Analytical chemistry on the nanometer scale
tip-enhanced Raman spectroscopy in the top-illumination
top-geometry

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Analytical Chemistry on the Nanometer Scale:  
Tip-Enhanced Raman Spectroscopy  
in the Top-Illumination Geometry

A dissertation submitted to

ETH ZURICH

for the degree of  
Doctor of Sciences

presented by

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accepted on the recommendation of

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Prof. Dr. Andreas Stemmer

2012
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## Abbreviations

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<td>µCP</td>
<td>microcontact printing</td>
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<tr>
<td>2-PySH</td>
<td>2-mercaptopyridine</td>
</tr>
<tr>
<td>4-PySH</td>
<td>4-mercaptopyridine</td>
</tr>
<tr>
<td>AFM</td>
<td>atomic force microscopy</td>
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<tr>
<td>APD</td>
<td>avalanche photo diode</td>
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<tr>
<td>BCB</td>
<td>brilliant cresyl blue</td>
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<tr>
<td>CCD</td>
<td>charge-coupled device</td>
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<tr>
<td>CVD</td>
<td>chemical vapor deposition</td>
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<tr>
<td>DPSS</td>
<td>diode-pumped solid-state</td>
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<tr>
<td>EDX</td>
<td>energy-dispersive X-ray spectroscopy</td>
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<tr>
<td>EF</td>
<td>enhancement factor</td>
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<tr>
<td>EM</td>
<td>electromagnetic field</td>
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<tr>
<td>EMCCD</td>
<td>electron-multiplying charge-coupled device</td>
</tr>
<tr>
<td>EtOH</td>
<td>ethanol</td>
</tr>
<tr>
<td>FAB</td>
<td>fast atom bombardment</td>
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<tr>
<td>FDTD</td>
<td>finite difference time domain</td>
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<td>FE</td>
<td>finite elements</td>
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<tr>
<td>FIB</td>
<td>focused ion beam</td>
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<tr>
<td>FWHM</td>
<td>full width at half maximum</td>
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<tr>
<td>HOPG</td>
<td>highly oriented pyrolytic graphite</td>
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<tr>
<td>IR</td>
<td>infrared</td>
</tr>
<tr>
<td>ITO</td>
<td>indium tin oxide</td>
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<tr>
<td>MeOH</td>
<td>methanol</td>
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<tr>
<td>Abbr</td>
<td>Term</td>
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<td>------</td>
<td>-------------------------------</td>
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<tr>
<td>NA</td>
<td>numerical aperture</td>
</tr>
<tr>
<td>NB</td>
<td>Nile blue</td>
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<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
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<tr>
<td>PALM</td>
<td>photoactivated localization microscopy</td>
</tr>
<tr>
<td>PDMS</td>
<td>poly(dimethylsiloxane)</td>
</tr>
<tr>
<td>PM</td>
<td>parabolic mirror</td>
</tr>
<tr>
<td>PML</td>
<td>perfectly matched layer</td>
</tr>
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<td>PMMA</td>
<td>poly(methyl methacrylate)</td>
</tr>
<tr>
<td>PMT</td>
<td>photomultiplier tube</td>
</tr>
<tr>
<td>PVA</td>
<td>polyvinyl acetate</td>
</tr>
<tr>
<td>PVD</td>
<td>physical vapor deposition</td>
</tr>
<tr>
<td>QCM</td>
<td>quartz crystal microbalance</td>
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<tr>
<td>RS</td>
<td>Raman spectroscopy</td>
</tr>
<tr>
<td>SAM</td>
<td>self-assembled monolayer</td>
</tr>
<tr>
<td>SEM</td>
<td>scanning electron microscopy</td>
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<td>SERS</td>
<td>surface-enhanced Raman spectroscopy</td>
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<td>SLG</td>
<td>single layer graphene</td>
</tr>
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<td>SNOM</td>
<td>scanning near-field optical microscopy</td>
</tr>
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<td>SPM</td>
<td>scanning probe microscopy</td>
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<tr>
<td>STM</td>
<td>scanning tunneling microscopy</td>
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<tr>
<td>STORM</td>
<td>stochastic optical reconstruction microscopy</td>
</tr>
<tr>
<td>TEM</td>
<td>transmission electron microscopy</td>
</tr>
<tr>
<td>TERS</td>
<td>tip-enhanced Raman spectroscopy</td>
</tr>
<tr>
<td>TTF</td>
<td>tetrathiafulvalene</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
<tr>
<td>VIS</td>
<td>visible</td>
</tr>
<tr>
<td>XPS</td>
<td>X-ray photoelectron spectroscopy</td>
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Abstract

Advances in nanoscale fabrication using top down techniques such as 28 nm lithographic processes in semiconductor industries or bottom up approaches using molecular self-assembly techniques, lately increased the needs but also the requirements for true nanoscale analytical techniques. Pure structural information is not sufficient anymore, but chemical information on the nanometer scale is required, not only by biologists and life scientists, but also chemists, physicists, engineers and material scientists, for example, to optimize solar cell nanostructures or to determine interactions on the nanometer scale between different polymer layers.

Tip-enhanced Raman spectroscopy (TERS) is a combination of Raman spectroscopy and a scanning probe microscopy (SPM) to simultaneously obtain both, high resolution topographic and chemical information within a single experiment. Here, a novel optical configuration is used (top-illumination and top-collection with high numerical aperture), which combines the advantage of a more efficient illumination and collection than side-illumination TERS with the ability to work on opaque samples as required for most cases outside biology.

The initial starting point of this thesis was the development and application of a new optical geometry for TERS with illumination and detection perpendicular to the surface from above - top-illumination gap-mode STM TERS. Proof-of-concept experiments, as well as first imaging results were achieved with electrochemically etched silver scanning tunneling microscopy (STM) tips on ultra-flat template stripped Au surfaces and spin coated dye films as sample molecules. The strongly confined and enhanced electromagnetic (EM) field between tip and sample is responsible for an increased Raman scattering intensity in gap-mode TERS, as well as the superior resolution. The increase in Raman scattering intensity allows either short acquisition times per spectrum in the 50 ms range or the detection of very few molecules, even from weak Raman scatterers.

The influence on the intensity of the EM field from the standard Au substrate, as well as unusual metal and non-metal substrates was studied both experimentally and theoretically yielding a wide range of usable metal substrates with reasonable field-enhancement. Using top-illumination gap-mode STM TERS, a resolution of \(< 12 \text{ nm}\) was determined in imaging experiments. The technique allowed detection of a few single molecules for resonant dye molecules. The detection of non-resonant
monolayer structures was shown on self-assembled monolayers (SAM) of thiols on Au. From the spectral information, unambiguous identification of the thiol SAM and even differentiation of two isomeric thiol SAMs was possible. Imaging experiments allowed to draw conclusions on density and purity of the SAM. Single layer graphene (SLG) transferred onto Au for investigation and also SLG straight from the production process, still on a Cu surface, were examined. The ability to identify the number of layers, the purity and the distribution of defects with great sensitivity was shown on the nanometer scale. Even signals suggesting the presence of a H-terminated graphene or a hydrocarbon contamination, previously not reported, were found. Most likely previous methods lacked the sensitivity to detect these contaminations. Amongst others, this work shows that TERS is already well suited to obtain scientific information from the nanometer scale, but some limiting technical factors, such as a lack of reproducible, and stable tips, missing databases for interpretation of collected signals and difficulties of signal attribution to sample, background or contamination, still need additional attention from the TERS community.
Zusammenfassung


der STM-Metallspitze und der metallischen Probe ist sowohl für die Signalverstärkung in TERS als auch die gesteigerte Auflösung der Technik verantwortlich. Die Steigerung der Signalintensität um mehrere Größenordnungen erlaubt entweder die Reduktion der Integrationszeit pro Spektrum bis in den Mikrosekundenbereich, oder die Detektion von sehr schwachen Ramanstreuen mit längeren Integrationszeiten. Der Einfluss des standardmäßig eingesetzten Goldsubstrates, sowie der Einfluss von anderen, eher unüblichen Metallsubstraten, auf das elektromagnetische Feld in der Lücke (gap) zwischen Spitze und Substrat wurde theoretisch und experimentell für eine große Bandbreite an Metallen, aber auch für einige Nichtmetalle untersucht.


1. Introduction

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1.1. Motivation

Continuous technical developments used in research as well as in industry, lead to the production of smaller and smaller structures; micro fabrication is nowadays slowly replaced by nanoscale fabrication. To create these structures, both, bottom up approaches like molecular self assembly or laser induced molecular deposition are employed as well as top down approaches such as reactive ion etching, laser induced patterning, focused ion beam (FIB), fast atom bombardment (FAB) or lithography processes with deep UV lasers.

A major problem for production and quality control is that only a small number of analytical techniques are able to sufficiently resolve objects or structures in this size regime. Most high-resolution surface analysis techniques are either electron microscopy based vacuum techniques like scanning electron microscopy (SEM) or transmission electron microscopy (TEM) or scanning probe methods (SPM) such as atomic force microscopy (AFM) or scanning tunneling microscopy (STM) delivering high resolution images, but no information on the chemical identity or the present state that the surface is in. Nevertheless, this chemical information is crucial for a lot of applications. Stressed silicon in a microchip can for example be correlated with the reliability of computer chips, the efficiency of solar cells is determined not only by the layering of different materials but also by their crystalline phases. Detection, and identification of functional groups on patterned monolayers is a prerequisite if a biochip production on this base should be considered. A further example is catalysis: only if the chemistry at the nanoscale can be investigated and understood, rational improvement and design of catalytically active sites will be possible. Answering any of these questions does not only require high resolution, but also chemical identification on the nanometer scale.

Label based high resolution optical methods, such as photoactivated localiza-
1. Introduction

Pulse activation microscopy (PALM), stimulated emission depletion microscopy (STED) and stochastic optical reconstruction microscopy (STORM) deliver a limited amount of chemical information and promise high resolution and strong signals \(^{[1]}\), but need to introduce labels for detection. The introduction of a fluorescence label can influence the formation of structures or the behavior of molecules on the surface and of course only labeled molecules can be detected. Furthermore, any unknown molecule on the surface will stay invisible to these techniques.

This shows that there still is a strong need for a label-free method, which can deliver high resolution chemical information at ambient pressure, ideally for both inorganic and organic materials. Tip-enhanced Raman spectroscopy (TERS) is an emerging further development of Raman spectroscopy and Raman imaging. Raman spectroscopy offers label-free chemical fingerprint information but is intrinsically limited in spatial resolution by the focusing properties of light and intensity from the intrinsically low Raman crosssections. The additional tip enhancement can resolve both these shortcomings by increasing lateral resolution and Raman intensity. On the one hand, this allows for faster spectral acquisition and thus imaging at reasonable frame times, on the other hand, very sensitive detection of few molecules down to the single molecule level is possible. This shows that there still is a strong need for a label-free method, which can deliver high resolution chemical information at ambient pressure, ideally for both inorganic and organic materials. Tip-enhanced Raman spectroscopy (TERS) is an emerging further development of Raman spectroscopy and Raman imaging. Raman spectroscopy offers label-free chemical fingerprint information but is intrinsically limited in spatial resolution by the focusing properties of light and intensity from the intrinsically low Raman crosssections. The additional tip enhancement can resolve both these shortcomings by increasing lateral resolution and Raman intensity. On the one hand, this allows for faster spectral acquisition and thus imaging at reasonable frame times, on the other hand, very sensitive detection of few molecules down to the single molecule level is possible.

Until now, two TERS geometries are commonly used: bottom-illumination TERS combines the advantages of high numerical aperture (NA) optics, tight focusing and very good collection efficiency, but needs transparent samples, and is thus often employed for biological samples. Side-illumination allows measurements without sample restrictions, except for the distance control limiting the sample roughness that can be tolerated. The drawbacks of side-illumination are a larger confocal illumination area and lower EM field density. A reduced collection efficiency due to the lower NA and excitation can be compensated by additional enhancement, in case a metal is used as tip and substrate, due to the so-called 'gap-mode' effect. As the gap-mode effect is limited to metals and strongly distance dependent, the choice...
of substrates is limited as well as the maximum sample thickness. A third, working TERS geometry with top illumination is based on a parabolic mirror as focusing element, reaching close to ideal focusing in air, yet as already very slight alignment errors result in loss of the entire signal, these systems are quite care intensive and need regular, sophisticated maintenance.

The main goal during this thesis was to create a better and more efficient system for experiments on opaque samples: top-illumination TERS. Microscope objective based top-illumination TERS combines compatibility with opaque samples and reasonably high NA for better excitation and collection from top instead of the side. Both, excitation and detection pathways are realized perpendicular to the sample and are combined with a STM distance control. The resulting gap between the metal substrate and the metal tip localizes and enhances the electromagnetic field as explained in detail in section 2.8. The STM distance control allows for simultaneous high resolution imaging (in principle down to atomic resolution) that is limited by tip size, sample roughness and instrument stability.

With the presented instrument, resolutions down to 12 nm have been achieved as well as TERS enhancements factors in the order of $10^7$. Reproducible full spectral imaging with fast frame times of roughly ten minutes for 64x64 pixels were acquired, but also high resolution images of 200x200 pixels were obtained (with correspondingly longer frame durations). With this tool at hand, self assembled monolayers of non-resonant thiols on metal surfaces (mimicking functionalized biochip surfaces) have successfully been identified and spatially localized. Precise localization and size determination of defects within graphene was a second important example, showing the broad potential application range for TERS in nanoscale sciences as well as nanoscale production.

### 1.2. General Introduction

The lateral resolution of an optical microscope is limited due to optical diffraction. According to the Rayleigh criterion, i.e., the smallest distance between two discernible microscopic objects, is given by eq. 1.1$^{[2,3]}$.

$$\Delta x = 0.61\lambda/NA$$

(1.1)

The lateral resolution $\Delta x$ depends on the wavelength $\lambda$ of the light employed for excitation and the numerical aperture NA of the objective lens. The simplified expression of $\Delta x \approx \lambda/2$ usually gives a good estimation for the resolution that can be theoretically achieved, resulting in $200 - 400 \text{nm}$ as the highest resolutions of microscopes working in the range of visible light.
In 1928 Synge\cite{4} proposed the idea to employ a small aperture for imaging surfaces with sub-wavelength resolution using visible light. An opaque metal screen with an aperture smaller than the wavelength of light (e.g., 100 nm) is illuminated from the back and scanned over the sample surface (see Figure 1.1(a)). If the distance between aperture and surface is within the near-field regime (a few nanometers), the resolution of such a microscope is not limited by optical diffraction but rather defined by the size of the aperture. First experimental evidence for this idea was achieved with 3 cm microwaves in 1972\cite{5}, and it took 12 more years until the realization of scanning near-field microscopes (SNOM) for the visible optical range became possible\cite{6,7}. In illumination mode SNOM, light is coupled into a fiber, which ends in a sharp tip that is scanned in close distance over the sample surface, most often using shear-force feedback (see Figure 1.1b)\cite{8}. Light can leave the SNOM probe only through a small aperture at the tip end, whose dimensions define the spatial resolution.

In the first years after its invention, SNOM was mainly applied to luminescence...
and fluorescence experiments, reaching lateral resolutions of a few tens of nanometers\cite{8}. The exceedingly low Raman cross-sections of many samples as well as the small light transmission and low damage thresholds of optical fiber probes hampered the development of SNOM-Raman for about 10 years. Tsai et al. demonstrated the collection of near-field Raman spectra for the first time in 1994\cite{10}, and in the following years mainly the groups of Batchelder\cite{11,12} and Hallen\cite{13,14} performed pioneering work in SNOM-Raman imaging. Later on, improvements in fiber probe fabrication and studies on combinations of SNOM-Raman with surface-enhanced Raman scattering (SERS) and resonance Raman scattering allowed applications of these techniques in chemistry and biology, but still the low throughput of fiber probes hampered the use of aperture-based near-field Raman imaging in many fields of application\cite{15,16,17}. Limitations caused by aperture fiber probes can be overcome by using apertureless near-field methods. After the invention of the STM in 1982\cite{18}, Wessel proposed the combination of surface-enhanced spectroscopy (Raman or second-harmonics generation) with STM in 1985\cite{19} (compare Fig. 1.2). In contrast to SERS, where the sample is coated onto a rough metal surface that provides enhancement of the electromagnetic (EM) field and of the Raman signals, in TERS the enhancing metal structure is brought into close contact with the sample from outside by a sharp full-metal or metal-coated tip. Surface plasmons excited at the apex of the tip act as an apertureless optical probe. When the tip is scanned over the surface, Raman microscopy below the diffraction limit becomes possible.

Interestingly, Synge had a very similar idea already in 1928. He proposed to use the light scattered by a small particle that is illuminated under total internal reflection from the back and scanned in close distance over the sample as excitation source for microscopy. Before publishing, Synge sent his ideas to Einstein, who in a short reply told him that the basic concept seems to be reasonable but the method Synge proposes for its implementation, Einstein found 'fundamentally unusable' ('prinzipiell unbrauchbar')\cite{20}. In a second letter, Synge came up with his original idea of using a small aperture in a metal screen or at the end of a quartz tip (that is very similar to aperture SNOM probes used nowadays) as a light source, and Einstein encouraged him to publish his ideas in a scientific journal, which ended up in Synge’s publication mentioned above\cite{4}. Wessel’s idea was to resonantly enhance the particle and to generate a locally enhanced EM field, an effect known from SERS. The particle that is for example mounted at the end of a tip is scanned over the sample surface and allows collecting images with nanometer-scale resolution. Wessel did not know about Synge’s idea, but was rather inspired by the invention of the STM and discovery of SERS. In 1989 Fischer and Pohl demonstrated a first proof of principle of this idea by imaging a metal film with 320 nm holes at a resolution of 50 nm using a laser-illuminated gold-coated polystyrene nanoparticle as
1. Introduction

Apertureless SNOM probe\cite{21}. It took 10 more years until the first spectroscopic applications of this idea were published. In 1999, Sanchez, Novotny, and Xie published experimental results of tip-enhanced two-photon fluorescence measurements\cite{22} and in 2000, the groups of Zenobi\cite{23}, Anderson\cite{24}, and Kawata\cite{25} independent have shown tip-enhanced Raman scattering for the first time.

1.3. Outline of This Work

The results of this PhD thesis are arranged in the following way:

The theory section is intended to introduce the physical basis for the conducted experiments and explain the working principle of TERS. The different necessary components for TERS as well as their available combinations to achieve TERS and their (dis-) advantages are briefly explained. This is followed by a short Materials and Methods section with the most important protocols for production of tips, substrates and samples.

Confocal Raman investigations in combination with different SPM or TEM/SEM methods are used in chapter 4 to characterize samples from several scientific fields. All these examples would benefit furthermore from a suitable TERS geometry that does not need conductive surfaces (e.g., top-illumination AFM-TERS), so they could be investigated in more detail or with better signal-to-noise ratios in the future. Chapter 5 describes and explains the setup of a new optical illumination configuration for TERS with top illumination and detection in detail, highlighting the initial expectations, as well as the road of development towards the now reliably working top-illumination gap-mode STM-TERS. A detailed system characterization and first high-resolution full spectral imaging is featured in chapter 6, as well as the determination of the resolution and the enhancement reached with a top-illumination gap-mode STM-TERS instrument. An investigation of the EM field in the gap between tip and sample in chapter 7 deals with the influence of the metal substrate onto the enhancement in the previously described top-illumination gap-mode TERS configuration. The gap-mode behavior of a resonant dye between a silver tip and different metal substrates is investigated experimentally. Complementary data from finite difference time domain (FDTD) and finite element (FE) simulations are used to better understand the influence of the metal film on the EM field in the gap. In chapter 8 further experiments on thiol-coated gold films demonstrate long-range imaging capabilities on the micrometer scale as well as the high sensitivity. The strong enhancement in TERS allows the detection and imaging of patterned self-assembled monolayers from non-resonant molecules, created by micro-contact printing. Samples of graphene on Cu and Au substrates were investigated in chapter 9. A strong emphasis is put on the detection of very small
defects and surface contaminations which can strongly influence the electronic properties and are thus crucial when considering graphene for applications in molecular electronics. Graphene is only one well known example of a highly researched new substrate, that until now can hardly be efficiently characterized on the nanometer scale by available analytical tools. As our investigations show, this gap can potentially be closed by tip-enhanced Raman spectroscopy. Finally a short summary and outlook are given to conclude this thesis.
2. Theoretical Background and State of the Art

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2.1. Confocal Microscopy

In this short section, a few basics, necessary to understand and calculate electromagnetic fields in the optical near field are briefly explained. For a more thorough background, full theoretical introductions can be found in several textbooks covering the topic in detail[26-29]. Laser beams used in our experiments (Helium-Neon lasers and frequency doubled Neodym-YAG solid state lasers) can be approximated by Gaussian modes and described by eq. 2.1. When focusing a propagating Gaussian laser beam, the radius of the beam waist is always above the diffraction limited \( w(0) \) in eq. 2.2 with the divergence angle \( \Theta_{div} = D_m/f \) (beam diameter \( D_m \) and focal length \( f \)). This focused beam will then form an Airy disk pattern in the focal spot as visualized in Fig. 2.1.

\[
E(r, z) = E_0 \frac{w_0}{w(z)} \exp \left( -\frac{r^2}{w^2(z)} \right) \exp \left( -ikz - ik \frac{r^2}{2R(z)} + i\zeta(z) \right) \quad (2.1)
\]

\[
w(0) = \frac{4\lambda}{\pi\Theta_{div}} \quad (2.2)
\]

If an object of interest is observed or excited using a microscope objective and it is located within the focused electromagnetic field of the laser, an interaction with the photons occurs. Yet, two close-by objects can only be discerned if the distance between them is sufficient. Should they be closer to each other, the signal from both will overlap and form a combined, broader signal. The Rayleigh criterion \( \sin \alpha = 1.22 \cdot \lambda/D \) defines the angular resolution of a system (i.e., the minimum angle \( \alpha \) that two points or objects need to be separated from each other, in respect to the detector, to be discernible), and can be approximated for a microscope by
the spatial resolution $\Delta l = 1.22 f \lambda / D \approx 1.22 \lambda / 2NA = 0.61 \lambda / NA$. This is the minimum distance of two objects that the microscope can resolve. In other words, two small objects (smaller than the wavelength) can only be distinguished, if their spacing is larger than $\Delta l$, the distance from the central maximum of the point spread function (PSF) of the exciting beam to the first minimum. A commonly used representation of a PSF is the pattern an ideal lens forms when illuminated by point source, is the Airy disk that can be seen in Fig. 2.1. In a diffraction limited optical setup using visible light, spot diameters of less than 200 nm cannot be reached with conventional focusing. The Airy disk of an ideal lens will form a number of higher order maxima, and thus illuminate a large area around the central spot, albeit with weaker intensity. In order to limit the detection of a microscope to just the central maximum, the collection area has to be restricted. A very efficient method to detect only photons from a small central area is confocal microscopy. The principle of confocal microscopy was already patented in 1957 by Minsky as shown in Fig. 2.2. By inserting pinholes in conjugated planes to the sample plane, within the optical pathway of the microscope, and focusing the light with a lens through those pinholes, the light reaching the detector originates from a restricted area, localized in all three dimensions. The illumination region on the sample is defined by the point spread function of the illumination beam, and the constriction in z-direction is realized by placing the detector behind a pinhole in an optically conjugate plane, filtering signals from below or above the sample plane.
2.2. Electromagnetic Field Simulations

In near-field optics, the Maxwell equations (eq. 2.3-2.6) are usually used as a starting point to describe or calculate electromagnetic fields. The different equations describe the divergence of the electric displacement field $\nabla \cdot D$, the magnetic field $\nabla \cdot B$, the electric field $\nabla \cdot E$ and the magnetizing field $\nabla \cdot H$, respectively.

\begin{align*}
\nabla \cdot D &= \rho_f \\
\nabla \cdot B &= 0 \\
\nabla \times E &= -\frac{\partial B}{\partial t} \\
\nabla \times H &= J_f + \frac{\partial D}{\partial t}
\end{align*}

A few deductions can be made from these equations. The electric field and the magnetic field are orthogonal to the propagation direction of the wave as well as to each other. Both electric and magnetic fields are proportionally related in magnitude by \( E_0 = c_0 B_0 \) with \( c_0 = 1/\sqrt{\mu_0 \epsilon_0} \) as can be deduced from the Poynting vector \( S = E \times B \). For more detailed calculations consult for example\cite{30}.

By computationally solving the Maxwell equations, using certain boundary con-
2. Theoretical Background and State of the Art

2.1. Finite Difference Time Domain (FDTD) Calculations

To describe a special system, an experiment can be simulated in form of a finite difference time domain (FDTD) calculation. In FDTD calculations, the Maxwell equations are solved to compute the electrical and magnetic fields alternatingly. Using a very short time step with the previous fields as starting values for subsequent calculations, the fields are calculated over a certain period of time (in the fs range). Starting with a field free simulation area, an electromagnetic field representing a light beam (usually a few fs long pulse) is introduced and the propagation of the beam as well as the intensity of the EM field is observed over time. The EM field intensity can then be compared to experiments and potentially be used for prediction of altered experimental parameters, once sufficient confidence in the simulation results is established.

In finite element (FE) simulations, a region of interest is discretized using a large number of tetrahedra (instead of rectangular areas in the case of FDTD). For each tetrahedron, partial differential equations are set up to accommodate the boundary conditions of the system and to match the magnitudes of adjacent tetrahedra. These equations are then solved with large computation effort, to calculate the strength of the electromagnetic field. The use of tetrahedra allows a more flexible setup of the simulation region and more efficient setup of the computational boundary. This reduces the overall computational cost of the simulation and allows more detailed or larger simulations. In these tetrahedral areas, the maxwell equations are solved directly or iteratively depending on the technique in use. Mostly a steady state solutions is acquired showing the intensities in the different areas. By changing the phase of the incoming wave, the movement of light within the system can be mimicked.

2.3. Atomic Force Microscopy

AFM advanced to be one of the most commonly used distance control methods in scanning probe microscopy due to its ease of use, its reliability and its applicability to a wide range of samples. The simple working principle is based on reflecting a laser beam from the top of a tip with a very fine cantilever usually in an angle of 45°. The reflected light is detected by a four quadrant detector allowing precise tracking by simple differential readout of the four quadrants. Upon twisting or bending of the cantilever due to an interaction with a surface (by Van der Waals, capillary, electrostatic, repulsive or magnetic forces), the position of the reflected beam on the detector changes and the intensity on each quadrant is detected by the system. By calculating the differences of the segments, the system can trace the movement of the laser beam and thus relate back to the cantilever movements. The laser deflection, in combination with a control feedback loop can be used to
keep the cantilever at a certain distance to the sample surface. When moved over a surface, a topography map can be created by keeping the tip at a fixed distance to the surface and monitoring the compensation movements necessary to keep a constant distance. Several main operating modes, contact and non-contact or semi-contact mode are currently well established for ordinary measurements. In contact mode, the tip is dragged across the surface with constant interaction with the surface, while in non-contact mode, a periodic oscillation in z-direction is used to probe the surface at every sample spot. Detailed information on the setup and the mentioned measurement modes and more elaborate AFM modes can be found in several textbooks including [31–33]. The main advantage of AFM over SEM is clearly the three dimensional topography information, the operation at ambient conditions or even in water. AFM can compete with SEM in terms of resolution (atomic resolution possible) and speed with up to video rate scanning speeds (for small areas). On the down side, the scan size is limited to the range of the employed piezo scanners, usually in the lateral range of 100 µm (and 10 µm in height). Additionally, thermal sample drift can pose problems especially in slow, high-resolution scans. Artifacts introduced by an irregular tip shape can be manifold and due to the tilted side walls of the tip, steep walls cannot be completely resolved.

