power, were 285 K and 307 K. These values were close to the ambient temperature within the experimental error-bars. So, the correction factor remained about the same for the tip-enhanced signal measured with red light. Unfortunately, the precision of the tip-enhanced signal measured with green light was not sufficient to estimate the correction factor and it has been assumed to be the same as in the micro-Raman measurements. The estimated temperatures are presented in Fig. 60. The tip-induced heating is significantly larger for the red light than for the green light. We measured enhancement factors of the tips, and calculated the contrast for each tip, according to the equation (5.1) in Section 5.1.1, which we repeat here:

\[ \text{Contrast} = \frac{I_C - I_W}{I_W} = \frac{I_{NF}}{I_W}, \]  

(5.1)

Fig. 60 Temperature as a function of the incident laser power with the wavelength 647 nm (a) and 514 nm (b): Squares present experimental data for the tip-induced heating (closed squares – Tip1; open squares – Tip2) and stars present experimental data when the tip is withdrawn. Lines present results of simulations for Tip1 (thick) and Tip2 (thin).
The contrast stayed constant during the whole set of measurements, demonstrating that no significant degradation of the tip apex took place during the measurements. The contrast values for 647 nm excitation wavelength were estimated to be 0.15±0.01 for Tip1 and 0.07±0.01 for Tip2. To estimate the enhancement factor for the optical signal, we need to take into account the ratio of the volumes contributing to the far-field signal, $V_{FF}$, and to the near-field signal, $V_{NF}$, [174]:

$$EF = \frac{Contrast}{\frac{V_{FF}}{V_{NF}}}$$

Assuming volumes are the same as presented in the Fig. 57, we estimated the ratio $V_{FF}/V_{NF} \approx 1600$. That gave us very modest enhancement of the Raman signal by the tips at $\lambda=647$ nm (consistent with the low contrast values), $EF \approx 240$ for Tip1 and $EF \approx 110$ for Tip2. For 514 nm excitation wavelength, the tip contrast values were equal to 0.19±0.03 for Tip1 and 0.16±0.03 for Tip2. The far-field volume for the green light was expected to be smaller than for the red light $(514/647)^2 \approx 0.6$. We found $EF \approx 180$ for Tip1 and $\approx 150$ for Tip2 in the case of illumination by green light.

Raman measurements without a tip demonstrated (Fig. 59) that Joule heating is considerably higher at $\lambda=647$ nm, consistent with the higher optical absorption of the polymer at this wavelength. The optical spectra of the tips (Fig. 61) show broad resonance around 700 nm with almost no signal at wavelengths below 500 nm. These spectra indicate that the enhancement of the optical field in red light should be much stronger than in green light. Indeed, the presence of either tip near the sample surface generates significant additional heating, especially under the red light illumination (Fig. 60). The apparent difference in tip-induced heating between red and green light is
consistent with the differences in the optical properties of the tips: Strong optical resonance in the red wavelength range should result in a strong enhancement of the electric field of the incident light with \( \lambda = 647 \) nm, while essentially no enhancement and no tip-induced heating is expected around \( \lambda = 514 \) nm (Fig. 61). This result, however, contradicts the estimated enhancement factors for these two wavelengths. We do not yet have a clear explanation for this observation and some possible reasons are discussed below.

Let us now compare the experimental results to the results of simulations. Simulations of the heating without any tip appear to be in good agreement with the experimental results at \( \lambda = 647 \) nm, while simulations slightly overestimate the heating in the case of light with \( \lambda = 514 \) nm (Fig. 59). This agreement of the model calculations with the experimental data with no adjustable parameters justifies the applicability of the simple model approach used in our analysis. An additional simulation parameter, the intensity enhancement factor for the small circle, was introduced to simulate the heating with a tip. Assuming that the enhancement of the incident laser light intensity by the tip is \( \approx (EF)^{1/2} \), we calculated the expected intensity enhancement to be \( \approx 15.5 \) and \( 10.5 \) for \( \lambda = 647 \) nm, and \( \approx 13.5 \) and \( \approx 12 \) for \( \lambda = 514 \) nm for Tip1 and Tip2, respectively. Comparison of simulations and experimental results for the tip-induced heating shows reasonable agreement for illumination with red light (Fig. 60a). However, the simulations strongly overestimate tip-induced heating in the case of green light (Fig. 60b). One of the reasons for this discrepancy may be the difference in wavelength used to estimate the intensity enhancements by the tips: We used contrast measured at the Stokes Raman signal, which was significantly red shifted relative to the laser wavelength (Fig. 61). The
heating, however, was generated at the laser wavelength. The difference in the optical signals generated by the tips at 647 nm and at the corresponding Raman line ($\lambda=716$ nm) is not significant for both tips (Fig. 61). However, the tip optical resonance decays strongly (by a factor $\sim 4$-5) between $\lambda = 558$ nm (Stokes Raman line wavelength) and $\lambda = 514$ nm for both tips (Fig. 61). Assuming tip-induced heating four times smaller results in good agreement between simulations and experiment also for green light.

Both the measured temperatures and those calculated from simulation are significantly lower than the degradation temperature of the PEDOT/PSS blend ($\sim 523$ K) and for most laser powers used are even lower than its glass transition temperature ($\sim 443$ K) [195]. The stability of the polymer at these temperatures has also been confirmed experimentally [196]. This explains the reproducibility of the results. It is important to note that the tips remained unchanged after all the experiments, as confirmed by the consistency of their contrast factors over the time of two weeks, as well as their morphologies after use. SEM images of the two tips after all the experiments are
presented in Fig. 62 and no noticeable damage is observed (the left edge of the first tip was not illuminated by the laser and exhibits a metal deposition defect).

Fig. 62 SEM images of the two tips after all the experiments. Tip1 is presented in “a”, Tip2 – in “b”.

Our experimental and simulation results agree qualitatively with the simulation results available in the literature. An example of a temperature profile as a function of distance from the center of the tip for the polymer film at a depth of 10 nm is plotted in Fig. 63. The variation of the temperature with the distance away from the tip in the horizontal plane is similar to the simulations results obtained by Downes and co-workers [169] for the “Au tip-air-mica substrate” interface and to the simple graph of temperature distribution in the gold substrate, shown by Zhang et al.[168].
Fig. 63 Simulated temperature distribution at 10 nm below the surface of the polymer layer at laser power 1 mW, assuming enhancement of the light intensity by the tip ~15.5.