2.4. Scanning Tunneling Microscopy

In STM, the same concept of a constant tip-sample distance is realized by a different interaction with the sample. When applying a bias voltage between a sharp tip and the sample, electrons can tunnel through the gap, once the distance is sufficiently small. The tunneling probability is strongly distance dependent, and decreases exponentially with increasing tip-sample separation. Thus the distance control is very sensitive. Tunneling over distances of up to $3 - 6 \text{ nm}$ has been shown in the literature. In standard measurements, a tunneling distance of $< 1 \text{ nm}$ can be estimated. Yet, there is no physical contact between tip and sample during the entire measurement. As the tunneling effect only occurs for the closest atoms to the sample, a smooth shape of the tip apex and the sharpness of the tip is not very important to keep a constant distance. Yet, for imaging, the macroscopic shape of the tip is important to prevent artifacts, as already mentioned for AFM. Both, the lateral as well as z-resolution of the STM is very good and atomic resolution can be achieved without major requirements on very flat surfaces. The drawbacks of the technique are a low tolerance to surface roughness and the absolute need for conductivity of the sample. In few cases, non-conductive samples can be coated by a conductive layer, but the coating step usually masks fine structures on the surface. Detailed information about tip production and STM in general can be
2. Theoretical Background and State of the Art

found in standard textbooks including\textsuperscript{[34–37]}.

2.5. Raman Spectroscopy

Raman spectroscopy is an optical spectroscopy technique usually used in the visible range between 400 and 800 nm. For efficient excitation of the sample molecules, laser beams are employed. A photon of the laser beam, interacting with a molecule can excite it to a higher energy level. If there is no energy level at a distance which matches the Bohr criterion $\Delta E = h\nu$, from present energy level of the molecule, the photon is re-emitted (scattered) nearly instantly on a time scale of $10^{-14}$ – $10^{-15}$ s. In most cases the same wavelength is re-emitted in what is called a Rayleigh scattering process. When a molecule from a rotationally excited state is further excited by the laser beam and the entire energy emitted upon relaxation, a shorter wavelength photon is emitted in an anti-Stokes Raman process. The spectroscopically more commonly used Stokes Raman signal originates from molecules in the vibrational ground state, conserving a part of the excitation energy and not relaxing back into the ground state but instead staying in a vibrationally excited state. The emitted, red shifted photons, are collected as part of the Stokes Raman spectrum. The information from Raman spectroscopy is thus similar to infrared (IR) spectroscopy a fingerprint of the molecular vibrations. However, in contrast to IR spectroscopy, due to the use of visible light, and due to the fact that water is a very weak Raman scatterer, water is not problematic. The selection rules in Raman spectroscopy are different from IR, as not a change of the dipole moment but a change in the polarizability tensor is a prerequisite for Raman scattering. The Raman bands in the fingerprint spectra are thus at the same position as in IR, but different band intensities are expected. Compared to IR, Raman spectroscopy is a complementary technique, with advantages for biological samples and better spatial resolution due to the shorter wavelengths in use. As well, the region of very small cm$^{-1}$ region is far easier to access in Raman (where only a good laser line filter is necessary) than in IR (to sufficiently excite the far-IR region, very strong coherent light sources are necessary, e.g., synchrotron radiation or free electron lasers). Due to the visible light range, Raman microscopy is far easier to realize than IR microscopy, and higher spatial resolutions can be reached. The main drawbacks of Raman spectroscopy are the low Raman crosssections (i.e., the low probability for a Raman process to occur) resulting in low signal intensity or need for high laser powers or very long collection times. The second limitation, common to all optical techniques, is the limited spatial resolution as the excitation light cannot be arbitrarily confined (compare section 2.1). More detailed explanations can be found in\textsuperscript{[38–40]}.
2.6. Surface-Enhanced Raman Spectroscopy

One way to increase the signal strength in Raman spectroscopy is surface-enhanced Raman spectroscopy (SERS). In this technique, the electromagnetic field of the excitation laser is confined between nanosized metal structures and used for more efficient excitation of the molecules. A simple method to reach a reliable SERS effect is vapor coating of thin silver films ($5 - 6 \text{ nm nominal thickness}$). These films form irregular island structures on surfaces which occasionally result in well-enhancing gaps between two metal particles leading to a large signal increase compared to confocal Raman spectroscopy for up to single molecule sensitivity\[41\]. Other approaches for irregular SERS substrates include the use of Ag nanoparticles, rough electrodes or roughness at edges of coated Ag-films. Although simple to produce, the signal enhancement of these structures is very unpredictable and inhomogeneous. Promising approaches to form regular arrays of well enhancing structures have been presented in literature, but no definite 'gold standard' has emerged yet. Most regular SERS structures still use silver\[42\] or gold\[43,44\] to produce enhancement. Simple coating procedures of surfaces masked by polystyrene spheres\[45\] up to very elaborate ion-etching\[46\] methods are in use to create SERS active patterns. Energy from an exciting laser is absorbed by the SERS structures, guided along the surface or within nanoparticles, e.g., by surface plasmons, leading to stronger EM fields in gaps or at edges similar to the effects observed in section 2.7. More information on the basic SERS principles can be found in\[47,48\], but especially the SERS substrate production still is a very active field of research\[49\] for which additional primary literature should be consulted.

2.7. TERS

Tip-enhanced Raman spectroscopy is a combination of confocal Raman spectroscopy with a metal structure to gather and localize light at the apex, where it is used as a nanometer sized light source. The metal structure is in most cases the tip of an AFM or STM, simultaneously used to confine the laser energy and keep the tip-sample distance distance constant. The arising advantages are a enhanced EM field under the tip (allowing down to single molecule Raman experiments) and the increased spatial resolution reaching 12 nm what can be reached in terms of resolution, intensity, but it is complicated numbers on 12 nm. The main components of a TERS system are described in more detail in section 2.8. Most often continuous wave lasers with wavelengths in the visible range and laser powers at the sample from the microwatt to milliwatt range, focused onto $< 1 - 5 \text{ \mu m}^2$, are employed for excitation. The tip is held in close proximity to the sample by a SPM feedback, such
as STM, AFM, or shear-force feedback. Such systems also allow scanning of the tip and/or sample laterally and thus enable precise positioning of the enhancing tip to selected locations on the sample or TERS imaging. A convenient way to realize such a TERS system is to combine a SPM with a confocal Raman microscope.

TERS signals are generated by sample molecules located within the optical near-field of a strongly confined and enhanced EM field at the apex of a metallic or metal-coated tip that is excited by a focused laser beam (for review articles on TERS, see for example refs. 50–53). Most often Ag or Au are employed as enhancing metals, and tip-sample distances in the range of $< 1 - 10$ nm are controlled by a feedback mechanism. TERS enables the collection of enhanced Raman spectra with the advantage over SERS that the substrate material is not limited to nano-rough metal surfaces or metal nanoparticles but the sample molecules can be at every metallic or non-metallic surface. The tip can be moved laterally relative to the sample surface. This allows bringing the enhancing tip to selected spots on the sample, and finally raster-scanning of the tip or the sample enables TERS imaging with a lateral resolution that is only limited by the size of the confined EM field. Thereby, the EM field is approximated by the size of the tip apex, typically ranging from approx. 10 – 50 nm 54–56. The lightning rod effect, an excitation of localized surface plasmon polaritons in the metal, or antenna resonances as well as combinations of these effects 50 can be used to rationalize the enhancement effect. The lightning rod effect is a purely shape-induced effect. Geometric singularities of an irradiated metallic structure, such as a very sharp tip apex, lead to highly localized surface-charge densities that can be the source of a highly enhanced EM field. The lightning rod effect is almost independent of the excitation wavelength. If the laser wavelength coincides with a localized surface plasmon resonance of the tip, the metal absorbs a part of the laser energy and surface plasmon polaritons are excited. These are collective vibrations of electrons, which create a localized and enhanced evanescent field around the tip apex. The surface plasmon resonance wavelength depends on shape and material properties of the tip. The third enhancement effect is also wavelength-dependent. Antenna resonances are enhanced if the tip dimension is equal to multiples of half the wavelength of radiation. Also in this case, an enhanced and highly localized electromagnetic field is observed at the tip apex. In practice, a combination of these effects occurs and the enhancement factor of a tip depends on material properties of the tip and surrounding medium, tip size and shape, as well as the laser wavelength employed for excitation. The enhanced EM field around the tip apex drops dramatically with increasing distance and extends only 10 – 20 nm in the direction of the sample 57. Thus TERS can be considered as a technique that is sensitive to the surface and a small sub-surface region of a sample. Additional enhancement can be achieved by taking advantage
of the near-field coupling between the tip and a metallic substrate, which is often referred to as 'gap-mode' TERS. Raman signals from molecules located between the dipole induced in the tip and a mirror dipole induced in the substrate are further increased by a factor of up to 100 compared to normal TERS, allowing even for single-molecule detection of resonantly excited dye molecules\cite{58-61}. The enhanced EM field leads to enhanced Raman scattering of the sample, and Raman scattered photons from the evanescent near field are again enhanced by the tip and scattered into the far field where they are collected by conventional optics\cite{50}. The collected light consists of two parts: the enhanced near-field spectrum that has been generated in the small sample region below the tip, and the far-field background that was generated in the whole illuminated sample volume, i.e., in the laser focus. For thin-film samples, opaque materials or weakly Raman scattering molecules, the far-field background is often not an issue, but it can blur TERS images of bulk samples with considerable optical penetration depth or consisting of moderate to strong Raman scatterers. Depending on the properties of the sample, there are different tip-illumination schemes possible: transparent samples allow the excitation of the tip by focusing light through the sample (bottom illumination), whereas in the case of opaque samples side or top-illumination schemes have to be employed. The performance of a TERS setup and tip can be compared based on the value of the contrast and enhancement factor. The following section explains their calculation and comparability.

### 2.7.1. Contrast and Enhancement Factor

The enhancement factor $g$ of the EM field is defined as ratio between EM field strength at the tip apex, $E_{\text{tip}}$, and the EM field strength at the sample surface without a tip in contact, $E_0$\cite{50} (eq. 2.7).

$$g = \frac{E_{\text{tip}}}{E_0}$$

(2.7)

Since light intensity depends on the square of the EM field strength, the enhancement factor of the excitation light intensity at the laser wavelength $\lambda_{\text{laser}}$ is $g_{\lambda_{\text{laser}}}$.$^2$. A TERS experiment relies on the enhancement of both, excitation light (at $\lambda_{\text{laser}}$) and Raman scattered light (at $\lambda_{\text{Raman}}$). Thus, the enhancement factor of TERS signals $EF_{\text{TERS}}$ can be given in eq. 2.8.

$$EF_{\text{TERS}} = g_{\lambda_{\text{laser}}}^2 \cdot g_{\lambda_{\text{Raman}}}^2 \approx g^4$$

(2.8)

The EM field enhancement factor is wavelength-dependent, mainly following the plasmon resonance spectrum of the tip. As long as the Raman shifts are small, we
can assume $\lambda_{\text{laser}} \approx \lambda_{\text{Raman}}$. In such cases, the TERS enhancement factor can be approximated with $g^4$. It should be pointed out that for an exact determination of the enhancement factor, for example to compare with numerical simulation data, differences between the field enhancements at $\lambda_{\text{laser}}$ and $\lambda_{\text{Raman}}$ should ideally be considered\[^62\]. In order to calculate TERS enhancement factors based on experimental data, two factors have to be determined. First, the contrast between the near-field and far-field signals is derived from enhanced and non-enhanced Raman signal intensities. Second, a geometry factor is calculated taking into account that the sources of far-field and near-field signals are of different size, i.e., a different number of molecules contribute to the two signals. There are two different definitions of the contrast used in the literature:

$$
C = \frac{S_{\text{tip}}}{S_0}
$$

(2.9)

$$
C = \frac{S_{\text{nearfield}}}{S_{\text{farfield}}} = \frac{S_{\text{tip}} - S_0}{S_0} = \frac{S_{\text{tip}}}{S_0} - 1
$$

(2.10)

where $S_0$ is the background signal without the tip present. Definition 2.9 is of more practical relevance, because it is a direct measure for the signal increase by the tip and the image quality that can be obtained in a TERS experiment (used for example in refs.\[^{23,63-66}\]). An example for a spectrum enhanced by TERS and a corresponding confocal Raman spectrum are shown in Fig. 6.4(c). These two spectra combined with the lateral confocal and TERS imaging resolution (for example from Fig. 6.4(a) and Fig. 6.5) are sufficient to calculate the enhancement factor by equations 2.9 and 2.10.

Especially in the case of relatively weak enhancement, the signal $S_{\text{tip}}$ measured with the tip engaged is superimposed on a relatively large non-enhanced background $S_0$ that is also present without a tip in close proximity to the sample. The background signal $S_0$ is generated in the whole sample area or volume that is irradiated by the laser focus, and therefore a strong background signal also blurs the resolution of the obtained image. Definition 2.10 is more precise when enhancement factors are calculated that are compared to numerical simulations or SERS measurements (used for example in refs.\[^{25,57,67-70}\]). This definition gives the ratio of near-field ($S_{\text{nearfield}}$) and far-field signal intensities ($S_{\text{farfield}}$). As can be seen, both definitions differ only by a value of 1. Thus, the difference between definitions 2.9 and 2.10 is only significant in the case of weak enhancement. The contrast can only be calculated when both, enhanced and non-enhanced signals can be determined at the exactly same place on a homogeneous sample. In cases where the far-field signal cannot be determined (e.g., monolayers of weakly scattering molecules) sometimes the upper limit for the signal intensity is calculated based on the noise level, which
we consider only as a very rough estimate which strongly depends on the calculation method for the noise level (e.g., standard deviation vs. peak-to-peak difference of the noise). For calculating the enhancement factor, the difference in source volume of far-field and near-field signals has to be considered\cite{23,25}. In the case of thin-film or opaque samples, the geometry factor can be approximated with the source areas. The far-field signal is generated in the whole laser focus, thus $A_{\text{far field}} = r_{\text{laser}}^2 \cdot \pi$ with $r_{\text{laser}}$ being the radius of the laser focus is most often used to calculate the far-field source area. The area from which the near-field signal arises is more difficult to estimate but is often approximated with $r_{\text{tip}}^2 \cdot \pi$, where the tip radius $r_{\text{tip}}$ can be determined by SEM, even though there is experimental evidence that the enhancing region is, at least in some cases, smaller than the tip apex\cite{57}. A more precise, but tedious way for the determination of the diameters of both areas would be to determine the lateral resolution of both, confocal Raman and TERS images. The TERS enhancement factor can be calculated according to eq. 2.11.

$$\text{EF}_{\text{TERS}} = C \frac{A_{\text{far field}}}{A_{\text{near field}}}$$ (2.11)

In the case of bulky samples with a considerable optical penetration depth, the far-field signal is generated in a volume, and thus the ratio between far-field and near-field volumes has to be calculated. For this, the height of the focus or the optical penetration depth (the smaller value of both has to be considered) as well as the extent of the enhanced field around the tip in $z$ direction have to be estimated. Generally, the calculation of the conversion factor between contrast and $\text{EF}_{\text{TERS}}$ is often based on many estimations, and thus comparisons between different studies and between experiment and theory are difficult. For a better comparability, enhancement factors should only be given together with an explanation of the calculation method and the estimated numbers. We propose to mainly use contrast factors for comparisons between different tips or studies, because they can be experimentally determined and they are of more practical relevance giving direct information about the TERS image quality. Pettinger et al. proposed an alternate way to determine the enhancement factor that has the advantage of being based only on experimentally determined values\cite{54,66}. They studied the temporal behavior of TERS signals of dye molecules and found an exponential decay of the form $e^{(-t/\tau)}$ due to bleaching. The ratio of the time constants $\tau$ of confocal and tip-enhanced bleaching gives the enhancement factor of the excitation light $g_{\text{laser}}^2$ (considered no differences in the lifetime due to heating or two photon-processes are present in either of the two cases). The enhancement of the Raman scattered light cannot be determined with this method, but with the approximation that both enhancements are approximately the same, the TERS enhancement factor can be approximated.
by $g_{\lambda \text{laser}}^4$. A more detailed description of methods for calculating the enhancement factor and their comparison can be found in [71].

2.8. Key Components of a TERS Setup

A combination of small numbers of different components are necessary to build a TERS setup. The first necessity is focused laser light. A part of the light energy has to be localized by a TERS active SPM tip which is in sufficiently close proximity to the sample to excite the sample molecules. The emitted light has then to be collected and analyzed. In this section, the different optical geometries, SPM techniques and according tips with their different combinations are critically evaluated upon advantages and disadvantages and possible areas of application.

2.8.1. Optical TERS Geometries

Challenges posed by a variety of samples can be met by using an appropriate optical geometry. Three different major types, bottom-, side- and top-illumination depicted in Fig. 2.3 have been used until now.

The choice of the illumination scheme determines the properties of the objective lens that can be used [72]. In the case of bottom illumination, very high NAs of up to 1.4-1.6 using oil immersion objectives can be employed, whereas objectives with considerable working distance often have to be used in side or top-illumination schemes, leading to NAs typically ranging from 0.28 to 0.55, with a maximum value of 0.7 that has been used in a top-illumination scheme [73]. As an alternative, parabolic mirrors (PM) as focusing element enable an NA of approx. 1 in top-illumination TERS. Laser wavelength and NA determine the size of the focus spot. A high NA allows focusing the laser more tightly around the tip, which reduces the far-field background contribution. For light collection from the sample, most often the same optics as employed for excitation are used. Here, the NA of the objective lens determines the collection efficiency, which is significantly higher in bottom illumination as compared to side and top-illumination schemes. The additional gap-mode enhancement that can be realized in side- and top-illumination setups outweighs this drawback, and the achievable signal in 'gap-mode' TERS (detailed explanation see section 2.8.3) is at least 5 times higher as in bottom-illumination setups, even when using oil-immersion objective lenses [72].

Bottom Illumination

The earliest versions of TERS in 2000 [23–25] all used a bottom-illumination geometry. In this geometry light is focused from below through a transparent support
2.8. Key Components of a TERS Setup

Figure 2.3.: Illustration of the different possible illumination and detection geometries for tip-enhanced Raman spectroscopy. Standard bottom and side illumination are displayed in the upper part, top illumination with parabolic mirror or lens based focusing in the bottom row. Figure adopted from [9].
onto the tip and the sample. The tip rests on top of the sample and interacts either directly with the laser focus or with its evanescent field. Often glass substrates are used as a sample support, but other sophisticated support layers like gold nano platelets\[74\] can offer special properties. A glass support allows for the use of refractive index matching oil between the substrate and a high numerical aperture (NA) objective with an NA $\geq 1.4$. As discussed, the higher NA allows a tighter focusing, thus more confined laser energy and a smaller illuminated background area. To gather a maximum amount of Raman signal from the sample, the same objective is usually used to collect signals emitted into the glass slide with a high solid angle of slightly above $135^\circ$ at NA 1.4, but a different collection direction would in theory also be possible. As feedback in this configuration, AFM and shear force feedback systems are ideally suited. Optically transparent, conducting substrates like indium tin oxide (ITO) or thin gold films were already used in 2002 to allow STM feedback\[75\]. Due to the large variety and flexibility in AFM instruments and confocal microscopes, bottom-illumination instruments are ideally suited to investigate biological samples. With the exception of a proof of concept\[76\] all experiments have been performed in a dry state, but it is possible to extend research towards biological specimen in native conditions. For these samples, the high NA helps to reduce thermal strain on the sample as well as confocal background signals.

**Side Illumination**

To overcome the limitation to transparent samples of the bottom illumination approach, in 2001 the first TERS instrument using AFM tips under side illumination has been presented\[63\] still with collection from the bottom. Several different approaches using AFM and STM with collection through the side-illumination optics have followed shortly after\[59,66,70,77-79\]. In these setups, the laser beam is focused using long working distance objectives onto the tip end, at angles usually between $45 - 70^\circ$, but partly up to grazing angles of $87^\circ$\[77\] relative to the tip axis. A long working distance is necessary to reach tip and sample, thus the NA of the objectives is in a range between 0.28 and 0.55. Due to the angle between the focal plane and the sample surface the laser focus is non-symmetric and further enlarged to approx. 2 – 3 $\mu$m. To compensate for the increased spot size, the energies used for side illumination often range between 5 and 10 mW. The collection potential of these systems is limited by the small angle of collection of the low NA lenses. The advantage is of course no limitation in the choice of sample support and the ability to work on opaque samples. Despite the low NA, these systems can benefit from the strong enhancement in the gap between two dipoles formed by the metal or metalized TERS tip and a mirror dipole in the metal surface when working on a metal substrate. The additional enhancement is in the order of $10^2$ even
2.8. Key Components of a TERS Setup

overcompensating the losses due to the small NA.

**Top Illumination**

The second option to work on non-transparent samples is an illumination directly perpendicular to the sample surface (epi-illumination), along the direction of the tip. It combines advantages out of side and bottom illumination. The NA is between that of the other two techniques and can be as large as 1, and the focusing is achieved in a symmetric fashion leading to a more predictable and tighter focus than side illumination. Top illumination combines this with a reflecting geometry allowing work on opaque as well as transparent samples. In this orientation, one of the biggest problems is the location of the tip between the objective and the laser focus. Depending on the position and size, the tip will block a certain amount of light during excitation as well as in the signal detection. To minimize this, the numeric aperture of the focusing element is key to see 'around the tip' and also collect the created signal more efficiently. Two different focusing elements have been used for this purpose the parabolic mirror and microscope objectives. As the tip is aligned in propagation direction of the laser, an excitation of the tip is most efficiently achieved by a longitudinal wave\[80,81\]. The use of higher order laser modes such as the so-called 'doughnut modes' has been shown for parts of the epi-top-illumination systems\[61,82\], but would most likely be beneficial for all of them. Top-illumination TERS is ideally suited to investigate opaque samples such as silicon based or crystalline samples as well as thick samples, where the losses in transmission would be too high. Thus these instruments could have increased impact in inorganic chemistry to investigate small crystalline phases at a nanoscale level or in the semiconductor industry for quality control.

**Parabolic mirror based focusing**

The parabolic mirror as a final focusing element has been presented in 2003 for near-field measurements by probing the near-field with a scattering tip,\[83\] and several TERS experiments were shown from 2007 on\[82,84,85\] with resolutions down to 15 nm and few molecule sensitivity\[61\]. For a PM setup, a small hole is drilled in the center of a PM to insert a shear-force tuning fork attached to a piezo tube for distance control and approach to a sample. The sample is located on a scanning stage tripod that can move the sample around the focal point of the PM. Practical issues such as the strong susceptibility to coma aberrations\[86\] pose high constraints on the alignment of the system before reasonable results can be obtained. Already minuscule misalignments of 0.01° are sufficient to render the system merely useless due to coma aberrations\[86\]. Once these requirements are met, purely considering the NA, the PM outperforms any lens based system in an air environment\[87\] with its superior NA of 1. A second issue with the PM is the sample size. As the light
has to pass around the sample to reach the PM, the sample has to be transparent or kept rather small (< 5mm). A production of larger PMs may be possible, but could be difficult due to the high precision necessary to keep the NA close to 1 and would also require similar sized glass lenses to expand the laser beam for illumination. As well, the longer distance to the focal point would require a longer piezo tube to bring the shear-force tuning fork to the focal point, making it more susceptible to external vibrations.

**Objective lens based focusing**
The more standard way of focusing the laser beam in top illumination is a microscope objective. These objectives sacrifice a little of the NA (0.7 compared to 1) in respect to the PM, but they are commercially easily available, and are much easier to handle. The first experiments were all conducted with objectives in the range of NA 0.45\cite{88-90} and mainly showed quite weak enhancements on silicon, most likely due to the major part of light blocked by the cantilever and the strong confocal background. The use of 0.7 NA objectives yielded far higher enhancements > 10^6 in gap mode and resolutions of 15 nm\cite{73} allowing high pixel number TERS imaging. Recently, a proof of concept using AFM feedback with top illumination, also able to perform TERS imaging was presented\cite{91}. Blocking a part of the excitation laser path on the side from which the AFM tip is inserted, an illumination under a small angle is realized to excite the metal coated AFM tip. Maybe due to blocking of the created Raman signal by the bulkier AFM cantilever, the contrast was close to the noise level and considerably lower than results presented on other state of the art instruments. An AFM feedback, once the optical performance can match other instruments, would eliminate the limitation to conductive surfaces, at the cost of the additional gap-mode enhancement.

### 2.8.2. SPM Feedback

The signal enhancement in TERS is strongly confined to the end of the tip. The dimension of the EM field is in the range of several to tens of nanometers. A very fast decay of the signal upon a tip sample distance increase was shown theoretically\cite{92,93} as well as experimentally\cite{58,94,95}. Thus the control of the tip position is crucial for TERS experiments. The precise distance control of SPM methods renders them ideal for this purpose. In TERS three SPM methods, AFM, STM and shear force have mainly been employed, each of them impacting the measurement in different ways.
AFM

As explained in section 2.3, the atomic force microscope relies on either the changed reflection of a laser beam of the cantilever backside due to a tip-sample interaction (contact mode), or a change in resonance frequency of the tip due to the proximity of the sample (semi-contact mode). AFM has no specific sample requirements and can work on virtually any surface with a roughness of up to several microns. In TERS, AFM contact mode has been used very successfully\cite{91,96,97}, with the advantage of a constant distance to the surface and thus a constant enhancement. In semi-contact mode, the tip oscillates and continuously changes the distance to the surface but has less sample interaction and causes less sample strain\cite{98-101}. Depending on the oscillation amplitude, the effective time within the enhancing near-field region is diminished remarkably. One option is to synchronize the laser excitation with the time of the tip in the near-field region\cite{102} with a shutter, but still the amount of signal per time is reduced with this technique. A second option would be a very fast detector, coupled to a lock-in amplifier to only detect during times when tip and surface are in close proximity, or to directly split up the detection in a near-field and a far-field contribution. Very low amplitude tapping (< 4 nm) can be used for TERS increasing the amount of time in near-field region\cite{103}. Due to the lower tip-surface interactions, measurements in semi-contact mode are preferential for delicate samples and likely to prevent pickup processes contaminating the tip. One limitation of both AFM modes is the cross-talk between the feedback laser of the AFM and the Raman excitation laser. Most commonly, 650 nm laser diodes are used for AFM feedback, and only recently 785 nm and 830 nm feedback systems have become available from AFM producers. The laser diodes still limit the usable range of excitation lasers for the Raman to the UV-VIS range.

STM

The second major distance control method in TERS is scanning tunneling microscopy (compare section 2.4) where a conductive tip is kept in tunneling distance to the surface. To enable tunneling, both tip and sample surface need to be sufficiently conductive, thus mostly metals are used as substrates as well as for the tips. The tunneling distance between tip and sample is usually considered to be in the region of a nanometer or less\cite{104}, positioning the sample closer to the EM field than in average AFM distance control. One advantage is good and cheap availability of tips, etched from metal wires for STM. The need for conductivity is most commonly solved by using opaque conductive substrates and thus limiting the remaining optical geometries as well as the choice of substrate.
Shear Force

In shear force distance control a metal tip is attached to a high frequency tuning fork (e.g., quartz crystal, 32.768 kHz, beneficial for the high quality factor) is used to detect the proximity of the surface by damping of the free oscillation, similar to semi-contact mode AFM. The major difference is the oscillation direction of the tuning fork laterally to the sample surface, which keeps the distance more or less constant. Shear force feedback is applicable to virtually any surface with low roughness and does not limit the excitation to the UV-VIS range like AFM. Drawbacks are the cumbersome tip-glueing procedures, a less stable feedback than AFM and the lateral displacement of a tuning fork during the oscillation, which strongly depends on the excitation energy and can be in the range of $5 - 30 \text{ nm}^{[105]}$. The excitation thus has to be carefully controlled to gather high-resolution images.

2.8.3. TERS Tips

The most crucial element to tip-enhanced Raman spectroscopy is the one that distinguishes it from confocal as well as surface-enhanced Raman spectroscopy: the tip. A full metal or metal coated tip is necessary to confine the laser energy into the EM field at its apex, excite the molecule and transform near-field information into a propagating far-field radiation by scattering. The chemical properties of this tip determine the long term stability and the mechanical stability of the tip. The lifetime of a tip is limiting the possible duration of a measurement and the mechanical properties are crucial for wear resistance and scanning quality. The material of the tip together with the shape determines the optimal frequency to excite the tip and thus the suitability of different laser wavelengths. The size of the EM field is usually estimated to be similar to the size of the apex, thus the tip also determines the TERS resolution and usually also relates to the SPM resolution.

AFM TERS Tips

For AFM feedback, different kinds of standard AFM tips are the basis for the TERS tips. Depending on the selected AFM mode (contact/semi-contact mode) silicon based tips with different resonance frequencies in the range of $10 - 400 \text{ kHz}$ are employed. These tips are vapor-coated with a film of a pure metal or a more complicated layer system of auxiliaries and a metal film for the enhancement, usually silver$^{[106-108]}$ or gold$^{[70,109]}$ (see Fig. 2.4(a)). Auxiliary layers can help tune the resonance frequency of the tip to the desired wavelength$^{[55]}$. The choice of metal not only influences the frequency by which it is efficiently excited, but also the chemical stability of the coating layer. According to their physical properties, silver tips should offer higher enhancements than gold tips, due to their lower imaginary
part of the dielectric function over the entire UV-VIS range\textsuperscript{[110]}, at the expense of chemical stability. Silver tips are only active during a limited time frame in the hour range and degrade by oxidation as well as contaminations from the ambient air or the sample\textsuperscript{[111]}. Gold tips have lower signal enhancement, but can be used for prolonged durations up to weeks with reasonable signal enhancement.

**STM TERS Tips**

The tunneling process of STM requires a high conductivity of the tip. As metal coated tips with dielectric cores are not conductive enough, mainly etched full metal tips are produced out of silver\textsuperscript{[58,112,113]} and gold\textsuperscript{[114–117]} (see Fig. 2.4(b)). Depending on the etching parameters (voltage, etchant, temperature,...) different tip shapes can be produced, strongly influencing the mechanical stability and the optical properties of the tip. The usually quick, simple and cheap etching process makes tip production far easier and less cumbersome, and, once a working protocol is established, allows the quick production of a larger numbers of tips to select from upon their TERS capabilities. Metal tips have a high aspect ratio at the tip apex, crucial for the focusing in top and side illumination. Reports show that the crystallinity of the bulk wire used for etching also seems to influence the properties and performance of the etched wire\textsuperscript{[118]}. Another option is the use of preformed nanowires\textsuperscript{[119]}, yet both the involved selection and attachment processes are cumbersome and delicate. In simulations, resonances of TERS tips are often reduced to a contribution of the spherical tip apex, and according to the resonances of gold and silver nanoparticles, gold tips should be more suitable for wavelengths in the red an near IR range.

**Gap-Mode TERS Using STM Tips**

When using STM tips, a further factor to keep in mind is the sample substrate. Metal surfaces have their own plasmon resonances which, due to the small distances of tip and surface, can form a plasmonically coupled system with the TERS tip. This coupled system is usually referred to as gap mode and can change the plasmon resonances in respect to a solitary tip (due to changed energy states\textsuperscript{[120]}). The temporally existent partial charge in the tip apex induces a mirror charge in the metal substrate\textsuperscript{[92]}. This can be considered as a system of very close-by dipoles which are known to strongly localize and enhance an EM field in between the two. The sample itself is located in between these dipoles, making ideal use of the EM field for excitation of the Raman signals.
Shear Force TERS Tips

Etched full metal gold tips, cut to an appropriate length, are commonly glued onto a quartz crystal tuning fork and used in shear force feedback. The additional weight of the etched tip reduces the frequency and the quality factor of the tuning fork and thus has to be kept to a minimum. A second option is to pull or etch glass fibers and coat them with a metal film or embed metal particles in the end of a glass fiber tip.

For AFM-TERS, most often commercially available silicon tips are coated with silver. Silicon tips are cost efficient and widely available in various shapes and with cantilevers covering a wide range of force constants. The surface plasmon resonances of silver nanoparticles have been shown to red shift, when they are attached to materials with high refractive indices\textsuperscript{[121]}. Silicon has a relatively high refractive index \( n = 4.4 \) causing the surface plasmon resonance of the enhancing silver layer to shift to higher wavelengths. This is unfavorable since in AFM-TERS often lasers with low wavelengths in the visible range (blue to green) are employed for excitation and thus, there is often very weak overlap between laser excitation and surface plasmon resonance of AFM-TERS tips. When tip materials with lower refractive index, such as silicon nitride (Si\(_3\)N\(_4\), \( n = 2.05 \)) or silicon dioxide (SiO\(_2\), \( n = 1.5 \)) were used with 488 nm excitation, significantly higher enhancement factors were obtained\textsuperscript{[108]}. The same effect can be obtained by applying a two-layer coating onto commercially available Si or Si\(_3\)N\(_4\) tips\textsuperscript{[55,122]}. Here, a dielectric material with low refractive index, such as SiO\(_x\) (\( n = 1.5 - 2.05 \), depending on \( x \)) or AlF\(_3\) (\( n = 1.4 \)), is coated onto the tip followed by a Ag layer. With such two-layer coatings, contrasts as high as 70 – 80 on brilliant cresyl blue (BCB) thin films as test samples and high yields of enhancing tips were obtained with 488 nm excitation\textsuperscript{[55]}. The stronger enhancement can be explained by a shift of the plasmon resonance frequency towards the laser wavelength, which was confirmed by numerical simulations by a finite element method for silver tips\textsuperscript{[123]} and using a dielectric coating layer\textsuperscript{[124]}. The lifetime of TERS tips, especially when silver-based, is limited due to oxidation and contamination and the accompanied change of the optical and plasmonical properties. SAMs could be used to protect TERS tips from oxygen in the air and additionally prevent other chemical reactions. A thiol SAM was successfully used to protect a cantilever from contamination during measurements in aqueous environments\textsuperscript{[76]}. Increased mechanical stability of metal-coated structures is known from silicon AFM tips, but can be further enhanced using a tungsten core with a metal coating\textsuperscript{[125,126]}. Possibly, other coatings could be used to tune tip properties. These coatings could be applied by means of atomic layer deposition, electrochemical processes, chemical vapor coating or even chemical reactions on the surface as seen by the self-assembly
of thiol monolayers. A way to influence both, enhancement and stability at once would be the use of alloys with special properties instead of standard metals for the enhancing layer. The main difficulty is to not only obtain superior mechanical and chemical properties but simultaneously keep the losses of energy in the desired excitation wavelength at a minimum to allow high enhancement factors.

**Specialized Tips**

Over time a number of non-standard tips have emerged which in part contain considerable efforts for production and make use of small metallic structures on the tip itself to localize and enhance the field at the tip apex. In 2005 Farahani et al. demonstrated a tailored nanostructure to enhance the EM field, forming a bowtie antenna at the end of an AFM cantilever\cite{127} (shown Fig. 2.4(c)). This enabled them to move an entire antenna structure around and locate it anywhere on the sample. The highest field enhancement is in this case located between the two arms of the antenna, where the sample cannot be located, yet the extending field, albeit weaker, can be used for near-field optical measurements. Several other methods to attach micro- or nanoparticles to SPM tips have since then been presented\cite{128,129}: for example by combining electron beam deposition, etch masks and Ar-ion milling, the attachment process can be avoided and reproducible metallic nanostructures can be created directly on a cantilever\cite{130}. A different approach is presented by Berwerger et al.\cite{72} using focused ion beam (FIB) milling. They created a grating coupler on the shaft of the tip, enabling them to launch a surface plasmon polariton towards the apex of the tip by illuminating only the shaft part but not the apex and the sample. The detection then selectively detects light emitted from the apex itself or molecules interacting with the created EM near field. A similar idea with different geometry has been presented by De Angelis et al.\cite{131,132} where they designed a photonic crystal cavity on the back of an AFM cantilever with a tapered waveguide attached in the center (see Fig. 2.4(d)). This waveguide transfers the illumination light focused on the back of the cantilever to the sample, thus also excluding any confocal background signals. Weber-Bergioni et al.\cite{133} used coaxial optical antenna scan probes in a bottom-illumination approach to create a plasmonically coupled antenna with the resolution of a sharp metallic tip by creating a circular gap around a central metallic pin protruding from the surrounding metal. It has yet to be noted that for efficient coupling, all structured or plasmonic tips have to be designed and tailored differently for every laser wavelength and do require a substantial amount of production infrastructure.
2. Theoretical Background and State of the Art

Figure 2.4.: Selection of available TERS tips. (a) Silver coated AFM TERS tip. (b) Etched full metal STM TERS tip. (c) Bowtie antenna on an AFM cantilever adopted from \cite{127}. (d) Photonic crystal cavity on a Si$_3$N$_4$ cantilever with attached tapered waveguide\cite{131}.

2.8.4. Optical Signal Detection

The direction of detection is, for convenience reasons, in most cases chosen to start equal to the excitation direction and is then separated using either notch filters or edge filters to discard the reflected laser and Rayleigh scattered light which is far stronger than the Raman shifted signals by several orders of magnitude. Nonetheless, separate detection optics were already successfully employed amongst the first instruments\cite{63} but may bear further potential of an altered signal collection taking a directional emission distribution into account for a far larger variety of instruments. After discarding of the reflected as well as the Rayleigh scattered light, the final detection was traditionally realized by an avalanche photo diode (APD). They offer a high detection efficiency and single photon counting ability, with a rather limited rate of photons per second. They were combined with wavelength filters to select certain areas from the Raman spectrum. Nowadays, the remaining Raman photons are spread out by wavelength using a spectrometer with single or multiple turrets hosting optical gratings. Ruled diffraction gratings disperse the incoming beam by wavelength onto the detector with an increasing resolution depending on the number of grooves/mm but decreasing transmission efficiency. Besides line gratings, holographic gratings can be used, especially useful for high resolution and high signal-to-noise applications. The dispersed photons are detected by a charge coupled device (CCD) or an electron multiplying CCD (EMCCD) with close to single photon counting capabilities and high efficiency in the visible range, able to detect an entire Raman spectrum with 1024-1600 pixels at once.

2.9. Point Spectroscopy and Imaging

The information content of Raman spectroscopy is very high, especially for smaller molecules. A single point spectrum can deliver information on the presence of several substances at once (as long as they can be distinguished spectroscopically and the signal level is sufficient). To extend the information one can gather from a sample, imaging of the surface can deliver information on the concentration and distribution of all detected molecules over the entire surface. This is usually achieved by scanning the surface and acquiring entire Raman spectra at each location.

2.9.1. Point Spectroscopy

Most TERS experiments are single point measurements. After selection of an area of interest on the sample (either from the optical image or from a previous SPM scan) the pre-aligned tip in the laser focus is located above this sample spot, and a spectrum is acquired over a certain period of time. Depending on the scattering
cross section of the sample, integration times can range from split seconds over usual times of several seconds up to minutes for very Raman-inactive substances. The yielded spectrum combines the enhanced spectral fingerprint information from the near field area as well as the confocal background signal from the area of the entire laser focus.

2.9.2. TERS Imaging

After single point spectroscopy, TERS imaging (each point in the image contains an entire Raman spectrum) is the logical next step. As the setups already include a scanning element to position the sample or tip, the main factors that formerly limited full spectroscopic imaging were the positioning accuracy and time constraints connected to the system stability. The inability to remain on a certain sample spot without movement due to thermal drift or mechanical instabilities, long time only allowed point spectroscopy or point-by-point line scans [58,82,134-136]. Several factors lead to the feasibility of full spectral imaging. On the one hand, the stability of the SPM systems increased continually, leading to smaller drift of the tip in respect to the sample. Concurrently, the progress in tip design lead to stronger enhancing tips and thus more Raman-scattered photons. As a third reason, the progress from APD to electron magnifying CCD detectors with close to single photon counting abilities allowed simultaneous detection of entire Raman spectra under low signal conditions. All this lead to a reduction of the necessary integration time per acquired Raman spectrum. Full spectral TERS imaging has been reported in literature in the past years with an increasing number of pixels [90,135,137-139] for up to 200x200 - 256x256 pixels [73,133,140] and increasing resolution of down to 12 - 20 nm [73,101,138,141,142] (4 nm were achieved in a special tip-pressure assisted procedure [136] limited to pressure sensitive bands). TERS imaging offers several obvious advantages, more information on the sample clear detection of shapes and borders but also includes a determination of the resolution in every image (when looking at edges or when examining small features of a sample). By imaging of known shapes (compare Fig. 8.6) or surface structures, and comparison of the AFM/STM feedback during imaging to a reference scan, artifacts and drift can be detected and if necessary, a compensation for these artifacts may be possible.
3. Materials and Methods

In this chapter the most important recipes used throughout this thesis for the preparation of tips, substrates and samples are listed, including details concerning handling and points where special care should be taken.

3.1. Tips

3.1.1. Ag Coated AFM Tips for TERS

There are two standard recipes used in our lab for coating AFM tips with silver for TERS (with 532 or 632.8 nm). Both are two layered, with a base layer of 20 nm AlF$_3$ or SiO$_x$ on Si$_2$N$_3$ AFM tips and a top layer of 30 nm of silver both deposited by physical vapor deposition (PVD) in a Bal-Tec Med 020 coating chamber (Leica, Wetzlar, Germany). All thickness values are nominal values, i.e., as measured on the surface of a quartz crystal micro (QCM) balance. The important factors to control are the purity of the silver (which should be $> 99.99\%$), the vapor pressure in the chamber (which should be $< 1 \times 10^{-5}$ mbar) and the speed of coating (around 0.1 nm/s). For well usable tips, a meticulously cleaned coating chamber, and a slow coating process are necessary to avoid carbon contaminations. To achieve homogeneous coating around the cantilever, the tip apex should point exactly towards the evaporation source. More details on production of AFM-TERS tips can be found in the thesis of Dr. B.S. Yeo$^{[143]}$.

3.1.2. Etched Ag Wires for TERS

The STM tips used in all STM-TERS and STM-TERS imaging experiments were produced with the etching setup shown in Fig. 3.1. The setup consists of a laboratory voltage generator set to 8 – 10 V attached to a custom made circuit$^{[144,145]}$ controlling the current switch-off after successful etching. The cathode consists of a 0.1 mm Pt wire forming a $\varnothing$2.5 cm loop; the anode consists of an Ag wire with $\varnothing$250 µm. This wire is immersed 1 cm into a volumetric 1:1 mixture of perchloric acid (HClO$_4$) and MeOH. A sufficient immersion depth of the wire is important to reach a sufficient current jump to trigger the circuit. The perchloric acid serves as etchant for the electrochemical oxidation of the silver and the methanol helps to
improve the surface quality of the etched tip by reducing gas bubble formation in the etchant solution. Important control parameters for the etching are the composition of the etching solution, the immersion depth as well as a central position of the Ag wire within the cathode loop and a perpendicular immersion of the tip into the etching solution. The quality of the tips can potentially be increased by a previous annealing step of the bulk silver wire to reduce the number of grain boundaries or by using a single crystalline wire, but this has not been pursued until now (this approach has been shown on Au tips in\textsuperscript{118}).

### 3.1.3. Pull-Cut Pt/Ir Wires for STM Imaging

For pure STM imaging, pull-cutted STM tips from Pt/Ir wire are used. These tips are simply produced by tightly grabbing the Pt/Ir Wire on one side with a pair of tweezers and cutting the wire at a steep angle with a sharp pair of scissors whilst pulling. Shortly before the wire is entirely cut through, the pulling force tears the remaining attached wire apart, forming a reasonably sharp tip-like end with only few exposed atoms. These tips can be used for imaging and are even sufficient to reach atomic resolution given adequate instrument scanning quality.
3.2. Substrates

3.2.1. Silicon Substrates

Polished Si(100) wafers (Si-Mat, Landsberg, Germany) were diced to an edge length of 1 cm using a diamond wafer saw for use as silicon substrates. The resulting saw dust was removed from the wafer dices before further use. The first step in the cleaning process was cleaning in a N$_2$ stream, followed by rinsing with or sonication in water to dissolve water soluble contaminants and ethanol or acetone for organic residuals and subsequent drying in a N$_2$ stream. If the substrate was intended for spectroscopic use, the Si dices were furthermore immersed into piranha solution (2:1 volumetric mixture of H$_2$SO$_4$ and H$_2$O$_2$) for at least 20 minutes before rinsing twice with deionized water and subsequent drying under a N$_2$ stream.

3.2.2. Oxidized Si Substrates for Graphene Imaging

Atomic layers of materials are usually transparent and invisible on a Si substrate. Using an oxide layer with a very specific thickness of either 90 or 280 nm, even very thin layers (atomically thin as in the case of graphene) can be visualized due to a changed interference pattern and a resulting color shift of the illumination light$^{146,147}$. This oxide layer was created by oxidation of a standard silicon wafer in an oxygen atmosphere under elevated temperatures. To obtain correct results, the native thickness of the oxide layer on the Si wafer was determined by ellipsometry. A native oxide layer of 1.6 nm is already accounted for in the following recipe targeting a 90 nm oxide layer. In a tube furnace with a slow oxygen flow, the Si wafer was heated for approx. 100 min with a temperature gradient of 600$^\circ$/h to 950$^\circ$C, kept for 100 min and cooled down to 750$^\circ$C within 40 min. At this point the oxygen stream was cut off and the sample slowly cooled to room temperature resulting in an approx. 90 nm oxide layer with typical purple/blueish color shown in Fig. 3.2.

3.2.3. Template Stripped Au Substrates

In order to minimize the influence of the substrate on a STM-TERS experiment, ideally very flat gold films are needed. Template stripped Au surfaces similar to$^{148}$ were produced by coating polished Si(100) wafers (Si-Mat, Landsberg, Germany) in a Bal-Tec Med 020 coating chamber at pressures below 1x10$^{-5}$ mbar with a 150 nm 99.99% Au film, evaporated by resistive heating at a rate $\leq$ 0.1 nm/s (a very small initial coating rate is crucial, as the Si/Au interface forms the sample surface at the end). The deposited gold film was bonded to piranha-cleaned microscope slide fragments (of approx. 1 cm width) using optical adhesive (NOA 61, Norland, Cranbury, USA) with subsequent UV curing and thermal treatment at 80$^\circ$C for
3. Materials and Methods

Figure 3.2.: 1 cm dice from a silicone wafer, oxidized to a 90 nm oxide layer, tailored for detection of atomically thin films (graphene). Under appropriate lighting conditions, a graphene layer is visible as a purple film under a microscope, on top of the Si/SiO$_2$ stack.

...) faster bonding to SiO$_2$. The gold films were mechanically stripped from the Si wafers by scratching around the sides with a razor blade and careful lifting of the glass slide from the Si wafer, immediately before use. This procedure yielded gold surfaces with an average roughness of $< 1$ nm as presented in Fig. 8.1.

3.2.4. Metal Coated Si Substrates

Metal coated Si substrates are produced by cleaning silicon substrates as mentioned, followed by subsequent coating of metals with a thickness of 150 nm at rates $\leq 0.1$ nm/s, as described above. If a very flat surface is necessary, template stripping should also be possible for various other metals than Au as long as the adhesion towards Si is not largely superior than in the case of Au.

3.2.5. Ag SERS Substrates

SERS substrates with silver as an active species can easily be created by thorough cleaning of any glass substrate (Si could potentially work as well, but has not been used) followed by subsequent coating of 5–6 nm (nominal thickness) of Ag at rates $\leq 0.1$ nm/s. At this thickness, silver does not form a continuous layer but an inhomogeneous film with islands showing strong SERS activity.

3.3. Samples

3.3.1. Substrates with a BCB or NB Thin Film

A thin dye film of Nile blue (NB) or brilliant cresyl blue (BCB) can be produced by placing a 10 µL droplet of a 5x10$^{-5}$ mol/L BCB or NB solution on a clean
substrate in a spin-coater, at an initial speed of $< 500 \text{ min}^{-1}$ and increasing the speed slowly to $2000 \text{ min}^{-1}$ and continue spinning to dryness. The thickness of the layer is sufficient for a low but still detectable confocal background signal in confocal measurements with a laser power around 100 $\mu$W, yielding approx. 1 count/s. The film is mostly homogeneous on the $\mu$m scale, but as shown in chapter 6, on the nanoscale large differences in the number of molecules can be present.

3.3.2. Titanium Dioxide Nanoparticles

Titanium dioxide ($\text{TiO}_2$) is a versatile substance, used in different applications ranging from simple wall painting or sunscreen (where structure and purity of the particles are negligible) to highly sophisticated thin film coatings for dye sensitized solar cells\cite{149}, FeRAM $\text{H}_2$ passivation layer\cite{150} or to form $\text{TiO}_2/\text{SiO}_2$ anisotropic reflectors\cite{151}. In these cases, purity, structure and crystalline phase play an important role and need to be observed on the sub-micrometer scale.

Anatase, rutile and brookite, the three most common types of $\text{TiO}_2$ can be distinguished by their Raman spectra. Confocal Raman spectroscopy allows to determine and assign the components and phases present in a diffraction limited laser spot of around 500 nm. However, the composition of particles on the nanometer scale would be of interest to determine if signals from a single nanoparticle, containing several crystalline phases originate from a number of tightly agglomerated smaller nanoparticles with pure crystalline phases or from (gradual or sharp) crystal phase changes within condensed nanoparticles. These investigations could be realized with an AFM top-illumination TERS instrument as the films are non-transparent but too thick for STM feedback.

A suitable way to disperse the $\text{TiO}_2$ particles for confocal Raman and a potential TERS measurements on a flat surface would be with sufficient space between them to be able to address single particles or single small clusters. A dispersion of 20 mg $\text{TiO}_2$ in 9.98 mL of $\text{H}_2\text{O}$ was prepared by 45 min sonication. 10 $\mu$L of the $\text{TiO}_2$ dispersion was diluted with 1 mL of 2% polyvinyl acetate (PVA) and sonicated for 1 min before spincoating onto acetone cleaned microscope cover glasses. This led to a suitable distribution of separated small $\text{TiO}_2$ particles. The distribution of particles after spincoating on the surface, was measured by AFM as shown in Fig. 3.3.

The confocal Raman spectra in Fig. 3.3 reveal, that the composition in the measured spots 1-5 differ substantially in amount of anatase and rutile. With $\text{TiO}_2$ nanoparticles, long integration times are necessary due to weakly scattering particles, limiting the possibility to map particle distributions. Signal enhancement using tip-enhanced Raman spectroscopy could solve this problem and increase the
resolution to around $10 - 20 \text{ nm}$, resolving internal structures of single particles.

### 3.3.3. Nano-Diamonds

In the field of TERS there is a definite need for a standard sample. This sample should provide information about the enhancement, efficiency, optical resolution and calibration of different TERS instruments as well as different TERS geometries and different TERS tips. The ideal standard sample has to be easily available, robust, should not bleach, and ideally allow fast and easy measurements with TERS as well as be sufficiently reproducible. Nanometer sized diamonds were one potential sample candidate to fill this void as inorganic nanoparticles offer the advantage of high stability and small size. Nano-diamonds were a promising candidate for TERS standardization due to several reasons:

- Easily available in sizes ranging from micrometers down to tens of nanometers.
- Sharp Raman signal at $1332 \text{ cm}^{-1}$ without additional bands.
- Topographic and optical contrast allows to locate the species.
- High thermal stability makes long term measurements possible, even in highly enhanced electromagnetic fields.
- No photochemical degradation, allowing high laser powers for fast spectra acquisition or imaging.
- Simple sample preparation - random distribution by spin coating a diluted dispersion of diamond nanoparticles (with more and less dense regions).

Synthetic diamond nanoparticles were obtained in three different sizes, $0 - 30 \text{ nm}$ (Microdiament AG, Lengwil, Switzerland), $0 - 100 \text{ nm}$ (Van Moppe & Sons s.a., Daellikon, Switzerland) and with a size of $0 - 500 \text{ nm}$ (DiaPlus, Oststeinbeck, Germany). Natural diamond nanoparticles were $0 - 500 \text{ nm}$ in size (DiaPlus, Oststeinbeck, Germany).

**Sample preparation**

All glass slides used were immersed in piranha solution (1:2 mixture of $\text{H}_{2}\text{O}_{2}$ and $\text{H}_{2}\text{SO}_{4}$) for at least $30 \text{ min}$ and subsequently rinsed with methanol and dried under a $\text{N}_{2}$ stream before use. The following options for sample preparation were investigated:
Figure 3.3.: (a) Literature from\textsuperscript{[152]}: Raman spectra of anatase (A), rutile (R) and brookite (B) (b) Confocal Raman spectra showing strong rutile signals in locations 2 and 4 as well as mostly anatase bands in locations 1 and 3. (c) AFM with 1 µm total height, and (d) Confocal optical image of TiO\textsubscript{2} sample.
3. Materials and Methods

Figure 3.4.: (a) AFM image and (b) linecut from a scratch through the polymer surface down to the supporting glass surface. Height of the polymer film is around 20 nm.

- Diamond nanoparticles on glass slides

Microscope cover slips were spincoated with a diamond nanoparticle solution as samples for TERS measurements. This approach produced particle agglomerates instead of a distributed of individual nanoparticles. Additionally the particles were moved by the scanning tip during AFM measurements on this sample. Even during low force tapping mode, the diamonds were shifted by the scanning tip.

- Diamond nanoparticles in polymer

Different concentrations of diamond nanoparticles were suspended in a solution of 2% PVA in H₂O. Small amounts of the solutions were then spincoated onto glass slides (usually around 10 µL). Polymer thin films with a thickness of around 20 nm were obtained (see Fig. 3.4). The diamond nanoparticles were protruding out of the thin film depending on their size (see. Fig. 3.5). TERS measurements of these films never showed enhanced spectra from the diamonds but, if at all, only signals from the polymer. A possible explanation would be that by the co-spincoating procedure, a film formed, coating all protruding diamonds with a thin layer of polymer. Due to the shallow dept of field in TERS, only this polymer layer may have been probed.

- Diamond nanoparticles attached by instant adhesive

Further approaches were based on a layer of spincoated instant adhesive combined with either sprinkled diamonds or spincoated diamond solution directly on the glue or transferred from a clean glass slide. AFM measurements showed
3.3. Samples

that the glue hardly solidifies without additional pressure and is thus not suitable for AFM. Applying pressure with a second glass slide irreversibly glues the latter onto the thin film. Also, a 10 s confocal Raman spectrum of the instant adhesive shown in Fig. 3.6 (cyanacrylate based) revealed typical bands of cyanacrylate. Thus, cyanacrylate based preparation methods have been abandoned completely.

- Diamond nanoparticles on sticky tape

A pick up of diamond nanoparticles spin coated onto a clean glass slide by a strip of double sided sticky tape attached to a glass slide was a further attempt. The sticky tape showed a very uneven topography with roughnesses of up to 200 nm height shown in Fig. 3.7(a). On top, the sticky tape exhibited a similar problem as instant adhesive, with an additional broad fluorescent background shown in Fig. 3.7(b).
3. Materials and Methods

Figure 3.7.: (a) 3D representation of the topography from diamond nanoparticles on sticky tape, measured by AFM and (b) Raman spectrum from the same area. The surface roughness is too large for the detection of diamonds and the fluorescent background as well as the cyan acrylate bands in the Raman spectra cover the typical Raman signature of diamond in spectroscopic measurements.

- Diamond nanoparticles picked up by a polymer film from a glass slide

The next attempt was to use a film of 100 μL spincoated PVA (2% in H₂O) to pick up diamond nanoparticles spin coated onto a clean glass slide. The pick up process seemed to be too inefficient to deposit a reasonable amount of particles in the polymer film.