Our work suggests that the heating of an optically absorbing sample in the presence of a tip is significant even with modest field enhancement by the tip and laser power around 1 mW. It should always be taken into account for samples which are more prone to thermal degradation or other temperature-induced effects. Further investigations of the dependence of heating on the optical properties could be carried out with tips made by different procedures and using different metals, e.g. silver, where the aforementioned optical properties would be significantly different [139].

5.3 TERS scanning.

Farfield Raman scanning, as a separate technique, allows one to obtain 2D mapping images of enhancement from different Raman active species. It is possible to find clusters of carbon nanotubes and differentiate them from empty substrate (Fig. 64a). While clusters of nanotubes down to 500 nm can be visualized, observation of a single nanotube is not possible due to the diffraction limit. Nearfield Raman scanning can
achieve this resolution. Unfortunately, it has been found that doing 2D mapping of CNTs with a regular AFM-based TERS system is hardly possible. While the contrast is great (Fig. 64b) and is more than enough to make it possible to distinguish between the farfield and nearfield signals, the CNT ruptures on the scale of a second and the nearfield Raman signal becomes equal to that of the farfield. This can happen due to one or a combination of the following reasons. First, the AFM tip is made to stand on a nanotube for a second when the Raman signal is collected. This can be enough to damage it. Second, the extreme enhancement, which is evident from Fig. 64b, could contribute significantly to the thermal decomposition of the nanotube in the area of contact with the tip even with laser power below 1 mW.

![Fig. 64. a) Farfield scanning of a 3x3 micron area of carbon nanotubes, wrapped with PMPV for better surface adhesion, dispersed over a clean Si wafer b) Comparison of G-band signals for a CNT with a gold TERS tip in contact (top) and without the tip (bottom)](image)

Based on our observations in the experiment, we can say that in order to successfully create TERS maps of carbon nanotubes, one should ensure the following: very low laser power at the focal spot (i.e. 0.1 mW or less) and tuning-fork set-up for tip
mounting, where the tip is essentially floating above the analyte and is not touching it. It was demonstrated [197] that such a set-up was indeed capable of CNT TERS imaging.

Fig. 65 a) AFM image of a cluster of “nanoraspberries” on a PEDOT/PSS film b) simultaneous nearfield map with Lorentzian fit area under the highest PEDOT peak ($\sim 1450$ cm$^{-1}$) plotted as the ordinate. 514.5 nm laser has been used for Raman scanning.

If the restrictions noted above are not as detrimental to the TERS mapping, an image with reasonable quality can be achieved. SERS experiments with silica-core silver-shell nanoraspberries partially embedded in a PEDOT/PSS film have shown that they can withstand commonly used laser powers ($\sim 1$ mW) without degrading. Their mechanical strength was also quite good as confirmed from numerous AFM experiments with both contact mode and tapping mode tips used. In Fig.65, the topography of a nanoraspberry aggregate on such a SERS sample is shown together with a nearfield Raman map. It should be noted that the highest enhancement on the Raman map corresponds to the area in the middle of the cluster which suggests the creation of a hot-spot in this pocket.
In this Chapter, several promising ways of creating TERS tips have been discussed and the properties of some sample tips were characterized. The effect of signal reflection from the tip side has been identified and quantified to a certain degree. It was also shown that metalized tips, especially those coated with silver, are easily degraded through chemical and mechanical interactions with the environment and the analyte. Coatings of silicon oxide and aluminum oxide have been evaluated as potential candidates for TERS tips protection. Both were found to be able to improve tip longevity, although the improvement in the case of silicon oxide was only moderate. Tips with alumina coatings were capable of extending the lifetime of TERS tips for more than a month when stored in dry or humid conditions. Chemical and mechanical degradation of tips has been discussed. Additionally, the presented experimental studies demonstrate significant tip-induced heating of the samples with optical absorption. The heating depends strongly on the optical properties of the tips and is minimal for laser wavelengths outside of the tip’s plasmon resonance. The magnitude of heating can easily reach 100K, even at laser power as low as 1 mW and modest tip enhancement. The experiment and simulation results are in good agreement with those in the literature. Finally, TERS mapping has been attempted for a sample of carbon nanotubes and successfully performed for a sample of silver nanoraspberries.
A single-walled carbon nanotube (SWNT) is formed by rolling up graphene along a so-called chiral vector to form a cylinder. The circumference of the SWNT is determined by its chiral, or sometimes called “rolling” vector \( C_h = na_1 + ma_2 \), where \((n, m)\) are integers known as the chiral indices and \(a_1\) and \(a_2\) are the unit vectors of the graphene lattice. Each rolling-vector leads to a specific carbon nanotube species that

![Diagram of chiral indices and rolling vectors for carbon nanotubes.](image)

Fig. 66. Schematic representation of the rolling vector \( C \) for carbon nanotubes. Reproduced with permission from [199]. Copyright 2008 American Chemical Society.
exhibits different chirality and diameter as shown in Fig. 66 [198]. Nanotubes with $n = m$ (known as armchair nanotubes) and those with $n - m = 3j$, where $j = 0, 1, 2, 3...$ are metallic at room temperature (red color in Fig. 66). Carbon nanotubes with $n - m = 3j + 1$ and $n - m = 3j + 2$ are semiconductors with a bandgap that varies inversely with the tube diameter. Nanotubes with $m = 0$ are known as zigzag nanotubes and can be either metallic or semiconducting. $\theta$ is the chiral angle.

SWNTs are known to exhibit remarkable tensile strength [200]. Their superior mechanical properties have attracted significant attention. One of the possible applications in industry is to use SWNTs as rubber reinforcement, where improved mechanical strength is desirable. Effective reinforcement, however, requires the nanotubes to be uniformly dispersed in the polymer matrix and to have good adhesion to the polymer matrix. Achieving a good dispersion remains to be a challenge, as SWNTs themselves tend to form strong aggregates.

Theoretical studies have shown that CNTs could exhibit high conductivity [201]. Theoretical values calculated for tubes with arm-chair conformations (see Fig. 66) could be many orders greater than those of copper. However, not only purely metallic zig-zag nanotubes are of great interest. Nanotubes marked with blue color in Fig. 66 could be even more useful in certain circumstances due to their semiconducting nature, that is, the ability to be conductors at some conditions and dielectric materials at others. Currently, one of the most promising methods of bulk SWCNT preparation is high-pressure CO gas synthesis (HiPco), which produces sample with a relatively narrow distribution of chiralities. In this process, CO is injected into a chamber at a temperature of $>1000^\circ$ C at a pressure of 30-50 atm and with catalytic clusters of iron [202]. Consequently, there is a
great need for a reasonably cheap, easily scalable and effective separation method which would provide such SWCNTs with a narrow distribution in chiralities, preferably with only one type of nanotube present.