- Diamond nanoparticles on top of a polymer film, pressed in

The next step was to use a dried film of 100 μL PVA solution (2% in H₂O) and spin coat the diamonds in a water solution on top of this film. The particles were embedded in a polymer film by applying pressure with a second glass slide from top. This led to nicely distributed particles. Yet not all these particles were attached firmly enough in order not to be displaced by the scanning tip.

- Diamond nanoparticles on top of a polymer film, pressed in and rinsed with small amounts of acetone

An analogous procedure to the above case was used, but with an additional step, where the resulting sample was rinsed twice with a small amount of solvent to get rid of loosely attached particles. This approach yielded a reasonable number of distributed diamond nanoparticles firmly attached to the polymer as seen in Fig. 3.8. In the image we can evaluate the height of bigger particles (protruding between 50 and 300 nm) and particles smaller than 50 nm which both exist in reasonable amounts. The bigger particles could serve as
targets for far field measurements, the smaller ones could be potentially used for resolution and near field enhancement determination.

The co-spincoating procedure, mentioned last, may result in a reasonable sample for TERS, but the diamond signal decreases when the size of the diamond becomes too small. This could be a result of falling below a certain phonon length, or due to the higher ratio of surface to bulk carbon. In this case, more $sp_2$ than $sp_3$ hybridized carbons on the surface and in the bulk, respectively, deliver only a carbon D oG band instead of the diamond band. It seems, diamonds with a size of 50 nm or more are necessary for the diamond signal to appear. These particles cannot be investigated by STM-TERS due to lacking conductivity, but may be suitable for a top-illumination AFM approach.

3.3.4. **Graphene on Oxidized Si, Produced by the Scotch Tape Method**

To produce graphene samples on oxidized Si for confocal Raman spectroscopy, a piece of highly oriented pyrolytic graphite (HOPG) is rubbed mechanically against the surface of the oxidized wafer. Parts of the HOPG stick to the surface as large clusters with many layers. By repeated stripping of the surface with a scotch tape, the layer number is reduced until ideally only a single layer remains on the surface\cite{153}. The first layer of a graphite flake (a graphene sheet) has a stronger interaction to the Si surface than to the subsequent layers of the scotch tape and thus remains on the surface. Identification of the layer number can either be realized by the interference color, the AFM height at edges or the Raman spectrum.
3. Materials and Methods

Although all three were shown to be suitable to detect the number of graphene layers, the safest way to determine a single layer is the change in the Raman spectrum compared to few or multi-layer graphene (shift and shape of the 2D-band, compare chapter 9).

3.3.5. Graphene on Template Stripped Au, Produced by the Scotch Tape Method

For STM-TERS experiments, oxidized silicon is unsuitable, being a good electrical insulator. The Si surface thus either has to be replaced by a gold film or another conductive material. Silicon oxide can still be used as a base layer, when coating an optically transparent thin film of gold. This has the advantage of superior visibility of the flakes, but sacrifices good conductivity due to the very thin gold layer. Otherwise, a template stripped Au surface can be used together with the scotch tape method to produce single layer graphene on Au. Yet, the detection and localization of the very small and scarcely distributed single layer graphene (SLG) flakes is then close to impossible. Even a STM detection is quite difficult due to the large areas that have to be scanned in order to retrieve a flake (often only few small flakes are found in an area of 100x100 µm²).

3.3.6. Microcontact Printed Thiols on Template Stripped Au

For the production of a patterned monolayer of thiols on a noble metal substrate, micro contact printing (µCP) of thiols on template stripped gold is ideally suited because of the chemisorption of the thiol to the gold and the flat surface of the Au film. A similar procedure to ref.154 is used to transfer a thiol monolayer onto the gold surface. To pattern the surface of the gold film, a microcontact printing stamp of suitable size can be used for the transfer. The stamp is inked with a droplet of a 10 mM ethanolic solution of thiol for 1 min, and washed with copious amounts of ethanol. The pattern is then transferred onto the gold film by placing the stamp with the pressure of its own weight onto the gold film for 10 s followed by careful lift-off, yielding efficient transfer with good surface coverage (compare Fig. 8.3).
4. Confocal Raman Investigations

Parts of this chapter are adopted from several publications.

M.Pacton, T.Schmid, G.Gorin, M.Massault and J.Stadler:
'Cretaceous Black Shale: a Window Into Microbial Life Adaptation'

L.Zhu, J.Stadler, T.A. Schmitz, F.Krumeich and R. Zenobi:
'Atmospheric Pressure Sampling for Laser Ablation Based Nanoscale Imaging Mass Spectrometry: Ions or Neutrals?'
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J.Puigmarti Luis, J.Stadler, D.Schaffhauser, A.Perez del Pino, B.Burg, P.Dittrich:
'Guided Assembly of Metal and Hybrid Conductive Probes Using Floating Potential Dielectrophoresis'
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In this chapter, three applications are presented to illustrate, how the information obtained from confocal Raman spectroscopy can be used to better characterize real-world samples from different areas of research. The samples show the combination of confocal Raman imaging with a structural information technique, AFM and SEM, respectively. All three samples originated from collaborations or small projects together with partners from different areas in analytical chemistry and geology. They show how the large information content of Raman spectroscopy can help to solve questions from synthetic chemists, biologists and others. Especially the combination of high resolution imaging data with the chemical information from spectroscopy helps to identify, characterize and understand small surface structures.
4. Confocal Raman Investigations

Figure 4.1.: Schematic setup of Au/TTF nanowire chip: Silicon based chip with two rows of 10 electrode pairs. Between the electrodes, nanowires are grown from a Au nanoparticle solution or a mixture of Au/TTF respectively as illustrated on the right hand side.

4.1. Confocal Raman Imaging of Au/TTF Nanowires

In the first example, J. Puigmarti-Luis provided several microchips as samples. As seen in Fig. 4.1 the chip consists of two columns of pointed electrodes. They were roughly 40 nm in height, 2 µm in width and several tens of µm in length, with a 1 – 2 µm gap and a large area in the back for electrical contacting. Between these electrodes, gold nanowires were grown from a Au solution or a solution of Au mixed with tetrathiafulvalene (TTF, structure in Fig. 4.2) respectively. Nanowire growth is used as an assembly method to locally form a hybrid material of Au/TTF. These materials are known for their tunable properties\[155,156\] and their particular value for the construction of novel multifunctional nanoscale devices\[157,158\]. In the first case (the Au/noTTF chip), pure gold nanoparticles have been grown from solution to nanowires between the electrodes. A drop (approx. 5 µL) of hydrogen tetrachloroaurate (0.2 mmol) in acetonitrile covered the entire chip. A sine wave of 5 V at a frequency of 1 MHz was applied between the bias electrode and the doped silicon substrate until the solvent was evaporated. On the second chip (the Au/TTF chip), TTF has been co-embedded in the nanowires, using a TTF/Au precursor solution in acetonitrile, forming very similar wires in between the electrodes. The formation of the exact compounds within the wires was in question as well as their potential Raman enhancement abilities. The electronic state of the TTF was determined to allow predictions of possible charge transfer reactions and the formation of TTF-Au hybrid composites.

Raman spectroscopy was used to characterize nanowires on both chips. In contrast to standard SERS experiments, where a rough noble metal surface or small particles made of silver or gold are used to enhance signals,\[159\] a known location was addressed using the nanowire chip and the enhancement of signals was con-
4.1. Confocal Raman Imaging of Au/TTF Nanowires

Figure 4.2.: Molecular structure of tetrathiafulvalene (TTF) which is co embedded in grown nanowires.

fined to the wires between the electrode pairs. In Fig. 4.3(a) and (b) 3D atomic force microscopy (AFM) images from a branched hybrid wire and a bare gold wire, respectively, are shown color coded with the optical response of the addressed structures, as determined by confocal Raman spectroscopy. The evaluation of the Raman map (in Fig. 4.3(d)) at the intensity of the $501\text{ cm}^{-1}$ band, a typical marker band of TTF$^{\text{+\cdot}}$, clearly proves that TTF signals are emitted from the hybrid structures located between the electrode pairs. The bare gold wires shown in Fig. 4.3(b) exhibits stronger signals than the background, as indicated by the brighter areas between the electrodes. A careful examination of the attributed spectra reveals, however, that the increase in signal levels in the TTF marker band region is due to an increased background and not due to a specific Raman signal from within the structure. The spectra do not show marker bands from TTF molecules, and only emit signals from enhanced carbonaceous contaminations (Fig. 4.4).

Furthermore, SERS from the stock solution, used to grow the micro- and nanowires, and that of the TTF-Au wire on the silicon oxide surface (Fig. 4.3(c) and (d), respectively) were compared. The SERS spectra from the TTF-Au solution clearly show typical Raman bands of TTF$^{\text{+\cdot}}$ at $501\text{ cm}^{-1}$, $748\text{ cm}^{-1}$, $1418\text{ cm}^{-1}$ and $1514\text{ cm}^{-1}$. These bands correspond well with the literature values of TTF on gold with peaks located at $506\text{ cm}^{-1}$, $748\text{ cm}^{-1}$ and $1418\text{ cm}^{-1}$. The band at $1514\text{ cm}^{-1}$ indicates that small amounts of TTF$^0$ are present in the solution. In Fig. 4.3(d), representative Raman spectra from a TTF-Au wire as well as a background spectrum from the chip are shown. Due to short integration times, the noise level of the spectra from the nanowires is higher than that of the background and the TTF-Au solution SERS spectra. However, five typical TTF$^{\text{+\cdot}}$ bands at $500\text{ cm}^{-1}$, $1288\text{ cm}^{-1}$, $1423\text{ cm}^{-1}$ and $1448\text{ cm}^{-1}$ as well as $1514\text{ cm}^{-1}$ were found in the area containing the hybrid structures, unambiguously proving the presence of TTF in these wires.

The AFM in this example allowed the determination of the exact structure of the nanowires and the chemical composition and charge state of the TTF was revealed by the confocal Raman information. The combination of both techniques yielded the additional information that the Raman signals were restricted to the exact
Figure 4.3: (a) and (b) 3D AFM topography images from two wires of TTF-Au and bare gold, respectively, grown between the contacting electrodes. The color represents the intensity of the Raman TTF marker bands across the positions probed in the AFM image. (c) Surface enhanced Raman spectra of TTF-Au stock solution at different positions on a 6 nm silver SERS substrate. (d) Confocal Raman spectra from enhanced TTF in hybrid wire areas (gray tones) and the background spectra form the pure chip (red).
4.2. Investigations of Black Shale Microbacteria in Microtome Cuts

In this section, microtome cuts of rock from the Urbino level of Monte Petrano, Italy, created during the Albian oceanic anoxic event (OAE1b), were investigated upon the physiology of microbacteria in inclusion of fossilized microbacterial organic matter. Conventional extraction technologies confirmed the presence of organic matter but lacked information on the origin from within the sample. A combined analysis of TEM and confocal Raman information is shown in Fig. 4.5. The TEM images allowed quick and precise localization of fossil bacteria features in the microtome cuts that resembled microbacterial shapes and energy-dispersive X-ray spectroscopy (EDX) measurements pointed out phosphorous and sodium within the structures. These features were subsequently investigated by confocal Raman spectroscopy upon the presence of typical Raman signatures from organic matter. The spectroscopic signatures from confocal Raman spectroscopy revealed local CH$_2$ bending modes at 1448 cm$^{-1}$ and C=C stretching mode at 1531 cm$^{-1}$ (typical for conjugated double bonds in carotenoids) within the bacterial features but not in the background confirming the origin of the organic matter from the bacterial shaped location of the nanowires and could be distinguished from potential background signals.

Figure 4.4.: All spectra are from a chip where pure gold wires were synthesized without TTF (10 s, 632.8 nm, 100 µW power). The red spectrum shows background signals from the pure Si chip where all bands can be attributed to Si. The black and gray spectra are taken from three points within the gold wire area, exhibiting a raised overall background without visible TTF bands. Weak bands from amorphous carbon are visible in the region of 1000 – 1600 cm$^{-1}$. This area can potentially enhance Raman signals, but no clear signatures are present due to the lack of analyte molecules.
inclusions. From the spectra, likely carotenoids as well as inorganic polyphosphates are suggested to be in the microbacteria. The Raman information on the polyphosphate lead to conclusions about the survival strategy in use by the microbacteria during that time as a reaction to the Albian oceanic anoxic event.

4.3. Investigation of Dye Deposition on a Glass Substrate

In a collaboration with L. Zhu, combined AFM and confocal Raman spectroscopy was used to investigate a deposited thick layer of dye molecules on a glass substrate. The amount of material collected by a sampling device from a laser ablation events should be quantified to determine the collection and transfer efficiency of the instrument. Using Raman spectroscopic information allowed chemical identification of the deposit with confocal Raman resolution and assignment to the ablated material. The high resolution information of the AFM on the size a height of the deposits allowed a coarse quantification of the amount of material deposited. Using the relation between signal intensity from the Raman and the thickness determined by AFM, we were able to show that the topography signal could not result entirely from sample molecules, but that additional dust particles from ambient were deposited in the center of the spot. The combined information allowed a more accurate estimate of the amount of deposited material more accurately, and thus the transfer efficiency of the specialized sampling system, used for the deposition, could be determined more precisely.
4.3. Investigation of Dye Deposition on a Glass Substrate

Figure 4.5.: Fossil bacteria structures imaged by TEM (a) and confocal laser-scanning microscopy (b). Raman maps collected at the exact same place as in (b) showing distributions of Raman intensities at 1448 cm\(^{-1}\) (c) and 1531 cm\(^{-1}\) (d), respectively. (e) A typical Raman spectrum collected in bacteria-like structures. In most cases, collection times of 10 min were necessary for sufficient signal-to-noise ratio. The 10x10 pixel mapping experiments took approximately 17 h and were carried out overnight.
Figure 4.6.: Optical microphotograph (a), intensity map of the Raman band at 582 cm$^{-1}$ (b), and high resolution AFM scan (c) of the material on the collection plate. This particular plate collected the material from a series of laser ablation events, forming a micrometer size crater. The insets in (a) are zoomed views of the center and the edge of the collection area by SEM (the SEM image of the edge is rotated clockwise by 90°).
5. Development of a Top-Illumination TERS System with Top Detection

The instrument that was mainly employed and further developed during the duration of the PhD thesis will be explained in detail in this chapter. The instrument, an Ntegra Spectra Upright (NT-MDT, Zelenograd, Russia) was purchased in 2007 and is a combination of a piezo scanning based AFM/STM system with a confocal Raman spectrometer. This system was installed with the intention to realize tip-enhanced Raman spectroscopy in a top-illumination top-detection configuration using AFM feedback. The intention was later on converted into a top-illumination instrument with STM feedback. In this part, the instrument will be described in detail including different operation modes and the signals that can be acquired. Furthermore, the path towards reliably working gap-mode STM TERS on this instrument will be reviewed.

This specific instrument was chosen for several reasons. First, high SPM accuracy, positioning stability, and high detection efficiency was planned to be used to allow more complicated TERS experiments and boost sensitivity of the method. The second, even more important argument for the choice of this exact instrument was the special illumination and detection geometry. Both, illumination and detection are realized from the top, perpendicular to the sample surface. Not having to pass the specimen of interest with the excitation or detection light opens up an entirely new field of application compared to bottom-illumination TERS: opaque samples. A different configuration, suitable for such samples is side-illumination TERS. The advantages of top illumination over side illumination are the symmetrical focus leading to a smaller confocal spot size and the higher energy densities in the focus as well as less illumination of the surrounding background areas (details in sect. 2.8.1). Additionally, the detection efficiency is increased by a higher numerical aperture in the detection pathway. A more detailed picture of the optical geometry is drawn in the following section.
5. Development of a Top-Illumination TERS System with Top Detection

5.1. Instrument Layout

The NT-MDT Ntegra Spectra Upright (displayed in Fig. 5.1) is divided into two main parts. The front part consists of a base platform with the scanning piezo on which the sample is placed, for performing AFM/STM. Above, the microscope head is located including a high numerical aperture (NA = 0.7), long distance focusing objective with a focusing distance of 6 mm (Mitutoyo, Kanagawa, Japan) as well as the AFM or STM tip including the respective adapters. Additionally, an AFM detector is located in the upper part of the head. Above the head, a 4-way turret is used to select the ratio of light steered towards a CCD camera with white light illumination for sample observation on top or in direction of the optical detection unit in the back of the instrument. In this 4-way turret, partially reflective mirrors with 5 to 100% reflectivity are located, allowing the optical observation of the sample and tip area and efficient transfer of the laser and Raman light respectively. In a second part of the instrument, the optomechanical unit, an edge filter is used to redirect Rayleigh scattered light onto a photomultiplier tube (PMT) (compare scheme 5.2). In conjunction with a piezo scanning mirror in the beam path this detector is useful to quickly gather information about the reflectivity of the sample and allows fast imaging of the surface even at low laser powers, as well as initial coarse alignment of tip and laser position.

In the optical detection unit, the excitation can be alternated between a 632.8 nm 10 mW Helium-Neon laser (LGK 7627, Siemens, Munich, Germany) or a 50 mW 532 nm diode-pumped solid-state (DPSS) laser (MLL532, CNILaser, Changchun, China) and the selected beam can furthermore be adjusted in polarization and intensity using a half-wave plate and a gradient neutral density filter respectively. A double slit, forming a pinhole which can be adjusted in terms of 3D-position and opening diameter can be used to ensure proper confocallity of the system at the entrance of the spectrometer. The spectrometer contains two bend mirrors and 150, 600 and 1800 lines/mm quadruple gratings as well as an additional echelle grating for measurements with high spectral resolution. The detection after the spectrometer can either be realized with a single photon counting PMT or an EM-CCD (Newton 971 UVB, Andor, Belfast, UK), which was customarily introduced into the system.

In terms of sample positioning and feedback, the sample is coarsely aligned with micrometer screws and fine sample movement as well as sample scanning is realized with a 100x100x10 µm³ piezo scanner. Feedback distance control in respect to the sample is ensured by either a STM or AFM module fitted into the optical detection unit at a fixed position.
5.1. Instrument Layout

Figure 5.1.: Front view of the NT-MDT Spectra Upright. Visible white light CCD camera on top, optomechanical unit for confocal measurements in the back, the piezo sample stage in the bottom left as well as the optical head for laser focusing, signal collection including the SPM tip retention.

Figure 5.2.: Schematic setup of the NT-MDT Ntegra Spectra Upright instrument. The entire schematic optical pathway from laser excitation in the bottom right, over to the sample scanning unit in the left, and back to the different detection options is displayed in the scheme on the right. A more detailed mockup of the focusing onto the tip and the sample can be seen on the left hand side. Description of the parts in the instrument and the optical pathway can be found in the main text. Scheme partially supplied by NT-MDT.
5. Development of a Top-Illumination TERS System with Top Detection

5.2. Operation Modes

A number of different operation modes can be used to collect different signals successively or simultaneously. The most commonly used scan modes will be explained here to show the reader how images were acquired.

In AFM mode, either a contact or semi-contact mode tip can be fixed in the AFM retention to scan the surface of an arbitrary sample with a topographic height of up to approx. 10 µm. Yet, the steepness of edges usually limits the reasonable scan height to around one to three micrometers in height when using scan speeds of 0.25 – 0.5 Hz. Several more sophisticated scanning modes (incl. force distance curves, two-pass methods, etc.) are available but were not of direct interest for our research. Only standard contact or semi-contact mode AFM with normal or very small amplitudes (<0.5 nm) was used (for pure AFM and AFM TERS).

STM feedback can be used to determine the topography of all types of conductive samples using different bias voltages between tip and sample. In STM mode, most commonly, constant current mode is used, keeping the tunneling current and with that the tip-sample distance constant during measurements (considering no change in conductivity in the gap). Within the STM holder, the STM tip is held by a mechanical spring and can only be moved together with the entire STM holder by micrometer screws in the optical head. All scanning movements have to be realized with the sample or laser scanning piezos, respectively.

A photographic image of an STM tip, close to approaching a Au sample is shown in Fig. 5.3. The etched silver STM tip is fixed by the retaining spring and approached onto the sample in an angle of roughly 45° from the backside. The sample is contacted with a metal wire, connected to the bias voltage outlet of the system via a flexible, thin, insulated copper wire (blue, in the front left).

For single point confocal Raman experiments, the laser is focused using the optical head and steered towards any desired location using the scanning mirrors or the piezo scanner. The Raman spectrum is then recorded using the EMCCD. In imaging experiments the lateral position is rastered in between spectra and coded with its respective position. The vendors of the instrument refer to piezo scanners for sample and laser scan as 'master' and 'slave' scanner, respectively, thus sometimes the measurements are referred to as 'master scan' (sample) and 'slave scan' (laser). In general, for confocal Raman imaging, sample scanning should be preferred over laser scanning (especially for larger scans) as the focusing of the lens is best in the center and also for the larger window of displacement available when scanning the sample (77x80 µm using the laser scanner compared to 130x130 µm with the sample piezo scanner).

As explained in section 1.2, to realize a tip-enhanced Raman experiment, a metal
5.2. Operation Modes

Figure 5.3.: Photographic image of the tip-sample area in an STM experiment. From the top, a 0.7 NA objective is reaching into the image, the tip is inserted from the top right and the sample is underneath, contacted from the bottom left to apply a bias voltage.

Figure 5.4.: Photographic image of the tip-sample area in an AFM experiment. The slightly bulkier AFM retention (in comparison to the STM holder) can be seen in the center of the image.
5. Development of a Top-Illumination TERS System with Top Detection

or metalized tip has to be introduced in the laser focus and approached to the sample using AFM or STM feedback. To ensure the proper location of the tip within the laser focus, a rough alignment can be realized using an image of the Rayleigh scattered light from the tip apex. Ideal alignment is best achieved by scanning the laser over the tip in feedback and recording the Raman signals from the background as well as the tip-enhanced area. Using this image, the laser can then be focused onto the spot with the strongest Raman bands from the sample, that is the contact point between tip and sample. From this point onwards, the position of the laser focus is fixed and locked onto the tip apex (as a reminder, the tip is anyhow fixed in space). Acquiring a spectrum or an image then works straightforward by displacing the sample between measurements with the TERS tip enhancing and localizing the Raman signals.

5.3. Software

An integral part of the instrument is the software, which can simplify experiments by automation, when well programmed. Nevertheless it can also hinder measurements, the instrument would in principle be capable of, by lack of specific signal collection options, timings, or commands.

The advantage of a single software in an integrated instrument from one producer is the handling of the entire instrument with a single user interface. If the same software controls for example the SPM as well as the spectrometer, efficient imaging is possible with minimal dead time. The NT-MDT software allows full control over the instrument SPM, the confocal Raman spectrometer as well as the white light camera for sample observation. Several parts of the instrument are automatized and software controllable such as the neutral density filters, the polarizers, the laser transmission for different wavelengths and selection of detection gratings. Also the movements of pinholes for optimization of the signal transmission can be controlled by the software. A further feature is the storage, import and export (including data evaluation of both SPM and spectroscopy data). This enables the combination of spectral and topographic signals yielding more information than the mere sum of both (as for example in section 4.6).

5.4. Approach 1: Top-Illumination AFM TERS

The first approach to realize top-illumination TERS was based on vapor coated AFM tips for enhancement. It was clear from the beginning, that standard AFM tips are not suitable for this experiment due to their 40 µm wide cantilever blocking both excitation light and detection path. Therefore, smaller, pointed cantilevers
with tips protruding to the front were used allowing top visibility of the tip (ATEC, Nanosensors, Neuchatel, Switzerland). A comparison of both tips in Fig. 5.5 gives a clearer idea why focused light from the top is blocked for standard AFM tips.

Experiments with several tips and on several different samples showed no or only negligible enhancement of the selected dye samples under investigation. Incorrect production of the tip was excluded by checking tips from the same batch with a working bottom-illumination AFM TERS instrument, successfully performing TERS experiments there. The optical alignment of the system and the focusing on the tip was extensively checked and optimized without success. Most recent experiments indicate that the low aspect ratio of of the angled tip, still leads to shadowing the apex from incoming light or even more likely block too much of the emitted Raman scattered light. First experiments with special, high aspect-ratio tips (OMCL-AC-160BN, Olympus, Tokyo, Japan) seem to allow AFM TERS with strong enhancement, even with top-illumination and top-collection.

5.5. Approach 2: Off-Center Top-Illumination AFM TERS

Several approaches have been made to attempt top-illumination TERS with top-collection using top visibility AFM tips. The first change in the system was based on the idea that illumination of only a part of the objective on the front side of the cantilever could result in a side-illumination like illumination, but still benefits from the symmetric collection all around the tip with high numerical aperture. In Fig. 5.6, the focusing lens is shifted indicating that the laser beam only partially covers the NA 0.7 focusing objective. Thus, the laser focus would increase in diameter, but partially hit the tip sideways. One effect would be the higher amount of light reaching the apex under high angles and thus a higher longitudinal field component.
By combining this shifted illumination with a polarization once along the tip axis and once perpendicular to it, even a conclusion about the magnitude of this effect could have been made. Yet, the change due to this shift was if at all only a small reduction of the shadowing of the tip onto the sample underneath the tip. No significant TERS enhancement could be observed in this configuration.

5.6. Approach 3: Side-Illumination AFM TERS

In order to try to locate the source for the previous failures, the excitation and detection channel of the TERS experiment were separated. Using a side illumination with a separate laser path and a low NA focusing lens, one step back towards known TERS setups was taken as illustrated in Fig. 5.7.

The excitation laser was coupled through a glass fiber directly onto a long working distance 0.2 NA aspherical lens to focus light onto the tip end from the side leading to an enlarged focal spot size. The adjustment of the laser focus had to be realized by micrometer screws. The expected advantages of this approach were an illumination from the side, certainly not blocked by the cantilever or the tip itself and thus better illumination of the apex of the tip. When illuminating a tip from a lower angle, the polarization of the light is largely along the direction of the tip (perpendicular to the surface) and should thus more efficiently excite the surface plasmon polaritons traveling along the tip towards the apex. This should result in a stronger electromagnetic field in the gap between tip and surface. The influence of the polarization could be quantified in this configuration by changing the polarization of the Gauss beam from perpendicular to the surface to parallel to
5.6. Approach 3: Side-Illumination AFM TERS

Figure 5.7.: (a) Photographic image of the side illumination attempt with top detection. Fiber optical laser coupling, directly into a NA 0.2 long distance focusing lens with a 532 nm laser. (b) Raman image of the 520 cm⁻¹ Si band from a silicon wafer illuminated by the side illumination show a focus size of ~ 5x10 μm. (c) Schematic illustration of the side-illumination TERS setup with top collection. Scheme partially supplied by NT-MDT.
the surface and compare the EM field in the gap in both cases.

Several disadvantages were incorporated into the system by the implementation of a side illumination:

- No shutter for the excitation beam: as the excitation was directly fiber coupled from the laser to the tip, no mechanical shutter was present in the setup. Short or defined acquisition times were still possible using gated detection, but the thermal strain on the sample increased by the continued laser irradiation.

- Reduced accuracy for laser focusing: the focused laser beam could only be moved manually via micrometer screws instead of the far more precise piezo mirrors in the system. As well, during vertical displacement of the fiber for beam focusing, the laser focus did not move perpendicular but in an anged fashion in respect to the sample surface.

- Larger, unsymmetrical laser focus (shown in Fig. 5.7): due to the smaller NA used and the angle in which the light beam was introduced, the laser focus did not only widen but also turned elliptical. The focus size was roughly 5 μm along the short and 10 μm along the long axis.

- Mechanical instability: the long fiber and the screw on the retainer for the side illumination reduced the overall stability of the illumination. Even after exchanging the optical head for a revised 2nd generation model with increased stability by an additional retention screw, the slightest touch on the fiber or the optical unit would result in visible shifts of the excitation laser.