In this Chapter we propose a promising method for SWCNT separation, such as polymer wrapping and sonication which offers an attractive approach to effective enrichment of specific CNTs in large quantities. Additionally, the changes of the physical properties that are introduced to the sample with different separation techniques will be also described.

6.1. Important aspects to be considered in studies of SWNTs

6.1.1. The introduction to Raman scattering of carbon nanotubes

There are several analytical tools which are sensitive to carbon nanotubes and are able to detect them in a solution. Among them, optical absorption, 2D fluorescence, and Raman scattering are the most convenient tools, as they can be used with as-prepared samples and the measurements themselves are relatively fast and simple. Of these three, however, Raman scattering appears to be the most effective technique, as it can resolve peaks from different nanotubes best and it also can probe different properties of the measured nanotubes at the same time. Multiple C-atom displacements for a single-walled carbon nanotube are Raman-active and can be identified with this technique. Moreover, many of the modes are quite intense, particularly the ones shown in Fig. 67.
Fig. 67. Raman-active normal mode eigenvectors (arrows) and frequencies (numbers) for a (10,10) nanotube. The red arrows indicate the magnitude and direction of the appropriate C-atom displacements. The unit cell (blue atoms) is shown schematically in the upper right-hand corner. From [203]. Copyright 2007. Reprinted with permission from AAAS.

The modes that are of particular interest are the Radial Breathing Modes (RBM, third in the bottom row, Fig. 67) and the so-called G-bands (first in Fig. 67). There are other bands, such as the D-bands at ~1300 cm$^{-1}$ and the second harmonics of the D-band, often in literature quite confusingly called the G’-band, and 2600 cm$^{-1}$ which provide some information on the defects of the tubes. These peaks are less useful, since their positions (1550-1600 cm$^{-1}$) are not different from the positions of the G-bands of such materials as amorphous carbon, carbon black and carbon fiber and thus cannot help distinguish among these materials. They can, however, help distinguish amorphous carbon, graphene and graphite and CNTs prepared at various temperatures.
In an important work by Rao et al. [204] which was confirmed by earlier theoretical calculations [205], it was found that Raman measurements using a laser with a fixed wavelength can probe only particular types of carbon nanotubes. The types depend on the wavelength of the laser used. This is due to the fact that SWCNTs exhibit resonant Raman and each set of nanotubes with a particular diameter/chirality will only be excited by absorbed light with an energy matching its interband transition, as shown in Fig. 68.

![Fig. 68. Calculated density of electronic states (DOS) of metallic (10,10) and (11,8) and semiconducting (12,7) tubes using tight-binding calculations with Fermi level positioned at zero energy [206].](image)

Recently, the chiral index (n,m) of about 50 nanotubes, including both metallic and semiconducting nanotubes, have been assigned on the basis of 3D Raman with the excitation energy ranging from 1.52 to 2.71 eV [207-209]. To determine the SWNT diameters in our samples, Raman spectra were acquired for the raw HiPCO nanotubes in the region assigned to the SWNT radial breathing mode (RBM) frequency (100-400 cm⁻¹) when the samples were excited at 647.1 nm and 514.5 nm (Fig. 69). The tube diameters
and their corresponding chiralities in the sample were calculated and listed in Tables 1 and 2, by using the relation:

\[ \omega(\text{RBM}) = C_1/d + C_2 \quad (6.1) \]

where \( C_1 \) and \( C_2 \) are empirical constants. The constants were taken from Reference 209: \( C_1=219.3 \text{ nm-cm}^{-1} \) and \( C_2=14.7 \text{ cm}^{-1} \). The results were complementary to the fluorescence study, as the later only detected individual semiconducting tubes [210].

Fig. 69. RBM Raman modes of solid raw HiPCO SWCNTs measured with a) 647 nm laser and b) comparison of the spectra measured by 647 (red curve) and 514 nm (green curve) lasers.
The 2D fluorescence data (scanning over a range of laser wavelengths and recording the emission signals) are plotted in Fig. 70 along with literature fluorescence data from a very similar sample [199]. The spectrum from the sample from Ref. 199 has shown several extra peaks which are absent in the spectrum from our sample. Several of the CNT peaks which corresponded to the 647 nm Raman spectra in our sample have been marked.

For results obtained using the 514.5 nm laser, tube diameters were determined based only on eq. 6.1 due to the absence of fluorescence peaks in the region (green line in Fig. 70). Then, based on the assumption of linear dependence of inverse diameters and frequencies of RBM modes [210], the chiralities of CNTs measured with the λ=514.5 nm
laser have been calculated. Taking the C-C distance to be 0.246 nm, the chiralities were determined as a best match using a simple procedure in Excel™, according to the formula:

\[ d = \frac{0.246}{\pi} \sqrt{n^2 + nm + m^2} \]  

(6.2)

For the 647 nm laser, the tube chiralities were determined based on both fluorescence and Raman data. The possible electronic transition peaks [210, 211] were taken into account, thus excluding some of the chiralities (some semiconducting tubes) which otherwise matched the Raman results. It is quite interesting that all of the peaks probed by the green laser corresponded to metallic nanotubes. This is in good agreement with the 2D fluorescence data which show that there are no absorption bands of semiconducting SWCNTs when the sample is excited at 514.5 nm.

Table 1. Parameters of SWNTs based on Raman RBM peak frequency for 647 nm laser.

<table>
<thead>
<tr>
<th>Frequency of RBM, cm(^{-1})</th>
<th>HiPCO SWCNs</th>
<th>Final sediment, after 10 steps</th>
<th>Suspension, after 7 steps</th>
<th>Assigned chirality, ((n, m))</th>
<th>Calculated diameter, nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>192.43</td>
<td>195.47</td>
<td>198.72</td>
<td>(12,6) met</td>
<td>1.243</td>
<td></td>
</tr>
<tr>
<td>217.11</td>
<td>218.37</td>
<td>(very weak)</td>
<td>(8,8) met</td>
<td>1.085</td>
<td></td>
</tr>
<tr>
<td>249.86</td>
<td>251.68</td>
<td>255.29</td>
<td>(10,3)</td>
<td>0.923</td>
<td></td>
</tr>
<tr>
<td>257.42</td>
<td>263.05</td>
<td>266.53</td>
<td>(7,6)</td>
<td>0.882</td>
<td></td>
</tr>
<tr>
<td>280.71</td>
<td>282.69</td>
<td>286.12</td>
<td>(7,5)</td>
<td>0.818</td>
<td></td>
</tr>
<tr>
<td>295.76</td>
<td>295.76</td>
<td>302.44</td>
<td>(8,3)</td>
<td>0.771</td>
<td></td>
</tr>
</tbody>
</table>

Note: “met” denotes metallic tubes
Table 2. Parameters of SWNTs based on Raman RBM peak frequency for 514 nm laser.