The side illumination approach resulted in a number of drawbacks and decreased system stability but did not yield a performance boost of the TERS signals as expected. This way the excitation could be ruled out as the main source of error in the setup. Thus the entire side illumination approach was abandoned and the detection pathway was targeted in more detail as the most probable remaining error source.

5.7. Approach 4: Top-Illumination STM TERS

After several changes of the illumination geometry, the most likely, remaining cause for the lack of TERS enhancement was a blocking of the light path by the AFM cantilever even though slim 'top-visibility' cantilevers have been used. To minimize the blocking of light and furthermore make use of additional gap-mode enhancement, the AFM distance control was discontinued in favor of STM distance control. Etched STM tips feature a smaller cone angle than the 'top-visibility' AFM tips,
thus block smaller amounts of incoming and Raman scattered photons. The advantages of STM are a very accurate distance control, coupled with high resolution structural information from the surface and, as the distance control works on metal surfaces, the presence of very localized excitation fields in the gap between tip and sample. The increased amount of free electrons in a pure metal tip and the better conductivity than a vapor coated metal film on Si could increase the lightning rod effect of the etched STM tip compared to an AFM tip. To realize STM distance control, a specialized STM holder allowing displacement of the STM tip using micrometer screws has been produced in collaboration with NT-MDT Russia. Using this holder, the STM tip can be coarsely positioned to be within the scanning range of the piezo driven laser scanners. These piezos are subsequently used to precisely align the laser beam to the tip apex. To ensure sufficient conductivity and flatness of the sample, TS gold (compare section 3.2.3) was used for an initial proof of concept. Later, the use of substrates was expanded to various other metal surfaces adopting to specific sample requirements (compare chapters 7 and 9).

5.8. First TERS Results and First TERS Imaging

First single point STM-TERS spectra were acquired in June 2009. In Fig. 5.8 signals from a TERS experiment on BCB on Au are displayed, showing a spectroscopic contrast between the confocal background signal and the enhanced TERS spectrum in the order of 60.

First STM-TERS imaging results were acquired in August 2009 on a spincoated sample of Nile blue on template stripped gold. In Fig. 5.9 the STM height signals in red is overlayed with the intensity of the main NB band in green showing inhomogeneous nanoscale distribution of dye molecules on the surface.

5.9. Unused Instrument Potential

Several options of the instrument are available but were either not in use or are not installed at the moment. An installed single photon counting PMT with wavelength selection via an exit slit can be used very efficiently for fluorescence detection. A further potential option for more precise scanning in the nanometer range would be the installation of a smaller piezo scanner with 10x10x1 \( \mu m^3 \) scanning range. These sample scanners offer smaller sample drift and better positioning reproducibility at the cost of reduced range. The instrument also allows more complicated SPM methods with one or several passes over the sample to probe specific other surface properties (magnetic, electric, surface potential, etc.).
Figure 5.8: Spectra from the first successful TERS enhancement of BCB on a Au surface collected with this setup. 10 s background spectrum (black) and 1 s TERS spectrum from the tip (red) in direct comparison exhibit a clear signal contrast of around 60.

Figure 5.9: First TERS imaging results acquired in August 2009 with high pixel number of 200x200 and a step size of 2 nm/pixel on a sample of spincoated NB dye on a template stripped Au film. The overlay shows the NB signal intensity in green and the STM height signal in red from the same sample area. The NB intensity is localized mainly in the trenches of the image where more dye is expected to remain during spin coating but also partially covers the elevated areas.

We present a new top-illumination scheme for tip-enhanced Raman spectroscopy (TERS) in a gap-mode configuration with illumination and detection in a straight-forward fashion perpendicular to the sample surface. This illumination focuses the light tightly around the tip end, which effectively diminishes far-field background contributions during TERS measurements. The setup maintains the entire functionality range of both the scanning probe microscope and the confocal optical microscope of the setup. For the first time, we show large (64x64 up to 200x200 pixels), high resolution TERS imaging with full spectral information at every pixel, which is necessary for the chemical identification of sample constituents. Using a scanning tunneling microscope (STM) tip and feedback, these TERS maps can be recorded with a resolution better than 15 nm (most likely even less, as discussed with Fig. 6.5). An excellent enhancement (∼ 10^7x, sufficient for detection of few molecules) allows short acquisition times (<< 1s/pixel) and reasonably low laser power (in the µW regime) yielding spectroscopic images with high pixel numbers in reasonable time (128x128 pixels in < 25 min). To the best of our knowledge, no Raman maps with similar pixel numbers and full spectral information have ever been published.

6.1. Introduction

The necessity to gain chemical information on the nanometer scale has steadily increased due to nanometer sized production in industry and increasing general
interest in nanometer sized structures. Structural information is nowadays easy to acquire using high resolution techniques such as atomic force microscopy (AFM), scanning tunneling microscopy (STM), scanning electron microscopy (SEM) or transmission electron microscopy (TEM). Yet these techniques are limited in terms of chemical information and also by the need for vacuum and electron beam stability of the sample. Vibrational spectroscopy offers more insight into the chemical properties of samples showing their molecular or lattice vibrations. Unfortunately optical spectroscopic techniques are limited in their spatial resolution, and, in the case of Raman spectroscopy, by the weak signal strength. Due to optical diffraction, the resolution is limited to roughly half the wavelength of light unless special near-field techniques are used.

In this article we consider a near-field technique invented in 2000\cite{23-25,161}, tip-enhanced Raman spectroscopy (TERS), as a tool to access information about the chemical composition on the nanometer scale. The metallic STM\cite{75} or metalized AFM\cite{23,162} tips can be used to confine and enhance the electromagnetic field from the illumination laser beam focused to the apex of the tip. The confinement allows spatial resolution down to tens of nanometers and the enhanced field aids to compensate for the intrinsically small cross section of the Raman process. With additional signal from the sample, much shorter acquisition times and a use of limited laser power are possible, reducing the strain on the sample.

Here we introduce a new optical configuration with an illumination directly from the top and an STM distance control, which allows the use of nontransparent samples while still maintaining a high numerical aperture (NA) and an approximate rotational symmetry of illumination. In comparison to side illumination TERS setups\cite{54,70,163,164}, the high NA of 0.7 reduces the background signals and confines the laser energy more efficiently to the tip. In comparison to epi-illumination TERS systems\cite{23,75,165}, the top-illumination/collection system is not limited to transparent samples but allows observation of opaque samples as well.

Until now, two working approaches and one proof of concept have appeared in literature for studying opaque samples by TERS, the side illumination setups mentioned already in\cite{54,70,163,164} and an approach based on a parabolic mirror (PM) as a focusing element\cite{82,84,85}. The PM has the advantage of providing a NA up to 1 and is thus superior in terms of focusing capability. However, the alignment of the beam and the PM is very delicate and difficult. Slight angular misalignment defocuses the laser beam significantly and renders the system unusable\cite{166}. Hence, this optical configuration is not widely used: only two research groups (Meixner, Pettinger) have successfully employed this approach for near-field experiments. The only working top illumination setup with lens based focusing previously described\cite{90} had a far lower NA and a special higher order laser mode.
(the so called "doughnut" mode) in combination with specially designed (resolution limiting) glass fiber tips on a tuning fork in AFM tapping mode feedback. Schultz et al. were able to present near-field signals with a contrast of around 2 and a resolution of 200 – 300 nm, which may have been limited by the increased background from the small NA used. The low enhancement only allowed determination of the near-field signal after subtraction of a far-field spectrum. We show here that strong near-field enhancement in top illumination can be achieved directly with standard Gaussian mode lasers and standard etched full-metal STM tips in the gap-mode configuration. The term 'gap-mode TERS' describes the use of the highly enhanced electromagnetic field, confined in between a metal tip and a metal surface, as an excitation source. This field is enhanced by an induced image dipole in the substrate, the lightning rod effect and surface plasmon resonance. The emitted Raman signal from the sample is enhanced again by the tip and scattered into the far-field for detection.

With this setup, we are able to show full TERS maps with a resolution better than 15 nm and acquisition times as short as 0.05 s per pixel. These short acquisition times can be realized based on an enhancement factor of $>10^7$ and allow maps of, for example, 128x128 pixels in less than 25 min.

6.2. Experimental

All experiments in this paper were conducted on a combined STM/Raman spectrometer (Ntegra Spectra, NT-MDT, Zelenograd, Russia, schematic see 6.1). The instrument consists of a 100x100x10 µm$^3$ piezo scanner for sample movement, controlled by an STM feedback unit, and allows full visibility of tip and sample simultaneously. The tip is held at a 30 – 50° angle with respect to the sample surface, can be positioned laterally and is approached by moving the sample into tunneling contact. The STM tip holder unit has been custom designed to fulfill these needs. From the top, a white light source with a CCD camera offers a magnified live view of the sample. This CCD can be used to monitor the sample, place the tip directly onto sample areas of interest, and coarsely align the laser to the tip. The incoming laser light from a linearly polarized 632.8 nm, 15 mW He-Ne laser is focused by a long working distance objective (100x NA = 0.7, Mitutoyo, Kanagawa, Japan) and can be scanned over the sample surface using piezoelectrically driven mirrors. Light emitted or scattered from the sample is collected by the same optics, separated by an edge filter, and directed into a quadruple grating Raman spectrometer (integral part of the NT-MDT system). The photons from the selected part of the spectrum are then acquired by an electron multiplying CCD (EMCCD), which is Peltier cooled to $-90 \degree$C (Newton 971 UVB, Andor, Belfast, UK).
Figure 6.1.: Schematic of the instrument (Ntegra Spectra, NT-MDT, Zelenograd, Russia): top-illumination TERS instrument with a 100x 0.7 NA objective (Mitutoyo, Kanagawa, Japan), 100x100x10 μm scanning piezo, 4 way grating turret with 150, 600, 1800 l/mm and echelle grating, EMCCD (Newton 971UVB, Andor, Belfast, UK), white light illumination, live view CCD, 632.8 nm He-Ne and 532 nm solid-state laser. Inset: enlarged view of the angled tip on the sample surface and the focused laser beam.
6.2. Experimental

For vapor coating of metals, a Bal-Tec MED 020 vapor coating chamber was used. Solvents were purchased from various suppliers (incl. Aldrich, Fluka, Acros) in analytical grade purity. As analytes, brilliant cresyl blue (BCB, Fluka) and Nile blue A (NB, Fluka) dyes, dissolved and diluted in methanol were used.

As a conductive sample support for STM, small squares of freshly cleaved mica were coated with 100 nm of gold (Bal-Tec, 99.99%) by resistive heating, using a deposition rate around 0.1 nm/s at pressures below $10^{-5}$ mbar. The structure of the gold coating was determined by AFM and STM and showed corrugation of less than 5 nm over the entire, otherwise homogeneous surface. Gold surfaces prepared in this way were then spin-coated with a solution of $5 \cdot 10^{-5}$ mol/L of BCB or NB in methanol. Any roughness of the gold film can lead to surface enhanced Raman (SER) activity. However, the Au surfaces did not show any SER activity.

For production of the STM tips, silver wire with a diameter of 0.25 mm and 99.99% purity (Aldrich) was sharpened by electrochemical etching. As an etchant, a mixture of 1:2 (v/v) perchloric acid (Riedel-de Haën, Germany) : methanol was prepared. The metal wire was cut to an appropriate length and immersed into the etching solution together with a 1 mm diameter gold wire loop as a counter electrode. A voltage of 10 V was applied between the electrodes and switched off by a custom made circuit\[144,145] for cutting the power. The etching was switched off within 10 ms upon a steep drop in current, which appears due to the formation of the tip and the loss of the immersed part of the wire. After etching, the tips were rinsed with methanol and checked visually under a Nikon 360x stereo microscope. Tips with a diameter of $< 100$ nm could be produced routinely, as shown in Fig. 6.2. This tip etching procedure yields tips that combine a sufficient sharpness and high enhancement with reasonable stability for STM scanning. For the TERS experiments, freshly etched silver tips were used and, unless otherwise stated, less

![Figure 6.2: SEM image of silver tip showing a radius of curvature at the apex of about 75 nm. (a) Overview showing pencil like shape of the tip, and (b) tip end; the black circle corresponds to a diameter of 75 nm.](image-url)
6. Nanoscale Chemical Imaging Using TERS

Figure 6.3.: 1086x758 nm STM scan of a flat highly ordered pyrolytic graphite (HOPG) surface with an etched silver tip, 2 nm step size. The image shows steps of graphite sheets on the HOPG surface with 0.67 and 1.17 nm height, corresponding to 2 and 4 layers of graphite. Graphic: line profile over the graphite sheets with a noise level of < 0.5 nm and a lateral resolution of < 10 nm during the scan (linear correction applied).

than 15 µW of laser power were focused onto the sample.

All Raman spectra were exported in ASCII format without any background correction for evaluation in Origin 8 (Origin Lab Corp., USA), 3D images were exported and evaluated using SPIP 5.0.1 (Image Metrology A/S, Denmark) and all 2D images were processed in the vendor software directly (NT-MDT, Zelenograd, Russia). For STM data, a linear line-by-line height correction was usually applied to compensate for drift during acquisition.

6.3. Results and Discussion

To determine the quality of the STM imaging, a sample of highly ordered pyrolytic graphite (HOPG) was scanned at 2 nm/pixel resolution (see Fig. 6.3). The scan shows steps with heights of 0.67 and 1.17 nm closely matching 2...3 layers of graphite (the height of freshly exfoliated graphite layers is 0.335 nm\textsuperscript{[168]}), which are clearly resolved. The line profile also documents an average roughness < 0.5 nm in z direction. Judged by the width of the steps in the line profile, a lateral resolution of < 10 nm was determined. Consecutive scans showed the reproducibility of the scans and a drift of 2.2 nm/min within 2 h. Sample drift strongly depends on the nature of the sample, the room temperature, as well as on the type of sample fixation and can thus vary between measurements.

The standard procedure for recording a TERS map involves the following steps: First, the etched tip and the laser have to be aligned. Therefore the tip is placed
Figure 6.4.: (a) 5x5 µm Raman map of the area around the tip in contact with a spin-coated sample of BCB (10 µL 5·10⁻⁵ mol/L) on gold, obtained by scanning the laser over the stationary tip in tunneling contact (tip introduced from the right hand side). Crosses indicate the locations of spectra reported in (c). (b) Intensity profile along the white line in (a), showing a focus size of 600 nm in diameter. (c) TERS spectrum taken at the center of the tip on the surface (red) and Raman spectrum obtained at an unenhanced area (blue) showing a contrast of > 20; 1 s/spectrum.

onto a homogeneous sample area and the laser focus is scanned over the tip using the piezoelectrically driven scanning mirrors. This results in a spectral intensity map from which the best alignment for tip and laser can be determined (see Fig. 6.4). Until present this had to be done manually via trial and error or guided by external microscopes in existing gap-mode configurations, with a far greater margin of error and frequent loss of signal [54,58,79]. With this mapping mode, a quick, easy, and reliable alignment is possible. Figure 6.4(a) illustrates this procedure: it shows enhancement by the tip while illuminated by the confocal laser, a slightly elongated circular shape (the tip was introduced from the right hand side in this image), and (b) the spot size of the focused laser of ~600 nm in diameter. The laser focus is aligned to the center of this spot (indicated by a red cross in Fig. 6.4(a)) and the sample is subsequently scanned underneath the fixed tip and laser position. During this scan, a complete Raman spectrum is acquired at every pixel. These spectra are saved as a 4D data set consisting of x,y position, spectral shift as well as the Raman intensities. This 4D map can then be reviewed spectrum by spectrum, or intensity maps of certain marker bands can be displayed revealing typical signatures of different chemicals.

When Raman maps are recorded, additional information is obtained, such as the topography from the piezo scanner, the error signals from the STM feedback, and the Rayleigh scattering intensity of the surface. The combination of a Raman intensity map and topography information is shown in Fig. 6.5. It can be used to correlate the topography and the chemical composition of the sample with high lateral resolution. The map in Fig. 6.5 was acquired on a 45 nm-thick gold film, spin-coated with 5 µL of 5·10⁻⁴ mol/L NB together with 5 µL of 5·10⁻⁵ mol/L
of BCB. Due to the similar Raman cross sections of these two compounds, the spectra mainly show the signals from the more concentrated NB. Fig. 6.5(a) shows the topography from the STM feedback during acquisition, Fig. 6.5(c) the Raman intensity of the band at $590\text{ cm}^{-1}$, and Fig. 6.5(b) is an overlay of the STM height (orange) and the Raman intensity in green. The scan direction in the map was from top left to bottom right. The areas of increased Raman intensity form coherent patterns within several lines of the scan, hence they cannot be attributed to random or short term fluctuations of the signal. The Raman intensity on the surface appears homogeneous in a confocal Raman scan, but when increasing the resolution using the TERS tip, we observed an increased Raman intensity of analytes inside small trenches ($2 - 3\text{ nm}$ depth) of the gold surface. The z-distance dependence of the Raman signal is very strong due to the strong localization of the electromagnetic field to the tip end and the coupling of tip and sample. Experiments and theoretical calculations have shown gap-mode enhancement to decay strongly within $5\text{ nm}$ and vanish within $15\text{ nm}$\cite{59,94,169,170}. Thus, the increased signals could at first glance be attributed to a movement of the TERS tip in z direction, i.e., due to to an improper STM feedback. However, in the circled regions, two topographically flat regions exhibit a strong difference in the Raman signal, contradicting that the intensity changes arise from a change in tunneling distance. Therefore, we attribute the intensity changes to concentration differences. In other words, not only nanoscale chemical identification but also quantitative information is available from these maps. The graph in Fig. 6.5(d) shows line profiles of the topography and the Raman intensity at the same position (indicated by the white line in Fig. 6.5(a)-(c)), clearly demonstrating two things: first, the highest intensity of the Raman signal is located directly over the shallow trench in the surface and second, the resolution of both STM image and Raman map in this measurement is well below $15\text{ nm}$. When comparing this resolution to the size of the STM tip (around $75\text{ nm}$ in diameter), the field enhancement in the gap is confined to only a fraction of the tip apex, clearly confirming its near-field origin (similar observations have been made experimentally from Hartschuh et. al.\cite{57} as well as calculations by Roth et. al.\cite{78} support this).

Not only the resolution but also the time required to record a TERS map is important, as the stability of the system (drift of sample, tip and laser alignment) limits the acquisition time. The TERS map in Fig. 6.5 consists of $64\times64$ pixels with a $2\text{ nm}$ step size (a $200\times200$ pixel image with $2\text{ nm}$ step size is shown in the supporting information). The step size in nanometer per pixel is not related to the optical resolution of the image but only indicates the traveling distance of the tip in between two recorded spectra. It is only limited by the positioning ability of the scanning piezos (well below $1\text{ nm}$ per pixel could be used). Both TERS maps
6.3. Results and Discussion

Figure 6.5.: TERS map of a 128x128 nm area of a spin-coated sample of 10 µL, 5 \times 10^{-5} \text{ mol/L BCB} and 5 \times 10^{-4} \text{ mol/L NB on gold, 64x64 pixels, 0.05 s per point. (a) STM image obtained during the scan. (b) Overlay of Raman intensity at 580 \text{ cm}^{-1} \text{ green, translucent) on the height signal (red). (c) Intensity of the 590 cm}^{-1} \text{ marker band of NB. (d) Height and intensity profile along the white line shown in (a)-(c) showing higher Raman intensity in a trench; white circles show areas of high and low Raman intensity without obvious topographic difference, ruling out distance fluctuations as origin of the intensity changes.}
were recorded with an acquisition time of 0.05 s per spectrum, showing that with a sufficiently 'hot' tip, short map times and high resolution Raman maps can be achieved. The overall time for a 128x128 pixel map with an integration time of 0.05 seconds is less than 25 minutes. Due to the much longer time needed, previous tip-enhanced Raman maps reported in the literature were limited in pixel number and, often, by tedious procedures of acquiring single spectra with subsequent manual repositioning of the tip (> 6h for 20x20 pixels\textsuperscript{[171]}, > 10 min for 16x16 pixels\textsuperscript{[172]}, > 22 hours for 64x64 pixels\textsuperscript{[135]}).

An additional benefit from a highly enhancing tip is that the thermal strain of the sample can be limited by using low laser power. The laser power used in the majority of the experiments was as low as 15 µW. Similar results could even be produced using only 4 µW of power, albeit at the cost of a longer integration time (0.5 s per spectrum). This shows that we can choose, depending on the type of sample and the information needed, an appropriate balance between acquisition time, spatial resolution and laser power.

During a series of measurements, targeted to map concentration differences of multiple dyes on a flat surface, consecutively or simultaneously spin-coated with diluted solutions of NB and BCB, temporal intensity fluctuations of the two dye signals were observed. High resolution TERS maps were recorded to investigate fluctuations of the ratio of the NB and BCB bands. In consecutive spectra within a single line and even while continuously monitoring the TERS signal from a parked tip strong fluctuations could be observed. Figure 6.6 shows four spectra taken during a TERS map of a gold surface spin-coated with NB and BCB. The spectra all show the typical bands of BCB (marker bands at 580 cm\(^{-1}\) and 1667 cm\(^{-1}\)) and NB (marker bands at 590 cm\(^{-1}\) and 1643 cm\(^{-1}\)). The spectra originate from locations separated by 2 nm (which is below the previously observed resolution limit), such that only minimal changes in intensity were expected. A drift larger than that can be ruled out due to the short time interval (0.2 s) between the acquisition of the spectra (less than 1 s passed during the acquisition of all four spectra). Nonetheless the band intensities of the 580 cm\(^{-1}\):590 cm\(^{-1}\) marker bands changed from 400:30 counts (red spectrum) to 160:800 counts (blue) to 500:70 (brown) counts and ended up with an intermediate ratio of 700:330 within less than a second. The overall enhancement during this period was fairly stable and cannot account for the large changes in relative intensity of the NB and BCB marker bands. This leads to the conclusion that the identity of the dye molecules within the enhanced area must have changed during the experiment. The possible causes for this behavior are diffusion of molecules on the surface due to laser heating, or the presence of only a few single molecules in an extremely small 'hot zone' under the tip apex. The latter could either be due to a low surface coverage of dye (unlikely, as we estimated a
significantly higher coverage) or, more likely, an even stronger confinement of the field than the 15 nm spatial resolution determined from Fig. 6.5. This would automatically lead to a low number of molecules contributing to the Raman signal, and also imply an even higher resolution and higher enhancement which is necessary to see a few single molecules. This interpretation appears to be reasonable: single molecule sensitivity for tip-enhanced Raman spectroscopy in gap-mode configuration has already been presented in the literature [58,59,173], with high enhancement factors of $10^6 - 10^8$.

To estimate a lower boundary for the field enhancement achieved with this setup, we calculate the enhancement factor using eq. 6.1.

$$EF = \frac{I_{NF}}{I_{FF}} \cdot \frac{A_{FF}}{A_{NF}}$$  \hspace{1cm} (6.1)

This approach considers the fraction of intensity from the far-field ($I_{FF}$) and the near-field ($I_{NF}$) from a single vibrational band (the so-called spectroscopic contrast), and divides it by the areas of origin ($A_{FF}, A_{NF}$), thus representing a signal intensity independent of the number of molecules probed. $I_{FF}$ and $I_{NF}$ can be determined from spectra taken with the tip engaged and retracted (Fig. 6.7) after normalization to counts per second. The resolution of the confocal far-field measurement can be deduced from the line profile in Fig. 6.4(b) as the tip will emit a strong Raman signal as long as it is irradiated by the laser spot. The near-field resolution can be determined from the smallest resolved features (appearing in several lines) in the inset of Fig. 6.5.

The strongest Raman signals (which are often chosen for evaluation) were around 21000 counts in 0.05 s. However, we consider it more correct for the calculations to use the averaged near-field intensity over an entire Raman map, amounting to $\sim 2000$ counts in 0.05 s. The area of the Raman map (512x512 nm) is very similar to the area probed by the confocal laser beam (600x600 nm), which thus probes a similar number of molecules. In Fig. 6.7, Spectra 1-2 were arbitrarily chosen from the map to show the spectral similarity and only slightly fluctuating enhancement throughout the map. The far-field contribution (Fig. 6.7, blue spectrum, taken at the position of the blue cross in Fig. 6.4(a)) yielded 350 counts in 50 s. To check the cleanliness of the tip, a 50 s spectrum from the retracted tip apex was recorded after the scan and is shown in spectrum 4. With a radius of the confocal spot of 300 nm, and a near-field diameter of 15 nm, which are both conservative estimates, we can calculate an average enhancement factor of $9 \cdot 10^6$ over the entire TERS map. Consideration of the strongest measured spectrum with 21000 counts in 0.05 s would have yielded an enhancements of $\sim 10^8$, yet the approach chosen seems more correct to us because the far-field spectrum also represents an average
Figure 6.6.: Four consecutive TERS spectra (adjacent pixels) from a Raman map of NB and BCB on gold (taken within 1s), showing strong changes in the intensity of the BCB and NB marker bands at 580 cm\(^{-1}\) and 1650 cm\(^{-1}\). Left Inset shows fluctuating intensities of the main bands: Pixel 1 - 2 - 3 - 4 yield 400:30 - 160:800 - 500:70 and 700:330 counts respectively. This behavior can be explained by an extremely localized field probing only few single molecules. \(\lambda = 632.8\,\text{nm},\ 15\,\mu\text{W},\ 0.1\,\text{s/pixel integration time, taken from a Raman map with 2nm step size and 128x128 pixels.}\)

Figure 6.7.: Raman spectra from a Raman map of NB on a flat gold surface. (red/orange) 2 random spectra, 0.05 s each on tip showing the spectral similarity within the maps. (blue) 50 s spectrum from the sample (tip retracted) to calculate the confocal Raman signal. (black) 50 s spectrum with the illumination laser focused on the tip end (tip retracted, after measurement) to check for cleanliness of tip after the measurement.
over this entire area. This difference shows that for the calculation of enhancement factors, the sample has to be selected very carefully; concentration differences on the sample can lead to strong overestimation of the enhancement.

To the best of our knowledge, no full spectral maps with similarly high pixel numbers have been presented in the literature. All previously presented Raman maps either were of much lower pixel number \[61,137,138,141,172\] or were from selected spectral bands only \[90,174,175\]. The work herein represents a very important step for tip-enhanced Raman spectroscopy, allowing the exact localization of substances due to their spectroscopic signature and concentration differences from their signal strength.

Top illumination in TERS offers a series of advantages and can in most cases compete with or outperform the results from known instruments. It combines the advantages of side and bottom illumination TERS instruments and is only limited by the need to work with conductive samples at the moment (STM feedback). An enhancement of \(~ 10^7\) (conservative estimate) is in line with the highest reported enhancement factors from the literature which range from \(10^5\) to \(10^7\) \[61,176\]. At this point it is necessary to mention that due to the different approaches of determining the enhancement factor, these values are not perfectly comparable and should at some point be redetermined in a standardized way \[71\].

The resolution of our instrument was determined to be better than 15 nm, which is also a conservative estimate as we took the full width of the smallest features in the measurements. Other authors use the step height or the full width at half maximum, which would yield about half of this size. Still, our value compares well to existing resolutions in the literature which are on the order of 15 to 20 nm \[61,141,177\]. Recently a value of 4 nm was published by Kawata et. al. \[136\]. However, in this special case, the resolution does not result directly from the illumination, but is the resolution of an induced band shift due to pressure exerted by the AFM tip. This limits their approach to molecules prone to exhibit a band shift upon exerted pressure and have limited choice of marker bands.

Most systems using gap-mode TERS achieve a high enhancement, but use a side illumination approach with illumination from a \(60 - 70^\circ\) angle with long working distance objectives with a NA in the range of 0.3 - 0.5 \[66,178\]. Due to the flat illumination geometry and the low NA, the far-field illumination region is large and the laser energy is less localized. The less localized intensity leads to higher background in the spectra and requires higher laser powers. This can lead to bleaching of the sample around the tip or photochemical degradation of the sample. The configuration presented here, with a circular top illumination not only reduces the laser spot size for less background but also confines the laser energy far more efficiently for tip excitation. Additionally, it later on offers the possibility to switch from a Gaussian
mode laser beam profile to the so-called doughnut modes for a strongly localized on-axis polarization component which could help excite the tip surface plasmons more efficiently.

Our system offers the full features of confocal laser scanning Raman microscopy, confocal Raman spectroscopy and back reflection laser intensity measurements. It allows a direct view of the sample for rapid identification of larger sample features and simpler laser alignment.

To conclude, we could show that it is possible to do gap-mode TERS imaging with silver tips on a gold surface using an STM feedback in a top illumination and top collection configuration. This setup achieves a resolution of $\leq 15\, \text{nm}$ both in STM and Raman mode and the enhancement factors compare to the best ones shown in literature. The necessary laser intensity has often been neglected until now, but here we can show the use of very low laser powers, in the low microwatt range, for the acquisition of clear Raman spectra in split seconds. This allows for the collection of high resolution Raman maps with the full spectral information in every pixel for chemical identification. We expect this to have strong influence on studies in the field of carbon nanotubes on metal, catalysis, molecular electronics or self-assembly on metal surfaces.

6.4. Acknowledgements

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7. Characterizing Unusual Metal Substrates for Gap-Mode Tip-Enhanced Raman Spectroscopy

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Johannes Stadler, Benedikt Oswald, Thomas Schmid and Renato Zenobi:
'Characterizing Unusual Metal Substrates for Gap-Mode Tip-Enhanced Raman Spectroscopy'.

In this article, the electromagnetic field in gap-mode tip-enhanced Raman spectroscopy (TERS) is investigated theoretically and experimentally for a range of commonly used and unusual metal and non-metal substrates. By approaching a metal tip to a substrate, both form a coupled system that confines the electromagnetic (EM) field created at the tip apex. The influence of the substrate onto the electromagnetic field enhancement is observed in a top-illumination gap-mode TERS setup for different metal substrates. These include Au, the most commonly used substrate but also a wide range of sparsely or previously unused TERS substrates (Cu, Ag, Al, Pd, Pt, Ni, Ti, Mo, W, stainless steel, Al₂O₃, SiO₂). Self-assembled monolayers of thiols and brilliant cresyl blue thin film samples are investigated experimentally on nine metal substrates, all showing considerable TERS enhancement. Using finite difference time domain (FDTD) and finite element (FE) simulations, the article provides a good estimate of the electromagnetic field enhancement for a wide range of substrates, for users to estimate how well a substrate of choice will perform in a gap-mode TERS experiment. The reduction in electromagnetic field strength $|E|^2$ compared to Au is less than an order of magnitude for many metals (Calculations: Cu 92%, Ag 81%, Ni 53%). This article experimentally shows that a wide variety of conductive substrates can be used, when one is willing to trade a fraction of the electromagnetic field enhancement. TERS was seen on all metal substrates incl. stainless steel, yet quantification was not always possible. These qualitative results were complemented with intensities from calculations. The wider variety of substrates will increase the applicability of TERS and evolve it one step further towards use in standard analytics.
7.1. Introduction

Tip-enhanced Raman spectroscopy (TERS) is a powerful emerging analytical tool for chemical analysis on the nanometer scale. It was developed simultaneously at the beginning of the century in the labs of Zenobi[23], Anderson[24] and Kawata[25]. TERS on the one hand benefits from the large information content of a spectroscopic technique, i.e., allows identification of molecules from their vibrational fingerprint spectrum. Yet, in contrast to conventional Raman spectroscopy it surpasses the two major drawbacks of Raman, i.e., the bad sensitivity due to the intrinsically small cross section of the Raman process and the limited resolution due to diffraction of light. This is achieved by introducing a metal or metal coated sharp tip into the illumination laser focus, and approach it to within a few nm of the sample. Several distance control methods can be used to control the position of the tip. In this approach, we employ scanning tunneling microscopy (STM) for which a conductive substrate is a prerequisite. Upon illumination of the TERS tip with a focused laser beam, a dipole is induced in the tip apex. Due to the the proximity of the tip to the metal substrate, an image dipole is induced in the metal. The strong, very localized electromagnetic field in the gap between the tip and substrate is used as a nanometer-sized light source for the experiments, hence the name gap-mode STM-TERS. In almost all published gap-mode STM-TERS results, Au has been used as a substrate. It combines several advantages, e.g., chemical inertia, sufficient substrate flatness[148] as well as small losses in the visible optical region. Using Au surfaces, an efficient enhancement can be reached in conjunction with etched full metal tips from Ag[58,112,113] or Au wires[114,116,117]. Yet, the production of very flat and pure Au substrates is cumbersome and expensive. Limitation to gold substrates is a drawback due to incompatibility with certain samples and also negates the possibility to investigate samples directly during production on their native substrates. In this article, the impact of the substrate metal on the TERS process should be investigated experimentally as well as theoretically. Previous calculations mostly investigated the influence of the dielectric constant of the tip[124], the shape[179] or the influence of the tip-sample distance[78,92,95] on the electromagnetic field in the gap between the tip and the sample. Calculations on the intensity of the EM field as a function of the dielectric constant have been presented by Zhang et al.[172] using a simplified single oscillator dipole model. For three different substrates, Au, Pt and SiO$_2$ coarse field simulations with tip were presented by Yang et al.[92]

Experimentally, Au is used in most experiments as sample support for STM-TERS experiments. It has been used in side illumination and top illumination experiments, with both, objective lenses and a parabolic mirror (PM) as focusing element[61,73,137,144,173,180]. Only a few single examples can be found in the liter-
7.2. Materials and Methods

Confocal Raman spectra were collected on a combined AFM/STM instrument that incorporates a quadruple grating Raman spectrometer (Ntegra Spectra, NT-MDT, Zelenograd, Russia), and an EMCCD camera (Newton 971UVB, Andor, Belfast, UK). The tip-enhanced Raman data was acquired in the top-illumination and top-collection gap-mode configuration, as described in detail by Stadler et al.\textsuperscript{[73]}

In TERS experiments, the excitation laser (632.8 nm, Helium-Neon) was carefully focused onto the approached STM tip. Subsequently, the laser was scanned over the contact area of tip and surface, and the TERS enhancement was used to optimize the overlap of the confocal laser beam with the TERS tip. The laser power at the sample in experiments with brilliant cresyl blue (BCB) was 15 µW, with thiophenol (PhS) 100 µW were used (unless specified otherwise). A collection time of 1 s per spectrum was used in imaging TERS experiments. For confocal Raman measurements, spectra from BCB were recorded, but with longer integration times. For PhS, spectra from the non-enhanced area in the TERS images were used for
7. Characterizing Unusual Metal Substrates for Gap-Mode TERS

Tip Production

STM tips were etched from silver wire with a diameter of 0.25 mm (99.99% purity, Aldrich). As an etchant, a mixture of 1:2 (v/v) perchloric acid (Riedel-de Haën, Seelze, Germany) : methanol was prepared. The metal wire was cut to an appropriate length and immersed into the etching solution together with a 5 cm loop out of 1 mm platinum wire as a counter electrode. A voltage of 8 V was applied between the electrodes, and switched off by a custom made circuit[144,145]. The etching was terminated within 10 ms of a steep drop in current, that takes place when the apex forms and the immersed part of the wire detaches. After etching, the tips were rinsed with methanol and visually inspected using a Nikon 360x stereo microscope. This tip etching procedure yields tips that combine sufficient sharpness and high enhancement with reasonable stability for STM scanning. For each TERS experiments, freshly etched silver tips were prepared unless stated otherwise.

Metal Films

Gold films were produced by coating 150 nm of gold onto silicon wafers, followed by a template stripping process similar to the one described by Hegner et al.[148] For silver and copper films, 150 nm of metal were deposited by physical vapor deposition onto a silicon substrate, previously cleaned in piranha acid. The following pure metal substrates were purchased (MTI KJ Group, Redmond, USA) and used as received: Al(111) (single crystal), Mo (polycrystalline), Ni (polycrystalline), stainless steel (SS301, polycrystalline), Ti (polycrystalline), and W (polycrystalline).

Sample Molecules

In separate measurement series, two types of samples were investigated. In the first series, a thin film of brilliant cresyl blue (BCB, Fluka) was deposited by spin coating 10 µL of a methanolic 5x10⁻⁵ mol/L BCB solution onto flat metal substrates: Au, Ag, Cu, Mo, Ni, stainless steel, Ti, W and Al. Spin coating is known to produce homogeneous layers on flat substrates, but as shown in[173], the nanoscale distribution can be heterogeneous and differ between samples but also between locations within the same sample. To eliminate the influence of inhomogeneities as an error source, thiophenol (PhS, Acros Organics) deposited directly on the TERS tip was used to probe the EM field in a second measurement series. Thiols are known to bind very strongly to metals and form self assembled monolayers (SAM). To keep the monolayers identical between measurements on several substrates, the SAM
was produced directly on the tip by dipping the freshly etched tip into a solution of \(10^{-4}\) mol/L PhS in EtOH for 30 s and subsequent rinsing with EtOH. In this way, the same SAM could be used for experiments on both the different metals as well as on the Au reference. Potential bleaching due to decomposition of the thiol molecules was investigated under irradiation with far stronger laser light (\(> 1\) mW) and found to be negligible.

**FDTD Simulations**

For 3D FDTD simulations, a commercial FDTD solver was employed (FDTD solutions, Lumerical Solutions). To correctly and efficiently simulate the near-field region, a truncated tip was positioned 1 nm above the metal substrates and excited by a focused Gaussian beam from the back. The simulated region had a size of 500x500x1500 nm\(^3\) with an adaptive conformal mesh and voxel sizes ranging from approx. 20 nm\(^3\) in the outer regions down to 0.1 nm\(^3\) in the gap between tip and sample (see. Fig. 7.1(a)). The simulation time was 50 fs. Detailed parameters for the tip are a 10 nm cone radius, 10° cone angle, and illumination from 1 \(\mu\)m distance to the tip apex with a \(\sim 4\) fs, 632.8 nm Gaussian beam pulse. The focusing of the beam pulse was simulated using a thin lens approximation with a \(\varnothing 5\) mm 0.7 NA lens and a \(\varnothing 3\) mm beam consisting of 500 plane waves to a size of approx. 650 nm forming our excitation focus shown in Fig. 7.1(b)). The metals for the tip and the surfaces are calculated using a dielectric constant fitted to tabulated data (CRC\(^{[185]}\) for the surface metals and Palik\(^{[186]}\) for the silver tip) at the simulation frequency. A conformal mesh was used in the calculation to better account for the change of refractive index in the metal-air interfaces within voxels.

**Finite Element Calculations with the Hades3D Code**

We employed the computational electrodynamics code Hades3D to analyze a detailed three-dimensional model of the TERS setup in the time-harmonic regime. The Hades3D code\(^{[187]}\) uses the latest Discontinuous Galerkin (DG) method and solves the electric field vector wave\(^{[188]}\), aka. `curl-curl`, (eq. 7.2) in the frequency domain/ time-harmonic regime.

\[
\nabla \times \frac{1}{\mu} \nabla \times \vec{E} - \omega^2 (\epsilon - \frac{i\sigma}{\omega}) \vec{E} = -i\omega \vec{J}_0 \tag{7.2}
\]

The Hades3D code discretizes eq. (7.2) on a unstructured, tetrahedral mesh in three spatial dimensions. Using tetrahedral elements allows for a flexible discretization of complicated, curved geometry avoiding the staircasing effect. In addition, using tetrahedral elements allows for level of detail modeling where geometrically
Characterizing Unusual Metal Substrates for Gap-Mode TERS

Figure 7.1.: (a) Setup of the 3D FDTD simulation of a Ag tip located 1 nm above a 150 nm thick, flat gold substrate. A Gaussian beam is introduced from top and a mesh refinement towards the gap with down to 0.1 nm$^3$ voxels is used for the simulation. (b) Focus of the Gaussian excitation beam calculated with the thin lens approximation. The focal diameter of approx. 650 nm corresponds well to experimental values.

delicate, but physically immensely relevant geometry needs to be discretized with small elements and larger elements are used elsewhere. When working in the time-harmonic regime, measured complex refractive index data$^{[185]}$ can be used directly. For the simulation of the TERS setup, identical geometry parameters were used for the tip. The simulation setup consisted of the three-dimensional tip that hovered over a hexahedral substrate block with a square cross-section. Here, in stark contrast to the FDTD analysis, the TERS setup was modeled with optimal efficiency. In particular, the TERS setup was fitted into an ovally shaped computational domain, not wasting tetrahedral elements. Even today, given seemingly unlimited compute power, it is essential to model nano-optical setups with the highest possible efficiency, since the solution of the complex linear systems that results from the DG discretization is a challenging task. Our model counted 4 million degrees of freedom, i.e., the linear system was a square matrix with 4 million rows and columns. A representation of the electromagnetic problem setup is shown in Fig. 7.2. The size of tetrahedral elements used for the discretization of the TERS setup comprised several orders of magnitudes, from a fraction of a nanometer, close to the tip, up to several tens of nanometers further away from the tip. The mesh was created with the open source GMSH$^{[189]}$ mesh generation program. The computational domain was transparently truncated with a 1st order absorbing boundary condition (ABC)$^{[188,190]}$. It is one of the strengths of the 1st order ABC to truncate almost any convex geometry in a computational domain; in our case it was an ovally shaped boundary. While the perfectly matched layer (PML) might re-
Figure 7.2: (a) Computational domain of the Hades3D FE calculations with a refined grid towards the tip apex. The green shell represents the outer transparent boundary, minimizing reflections with an optimized shape to reduce computational cost. (b) $|E^2|$ intensity of EM field upon illumination with a 632.8 nm laser from the top.

To reduce spurious reflections even further, its practical application is usually restricted to hexahedral shapes; thus there is a tendency of wasting unnecessary tetrahedral elements. The electromagnetic problem is excited through an incoming transverse-electric-magnetic (TEM) wave at a wavelength $\lambda = 632.8$ nm.

7.3. Results and Discussion

Experimental Determination of the EM Field

To experimentally investigate the EM field in the TERS gap, resonant BCB molecules were used to probe the field intensity. On all surfaces, a thin layer of dye molecules was homogeneously distributed by spin coating. With an approached tip, the laser was then scanned over the contact area. The tip then probes the scanned laser focus, resulting in TERS enhancement when both overlap. At every pixel a Raman spectrum was collected resulting in an enhancement map of an area at and around the tip. The signal collected on the CCD camera is mainly influenced by three factors, the number of molecules contributing to the signal, the enhancement of the tip in use and the influence of the metal substrate on the field in the gap. The distribution of molecules on the surface of the substrate was expected to be homogeneous. To compensate for differences between different metal substrates, confocal reference spectra were collected. To evaluate the Raman intensity in the spectra, the luminescence background of the spectra was subtracted to yield pure Raman contributions. The contrast factor between two Raman bands (confocal
7. Characterizing Unusual Metal Substrates for Gap-Mode TERS

TERS signal) was calculated according to eq. 7.1, to eliminate the influence of the density of molecules on the surface. The contrast factors calculated from two different Tip enhancement maps (metal/Au) were then divided to cancel the influence of the specific enhancement of the tip in use. The relative intensity of a specific metal compared to Au should now be neither sensitive to the tip used nor be influenced by the number of molecules on the surface, and thus represent the pure contribution of the metal surface to EM field strength in the TERS gap.

In Fig. 7.3, two single spectra from within the TERS hot spot of the same tip on a Cu and a Au surface are shown, as well as the corresponding confocal Raman spectra (from the same area, collected with tip retracted after refocusing on the surface). From these four spectra, both contrast factors for Au and Cu can be calculated as shown in eq. 7.3. Thus, based on these four spectra, the contrast of the tip on Cu was 21% of the contrast of the same tip on Au (i.e., the contrast was smaller by a factor of 5). For obtaining the values in Table 7.1 the same approach, with several TERS spectra from the TERS hot spot and background spectra from confocal images, was used.

\[ C_{Cu} = \frac{S_{\text{nearfield, Cu}}}{S_{\text{farfield, Cu}}} = \frac{410 \text{ cts}}{60 \text{ s}} = 154 \]

\[ C_{Au} = \frac{S_{\text{nearfield, Au}}}{S_{\text{farfield, Au}}} = \frac{370 \text{ cts}}{60 \text{ s}} = 740 \quad (7.3) \]

Exemplary Calculation:

\[ \frac{C_{Cu}}{C_{Au}} = \frac{154}{740} = 20.8\% \]

Analogous evaluations were done for Ag, Cu, Ni, Ti and Mo with several tips each, resulting in the TERS(BCB) values in Table 7.1 and the blue triangles in Fig. 7.5. Repetitions of these experiments showed only little reproducibility, as indicated by the large error bars. The cause for this are most likely nanoscale concentration differences on the metal surfaces. This behavior has already been observed in other samples, even if they appear homogeneous in confocal Raman measurements[73].

To eliminate effects due to nanoscale concentration differences when probing the molecules, a self assembled monolayer of PhS was deposited on the etched silver tips. The same tip and thus the identical monolayer was then used in consecutive experiments to again probe the EM field in the TERS gap on Au and other metals. Potential bleaching was found to be negligible, even upon irradiation of the tip with > 1 mW over 1 h. Results from PhS on a Ag tip approached to Ag and Ni are presented in Table 7.1 showing far better agreement with the results from simulations. The data points represent the intensity on Ag and Ni respectively each divided by the intensity of the same tip on Au. The measurements on Ni
7.3. Results and Discussion

Figure 7.3.: (a) 1s TERS spectra from BCB on Au (red) and Cu (red), with the corresponding 60s confocal Raman spectra, all recorded using the same tip. Spectra are offset for clarity and three spikes were removed from the confocal spectrum on Cu. (b) TERS point spectra from PhS on the tip in contact with different metal substrates: Tip on Ag (red), Ni (green) and Al (blue) all showing clear signatures of PhS from the SAM on the tip. Intensities differ due to different enhancements of the tips in use for the respective experiment.

and Ag show signal intensities of 72 and 78% compared to that on Au with the same tip, and thus clearly indicate the suitability of these substrates for TERS (a visual representation for the different metals is given in Fig. 7.5 (supplementary information)). A drawback of using a thiol monolayer as a probe for the EM field is the overall low signal intensity. In many experiments on metal surfaces, specific bands of PhS were detected but could not be adequately quantified due to the high noise level. Although an experimental quantification was not possible in all cases, a similar behavior in TERS with small losses within one order of magnitude for all metal surfaces, can be expected. This is also supported by simulations. For non-metal substrates, far higher losses are to be expected, making conductive Si surfaces or ITO glasses a poor choice for gap-mode TERS experiments. Experiments on non-metal substrates (ITO) support this estimate (data not shown). The gap-mode effect (comparison of a tip in free space without surface and a tip on Au) delivers an additional enhancement of 20-40 in the EM field intensity $|E|^2$, with even stronger impact on the Raman intensities, which increase by a factor of 50 to > 300.

The comparison of the two different sets of TERS experiments on BCB and PhS shows major differences on the same metal substrates. Potential sources for these differences are potential bleaching of the BCB dye, nanoscale differences in the distribution of BCB, but also the location of the PhS molecules on the tip rather than on the surface. Although only a nm wide, gradients in the EM field between tip and surface could account for part of the differences in between experiments.
Table 7.1.: Comparison of the electromagnetic field enhancement in the gap between various metals and a silver tip, using two different simulation techniques and two different experimental approaches. All theoretical values have been normalized to the value of a tip on Au. Experimental values were normalized to the intensity measured on a Au substrate using the same tip.

<table>
<thead>
<tr>
<th>Metal</th>
<th>$E^2_{\text{FDTD}}$</th>
<th>$E^4_{\text{FDTD}}$</th>
<th>$E^2_{\text{FE}}$</th>
<th>TERS (BCB)</th>
<th>TERS (PhS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Au</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Cu</td>
<td>0.92</td>
<td>0.848</td>
<td>-</td>
<td>0.16±0.11</td>
<td>-</td>
</tr>
<tr>
<td>Ag</td>
<td>0.81</td>
<td>0.654</td>
<td>0.559</td>
<td>0.23±0.05</td>
<td>0.77</td>
</tr>
<tr>
<td>Al</td>
<td>0.63</td>
<td>0.402</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pd</td>
<td>0.61</td>
<td>0.375</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pt</td>
<td>0.55</td>
<td>0.297</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ni</td>
<td>0.53</td>
<td>0.281</td>
<td>-</td>
<td>0.07±0.06</td>
<td>0.72</td>
</tr>
<tr>
<td>Cr</td>
<td>0.51</td>
<td>0.264</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ti</td>
<td>0.45</td>
<td>0.199</td>
<td>-</td>
<td>0.12±0.14</td>
<td>-</td>
</tr>
<tr>
<td>Mo</td>
<td>0.44</td>
<td>0.194</td>
<td>-</td>
<td>0.11±0.16</td>
<td>-</td>
</tr>
<tr>
<td>W</td>
<td>0.41</td>
<td>0.166</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Al$_2$O$_3$</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SiO$_2$</td>
<td>0.05</td>
<td>0.003</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tip only</td>
<td>0.05</td>
<td>0.003</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Computational Calculation of the EM Field**

The finite difference time domain (FDTD) method has been developed over the last decade and now reached considerable maturity. Over the course of the years, a wide span of applications has been analyzed successfully with the FDTD approach: from nearly static problems up to the optical region of the electromagnetic spectrum. On the other hand, there is increasing evidence, e.g.\[191\], that the FDTD method is not up to the task of reliably modeling nano-optical problems. Even worse, the publication\[191\] concludes that the FDTD method would not converge to the true solution, due to the staircasing approximation, when discretizing curved geometry, even when the voxels in the Cartesian sampling grid become negligibly small. Thus, FDTD increasingly becomes a side-track method for nano-optics since it struggles with the wide span of characteristic length scales typically encountered there. In marked difference to microwave electromagnetic problems where the size of the elements usually amounts to $\lambda/10$ or $\lambda/20$, in nano-optics, the size of elements easily tends to $\lambda/1000$ or even smaller. Additionally, the radius of curvature for geometry encountered in nano-optics is on the order of a few nanometers only. Consequently, the discretization of geometry needs to adapt. This is where the Discontinuous Galerkin variant\[192\] of the finite element method has its advantages;
given good mesh generation software it is a manageable task to create a mesh with level of detail, whose element sizes varies over several orders of magnitude. Thus, a good approximation of curved geometry becomes available. Solving the resulting discretized system, i.e., a large, sparse complex symmetric matrix, is a formidable task\cite{192} which in itself is an active research topic. However, there is a silver line over the horizon\cite{193} that will soon allow to solve ultra-large linear systems, with several tens of millions of degrees of freedom, efficiently; this will eventually enable the accurate and reliable analysis of realistic structures with confidence. In conclusion, we maintain that the finite element method is the method of choice, when analyzing complicated geometry and material systems is of the essence.

A first set of calculations was designed to reproduce the known field distribution in TERS, to ensure credibility of the FDTD results. Therefore, 3D FDTD field distributions from similar systems calculated in the literature\cite{78} were recalculated using our model. Very similar results were found, as shown in Fig. 7.6 (Supporting Information). In a second step, the influence of the gap distance in our system was systematically varied with a surface approaching the tip from infinite distance down to 0.25 nm. An animation of $|E|^2$ in the x,z plane from the simulation with decreasing distance is given in Fig. 7.7 (Supplementary Information). The behavior of the EM field in the movie compares well to known distance dependence in TERS\cite{78,92,95}. It clearly shows increased fields around the tip apex already without the surface, as well as a further localization and increase of the field upon approach of the metal surface. The reason for better confinement and the rising magnitude of $|E|^2$ is the induction of a mirror dipole in the metal surface by the dipole in the tip apex. A potential explanation for the increased fields could be efficient coupling of the surface plasmons of tip and surface.

Fig. 7.4(a) displays view of a Ag tip in tunneling distance (1 nm) to a gold surface with a logarithmic scale. Surface plasmons polaritons with increasing intensity can be seen on the side of the tip approaching the apex. In the animation in Fig. 7.9 (Supplementary Information) the phase of the calculated finite elements (FE) solution is varied from $-180^\circ$ to $180^\circ$ to illustrate the movement of the plasmons along the side of the tip towards the apex, creating a very strong and localized EM field in the gap. In the linear intensity view in Fig. 7.4(b), the very strong localization of the field leading to increased resolution in TERS is visualized.

The influence of the metal substrate was determined from the maximum intensity of the $E^2$ field in the FDTD and FE calculations. The calculated values are plotted in Fig. 7.5. The numerical values in Table 7.1 show that the loss of EM field intensity, when replacing the most strongly enhancing and most frequently used Au surface by a different metal, is in the region of a factor of only 1.5 to 3 for metals, but considerably higher for non-metals. A visual comparison of the EM-field
distribution in the gap for different metals calculated by FDTD is shown in Fig. 7.8 (Supplementary Information). Both calculation methods and practical experience support this, although minor differences between the calculation methods do remain. When compared to an experimental Raman intensity, the electromagnetic field intensity $|E|^2$ cannot be used directly, but has to be squared, as the measured Raman intensity scales with $|E|^2_{\text{Laser}} \cdot |E|^2_{\text{Raman}}$. Although the frequency of the Raman scattered light changes within the same experiment for different bands, it is still reasonably close to the laser wavelength, as an approximation, both can be considered equal and the enhancement can be calculated as $|E|^4$. The approximation can be one explanation for some remaining differences between experiment and calculations. The more correct approach using the effective Raman wavelength cannot be incorporated in the calculations at present.

7.4. Conclusions

Results from both, experiments and calculations, were used to investigate the suitability of different metal and non-metal surfaces for use in TERS. Using a thin dye film, enhancement could be shown on all experimentally investigated metal substrates, but not yet on non-metal substrates. The experimental quantification of the electromagnetic field turned out to be difficult due to low signal intensity or inhomogeneous distribution of molecules on the nanometers scale. Yet, the experimental proof for the presence of the enhancement can be combined with the numerical values from two independent sets of simulations. Both simulations sug-
7.5. Supplementary Information

guest TERS intensities on metal substrates to be within one order of magnitude of those of Au. This clearly shows that not only Au, but a number of other metal substrates is well suited for TERS experiments and can be used when gold has unwanted specific interactions with the sample (especially when Au−S interactions are undesired), when it is difficult to transfer a sample onto the gold substrate or simply because it is too expensive. The drawbacks of changing the substrate are in most cases a slightly reduced conductivity resulting in a less reliable feedback during STM scanning and occasionally rougher surface topography.

Potentially, two additional parameters could improve the TERS enhancement using metal substrates: on the one hand, using a suitable laser wavelength, the losses from Im(ε) in any dispersive metal can be reduced. At the same time, if wavelength can be optimized to efficiently excite the resonance frequency of the coupled tip-sample system, any sample can be measured more efficiently. Determination of the coupled influence of both these effects using a tunable laser source in the visible range could prove this. Such measurements could furthermore be combined with 3D simulations to quantify the contributions of both effects to the total field enhancement.

Envisioning a more general use of TERS, a wide range of affordable substrates is necessary to allow more widespread application of the technique. If a very cheap, flat, e.g., stainless steel sample support could be used for most experiments where the intensity gain from gap-mode TERS does not have to be optimal, consumable costs for TERS would be greatly reduced. Samples present on any working metal substrate, e.g., for monitoring the production of chemical vapor deposition (CVD) graphene on Cu, could be investigated directly by TERS during production without further processing. This could bring TERS one step further towards an established standard for everyday routine analytics.