<table>
<thead>
<tr>
<th>Frequency of RBM, cm&lt;sup&gt;-1&lt;/sup&gt;</th>
<th>HiPCO SWCNTs</th>
<th>Sed10, solid</th>
<th>Sus7, in solution</th>
<th>Assigned chirality, (n, m)</th>
<th>Calculated diameter, nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>187.36</td>
<td>189.77</td>
<td>N/A</td>
<td>(16,1)/(11,8) met</td>
<td>1.270</td>
<td></td>
</tr>
<tr>
<td>208.18</td>
<td>208.33</td>
<td>N/A</td>
<td>(10,7)/(11,5) met</td>
<td>1.133</td>
<td></td>
</tr>
<tr>
<td>219.50</td>
<td>219.80</td>
<td>N/A</td>
<td>(12,3) met</td>
<td>1.071</td>
<td></td>
</tr>
<tr>
<td>233.12</td>
<td>231.50</td>
<td>N/A</td>
<td>(9,6) met</td>
<td>1.004</td>
<td></td>
</tr>
<tr>
<td>249.17</td>
<td>249.35</td>
<td>N/A</td>
<td>(12,0) met</td>
<td>0.935</td>
<td></td>
</tr>
<tr>
<td>263.94</td>
<td>264.22</td>
<td>N/A</td>
<td>(8,5)met</td>
<td>0.880</td>
<td></td>
</tr>
<tr>
<td>272.60</td>
<td>273.40</td>
<td>N/A</td>
<td>(9,3) met</td>
<td>0.850</td>
<td></td>
</tr>
<tr>
<td>316.74</td>
<td>(very weak)</td>
<td>N/A</td>
<td>(8,2) met</td>
<td>0.726</td>
<td></td>
</tr>
</tbody>
</table>

It was impossible to measure the Raman signal from the carbon nanotubes samples in solution with the green laser, as they have exhibited significant background fluorescence. Thus, only the solid samples have been measured.

6.1.2. Shifting of peaks from interactions of CNTs with different chemical species

The studies of SWCNT samples with fluorescence and Raman spectroscopies have revealed that there is some difference in their electronic characteristics between as-prepared SWCNTs and SWCNTs in solutions (or dried from solution), see Fig. 71. The emission peaks of SWNTs-PmPV in THF were consistently red-shifted, by about 30-40
nm, in comparison to the corresponding peaks of a SWNT suspension in H₂O. In addition, the excitation maximum (λ_{ex}) of SWNT-PmPV in THF was red-shifted by about 15 nm, revealing that there is a strong π-π electronic interaction between the nanotubes and PmPV chains that are tightly wrapped on the nanotube surfaces. Such a shift has been observed in 2D Raman analysis [212], where excitation energy for the most studied wrapped nanotubes shifted upwards, when compared to as prepared CNTs. This is different from our results and other literature results [199]. However, this disagreement can be explained in terms of different nanotube types and different wrapping agents used.

The existence of such a shift is rarely identified by the authors and little to no explanation of it is usually given. Several authors, however, identified the change of RBM peak positions for nanotubes after interaction with other substances [213-215]. It was argued [215] that this change is due to the surrounding polymers creating a
difference in the local dielectric environment where the increased dielectric screening causes a reduction of the electron-electron and excitonic Coulomb interactions. The reduction, in turn, is caused by the formation of impurity states below the conduction band of the nanotubes. Moreover, it was stated [214] that all of the observed shifts were to higher frequencies, which disagrees with the model of strain-induced shifts. In [215], it was noted that all of the shifts for filled tubes (i.e., with species trapped or absorbed inside) were of same magnitude and direction for different molecules of different sizes, but similar chemistry. The strain-induced shifts are expected to produce both positive and negative shifts varying in magnitude and sign with the chiral vector [216]. In the work of Lee et al. [213], fullerene (C60) molecules were placed inside carbon nanotubes with noticeable subsequent local expansion of tube diameter. The peak shifting was attributed to straining, according to the authors, though it could have been attributed to fullerene-nanotube interactions with equal confidence. Very little correlation has been found with the model that describes the strain-induced frequency shifts [216]. That is, for tubes with \( n - m = 3j + 1 \) and \( n - m = 3j + 2 \), the shifts are supposed to be in different directions (increase or decrease of the bandgap). All of the emission peaks of the tubes in our 2D fluorescence experiments red-shifted when compared to the literature result of non-wrapped tubes. Moreover, for different angles of the rolling vector, the shift should be different, which generally is not the case for our samples. Both observations were viewed as a significant proof of the “formation of impurity states lowering the bandgap” theory. Regardless of the mechanism, the shifting of absorption bands, identified from our fluorescence experiments, indicates the strong interaction of PMPV chains with the carbon nanotubes.
In addition to the shift of the absorption bands, our results show significant shifting of RBM modes due to interactions with various species at different stages of SWCNT purification, which is in good agreement with recent literature results [217]. Unlike with the absorption bands, the shifts happen even due to interactions with the solvents.

![Graph](attachment:image.png)

**Fig. 72.** Shifts of different RBM modes to higher frequencies relative to those of HiPCO as prepared SWCNTs. Influence of chemical purification and polymer wrapping on CNTs’ RBM (a) and influence of various solvents on RBM (b). The spectra were measured with 647 nm laser.

Our results (Fig. 72) show that all of the RBM modes of SWCNTs exhibit shifts to the higher frequencies with no exceptions. This happens for all types of chemical treatments (Fig. 72a) and solvents (Fig. 72b) studied. One explanation for the shifts for the polymer-containing samples would be the π-π interactions between the CNT and the polymer adsorbed on its surface. When the polymer was partially removed by the process of soaking in big amounts of THF (dozens of mg CNT per liter THF) and centrifuging three times for 1h each time, the shifts became smaller. However, samples which never reacted with polymer chains gave very similar shifts. Apparently, when CNTs are placed
in a solvent, their electronic structure changes, which, in turn, changes the effective spring constant of the vibration.

In addition to that, the G-bands were also shifted and/or changed for nanotubes interacting with different solvents and/or polymers. However, these bands are not necessarily shifted to the red zone. In fact, the direction and magnitude of the shifts were not even correlated with one another. A recent study [218] on the straining of CNT tubes showed that the G and G’ bands are significantly down-shifted with strain parallel to the main axis of the tube. Moreover, these shifts are expected to be highly correlated to the level of the strain. A recent study [219] has demonstrated with two different techniques (heating in a cryostat and variations of laser power) that G and D bands for multiwalled CNTs strongly depend on temperature. A change of temperature by ten degrees induced a downshift of slightly less than 1 cm⁻¹. When the laser power has been changed for our samples, noticeable shifts of G bands and even RBM modes have occurred, as seen in Fig. 73 (the sample was equivalent to the one shown as a blue line in Fig. 74b).

The 2D fluorescence and Raman experiments have shown that the peaks of the SWCNTs are highly sensitive to multiple different factors and not only their position, but their relative intensity can change significantly without any change of the CNT sample composition.
Fig. 73. The influence of laser power on RBM peak position (a, b), the G-band position (c) and temperature of the nanotubes (d). The red solid lines are guides for the eye. Each color in (b) corresponds to a different power at sample.

6.1.3. Destruction of carbon nanotubes

Very little attention has been given in the literature to the phenomena of nanotube destruction during all the purification procedures currently employed by researchers, although it is likely that this problem is recognized by many. Destruction can happen during prolonged treatment with concentrated nitric acid. This has been suggested previously [220] and is also confirmed by our results (Fig. 74) which show that nitric acid
treatment removes the majority of small tubes as is evident from Raman RBM spectra with both 514.5 and 647 nm lasers. While it is still possible that the destruction happened on the basis of tube diameter and was not related to the metallic or semiconducting nature of the nanotubes, the fact that the majority of the surviving tubes were metallic, according to Tables 1 and 2, shows that this purification most likely removes both the smallest and semiconducting tubes.

Fig. 74. The result of concentrated nitric acid purification on as prepared HiPCO SWCNTs. Raman RBM spectra with 647 nm (a) and 514.5 nm (b) lasers.

After the tubes were purified by acid treatment, they could be further destroyed during the following processes of chemical separation, in which the nanotubes are reacted with different agents in solution. For proper nanotube dispersion in solution, sonication is commonly used. Surprisingly, to the best of our knowledge, very little to no information on this type of destruction has been published in the literature. Goyanes et al. [221] observed destruction of multi-walled CNTs in a mixture of concentrated nitric and sulfuric acids, but failed to acknowledge the destruction by sonication. In our extensive
studies, such destruction was always observed. The effect of destruction of tubes in our samples by sonication is shown in Fig. 75a, which shows that the metallic (8,8) tubes are preferentially destroyed, though as well as some (12,6) tubes are destroyed. The destruction of the (12,6) tubes is less obvious due to the normalization to the (12,6) peak. Results with the 514.5 nm laser show a similar trend with all metallic peaks decreasing in relative intensity (Fig. 75b). Based on these results, destruction by sonication mainly affects metallic tubes and depends very little on their diameter.

Fig. 75. The effect of prolonged sonication on raw SWCNTs evidenced by changes in the spectra measured with 647 nm (a) and 514.5 nm (b) lasers.

In addition to destruction by sonication, Huang et al. [222] has reported that metallic single-walled carbon nanotubes in carbon nanotube thin film can be destroyed in preference to their semiconducting counterparts when the wavelength and power intensity of the irradiation are appropriate and the carbon nanotubes are not heavily bundled. This was later confirmed by Zandian et al. [223], who have also suggested that this method can be used for selective removal of resonant metallic nanotubes from the sample. An even more effective method for preferential destruction of metallic nanotubes has been
proposed [224], by which means the contents of semiconducting nanotubes in the samples has been increased from 50% to nearly 95% after an hour or irradiation.

Fig. 76. The difference between the initial spectrum and spectrum after 2 min 647 nm laser burning, with the spectra normalized using the second peak: RBM (a) and G (b) bands.

As can be seen from Fig. 76, in our samples the metallic (8,8) and (12,6) peaks have been mainly affected by the heating induced by a 647 nm laser. The (10,3) tubes were also affected. All other semiconducting tubes suffered significantly less. The destruction of tubes also occurred when illuminated with a 514.5 nm laser. However the quantification was more difficult, as all of the peaks were decreasing at relatively the same rate.

Metallic nanotubes can also be destroyed by microwave irradiation, as shown by Shim et al. [225]. Finally, nanotubes can be destroyed during electron irradiation in electron microscopes (SEM, TEM) [226], though it is unclear whether metallic nanotubes are also destroyed in this case or there is no particular preference in the threshold value of electron irradiation for removal of different types of CNTs.

Our experiments have indicated that carbon nanotubes can be easily destroyed with prolonged sonication and laser heating. The semi-quantitative nature of the
experiments helped to determine what types of nanotubes can be destroyed and in which quantities with the common purification techniques.

6.2. Purification of different CNT types by different methods and their combinations

In a review article [227], different ways of non-covalent functionalization of single-walled carbon nanotubes have been described in detail. Among other advantages, remarkable solubility of functionalized nanotubes has been noted. Such advantage has not remained unnoticed when Maeda et.al. [228], who applied this phenomenon to facilitate large-scale separation of single-walled CNTs. The separation was evident, however no detailed theoretical explanation has been provided.

It appears that in most SWCNTs separation procedures based on dispersion with different surfactants or short molecules [209, 228, 229], lighter nanotubes with smaller diameters can be enriched in supernatant. Although this phenomenon can be related to preferential interaction of different organic molecules with specific types of nanotubes, this effect could be possibly overshadowed by one caused by their density difference. There is evidence in support of this statement in the literature. In Ref.[230] it has been reported that nanotubes have been quite effectively separated based on their densities. In many papers about SWCNTs separation ultracentrifugation in excess of 50000 g and higher was used, which helped with separations significantly [209, 214, 228, 229]. So far, however, apart from the article of Arnold et.al. few have recognized the importance of nanotube density in their separation.
The nondestructive separation of metallic SWNTs has been demonstrated by using an alkyl amine [213, 228], porphyrin [231], pyrene [232] and DNA [233]. In these reports, the added chemical reagent exhibits different affinity toward different types of SWNTs, thereby discriminating them for separation. Recent studies show that the amine-assisted method can effectively enrich the metallic SWNTs to ~87% purity [228], as calculated by the authors. However, the calculation was based predominantly on Raman measurements with one laser wavelength, which cannot describe the overall composition of the sample.

6.2.1. Purification of different CNT types by different methods and their combinations

Different polymers show the ability to discriminate among nanotube species in terms of either diameter or chiral angle [234]. As a soluble π-conjugated polymer, poly[(m-phenylenevinylene)-alt-(p-phenylenevinylene)] (PmPV) represents another interesting system to interact with SWNTs. Several studies of wrapping SWNTs with PmPV derivatives [235-237] suggest selective interaction with semiconducting SWNTs (diameters of 1.28 and 1.35 nm) [237]. Little is known, however, about the origin of this intriguing selectivity.

One of the methods used to estimate the flexibility of polymer chains involves observing the rate of viscosity change with molecular mass. The equation describing the dependence of the intrinsic viscosity of a polymer on its relative molecular mass (molecular weight) has the form:

\[ \lbrack \eta \rbrack = K \cdot M_r^\alpha \quad (6.3) \]
The Mark-Houwink exponent $\alpha$, which shows how fast the viscosity increases with increase of the molecular mass, for PmPV in solution is determined to be $\alpha \approx 1.0$ [238]. This value indicates a somewhat stiff coil (for comparison, poly(vinyl chloride) has 0.92 in THF), but is far from the limit of 2 for a rigid rod [239]. Thus, the semirigid $\pi$-conjugated polymer backbone has certain flexibility to adjust its conformation during wrapping SWNTs. In order to understand the potential role of polymer conformation in the SWNT wrapping, a molecular modeling study using the AM1 method following a faster MM+ modeling, by using HyperChem™ (version 8.0), has been performed [240] which reveals that the polymer adopts a helical conformation with a cavity size of about 1.3 nm (Fig. 77).

Fig. 77. Molecular modeling of PmPV oligomer (cyan color) wrapped on a (8,7) SWNT ($d = 0.988$ nm, brown color). The nanotube fits the molecular geometry of the bent PmPV conformation (a), and the polymer adopts a helical conformation during the wrapping interaction (b).

In addition, the phenylenevinylene (PV) fragments are arranged either perpendicular or parallel to the SWNT surface to facilitate $\pi-\pi$ interactions. One of the fundamental issues is how well the tubes can be held in the predefined cavity by the
helical conformation of PmPV, as this will have direct impact on the selectivity of tube wrapping and the stability of the resultant wrapping. An intrinsic property of SWNTs is that they tend to stay in a bundle. Previous studies relied on a single stage of sonication to break up the nanotube bundle which, was likely to lead to wrapping of bundled SWNT by PmPV [229]. In the current study, the SWNT sample has gone through a sequence of sonication and centrifugation to optimize the probability for individual SWNT wrapping. The wrapping process was monitored in every stage to understand the wrapping of SWNT by PmPV.

Visible-near-infrared (Vis-NIR) absorption spectra were acquired from the supernatant solutions to monitor the change in the populations of different tubes. The individual peaks in the Vis-NIR spectrum can be attributed to the valence-to-conduction band electronic transitions which depend on the size and chirality of the nanotube. The absorption spectra of supenatant solutions (Sup-1 to Sup-7) (Fig. 78a) revealed the details for semiconducting tubes which exhibit the electronic van Hove transitions E11 (between the first order discontinuities in the DOS of nanotubes, 900-1600 nm) and E22 (between second order discontinuities, 700-900 nm). The absorption peaks at 1410 and 1550 nm were attributed to (11,7) and (11,6) SWNTs with diameters of 1.25 and 1.19 nm, respectively [230]. In the original sample, the peak intensity at 1410 nm was weaker than that at ∼1280 nm. However, the relative peak intensity at 1280 nm decreased as the sonication-centrifugation process was repeated. In the solution of Sup-7, the absorption peak at 1410 nm became predominant, showing that PmPV polymer had the ability to selectively wrap certain nanotubes.
To gain further understanding into this process, a freshly prepared suspension of SWNTs in polymer solution was subjected to different length of centrifugation times. During the high speed centrifugation, some of the nanotubes precipitated gradually from the suspension. Although the poorly dispersed tubes had a greater tendency to come out, some of the well-dispersed tubes could also be forced out of the polymer solution. It was interesting to notice that the tubes which gave rise to the 1410 nm absorption were less affected by the centrifugation than were the ones corresponding to 1280 nm, for which absorption gradually decreased with increasing centrifugation time (Fig. 78b). This result further confirms the assumption that PmPV polymer had a preference to wrap the nanotubes that gave 1410 and 1550 nm absorption and had diameters of 1.19 and 1.25 nm, respectively. Molecular modeling also showed that the PmPV chain could adopt a coil conformation with a cavity size of about 1.3 nm (Fig. 77). Clearly, the tubes of comparable diameter would fit into this cavity well, thereby allowing an intimate interaction between the nanotube surfaces and wrapping polymer chains. Due to tight
wrapping around the nanotube, the polymers chains would not be easily separated by centrifugation.

The supernatant solution was not stable, but settling of the tubes took many days. When the Sup-5 solution was left at room temperature for a week, the absorption peak at 1280 nm was found to decrease in a manner similar to that seen in Fig. 78b. The impact of sample settling for one week was equivalent to about 12 h centrifugation. The freshly prepared Sup-5 was transparent. After being kept for a few weeks, some tuft-like precipitation was formed from the Sup-5 solution (Fig. 79). Resonication for 3 h could easily redisperse the SWNTs back into solution. The results clearly indicated that the wrapping process was reversible.

![Fig. 79. Images of sup-5 as prepared (a), after settling for two weeks (b), exposure to a 40 W lamp for 5 s (c) and 10 s (d), and then followed by sonication for 3 h (e).](image)

It was noted that the precipitation process could occur in a shorter time period (in about 12 h) upon irradiation with UV-visible light. As seen in Fig. 80, a large portion of the tubes dropped out of the solution after just a few hours of irradiation. The SWCTs corresponding to absorption peaks at 1410 and 1550 nm were less affected by irradiation. It was likely that the irradiation altered the conformation of the wrapping polymer,
thereby speeding up the unwrapping process and allowing the tubes to slip out more easily. When the nanotube diameters closely matched the cavity created by the helical conformation of PmPV, the polymer chains were more tightly wrapped on the surface of the tubes.

Fig. 80. Absorption spectra of freshly prepared suspension (Sus-5) before (solid line) and after 3 h irradiation (dashed line). The spectra were normalized to the peak at 1410 nm for clarity.

This local chain rigidity, which is dependent on the wrapped nanotube diameters, hampered the chain movement triggered by irradiation. The polymer which was wrapped on the tubes of smaller diameters allowed the chain segment motion to occur relatively easily in response to irradiation. In summary, results from both centrifugation and photolytic conditions showed that the stability of nanotube suspensions in PmPV solution was dependent on the tube diameters and chiralities. The slower precipitation rate observed for the tubes which gave absorption peaks at 1410 and 1550 nm leads to the conclusion that (11,6) and (11,7) SWNTs had stronger interactions with the PmPV than did the others. The tuftlike precipitation formed initially came out as a whole to settle at
the bottom (Fig. 79b). When an incandescent lamp of 40 W was positioned at a close distance (∼15 cm) to the vial containing the suspension, the entire SWNT mass floated up when the lamp was turned on and settled down when the lamp was off. This observation indicates that the polymer and nanotubes entangled with each other during the precipitation. Each polymer chain became wrapped around multiple tubes in order to hold them in close proximity. The presence of multiple polymer chains on a single nanotube or nanotube bundles is also plausible which is consistent with the formation of a thick PmPV coating on SWNT, as seen in the AFM image of SWCNTs with a relatively big amount of polymer (Fig. 81). The nanotubes are clearly seen in the phase image, as they are much harder, than the surrounding PMPV.

Fig. 81. AFM height (a) and phase (b) images of SWCNTs/PMPV spun cast on a precleaned Si wafer from Sus-5. Image scan 5x5 microns, tapping mode.

The original SWNT sample was compared with the Sed-8 which had gone through repeated extraction with PmPV solution, sonication, and centrifugation processes. Fig. 82a shows that the content of metallic tubes in the Sed-8 was reduced while that of semiconducting ones was enhanced. The depletion of metallic tubes in the
sediment indicated that the metallic tubes were enriched in the supernatant. The reduction in the intensity of the characteristic Raman G-band (Fig. 82b) further confirmed the reduction in metallic tube content in the sediment sample.

The original SWNT sample displayed a relatively broad G-band which consists of two superimposed peaks at ~1550 and 1587 cm\(^{-1}\). The broadened “G-“ (the low frequency side of the G-band) feature, which is characteristic of metallic tubes, indicated their higher content in the original SWNT sample. The narrower and weaker “G+” (the right side of the G-band) observed from the Sed-8 sample indicates a significantly lower metallic tube content.

Fig. 82. Raman RBM (a) and G-bands (b), measured for raw SWCNTs and sediment samples with 647 nm laser.

The Raman D-bands at 1350 cm\(^{-1}\), which are defect sensitive for carbon nanotubes, were nearly identical for the original and Sed-10 samples (Fig. 82b). Therefore, no detectable defects were introduced into the nanotubes during the repeated separation procedures.
Examination of a series of suspension samples revealed that the selectivity in wrapping SWNTs by PmPV was dependent not only on the type of tubes, but also on the individual tube chirality indices \((n,m)\). The Raman spectra for the series \((\text{Sus}-n)\) were normalized against the solvent peak for comparison, since the concentration of PmPV solution remained constant. As shown in Fig. 83a, the relative intensity of the first RBM peak at around 198 cm\(^{-1}\), which corresponds to the population of metallic \((12,6)\) nanotubes, decreased significantly (24.1\%) from Sus-1 to Sus-7 (normalized to the total peak area). A plot of the RBM peak area over the entire Sus-series (1 through 7) revealed a linear correlation (Fig. 83b). In contrast, the relative intensities of other RBM peaks, which are attributed to semiconducting nanotubes, increased slightly but linearly over the Sus-series. As illustrated in Scheme 3, the nanotubes present in the suspension sample Sus-\(n\) should be equal to that in the preceding sediment sample Sed-(\(n-1\)), since all the tubes in the sediment were well dispersed into the suspension.

Fig. 83. Raman RBM of Sus-1 and Sus-7 (a) and the variation of normalized area of each Raman peak in consecutive suspension samples (b).
In other words, the observed enrichment of semiconducting tubes in the suspension was equivalent to the enrichment in the sediment, which agreed well with the study of dry samples (Fig. 82). The areas of the (8,8) tube RBM peaks were not used in the calculation, as the amount of the (8,8) tube was highly dependent on other factors, such as destruction by sonication. The results from the suspension study, therefore, further confirmed the conclusion that metallic tubes were enriched in the supernatant, while semiconducting ones were enriched in the sediment. The assumption of good dispersion was confirmed by examining the Raman G-band (1500-1600 cm\(^{-1}\)). The heights of the Raman “G–” peak (1520-1560 cm\(^{-1}\)), which corresponds to bundled metallic nanotubes, were indistinguishable for all the Sus samples (Fig. 83a), indicating the absence of the nanotubes bundling. All the samples exhibited the same feature that the very weak G- peak was nearly buried in the baseline of the G+ peak (at \(\sim 1587\) cm\(^{-1}\)) of the semiconducting nanotubes.

6.2.2. Purification of different CNT types by a combination of nitric acid treatment, prolonged sonication and polymer wrapping

According to the Raman measurements with two lasers, the biggest nanotubes were around 1.27 nm in diameter. This result has been confirmed with the 2D fluorescence and absorption data. The Raman instrument allowed acquisition of signal down to 100 cm\(^{-1}\) with high accuracy. If equation (6.1) for Raman data is used, the maximum diameter of nanotubes that could be present in the sample would be about 2.34 nm. Based on literature results from similar initial samples produced in Rice University
(e.g. [199, 211]), there should be no nanotubes above 2 nm and the average diameter would be around 1 nm. In this section, we have studied how a combination of several methods, namely nitric acid treatment, sonication and polymer wrapping, could provide a simple and convenient method for SWCNT purification to achieve a sample with a very narrow size distribution of metallic tubes.

In Fig. 75 the breakage of semiconducting nanotubes by concentrated nitric acid has been shown, which resulted in larger tubes preferentially enriched. The trend is obvious, however a noticeable amount of smaller tubes is still present in the sample. After the nitric acid treatment, a different polymer has been used – poly[[(p-phenylene-ethynylene)-alt-poly(p-phenylene-vinylene)] or PPE-PPV, which was expected to be more effective than PMPV. The incorporation of triple bonds into the PPV backbone increases the oxidation and the reduction potentials, thus making this polymer a potentially good electron transporting material [241].

After wrapping of some amount of acid-purified SWCNTs with PPE-PPV, the sample was left to precipitate and the solvent (THF) was allowed to completely evaporate in a vacuum hood without any further purification. The solid sample was not homogeneous and the ratio of (12,6) and (8,8) peaks was quite different for nearly every spot probed.

A sequence of spots has been analyzed by Raman by focusing the beam deeper into the solid sample for each spot (Fig. 84). From Fig. 84a it is seen that the magnitudes of the peak shifts are the same for the (12,6) and (8,8) tubes regardless of the spot chosen. Fig. 84b shows that the amount of (12,6) tube increases with depth, as denoted in terms of "steps". One step is equivalent to roughly a change of several hundred nanometers in
the depth of probing. At the same time, this increase correlates with the increase in the absolute value of the peak shift or, in other words, the degree of CNT-polymer interaction. Based on these data we can state that the polymer interacts more strongly with (12,6) tubes.

Fig. 84. Peak positions (a) and peak ratio (b) for the different depths in the sample. The CNT composition is seen to vary with depth.

Using this knowledge, a sample of acid-treated HiPCO SWCNTs was purified similarly to the sample in the previous section, i.e. through several steps of sonication and redispergation in THF-polymer solution. The Raman spectrum from the final Sup-3 sample is compared with that of the initial sample in Fig. 85.

The purified sample contained a very narrow distribution of metallic nanotubes with the majority of the diameters ranging from 1.27 to 1.133 nm, based on the Raman data with two different lasers. However, comprehensive 2D Raman scanning over multiple wavelengths would give a clearer picture of the distribution. The nanotubes, purified in the way outlined above, could be potentially very effective for such applications as filters. Recent works [242] have demonstrated that CNT filters can
provide a valuable tool for fast separation of different materials based on nanotube

diameter rather than on tube end material recognition or interactions with the outer surface. On the other hand, quantifying transport through an individual tube in a MWNT membrane is difficult, because MWNTs are prone to blockages, in particular by “bamboo” structures and catalyst particles that can migrate to and obstruct the nanotube interior [243-245]. Thus, single-walled metallic nanotubes with a narrow distribution of diameters can prove a very valuable asset for this application.

The experiments performed on single-walled carbon nanotubes undergoing different interactions have shown that reliable data acquisition is quite complicated. It was found that changes occur to the position, intensity and shape of the peak in Raman, fluorescence and optical absorption depending on the interaction. The metallic nanotube content was shown to decrease with sonication and laser illumination. A sequence of polymer wrapping experiments produced sediments with an increasingly higher content
of semiconducting nanotubes. Finally, it has been shown that a sequence of nitric acid treatment, prolonged sonication and polymer wrapping has resulted in a sample of metallic nanotubes with a narrow distribution of diameters. The results lead to the conclusion that the creation of effective SWNT procedures is possible with the aid of two-dimensional Raman and fluorescence measurements.
CHAPTER VIII

SUMMARY

In this Dissertation, Raman spectroscopy, as well as other supporting spectroscopy techniques, has been employed as a powerful research tool for the characterization of properties of different nanoscale materials. In the fourth Chapter, several important features, characteristic for SERS substrates, have been identified: high enhancement with even moderate laser powers often results in degradation both of the polymer layer and the metalized substrate. Also, high enhancement might facilitate adsorption of chemical species onto the metal particles, drastically decreasing their effectiveness. This can be overcome by the introduction of a “protection” layer which decreases the extent of oxidation and sulfidation of silver substrates thus significantly increasing their lifetime. Experiment has shown an exponential decay in enhancement with increase in separation of metal and polymer analyte layers which limits the range of thicknesses viable for such protection layers. We identified the factors increasing or diminishing the enhancement which is crucial for the creation of more effective SERS substrates. We have observed highly crystalline particles with sharp features or rough surfaces to give highest enhancement. In this Dissertation, we suggest that aggregates of small faceted particles, generally triangles, are responsible for the reproducible formation of “hot spots” in SERS substrates based on the obtained images of the actual
morphologies of these spots. On the other hand, single particles and rod aggregates were shown to produce no blinking.

In Chapter 5, we discuss several promising ways of creating TERS tips and characterize the properties of some of them. The effect of signal reflection from the tip side has been identified and quantified to a certain degree. It was also shown that metalized tips, especially those coated with silver, are easily degraded through chemical and mechanical interactions with the environment and the analyte. Coatings of silicon oxide and aluminum oxide have been evaluated as potential candidates for TERS tips protection. Both were found to be able to improve tip longevity, although the improvement in the case of silicon oxide was only moderate. Tips with alumina coatings were capable of extending the lifetime of TERS tips for more than a month when stored in dry or humid conditions. Chemical and mechanical degradation of tips has been discussed. Additionally, the experimental studies presented demonstrate significant tip-induced heating of the samples with optical absorption. The heating depends strongly on the optical properties of the tips and is minimal for laser wavelengths outside of the tip’s plasmon resonance. The magnitude of heating can easily reach 100K, even at laser power as low as 1 mW and modest tip enhancement. Knowledge of the tip-induced heating is very important for measurements of samples which have a relatively low glass transition temperature or can undergo some changes when heated. It is even more important for the case of biological samples, like proteins, as they can denature and be rendered useless. The experiment and simulation results are in good agreement with those in the literature.

Extensive studies of known CNT separation procedures have shown that very little is known about the real mechanisms of separations. Destruction of CNTs has been
found to be present on most steps of their separation and even characterization. Our experiments have revealed that the positions, intensity and shapes of the peaks in Raman, fluorescence and optical absorption change significantly when CNTs interact with the solvent, polymer or both. In particular, different solvents and polymers can cause different red-shifting of the RBM peaks which was quantified. These peak shifts can influence the conclusions derived from the separation results and thus, special attention should be given in such instances. Such changes, however, in the future will allow multiple observations to be made from a single Raman or fluorescence spectrum. The metallic nanotube content was shown to decrease with sonication and laser illumination. A sequence of polymer wrapping experiments produced sediments with an increasingly higher content of semiconducting nanotubes. As a final result of Chapter 6, it has been shown that a sequence of nitric acid treatment, prolonged sonication and polymer wrapping has resulted in a sample of metallic nanotubes with a narrow distribution of diameters.
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