Acknowledgements

We want to acknowledge P. Leidenberger as a co-developer of the Hades3D code family.

7.5. Supplementary Information
7. Characterizing Unusual Metal Substrates for Gap-Mode TERS

Figure 7.5.: (black) Calculated $|E|^2$ intensity of the EM field with both calculations, FDTD of all 14 surfaces and some FE calculations (green) using Hades3D. (gray) Calculated Raman Intensity $|E|^4$ from FDTD. (blue) experimental contrast factor $C_{metal}/C_{Au}$ from spin coated BCB, calculated from the hot spots of several TERS images including error bars. (red) Contrast factor $C_{metal}/C_{Au}$ from a SAM of PhS on a tip approached to the respective metal.

Figure 7.6.: Comparison of calculations by Roth et al.\cite{78} (left) and analogous calculations with our FDTD approach (right). Both simulations show virtually identical intensity distributions for all field components. The FDTD approach seems to well reproduce the calculations from the literature and deliver reasonable results.
Figure 7.7.: Animated sequence of field distributions calculated by 3D FDTD from a \( \varphi 20 \text{ nm} \) Ag tip approaching a Au surface, illuminated by a focused Gaussian beam from the back. Upon approach of the tip, the EM field increases by a factor of 50 in intensity and gets spatially compressed into the tip-sample gap. Numerical errors occurring at the curved boundary of the tip are typical for FDTD calculations even though a conformal mesh has been used.

Figure 7.8.: Animated sequence of field distributions calculated by 3D FDTD from a \( \varphi 20 \text{ nm} \) Ag tip approaching different non-metal and metal surfaces, illuminated by a focused Gaussian beam from the back. All intensities of metal substrates are within a single order of magnitude. The general influence of the gap-mode compared to a solitary tip is between one and two orders of magnitude. The numerical values of intensities on single metals are given in Fig. 7.5.
Figure 7.9.: Animated finite elements calculation using Hades3D\textsuperscript{[194,195]} of the EM field in a gap mode configuration. For the animation, the phase of the incoming Gaussian beam was swept from $-180^\circ$ to $180^\circ$. Clear visibility of the incoming wave illuminating the tip from the backside and a concentration of energy at the tip apex.
8. Tip-Enhanced Raman Spectroscopic Imaging of Patterned Thiol Monolayers

Publication appeared as J. Stadler, T. Schmid, L. Opilik, P. Kuhn, P. S. Dittrich and R. Zenobi:
'Tip-enhanced Raman spectroscopic imaging of patterned thiol monolayers',

Full spectroscopic imaging by means of tip-enhanced Raman spectroscopy (TERS) is used here to measure the distribution of two isomeric thiols (2-mercaptopyridine (2-PySH) and 4-mercaptopyridine (4-PySH)) in a self-assembled monolayer (SAM) on a gold surface. From a patterned sample created by microcontact printing, an image with full spectral information in every pixel is acquired. The spectroscopic data is in good agreement with the expected molecular distribution on the sample surface from the microcontact printing process. Using specific marker bands at 1000 cm\(^{-1}\) for 2-PySH and 1100 cm\(^{-1}\) for 4-PySH, both isomers can be localized on the surface and semi-quantitative information can be deduced from the band intensities. Even though nanometer size resolution information is not required, the large signal enhancement of TERS is employed here to detect a monolayer coverage of weakly scattering analytes that are not detectable with normal Raman, emphasizing the usefulness of TERS.

8.1. Introduction

Chemical characterization of surface adsorbates is of great interest for several areas of research. The composition of biological membranes or artificially structured surfaces used in molecular electronics determines their properties as well as their function. However, the characterization is difficult due to the small size and the low number of molecules these structures consist of. Most techniques like nuclear magnetic resonance (NMR), infrared (IR) spectroscopy and Raman spectroscopy (RS) lack the necessary spatial resolution while others like scanning tunneling microscopy (STM) or scanning electron microscopy (SEM) do not provide enough chemical information. Furthermore, the limited amount of analyte results in weak
signals rendering characterization even more difficult. Ideally, information should be gathered with minimal disturbance of the molecules - which rules out any kind of labeling, but also shows the need for an ambient pressure technique.

Tip-enhanced Raman spectroscopy (TERS) has been developed to obtain chemical information with very high spatial resolution\(^{[61,141,177,178]}\) or chemical information from very few, in some cases even single molecules\(^{[58,59,173]}\). The technique uses a metal or metalized AFM/STM tip to confine the laser energy focused by a confocal microscope objective and to act as a "nano-torch" to locally excite molecules underneath it and enhance their Raman signals. Here we demonstrate an application of TERS which particularly exploits its signal enhancing effect anywhere on the sample surface. Due to the increased signal, TERS can detect small amounts of analyte in a short time, allowing acquisition of Raman images of a surface area covered with weakly scattering molecules. The information from such a Raman image is used here to chemically identify and localize two different thiol isomers in an inhomogeneous self-assembled monolayer (SAM). In this work the lateral resolution of TERS is not used to its fullest potential, yet, benefiting from the signal enhancement, weak scatterers can be identified over a larger area.

Thiols are used for several purposes. They can form a very thin protective layer on metal surfaces\(^{[196]}\) or can be employed in sensorics\(^{[197,198]}\). Moreover, thiols have been suggested as components in molecular electronics\(^{[199]}\). Thiols are commercially available in a wide chemical diversity and can easily be linked to a variety of (bio-)molecules using simple chemistry. Thus, they may be used to pattern and functionalize entire surfaces or certain surface areas. Here, we modified selected areas on a gold surface by a thiol and in a second step, the remaining substrate was covered by a secondary thiol film. This type of surface can act as a basis for biosensors\(^{[200,201]}\).

To produce patterned SAM structures on a gold surface, microcontact printing (µCP) is the tool of choice. It originated in the lab of Whitesides in 1993\(^{[202]}\) and provides cheap, quick and easy access to patterned surfaces after the initial production of a microfabricated master that can be molded multiple times to create stamps. The pattern on the surface allows us in a first step to check if a patterned region can be discerned from the bare substrate using TERS. In a further step, the functionalization of the bare substrate with a secondary analyte shows, that two very similar analytes can be told apart and localized.

For our experiments we chose 2- and 4-mercaptopyridine (2-PySH and 4-PySH), which are used to modify electrode surfaces in protein electrochemistry\(^{[203]}\). In a non-destructive experiment, the spectral signature of both isomers was employed to map their distribution on the sample surface using TERS in a gap mode configuration. The term 'gap mode' describes the use of a metal tip for TERS in close
proximity (< 5 nm distance) to a metal surface with the analyte in between the two. In this geometry, a very strong, highly localized electromagnetic field is formed in the small gap between the metal tip and the substrate, leading to a strong signal enhancement and a well-localized signal source [78,80,107,145,167]. The extent of the enhancement and, along with that, the intensity of the measured Raman signal strongly depends on the tip-surface distance [59,94,169,170]. Fluctuations in the tip-sample distance can lead to considerable Raman intensity changes, thus flat gold films are an ideal substrate to minimize the STM feedback changes and distance related artifacts.

Previous studies on self-assembled thiol films were conducted using X-ray photoelectron spectroscopy (XPS), AFM [201], STM [203-205], and Ellipsometry [206] as well as Raman spectroscopy [137,207]. Single point TERS experiments have already been presented by our group in a study of the spectral and binding properties of 4-PySH on gold [163]. By using TERS, the topography and the chemical composition of molecular monolayers can be measured simultaneously during Raman imaging with high lateral resolution, around 15 nm, as demonstrated for areas of less than 500x500 nm² [73]. As shown here, large sample areas can also be measured to gain information from coarser structures. Traditionally this is a scale where confocal Raman microscopy has its strengths, but due to the low signal intensity from molecular monolayers, an enhanced Raman technique is necessary to determine the chemical identity of the molecules. In surface-enhanced Raman spectroscopy (SERS) experiments (with a rough Ag film as a substrate, produced by vapor coating with randomly located enhancement hot-spots), the necessary enhancement can in principle be reached. We attempted to visualize the distribution of molecules using SERS, but this did not yield satisfying results. However, the large signal-to-noise ratio in every pixel of a TERS image allows one to obtain enough information to distinguish a full monolayer from a few scattered molecules on the surface that contribute to the overall signal and thus allows to visualize patterned monolayer structures.

In this article, we show that TERS can be used to image chemically heterogeneous surfaces without the need for labeling, even when adsorbates have very similar properties, i.e., a monolayer consisting of both, 2-PySH and 4-PySH. With TERS, both isomers can be localized on the surface by their spectroscopic signature and even rough information on the surface coverage can be obtained from the intensity of characteristic marker bands within the spectra. Due to the strong enhancement, this can even be done with a (sub-)monolayer of non-resonant molecules. Similar to AFM and STM, TERS images can be acquired with different step sizes to gather information about objects or structures on the nanometer or micrometer scale.
8.2. Experimental

All spectra were acquired by a combined AFM/STM connected to a quadruple grating Raman spectrometer (Ntegra Spectra, NT-MDT, Zelenograd, Russia) coupled to an EMCCD (Newton 971UVB, Andor, Belfast, UK). This top-illumination TERS setup has been described in detail in[73].

Template-stripped gold films have been created similar to the description in[148] by coating polished Si(100) wafers (Si-Mat, Landsberg, Germany) in a Bal-Tec Med 020 coating chamber at pressures below 1x10^{-5} mbar with gold (99,99%, Leica, Wetzlar, Germany) evaporated by resistive heating at a rate \( \leq 0.1 \text{ nm/s} \). The deposited gold film was bonded to clean microscope slide fragments using NOA61 (Norland, Cranbury, USA). The gold films were mechanically stripped from the Si wafers and used immediately.

STM measurements were made to characterize the properties of the surface before sample preparation. In Fig. 8.1(a) a 10x10 \( \mu \text{m}^2 \) STM scan shows that during evaporation of the gold, under the given conditions (see experimental), flat flakes of around 500 nm form as well as some single crystalline domains in the top left and right central area. A more detailed 500 nm scan in Fig. 8.1(b) reveals small surface corrugations on a well ordered crystalline patch from Fig. 8.1(a) with an average roughness of \(< 1 \text{ nm} \) in height.

Microcontact printing (\( \mu \text{CP} \)) can be used to transfer a monolayer of thiols onto a noble metal substrate as described in[154,208]. A similar procedure as in ref.[154] was used here to transfer a monolayer of 2-PySH onto the gold surface. To pattern the surface of the gold film, a \( \mu \text{CP} \) stamp with differently sized elevated circles in hexagonal arrays was used. In Fig. 8.2 a fluorescence image of the stamp layout is depicted. The stamp consists of an array of 650 \( \mu \text{m} \) wide hexagons, filled with flat circles of decreasing size (from top to bottom) and decreasing surface coverage (from left to right). The height of the elevated features of the stamp is defined by the thickness of the photoresist on the master (2.1 \( \mu \text{m} \pm 0.1 \mu \text{m} \)). The master for \( \mu \text{CP} \) stamps was fabricated by standard photolithography[202,209,210]. Briefly, a positive resist (AZ1518) was spin-coated to a height of 2.1 \( \mu \text{m} \pm 0.1 \mu \text{m} \) onto a silicon wafer, exposed through a sub micrometer resolution chrome mask and developed. After overnight silanization, poly(dimethylsiloxane) (PDMS), was mixed in a 10:1 ratio with curing agent, poured onto the master, degassed and cured in an oven at 80 °C overnight. The cured PDMS mold was cut into stamps.

For \( \mu \text{CP} \), a droplet of a 10 mM ethanolic solution of 2-PySH was placed on the stamp for 1 min, and washed with copious amounts of ethanol. The pattern was then printed onto the gold film by placing the stamp with the pressure of its own weight onto the gold film for 10 s followed by careful lift-off.
8.2. Experimental

Figure 8.1.: STM topography of a template-stripped gold surface (a) 10x10 µm² topography scan showing single crystalline gold areas (top left) and a typical grain size of around 1 µm. (b) 500x500 nm² image of the gold substrate showing slight corrugations. Line cuts (c) and (d) show peak-to-peak height differences of around 1 – 2 nm and a noise level below 1 nm.

Figure 8.2.: (a) Scheme of the µCP process. Incubation of the stamp in ethanolic solution with subsequent printing on a template-stripped gold surface. (b) Magnified bright field image of a µCP stamp for thiol deposition. The stamp consists of 650 µm hexagons filled with regular arrays of circles of different sizes and separation. Circles from top to bottom have diameters of 25, 12 and 5 µm with increasing fill factors and separation from left to right. Inset: magnified white light image from the circular elevations within a single hexagon.
The filling of the bare gold areas by 4-PySH was done by covering the entire printed gold film with an 10 mM ethanolic solution for 10 s followed by thorough cleaning with ethanol.

TERS tips were fabricated by electrochemical etching of silver tips (99.99% Ag wire, 0.25 mm, Aldrich) similar to \cite{144,145} in a solution of 1:1 to 1:2 (v:v) of perchloric acid (Riedel-de Haën, Seelze, Germany) : methanol with an etching voltage of 8 V. After etching, tips were rinsed with methanol to remove residues of the etchant. Etched tips were produced shortly before the experiments, not exceeding 4h of exposure to room air. Tips with suitable shape for STM scanning as well as TERS activity were chosen by visual examination under a 360x stereo microscope (Nikon, Amstelveen, Netherlands).

For the TERS experiments, tips were carefully approached to the sample and checked for Raman activity using a 632.8 nm Helium-Neon laser. Selected tips were then aligned using the laser scanning mirrors \cite{73} and subsequently used for TERS mappings. For each TERS map, laser power and exposure time per spectrum were adopted according to the enhancement of the tip and the activity of the analyte. The used laser power ranged between 0.1 − 2 W and collection times of 0.1 − 2 s per spectrum were chosen to yield a sufficient signal-to-noise ratio for the investigated Raman bands. Each pixel in the TERS experiments corresponds to one spectrum from an area of roughly 25 nm in diameter. In measurements with larger pixel to pixel distances, only the probed area contributed to the respective Raman spectrum.

8.3. Results and Discussion

Flat template-stripped gold thin films with a thickness of 60 − 200 nm on glass were used as substrates for our experiments and functionalized with thiols using micro contact printing (for details see the experimental section). In a first step, the efficiency and coherence of the transferred 2-PySH thiol monolayer was investigated using AFM. Figure 8.3 shows height and phase images of two AFM scans. The 30 µm overview scan in Fig. 8.3(a) shows a clear phase difference between the bare gold surface and the circular µCP thiol covered areas. The corresponding height image in (b) exhibits the same pattern, with the small elevations from the thiol layer nearly disappearing in the system's background noise. The enlarged 10 µm scan in (c) and (d) indicate a continuous smooth printing with few defects within the printed areas. The height signal now shows the elevation of the thiols a little more clearly, suggesting a step height < 1 nm, in agreement with existing literature \cite{211−213}. Due to the slight curvature of the underlying gold surface this value cannot be determined more exactly.

Attempts to spectroscopically visualize the thiol layer with confocal Raman spec-
Figure 8.3.: (a,c) 30/10 μm AFM tapping mode phase images of μCP 2-PySH on a gold surface. (b,d) Corresponding height images show a slight elevation of the thiol layer, nearly disappearing in the noise, suggesting a height between 0.5 – 1 nm. The phase image clearly illustrates the different surface properties of the printed thiol in comparison to the pure gold surface.
TERS Imaging on Patterned Thiol Monolayers

troscopy failed due to the intrinsically weak cross section of the Raman process and the small number of molecules forming the investigated monolayer. No typical Raman bands were seen with excitation for 6x10s by 3mW of a 632.8 nm laser in confocal measurements. The absence of signals from decomposition products (carbonaceous decomposition products usually scatter strongly) leads to the conclusion that the SAM was not destroyed by the high laser power, but that the intensity of Raman signals from the intact monolayer was too weak to be detected.

In experiments on Ag SERS substrates (nominal thickness 6 nm), the printed patterns could not be detected and localized by confocal RS. Either no Raman signals at all or homogeneous signals from all over the substrate were detected (data not shown). Due to the strong but inhomogeneous enhancement by single sites on a typical SERS substrate, it is most likely a very small fraction of molecules diffusing on the Au surface during production of the samples that dominated the spectra, preventing a localization of the molecules. Another possible explanation is that the roughness of the SERS substrate interfered with the patterning process we used (compare Fig. 8.2).

In Figure 8.4, a tip-enhanced Raman experiment is presented. An etched silver tip was used to probe the surface and enhance the Raman signals from the thiol monolayer. A 64x64 pixel map with 10x10 µm² and 156 nm/pixel with full spectral information at every pixel was acquired with a 632.8 nm laser at a power of 300 µW and an acquisition time of 2s per spectrum. Figure 8.4(a) shows the intensity distribution of the 2-PySH marker band at 1000 cm⁻¹ indicating the presence of 2-PySH on the surface. The circular structures from the µCP can be seen clearly and distinguished from the pure metal background. The experiment does not destroy the thiol on the surface due to the low laser power and the contact-free nature of the STM. The AFM phase image in Figure 8.4(b), taken after the TERS map from the same sample region still shows the intact thiol patterns. Fig. 8.4(c) shows a 120s reference SERS spectrum of 2-PySH (blue, rescaled) and two 2s TERS spectra from the positions indicated in (a) taken on the thiol layer (red, offset for clarity) and on the bare gold surface (black). The spectra clearly show the typical signals of the 2-PySH in the red curve and their absence in the black curve. The background is caused by the Ag TERS tip.

A µCP 2-PySH surface was then incubated in a 10 mM ethanolic solution of 4-PySH for 10s, to fill the bare gold surfaces between the covered areas with 4-PySH. The result is a mixed monolayer of two thiols with very similar properties distributed on the surface in a well-defined pattern. The AFM (a) phase and (b) topography images in Fig. 8.5 demonstrate that it is not possible to differentiate the two isomers on the surface based on these AFM experiments alone, due to their similar surface properties (height, friction).
Figure 8.4.: (a) 10 µm tip-enhanced spectroscopic image of μCP 2-PySH on gold. Evaluation of a thiol marker band intensity at 1000 cm$^{-1}$ yields a spot size of 5 µm with 4.1 µm spacings. (b) 50 µm phase image of the same area shows intact thiol structures after acquisition of the spectroscopic image in (a). (c) 120 s reference SERS spectrum of 2-PySH (blue) and 2 s TERS spectra from (a) on the thiol (red) and on the bare gold surface (black). Spectra have been offset (red) and rescaled (blue) for better visibility.
8. TERS Imaging on Patterned Thiol Monolayers

Figure 8.5: 20 μm tapping mode AFM image of μCP 2-PySH on gold immersed in 4-PySH to fill gaps. (a) Phase image (b) topography - no discernible patterns on the surfaces between the two thiols can be seen. The roughness in (a) shows the typical topographic structure of the underlying gold film. The central feature originates from an impurity on the sample surface. The height of the image (excl. the central feature) is around 2 nm.

One possible but very tedious way to distinguish the two thiols used would be high resolution STM (which usually is size limited to the nanometer range) to search for typical molecular patterns in the SAM structure. However, both thiols can assemble in several different structures\textsuperscript{203,204} and a possible mixing of both thiols would lead to further complications for STM. By using TERS imaging instead and therefore gaining chemical contrast, the distribution of the two different thiols on the surface can be determined. Figure 8.6 (a) and (b) show the background corrected intensity maps of the 2-PySH and 4-PySH marker bands at 1000 cm\textsuperscript{-1} and 1100 cm\textsuperscript{-1}, respectively. To exclude that changes in enhancement during the experiment are responsible for the contrast (e.g., due to changes in tip-sample distance), the ratio between the two marker bands is also shown in Fig. 8.6(c). A quite uniform distribution within the printed areas and only weak fluctuations of this ratio in the filled areas can be seen. The evaluation shows that the printed thiol patterns have a diameter of 12 μm and a 3.5 μm spacing, in agreement with the 12 μm hexagon with a 55% coverage on the μCP stamp. Figure 8.6(d) shows 0.5 s TERS spectra from the printed 2-PySH area (red) and the area filled with 4-PySH (black). All bands from the black spectrum correspond well to the black 10 s reference SERS spectrum from 4-PySH. In the red TERS spectrum from 2-PySH we can see all the bands from the 120 s reference SERS spectra from 2-PySH, but also an additional peak at 1100 cm\textsuperscript{-1} from the strongest band of 4-PySH. Considering
that during preparation the entire sample is immersed in 4-PySH to fill the gaps from the µCP, a certain amount of 4-PySH can be expected to embed in the area printed with 2-PySH. Additionally, the 60 s confocal background spectrum (blue) from the 2-PySH thiol monolayer shows that the Raman signal of the monolayer is too weak to be picked up by a confocal measurement.

8.4. Conclusions

Full spectral imaging using TERS can be used to visualize the distribution of two very similar non-resonant thiols within a single monolayer on a gold film. Two isomeric thiol species were differentiated and localized on the sample surface using their spectroscopic signatures. The investigation of monolayers could be useful in the analysis of catalytic processes in heterogeneous catalysis\textsuperscript{[53]} where the investigation of single active sites or the processes within a monolayer require signal enhancements from TERS combined with the ability to localize this enhancement on selected surface sites. The enhancement shown here can be conservatively estimated using the band intensity contrast between the confocal and the tip-enhanced case (< 50 cts in 60 s confocally and 500 cts in 0.5 s for TERS), corrected by the area of origin known from previous experiments\textsuperscript{[73]} (500 nm for confocal Raman and 25 nm in TERS) to be in the order of $10^5 - 10^6$. 
Figure 8.6.: (a)-(c) 30x10 µm² tip-enhanced spectroscopic images. (a) Intensity of the 2-PySH marker band at 1000 cm⁻¹. (b) Intensity of the 4-PySH marker band at 1100 cm⁻¹. Images show complementary patterns with higher intensity of the µCP thiol in the circles and higher intensity of the thiol used to fill the gaps around the circular areas. (c) Intensity ratio of the two marker bands. (d) 0.5 s TERS spectra from the printed 2-PySH area (red) and the area covered with 4-PySH (black). Small residues of 4-PySH in the red curve are visible at 1100 cm⁻¹. (e) 120 s reference SERS spectra from 2-PySH (red) and 10 s SERS spectrum of 4-PySH (black) and a 60 s confocal background spectrum (blue) from a 2-PySH thiol monolayer.


Electronic properties in different graphene materials are influenced by the presence of defects and their relative position with respect to the edges. Their localization is crucial for the reliable development of graphene-based electronic devices. Graphene samples produced by standard CVD on copper and by the scotch-tape method on gold were investigated using tip-enhanced Raman spectroscopy (TERS). A resolution of < 12 nm is reached using TERS imaging with full spectral information in every pixel. TERS is shown to be capable of identifying defects, contaminants, and pristine graphene due to their different spectroscopic signatures and of performing chemical imaging. TERS allows the detection of smaller defects than visible by confocal Raman microscopy and a far more precise localization. Consecutive scans on the same sample area show the reproducibility of the measurements, as well as the ability to zoom in from an overview scan onto specific sample features. TERS images can be acquired in as few as 5 min with 32x32 pixels. Compared to confocal Raman microscopy, a high sensitivity for defects, edges, hydrogen-terminated areas or contaminated areas - in general for deviations from the two-dimensional structure of pristine graphene - is obtained due to selective enhancement as a consequence of the orientation in the electromagnetic field.

9.1. Introduction

Since the discovery of buckminsterfullerene in 1985[214], a growing interest in carbon modifications yielded a variety of carbon allotropes with special shapes and properties, such as single- and multiwalled carbon nanotubes, 'bucky balls', lonsdaleite, carbon nanobuds and nanofibers, graphene whiskers, graphene ribbons and multi- and single layer graphene (SLG). After the discovery of graphene in 2004,[215]
Novoselov et al. demonstrated a simple production method called the scotch-tape technique\textsuperscript{[153]} that made graphene easily available to researchers around the world. Graphene is nowadays not only subject to intense basic research but also made its way into different applications such as printed circuits\textsuperscript{[216]}, transistors for frequencies up to the GHz range\textsuperscript{[217,218]}, supercapacitors\textsuperscript{[219]}, or ultrafast photodetectors\textsuperscript{[220]}. Electronic devices are usually produced either from SLG produced by the scotch-tape technique and deposited on SiO\textsubscript{2}/Si for identification with consecutive top-gating or from graphene films that are first produced by CVD on Cu films\textsuperscript{[221]} and then transferred onto other substrates by dissolving the Cu support layer and transferring the graphene film using a PMMA layer\textsuperscript{[222]}. The electronic properties of these graphene-based devices strongly depend on a correctly designed graphene sheet. Lattice defects at the ribbon edges are expected to contribute to Anderson-localization\textsuperscript{[223]} and disorder within the sheet or nanoribbon of graphene can, for example, influence the band gap; charged impurities and ripples are relevant when the mobility of electrons is of importance for the manufactured device\textsuperscript{[217]}. Furthermore, selective placement of extended defects can strongly enhance conductivity of graphene nanoribbons,\textsuperscript{[224]} enabling them to process information or carry spin polarizing current\textsuperscript{[225]}, making a localization of defects compulsory for characterization.

Several analytical techniques such as electron microscopy (EM), scanning tunneling microscopy (STM), or atomic force microscopy (AFM) as well as Raman spectroscopy (RS) have been used to study the properties of graphene and image its structure\textsuperscript{[226–232]}. Due to the clear and pronounced vibrational spectra of graphene, Raman spectroscopy is ideally suited to investigate graphene\textsuperscript{[233]} and delivers information about the number of layers\textsuperscript{[234,235]}, strain\textsuperscript{[236–238]}, temperature, as well as substrate effects\textsuperscript{[239–241]}, electronic properties\textsuperscript{[242–244]}, edge configurations,\textsuperscript{[245–247]} and defects within the graphene structure\textsuperscript{[248–250]}. The easily detectable Raman bands within graphene include the disordered band at \( \sim 1350 \text{ cm}^{-1} \) (D-band), the graphitic band at \( \sim 1580 \text{ cm}^{-1} \) (G-band) and a second-order iTO phonon mode at \( 2630–2700 \text{ cm}^{-1} \) (2D-band). Additionally a (normally Raman inactive) out-of-plane mode at \( 867 \text{ cm}^{-1} \)\textsuperscript{[251]}, several weak combination modes and second-order overtones do exist\textsuperscript{[252]} but are usually too weak to be observed in confocal Raman experiments.

Tip-enhanced Raman spectroscopy (TERS) yields the same information as confocal Raman spectroscopy but outperforms it by far in terms of lateral resolution (increased selectivity) as well as sensitivity (increased signal strength). This is realized by placing a metallic or metal-coated scanning probe microscopy (SPM) tip into the excitation laser focus of a confocal Raman microscope, which confines a part of the laser energy in a very small region underneath the apex of the tip by
local enhancement of the electromagnetic (EM) field. With this localized, strong EM field, molecules in a small area are excited, and the resulting enhanced Raman signals report chemical information from this region which is on the order of tens of nanometers. Some TERS experiments on graphene have already been presented in the literature\cite{117,135,175,253}. Hoffmann et al. have shown that Raman signals of graphene can be enhanced by TERS; Domke et al. showed spectra from graphene as an adlayer on 6H-SiC, and Saito et al. showed a TERS image identifying the layer numbers by the signal intensity and correlated it to STM scans. With the exception of the work by Saito, the above only presented point spectra of mixed single and multilayer graphene. Saito et al. showed spectral images but only focused on determining the layer number of graphene from the signal loss due to additional graphene layers. Snitka et al. added data on the shift of different bands under pressure. None of the above works detected defects or made use of the fact that graphene perpendicular to the excitation direction is only weakly enhanced. Nanoscale detection of defects in carbon-based materials such as carbon nanotubes has been presented before\cite{139,254,255} but not on graphene.

In this report, we present a TERS study of graphene on conductive substrates used in research and production. Those suitable for STM distance control, CVD graphene on Cu, and graphene on Au produced by the scotch tape method were investigated making use of the high spatial resolution, the spectroscopic information, and the selective enhancement in TERS imaging. Bands associated with pristine graphene, hydrogen terminated graphene, or from contaminations on top of the graphene film, as well as structural defects within the graphene, were assigned and localized with sub-diffraction-limited resolution. The enhancement of the Raman signals allows TERS to detect very small defects and localized contaminations that would be missed with confocal Raman spectroscopy, which emits less Raman photons. Simultaneously, the graphene bands are only weakly enhanced, allowing detection of the presence of a graphene layer without it interfering with the defect detection. These experiments could help to verify the intended structures of graphene layers produced for electronic devices. By identifying the exact location of defects, electronic properties, e.g., in single carbon nanoribbons could be explained, or line defects that are used to increase the conductance along a specific path\cite{225} could be imaged. High-resolution STM has until now been used to find single atomic mismatches in the graphene lattice, but due to the necessary atomic resolution, the scan sizes usually only cover some tens of nanometers. Using the Raman signature instead of atomic resolution to detect defects, TERS can cover larger areas than STM, on the order of hundreds of nanometers to micrometers, but with additional molecular information compared to the pure structural information in STM. Defects on a scale of tens of nanometers can be detected and localized to
within < 12 nm.

9.2. Experimental

Samples of graphene on gold were produced by coating > 50 nm of gold onto silicon wafers, followed by a template stripping process similar to the one described by Hegner et al.\cite{148}. Freshly cleaved graphite was rubbed against the exposed gold surface to transfer graphene, and excess graphite was removed by the scotch-tape method\cite{215}. Samples of graphene on copper consisted of commercially available SLG on a 20 µm copper foil (Graphene Laboratories Inc., Ronkonkoma, USA) produced by CVD\cite{221}.

Silver tips for the TERS experiments (99,99% Ag wire, 0.25 mm, Aldrich) were etched similar to previous reports\cite{144,145} in a solution of 1:1 to 1:2 (v/v) of perchloric acid (Riedel-de Haën, Seelze, Germany)/methanol, with an etching voltage of 8 V, and preselected using a 360x stereomicroscope (Nikon, Amstelveen, The Netherlands).

Raman spectra were collected on a combined AFM/STM instrument connected to a quadruple grating Raman spectrometer (Ntegra Spectra, NT-MDT, Zelenograd, Russia) equipped with an EMCCD (Newton 971UVB, Andor, Belfast, UK). The tip-enhanced Raman data were acquired using top-illumination and top-collection as described in detail by Stadler et al.\cite{73}.

For each TERS experiment, the laser (632.8 nm helium-neon) was carefully focused and locked onto the approached STM tip and the sample scanned underneath both. The laser power at the sample was usually between 0.1 – 2 mW, and a collection time between 0.1 and 1 s per spectrum was used.

The acquired 4D TERS imaging data (x, y, Raman shift, intensity) was processed directly in the proprietary instrument software and exported as images or ASCII data. No background corrections were made for the spectra shown except for the mentioned changes in Fig. 9.3. For the Raman images, band intensities with respect to the surrounding background were determined and color-coded. A line-wise linear slope correction was applied for the STM images to correct for sample drift in the z-direction.

9.3. Results and Discussion

A sample of graphene produced by CVD, grown on a 20 µm Cu foil, was imaged by STM (Fig. 9.1(a),(b)) and confocal Raman microscopy using ~ 3 mW, 632.8 nm excitation by a HeNe-Laser (Fig. 9.1(c)). The STM image shows the typical large copper grain structures and graphene covering the entire copper substrate with
smooth as well as slightly corrugated surface features. The more highly resolved image in (b) shows areas with pristine graphene exhibiting the typical triangular moire patterns (see inset) similar to observations on Cu and Ni\textsuperscript{[227,228,256], also, edges and folds of the graphene, which may result from the structure of the underlying copper foil,\textsuperscript{[256]}} are visible. The resolution of the confocal Raman image (c) is around 1 \( \mu \)m - close to the diffraction limit for this wavelength using a 0.7 NA objective and was taken from a close-by area. This 64x64 pixel image was acquired using a 0.25 s/spectrum integration time. Fig. 9.1(d) shows an averaged spectrum over the entire confocal image yielding 200 counts per second for the graphene 2D-band at 2634 cm\(^{-1}\). This band can be unambiguously assigned to SLG by its intensity and position under 632.8 nm laser excitation\textsuperscript{[257,258], all indicating the presence of a single layer of graphene on the metal substrate. During this and other confocal scans, no signs of CH bending or stretching modes at the typical positions 1450 cm\(^{-1}\) and 2800 – 3000 cm\(^{-1}\), respectively, were detected. Occasionally, very weak signs of defects within the graphene structure were observed as indicated by the slight shoulder in Fig. 9.1(d) on the low energy-side of the 1570 cm\(^{-1}\) band.

Due to the use of opaque substrates in the production processes of graphene, only a top illumination TERS configuration\textsuperscript{[73]} is suitable to investigate these samples. The same sample as for the confocal experiments was imaged on a scale of 640x640 nm\(^{2}\) (the size of a single confocal laser spot), now using TERS imaging (Fig. 9.2). A tip-enhanced Raman spectrum was acquired at every sample location and evaluated for the intensity of certain Raman bands or Raman fingerprint patterns. To ensure sufficient reproducibility of the experiments, the same sample spot in Fig. 9.2 was imaged several times using the same tip, with a step size of 10 nm and 0.25 s/spectrum. Fig. 9.2(a) shows a 640x640 nm\(^{2}\), 64x64 pixel overview image followed by a zoom of the central part and two consecutive 32x32 pixel images of the same area (b-d). Using a short acquisition time, all images were acquired within 35 min, which minimizes but does not eliminate drift. The spectral information content from each Raman image was evaluated and plotted as color-coded intensity maps of the SLG marker bands. The images show the intensity distribution of the graphene 2D-band at 2634 cm\(^{-1}\) in green and the intensity between 2800 – 3000 cm\(^{-1}\), typical for CH stretching modes, in blue. The 32x32 pixel scans clearly show that the measurements are quite reproducible, showing only slight differences and some sample drift. White lines were added to guide the eye. The triangular blue structures in the upper parts change between the images and additional CH-terminated structure appears in Fig. 9.2(d), the origin of which we cannot fully explain at present. One possible reason for the changes could be thermally induced changes by the localized field of the tip. An important observation is that the areas covered by pristine graphene and CH-terminated carbon species
Figure 9.1.: (a,b) STM images from CVD graphene on Cu, 20 µm overview scan and 5 µm zoom showing coarse copper grains and partially folded graphene on top. (c) 64x64 pixel confocal Raman image (0.25 s/spectrum, 65 µm) from a different area, showing the Raman intensity of the graphene 2D-band. (d) Averaged Raman spectrum from (c) showing only the graphene G- and 2D-band, no CH bending- or stretching modes, and only a weak contribution from graphene D-band. Intensity in the spectrum normalized to 1 s integration time. In (a,b), brighter color corresponds to an increase in height; in (c), it indicates an increased signal strength of the graphene 2D-band at 2634 cm$^{-1}$. 

are clearly anticorrelated. Both signals complement each other well and do not give any indication of graphene-free areas. The coverage agrees with previous reports stating that a continuous film over the entire substrate is produced by the CVD production method\cite{221}. Yet, no reference to the presence of CH species in these films exists in the literature, most likely because confocal Raman spectroscopy is not sensitive enough to detect them. The weak interactions between graphene and the copper surface allow the graphene film to bridge grain boundaries\cite{256}. In comparison to the weak confocal Raman signals (with < 200 counts per second), the enhanced TERS band intensities range between 4000 and 8000 counts per second for the 2D mode of SLG and 8000-16000 counts per second for the CH stretching modes, showing the mainly near-field contributions in TERS spectra. The spectroscopic contrast (factor by how much band intensities increase in the presence of the TERS tip)\cite{73} is between 40 and 80 for SLG while the confocally invisible CH stretching modes are subject to an even higher enhancement, possibly due to their polarizability along the enhanced EM field between tip and surface. An exact value for the enhancement cannot be determined without the far field signal. The lower enhancement for SLG was already observed by Domcke et al.\cite{253} and can be explained by the fact that the in-plane vibrational modes of flat graphene are polarized strictly orthogonally to the excitation direction between tip and substrate and the detection direction. Thus, the intensity of the graphene 2D signal is most likely strongly dependent on the structure of the substrate; that is, a higher intensity results when graphene is slightly tilted. Defective graphene areas seem to be less affected by the excitation direction, maybe due to broken symmetry. In comparison to the confocal Raman spectrum (Fig. 9.1(d)) that hardly shows any signs of defects, the TERS spectra (e.g., the red curve in Fig. 9.4) clearly reveal the spectral signature of the D band, and only a small SLG background. This shows that TERS detects these defects far more efficiently than confocal Raman spectroscopy. In confocal RS the defect covers only a very small portion of the large laser focus surrounded by lots of graphene, in contrast to TERS where a defect can easily be larger than the probed spot size and thus accounts for all of the acquired signal. Thus the relative intensities of defects in graphene appear far smaller in confocal experiments and can easily be lost in the background. To check SLG for the presence of defects, this is quite advantageous as it emphasizes the defects, contaminations and folds that should be detected, without creating an overwhelming SLG background signal.

A different area on the same sample was imaged with 64x64 pixels, 0.25 s/spectrum on the scale of 640x640 nm²: Fig. 9.3 represents intensities of selected marker bands from within this image, and typical point spectra are shown in Fig. 9.4. Fig. 9.3(a)-(d) shows different maps of band intensities within a single Raman image.
Figure 9.2.: (a) 64x64 pixel, 640x640 nm² Raman image of graphene on Cu, 0.25 s/spectrum (b) 32x32 pixel zoom from (a) (c,d) consecutive 32x32 pixel images. (a-d) All images show color coded band intensities of the graphene 2D-band (green) superimposed on the CH stretching mode (blue). White lines were added to guide the eye. A sample drift of around 20 – 40 nm toward the top right is visible. Further differences between the measurements could occur due to enhancement fluctuations, noise, or thermally induced changes, which could all affect the displayed band intensities.
All spectra for Fig. 9.3 were background-corrected and corrected for enhancement fluctuations by normalization to the local enhancement (using the Raman signals around 130 cm\(^{-1}\) that can be assigned to silver oxide present on the silver tip). The images show the intensity of (a) the 2D-band at 2634 cm\(^{-1}\) typical for SLG, (b) the graphene G-band at 1580 cm\(^{-1}\), (c) the D-band at 1350 cm\(^{-1}\), and (d) the CH stretching mode at 2800 – 3000 cm\(^{-1}\). In the upper left part, an area of pristine SLG dominates all the spectra, showing neither defects nor CH contamination or edges (Fig. 9.4, solid blue line). In the bottom right half of the image, two areas can be distinguished. The diagonal feature reaching from the middle toward the edge of the frame exhibits signals from D-, G- and the 2D-band, which leads to the conclusion that this is a single layer of graphene with either weak defects or contamination (Fig. 9.4, dashed red line). The areas left and right of this diagonal feature show some G-band and mainly CH stretching and CH bending mode intensity (Fig. 9.4, dotted green line). This leads to the conclusion that this area contains hydrogen-terminated carbon atoms from either a graphene edge region or an organic contaminant. A contaminant, however, should still exhibit SLG signals to some extent, and the edge region should be considerably smaller. A third possibility is thus the presence of partly H-terminated graphene, which is left over from the production process.

The detected defects in Fig. 9.3(c) highlighted by white circles are restricted to areas only 100x150 nm\(^2\), 100x250 nm\(^2\) and 50x100 nm\(^2\) in size. This underscores that we can benefit from the high resolution of TERS to determine the exact size and position of defects. Moreover, due to the strong enhancement, it is possible to detect these small, weakly scattering defects.

From a thin uniform graphene film, one would intuitively expect a constant Raman signal over the entire measurement. Yet, as discussed above, several effects in a TERS measurement can be superimposed on the constant signal. First, small fluctuations in the feedback change the local EM field strength and thus the Raman intensity. Even more importantly, surface corrugations of the substrate, as well as slight changes in the orientation of the graphene lead to a better overlap of the EM field used for excitation with the polarizability of the graphene modes. Although excited by a focused Gaussian beam, a considerable amount of polarization of the EM field along the direction of the tip axis can be expected in gap mode\(^{[107,137,259]}\), which does not efficiently overlap with the in-plane Raman modes of the graphene. Depending on the angle and thus the overlap, the detected local intensity of certain SLG flakes changes dramatically (compare Fig. 9.5).

We also investigated samples produced by the second widely used production method, the scotch-tape method. A graphene sample was prepared according to standard protocols\(^{[215]}\) and deposited on template stripped gold. TERS imaging
Figure 9.3.: for representations of the same 64x64 pixel, 640x640 nm$^2$ Raman image of graphene on Cu, 0.25 s/spectrum. The four images show the background and enhancement corrected band intensity of the (a) 2D-band at 2634 cm$^{-1}$ typical for single layer graphene, (b) graphene G band at 1580 cm$^{-1}$, (c) D band at 1350 cm$^{-1}$ and (d) CH stretching mode at 2800 – 3000 cm$^{-1}$. Image (c) shows clear localization of graphene defects (white circles) and the complementary behavior of the 2D and CH bands. x, * and ◊ depict the locations of the spectra shown in 9.4.
9.3. Results and Discussion

Figure 9.4.: Tip-enhanced Raman spectra of graphene on Cu, 0.25 s, from Fig. 9.3 (indicated by \( \times \), \( \diamond \)) showing typical spectra for single layer graphene (\( \times \), solid blue line), a defect with a pronounced 1350 cm\(^{-1} \) band (\( \ast \), dashed red line) and a spectrum from an area with contaminated or hydrogen terminated graphene exhibiting strong CH bending (1450 cm\(^{-1} \)) and stretching modes (2800 – 3000 cm\(^{-1} \), \( \diamond \), dotted green line).

was performed on sample areas preselected in an optical microscope containing thin graphene flakes. Fig. 9.5 shows a 100x100 pixel, 400x400 nm\(^2 \) TERS image taken in a preselected area. The green color again represents the intensity of the 2D-band from SLG while the red color shows the D-band intensity typical for sample defects. Within the image, a small, very intense SLG area can be found (white dashed circle, and Fig. 9.5(b), green spectrum). The surrounding area still exhibits SLG signals, albeit far weaker (by a factor of \( \approx 20 \)). Due to the high contrast, these signals are hardly visible in this two-colored representation. Two defects within the surrounding graphene layer were identified due to their spectroscopic signature depicted in Fig. 9.5(b) (red spectrum), and marked with white circles. The dimensions of these defects are 75x45 nm\(^2 \) and 55x25 nm\(^2 \), respectively. The spatial resolution of the Raman map was determined at two separate locations in the image, indicated by white dotted lines. The FWHM of these curves was determined by fitting the line profiles of the 2D- and the D-band with a Gaussian to be 10.6 nm and 11.8 nm FWHM, respectively. The spectra collected in the upper and the right part of the image exhibit a different shape and intensity and do not show the 2D-band of SLG. This indicates a graphene free region with signals from amorphous carbonaceous material (black spectrum in Fig. 9.5(b)).
Figure 9.5.: (a) 100x100 pixel, 400x400 nm$^2$ Raman image, of graphene on template-stripped gold, produced by the scotch tape technique, 0.3 s/pixel. Band intensity of 2D-band at 2662 cm$^{-1}$ (green) typical for SLG superimposed with intensity of graphene D-band at 1350 cm$^{-1}$ (red). Areas with strong defects are visible (white circles, 75x45 nm$^2$ and 55x25 nm$^2$) as well as a small area with intense graphene signals (dashed white line). The weaker graphene signals in most of the background are not visible due to the enormous contrast. The resolution of this image has been determined by a Gauss fit on the white dotted lines as 10.6 nm and 11.8 nm FWHM respectively. (b) Characteristic Raman spectra from the SLG (green), one defect area (red), and the graphene free background (black).
9.4. Conclusions

An investigation of graphene, based on the TER spectroscopic signatures of pristine, defective, and contaminated / H-terminated graphene, has been presented with a resolution of $< 12 \text{ nm}$. The expected single-layer thickness was confirmed from the position of the 2D Raman band. The dependence of the Raman enhancement on the orientation of graphene was found to reduce the signal intensity from graphene itself, allowing to easily localize defects, folds, and contamination signals on a moderate SLG signal background. Confocal Raman spectroscopy, in contrast, has 1-2 orders of magnitude poorer spatial resolution and exhibits identical intensity for flat and corrugated areas. It thus often neglects small defects or folds within large pristine graphene areas. Small defects inside continuous single-layer graphene films were identified with TERS and localized with high precision. The high signal-to-noise ratio in the spectra (due to the contrast factor of up to $>80$ with respect to confocal Raman microscopy) implies that, by using TERS, even smaller defects at the resolution limit on the order of $10 - 15 \text{ nm}$ can be localized. This detection and localization of small defects will be crucial for the production of reliable electronic structures and devices due to their influence on the electronic properties of graphene structures (e.g., nanoribbons). The presented measurements were conducted on two different samples based on gold as well as copper, produced with standard commercial graphene production methods in use. They promise a potential use of tip-enhanced Raman spectroscopic imaging to control the quality of graphene layers on a wide variety of substrates before or after transfer onto Si for use in molecular electronics.
10. Summary and Outlook

This work described the development of a new optical configuration for tip-enhanced Raman spectroscopy (TERS). The information normally obtained from Raman spectroscopy and microscopy experiments allows a localized determination of the chemical composition on a sample surface with diffraction limited resolution. Combination of Raman spectroscopy with different high-resolution scanning probe methods, as described in chapter 4, offers very advanced characterization of a sample surface. The combination of chemical and topographic information yields information on the chemical identity of topographic features, or on the location of specific molecules. Several examples show the importance of these combined measurements, even with confocal Raman spectroscopy being limited in signal intensity and spatial resolution. A key strength of tip-enhanced Raman spectroscopy is exactly the solution to these two problems. The strong electromagnetic field underneath the tip apex increases the Raman signal intensity in a few nanometer sized area, and thus simultaneously solves both major shortcomings of confocal Raman.

The optical illumination configuration of a TERS system determines what types of samples can be used, but also is key for the efficiency and the reachable enhancement of the system. The strength of the presented top-illumination top-collection approach is to combine the ability to work on opaque samples with illumination and collection using reasonably high numerical aperture. In comparison to a side-illumination approach, this leads to a significant decrease in background illumination and a considerable increase in collection efficiency from the tip area. Experiments with dye molecules coated onto Au substrates showed the ability to confine the Raman signals to an area < 12 nm, and reach enhancements comparable to top end instruments with other optical configurations such as side or bottom illumination TERS. With the strong electromagnetic field, fast detection of Raman signals was possible, which allowed imaging with high pixel numbers of 64x64 or even 200x200 pixels in < 15 min and < 2 h, respectively. The high pixel numbers could only be reached with acceptable signal to noise ratio in the spectra due to sufficient photon flux, using the enhancement of gap-mode TERS in STM feedback.

The influence of our standard TERS substrates made from template stripped gold on the electromagnetic field was compared to other metal substrates, not commonly used in TERS experiments, such as Ag, Cu, Al, Pd, Pt, Ni, Ti, etc. This comparison
of both experimental data and simulations lead to the conclusion that a wide range of metal substrates is eligible for TERS experiments at the sacrifice of less than an order of magnitude of electromagnetic field strength in the TERS gap. A wide range of substrate materials offers the choice to prevent or exploit specific interactions with the sample (Au-S for example), but more importantly, can spare the need of sample transfer, but instead allows investigations on native substrates.

The work on thiol monolayers was an important step, showing the potential of TERS to specifically use its signal enhancing nature to sensitively detect and localize small amounts of molecules on large surfaces and even visualize them by TERS imaging. Thiol SAMs, a model for an intermediate in the production process of potential biochips, were not only imaged to reveal the surface coverage and intactness of transferred structures, but contaminations, even consisting of structural isomers could be identified and distinguished during Raman imaging. In these experiments, the resolution of TERS has not been used to its fullest potential. The step sizes between spectra were in the range of hundreds of nanometers. The large microcontact printed structures in the micrometer range could only be imaged with reasonable pixel numbers using a larger step size. The more important aspect in this part was the use of the high electromagnetic field enhancement to be able to measure a monoatomic layer of non-resonant, weak Raman scatterers. Judging from the high resolution proven in experiments with dye films, presented earlier, an imaging of nanometer sized structures should also be possible. This was simply not realized because of missing technologies to reproducibly pattern surfaces in this size regime. Investigations of single layer graphene showed the strength of this TERS configuration to investigate the purity and structure of graphene on a nanometer scale. High resolution measurements only yields reasonable information if the sensitivity is sufficient to also detect chemical changes in the same size regime. This specific illumination showed to be very potent for the detection of defects in graphene, even amongst the well visible background signal of single layer graphene. Furthermore hydrogen termination or hydrocarbon contaminations on graphene were found, previously undetected in conventional Raman studies, most likely due to a lack in sensitivity. Another reason for the efficient contamination and defect detection in this experiment was the polarization of the exciting electromagnetic field that my have overlapped more efficiently with the Raman tensor of the contamination and less with the perpendicular oriented single layer graphene. The intensity of the pristine graphene was thus considerably reduced and did not overwhelm the Raman signals from the hydrogen or carbonaceous contamination. The detection of previously unknown structures again emphasizes the advantages of a spectroscopic method in respect to other high resolution methods, that rely on switching or stochastical events of fluorescent labels, as those methods are unable
to detect unexpected (and thus not labeled) molecules.

Logical follow-up experiments for the near future would be of instrumental, experimental and theoretical nature. On the instrument side, a number of improvements or developments are very important. The quality and reproducibility of STM tips should be investigated. An exact determination of tip shape and diameter will help characterize the success of the etching process. A permanent control over the I/V curves during etching, accompanied by a more precise circuit to switch off the etching current should result in a higher yield of highly enhancing tips and provide information about tip quality and success of the etching process. The development of a setup to measure the plasmon resonance of a TERS tip from its absorption spectrum would allow precise optimization of the tip or correct choice of excitation laser. Such a system could be based on a SPM on top of a total internal reflection setup with the tip absorbing light from the near-field on top of the total internal reflection crystal. Besides optimization of silver tips, the use of Au tips should be considered, and development of suitable tips for use with a 532 nm or even 488 nm laser should be pursued.

A direct improvement of the STM quality and positioning accuracy of the system could be reached by replacing the scanning piezo with a 10x10x1 µm^3 scanner. This would furthermore improve positioning accuracy during TERS imaging.

Initial results for top-illumination top-detection AFM TERS with high aspect ratio tips are very promising. Experimentally, the use of AFM feedback would allow to continue work on titanium dioxide and diamond nanoparticles or further resolve the work on inorganic solar cell materials. Also, the search for a suitable standard sample should be considered, as there is a persistent need for appropriate validation of instrument performance.

Theoretical modeling of exact system parameters should be considered as a prediction method for future instrument changes. Once the necessary level of detail for qualitative or even quantitatively correct electromagnetic field simulations is known, the evaluation of small changes in the experimental conditions are a mere question of computational power. By simulation of a systematic variation of instrumental and experimental parameters, good starting values for optimal experiments could be generated, taking specific sample properties into account.

To elevate TERS to the next level, stepping out of development labs into everyday lab practice, a more reproducible tip production yielding strongly enhancing, stable tips, not prone to carbon contaminations will be necessary. Tip design, tailored to fit a certain wavelength, different tip materials (maybe even alloys), potential tip protection against oxidation or contamination and background suppression by special tip design or spatially restricted illumination for background free measurements may become important milestones along the way of TERS. In the future,
TERS could potentially replace common confocal Raman spectroscopy, whenever good resolution or strong signals are necessary. Additionally, a deeper understanding of processes, occurring during experiments will prove crucial to understand and interpret gathered data. The influence of the strong and localized EM field on the Raman tensor, the role of nanoscale heating, and the influence of the tip on the emission of Raman photons will need to be investigated further. Carbonaceous or other contaminations will either have to be avoided during tip production and within measurements, altogether, or developing ways to distinguish these signals from true sample signals will be crucial to gain confidence into the collected data, especially when investigating complex sample materials as for example in life sciences.

In the far future, a number of potential long term goals for TERS would be quite interesting to pursue. One could imagine using arrays of TERS tips, plasmonically coupling light from the backside of these tips to excite a number of locations at once. The simultaneous detection of rows of pixels should be straightforward using vertically distributed zones on a CCD detector. For an array detection, maybe a solution including fiber coupling or the implementation of micro lens arrays in the optical pathway would be necessary. Two options could be imagined with this configuration: on the one hand, frame by frame imaging easily leading to video frame rate TERS would be possible. The second option would be a sub-sampling of the areas in between individual array tips, yielding very high resolution images over large areas.

A further ’nice to have’ addition to TERS instruments would be coherent, strong white light illumination. After an initial determination of the plasmonic properties of each tip from an absorption curve, the ideal excitation wavelength could be used for highly efficient TERS experiments. Furthermore this illumination source would easily allow to sidestep problems with fluorescence backgrounds.

A last challenge for the future of TERS would be acquisition of 3D-TERS data. May it be in a destructive way similar to 3D SEM imaging using sequential sputtering or microtome sample ablation, or by floating a precisely maneuverable nanomachine with an attached TERS tip through biological samples - both would yield incredibly valuable new information of chemical distributions on the nanometers scale. This would relieve TERS from one property that often also is a strength, but in the long run could turn out to be its biggest limitations, its surface sensitivity.
Bibliography


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Bibliography


A. Declaration of Originality

I hereby declare that the written work I have submitted entitled is original work which I have authored and which is written in my own words. The contributions of the co-authors of used publications are listed below in order of appearance.

Prof. Renato Zenobi is professor for Analytical Chemistry, Department for Chemistry and Applied Biosciences, ETH Zurich, supervised the entire PhD thesis and co-authored all publications.

Dr. Thomas Schmid is a senior scientist in the group of Prof. Zenobi, Department for Chemistry and Applied Biosciences, ETH Zurich, and co-authored all publications. Part of the introduction was written together with him for the review paper in Nanoscale.

Dr. Benedikt Oswald calculated and designed the finite elements simulations and authored the experimental part on finite elements of the publication submitted to the Journal of Raman Spectroscopy and assisted with data evaluation.

Prof. Petra Dittrich is professor for Analytical Chemistry, Department for Chemistry and Applied Biosciences, ETH Zurich, and supervised the production of the microcontact printing stamps for experiments with patterned thiols.

Phillip Kuhn produced and supplied the microcontact printing stamps for experiments with patterned thiols and authored the experimental part on microcontact printing in the publication from Beilstein Journal of Nanotechnology.

Lothar Opilik assisted in experiments and discussions about the microcontact printing of thiols published in Beilstein Journal of Nanotechnology.

Johannes Stadler
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C. Curriculum Vitae

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Title: 'Analytical Chemistry on the nanometer scale: tip-enhanced Raman spectroscopy in the top-illumination geometry'
Topics: nanoscale chemical analysis, tip-enhanced Raman spectroscopy, Raman imaging, AFM, SNOM, STM, confocal Raman spectroscopy, complementary ab-initio and FDTD

05/08 - 05/09 Roche Research Foundation fellowship for PhD thesis

11/06 - 05/07 Diploma thesis, University of Tuebingen
Topics: surface- and particle enhanced Raman spectroscopy, confocal microscopy, near-field optics, microscope set-up and optimization using higher-order laser modes

10/02 - 10/06 Studies of chemistry, University of Tuebingen

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D. List of Publications and Talks

First Author Publications:


Co-Author Publications:


Talks:


