ENLIGHTENING THE NANOWORLD
A dissertation on OPTICAL NANOSCOPY

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Presented by
RAOUL MATTHIAS STÖCKLE
MASTER OF SCIENCE, UNIVERSITY OF KENT AT CANTERBURY, UK
BORN JANUARY 10, 1974 IN ZÜRICH, SWITZERLAND

Accepted on the recommendation of
PROF. DR. RENATO ZENOBI, EXAMINER
DR. VOLKER DECKERT, CO-EXAMINER
PROF. DR. ALEXANDER WOKAUN, CO-EXAMINER

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Für Isabelle
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Abstract

The need to investigate samples with sub-micron dimensions has been a matter of interest in fields ranging from material science to cell biology for some time. The classical method of analysis uses electromagnetic waves, often at wavelengths in the visible spectrum, where investigations can be carried out under ambient conditions. Unfortunately, current methods with high chemical contrasts (e.g. IR, Raman, MS, etc.) are so far restricted by the diffraction that takes place due to the wave nature of light. This limitation can be overcome by means of Scanning Near-Field Optical Microscopy (SNOM), where light is not focused but rather apertured down to sub-wavelength dimensions.

Towards the goal of gaining molecular information on a nanometer scale, luminescence spectroscopy, vibrational spectroscopy, and mass spectrometry have been combined with SNOM. For luminescence and vibrational analysis, the SNOM probe is used to irradiate the sample with a laser beam, while the emitted, respectively scattered radiation is collected and analysed. Experiments show that this method is also capable of working in liquid environments, which is especially useful for various biological applications. For mass spectrometric analysis, the SNOM probe has been operated as a "nano-sampling tool", for delivering pulsed laser radiation to the sample surface. These laser pulses cause ablation of molecules from the sample surface that then have been transported and analysed in a sensitive mass spectrometer.

For all analytical methodologies described above, high transmission optical fibre probes have been fabricated by a newly developed etching procedure. However, instead of using tapered optical glass fibres to deliver the light to the sample surface, metallic probes where the light is focussed from the side or from below onto the sharp tip can also be employed. Electrostatic and electro-dynamic effects cause an extraordinary filed enhancements of several orders of magnitude in a localised zone underneath the tip. This effect is well known from Surface Enhanced Raman Spectroscopy (SERS) investigations, where roughened silver surfaces have been used to induce extremely high local fields. Preliminary experiments show that using a metallic tip for local field enhancement allows the application of SERS in a general way because elaborate sample preparation is avoided. Furthermore, quantitative investigations become possible, since the employed enhancement remains constant over the entire measurement. Optical analysis of surfaces, therefore, becomes possible at various environmental conditions, with ultra high spatial resolution.
Zusammenfassung

Ein Haar ist mit blosem Auge erkennbar, es hat einen Durchmesser von 0.05mm. Die heutige Chemie hat diese Dimensionen längst hinter sich gelassen: Sie forscht in Größenordnungen, die 10'000 mal kleiner sind als der Durchmesser eines Haares. Ein normales Mikroskop versagt hier seinen Dienst. Bisher galt deshalb: Wer chemische Analysen mit sehr guter räumlicher Auflösung vornehmen will, muss entweder eine winzige Probe entnehmen und diese mit hochempfindlichen Methoden untersuchen, oder als schonende Alternative einen Lichtstrahl auf eine sehr kleine Region der Probe richten und das gestreute Licht spektroskopisch, d.h. aufgeteilt nach Wellenlängen, analysieren. Einziger Nachteil: Die sogenannte Beugungslimite setzt der herkömmlichen optische Analyse klare Grenzen. Der Lichtstrahl kann im besten Fall auf etwa die Hälfte seiner Wellenlänge scharf gestellt werden (Beugungsgrenze).


Mit der hier beschriebenen Methode gelang es des weiteren, optische Raman-Spektren mit extrem hoher örtlicher Auflösung aufzunehmen. Raman gestreutes Licht gibt Auskunft über die Schwingungen von Molekülen. Der Vergleich mit bereits bekannten Daten ermöglicht eine eindeutige Identifikation des untersuchten
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Stoffs. Die Methode gilt allerdings als besonders "lichthungrig", d.h. der sogenannte Raman-Streuprozess ist sehr klein. Ein Trick hilft hier weiter: Der Streuprozess steigt um mehrere Größenordnungen, wenn die zu beobachtenden Moleküle auf einer speziellen Trägersubstanz (z.B. Silber) plaziert werden. Versuche auf selbst-hergestellten Silberinseln sollten die Ursache dieses noch ungeklärten Phänomens aufklären helfen.


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Chapter 1

INTRODUCTION
1.1 Seeing is Believing

Historically, the ability to envision something was often put into the same category as understanding.\(^1\) It was only a matter of time for the human being to invent instrumentation to clear the naturally placed hurdle of restricted vision. The curious nature of the inventors drove the venture into smaller and smaller regimes in order to discover novelties and expand their knowledge and understanding of their environment. Long after the invention of telescopes and microscopes, the search for new tools to study microstructures inherent to both dead and living matter continues to boldly go where no one ever has gone before.\(^2\)

1.2 History of the Light Microscope

The invention of the first optical microscopes did not have a great impact on life science at first. Although the word “Microscope” was first coined by members of the “Academia dei Lincei” a scientific society which included Galilei, the microscope was not a scientific tool, but rather owned by the upper-class as a recreational toy.\(^3\)\(^4\) It was not clear at that time that the microscope would ever be useful to make scientific discoveries.

While the 18th century produced some great mechanical improvements for the microscope, making it much more sturdy and easy to use, the images obtainable at modest magnifications (40x to 50x) remained rather blurry due to optical problems such as chromatic and spherical aberration. By using only a single, high-power, quality lens, Leeuwenhoek found he could get much clearer images than with compound microscopes. A recent study of the remaining Leeuwenhoek microscopes shows their magnifications to be from 50x over 200x, with resolutions as good as 2\(\mu\)m. Until around 1800, the compound microscopes could only resolve as well as around 5\(\mu\)m.

With the achromatic and Lister-corrected objective invented, progress in resolution of microscopes finally became a concern. Early in this century the microscope makers developed standardised ways to compare resolution of objectives every time they made a new style. They found a number of objects in nature, such as diatoms, which have repeating structures near the resolution of their objectives, and so by looking at these objects with different objectives, it became clear when one had slightly better resolution.
1.3 The Diffraction Limit

Even with chromatic and spherical aberration solved, there is one more factor involved with making a microscope as good as physically possible: angular aperture. While it would not become proven and well understood until Ernst Abbé published a paper in 1873, physical laws dictate that the minimum resolving distance ($\Delta s$) is related to the wavelength of light ($\lambda$) divided by a number known as the Numeric Aperture (N.A.), which is proportional to the angle of the light cone (2$\alpha$) formed by a point on the object, to the objective (Figure 1.1; Appendix A). For a short and comprehensive mathematical derivation see for instance Ref. [40]; for a full mathematical exploit see Ref. [6]:

\[
\Delta s = \frac{0.61 \cdot \lambda}{\text{N.A.}} = \frac{0.61 \cdot \lambda}{n \cdot \sin(2\alpha)}
\]

Stated in simpler terms: in order to get the maximum resolution from a microscope, the objective must collect as large of a cone of light as possible from the object.

Even though the above formula would not be known for many decades, just by randomly making various objective designs, then keeping the designs which seemed to have better resolution, the resolution of microscopes improved greatly. By the

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\[ \Delta s = 0.5 \cdot \lambda \cdot (\text{N.A.}) \] [Abbé Barrier].

---

† Calculating the maximum possible spatial resolution of an optical system requires an arbitrary definition of what is meant by resolving two features. In the Rayleigh Criterion, it is assumed that two point sources can be resolved when the centre of the Airy disc from one overlaps the first dark ring in the diffraction pattern of the second. In principle however, the Rayleigh criterion is only valid for self-luminous incoherent sources (e.g. stars viewed through a telescope).

Abbé’s definition of the resolution limit accounts also for coherent light sources across the object field and can be used to calculate the diffraction on also complex objects. The resulting expression for the ultimate resolution limit is surprisingly similar to the Rayleigh criterion:
1850ies, the major makers had reached the theoretical limit of resolution with a free air objective. Hence, by this time, microscopes could distinguish two points as different points if they were at least 0.28\(\mu\)m away from each other. In effect, what they were doing was maximising the angle of the light from the object to the objective, but they did not necessarily realise why this was helping.

It was in the 1870ies, when experimenting with water immersion objectives (which increase the refractive index \(n\)), Ernst Abbé, working for the German maker Carl Zeiss, elucidated the formula for which he is famous (and brought Zeiss to the forefront in microscope technology.)

By the 1880ies, using oil immersion objectives, a N.A. of 1.4 had finally been reached, allowing light microscopes to resolve two points distanced only 0.2\(\mu\)m apart. With the use of very unusual immersion fluids, more elaborate optical set-ups (4\(\pi\) Microscopy), exact knowledge of imaging properties, ultraviolet light, or prior knowledge on the sample (deconvolution), this limit can nowadays be pushed to below 0.1\(\mu\)m.

### 1.4 Beyond the Diffraction Limit

The application of using increasingly short wavelengths in the far UV or even X-rays regime requires elaborate instrumentation and is not practicable for a vast number of samples due to their highly energetic radiation.\(^7\)

The shift to serial data acquisition by raster scanning techniques and the use of particles other than photons (electrons, ions, molecular beams, etc.) ultimately led to the invention of the electron microscope by Ernst Ruska\(^8\) in 1931.\(^9\) This new instrument permitted biologists, for the first time, to view viruses and other detailed structures inside cells. Many of these new techniques provided images that were 1'000 times more detailed than what was being seen by light microscopes. A multitude of analytical devices like Secondary Ion Mass Spectrometry (SIMS), Electron Energy Loss Spectroscopy (EELS), Auger Electron Spectroscopy (AES), etc. followed, some of which allowing even (near-) atomic resolution while still offering a wealth of chemical information on the sample surface (Figure 1.2). On the other hand, intrinsic limitations like the vacuum requirements, static charges, radiation damage, expensive apparatus, elaborate sample preparation, restriction to elemental analysis, etc. restricts the widespread use of non-optical techniques.

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\(^7\) Was awarded the Nobel Prize in 1986
Figure 1.2. Resolving power of common nano-analysis techniques. The green bars denote techniques capable of investigating samples at ambient conditions: Infrared Absorption/Raman, Scanning Near-field Optical Microscopy (SNOM), Atomic Force Microscopy (AFM), and Scanning Tunneling Microscopy (STM); the red bars denote methodologies requiring vacuum conditions: Laser Ablation Mass Analysis (LAMA), Secondary Ion Mass Spectrometry (SIMS), Auger Electron Spectroscopy (AES), Scanning Electron Microscopy (SEM), Tunneling Electron Microscopy (TEM), and Electron Energy Loss Spectroscopy (EELS).

Optical techniques, however, can be used for a much broader range of samples. Their universality originates from their non-invasive character, ease of use, extensive zoom capability, possibility of real-time observation due to the high temporal resolution, safety, comparably low cost, single-molecule detection limits, specificity, invariance to the experimental environment, and the relatively non-perturbing nature of the radiation used. Obviously, the search for novel concepts to overcome the fundamental restriction in optical resolution attracted the attention of a large number of scientists interested in combining nanoscale analysis with optical spectroscopy.
The concept for optical microscopy in the near-field was first presented in 1928 by Edward H. Synge when he suggested a “Method for extending Microscopic Resolution into the Ultra-Microscopic Region”. The key idea was to laterally constrain the illumination by an opaque shield with a small hole in it, rather than by diffraction optics. Any incident beam of light at this aperture will, at first, be confined to the dimensions of this opening. If the aperture is of sub-wavelength dimensions, the light will rapidly diffract in all directions, but there will be a region in the vicinity of the aperture, where the beam retains the approximate dimensionality of the hole. Furthermore, the major component of the electromagnetic field of this sub-wavelength sized light source is of an evanescent character, i.e. unlike in conventional optics, the light waves do not propagate through space, but rather are localised near the aperture, readily decaying in strength with increasing distance from their origin. Assuming such a nanoscopic light source could be created, one could raster scan it over the sample surface in a manner similar to well established techniques like Scanning Tunneling Microscopy (STM): a two-dimensional image can be built up serially, one point at a time.

Since the evanescent electro-magnetic field typically does not extend farther than about the dimension of the aperture itself, the objects of interest have to be brought into very close proximity to the hole, the so called optical “near-field”. Any material within this optical near-field can interact with the electromagnetic radiation and scatter transverse propagating light, which can be detected using conventional optics placed in the “far-field”.

Figure 1.3. Confining light by a sub-wavelength aperture
1.5 How Large is the Optical Near-Field?

In principle, the theory of the optical near-field is straightforward, only requiring the solution of Maxwell's equations for the geometry of the opaque shield and aperture as border conditions. The first rigorous calculation for a circular aperture in an ideally conducting, infinitely thin screen was performed by Bethe in 1944.\textsuperscript{12} Six years later, some errors in his model were corrected in a paper by Bouwkamp\textsuperscript{13}. Since, the Bethe/Bouwkamp's solution has been used by several authors to approximate the fields emitted by apertures of various shapes and dimensions.

The dimension of the optical near-field is essentially dependent on the aperture size and optical wavelength used. The field decays vertically with increasing separation from the aperture in an exponential like manner.\textsuperscript{9} Within the Bethe/Bouwkamp solution, the field distribution is smeared out already 5nm behind the aperture and is therefore different from the fields inside the aperture.\textsuperscript{15} Commonly, the optical near-field is assumed to be within a distance of less than about 20nm from the back-illuminated aperture. The presence of a dielectric medium (e.g. glass, water, substrate) can, to a certain extent, conduct the electro-magnetic field, in which case the aperture can no longer be regarded as an independent light source.\textsuperscript{15}

![Figure 1.4. The optical near- and far-field of a back-illuminated, tapered aperture. Ideally, only objects within the optical near-field can interact with the electromagnetic evanescent waves and scatter light that is detectable in the far-field by conventional optics.](image)

\textsuperscript{9} The model for "radiating antenna" predicts a $6\theta$ power relationship.\textsuperscript{14}
Horizontally, the optical field is limited to about the aperture dimension with an exponentially decaying field intensity at its borders. An aperture with a diameter of about 100nm, therefore, will roughly give rise to an optical resolution of about 100nm. Since the aperture acts as a point source having a finite amount of radiation in all directions (in principle also in the backward direction), the near-field light can easily be distinguished from other radiation, like stray light or normal propagating radiation, by detecting it at angles larger than the one of total internal reflection ("forbidden" light near-field microscopy).16

The dimensions of the aperture have a much greater impact on the transmissivity of light through the aperture. Depending on the mathematical model used and energy transport channels considered, the emerging light intensity I through an aperture with radius r varies between:17

\[ I \propto r^5 \cdot \exp\left(-\frac{1}{r}\right) \quad [\text{for } r \ll \lambda] \]

and,18 for the case of the photons in the glass taper being transformed into excitons, followed by the retransformation of these energy quanta at the tip aperture into evanescent photons:19

\[ I \propto r^5 \quad [\text{for } r \ll \lambda] \]

The derivation of a practical "thumb of rule" has been found to be difficult since a number of parameters such as the aperture thickness (length), shield material, etc. have to be included in the theoretical considerations. For most cases, the transmitted light intensity through a sub-wavelength hole should scale as15,20-22

\[ I \propto r^4 \]

A decrease in aperture diameter from 100nm to 20nm, therefore, decreases the transmitted light intensity by a factor of 625; a reduction to 10nm reduces the residual light intensity by a factor of 10'000.
1.6 Technological Difficulties and Solutions

A series of technological difficulties prevented scientists from converting the idea of Synge into a working instrument for several decades:

(i) To compensate for the immense light losses at the aperture a very intense light source is essential. Originally, the use of a (high pressure) carbon arc lamp was suggested by Synge. Since the realisation of the first experimental laser about 40 years ago, a wide and still rapidly expanding variety of lasers have been developed. Nowadays, commercially available laser systems satisfy the requirements for near-field optical microscopy.

(ii) Another technical difficulty lies in the fabrication of a suitable aperture. As the aperture has to be brought into very close proximity to the sample only extremely flat substrates can be measured with a planar aperture. For this reason it is necessary to use sharply tapered screens in order to track rougher surfaces. In 1984 Dieter W. Pohl and co-workers published a working near-field optical microscope. Their aperture consisted of a sharp metal-coated pyramidal piece of quartz with a tiny opening in the metal coating at the tip apex. Shortly thereafter, working independently, Aaron Lewis and co-workers proposed a similar set-up, but with a tapered aperture made by electron beam lithography. The design of sharpened glass “needle-like” probes was further advanced by Aaron Lewis, Eric Betzig and co-workers who proposed to use tapered and metal-coated micropipettes and tapered optical fibres. Since, a large number of publications on improved aperture designs and their fabrication have appeared. Still, the formation of an
optimal near-field optical aperture has not yet reached the theoretical limits. An extensive discussion on the design and fabrication of near-field optical apertures can be found in chapter 2.

(iii) The accurate positioning of the aperture over the sample surface is of high importance. Furthermore, to maintain a constant light intensity at the sample surface the aperture-sample separation must be kept constant within a few Ångstroms during imaging. This necessitates some form of feedback between aperture and sample (Figure 1.5). With the invention of the Scanning Tunneling Microscope in 1982 by Heinrich Rohrer and Gerd Binnig† a breakthrough in controlled nano-positioning was achieved. The use of piezoelectric actuators and the rise of powerful desk-top micro processors allowed for atomic precision positioning. Various feedback mechanisms associated with the development of the so called scanning probe technologies allow to position, control, and raster-scan the near-field optical aperture close to the sample surface while the optical signal can be recorded independently, point by point.

1.7 Scanning Near-field Optical Microscopy (SNOM)

Once a tip-shaped, sub-wavelength-sized light source can be raster-scanned over the sample surface, the way for optical surface analysis on the nanometer scale becomes practicable. The family of techniques that make use of a nanoscale optical near-field capable of raster-scanning a surface is collectively termed “Scanning Near-field Optical Microscopy” or “SNOM”. With SNOM, high resolution measurements can be made under a wide range of conditions (vacuum, liquid, and gas from four to several hundred degrees Kelvin) revealing chemical information of virtually any surface.

A variety of optical configurations and detectors have been utilised to collect and analyse the light scattered from the sample underneath the scanning near-field optical probe. The electromagnetic radiation employed spans from microwave frequencies, infrared, visible to the ultraviolet region. Depending on the wavelength and detectors used, a multitude of information on the chemical structure of the sample surface can be gained: Inter-atomic distances, dipole moments, nuclear

† Were awarded the Nobel Prize in 1986.
or molecular interactions, bond force constants, molecular charge distributions, bond dissociation energies, molecular orientations, inter- and intra-molecular vibrations and rotations, and electronic transition and excitation states.$^{33,34}$

The coupling of a mass-spectrometer with a scanning near-field optical set-up, as described in a later chapter of this work, represents the first non-optical spectroscopy technique in SNOM. Judging from the speed with which the young field of SNOM develops, many more exciting interfaces to advanced detection schemes can be expected.

Besides the potential for chemical analysis, SNOM allows for optical modification of surfaces with high resolution paving the way for optical high-density data storage$^{35,36}$ and nano-lithography$^{37-39}$ - under ambient conditions.

In conclusion, SNOM is a universal yet distinctive tool for modern surface nano-analysis combining the benefits of optical spectroscopy with those of well established scanning probe techniques. With this novel technique, a spatial resolution below 100nm can now routinely be achieved. However, it is crucial for certain applications to interface complementary detection schemes as well as to extend the applicability of SNOM to diverse environmental conditions. Furthermore, the improvement of spatial resolution by another order of magnitude is indispensable, especially in the demanding fields of micro biology and semiconductor technology.
1.8 References


Chapter 2

INSTRUMENTATION
2.1 Laboratory Requirements

When it comes to the investigation of nanometer sized regions, temporal drift becomes increasingly important. The source for spatial drift often lies in temperature gradients induced by initial non-equilibrium conditions (e.g. hot sample on a cold table) or temperature fluctuations during the measurement. For this reason an elaborate recirculating cooling system was installed in the SNOM-laboratories keeping the environmental temperature constant (±0.1°C), optionally between 17°C and 25°C.

Also of great importance, external vibrations can detriminetally affect the resolution of SNOM. To ensure a constant separation between probe and specimen and a precise lateral positioning of the tip over the sample surface, the instrument has to be shielded from external vibrations in the range from below 1Hz to above approximately 200kHz. To decouple the SNOM from mechanically generated vibrations (originating from computer ventilation, people walking, pumps, etc.) the microscopes were mounted on a vibration isolated optical table [VH-3036-OPT from Newport, Irvine (CA), USA; Micro-g 63-564 from TMC, Peabody (MA), USA]. All air-fans within the electronic control units were replaced by sound damped ventilators. To further reduce the number of vibrational sources the SNOM laboratory was moved, and later experiments were conducted in the basement of the building. To attenuate the effects of sonic waves (talking, ventilation noise, etc.), all walls of the laboratory were covered with sound-absorbing material [Dämpfungsplatten 342823-76 from Hobbytronic, Oberburg, Switzerland; Schallschluckmatten genoppt Typ S232 & Verbundschaum mit Vlies X8 Typ S482 from Sigerist, Schaffhausen, Switzerland] whereas two layers of carpet topped the concrete groundwork. Additionally, selected parts of the room and the instrument itself were covered with sound absorbing thin boards [Schalldämm-Matten Typ S401/SK from Sigerist, Schaffhausen, Switzerland] for maximum sound isolation. Heat producing, noisy instrumentation like the (air-cooled) laser systems, and rotary pumps were placed outside the measuring room connected with the SNOM via feed-through holes in the walls.

As discussed in the previous chapter, only very small light intensities are transmitted through the subwavelength aperture. Any stray light (even from uncovered LED’s) reaching the optical detectors would therefore obscure the feeble SNOM signal. To diminish unwanted reflections the interior of the SNOM laboratory has been painted in black. Heavy curtains within the laboratory and in front of the entrance doors help to prevent undesired light from reaching the sensitive detectors.
For delicate measurements, all computers can be controlled remotely via the intranet. In this way, high-resolution analysis can be performed without external perturbations.

2.2 SNOM Instrument Design

Most of the experiments described in this work were carried out on modified commercial near-field scanning optical microscopes [Aurora & Lumina from TopoMetrix (now ThermoMicroscopes), Santa Clara (CA), USA]. The instruments consist of four major components:

1. The Electronic Control Unit (ECU/ECU-plus) provides the electronic signals that control the scanners, positioning devices, and amplifiers in the microscope stage and chassis. The x-y-z data of the piezo scanners is collected by the ECU, converted to digital data, and fed back to the computer for processing.

2. The computer (CPU) acts as the user interface to steer the microscope and is used for data acquisition and later analysis.

3. A CCD camera and video monitor allows to observation of the sample and tip interaction macroscopically, facilitating the experimental preparations.

4. A microscope, which consists of three major components: the head, sample-stage, and optical section. The head incorporates the tip mounting assembly, the laser and detector for the distance feedback control, the z-height positioning screws for the detector, and tip-sample separation control. The sample-stage unites the coarse x-y sample translator with the x-y-z piezoelectric scanner assembly. The optical section incorporates the detection optics (lenses, fibres, microscope objectives), optical filters and polarisers, beam-splitters, mirrors, optical fibres, and high sensitivity optical detectors. Depending on the SNOM-measurement, the optical section is heavily modified to suit the needs for the spectroscopic analysis.

2.3. Scanning Unit

Within the head and sample-stage, piezoelectric actuators allow for an extremely precise three-dimensional movement. Because of the properties of the ceramic material, the physical dimensions of the piezoelectric tube scanners (Lumina) or
tripod scanners (Aurora) change depending on the applied electric potential across the electrodes on opposing faces. By applying appropriate voltages to the piezo-scanners either the tip (Lumina) or sample (Aurora, Lumina) can be moved with sub-nanometer accuracy. The total scan range in the x-y plane is approximately 100\(\mu\)m for the head scanner and 50\(\mu\)m for the sample-stage, respectively, while the range in z-direction is for both actuators circa 10\(\mu\)m. To control piezo creep and hysteresis, so called “linearisers”\(^{+}\) have been included in the stacked piezo scanners (head and sample-stage) of the Lumina for improved accuracy.

Generally, higher resolution imaging can be performed when scanning the head rather than the sample-stage since less mass is moved. For most SNOM-experiments, however, it is for obvious reasons crucial to keep the optical line between probe and detection optics fixed. For this reason most near-field optical experiments described in later chapters have been carried out by scanning the sample-stage, while experiments not involving an optical detection were preferably carried out in the tip-scanning mode. Scan rates between 0.05 and 10Hz\(^{\dagger}\) allow for imaging surfaces from several hours down to few tens of seconds.

### 2.4 Feedback Mechanisms

The probe-to-sample separation should be less than the aperture radius to be within the optical near-field. This separation is usually sensed and regulated during scanning, which permits the probe to track the topography of the sample. A sensor signal proportional to the separation is generated and applied to the input of a feedback circuit. The output from the circuit drives the piezoelectric actuators either in the microscope head (tip scanning mode) or sample stage (sample scanning mode) to regulate the separation.\(^{1,2}\)

A series of techniques have been developed to enable imaging of both conducting and non-conducting surfaces:

Some less frequently used techniques are based on the measurement of the intensity of the interference pattern of the light directly emitted by the fibre tip and that reflected from the surface.\(^{3,6}\) In a similar way, Moerner et al. used the background fluorescence increase as a measure for the near-field optical coupling of the SNOM-
The distance control mechanism particularly suited for SNOM probes and which can also be used to investigate samples requiring liquid ambients is the shear-force distance control mechanism. It was introduced simultaneously by Betzig et al.\textsuperscript{16} and Toledo-Crow et al.\textsuperscript{17} in 1992. The shear-force technique is implemented as follows: The probe is vibrated parallel to the sample surface with a dither amplitude at the aperture end of the probe of a few nanometers. The frequency of the driven vibration is at one of the probe’s mechanical resonances. As the probe approaches the sample surface, it is influenced by shear forces that damp the resonance and cause the dither amplitude to decrease and the dither phase to shift monotonically as the separation decreases. The use of a laser spot diffracted from the fibre probe\textsuperscript{16} (used with the Aurora) or interferometric techniques to measure the vibration amplitude have the disadvantage that additional stray light is brought into the vicinity.

![Diagram of AFM feedback mechanism](image)

**Figure 2.1. Schematic of AFM feedback**
of the aperture and that accurate alignment of these systems with respect to the probe is necessary. An alternative method is to use piezo-electric materials, which generate a piezoelectric voltage proportional to the amplitude of the oscillation.\(^{18,19}\) Based on this idea, the use of tuning forks for detecting the probes amplitude was introduced (used with the Lumina, figure 2.2).\(^{20}\) The fibre is attached to one arm of the tuning fork and the tuning fork is oscillated at resonance. When the probe approaches the sample surface, a decrease in the oscillation amplitude and a shift in the oscillation phase of the tuning fork is observed.\(^{21,22}\) Therefore, sensing the dither amplitude or phase enables one to regulate the probe-to-sample separation and to scan the sample in the “constant shear-force” mode.\(^{15,21,120}\) The real origin of the decrease in vibration amplitude is not well understood and is the subject of much qualitative speculation in the literature.\(^{15,23-27}\)

### Mounting of the Fibre Tips

**Aurora** – The tapered and metallised optical fibre probe has to be introduced (cleaved fibre end first) and fixed\(^1\) into a shortened capillary tube (1μl) so that the tip apex is about 1mm away from the capillary end face. The capillary is then mounted (glued or screwed) to a magnetic stainless steel holder such that the tip is about 5mm from the steel holder. Once the SNOM probe is fixed in a holder, it can easily be mounted in the tip assembly holder of the instrument.

**Lumina** – The mounting of the optical fibre probes to the tuning fork requires good eyes and calm hands. Minute quantities of a somewhat slower hardening glue [Sekundenkleber Gel auf Acrylbasis, Migros, Switzerland] was preferably used to attach the fibre to one arm of the tuning-fork at one or more points. For achieving higher Q-factors with the tuning-forks, a small amount of glue was additionally applied on the other arm of the tuning-fork. The use of plasticine helped to position

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\(^1\) Several glues have been tried out. Best results were obtained with acrylic super-glue [Sekundenkleber (liquid or gel) from Migros, Switzerland]. For high-resolution imaging (<2μm) or for measurements in liquids two-component or UV-hardening glues have to be used, since out-gassing of the glue can lead to significant drifts during measurements or kill living bio-samples.
the fibre and tuning-fork. The tip holder assemblies can be recycled by dissolving
the glue with organic solvents (e.g. acetone).

2.5 Optical Collection Schemes

In principle, the light scattered from a sample in the optical near-field of the
SNOM probe can be collected in various ways (Figure 2.3). Most commonly, an
(oil-immersion) microscope objective is placed below the sample with the focal
point at the tip aperture. This constellation permits the use of oil immersion
objectives, and in this way, the light can be collected from a very large cone of
space. However, this necessitates the use of thin and transparent samples.

For opaque or thicker samples, the light must be gathered in reflection, i.e. in the
half-space of the SNOM probe. This can be performed by a long-working distance
microscope objective or a cleaved optical fibre, which has to be positioned in close
proximity to the SNOM tip. Experiments on test grids have shown that both approaches are
comparable in sensitivity and practicability. In contrast to the
detection in transmission, the
light path to the collection optics
can be obstructed by a
topographic feature of the
sample. Ways to extract this
topographic coupling for
spectrally resolved near-field
optical signals are discussed in
more detail later (Chapter 2.7).

A third way for light collection
is, although the least sensitive,
also the least affected by
topographically induced
artefacts. In the so-called
collection/illumination mode or
"Dual-Mode" detection
scheme, the light scattered from
the sample surface is collected by

![Figure 2.3. Detection pathways which have been investigated: Reflection optics, collection fibre, transmission optics, and dual-mode.](image)
the same aperture back through the optical fibre of the SNOM probe. The spectroscopically altered light (shifted in wavelength, polarisation, phase, etc.) can easily be separated from the illumination wavelength at the rear end of the optical fibre by beam-splitters, dichroic mirrors, or other optical components. Obviously, the overall attenuation of the detectable light is enormous, since the light has to pass the sub-wavelength sized aperture twice. This explains why this technique has, to date, only been used for fundamental investigations and not for real-life applications.

2.6 Measuring Modes

One of the two near-field optical microscopes in our laboratories has an Atomic Force Microscope (AFM) added to it, allowing characterisation of surface morphologies with very high lateral resolution (<10nm). To accomplish this, a SNOM scan-head of the Lumina can be exchanged with the AFM scan-head. AFM can be divided into two primary scanning modes, “contact” and “non-contact”, which simply refers to whether or not the scanning probe actually comes into physical contact with the sample surface. In the contact mode the AFM tip (usually a microfabricated silica or SiN₃ tip) is pressed with constant load onto the surface during scanning. A derivative of standard contact mode AFM, the Lateral Force Microscopy (LFM), can be used to measure frictional properties of the surface: As the probe tip is scanned across the sample surface, the friction between tip and sample causes the cantilever to flex laterally. This flexing, or torsion, can be detected by the four-quadrant photo-detector used for the standard feedback circuit. For example, boundaries between different materials with only minimal topographic variances can be detected with this mode. To some extent, surface modification (scratching, pushing particles, etc.), is inevitable in contact AFM.

The non-contact AFM mode is a much more gentle way of investigating surfaces. Like the shear-force feedback mechanism the cantilever on which the tip is mounted is oscillated at a resonance frequency, only that this time, the vibration is applied vertically (in z-direction). Again, the attractive or repulsive forces between tip and sample surface will change the oscillation amplitude and phase of the vibrating cantilever. These changes can be detected and used by the feedback loop to produce topographic data. Since the tip and sample are not (or only for short times and without lateral motion) in contact, this mode can also be used to image poorly fixated, soft, or fragile samples such as small particles, polymers, or biological specimens.
The optical transmission or reflection signal could always be gathered in parallel or independently from the scanning-probe mode used. By coupling an external laser source to the optical collection system, a low-resolution optical image of the sample can be acquired without the use of SNOM-probes. The use of pinholes and other optical components allows for sample imaging with near-diffraction limited resolution. This homebuilt “Laser Scanning Confocal Microscope” could be used in combination for example with the AFM yielding high-resolution topographic images with coarse, but with some resolved optical information. Moreover, the ability to tightly focus laser light onto AFM probes from below or the side has shown to be indispensable for the advanced near-field optical technique described in chapter 6.

As described in the first chapter, spatially resolved molecular information, hence optical resolution beyond that of conventional optical microscopy can only be accessed by SNOM. For most applications presented in this work the SNOM probe was scanned with a “constant gap” between tip and sample surface. This ensures - neglecting topographically induced artefacts - that at each measurement location the equivalent amount of light is coupled from the optical near-field to the sample. This has the added advantage of providing simultaneous topographic and optical data.

Another way to image surfaces is to perform scans at a “constant height” without operation of the feedback control. The auxiliary feedback is only used to approach the tip to the sample and to evaluate an operating height of the tip during a scan where no crash occurs. By a scan without feedback control, one can obtain an upper limit for the resolution in SNOM imaging virtually free from topographic coupling. This mode, however, is only suitable for very flat surfaces and requires very stable instrumentation to prevent the tip from accidentally crashing into the sample surface.

### 2.7 Topographic Artefacts

Frequently, optical signals acquired with scanning probe techniques show a correlation with structures in the topographic image. This undesired cross-talk arises from the nature of the constant gap mode used in many SNOM systems. The application of constant height scans, where no tip motion normal to the sample surface occurs, can be used to overcome this problem. However, this method is only useful if the surface roughness is below 5 to 10 nm, otherwise the near-field condition is not fulfilled. In the case of not ideally flat samples, the full resolution
potential of SNOM can only be obtained employing an independent feedback. Here, criteria have to be found in order to discriminate between true and apparent resolution in SNOM images. Such criteria may be based on an analysis of the SNOM image and of the simultaneously recorded topographic image of the sample on one hand and the dependence of the optical signal as a function of distance between probe and sample on the other.29

The topographic coupling can be extracted if the detected near-field optical signal is spectrally resolved. To a first approximation the sample composition and optical enhancing factors contribute to the spectrally shifted near-field signals such as fluorescence or Raman, while the optical near-field signal at the illumination wavelength is a function of the sample reflectivity, sample absorption, and topography. The latter can obstruct the light path to the detector and influence the tip-surface separation (Figure 2.4). In the near-field spectroscopy experiments, the reflected signal can thus be used to gauge these factors. By simply dividing the spectrally shifted signal intensities by the signal intensity at the illumination wavelength, the topographic coupling to the optical image can be deconvoluted. Possible limitations of this procedure can be the differences in sample reflectivity due to chemical irregularities on the surface or the change of sample absorptivity in the measured spectral range.32

2.8 Image Resolution

As far as image definition is concerned, many factors play a role in the final result. On one hand, one must define the criterion determining the quoted
“resolution”. The Rayleigh criterion defines the resolving power by the system’s ability to produce an image, which separates two points or parallel lines on the object. Frequently, the resolution in SNOM imaging is defined by the distance at which an intensity maximum falls to half of its magnitude (step-resolution), giving rise to rather "optimistic" values. Other definitions involve point-spread functions (Fourier analysis), cut-off frequencies, or object separation considerations.

On the other hand, the resolution in an SNOM can, in principle, only be defined for a particular instrument (tip included) and a particular sample. Because of the strong tip-sample interaction and the nature of the imaging process, an instrument that delivers a particular resolution on one sample may not deliver the same resolution on another sample.

Morphological Resolution

The maximum achievable morphological resolution in the x-y plane is established by the geometry of the (SNOM) probe itself. When imaging extremely flat surfaces, resolution is determined by the diameter of the atom (or atoms) at the probe’s tip. Thus, the macroscopic probe tip structure is not critical in atomic resolution imaging with an atomically flat sample. When imaging larger surface features, however,

Figure 2.5. AFM topographs acquired on the Lumina instrument. The images show laser-lithographically produced groves in a polymeric photoresist. [Sample kindly provided by Thomas Lippert, PSI Villigen]
image resolution and overall quality are determined by the probe geometry.\(^2\)

With high-resolution AFM tips [Olaf Walter, Germany], the ultimate resolving power of the newer Lumina microscope has been evaluated on test samples (Figure 2.5). The major limitation has been found to be the scan-stability. Small temperature variations, drafts (people moving, ventilation, etc.), or vibrations resulted in distorted images. Although extreme measures have been taken to suppress external disturbances (see also chapter 2.1), problems remained for scan-ranges below about one micron probably due to the large dimensions of the piezo-actuators (allow scan-ranges of up to 100µm) and the bulky, massive design of the instrument. Selected high-resolution AFM images of a laser-lithographically modified thin polymer film acquired on the Lumina are shown in figure 2.5. High aspect ratio contact-mode AFM tips were used to image the very steep changes in the sample. The achieved resolution is estimated to be around 10nm.

Images close to the theoretical resolution limit (ultimately defined by the tip dimensions) have been achieved on systems dedicated entirely for AFM [Digital

![Figure 2.6. High-resolution AFM topographs showing an HPI layer of Deinococcus radiodurans. The inset shows a correlation averaged unit of opened protomers in the right panel. The images have been acquired with an MultiMode SPM [Digital Instruments] at the Biozentrum in Basel.](image-url)
Instruments] at the Biozentrum in Basel. Figure 2.6 shows high-resolution AFM topographs of the hexagonally packed intermediate (HPI) layer of *Deinococcus radiodurans*. One can clearly see the units consisting of a large central pore formed by six assembled protomers. The function of the surface layer is not understood, but it is thought to protect the cell from hostile factors of the environment, and might also be responsible for the uptake and release of nutrients and cellular signals.\(^{37,38}\) On careful observation, one can even distinguish between the open and closed conformations of the pores. An average image of open pores, created by correlation averaging of selected proteins [Semplus], is depicted in the inset. This proves the possibility to acquire near-atomic resolution topographies under physiological conditions of fragile (and “living”) bio-molecules with standard, commercially available scanning probe microscopes.

### Optical Resolution: Confocal Microscopy

Conventional (lens-based) optical microscopy is theoretically limited in resolution to no better than one-half of the wavelength \((\lambda/2)\) of the light being used; about 0.2\(\mu\)m for the shortest visible wavelength (blue).\(^1\) This limitation is a result of diffraction that takes place in the microscope because of the wave nature of light. To approach this frontier good optical microscopes with objectives exhibiting high N.A. are needed (See also chapter 1.3).

In Scanning Confocal Microscopy (SCM), a diffraction limited spot of light is scanned across the sample. To further narrow the depth of focus a small pinhole is placed at the secondary focus of the objective lens, consequently, only light from the focal plane of the sample is passed through this aperture. This has the advantage of obstructing most of the light reflected from out-of-focus objects and other stray light. Because the focal plane is narrow, the microscope stage can be raised or lowered to optically section the sample producing a 3D image of the specimen.

---

\(^1\) In advanced 4\(\pi\)-confocal microscopy, resolutions of below 100nm (approaching 30nm) can be achieved. The increased resolving power can be achieved by a combination of the following: two-photon excitation fluorescence, collection in two half-spaces (4\(\pi\)), laterally confining the resolution by partially frustrating fluorescence emission with a second laser illumination source, illumination interference in 4\(\pi\), and deconvolution of the image using know point-spread functions of the objectives used.\(^{39-41}\)

Optical resolution below 200nm can also be achieved using so called “Solid Immersion Lenses” (SIL). The lenses need to be in direct contact with the sample to make use of their extremely high N.A. of well above 2.\(^{42-46}\)
To determine the optical resolution of the collection optics and for spatially resolved far-field optical measurements, a test sample consisting of five 1\(\mu\)m wide aluminium stripes evenly separated by 1\(\mu\)m on a quartz microscopy slide was characterised (Figure 2.7). The resolution pattern was illuminated at 488 nm [air-cooled argon ion laser] through a high N.A. (1.40) oil immersion microscope objective [Nikon] from below. The detection was performed through the same objective by an Avalanche Photo Diode (APD) [Hamamatsu] via a beam-splitter and an optical fibre. As can be seen in figure 2.7, all stripes could be resolved completely. From this image one can deduce the (far-field) optical resolution of our instrument to be better than 1\(\mu\)m.

Figure 2.7. Confocal image of 1\(\mu\)m wide aluminium stripes on a glass cover slide.
For a more precise estimate of the resolving power of the home-built confocal set-up, fluorescent beads were examined (Figure 2.8). For this, 93nm diameter carboxylate-modified latex spheres fluorescing in the yellow-green region (max. absorption 490nm; emission 515nm) [FluoSpheres L-5221, Molecular Probes Europe BV, Leiden, Holland] were spread onto a microscope slide after dilution of the stock solution (2% solids in distilled water with 2mM sodium azide) by 1’000.

Figure 2.8. High-resolution confocal fluorescence image of dye-doped latex-spheres (o 93nm)
The excitation was performed at 488nm by the same argon ion laser. Again, the 60x oil-immersion objective was used for illumination and collection. The fluorescence was detected by the APD after being separated from the illumination laser line by coloured-glass filters [Nikon]. A resolution of approximately 200nm was observed for ideal conditions, remarkable close to the theoretical limit (ca. 175nm).

**Optical Resolution: Near-field**

In order to be able to determine the optical resolution of an image acquired by SNOM, the origin of the apparent optical contrast has to be understood. Often features appearing in images with sub-wavelength dimensions involve a complicated interaction of the object with the optical field associated with the tip. In general, the near-field optical resolution is determined mainly by the size of the tip (aperture) size and its distance from the sample. Other factors influencing the imaging resolution, like the pixel resolution, sampling frequency, D/A-A/D conversion, etc, are mostly of a theoretical nature.

Highly resolving SNOM probes have highly confined optical near fields and, thus, have to be brought very close to the sample surface, increasing the sensitivity to artefacts. Moreover, highly confined optical fields are not only strongly sensitive to variations in the probe-sample separation, but generally also exhibit much weaker intensities demanding highly sensitive detectors or long measuring times. For these reasons one has to find a compromise between spatial resolution and depth of optical contrast/measuring time.

**Figure 2.9.** The optical skin depth of the metals used to define the aperture ultimately limit the maximum achievable resolution. The graph shows the optical transmission of visible light through different metals (Adapted from [57]).
Another factor that fundamentally limits the aperture probe near-field optical microscopy is the fact that the spread of the near-field of an aperture is determined not only by its size but also by the skin depth of the surrounding metal (Figure 2.9).\textsuperscript{57} Despite the technical difficulties in producing apertures smaller than about 30nm and minute optical transmission factors for such apertures, no increased resolving power can be achieved with openings smaller than about 15nm. Whereas spatial resolution has been claimed with optical contrast close to 10nm, confirmed resolving powers remained in the range of 30 to 50nm.\textsuperscript{8,58-61} Instrument resolution below that has only been achieved with so-called "aperture-less" SNOM configurations where no sub-wavelength aperture is present (Chapter 6).\textsuperscript{62-64}

2.9 SNOM Probes

Probably the most delicate component in SNOM is the nanoscopic tip itself. The development of advanced SNOM probes and their efficient fabrication are certainly the most important steps for further progress in near-field microscopy.

General Concepts

The aperture dimensions of an ideal SNOM probe should be as small as possible, but still exhibit maximum optical intensity at the tip apex. Various theoretical models have been established suggesting specific tip shapes for optimal transmission properties. Novotny et al. calculated the spot size\textsuperscript{1} and power transmission through the probe in relation of the tip radius, taper angle, and metal thickness in front of the tip.\textsuperscript{53,54,65} Generally, larger cone angles of the taper drastically improve the overall transmission coefficient, since the mode propagating furthest in the tapered waveguide (HE\textsubscript{11}) is cut off closer to the probe’s aperture.\textsuperscript{53,61,66} On the other hand, the spot size increases rapidly for taper angles greater than 100°.

In conclusion, taper angles in the range of 60° to 100° seem to offer optimum conditions for transmission and spot size. Further, to guarantee a single spot (one field maximum), the highest curvature must be at the apex of the probe.\textsuperscript{53}

\textsuperscript{1} i.e. highly concentrated optical near-field in three dimensions
Glass Fibre

Most SNOM probes still consist of commercial optical glass fibres that were elongated and/or etched at their extremity to obtain a very small scattering centre at the apex. A subsequently applied metal coating prevents the light from leaking through the tapered sidewalls (Figure 2.10). There are many kinds of optical fibres with miscellaneous properties and fields of application. An optical fibre consists of three concentric elements: The core, the cladding, and the outer coatings, which sometimes are separated in two distinct layers, the hard polymer coating and the polymer buffer (Figure 2.11).

The core is the light transmitting portion of the fibre. Due to their wide spread use, their high quality, and their optical properties in the visible region, silica-core fibres have shown to be most appropriate for SNOM applications. Depending on the optical modes transmitted by the glass core, the fibres can grouped in three main classes: Single-mode, multi-mode graded-index, and multi-mode step-index fibres. In single-mode fibres only the fundamental zero-order mode (TEM₀₀) is transmitted. The light beam travels straight through the fibre with no reflections from the core-cladding sidewalls at all. The core diameters of multi-mode fibres are much larger than single mode fibres. As a result, higher order modes are also propagated. The core in a graded-index fibre has an index of refraction that continuously decreases radially from the centre to the cladding interface. Consequently, the light travels faster at the edge of the core than in the centre. Different modes travel in curved paths with nearly equal travel times. This greatly reduces modal dispersion in the fibre. Therefore, graded-index fibres have bandwidths which are significantly greater than step-index fibres, but still much lower than single mode fibres. The core of a step-index fibre has a uniform index of refraction right up to the cladding interface where the index changes in a step-like fashion. Because different modes in as step-index fibre travel different path lengths
in their journey through the fibre, data transmission distances must be kept short to avoid considerable modal dispersion problems. However, in tapered multi-mode fibres, where the core diameter is rapidly decreasing, one mode after the other runs into cut-off, until only the final $HE_{ij}$ mode is still propagating (for core diameters below ca. 250nm). For this reason, all three classes of fibres can be used for SNOM experiments. Other important factors like (wavelength dependent) attenuation, numerical aperture, dispersion, bandwidth, and maximum power transmission have to be considered prior to selecting an optical fibre for SNOM-tip fabrication. Optical fibres with high transmission coefficients down to the deep UV region (240nm) exhibiting a low fluorescence and Raman background are readily available.

The cladding is usually made of the same material as the core, but with a slightly lower index of refraction (usually about 1% lower). The difference in refraction index is usually created by adding dopants like Boron, Fluoride, Phosphorus, and Germanium during fabrication. This index difference causes total internal reflection to occur at the index boundary along the length of the fibre so that the light is

![Figure 2.11. Schematic of a (step-index) optical fibre. Total internal reflection allows light to remain inside the core of the fibre.](image_url)
transmitted down the fibre and does not escape through the side walls. The glass cladding not only guarantees good optical properties of the wave-guide, but is also responsible for high optical destruction thresholds.

The coating usually comprises one or more coats of a plastic material to protect the fibre from the physical environment. The inner hard polymer coating increases the tensile strength and counteracts material fatigue, whereas the outer polymer buffer protects the fibre from mechanical wear and chemical corrosion. These outer coatings are no longer used at the tapered region of the SNOM probes and have to be removed prior to metallisation. The coating can be removed either mechanically, chemically, or pyrotechnically. Most commonly, the polymer jacket is mechanically stripped before taper formation† with the use of special fibre pliers. A less harsh method for coating removal is by dipping the cleaved fibre ends into suitable solvents. Since not all fibre jackets dissolve in organic solvents (e.g. dichloromethane, chloroform, etc.††) easily we changed to hot (120°-140°C) concentrated sulphuric acid which usually removes all types of coatings within 2 to 15 minutes. In this way, the coatings can be removed reproducibly in a much gentler manner. A quick and effective way to remove the polymer overlayers is to burn them with a flame. The use of natural gas (propane, butane) is recommended over the use of a lighter flame or torch since less carbon residues usually contaminate the bare glass fibre. With the flame removal approach, care has to be taken not to modify the optical properties of the fibres (dopant concentrations, etc.) due to excessive heating at high temperatures (e.g. >1300°C for LaserComponents fibres). Further, to decrease residual carbonisation on the bare fibre, any dust or debris has to be removed by wiping it off with isopropyl alcohol or other solvents like “Dynasolve 100” [Dynaloy Inc, Hanover NJ, USA] before burning. One has to ensure that no liquid remains on the surface, since any small droplets will cause pitting. Once the cladding is removed, either wiping with isopropyl alcohol or dipping the blank fibres in wettants (e.g. Triton X100 [Fluka, Switzerland] will remove any remaining carbon residues. All three methods have resulted in bare glass fibres suitable for SNOM tip fabrication. The chemical removal of the fibre jackets has been chosen for routine manipulations since complete batches of fibres (usually eight) can be treated simultaneously in an easy and very reproducible way.

† It seems that the coating can also be removed after taper formation by pulling the tube-end carefully with tweezers after loosening the coating in an organic solvent (e.g. chloroform).

†† The use of acetone is not recommended since some fibres seem to corrode slightly, yielding in a rough glass surface.
Taper-formation: Melt-Drawn Tips

Diverse fabrication process of SNOM tips lead to various shapes that were not very reproducible. The large majority of commercial SNOM tips are fabricated by the simultaneous heating and pulling to the rupture of the glass fibre. The adaptation of the method of pulling capillaries to fine tips (widely used in microbiology) to optical fibre tips was first performed by Betzig et al. Heating is usually either induced by irradiation with an IR laser or resistive heating by a small coil of wire. The taper angle and the diameter of the flat end of the tip (from 20 to 200nm) can be determined by the pulling parameters. A detailed description of the parameters influencing the fabrication of aperture probes was first given by Valaskovic et al.

High-resolution, high throughput SNOM probes are difficult to manufacture and have to be produced and selected manually. To compare the widely used heat-pulling process with the newly developed chemical taper-formation procedure, a heat-pulling device has been constructed (Figure 2.12): A glass fibre is fixed onto two metal blocks of which one is pulled by a well defined force (spring) along the main axis. The heating of the glass fibre is induced by driving high electrical currents (5-30 amperes) through a thin metal coil. Because not only the timing and intensity of the heating and pulling, but also the dimension of the heated area plays an important role in the resulting tip shape, very thin metal filaments were chosen for local heating. Initially, most of the fibres pulled exhibited two characteristic taper

![Figure 2.12. Schematic of the fibre-puller designed for heat-pulling SNOM probes from optical fibres.](image)

† The heat gradients at the fibre have been increased by using only singly coiled thin (0.05mm) metal wires. A further improvement can be expected when heating a thin metal foil with an aperture through which the fibre is fed, rather than filaments.
regions (Figure 2.13). This is often found when the heating power during pulling is set too low.\textsuperscript{70} It is assumed that for these cases the heat dissipation of the glass is high enough to form an anisotropic viscosity distribution through the glass fibre along its cross-section.\textsuperscript{71} Theoretical considerations show\textsuperscript{69,72} that this problem can be overcome by either increasing the heating power, or terminating the heating when the fibre radius becomes smaller than a certain fibre-dependent size. With the use of an electromagnet, the exact starting time of pulling could be controlled, enabling the creation of excess heat at the fibre before pulling. To further improve the tip shape characteristics the heating during pulling was performed non-continuously, with oscillating intensity fluctuations. A homebuilt control-board allowed to define pre-heating intensity and time, and the repetition rate of heating and peak-intensities during pulling. In order to increase the maximum heating power, a variety of heating filaments (Table 2.1) have been tried out, all of which were insufficiently stable for pulling large quantities of fibres: The filaments were destroyed either by oxidation\textsuperscript{†} or had melting points too low for optimal heat-pulling conditions. The use of an inert gas atmosphere (Argon, nitrogen, or vacuum, respectively) to protect the heating filament has been investigated. The time for the production of a single SNOM probe under these conditions, however, increased significantly rendering the method impracticable.

### Table 2.1

<table>
<thead>
<tr>
<th>Metal</th>
<th>m.p. [°C]</th>
</tr>
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<tbody>
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<td>Aluminium</td>
<td>660</td>
</tr>
<tr>
<td>Copper</td>
<td>1083</td>
</tr>
<tr>
<td>Platinum</td>
<td>1772</td>
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</tr>
<tr>
<td>Platinum/Rhodium</td>
<td>1830-1855</td>
</tr>
<tr>
<td>Rhenium\textsuperscript{†}</td>
<td>3180</td>
</tr>
<tr>
<td>Tungsten\textsuperscript{†}</td>
<td>3410</td>
</tr>
</tbody>
</table>

\textsuperscript{†} Tungsten (and Rhenium) starts to oxidise rapidly at higher temperatures (>500°C) forming gaseous WO. Coating the tungsten wire with protection layers (Glass, ceramics, oxides) showed to be impracticable.
A series of SNOM probes produced by heat-pulling were characterized regarding tip shape, taper quality, and transmission properties. Our findings were comparable with those of other groups. The pulling method yielded very smooth fibre tapers with an only minimal roughness at the glass surface, which positively influenced the quality of the subsequently evaporated metal layers. Furthermore, due to the creation of flat end faces at the apex, the formation of well-defined apertures by succeeding metal deposition is greatly facilitated. In particular cases, transmission factors approaching those of alternatively tapered fibres (10^-4 to 10^-3) have been achieved.

Usually, however, the heat-pulled fibres featured rather small taper angles (below 20°) leading to long and thin SNOM tips with only poor optical transmission properties (∼10^-3 to 10^-6; Figure 2.14). Another disadvantage of the heat-pulling method over other tapering schemes is the long time entailed in producing single SNOM tips one after another.

**Figure 2.14.** The ideal tip shape of SNOM probes have rather large cone angles like in (A). Heat-pulled SNOM probes mostly exhibit long and thin tapers like in (B) with only poor optical throughputs.

**Taper-Formation: Chemical Etching (Protection Layer Method)**

In 1983 D. R. Turner patented a method for chemically tapering optical glass fibres to efficiently couple them in fibre connectors. It was more than 7 years later that this method was discovered for the fabrication of SNOM tips. The tip formation takes place at the interface between the glass and the surface of the etching solution (hydrofluoric acid, HF). The glass in contact with the etching solution is slowly dissolved. Thereby, the height of the meniscus formed at the glass-liquid-air interface gradually decreases because it is dependent on the contact angle of the immersed glass, ultimately resulting in a glass taper above the etching solution (Figure 2.15). The initial height of the meniscus is also dependent on the surface tension of the materials involved. Consequently, designing the material composition allows for control of the resulting taper shapes. The custom fabrication of fibres is very expensive and elaborate. Therefore, the material compositions of the commercial glass fibres used have to be regarded invariable. On the other hand, the etching solution can be modified much easier by adjusting the concentration, or by adding salts or other substances (e.g. lubricants, detergents, etc.). Generally, the surface
tension is decreased when ions (this includes ions from solvated HF) are added, resulting in a less pronounced meniscus formation. Consequently, larger cone angles can be obtained when adding (buffer-)salts to the etching solution. Some groups have investigated the influences of additives in the etching solution and did find a weak correlation between buffer-salts and taper angle.\textsuperscript{79,80} When salts are added, there seems to be no change in the surface structure of the tips, most likely since the diffusion of the dissolved glass away from the fibre takes place much faster than its corrosion. Further, despite affecting the duration of the etching process, the HF concentration seems to have no effect on the tip shape or surface roughness.\textsuperscript{81,82} This is important because of the extreme toxicity of HF and, therefore, the necessity of using only low concentration solutions. As a consequence, only diluted HF-solutions (<50\%) were utilised. For routine tip fabrication a stock HF-solution (43\%, puriss) [Fluka, Switzerland] has been used.

The relative surface tensions involved at the air-glass-etchant interface can also be modified by adding an organic overlayer. In this way, the wetting of the glass by the overlayer is in direct competition with the good glass-adhesion property of the hydrofluoric acid solution. The ability of the added organic solvent to wet the glass surface, therefore, is a measure for the initial meniscus height and, therefore, for the resulting tip shape. As a consequence, the use of an organic overlayer not only protects the instrumentation from the corrosive HF vapours, but also leads to SNOM tips exhibiting larger cone angles.\textsuperscript{81} Bertrand Dutoit\textsuperscript{81} and Claude Philipona\textsuperscript{87} have investigated the influences of various organic solvents on the taper angle characteristics. However, the observed dependencies could only partially be reproduced (Graph 2.1). Since the final tip shape depends only partially on the type of organic solvent used, only qualitative results are given for the observations made in our laboratories. Due to its low toxicity and low solubility in HF, cyclohexane

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.15}
\caption{Schematic of the "Protection layer method" by Turner to produce tapered probes from optical fibres.}
\end{figure}

\textsuperscript{1} Solvents which have relatively low glass-adhesion properties compared to air and would result in smaller cone angles, generally dissolve well in hydrofluoric acid and therefore cannot be used. For practical reasons one abstains from controlling the atmosphere (high pressures, He, N\textsubscript{2}, etc. would result in smaller cone angles).
Graph 2.1. Cone angles vs. type of overlayer. Bertrand Dutoit’s (BD) observations in the Lausanne and Zurich laboratories, respectively, and our qualitative findings at the Zurich laboratories differed significantly, probably due to the big number of factors involved in the taper formation process.

was preferably used for the production of SNOM tips. To ensure an equilibrium state between cyclohexane and hydrofluoric acid, the organic solvent was added at least 10 minutes before the glass fibres were immersed. Although the thickness of the overlayer should have no influence on the taper formation, one has to take care to use enough solvent to avoid surface phenomena effecting the etching process. The use of organic solvents (e.g. thionylechloride [1.67kg/l] or chloroform [1.47kg/l])\(^1\) which are heavier than concentrated hydrofluoric acid [1.17kg/l] necessitates the introduction of the glass fibres from below (Figure 2.16). This so called “Reverse Etching” scheme\(^3\) seems to be less prone to external disturbances (vibrations, evaporation of organic

\(^1\) Only few HF-insoluble solvents can be used for reverse etching. However, “heavy” organic solvents can be mixed with less dense solvents to create “new” layers.
solvent) but also requires elaborate experimental preparations. Diverse parameters, like the relative wetting characteristics or the solubility of the etching solution (HF) in the organic solvent obscure the prediction of theoretical correlation between type of overlayer and resulting cone angle.

Another possibility to modify the tip shape is to control the temperature during etching. Many factors like the corrosion rate, meniscus height, or HF solubility in the organic protection layer, are more or less dependent on the temperature, thus, no exact prediction of the resulting cone angle can be made. Since the corrosion rate increases comparably fast with higher temperatures, larger cone angles and shorter formation times can be expected when etching at higher temperatures. More importantly, the temperature has to be kept constant during the whole taper formation process to avoid irregular tip shapes. In figure 2.17 the influence of temperature gradients during etching is depicted. Only when the temperature is held constant a conical shaped SNOM tip can be obtained (A). When the temperature increases during etching a parabolic tip shape is achieved, because the glass is dissolved faster at higher temperatures, gradually increasing the taper angle (B). In contrast, a “concave” tip is obtained when the temperature drops during the etching process (C). A tip shape of the type “D” is produced when the temperature is fluctuating. Transmission measurements have shown that SNOM tips of the type “C” are much worse than those of “A” and “D”. Theoretically, SNOM probes of the form “B” should have ideal optical throughputs.

Small vibrations during etching can produce little steps on the tip surface. This was one of the main reasons why thermostatic baths could not be used routinely for tip production. The attempt to average the vibrations by deliberately adding random noise (sub-sonic bath, stirring) failed. Consequently, the etching apparatus was placed
on vibration damped material (sand, polymeric foam, cork), and the access to the etching premises was locked during the entire etching process.

Impurities also hinder the fabrication of good SNOM probes, often yielding inhomogeneous and irregular tip shapes. For this reason, only pure solvents and acids were used for etching. The optical fibres were pre-cleaned by dipping them into organic solvents (initially acetone, but changed later to spectroscopic grade ethanol) and de-ionised water. After etching, any excess HF was washed away by dipping the tapered fibres into de-ionised water. The ready tapered fibres were transferred immediately into high vacuum (10⁻⁶ mbar) for subsequent metallisation, in this way reducing the potential of contamination through airborne particulate matter.

The tip formation by the protection layer method requires to introduce the glass fibres vertically into the etching solution. Even small tilt angles would result in asymmetrical tip shapes. Guiding notches with the newly designed tip-holders (Appendix C) and a spirit-level helped to position the fibres upright.

A relative motion of the glass fibre can be used to control the cone angle: When rotating a tilted glass fibre, theoretically, shorter taper lengths can be achieved. Likewise, the gradual immersion of the glass fibre into the etching solution would lead to a larger cone angle. Unfortunately, the required motor introduces vibrations which would lead to rough glass surfaces. The addition of hydrofluoric acid during etching would lead to a similar result, however, due to technical difficulties this variation was not investigated further.

Although the tip formation process is self-terminating, the tips have to be removed from the etching solution soon after complete taper formation, else the tip apex become dull. Possible explanations can be the expansion of the etching solution due to a temperature increase, surface vibrations, or a HF concentration gradient reaching into the organic overlayer. The exact length of the etching-interval is strongly temperature and fibre type dependent. The time-spans for distinct temperatures have been recorded routinely allowing for approximate predictions on the specifically required etching time.

The major advantage over heat-pulling SNOM tips is not only the fact that, in principle, larger cone angles (over 40°) can be achieved, but also the possibility to fabricate several probes simultaneously. With the newly designed tip holders (Appendix C), batches of up to eight tips could be manufactured in one process. Although identical parameters applied to a batch, there usually were variations between the etched tips. Only 40 to 60% of the tips were suitable for further processing, whereas the other tips showed significant impairments when viewed
under magnification. Furthermore, in contrast to the heat-pulling method where the proportions of the core and glass cladding is expected to remain unchanged even at nanoscale dimensions, the etching method yields an unchanged core diameter. As a result, the influences of the metal coating, like the mode spreading effect, starts to be important at smaller wave-guide diameters closer to the aperture.

Taper Formation: Tube-Etching

A well known problem of tips etched by the Turner method is the sensitivity of the tip shape to environmental influences such as vibrations, temperature drifts, etc. during etching, resulting in a glass surface with a considerable roughness. This roughness and the asymmetry of the tip apex are generally held responsible for pinholes in the subsequently applied aluminium coating and ill-defined optical apertures, respectively.

A novel fabrication method called “tube-etching” has been developed, significantly improving the overall tip quality.\(^{61,101,121-123}\) The mechanism of the taper formation is based on the chemical etching of the glass fibre within its polymer coating: Instead of removing the polymer coating from the optical fibre before etching, the cladded fibre end is dipped into the hydrofluoric acid solution (Figure 2.18). Consequently, the whole etching process takes place inside a hollow cylinder formed by the fibre’s protective polymer jacket that withstands degradation by HF.

Tip formation was found to follow two different pathways depending on whether the fibre’s polymer coating is permeable for HF or not.\(^{\dagger}\) Nevertheless, similar tips were obtained independent of the taper formation pathway involved.

If no HF can penetrate through the coating [HCG-M0100T-14, inner core diameter 100\(\mu\)m and HCG-M0200T-14, inner core diameter 200\(\mu\)m from LaserComponents],

\(^{\dagger}\) To check the permeability of the polymer coating for HF, for each type of fibre a closed fibre loop was dipped into a vessel containing HF. For the two multi-mode fibres from LaserComponents no etching inside the plastic jacket was observed, whereas the other fibres showed severe thinning of the fibre core after 60 minutes in HF, due to HF diffusion through the polymer coating.
the tip formation starts at the lower end of the fibre. No thinning of the glass in the upper region of the fibre is observed. Once a tip is formed, the tip shape is maintained while the tip shortens inside the tube. A schematic of the etching process is shown in figure 2.19. Video frames acquired during the etching process are depicted as insets. The tip quality in terms of sharpness and smoothness does not deteriorate upon further etching; the tube etching process is found to be self-limiting (in contrast to the self-terminating process found in the protection layer method by Turner). The scanning electron microscopy (SEM) images in figure 2.20 show aluminium-coated optical probes etched for 90 and 130 minutes, respectively. It is evident, that the taper angle and the surface quality are insensitive to the etching time. This result compares very well with the video frames in figure 2.19.

Figure 2.19. Schematic of “Tube-Etching” for non HF-permeable fibre coatings. The tip formation starts at the fibre end. The insets show selected frames acquired during etching.

*Etching was carried out in a Teflon vessel, equipped with two sapphire windows on opposite sides, allowing observation of the taper formation process with a slow scan charge-coupled device (CCD) camera. Pictures were taken every 30 seconds. The resulting time-lapse movie of the etching process facilitated the detailed investigation of the tip formation pathways.*
Figure 2.20. Electron micrographs of metallised, tube-etched SNOM tips. The tip quality does not depend on the etching duration. Tube-etching is a self-limiting process.

For the HF permeable protective polymer coating [FS-SN-3224, inner core diameter 3.36\(\mu\)m from 3M; 40-692.11, inner core diameter 3\(\mu\)m from Cabloptic; 91-9116.136, inner core diameter 3\(\mu\)m from Alcatel], the glass fibre is thinned regularly inside the plastic jacket due to diffusion of HF through the jacket. A preliminary tip formation at the position of the interface between the HF solution and the organic overlayer can be seen in figure 2.21. This is possible due to a gradient in the lateral diffusion along the tip in the meniscus region. The final tip formation takes place above the interface after complete removal of the thinned part. It should be noted that above the interface,

Figure 2.21. Schematic of the “Tube-Etching” process for optical fibres exhibiting HF-permeable polymer jackets. The upper row of insets, show selected video frames taken at the solvent interface, whereas the lower insets show the characteristic thinning of the fibre at the fibre endface.
lateral diffusion of HF through the jacket is no longer possible. Therefore, one can conclude that the tip in this region is formed by the same mechanism as in the case of the impermeable polymer coating.

Presumably, micro-convection inside the tube, probably in combination with transient capillary effects, is responsible for the tip formation (Figure 2.22). A similar mechanism was also postulated by Unger et al.\textsuperscript{84} for other fibre materials. Initially, due to geometrical constraints (A), it is expected that the outer regions of the fibre are etched slightly faster than the centre. This is attributed to the fact that at the rim of the glass cylinder, HF supply occurs out of a larger volume as compared to the central region. This effect starts the formation of a conical shape (see also the first inset in figure 2.19). As soon as a preliminary taper is formed, convection starts to

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{taper-formation.png}
\caption{Proposed mechanism for the taper formation in tube-etching. See text for details.}
\end{figure}
deliver HF to the upper region of the cone (B). This convection is driven by concentration gradients caused by the etching process itself and the gravitational removal of the reaction products.\(^8\)

\[
\text{SiO}_2 + 4 \text{HF} \rightarrow \text{SiF}_4 + \text{H}_2\text{O} \\
3 \text{SiF}_4 + 2 \text{H}_2\text{O} \rightarrow \text{SiO}_2 + 2 \text{H}_2\text{SiF}_6
\]

The influence of gravity on the tip formation process was checked by etching the fibres at various angles. Under such conditions asymmetric tip shapes were obtained.

Within the convection model, the tip geometry is expected to be determined mainly by the relative magnitude of lateral diffusion and convection as well as the temperature dependence of the etching rate. In the etching region, HF is consumed and the reaction products are transported away by gravity. The diffusion of products and educts increases linearly with temperature. The etching rate increases strongly with temperature. An increased diffusion is expected to lead to larger cone angles because of a more isotropic etching of the tip. An increased etching rate is likely to decrease the cone angle because the fresh HF delivered by convection may already be used up at the upper part of the cone before it reaches the apex region. Since both effects have an opposing influence on the resulting tip shape, consequently, a maximum cone angle is expected to be obtained for an intermediate temperature.

A similar explanation may also hold for the concentration dependence: Concentration changes will influence the reaction rate, and therefore, result in an optimum concentration for a given temperature.

To investigate the influence of the HF concentration and etching temperature on the taper quality and geometry, a series of etching experiments with dilute HF solutions at different temperatures was performed. An organic overlayer (e.g. p-xylene, iso-octane, cyclohexane) was used to protect the fibre mounts from the corrosive HF vapour. It is important that the solvent does not attack the polymer coating and is neither soluble in nor chemically reacts with HF. This overlayer had no influence on the tip formation process itself.\(^\dagger\) The system used in our experiments had a cover (in our case the tip holder itself) to create a closed environment inside the etching container (no exchange with the outside environment). This avoided uncontrollable external influences like drafts, evaporation/condensation, etc. from

\(^\dagger\) Other groups have reported to get more reproducible results and when using a thick (>5 mm) overlayer.
except for the Alcatel and Cabloptic single-mode fibres, all tested fibres yielded extremely smooth glass surfaces after etching. For a given fibre type, the cone angle can to some extent be controlled by varying the etching conditions. However, the main influence on the cone angle seems to result from the actual fibre type; For the same etching parameters the cone angles varies significantly.

The most striking advantage of the tube-etching method is the high quality of the tips. In figure 2.23, a comparison between a typical tip prepared by the Turner method and a characteristic tube-etched tip is shown. While the taper angle is quite similar for both techniques, the tube-etched tips are much smoother, evidently due to the fact that the taper formation is no longer a perturbation-sensitive surface phenomenon but rather takes place in a protected container.

This is even more evident in the SEM images of the glass surface recorded in close proximity to the tip apex (Figure 2.24). The glass surface of the tube-etched

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**Figure 2.23.** Optical micrographs of a tip etched by the protection layer method by Turner (left panel), and a tip etched by the tube-etching method (right panel). Where the conventionally etched fibre taper shows significant kinks, the tube-etched tip has a smooth and homogeneous taper region.

**Figure 2.24.** Electron micrographs of the glass surface in close proximity to the tip apex. Left panel: Protection layer etched tip; Right panel: Tube-etched tip.

† For improved imaging contrast the tapered glass fibres have been sputter coated with a thin platinum layer (5nm)
glass fibre is highly homogenous and virtually free of dips. In contrast, the tip produced by the protection layer method shows craters with diameter up to several hundred nanometers in the strongly corroded surface.

In particular, the dramatically increased smoothness of the tips is nicely reflected in the quality of the subsequently deposited ~100nm thick aluminium layer (Figure 2.25). In case of the tube-etched tips, the applied metal coating is virtually free of side holes. Their far-field transmission ranged from $2 \times 10^4$ to $5 \times 10^3$ for aperture diameters between 80 and 120nm.

![Figure 2.25. Electron micrograph of a ready to use SNOM tip fabricated by tube-etching an optical fibre. The inset shows a metallised SNOM tip prepared by Turner's method. The smoothness of the metal coating and aperture definition are far superior for tips fabricated by tube-etching.](image)

As a result of these experiments, it was found that tube-etching is a highly reproducible and efficient method to produce high-definition near-field optical probes with large cone angles and smooth, sidehole-free aluminium coatings. The method is tolerant against environmental perturbations such as temperature changes and vibrations. The fact that etching time does not influence the tip quality makes the handling of the process straightforward and easy. Furthermore, the yield of usable tips after etching is around 80-90% for tube-etching compared to 40-60% for the Turner method.

**Selective Etching**

Another route to obtain small point diameters and wide cone angles is to fully immerse the bare glass fibre into buffered hydrofluoric acid solution. Additives,
like complexing agents reduce the etching rate of the fibre core, resulting in cones with wide angles. In general, cone angles decrease with increasing doping concentration (e.g. GeO₂) in the glass core (graded-index multi-mode fibres). These methods require a precise time control or an in situ control of the etching process, realised by precisely measuring e.g. the decrease of the immersed fibre diameter. The group around Ohtsu succeeded in matching the chemical etching with the glass specifications. This method produces high-throughput optical near-field probes, however, the custom fabrication of optical fibres with well defined dopant gradients is expensive and requires specialised equipment.

Several groups have proposed schemes that combine heat-pulling and conventional chemical etching techniques (selective etching, Turner method) for the production of SNOM tips. In this way, they attempt to profit from the advantages inherent to the individual methods, like smooth glass surfaces and large cone angles, respectively.

Metallisation & Aperture Formation

In the taper region, where the waveguiding properties of the optical fibre breaks down, one or more metal-layers have to be applied around the sides of the probe to confine the light so that it only exits at the aperture at the very end of the tip.

Usually aluminium is chosen because of its excellent light reflecting properties in the visible region of the electromagnetic spectrum (See graph in figure 2.9), which reduces the amount of metal needed to confine the light. The opacity of the metal films, however, is not only depending on the reflectivity properties of the material used, but also strongly depends on the film’s homogeneity. Unfortunately, the formation of thin and smooth aluminium films is a difficult task. Evaporation of aluminium, for example, in the presence of contaminants, oxygen, or water most likely leads to rough films prone to pinholes where light can leak through the coating deteriorating the near-field optical properties of the probe. A vast number of factors have a great influence on the smoothness and homogeneity of the resulting metal film. A part of this work was to explore the effects that various coating conditions have on the quality of near-field optical tips.

Essentially, two pathways exist to coat tapered fibres. Either the aperture formation is created during metal deposition, or the tips are coated completely and the aperture is formed in a second step by selectively removing the metal at the tip apex.

The most common method of forming the aperture is during the evaporation of the metal. For this, the tapered fibres are rotated at an angle relative to a directed
metal vapour beam (Figure 2.26). Thereby, no metal is deposited at the flat end face of the fibre due to a geometrical constraint. For an infinitely sharp edge at the tip apex, the shadowing effect should result in an aperture sized as big as the end facet of the tapered probe. However, in reality the tip apex is not always well defined. Consequently, the final aperture dimensions depend also on the tilting angle under which the metal deposition is taking place. Note that, in principle, the larger the inclination angle, the smaller the relative metal deposition rate. As a consequence, longer evaporation times are required at distinct evaporation rates for larger tilt and cone angles. An estimate of the resulting film thickness \( d \) on the tapered fibre probe can be calculated from (Appendix B):

\[
d = \frac{E \cdot t}{\pi} \cdot \cos(\theta) \cdot \cos\left(\frac{\alpha}{2}\right)
\]

Estimation of film thickness on the tip

Where \( E \) stands for the absolute deposition rate on a plane parallel to the evaporation source [nm/s], \( t \) denotes the evaporation time [s], \( \theta \) is the inclination angle of the rotating fibre, and \( \alpha \) stands for the full cone angle of the tapered glass probe.\(^\dagger\) As a rule of thumb, long tips which have been metal coated at a grazing incidence exhibit a film thickness approximately three to four times less than comparable films on substrates placed parallel to the source. No metal will be deposited on the taper walls when \( \theta \) and \( \alpha/2 \) add up to over 90°.

\(^\dagger\) Typical values are \( E=10\,\text{nm/s}, t=30\,\text{s}, \theta=10^\circ, \) and \( \alpha=30^\circ \) leading to a coating thickness of approximately 90nm.
In theory, higher evaporation rates lead to smoother metal coatings, since there is a decreased amount of time is given for the condensing metal to agglomerate around centres of crystallisation. On the other hand, the tip is heated more at higher evaporation rates, since the heat dissipation from the tip apex is very limited in the vacuum environment. Accordingly, the diffusion rate of the metal on the surface increases, leading to undesired island formation. To counteract the increase in surface diffusion rate, external cooling of the tips can be employed. Although cooling during metallisation is technically difficult, it can be promoted by:

- Positioning the fibre probes as far from the evaporation source as possible, to reduce the inner (kinetic) energy of the metal vapour and radiation heating by the hot evaporation source.
- Cooling the vacuum recipient (water cooling).
- The use of shutters limits the heat radiation between the fibre probes and evaporation source.
- The deposition of several thinner layers allows the fibres to cool down between metal deposition phases.
- The application of different types of materials hinders the crystallisation and, therefore, the grain formation. The use of several materials instead of just aluminium also has other implications on the resulting tip quality. This will be discussed in more detail in the next section entitled “Metallisation & Optical Destruction Threshold”.

Many findings have also been employed for the production of rough noble-metal surfaces for surface-enhancing Raman investigations (Chapter 4). The detailed study will be presented in the later chapter. In summary, lower vacuum pressures, higher evaporation rates in combination with short evaporation time intervals, larger source-tip separations, and the use of different materials (e.g. Ti, Ni, etc.) in intermediate layers lead to smoother metal coatings.

The possibility to create apertures after metal deposition enables the use of other, non directed, metallisation schemes. Recently, a wet chemical process to coat tapered fibre tips with silver has been presented. More commonly, the SNOM tips can be coated by sputter-coating. In contrast to resistive or e-beam coaters which produce a directed vapour jet, the whole recipient is filled with a metal vapour cloud when sputtering, which metal-coats the fibres from all directions (Figure 2.27).

The comparison between sputtered metal coatings and those produced by resistive heating of a metal filament shows a significant difference in homogeneity and
smoothness of the resulting metal surface (Figure 2.28). On average, the silver islands are much smaller and more densely packed from the sputtering method. Further, the smaller island size distribution decreases the possibility of unwanted “leaks”. Additionally, the cooling of the fibre tips is greatly facilitated as the fibre tips do not need to be rotated anymore. Finally, in contrast to conventionally coated fibre tips where impurities and irregular tip morphologies most often produce pinholes due to geometrical shading, no such pinholes are found with sputter coated tips (Figure 2.29.).

Figure 2.27. Metallisation schemes like “sputter-coating” deposit the metal from all sides onto the fibre taper.

Figure 2.28. Comparison between e-beam evaporation (left) and sputter-coating (right). The electron micrographs show the metal surfaces after silver deposition on glass slides.

Sputter-coating [Baltec MED 010]: 150nm silver was sputtered at a rate of 2 to 5Å/s onto several etched glass fibre tips. The source-tip separation was around 10cm. The sputter gas (Ar, 10\(^{-3}\)mbar) was introduced after complete evacuation (10\(^{-6}\)mbar) of the vacuum chamber. The ground plate, where the fibres were positioned, was cooled down to liquid nitrogen temperature in order to reduce the diffusion rate of the condensed silver vapour, thus, preventing the formation of larger silver grains due to agglomeration at nucleation sites.

e-Beam evaporation [Balzers BE 500]: 800nm of silver was evaporated at 15Å/s in two steps on fibre tips rotating at 2Hz in 30cm distance from the evaporation source. The pressure in the recipient before metallisation was 1.0·10\(^{-7}\)mbar.
The formation of the aperture after metal deposition can be performed in various ways. Most crudely, Pohl et al.8 “opened” their completely coated tips by pressing them on a hard substrate, tearing away the metal at the tip apex. A more controlled method is based on the electrolysis between a silver metallised probe and an electrolytic glass. This “solid electrolytic etching” removes the metal selectively at the tip apex, while the optical transmission properties can be monitored simultaneously.97 Concurrently, Pilevar et al.98 and Veerman et al.99 published a micro-machining method to precisely cut the frontmost metal coating, in this way generating a perfectly smooth flat end face with a well defined aperture. Such probes, modified by a Focused Ion-Beam (FIB), exhibit defined aperture diameters down to 20nm. To investigate the possibilities of FIB-milling a feasibility study has been carried out with “closed” SNOM tips at the Paul-Scherrer Institute in Villigen. Although the instrumentation used had only a limited ion-beam confinement (approximately 150nm), the first micro-fabricated apertures have been created (Figure 2.30). The increasing availability of FIB-instrumentation will most likely allow to routinely shape fibre based SNOM probes in the not too distant future.
Metallisation & Optical Destruction Threshold

To date, the light intensity at the tip apex has been improved by optimising the taper formation procedures in order to get probes exhibiting larger and more homogeneous cone angles. Another way to increase the overall light intensity at the tip apex is to improve the coupling of laser power into the cleaved fibre. However, this is only possible up to the destruction threshold of the tip. Consequently, considerable effort has been put into improving the tip stability allowing for higher maximum light outputs. To ensure a localised, sub-wavelength sized light source at the tip apex, aluminium coating is usually evaporated onto the fibre material. The production of very smooth metal layers in the required dimensions, however, is not trivial. The opacity of the resulting thin films is not satisfactory, mainly due to the formation of metal grains. Other problems inherent to standard aluminium films, are the low destruction threshold limit and only little resistance to mechanical wear. To improve these shortcomings, adhesion layers have been applied between the glass fibre and the aluminium coating. A comparison of such metal films is presented here. The evaporation of different layers of metals such as aluminium in combination with titanium, chromium, cobalt, or nickel has been found to enhance the overall tip stability and produces smoother surface structures allowing to minimise the required metal film thickness.

In a previous section, the taper formation procedure called "tube-etching" has been introduced. Fibre tips produced by this technique have cone angles as large conventionally etched probes, but exhibit much smoother taper shapes and glass surfaces. The optical destruction threshold experiments presented here further demonstrate the importance of defect-free, smooth glass tapers for near-field probes with high optical destruction thresholds.

Eighty fibre tips were used in the optical threshold experiments. The light of a frequency doubled Nd:YAG laser [532nm; GCR-230, Spectra-Physics] was split (20:1) and the main part of the light was focussed onto a freshly cleaved glass fibre of the same type as the fibre probe under investigation (Figure 2.31). This glass fibre was then coupled via a fibre splicer [CamSplice assembly tool from Siecor] to the near-field optical probe. A neutral-density (ND) filter and a polariser allowed to

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1 The fibre probes were manufactured either conventionally, by the protection layer etching method or by the newly introduced "tube-etching" technique. Sixty centimeter long pieces of a multi-mode glass fibre with 100 μm core diameter (HCG-M0100T-14) from Laser Components were used. Additional experiments were carried out with single-mode 3μm core diameter glass fibres (40-692.11) and multi-mode 9μm core diameter glass fibres (Telecom. Standard 1550 nm) from Cabloptic.

(Continued on next page)
The near-field apertures were produced by evaporating metal layers onto the tapered glass fibres leaving a sub-wavelength-sized void at the tip apex. The metallisation was carried out in a mini-coating system [MED020, Baltec] by heating a tungsten filament at pressures between $3.1\times10^{-6}$ and $2.4\times10^{-5}$ mbar. Batches of eight tips were fixed in a cylindrical holder, which was rotated at approximately 1Hz. The fibres were mounted at an angle of 75° with respect to the evaporation direction. The tip – evaporation source distance was 20 and 23cm, respectively. To obtain smooth metal films, a high evaporation rate between 10 and 15nm/s for aluminium and a lower rate between 0.05 and 0.2nm/s for titanium and chromium was adjusted manually. The overall film thickness, as determined by a quartz crystal micro-balance [QSG 060, Baltec], was between 120 and 150nm leading to a fibre probe coating of approximately 40 to 50nm, depending on the cone angle of the fibre probe. The minicoating system in combination with a mechanical shutter enabled the evaporation of two different metals independently. A first series of tips were metallised with aluminium only. In a second series, a few monolayers of an adhesion metal (Cr and Ti) were applied prior to aluminium deposition. Finally, a series of tips were coated with 9 to 10 alternating metal layers of a few monolayers of Ti or Cr, respectively, and 25 to 35 nm of aluminium, whereas the top and bottom layer of these mixed-metal tips were Ti or Cr, respectively.

All tips were checked by standard optical microscopy. The optical transmission properties were evaluated in the far field by coupling in ca. 100μW continuous-wave (cw) laser light at 632nm [He-Ne 1201-2, Uniphase] into the cleaved fibre end of the probes. The cone angles of the tips have a strong influence on the optical destruction limit. For this reason, only tips with similar cone angles, between 20 and 30 degrees, were used for comparison. From selected tips, high-resolution scanning electron micrographs were taken on a Hitachi S-4100 before and after determining the optical destruction limit.

Figure 2.31. Experimental set-up for the destruction threshold determination of the SNOM tips. See text for details.
control the pulse energy precisely. Single pulses with increasing power were used for the experiments. The light emitted from the sub-wavelength aperture of the fibre probe was detected by a fast large-area silicon pin diode [S3204-05, Hamamatsu] coupled to a 500 MHz digital oscilloscope (9350A, LeCroy). The smaller portion of the light from the beam splitter was used to monitor the energy of each pulse. For this purpose, the beam was focussed onto a high-speed photo detector [DET200, Thorlabs], coupled to the second channel of the oscilloscope. The light emitted from the fibre probes was normalised by dividing the detector signal (channel 1) by the reference signal (channel 2) using the built in mathematical functions of the oscilloscope. The normalised signal was then transferred to a personal computer for data processing. The normalised detector signal increased with increasing laser power linearly up to the destruction limit of the tip. There, a jump in the measured signal indicated the destruction of the near-field probes. Following this jump in intensity, the measured detector signal increased again linearly, but shifted to higher intensities with respect to the one of the non-destroyed fibre tip. To confirm the physical change of the tip, a second series of laser pulses with increasing power was coupled into the probe. Expectedly, no jumps in the signal intensities were observed, whereas the shift to higher transmission also remained for low laser power intensities. The light intensities were calibrated at the fibre splicer assembly using a joulemeter [PSV-3102 V2/TMP-300, Gentec] and an optical power meter [Model 818-UV/OD3/840, Newport], respectively. After each experiment, the coupling efficiency of the fibre splicer was checked by measuring the transmission of the then freshly cleaved fibre.

Scanning electron micrographs of the fibre probes destroyed by excessive laser powers reveal that the destruction of the tip is a result of the metal coating being ripped off, whereas no obvious destruction of the glass core is observed (Figure 2.32). With a few exceptions, we found for each tip only one step in the measured detector signal, i.e. the tips were destroyed at one distinct laser power and no additional changes occurred upon further increasing the laser power after this first physical change.

The experiments show that the optical destruction threshold of the tube-etched fibre tips is about three times as high as compared to identically metallised, but conventionally etched near-field probes (Graph 2.2). One of the main benefits of the tube-etched fibre probes is their smooth glass surface, while conventionally etched fibres often show rather rough surfaces since the taper formation takes place at a solid-liquid-liquid interface that is sensitive to perturbations. Since any local defect at the glass surface would act as a nucleation site during metal deposition and can therefore additionally amplify the roughness and inhomogeneity of the
resulting metal coating, a smooth glass surface is favourable. Regarding the physical properties of the two types of tips, we conclude that the rupture of the metal coating originates at a defect site close to the tip apex, where the local energy density is the highest. Further evidence for this hypothesis can be found from the relative standard deviation of the destruction limits measured for the two types of tips: the high reproducibility of the smooth taper shapes and glass surfaces found for tube-etched tips leads to a significantly smaller relative standard deviation compared to conventionally fabricated probes, i.e., in contrast to conventionally etched tips, the destruction thresholds of correspondingly prepared tube-etched tips have similar values.
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Graph 2.2. Destruction thresholds of conventionally etched tips (Turner method) and tube-etched tips. The relative deviation for the conventionally etched tips is much larger than that of tube-etched tips. All tips, independent of their metallisation scheme, have been included for evaluation.

Typically, the metal coating was detached from the glass core in the form of thin flakes. From this, one can conclude that despite the good cohesive properties, the metal-glass adhesion is still insufficient. To improve the glass-aluminium adhesion, the etched fibre probes were exposed to concentrated ammonia and sodium hydroxide prior to metal deposition. This increases the surface charge and thus leads to a superior binding of the metal coating. However, in these preliminary experiments no improvement was observed. In consecutive experiments, an alternative idea to improve the stability of the metal coating was to use an adhesion layer between the aluminium coating and the glass (Figure 2.32). The use of an adhesive layer should also have the additional advantage of acting as a buffer region during transient heating, due to its intermediate heat expansion coefficient. For these purposes, transition metals of the fourth period are used. Titanium, chromium, nickel, and cobalt all showed good adhesive properties in qualitative scratch tests on metallised microscopy slides.

Fibre tips with an adhesion layer between the glass and the aluminium coating generally had a higher optical destruction threshold (Graph 2.3). The increase is more prominent for the tube-etched tips than for conventionally, two-layer etched probes. Tube-etched tips with few monolayers of titanium reached a destruction
The use of an adhesion layer between the glass and the aluminum coating increases the destruction limit of the SNOM tips. The effect is much more prominent for tips tapered by tube-etching.

threshold of 173±10 μJ [10ns pulse, λ=532 nm], whereas tips coated with chromium reached in average a slightly lower value of 163±14 μJ. Compared to tube-etched tips without any adhesion layer, this is an average increase of 42% and 34%, respectively. Although a slight increase in the destruction limit could be achieved for conventionally etched tips by applying an adhesion layer, the gain was much less significant and the value obtained for the average threshold of bare aluminium coated tips of 68±15 μJ even lies within the error margins of the titanium (70±19μJ) and chromium (69±18 μJ) co-coated tips. The outcome of the experiments showed that a few monolayers (<1nm thickness) were sufficient to increase the destruction threshold. Thicker adhesive layers (3nm) showed no further improvement.

Aged fibre probes (>6 months) coated with aluminium that were stored in boxes, but otherwise at ambient conditions sometimes show severely corroded surfaces. However, their optical destruction threshold did not change to a significant extent. To decrease of the overall coating thickness the application of a protection layer on

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*The oxide formation is generally accompanied by a decrease in film reflectivity and an increase in the film roughness, both of which can dramatically affect the performance of near-field probes.*

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top of the aluminium coating may become necessary. A series of tube-etched tips with an additional titanium coating on top of the aluminium coating have been prepared and will be compared with bare aluminium coated tips in future experiments.

Mixed metal coatings consisting of alternating thick (ca. 10nm) aluminium and thin (few monolayers) transition metal layers greatly increase the optical destruction threshold of the near-field optical probes (Graph 2.4). As with the adhesion layers, the greatest improvement was observed when using intermediate titanium layers. (Graph 2.5) With five alternating layers of titanium and aluminium the optical destruction threshold of tube-etched tips reached 276±6 µJ corresponding to an increase of over 125% compared to bare aluminium coatings. The combination of aluminium and chromium yielded in tips with a destruction limit of 238±26 µJ (+95%).

The use of intermediate metal layers is predicted to fulfill two functions. Firstly, the transition metal layer strongly perturbs the grain formation and crystallisation of the aluminium coating. Therefore, more amorphous layers with less pronounced defect centres are formed, consequently leading to higher optical destruction limits. Secondly, due to the large adhesive and cohesive properties of the investigated transition metals a general increase in mechanical stability of the metal coating is found.

Graph 2.4. Multi-layer coatings further improves the destruction threshold of tube-etched tips.
Graph 2.5. The type of intermediate layers has an effect on the destruction threshold level of tube-etched tips. Multi-layer coatings with titanium are most suitable for high-power measurements.

The experimental findings strongly promote the use of fabrication techniques such as heat-pulling or tube-etching that result in smooth and defect-free near-field optical fibre probes. Presumably due to the improved surface properties of the glass and the subsequently deposited metal coating, a destruction limit of $122\pm15\ \mu\text{J}$ was obtained, almost twice as high compared to the rougher, conventionally etched fibre probes. To further improve the optical stability of the fibre probes, an adhesion layer, for example chromium or titanium, can be applied prior to the aluminium

Figure 2.32. Schematic of the possible metallisation schemes. Multi-layer metal coatings are the most favourable due to their high destruction threshold and smooth, pinhole free coating.
deposition. Finally, the deposition of alternating thin titanium and thick aluminium additionally reduces the grain formation and crystallisation within the metal coating leading to firmer optical probes. In this way, an optical destruction threshold of 276μJ was achieved, corresponding to an improvement in stability of over 400% compared to conventionally fabricated near-field optical tips. The use of such probes will therefore allow a four-fold scan time reduction in future near-field spectroscopic measurements.

Micro-Fabricated Tips

Structures other than tapered optical fibres, for example cleaved crystals⁵, semiconducting structures¹⁰, tetrahedral tips⁵⁹, and coaxial structures¹⁰⁴-¹⁰⁶ are employed by other groups for sub-wavelength imaging, but they still account for only a small minority in the near-field optical society.

With the rise in micro-machining techniques, micro-fabricated probes are becoming increasingly popular.¹⁰⁷,¹¹⁰ They potentially allow for mass-production and integration into existing AFM instrumentation. The shape of the tips can be designed almost freely, allowing for maximum light transmission at increasingly small aperture dimensions (<30nm). Most commonly, the micro-fabricated SNOM probes consist of a hollow metallic pyramid with a circular aperture at the apex, attached to a silicon cantilever.¹⁰⁹,¹¹¹-¹¹³

A class of micro-machined probes contain an integrated signal transducer (Schottky diodes, but also pH sensors, thermocouples, etc). Such “active” probes allow for the complete omission of the detection pathway,²⁹,³³,³⁴,¹¹⁴-¹¹⁶ significantly facilitating the instrumental set-up. However, the required broad illumination of the sample is not suitable for a sensitive specimen and, potentially, can give rise to various artefacts.

Nanoscale light sources can also be created by attaching fluorescent molecules¹¹⁷,¹¹⁸ or other “self”-luminescent centres¹¹⁵,¹²⁴ (e.g. electro-luminescence of Schottky barriers) onto AFM tips. Technical difficulties in the probe fabrication and the short lifetime of such tips (e.g. bleaching of the active molecules), however, still limit their widespread application.

Although, micro-fabricated SNOM probes offer a variety of advantages over manually produced fibre tips, the design and manufacturing requires advanced and expensive micro-machining instrumentation. The first near-field optical images acquired by micro-machined tips have been published,²⁹,³³,³⁴,⁸⁶ though generally, such tips are not yet commercially available.
Characterisation of SNOM Probes

Before the tapered and metallised fibre probes were mounted to the tip holder assembly of the microscopes, they were routinely checked by a light microscope. The tips were characterised and classified according to their shape, taper continuity, sharpness, far-field interference pattern, and optical throughput (See figure 2.33). The characteristic parameters were recorded together with the information on the fabrication conditions in a database [FilemakerPro 4, Claris] allowing to track all the parameters of a distinct probe electronically.

From selected tips, high-resolution scanning electron micrographs [Hitachi S4100 at the CSEM Badenerstrasse, Hitachi S-700 at the Institute of Microbiology ETHZ, and for high-resolution images Hitachi S-900 at the Institute of Microbiology ETHZ] were taken allowing us to estimate the aperture diameter and homogeneity of the coating.

The evaluation of the near-field optical resolution using test patterns is time consuming and bears the high risk of destroying a high-quality tip in the procedure. The best measure for the probe quality remains to be the SNOM experiment itself. Together with the knowledge on the sample morphology and chemical contrast mechanisms involved, an optical resolution and throughput could be estimated, ultimately defining the overall probe quality.

Figure 2.33. Optical micrograph of a SNOM probe immersed in a diluted Rhodamine (Fluorescent Dye) solution. The point-like light source illuminates the dye at 488nm; The fluorescent emission is in the green (530nm) region. Note the wide angle of re-emission (reflecting the electromagnetic field intensity distribution).

† With the completion of this dissertation, more than 1'000 SNOM probes have been fabricated and characterised.
References


Enlightening the Nanoworld


...shear-force control in near-field scanning optical microscopy... High efficiency: dual collection mode...
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[45] See Ref. 44.


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[120] See Ref. [1].


Chapter 3

Near-Field Optical Spectroscopy
3.1 Contrast Mechanisms

The strength of near-field optical microscopy over other scanning probe techniques is that it allows the observation of a whole variety of sample properties. In SNOM a choice of various, sometimes complementary, optical contrast mechanisms are available to investigate surfaces. In principle one can distinguish between three main contrast mechanisms:

- Monitoring the **light intensity** allows the monitoring of changes in transmittivity, reflectivity, or index of refraction.

- Information on the orientation of surface molecules and sample birefringence can be obtained by observing **polarisation contrasts**. This group also accounts for various other analytic techniques involving phase variations (and amplitude modulations) of the electro-magnetic field, which, for example, are used in interferometric imaging.

- The **wavelength contrast** inherent to all chemically inhomogeneous surfaces allows for spectroscopic investigations. Chemical information can be obtained by numerous spectroscopic techniques such as light-absorption (IR, visible, and UV), luminescence (photo- as well as electro-excited), and Raman.

3.2 Intensity Contrast

The integrated amount of light gathered by the collection optics and detected by a broadband detector provides information about the transmission, reflectivity, or changes of index of refraction in general. The intensity contrast is conceptually the simplest imaging mechanism, but does not provide a deep chemical insight to the sample under investigation. Furthermore, monitoring just the intensity of the signal is particularly susceptible to topography artefacts, and care has to be taken when interpreting the data.

**Transmission**

Thin or transparent samples are preferably measured in transmission. In this arrangement high numerical aperture oil immersion objectives can be used to collect the scattered light, increasing the efficiency of the optical collection set-up.
The detected contrast arises from different (surface) materials having invariant optical densities, absorption properties, or reflection characteristics. In figure 3.1 a shadow-mask composed of small aluminium particles of about 250nm in diameter is shown. In the left panel, the topographic image clearly shows the hexagonal structure of the mask: high places are tinted bright, the deeper regions are coloured in a darker red. Virtually all light illuminating an uncovered substrate surface passes through it without much retention, whereas the light hitting an (opaque) particle is either absorbed or reflected. In theory, it does not make any difference whether the illuminating source is laterally confined or the detector is laterally highly resolving. Thus, such masks are commonly used as calibration standards in near-field optical microscopy.
one would expect a similar result with (i) a point source used in conventional SNOM in combination with a large detector, or, (ii) a wide-field illumination in combination with a laterally highly resolving detector. Consequently, places with metal particles lead to dark areas in the optical transmission image (Right panel figure 3.1, figure 3.2.). This experiment shows the ability to optically distinguish between different absorbing or reflecting materials in the lateral range well below the incident wavelength.

Reflection

Thick or non-transparent samples have to be measured in the reflection mode. The collection of the scattered light can be performed either by long working-distance microscope objectives, back through the tapered SNOM fibre (“Dual-Mode”), or by a cleaved optical fibre positioned in close proximity to the near-field source. To evaluate the performance of the SNOM apparatus in reflection mode a shadow-mask was prepared by evaporating a thin silver film over 450nm sized spheres dispersed on a microscopy quartz slide. The subsequent removal of the spheres produced a periodic structure. Figure 3.3 depicts the topographical and optical image,† which is obtained when collecting the near-field scattered light with a microscope objective placed at 45° relative to the SNOM probe. On careful

![Reflection Image](image_url)

**Figure 3.3.** Reflection image obtained from periodic metal structures. The detection has been performed with an ELWD microscope in 45°. Note, that the topographical and optical registry is not exact. The crosses and circles are guides for the eye.

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1 Measurements were performed on the Aurora instrument with 488nm incident laser radiation. The scattered light was detected by a photo-multiplier tube (PMT) after removal of the diode laser light (670nm) used for the feedback control of the SNOM probe.
Chapter 3 - Near-Field Optical Spectroscopy

Figure 3.4. Morphologically induced shading can lead to imaging artefacts in reflection SNOM.

observation, one can see that the topographical and optical images do not match entirely. The partial displacement of their registry most probably arises from the fact that the metal particles are not perfectly spherical, but can exhibit an irregular surface structure promoting or hindering the reflection towards the collection optics (Figure 3.4).

Figure 3.5. The morphological shading can clearly be detected in this reflection SNOM image. The optical detection was performed by a cleaved fibre placed on the left side of the probe. The total topographic contrast is 68nm (left image).
To show this phenomenon more clearly, a very blunt SNOM probe but with a small aperture at its apex, was used to image the same substrate as described above on the Lumina. With such a tip, only large topographic features are being followed by the probe, whereas small surface irregularities are averaged. The reflected light was collected by a cleaved optical glass fibre [100µm multi-mode from Laser Components] and subsequently detected by the standard photo-multiplier tube of the Lumina microscope. The collection fibre was positioned by a hydraulic micromanipulator [Nashirige, Nikon] within few micrometers of the SNOM probe at a grazing angle (15°) to enhance the predicted shading effect of the metal particles. In the optical image of the figure 3.5, one can clearly see the dark regions at the back side of the particles. The sides of the surface features pointing towards the collection fibre result in bright regions in the optical image. Although the sub-wavelength sized metal particles are not resolved in the topography image, they still appear in the optical image.

While interpreting the data obtained from intensity measurements, it must be remembered, that the measured signal is a function of the tip-sample interaction. For this and other reasons, this imaging technique is particularly prone to a variety of artefacts (See also “Limitations of SNOM...” in chapter 4.6). Further, intensity sensitive microscopy does not offer a high chemical information content of the specimen examined. However, the results obtained with these preliminary experiments have been very valuable for the “artefact-free” investigation of morphologically complex surfaces. For instance, all (surface-enhanced) near-field Raman measurements were routinely corrected for reflectivity variations to extract quantitative Raman intensity values from the morphologically convoluted optical image (Chapter 4).

3.3 Polarisation Sensitive Microscopy

The use of optical polarisers on the detection side of the instrumental set-up and the control of the polarisation state of light incident on the specimen can reveal various kinds of contrast to otherwise optically isotropic objects. This polarisation contrast is induced by dichroism (the material shows selective absorption of one or two orthogonal polarisation directions) or birefringence (the material shows different index of refraction for the two orthogonal polarisation directions). In addition,

† Well-known examples are dichroic polarisers or the birefringent crystal calcite, which forms double images of objects observed through it.
some materials show a distinct response on illumination by polarised light in combination with magnetism.\textsuperscript{10,11}

In order to perform polarisation sensitive spectroscopy, the state of polarisation has to be conserved in the fibre of the SNOM tip. Advanced SNOM tips fulfill these requirements,\textsuperscript{10} as extinction ratios well above 100 were measured for SNOM probes transferring linearly polarised light.\textsuperscript{13,10,12,17}

In principle, the direction of input polarisation can be kept either fixed or modulated. In practice, for technical reasons it is easier to work with a \textbf{fixed input polarisation direction}. The contrast in the optical image can easily be adjusted by controlling the state of polarisation of the incident laser radiation and the relative angle of the polariser at the detection side. A Langmuir-Blodgett film containing polydiacetylene (LB-PDA) has been prepared for high-resolution polarisation sensitive imaging. Optical far-field and near-field images depend strongly on the incident polarisation (Figure 3.6). Dark crystals turn bright and vice versa on changing the direction of incident polarisation. Such organic crystals of PDA have been extensively investigated due to their non-linear optical properties.\textsuperscript{18-20} The near-field optical investigations exposed the optical contrast mechanism involved in polarisation sensitive near-field optical microscopy of morphologically flat samples.

\textbf{Varying the linear input polarisation over all possible directions} offers additional information over working with one fixed polarisation. In a modulation scheme, the input polarisation is varied continuously over 180 degrees, and the sample response to all these different linear polarisation states is recorded. Experimentally, the modulation can be realised by an electro-optical modulator and a quarter-wave plate. However, fibre birefringence compensation is even more important than for fixed polarisation experiments, and depolarisation by the SNOM probes is a severe problem.\textsuperscript{10,21}

\textsuperscript{1} Magneto-optics describes the interaction of optics with magnetism. The Faraday effect describes the change of polarisation of light transmitted through a magnetised sample; the analogous effect in reflection is the magneto-optic Kerr effect which has huge applicability in technology, such as for magneto-optical storage (MO disks). The Faraday effect is a circular birefringence effect: depending on the magnetisation and the material properties and thickness, a rotation of the transmitted linearly polarised light can be observed.\textsuperscript{20}

\textsuperscript{11} Fibre probes often show asymmetries in taper shape and aperture, which lead to a partial depolarisation of the injected light. However, one direction of polarisation can usually be found where the fibre depolarisation is small, allowing to perform polarisation contrast imaging with reasonably small instrumental signal background.
Far-field Laser Scanning Confocal Microscopy

Polarisation Sensitive Scanning Near-field Optical Microscopy

**Figure 3.6.** Polarisation sensitive microscopy images of Langmuir-Blodgett films containing polydiacetylene. The images have been acquired with a confocal laser scanning microscope (left panel) and SNOM (right panels). In SNOM, the upper and lower panel have been acquired from different sample locations. Note that as a consequence of rotated polarisation state, the cracks in the films show up dark in the upper image, whereas they are bright in the lower image.

### 3.4 Wavelength Sensitive Spectroscopy

If the optical SNOM signal is wavelength dependent, it can be used to investigate the chemical properties of the sample surface. Spectroscopic measurements can be achieved by observing the output signal during a wavelength scan of the incident radiation (Excitation or Absorption Spectroscopy) or by keeping the input
wavelength fixed while measuring the spectrally variant output signal (Emission or Luminescence Spectroscopy).

**Absorption Spectroscopy**

In principle, conventional absorption spectroscopy (with fixed or tuneable laser source) can also be performed with SNOM. However, technical difficulties have restricted its widespread implementation in near-field optical microscopes. The difficulty of detecting weak absorption features with transmittance methods due to the otherwise generally large background intensity precludes routine analysis. The transmission characteristics of the nanometer-scale apertures of SNOM tips are strongly wavelength dependent. Further, the optical fibres used for excitation introduce a significant but fibre type dependent amount of absorbing material. Laborious pre-calibration of each near-field probe would be necessary even for qualitative investigations. Finally, one has to keep in mind that the available field used in imaging is a function of the object-probe system. Consequently, the measured SNOM signal intensity can vary significantly with tip-surface separation or local dielectric properties of the sample, complicating quantitative absorption spectroscopy with near-field optics. For these reasons only very few wavelength-dependent absorptivity investigations have been performed to date.\(^{22,23}\)

Nevertheless, infrared (IR) absorption spectroscopy has become popular for methodical investigations using the near-field technique since technical demands on the sub-wavelength sized aperture can be fulfilled more easily.\(^{24-27}\) Further, IR-absorption (or FT-IR) investigations have the potential to provide a wealth of information on the structure and chemistry of the examined sample.

The lack of high transmission optical fibres, the cost of a tuneable IR sources, and the rather low sensitivity of broadband IR detectors limits the applicability of aperture based scanning probe systems. With aperture-less SNOM configurations (Chapter 6), many of the restrictions can be overcome. Only recently, Knoll and Keilmann demonstrated an aperture-less near-field IR absorption measurement on a composite polymer film resolving polystyrene (PS) and polymethylmethacrylate (PMMA) with a lateral resolution below 100nm.\(^{28}\)

**Luminescence spectroscopy**

Chemical nano-analysis with SNOM is most commonly performed with so called emission spectroscopy techniques. Atoms and molecules, which absorb light, are excited electronically. The excited atoms or molecules can undergo a radiative decay
process, re-emitting photons. These light quanta are re-emitted with less energy, and therefore, can be detected spectrally shifted (i.e. at longer wavelength) with respect to the incident radiation frequency (Figure 3.7). Depending on the energy transitions involved, one distinguishes between fluorescence ($S_1 \rightarrow S_0$; fast decay of the excited state: $<10^{-7}$ seconds) and phosphorescence ($S_1 \rightarrow T_1 \rightarrow S_0$; slow decay of the excited state: milliseconds to hours).

The molecular fluorescence spectrum reveals the energy states of the molecular vibrations in the ground state. Experiments performed with a controlled state of incident polarisation can be used to determine the orientation of the molecules. Often, fluorescence is used to detect intermediate species in chemical reactions, or to learn about the reaction kinetics in general. Furthermore, fluorescence can be employed to probe the population state of electronic energy levels of molecules, while the decay rate of the fluorescence provides information on the energy dissipation channels intrinsic to chemical systems. Laser-induced fluorescence is widely used for chemical specification in production lines (pharmaceutical industry, food production, etc.) and for online analysis in general, commonly in combination with separation techniques such as chromatography or electrophoresis. The possibility to specifically “tag” biological species with fluorescent compounds greatly facilitates modern diagnostics.

In the last few years several groups have developed the capability to detect and identify single fluorescent molecules at room temperatures in solids, solutions, or on surfaces. Because typical molecules absorb and emit only approximately $10^5$ photons before photobleaching (Chapter 3.5), very sensitive detection is necessary. SNOM has the sensitivity to perform single-molecule spectroscopic and dynamic measurements at room temperature.

More important, the combination of fluorescence spectroscopy with an imaging technique like SNOM allows determination of specific molecular systems with very high spatial resolution. In figure 3.8.a, polymer embedded latex beads ($\phi$ 100nm)
The excitation was performed at 488nm. Separation of the fluorescence was performed with coloured glass filters.

Figure 3.8. Topography and fluorescence images acquired by SNOM: Matrix embedded latex beads (a), polystyrene spheres on mica (b), Langmuir-Blodgett film containing PDA (c), and microcontact-printed stripe of TRITC–labelled cytochrome c on PMMA (d). (See text for details).
have been imaged by fluorescence-SNOM. While completely embedded spheres cannot be detected in the topograph, they are resolved in the optical image. The beads at the sample surface give rise to higher signal intensities compared to those embedded deeper in the surface layer. Correspondingly, beads with higher intensities have generally been resolved with a higher optical resolution due to their smaller separation from the near-field probe during imaging. Note that the high elevations in the topographic image all show a characteristic double feature (two adjacent spheres). This is a commonly seen artefact arising from probes with two distinct tips, which are imaged by comparably sharp sample features. While the bad tip did not provide high quality topographs, an optical resolution below 70 nm was still obtainable.

Figure 3.8.b shows the topography and fluorescence signal of stained polystyrene spheres (ø 250 nm) on mica. Some of the beads do not show any fluorescence and, most probably, have been emptied (i.e. lost the dye) in the substrate preparation process. Although the ability to resolve single fluorescing beads from neighbouring ones may arise from topographic coupling, the achieved optical resolution was again estimated to be below 70 nm (not shown).

The polydiacetylene (PDA) used for polarisation sensitive SNOM measurements (Chapter 3.3) have been investigated also by fluorescence spectroscopy (Figure 3.8.c). As expected, the near-field fluorescence signal intensity was a strong function of the incident state of polarisation. The crystal in on the left side of the image very likely has a different orientation compared to the fluorescence emitting right crystal: When turning the incident polarisation, the left crystal starts to light up while the one on the right side darkens (not shown).

Sample specimens having no suitable “native” fluorescence can be labelled covalently with small dye molecules or semiconductor nano-crystals. Various staining techniques exist to site-specifically label bio-molecules exist, and are commonly employed in bio-analysis. Figure 3.8.d shows one of several microcontact-printed stripes\(^5\) of tetramethyl-rhodamine-isothiocyanate (TRITC, Rhodamine B) –labelled cytochrome c on PMMA. The 16 µm wide and 26 µm separated stripes are only about 3 nm high. Optical contrasts, therefore, should entirely arise from chemical variances and not from topographical coupling. The preliminary fluorescence measurements on this sample examined the suitability of the substrate for the determination of the resolving power of tip-enhanced Raman spectroscopy investigations (TERS, Chapter 6).\(^6,7\) While unlabelled micro-patterns (Cytochrome c without TRITC) could not be detected by SNOM, the labelled structures suffered strongly from photo-degradation at incident wavelengths of 488 and 514 nm (ca. 1 mW), respectively (not shown). The contact-printed patterns proved
to be very fragile and were easily modified by atomic-force measurements conducted in contact mode (not shown). The investigated contact-printed patterns, therefore, were not suitable for TERS experiments. However, the use of different dye-labels (e.g. brilliant cresyl blue; BCB) could prove to be more suitable for TERS experiments.

**Biological Applications**

Mainly due to its non-invasive character, the ability to study samples in their native environment, high sensitivity, high information content, and high time resolution, optical spectroscopy remains to be the key technique to investigate biological samples. A significant limitation in gaining insight into small compounds of biological systems (individual cells) like membranes, filaments, chromosomes, neurones, etc. *in vivo* has been overcome with the advent of SNOM. The main difficulties in performing near-field imaging of cells are the following: the scattering of the optical signal in the cell, the difficulties in keeping the sample unchanged during measurement, and the need of relatively large scan ranges in the z-axis.

Since the set-up used for our bio-SNOM experiments (Lumina) was built on a microscope, a particular cell could be pre-chosen from a culture of hundreds of cells. The home-built confocal laser-scanning set-up, additionally, could be employed to check the optical activity of the labelled specimens on a coarse scale. Figure 3.9 shows shear-force topography and near-field fluorescence from green fluorescent protein (GFP)-labelled fibroblast cells in air. The cells were prepared by growing

*Figure 3.9.* Shear-force topography and fluorescence images of GFP-labelled fibroblast cells in air.
them directly on glass cover slides and subsequent labelling by Anja Vinckier and Urs Ziegler (University Zürich).

SNOM is by its nature a surface analysis technique, therefore, one is limited to image the outer surface of living cells only. For a better understanding of protein structure and function at the inner membrane surface, the cells have to be “opened” to be accessible for high resolution imaging by SNOM. Basal cell membranes from Madine-Darby canine kidney (MDCK) cells have been prepared with a lysis-squirting protocol by Anja Vinckier and Urs Ziegler.53 Intact cells grown on glass substrates are cleaved revealing the inner basal cell membranes and still attached cytoplasmic components. These cell membranes remain firmly fixed to the substrate over the whole cellular area. Filaments of the actine cytoskeleton were subsequently stained with fluorescein-5-isothiocyanate (FITC) labelled phalloidin. The substructure obtained in near-field fluorescence images was not observed with the confocal microscope (Figure 3.10). This improvement in resolution opens the

Figure 3.10. Far-field confocal (left panel), and SNOM images (centre and right panel) of FITC-labelled actin filaments of MDCK cells. The two selected SNOM images were acquired by independent measurements at different sample locations. The lateral optical resolution is estimated for both images to be approximately 100nm. Confocal microscopy was performed by Urs Ziegler, University Zürich; the lower of the two SNOM images was acquired by Dieter Zeisel, ETH Zürich.
possibility of investigating native protein structures on the inner side of membranes under physiological conditions, giving insight into the function and organisation of membrane proteins at the inner side of basal cell membranes. For example, some proteins like the sodium phosphate transporter type II in MDCK cells are no longer equally distributed after lysis-squirt. The determination of the local transporter distribution and their diffusion kinetics under physiological conditions is of great interest and the first experiments in this direction have been performed in our laboratories. For this experiment, the sodium phosphate transporter type II proteins have been labelled with a polyclonal rabbit antibody followed by a goat anti rabbit Texas Red antibody by Urs Ziegler. The red emission of the transporter proteins could be recorded separately with the green emission of the actin filaments allowing determination of the relative position of the transporter and filaments. However, to facilitate these preliminary SNOM measurements, the bio-sample was fixated and dried. Ongoing experiments aim to investigate living cells with an unperturbed biological activity (Chapter 3.6).

3.5 Bleaching

The excess energy of electronically excited molecules is not only discarded by radiative decays, but more commonly is transferred into vibrational, rotational, and translational motion. In other words, the decay pathways convert the excitation energy into heat, which ultimately leads to the destruction of the molecules (thermal degradation). Furthermore, excited molecules may also directly take part in a chemical reaction with neighbouring molecules. This is referred to as “photo-bleaching” and presents a big problem in the spectroscopic investigation of light-sensitive samples.

In SNOM experiments, the excitation beam cross section is greatly reduced compared to conventional far-field spectroscopy. In near-field microscopy, only the region of the sample under immediate investigation is illuminated, sparing other molecules from photo-damage.

On the other hand, controlled photo-bleaching can be used for chemical explorations, revealing the energy dissipation channels of molecular systems. Selective photo-bleaching can also be a desired phenomena, for example, in the chemical surface modification or nano-writing. Figure 3.11 shows a glass surface covered with latex spheres (ø 100nm) stained with nil red dye. At selected points, the SNOM tip has been placed over the surface for several seconds, so that the dye is photo-bleached. In a second scan, the same tip images the surface while detecting
difficulties. First, the increased motility (e.g., typically 4-20 nm/s for fibroblasts) and the large thickness change (typically 10-20 nm) of living cells with time require the acceleration of the imaging process (i.e., increase scan speeds or lower pixel resolution). Secondly, the total internal reflection on the liquid-air interface necessitates the detection to be performed in transmission or in “dual-mode” or to use optical fibres fully immersed in the liquid phase for detection. More importantly, the immersion of the probe into liquid causes additional viscous damping, significantly reducing the tip-sample interaction forces and therefore lower the sensitivity. When operated in air, only the mode where the tines of the

Figure 3.12. The frequency response of a typical SNOM tip mounted on a tuning fork. Different vibrational modes are excited depending whether (a) the tip is in air; (b) at the liquid surface; (c) immersed in the liquid; (d) the tuning-fork is immersed in the liquid; (e) the tip is in feedback with the sample surface. A suitable resonance peak for liquid SNOM experiments is usually the one where both tines move in the same directions (around 66 kHz for this tip). The inset in the top spectrum shows a magnification of the perfectly symmetric resonance peak when operated in air.

† Elaborate designs of liquid cells have been proposed for SNOM measurements in liquids allowing for detection in reflection, but all suffer from significant sensitivity losses.

§ For the liquid-SNOM investigations, over thirty different SNOM tips were examined.
fork move in opposite directions is excited, whereas the other vibrational modes are not present (Figure 3.12). This resonance peak broadens and drops in frequency after immersion in liquid while other modes increase in intensity. The strong reduction in intensity of the original mode (observed at around 90kHz) is a function of the lowered Q-factor and the phase shift of the vibration. As a consequence, the frequency spectrum of the oscillated tip changes significantly. Most prominently, the oscillating mode where the tines move in the same direction is developed (the peak around 66kHz rises). For measurements in liquid environments, this new mode has usually been chosen for distance regulation. The variation of the frequency spectrum becomes even more severe when not only the tip, but also the tuning fork is immersed into the liquid. Thick liquid layers may be employed to minimise surface effects of the liquid layer on the specimen or to prevent the water layer from accidentally evaporating off, irreversibly damaging the biological sample. Care has to be taken that the glue used to mount the fibre tip to the tuning fork does not release chemicals into the buffer solution, which may harm the biological system and change the resonance frequency of the tip. Furthermore, depending on the feedback detection scheme, the tines have to be electronically isolated from each other (e.g. with hairspray). In our system, the activation of the vibration of the tuning fork has been performed mechanically (rather than electronically), by shaking the whole tuning fork at resonance with a remote piezo actuator.

Since the feedback in liquids is very sensitive to changes in the system (amount of liquid, immersion depth, etc.; figure 3.13), the tip-sample approach has to be conducted in several steps. Between each approaching step, like the immersion of the tip or the immersion of the tuning-fork, a complete frequency sweep has to be performed to be able to adapt the detection frequency of choice. To avoid accidentally colliding the tip with the samples, the adjustment of the detection frequency has to be repeated more frequently with decreasing tip-sample separation where large changes in the spectrum occur. Obviously, the process of bringing the tip into feedback is much more laborious and time consuming in liquid than in air. For this reason one should take care to select the desired region of interest by wide-field microscopy before approaching the specimen surface. Once the tip is in feedback, the imaging should be performed immediately, since even small changes in the system like the evaporation of water can alter the feedback conditions, and therefore, the tip-sample separation (Figure 3.13).

† A shift in phase leads to a different shape of the modulation in the phase sensitive frequency sweep.

‡ Acrylic super-glues have shown to be impracticable for this reason. UV-hardened polymer glues and two component glues have been used instead for liquid SNOM measurements.
Figure 3.13. Even small changes of the sampling conditions can shift the resonance frequency, alter the phase state of the oscillation, and change the $Q$-factor of the feedback system. Consequently, the tip-sample separation can differ significantly with time compromising SNOM imaging. The two upper panels show the influence of the amount of water in the liquid cell (Water added (top) and removed by evaporation (centre), respectively). The bottom graphs show one of the technical difficulties during the tip-sample approach: the lowering of the tip by only a few micro-meters leads to a intensity decrease of over one-half when measuring at a fixed oscillation frequency. The triangles in the graphs remain at the same frequency at each panel and are guides for the eye.
Liquid Cell Design

A constant liquid level during the entire measurement is crucial. To achieve this without introducing external vibrations, a liquid cell connected to a large water reservoir has been designed. The cells consisted of a polymer ring ($\Delta z = 2\text{mm}$, $\sigma = 2\text{cm}$) which has been fixed electrostatically on a thin ($70\mu\text{m}$) microscopy slide. Two channels on the sidewall of the polymer ring were connected with a large water reservoir (1 liter) by a flexible polymer tubing. The water reservoir could be adjusted in height by a micrometer displacement stage, allowing the water level in the liquid cell to be set. Water evaporating from the open liquid cell was continuously replaced from the large reservoir. Still, manual re-adjustments were necessary before each image acquisition (ca. every 10 minutes). A further difficulty was the diffusion of the ions in the buffer solution to the water reservoir, which slowly depletes the salt concentration in the measuring cell. The biological specimens used, however, proved to be fairly robust in this respect, and survived even when investigated in pure water for several hours.

Measurements on Intact Cells

Preceding measurements on basal cell membranes of Madine-Darby canine kidney (MDCK) cells allowed for successful fluorescence imaging by SNOM. For the corresponding liquid-SNOM experiments, intact MDCK cells were used. The cells were cultivated directly on the (pre-treated) microscopy slides of the liquid cells. Subsequent fixation and labelling of the actin cytoskeleton with FITC stopped further growth and made the cells stable for preliminary scanning probe investigations. Figure 3.14 shows eight fluorescence SNOM images of three different cell surfaces. Similarly to the experiments on the matrix-embedded spheres (Chapter 3.4: Luminescence Spectroscopy), the actin filaments at the cell surface were imaged with a greater optical resolution than those deeper in the surface. The optical resolution approaches $100\text{nm}$ in (a.) and (b.) and is still well below the incident wavelength (488nm) in (c.) and (d.). The latter two images have been taken from the same cell surface region in succeeding runs. Some variations are already detectable in those two images demonstrating the motility of the biological system. The interpretation of the optical images becomes difficult at this stage. The relation of depicted features to known biological structures is further complicated, since the SNOM images describe a projection of a 3-dimensional membrane rather than planar cross-sections of a cell as in conventional microscopy. The combination of laser scanning confocal microscopy with SNOM in one instrument would provide complementary optical images of the same sample, significantly facilitating the interpretation of the high-resolution images acquired by SNOM.
Figure 3.14. Four selected fluorescence SNOM images (upper panel) and the subsequently acquired magnified image (lower panel) of a cell surface of intact MDCK cells. (c) and (d) have been taken from the same cell surface in proceeding runs. Note that the optical images notably changed for the latter two measurements within about ten minutes that lie between two consecutive runs.

Figure 3.15 depicts the simultaneously acquired topography and fluorescence response of two cell surfaces. The surface roughness makes imaging especially difficult to be followed by the large SNOM probe, suggesting the use of high-aspect ratio tips. Those tips, however, suffer from poor optical transmission, and thus, demand longer measuring times. These

Figure 3.15. Corresponding fluorescence SNOM and shear-force topography images of MDCK cells. Note the high surface roughness of the cell.
images, however, are proofs of principle that it is possible to investigate intact cells under physiological conditions.

The ultimate objective is the in vivo monitoring of processes involved in channel or pore formation in native, unfixed basal cell membranes. The first step involves the observation of labelled sodium phosphate transporter proteins. The optical contrast and resolvability of the transporter proteins is expected to be even better due to their closer proximity to the outer membrane surface compared to the already detected actin filaments.

**Future Application**

In medical applications, photodynamic therapy (PDT) is widely used these days to remove cancer cells from the surrounding tissue selectively. Externally added dye molecules agglomerate preferably in malignant cells. A slight shift in absorbance is often detected when the dye is added to malignant rather than healthy cells. Moreover, illumination of these cells with the appropriate laser wavelength leads to their destruction. However, the mechanism and exact location of aggregation is still unclear. With a nano-analytic technique like SNOM further insight into the mechanisms of the dye aggregation can be obtained, aiding the optimisation of currently used PDT sensitizers.
3.7 References


Chapter 3 - Near-Field Optical Spectroscopy


Chapter 4

Near-Field Raman Microscopy
4.1 Vibrational Spectroscopy

The combination of fluorescence with SNOM enables the chemical investigation of surfaces with a very high lateral resolution. However, as discussed in the previous chapter, fluorescence SNOM results in photo-degradation and provides the experimentalist with only limited chemical information, rendering the determination of unknown sample surfaces almost impossible. Several other spectroscopy techniques are available for use with SNOM, among which Raman and infrared absorption spectroscopy are the most prominent. Both approaches explore the energy levels of molecules, providing information on the vibrational states\(^1\) and thus the structural units.

Raman versus IR Spectroscopy

Molecular vibrations can be observed with either IR or Raman spectroscopy. Whereas in IR spectroscopy, the absorption of the sample as a function of frequency is measured, the inelastically scattered light from laser radiation incident on a sample is monitored in Raman spectroscopy. The origin of the spectra acquired by Raman spectroscopy differs markedly from the ones obtained by IR absorption spectroscopy: Raman relies on the polarisability of the sample molecules, whereas IR spectroscopy depends on the change in dipolmoment. Consequently, Raman spectroscopy has inherent advantages and disadvantages over IR absorption spectroscopy:

- Since the selection rules for Raman and IR absorption spectra are different, some vibrations are only Raman-active (e.g. many totally symmetric vibrations) while others are only IR-active.

- Polarisation sensitive measurements or the use of the resonance Raman effect can provide structural information not accessible for IR spectroscopy.

- Water is a weak Raman scatterer, therefore, spectra of samples in aqueous solution can be obtained. This is especially important for the chemical investigation of biological samples. Likewise, Raman spectroscopy can also be performed through glass (e.g. walls of containers, microscopy slides, etc.).

The lack of broadband, high transmission optical fibres for the IR region, the cost of high-power tunable IR sources, and the rather low sensitivity of available

\(^{1}\) Both spectroscopy techniques can also provide other chemical information on the sample molecules (e.g. rotational states, orientation, surrounding environment, etc.) when examined with specialised set-ups.
The Raman Effect

In Raman spectroscopy, the radiation scattered from the sample upon strong irradiation by a laser is detected. Most photons undergo elastic scattering so that the frequency emitted is equal to the incident radiation (Rayleigh scattering). Some of the incident photons, however, collide inelastically with the sample molecules (give up their energy) and emerge with a lower energy constituting the lower-frequency “Stokes” radiation (Figure 4.1 and Figure 4.2). Other photons induce a transition from vibrationally excited states of the ground broadband IR detectors limits the application of IR absorption spectroscopy in SNOM. Accordingly, Raman spectroscopy is the superior method for obtaining chemical information on a nanometer-scale.

Figure 4.1. The Raman scattered photons constitute to signal bands shifted relatively to the incident radiation wavelength (ν₀). Note that due to different population states of the energy levels, usually more photons emerge with less energy (Stokes shift) and less photons gain energy (anti-Stokes).

Figure 4.2. Transitions between the molecular energy levels probed in fluorescence, IR absorption, Raman, and resonance Raman spectroscopy. In contrast to conventional Raman, the electrons get excited to the energy level of the S₁ state in resonance Raman. Thereby, the scattering efficiency is significantly enhanced. Note, that in condensed matter fluorescence and resonance Raman, in principle, can originate from equivalent electronic transition.
state, in this way emerging as higher-frequency “anti-Stokes” radiation. The frequencies of Raman scattered photons differ from the incident radiation on the sample and, therefore, can be detected with suitable instrumentation. The intensity and relative shift of frequency provides information on the energy transitions inherent to the sample molecule.\textsuperscript{1,6}

The Raman effect is a very weak phenomenon: only about one out of \(10^8\) photons is scattered inelastically. Several factors have an effect on the Raman activity of specific molecular vibrations. The gross selection rule for vibrational Raman transitions is that the polarisability\textsuperscript{4} should change as the molecule vibrates. However, it is quite difficult to judge by inspection when a vibration induces a change in polarisability, and if, by what measure. The mathematics involved for an explicit determination of Raman activity is based on group theory considerations and is reviewed elsewhere.\textsuperscript{1-4,7} Another factor influencing the intensity of the observed Raman lines is the population of the energy level from which the transition occurs. This can be seen, for example, when comparing the intensity of corresponding strong Stokes and weak anti-Stokes lines, or by influencing the Boltzmann distribution of the energy population by changing the temperature. The detailed theory further shows that there is also a fourth-power dependence of the Raman scattering efficiency on the incident radiation frequency.\textsuperscript{1,3,8,9}

4.2 Instrumentation and Experimental Techniques

A basic Raman microscopy instrument has been constructed consisting of the inverted microscope from Lumina [Diaphot 300, Nikon] that was coupled to a Raman spectrometer. The optics for collection, filtering, polarisation control, etc could be used for both micro-Raman and SNOM Raman investigations. For micro-Raman, the sample illumination was analysed in a back-scattering configuration through the (oil-immersion) microscope objective of the inverted microscope, whereas in SNOM, the illumination was through a SNOM probe in transmission mode. Figure 4,3 depicts a typical micro-Raman and SNOM Raman spectrum of Rhodamine 6G on a roughened silver film acquired with the home-built Raman set-up.

\textsuperscript{1} The polarisability of a molecule is a measure of its ability to distort in response to an applied field.
In the following paragraphs, the components of the illumination (Figure 4.4) and detection (Figure 4.5) set-up, and their spectroscopic implication are discussed.

**Wavelength**

Several reasons exist to change the excitation wavelength in Raman experiments.1\(^2\),5,6,10,11

- As the excitation wavelength changes, the Raman scattered wavelength changes accordingly, since the energy difference between excitation and scattering remains constant (Figure 4.1). Thus, spectral features originating from Raman scattering can easily be distinguished from other emission sources (e.g. plasma lines of laser, fluorescent lamp lighting, etc.) by changing the incident wavelength.

- Another motivation for changing the incident wavelength can derive from the chemical properties of the sample. The probability of scattering a Raman photon (quantum efficiency) is often as low as \(10^{-8}\), much lower than the quantum efficiency of, for example, fluorescence, which can be almost unity for certain materials. As a consequence, it is very difficult to detect the feeble Raman lines on a fluorescent background. The shift of the incident radiation to longer wavelengths (red, near IR) generally reduces the molecular absorptivity of the sample, and hence the undesired fluorescence. In contrast, the excitation with shorter wavelengths (blue, UV) increasingly separates the fluorescence and Raman spectra enabling to spectrally separate the two effects with optical components in the detection pathway.

- The Raman scattering efficiency increases significantly when an irradiation frequency is close to an intense optical absorption band of the sample is chosen.

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**Figure 4.3.** Typical far-field (upper trace) and near-field (lower trace) Raman spectra in arbitrary units of Rhodamine 6G (inset) on a roughened silver film. Signal peaks below 500 wavenumbers originate from the underlaying glass support. Excitation was performed at 488 nm for 10 seconds (FF, 1 mW) and 4 x 1 min (NF, ~1 mW), respectively. The assignment of the peaks to specific molecular vibrations can be found in literature.62
Further, the selection rules in so-called resonance Raman spectroscopy (Figure 4.2) change, so that certain transitions, which are normally weak, become allowed, thus providing extra information in the spectrum.\cite{6,10,12,13} Finally, structural information on bigger sample molecules can be obtained, since the vibrational Raman lines located close to the absorbing chromophore are amplified the most.\cite{14} Despite the problems caused by interference due to absorption (bleaching) and fluorescence, resonance Raman can be a useful tool for molecular investigation on surfaces.

- There is also a strong dependence of the Raman intensity on the excitation frequency: The Raman scattering cross-section of molecules\cite{15} increases by the fourth power with frequency.\cite{1,3,8,9} Further, the optical transmission through SNOM probes theoretically increases with smaller wavelengths. Therefore, much higher near-field Raman signals are expected when measuring at higher frequencies.

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**Figure 4.4.** Components in the illumination pathway of the Raman set-up. (The waveplates (retardation plates) can optionally be exchanged by fibre paddles.)

**Figure 4.5.** Components in the detection pathway of the Raman set-up. The notch filter is only used when measuring with AFM feedback (Lumina); the feedback laser used with the Aurora instrument was removed with a dichroic mirror instead of the notch filter.
With the available laser sources and detectors, we were first able to perform experiment at three different incident wavelengths only, namely 488, 514, and 632nm. This major technical limitation has recently been overcome with the acquisition of a new laser system [Lexel 95 SHG from Polytec, Waldbronn, Germany] providing well over 2W at 514nm and a double-monochromator CCD spectrograph [Acton UV from Polytec]. Future Raman investigations can, therefore, be performed with high laser powers at a choice of wavelengths.

Monochromatic Illumination

Raman spectroscopy requires an excitation beam that is spectrally pure, since scattered frequencies appear very close to the excitation frequency. Gas lasers have plasma line emissions that can occur in the same spectral region as sample vibrational modes. Furthermore, the glass fibres connecting the remote laser sources with the SNOM introduce additional frequencies in the observed spectral range by Raman scattering. For these reasons, a narrow band-pass filter (plasma filter, interference filter) that spectrally trims the excitation wavelength of interest has been inserted in the beam before the light illuminates the sample.

Polarisation

The assignment of Raman lines to particular vibrational modes is aided by noting the state of polarisation of the scattered light. Totally symmetrical vibrations give rise to polarised Raman lines in which the incident polarisation is largely preserved. In contrast, not completely symmetric vibrations give rise to “depolarised” lines since the incident radiation also generates radiation in the perpendicular polarisation direction. Raman lines, which demonstrate significantly reduced intensity when turning the polarising filter in the detection pathway from parallel to perpendicular to the polarisation of the incident beam can, therefore, be assigned to symmetrical vibrations (Figure 4.6). The orientation of the polarisation of the illumination can be employed for additional structural information and, therefore, should be controlled. Because the output of the utilised argon ion lasers was linearly polarised, optical elements introduced between the laser source and SNOM fibre allowed to

Available laser lines are 528.7nm (420mW), 514.5nm (2400mW), 501.7nm (480mW), 496nm (750mW), 488.0nm (1800mW), 476.5nm (720mW), and 457.9nm (420mW). Optional intra cavity frequency doubling provides additional lines at 257nm (200mW), 244nm (100mW), and 229nm (10mW).

For gases, liquids, and single crystals
define the polarisation orientation at the near-field aperture. The laser light was guided to the SNOM by long (4-6m) optical wave-guides, as the laser sources have been located in a separate room for temperature and vibration isolation (Chapter 2.1). In general, light becomes elliptically polarised as it passes through the fibre. Two different techniques have been employed to control the polarisation of the light before coupling into the SNOM fibre. First, a quarter-wave plate can be utilised to convert the electromagnetic field into a linear polarisation state. Subsequent usage of a half-wave plate allows then the electromagnetic field to be rotated. Another way to change the state of polarisation is to apply external stress to the connecting fibres. This can easily be performed usually with three fibre paddles, in which the fibre is coiled a prescribed number of loops. The bending of the three loops relative to the neighbouring ones results in stress-induced birefringence, thus, changing the polarisation state of the emitted laser light. Therefore, the utilisation of retardation plates or fibre paddles allows any state of polarisation to be achieved.

Raman Signal Separation and Detection

Light at the incident wavelength (Illumination beam, Rayleigh scattering) was suppressed by two holographic notch filters [Holographic notch-, super notch-, and super notch plus- filters for various wavelengths from Kaiser Optical Systems,

\* Retarder plates change the polarisation of light by varying the phase between the two constituent orthogonal linear polarisation states of elliptically polarised light.\* 

\*\* The desired birefringence is induced by the loop in the fibre, not by twisting the fibre paddles.
Ann Arbor, MI] right after the collection optics and within the detection unit (spectrograph). The use of a notch-filter in front of the fibre, which connects the SNOM and the spectrometer, significantly reduces Raman scattering originating from the glass of the wave-guide. In the Aurora SNOM, the sample-tip separation was controlled by a built-in “optical bouncing” shear-force feedback mechanism. The signal arising from the laser diode that was employed for this was separated from the SNOM signal by a dichroic mirror.

The filtered Raman SNOM signal was imaged onto a high transmission fibre [HCG-M0100T-14 (100µm) and Laser Components HCG-M0200T-14 (200µm) from LaserComponents, Olching, Germany] coupling the SNOM with the Raman spectrometer. The aperture of the fibre acts as a spatial filter, suppressing stray light. To improve this spatial filtering, an optional pinhole (25µm, 50µm, or 100µm) could be introduced in a confocal scheme in front of the fibre. The Raman spectrometer consists of a holographic grating as the dispersive element [Holospec f/1.8i, Kaiser Optical Systems] coupled to a liquid nitrogen-cooled charge-coupled device (CCD) camera [SDS 9000, Photometrics] containing a thinned, back-illuminated chip with 256x1024 pixels. The use of such a spatial measuring device allows for simultaneous and independent monitoring of a wide spectral regime. A 100µm slit in the spectrometer yields a spectral resolution of below 10 cm⁻¹.

The software used to control the SNOM software [TopoSPM version 3.06 to 4.1 from TopoMetrix] was linked with the spectrograph software [MAPS 2.0 from Photometrix Ltd.], allowing data acquisition at any point of a previously acquired shear-force image of the SERS substrate or during SNOM imaging.

Power

For “light-hungry” applications such as Raman spectroscopy, SNOM tips with high optical transmission factors are essential. The improved destruction-threshold of the tips (Chapter 2.7) allows coupling laser powers of up to 10-15mW into the rear end of the SNOM fibre. However, a more typical laser power for SNOM fibre probes was usually only on order of few milliwatts (0.5-3mW as determined by an optical power meter [Model 818-UV/OD3/840, Newport]), resulting in sub-microwatt light intensities in the near-field of the SNOM probes. This was for two reasons: On one hand, the initially available argon ion lasers had peak powers of only about 50mW (at 488nm). Taking into account the initial losses through coupling the connecting fibre, plasma-filters, retardation plates, and lenses for focussing to the SNOM fibre, only about 10mW remained available. On the other hand, the laser stability has shown to be better when it is not operating at maximum power.
The recent acquisition of a new laser system [Lexel 95 SHG from Polytec, Waldbronn, Germany] providing well over 2W at 514nm, will allow future SNOM Raman experiments to be performed close to the destruction threshold of the SNOM probes. In that way required measurement times will be shortened significantly.

4.3 Surface Enhanced Raman Spectroscopy (SERS)

The main disadvantage of Raman spectroscopy, as stated previously, is the very small scattering intensity. In general, the differential Raman scattering cross-section is in the range of $10^{-28}$ to $10^{-31}$ cm$^2$ s$^{-1}$ molecule$^{-1}$, which is particularly small compared to the ones found for UV absorption ($10^{-18}$), IR absorption ($10^{-20}$), fluorescence ($10^{-19}$), or Rayleigh scattering ($10^{-26}$), respectively.$^{17}$ One way to enhance the Raman scattering cross-section by several orders of magnitude is by using irradiation wavelengths with energies close to an electronic transition of the molecule, where the Raman polarisability shows resonance behaviour (Resonance Raman Spectroscopy). As discussed earlier, Resonance Raman suffers from a variety of problems such as photo-induced degradation and interference from fluorescence, and is therefore, suitable for selected applications only.

![Figure 4.7. AFM topograph of a typical SERS surface. Sample molecules adsorbed on such noble metal surfaces show tremendously increased Raman scattering cross-sections.](image-url)
The discovery of a "new" Raman effect in 1974 by Fleischmann et al.\(^1\) generated considerable excitement in the physics and chemistry communities. Molecules adsorbed on appropriately prepared metal surfaces display Raman cross-sections several orders of magnitude greater than the corresponding response for an isolated molecule of from a solution. Tremendous enhancement factors exceeding a trillion-fold increase\(^2\) over normal Raman scattering efficiency have been reported for molecules adsorbed on rough silver surfaces (Figure 4.7). Such enhancements generally enable the investigation of sub-monolayer coverages of adsorbed species and even single molecules\(^3\) by Raman spectroscopy. The possibility to characterise substrate-adsorbate interaction with a small number of molecules is of particularly great interest in the fields of catalysis (or surface chemistry\(^4\) in general), one of the main driving forces behind SERS research.\(^5\)

**SERS Mechanism**

Despite great efforts, the mechanism of SERS remains as yet not completely understood. Yet, it is generally agreed that different mechanisms contribute to the strong enhancement of the Raman signal, depending on the SERS-active substrate and on the electronic structure of the adsorbed molecule. The intensity of Raman scattering \(I\) is proportional to the molecular polarisability \(\alpha\) and the incident electric field \(E\) upon the sample molecule \[I = (\alpha \cdot E)^2\]. The different mechanisms can be broadly divided into two groups: the "chemical" enhancement (effecting \(\alpha\)) and the electromagnetic effect (influencing \(E\)).

Chemical enhancement is a short-range effect (few Å) and presumably originates from charge-transfer or bond formation between the adsorbate and metal, which can increase the molecular polarisability.\(^6\) This effect is not well understood, especially since the proposed mechanisms only changes the nature of the adsorbate, and therefore, the scattering cross-section of the molecule, which, in theory, solely accounts for an overall enhancement of about 1'000.

Electromagnetic enhancement occurs because the local electromagnetic field at the surface of a metal is significantly changed from that in the field from the incident radiation.\(^7\) This effect is most pronounced with fine metal particles or rough metal surfaces.\(^8\) Light incident at such surfaces can excite conduction electrons in the metal, generating a surface plasmon resonance. As a consequence, the particles

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\(^1\) The unexpected high Raman intensity, which was measured for pyridine on silver electrodes was first attributed to a simple increase in surface area. It was in 1977 when Jeanmaire and Van Duyne\(^9\), and Albrecht and Creighton\(^10\) independently suggested a previously unknown enhancement effect.
(or rough surface features) become polarised, which significantly increases the local electromagnetic field compared to the field of the incident radiation. The magnitude of the electromagnetic enhancement depends critically on the size, shape, and spacing of the metallic nano-structures on the SERS substrate. Therefore, the investigation and determination of structural parameters responsible for high surface enhancements is very important for the practical applications of SERS as well as for a better understanding of the nature of the surface enhancement. However, due to limitations in spatial resolution only a few direct measurements on metal structures have been performed.

The combination of SNOM with Raman spectroscopy allows to investigate such surfaces with a sufficiently high resolving power. Obviously, a detailed knowledge of the morphology of the Raman enhancing surfaces is necessary for a quantitative comparison with the theoretical predictions. Since it is difficult to include the interaction effects of nearby metal protrusions in the theory, experiments on well separated silver islands as well as on small metal clusters will contribute to a better understanding of the model for the Raman enhancing effects. This necessitates the fabrication of highly active SERS substrates suitable for simultaneous morphological and optical characterisation with high lateral resolution.

In the following sections, the production of such SERS substrates allowing near-field investigation of the enhancement effect is described. To be able to perform quantitative measurements and to facilitate later data interpretation, several conditions for the substrates have to be met. First, to avoid interference from neighbouring metal protrusions, substrates with widely separated metal islands must be created. Secondly, the exact morphology of the sample has to be known. This includes the knowledge of possible fine structure of the metal islands. Thirdly, the local surface coverage of the molecular adsorbate must be known in order to exclude Raman signal variations originating from local dye concentration irregularities.

† Newer theories centre on fractal geometries of rough metal surfaces.
4.4 Production of Isolated Silver Islands as SERS Substrates

SERS Substrate Fabrication Techniques

By choosing suitable experimental conditions during substrate fabrication, particle sizes and shapes, as well as the inter-particle distances can be controlled such that well separated silver islands are formed that still show high Raman enhancement.

Different techniques have been reported for nano-structuring SERS substrates: electrochemical roughening of electrodes, preparation of metal colloids or films by chemical or photochemical reduction of the corresponding salts. SERS substrates prepared by etching a silver foil with concentrated nitric acid has also been reported. Another way of preparing roughened metal surfaces for SERS is by laser ablation. An advantage of preparing SERS active substrates in this way over (electro-) chemical reduction procedures is that the surface of the resulting metal particles is free of organic or ionic contaminants and tends to be more stable. Van Duyne et al. describe a preparation of Ag films over polymer nano-spheres producing highly SERS active substrates. Another controllable way of fabricating SERS substrates is by means of micro-lithography. In this manner, uniformly sized and shaped silver particles are controllably created allowing for clear observations of the excitation resonances created by different incident laser wavelengths. The findings reported strongly support the plasmon resonance theories of SERS.

Vapour deposited metal island films are among the most widely used substrates due to the simplicity of the preparation of the substrates, their high stability, high SERS activity, and the ease of controlling the experimental parameters. For these reasons, this preparation technique was preferred for the production of SERS substrates consisting of isolated silver islands.

Substrate Pre-treatment

A comparison was carried out between substrates produced on untreated glass and substrates where the support glass was rinsed thoroughly with hydrofluoric acid 40% (Fluka) or silanised with dimethyl-dimethoxisilan [DMOS] (Fluka) prior to metal deposition. No reproducible differences in the resulting Ag film were observed.

† Fluorescence free, polished glass microscope slides [Merck] were pre-cleaned with acetone [puriss grade, Fluka] and methanol [puriss grade, Fluka].
Figure 4.8. AFM topographs of SERS substrates prepared on a clean microscopy slide (left) and on a glass slide which has been additionally immersed in concentrated hydrofluoric acid (40%) for several minutes. The pre-treatment of the glass slides did not show a reproducible effect.

observed,\textsuperscript{1} in agreement with studies by Semin and Rowlen (Figure 4.8).\textsuperscript{91} In addition, heating of the support slides up to 300°C prior to metallisation showed no measurable effect on the resulting surface morphology of the substrates.

Deposition geometry

A series of glass slides were metallised at different distances from the evaporation source at pressures below 10\textsuperscript{-4}Pa.\textsuperscript{11} With a decreasing separation between the evaporation source and the sample plate during evaporation, the mean island height increased (Figure 4.9 and Graph 4.1). This observation matches the findings of Schlegel and Cotton: The closer the substrates are to the evaporation source, the higher the evaporation beam is confined. As a consequence, the relative deposition

\textsuperscript{1} Topographs were acquired by AFM measurements on the Lumina. High-aspect ratio SiAl-C tips with a tip radius <10nm and a force constant of 0.2 N/m [Olaf Walter, Germany] were used for imaging in contact-mode. The load force was kept relatively low during all scans. Of each SERS substrate an overview scan of 5x5μm was taken prior to imaging a representative 1x1μm sized region. For comparison, SEM images were taken on a Hitachi S-4100 up to a magnification of 110,000x. The SERS substrates examined showed a homogeneous island distribution over the whole sample.

\textsuperscript{11} Vapour deposition was performed with a commercial coating system [BE 500, Balzers]. The average film thickness was generally 5nm, as determined by a quartz crystal micro-balance (QCM) [Telemark]. The thickness was within good agreement with the AFM measurements. The evaporation rate was set to 0.02 nm/s. The relative error of the deposition rate was below 10% as determined by comparing the overall deposited thickness over the time of evaporation with the values given by the QCM. Through water cooling of the recipient and the use of mechanical shutters, the deposition process was kept as reproducible as possible.
rate is higher, thus, the individual aggregation of the silver particles is disrupted by the higher number of incoming atoms.

An important factor for the formation of silver islands is the temperature of the support plate during metallisation. For reasons of radiative heating, we restricted our investigations of the source-sample separation dependence to relatively large distances. Therefore, only marginal heating of the substrates is expected even for the smallest separation of 15 cm.\(^92,93\) Through the use of mechanical shutters and a water-cooled vacuum chamber, no substantial fluctuation of the substrate's temperature is expected.

Empirically, glass slides metallised\(^*\) normal to the evaporation direction have been found to produce better-defined silver islands, compared to glass slides held

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\(^*\) If not stated differently, the support plates were metallised at a pressure of \(10^{-4}\) Pa, 35 cm above an e-beam evaporation source.
Parallel to Evaporation Source  45° Tilted to Evaporation Source

Figure 4.10. Topographs of SERS substrates fabricated by metal deposition on a glass slide perpendicular to the evaporation direction and tilted by 45°, respectively.

at an angle with respect to the source (Figure 4.10). A similar correlation between the deposition angle and the resulting substrate morphology was also found. The origin of this effect is still controversial, although similar observations have been made by crystallographers who found different growth behaviour of metal films on different crystal faces of the same metal.

Metal Deposition Rate

We hypothesised that a significant parameter in the formation of the silver islands is the translational and internal energy of the impinging silver atoms and clusters. To investigate this, the source temperature was varied, resulting in an evaporation rate that varied between 0.05 Å/s and 20.0 Å/s. We found that higher evaporation rates (i.e. higher temperatures) of silver lead to smoother surfaces with a less well defined island structure, while substrates that were metallised at very low deposition rates show, on average, higher and more separated silver islands. The residual

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1 For the angular dependence measurements, the glass slides were fixed directly above the source at various angles with respect to the evaporation direction, keeping the source-sample distance constant.

2 Metallisation was performed using a mini-coating system [MED 020, Baltec]. The metallisation was carried out by heating a tungsten filament under an argon atmosphere at 0.1 Pa. The sample-evaporation source distance was 12cm. Mechanical shutters in combination with a micro-balance [QSG 060, Baltec] ensured a controlled metallisation process. Each metallisation was carried out in batches of four support plates.
pressure in the deposition chamber can also influence the energy of the metal vapour. At lower chamber pressures, the collision-induced cooling of the silver in the gas phase should be reduced, i.e. at lower pressures, "hotter" silver particles are deposited. To examine this, argon was introduced as an inert gas. At an elevated argon pressure during deposition (10^{-1} mbar), a more distinct island formation was observed, in agreement with our hypothesis.

Differences in source temperature can also easily explain seemingly conflicting results. For example, Schlegel and Cotton\textsuperscript{92} found that with an increase in the evaporation rate, the silver particles are less defined and begin to merge, while films deposited at very slow rates show distinct, well-separated particles. Later work done by Van Duyne et al.\textsuperscript{87} did not show a dependence of the substrate morphology influence on the evaporation rate. A crucial difference in these studies was that the evaporation source was located much closer to the target in the work by Schlegel and Cotton,\textsuperscript{92} such that the source temperature had a much larger influence on the resulting Ag film.

**Post-deposition Temperature Annealing**

The heating of freshly metallised substrates not only changes the size, shape, and inter-island spacing of the silver particles, but also leads to a more uniform island distribution throughout the sample (Figure 4.11). A time-resolved study of the temperature driven silver island formation was carried out using annealing temperatures of 200°C to 300°C.\textsuperscript{1} The AFM images in figure 4.12 illustrate clearly how distinct silver islands form from relatively smooth metal films when heated for 10, 20, and 40 seconds, respectively. Statistical particle analysis confirmed that with prolonged heating more and more isotropic islands are formed. The average height of a silver particle increased from less than 3nm for the untreated substrate (A) to over 70nm for the substrate after complete island formation (D). The average number of particles in a 1μm\(^2\) area decreased on average from 100 to 20 particles; the average particle diameter increased from below 30nm to 150nm, whereas the average inter-island spacing increased from nearly zero to over 100nm. Similar trends were found for all examined substrates, independent of metallisation rate and mean film thickness.

\textsuperscript{1} Three more series were annealed on a hot plate [MR 2002, Reinolph], which was kept between 200°C and 300°C.
As predicted by the electromagnetic theory, the formation of a more isotropic surface structure is mirrored by the optical absorption spectra of the metal substrates. The extinction bands shown in figure 4.12 reflect a typical behaviour due to annealing of the substrates:† The broad extinction spectrum of an untreated substrate narrows more and more, yielding an absorption maximum corresponding to the dominant surface plasmon frequency, which is directly dependent on the metal island size present.95,96 Upon annealing, the extinction band first shifts toward shorter wavelengths below 450nm. With prolonged annealing, the band experiences a smaller bathochromic shift of a few tens of nanometers bringing its maximum back to wavelengths close to 460nm.

Earlier studies demonstrating the effect of annealing on optical properties, surface structure, and SERS effectiveness for thin evaporated silver films have been carried out by means of AFM and UV-vis spectroscopy.86,91,97-99 The explanation provided for the formation of metal islands upon annealing is usually based on differences in surface energy of the involved materials.87 Another explanation is offered by McCarthey,100 who suggests that the silver particles break thermally to form islands at new nucleation sites.

† Since the absorption spectra were not corrected for scattering and reflection losses, in the following the term “extinction” rather than “absorption” will be used. The extinction spectra of the SERS substrates were measured with a UV/VIS spectrophotometer [Uvikon 940, Kontron Instruments]. The spectra were taken between 380 and 780 nm at normal incidence. A cleaned, non-metallised glass slide was used as reference.
Figure 4.12. Effect of annealing of the SERS substrates. The island size distribution narrows, while the average island size grows, forming more uniformly shaped, distinct islands separated by void spaces of bare glass. In the left column AFM images (1x1 μm) are shown of an unannealed ("as-deposited") SERS Substrate (A), a substrate heated to 200°C for 10 sec (B), a substrate heated to 200°C for 20 sec (C), and a substrate heated to 250°C for 40 sec (D). In the middle column, the corresponding island size histograms (relative abundance vs. island diameter) are depicted. The statistical evaluation was carried out for a 5x5 μm-sized area. In the right column, UV/vis extinction spectra of the corresponding substrates are shown.
**Long term heating and substrate stability**

A series of metallised substrates were heated additionally to 200°C in a dry oven [TV 15U, Memert] for 25 minutes to 22 hours. No significant structural changes of the SERS surfaces could be detected between these samples and reference substrates heated for 1 minute only. Similar results were found for substrates additionally heated to 130°C, 300°C, and 400°C. As expected, the time used for reaching the equilibrium state decreased with higher temperatures in agreement with the surface diffusion model. Untreated metal substrates stored at room temperature roughened slowly changing their colour from purple to yellow. This uncontrolled modification, however, did not lead to substrates bearing high SERS activity. Annealed samples did not degrade further, even when stored under ambient conditions for several weeks.

**Post-deposition Chemical Treatment**

A series of substrates were treated chemically after silver deposition by exposing the substrates to HCl vapour for a few seconds. A hypsochromic shift of the extinction band was found similar to that of thermally treated samples. The change of island size, shape and spacing was examined by means of AFM (Figure 4.13). The effect of adding halide ions such as Cl⁻ is not restricted to a change in the surface morphology, but it also induces a change in the surface chemistry. Supporting the “active-site” SERS models, Hildebrandt and Stockburger have shown that certain binding sites for rhodamine 6G dye molecules are only available in the presence of chloride ions.

![AFM images showing the effect on “chemical annealing”. Unannealed (“as deposited”) SERS substrates (top) experience a change in morphology on exposure to HCl gas (bottom). The average island height increased here from few nm to over 40nm.](#)

† Vapour pressure of concentrated hydrochloric acid [Fluka] at 23°C.
However, the overall Raman enhancement factors of the chemically treated substrates we prepared was not as high as those for heat-treated substrates. Furthermore, the morphological change of the SERS surfaces could not be carried out in a controlled fashion. Consequently, only annealed SERS substrates were investigated in further experiments.

**Film Thickness and Island Density**

For substrates with only very small metal coverages, completely isolated silver islands were obtained, in contrast to thicker metal films, where no bare glass can be observed (Figure 4.14). The average inter-island spacing and the average number of islands per μm² after annealing, therefore, are strongly dependent of the total metal surface coverage.

Although it is known that smooth surfaces (Area RMS < 5nm) can be obtained with thick (>1μm) metal films, island formation is always observed when dealing with thin silver films (<50nm), where the metal-substrate interaction still plays an important role. As a result, isolated silver islands can be obtained by reducing the overall metal coverage of the substrates. The maximum mean film thickness that allows the formation of separated silver islands, however, depends on the evaporation and post-treatment conditions. In our experiments, most substrates with a mean metal film thickness below 1.5nm showed sufficiently separated Ag islands for their isolated optical investigation.

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"Area RMS" is defined as the square root of the mean value of the squares of the distance of the heights from the image mean value:

\[
RMS = \sqrt{\frac{1}{N} \sum_{i=1}^{N} (Z_i - \bar{Z})^2}
\]
Figure 4.14. AFM images showing the influence of the mean film thickness on the SERS substrate morphology. Annealed substrates with Ag coverages of 6.0 nm (a.), 1.0 nm (b.), and 0.5 nm (c.), respectively. The insets show a magnified region of the corresponding substrates. The island heights are on average 40 nm (a.), 6 nm (b.), and 5 nm (c.), respectively.
4.6 Sample Application and Characterisation\textsuperscript{73}

To investigate the SERS activity of the prepared thin silver films, rhodamine 6G (Rh6G, $\lambda_{max}=524\text{nm}$) [Fluka] was chosen as test substance. We have found that the application of a diluted dye solution by means of pipetting, if done carefully, leads to the desired low surface coverage (Figure 4.15).\textsuperscript{4} Maskevieh et al. reported a degradation of the SERS substrates upon application of certain organic solvents, including methanol and ethanol.\textsuperscript{97,104} This is probably due to the small amounts of solvent and short exposure times involved in the pipetting process. No structural modification was observed after coating our substrates with diluted Rh6G. With the technique used here, an average of 95 dye molecules was located within a spot of 100nm diameter.

\textbf{Figure 4.15. Schematic of the sample application procedure.}

For quantitative optical measurements in the near-field, the exact local dye coverage has to be determined, since stronger Raman signals can also arise due to higher local concentrations of the adsorbate. A series of friction force measurements were carried out to prove the uniformity of the dye layer. As can be seen in figures 4.16 and 4.17, a clear distinction between the dye-covered surface and the clean silver surface can be made. In the centre of the drop-coated spot, lower dye coverage

\textsuperscript{4} Stock solutions with concentrations of $1\times10^{-3}$ M and $1\times10^{-5}$ M were prepared by dissolving the dye in HPLC grade methanol [T.J. Baker].
Figure 4.16. Friction-force image of the centre of a rhodamine 6G drop on a SERS substrate. The upper panel is showing the topography (A), forward friction-force (B), and the error signal of the feedback loop (C). The lower panel shows a zoomed in region of the topography (D), the forward friction force (E), and the backward friction-force (F). A greater interaction between the AFM tip and the dye film forces the AFM tip to lag behind, which gives rise to lighter regions in the friction-force images (forward scan direction, B and E). In the backward scans (F) a contrast reversal is observed, i.e. the dye forms darker regions, confirming the purely chemical origin of the measured contrast. The total topographic contrast is 50 nm in (A) and 12 nm in (D), respectively.

is observed than at its border. There the dye aggregates to thin layers separated by the bare metal coating. Presumably, the Rh6G films maintain their integrity because of their natural coherence and their tendency to bridge even rather large surface irregularities. The film thickness of the dye in the centre of a spot does not differ significantly from the one measured at its border, where generally a continuous, un-ruptured film is observed. The absolute film thickness remains constant throughout a given SERS substrate, but varies slightly between 2 and 4 nm from
one sample to another. This variation is possibly caused by a change of repulsive and attractive AFM probe-sample forces, since no evidence for a multi-layer formation of the dye could be found. The interpretation of the friction force measurements is that the dye applied onto the SERS substrate crystallises to continuous monolayers, leaving voids where no dye is present at all. In addition, the dye aggregates uniformly over the surface, independent on the underlying material (glass, silver). Furthermore, the thickness of the additional adsorbate film remains constant over the whole spot investigated. Consequently, the near-field Raman signals always originate from approximately the same number of dye molecules, and fluctuations of the Raman signal intensities at different measuring sites must be exclusively caused by different enhancement factors of the underlying substrate.

4.5 Micro-Raman Measurements and SERS Activity

Determination of the SERS Enhancement Factor

In order to determine which substrates have highly active SERS silver islands, the ensemble-averaged enhancement value of the entire substrate must be high enough to ensure that single silver particles statistically show high enhancement
factors (EF). As the overall EF is strongly correlated with the total metal surface coverage, due to the increase of enhancing sites with the number of islands present, a relative, site corrected EF value has to be used. For this purpose, the normalised far-field Raman data was divided by the island size weighted average number of silver islands per \( \mu \text{m}^2 \) prior to comparison.

Several procedures for estimating absolute EFs have been described in literature. A direct comparison of Raman intensities with and without the SERS substrate would allow a realistic estimation of the absolute EF. Unfortunately, resonantly excited Rh6G fluoresces with a high quantum yield, which complicates Raman measurements without the surface enhancement. The use of a reference substance (e.g. methanol) with a known Raman cross section gives, to our knowledge, the best estimate of an absolute EF for rhodamine.\(^{109}\) However in our case, we would not be able to determine the relative local concentrations of the dye and the reference substance simultaneously on single metal islands. Finally, a comparison of the fluorescence cross section with the Raman cross section,\(^{71}\) or an estimation by comparing bulk or liquid measurements with surface measurements\(^{82,87,110}\) may be used.

We performed, for the first time, a direct estimation of the EF from SERS measurements by comparing the resonance Raman scattering intensity of Rh6G on bare glass and on SERS substrates:

From the micro-Raman measurements, it seems that for sub-monolayers of Rh6G on bare microscopy slides, a great part of the fluorescence is quenched, and, at the same time, the bleaching of the dye is greatly reduced. These Raman-active and photo-stable molecules, which are thought to be in a protected micro-environment,\(^{71}\) can be illuminated with power densities of 3kW/cm\(^2\) for up to one minute before photo-bleaching occurs. By measuring at various positions we collected enough Raman scattered light for the calculation of absolute, average EF by comparing SERS measurements with measurements of Rh6G on un-metallised glass substrates. In this way, we overcame many of the shortcomings of the conventionally used procedures. The rates of degradation depend on the morphological and optical surface properties of the investigated samples under illumination. Hence, some uncertainty remains about the exact number of dye molecules involved in the Raman scattering process. A comparison of the Raman intensities should, therefore, be further corrected by a factor expressing the photo-stability of Rh6G on metallised and bare glass substrates. Still, to first approximation, conservative estimations for the SERS substrates have been obtained, assuming the degradation rates for all substrates to be identical.
SERS Activity and Island Shape

We found an increase in the SERS activity with increasing silver surface coverages. This is a consequence of the increasing number of enhancing sites in combination with the formation of larger scale roughness features which additionally shift the surface plasmon resonance frequencies, towards longer wavelengths, thus, closer to the incident laser wavelength. These findings support the electromagnetic theories\cite{ref1,ref2,ref3,ref4}, which rely on large-scale roughness features rather than on the atomic-scale roughness. Although the particle sizes are only moderately small compared to \( \lambda \), and the dipolar approximation used in the electromagnetic model is not strictly valid, to first approximation the interference effect of the wavelength of light as it approaches the size of the particles (retardation) can safely be neglected.\cite{ref5} Furthermore, according to the plasmon resonance theory, the energy of an adsorbed photon is localised in a small volume defined by the shape and size of the metal protrusion. For particles located in close proximity to each other the interaction between the resonances delocalises the photon energy among them, reducing the local field enhancement. In the case where higher surface coverages lead to continuous metal films rather than an assembly of island structures, smaller local field enhancements are expected.\cite{ref6,ref7}

It has been shown theoretically\cite{ref8,ref9,ref10,ref11} and empirically\cite{ref12} that high enhancement values can be obtained when irradiating the SERS substrates with laser wavelengths close to the surface plasmon frequency. Therefore, for high enhancement, the metal islands have to be adjusted in size and shape such that the resulting plasmon resonance frequency overlaps with the wavelengths used, 488 nm or 514 nm, in our case.

The enhancement for silver spheroids is not only dependent on their overall size, but also of their aspect ratio.\cite{ref8,ref9,ref10,ref11} This ratio (R) is defined as the minor axis or particle height divided by the major axis or particle diameter. In our experiments, higher SERS enhancement factors were obtained with silver islands having a high minor-to-major axis ratio. For relatively high surface coverages (i.e. mean film thickness of 4-6nm), R-values between 0.28 to 0.32 were obtained. With decreasing surface coverage, the R-values decreased to below 0.05. As expected, substrates covered with particles exhibiting low R values did not show a high SERS activity when excited at 488 and 514nm, respectively. These findings are consistent with the theoretical predictions: the maximum electromagnetic enhancement has been predicted to increases in magnitude and to shift towards longer excitation wavelengths as R increases.\cite{ref13}
Substrate Selection

Morphologically, the most suitable substrates with isolated silver islands were those prepared by subsequently annealing films after depositing 1nm silver at a rate of 0.01nm/s (Figure 4.14.b). However, the islands are on average only 6nm high and therefore have a very low R-value of approximately 0.05. We assume that this was the reason for a low overall SERS enhancement (ca. 300) when excited at 488 nm. Comparably low EF were found for SERS substrates with similar or smaller mean film thickness.

A higher average EF of ca. $1.1 \times 10^4$ was found for a 4nm thick, completely annealed substrate as shown in figure 4.18. The evaporation rate of 0.1nm/s is comparable to the one of the 6nm thick sample described previously. The islands are separated typically more than several hundred nanometers. Electromagnetic interferences from neighbouring islands are consequently expected to be negligible. The shape and size of the silver islands were such that the average surface plasmon resonance frequency overlapped with the frequency of the incident laser. In this case, the UV/Vis-extinction spectra of the substrate showed a peak maximum around 450nm, sufficiently close to the excitation wavelength of 488nm. Furthermore, the metal particles exhibited relatively large minor-to-major axis ratios ($R \approx 0.4$), which in theory should lead to larger enhancement factors. In figure 4.3, a micro-Raman spectrum of Rh6G on this substrate is depicted. An average enhancement factor of $11'000 \pm 1'000$ was measured for this substrate. This sample was preferred to others with similar SERS activity also due to the large major axis of the isolated islands, which facilitates optical investigations of isolated Ag features. For these reasons, the ensuing near-field investigations of isolated single silver islands were performed on this substrate.

Figure 4.18. Pseudo 3D rendered AFM topography image from a region with high island density of the SERS substrate selected for the near-field Raman investigations.
4.6 Near-field Raman Investigations

Correction for Topographic Coupling of the Near-Field Optical Measurements

Frequently, optical signals acquired with scanning probe techniques show a correlation with structures in the topographic image. This undesired cross-talk arises from the nature of the constant gap mode used in many SNOM systems. The application of constant height scans, where no tip motion normal to the sample surface occurs, can be used to overcome this problem. However, this method is only useful if the surface roughness is <5-10nm, otherwise the near-field condition is not fulfilled. For SERS substrates showing an inherent roughness of few tens of nanometers, the topographic coupling can be extracted if the detected near-field optical signal is spectrally resolved. To a first approximation, the sample composition and optical enhancing factors contribute to the spectrally shifted near-field signals, like fluorescence or Raman, while the optical near-field signal at the illumination wavelength is a function of the sample reflectivity, sample absorption, and topography. The latter can obstruct the light path to the detector and influence the tip-surface separation. In the near-field Raman experiments, the reflected signal can be used to gauge these factors. By simply dividing the Raman signal intensities by the signal intensity at the excitation wavelength, the topographic coupling to the optical image can be deconvoluted. Possible limitations of this procedure, such as the differences in sample reflectivity due to chemical irregularities on the surface or the change of sample absorptivity in the measured spectral range is negligible for chemically homogeneous substrates. Therefore, all Raman measurements discussed in the following paragraphs were routinely normalised by the sample reflectivity in the corresponding spectrum.

Due to the large tip apex of the SNOM probes (including the metal coating up to 200nm), fine structure close to larger metal islands cannot be resolved. Although such fine structure would comprise only a small portion of the total mass of the film, and although no appreciable SERS is generally observed from such small islands, their presence could complicate later interpretation of the data. The morphological studies of the SERS substrates by AFM, however, revealed no fine structure on the silver islands. Additionally, even when using very sharp AFM tips with apex curvatures below 5nm there was no evidence of fine structure. The absence of sub-structures on the silver islands is most probably a direct consequence of the annealing process during the substrate preparation since substrates that were not annealed exhibited fine structure on the metal protrusions as well as on the glass surface.
Near-Field Raman Measurements

In figure 4.19 a topography image of a SERS substrate acquired by SNOM is depicted. In a first scan, the substrate’s morphology has been measured by the shear-force mechanism. The SNOM probe was then parked at selected positions across the two silver islands in the centre of the 1×1μm sized section. For clarity, the positions have been marked in the figure by black circles. The diameter of the circles (ca. 70nm) indicates the approximate optical resolution obtained by the SNOM tip. The resolving power was determined by inherent spectral variances in the Raman spectra of adjacent locations: when taking Raman data at locations less than 100nm apart, very different spectra were sometimes obtained, which proves that spectral changes can be resolved with <100nm accuracy. Scanning electron micrographs of the SNOM tips confirmed the estimated optical resolution (Figure 4.21). Assuming monolayer dye coverage, a spot size of 70nm diameter corresponds to approximately 50 Rh6G molecules assuming exactly one monolayer. Each spot was illuminated for four times one minute through the SNOM tip yielding Raman spectra like the ones depicted in figure 4.3, figure 4.19, or figure 4.23, respectively.

Figure 4.19. Shear-force image of a specially prepared SERS substrate consisting of isolated silver islands. At specific locations marked with circles, the SNOM tip was placed in a second scan to acquire near-field Raman spectra, like the one depicted for a selected location. The circles are 70nm in diameter and represent an estimate of the optical resolution obtained with the SNOM tip used.

The background signal originates from fluorescence and from the spectral response of the CCD-camera. Raman signals below 500 cm⁻¹ can also originate from the underlying glass support and from the glass of the SNOM probe itself.

As a measure for the scattering enhancement factor, the normalised and background corrected Raman peak intensity at 1650cm⁻¹ was selected, corresponding
Figure 4.20. The pole heights above the imaginary level denote the measured relative signal intensity of the Raman peak maximum at 1'650 cm\(^{-1}\). Each segment of the poles corresponds to an EF of 1'000 compared to average Raman intensities measured on unmetallised glass slides.
to the aromatic C-C stretch vibration of Rh6G. The magnitude of the band was directly compared with the average value obtained for unmetallised, Rh6G coated substrates. The calculated EFs were superimposed on the topographic image in forms of poles (Figure 4.20). Each segment of the poles corresponds to an additional enhancement of 1000 times. Local enhancement factors of 2,000 to well above 6,000 were measured at different locations. Variations in the Raman intensities of over 50% were observed for two points separated by less than 100 nm.

We still cannot explain the finding that the locally measured Raman enhancements never reached the average values measured in the “far-field” micro-Raman experiments. Perhaps “hot spots” with much higher EFs exist elsewhere on the substrate or cumulative effects of neighbouring islands play a role.

For the measurements carried out it seems that the SERS activity decreases slightly on top of the silver particles in comparison with their borders. For the incident laser light, the dipole resonance of an oblate silver ellipsoid (R<1) is polarised in the plane of the protrusion, parallel to the substrate surface. Thus following the theory, one would expect that the Raman enhancement will only take place at the edges of the islands. Qualitatively, our findings match the prediction of the electromagnetic models. However, we also find enhancement factors higher than the theoretical limit predicted by the Mie resonance model, i.e. for locations separated by more than a wavelength from the metal particles.

**Limitations of SNOM for SERS Investigations**

There are various problems and limitations when studying the field enhancement of SERS substrate with near-field optical methods. Some of these are fundamental, while others have already been overcome in our laboratory. They are discussed in the following sections:
Limited resolution: No decrease in the Raman scattering efficiency on top of silver particles was observed for some islands, for example the one on the right side in figure 4.20. This can be explained by the smaller lateral dimensions of those protrusions: the relatively long range electromagnetic enhancement of the borders in combination with the limited resolving power of the currently available SNOM tips average the observable field distribution for smaller particles. To be able to explore conventional SERS substrates with metal islands of less than 50nm diameter, a higher optical resolution would be required. With increasing optical resolution the aperture size of the SNOM tip decreases, rapidly reducing the optical power at the tip apex. According to Bouwkamp’s solution, a reduction of the SNOM tip aperture size from 100 to 20 nm leads to a decrease in transmission of over eight orders of magnitude. This would increase the measuring time per spot by a factor of several millions, making Raman imaging of small areas impractical.

Sample drift/degradation: The use of SNOM tips with high optical throughput allows for shorter measuring times. Relatively good signal-to-noise ratios were obtained for measuring times of approximately one minute. Raman imaging of a 1x1μm sample with an optical resolution of 70 nm can therefore be carried out within less than four hours. Instrumental drift for a period of several hours can be controlled. On the other hand, optical sample degradation can occur in this time frame. The rate of degradation is significant for determining quantitative values and therefore has to be either suppressed or controlled.

Registry of the optical and topographical image: The resolution of the topographic images is below 30 nm, probably due to a sharp protrusion at the apex of the SNOM probe. Such protrusions are responsible for a variety of artefacts, of which a lateral displacement of the optical and the topographical image is the most important (Figure 4.22). The effective position of a Raman measurement can be displaced by several tens of nanometers from the topographic co-ordinates. Therefore, a direct correlation between topography and Raman intensity has to be made with caution. Methods to overcome this problem have been proposed recently.

Figure 4.22. Schematic showing a common artefact inherent to aperture based SNOM. The optical and topographic images can be displaced by several tens of nanometers due to their different point of origin.
**Sample concentration dependence:** A highly controlled sample application or alternatively a thorough sample characterisation is necessary to deconvolute sample concentration effects from the surface enhancement effect. In the described experiments, the used dye Rh6G formed homogeneous monolayers facilitating later data interpretation. The use of alternative Raman scatterers might lead to less homogeneous surface layers.

*Figure 4.23.* For unknown samples, it is difficult to judge whether the increase in Raman intensity occurs due to higher EF or locally higher sample concentrations.

**Topographic coupling in the near-field optical image:** For SERS substrates with high surface roughness, *constant-height scans*, where the tip is held at a fixed distance from the average sample height, are not feasible. Topographic coupling of the optical image originating from the *constant-gap scan mode* applied instead therefore must be deconvoluted. The decoupling using spectrally resolved information, however, is only an approximation and does not include all possible contributions of the morphology to the optical near-field signal (See chapter 2.7).

*Figure 4.24.* Topographic coupling in the SNOM image can arise from the fact, that the sample-tip separation is sensed by a protrusion of the metal coating, and not from the optical aperture of the tip.
No direct correlation of conventional SERS measurements with near-field optical investigations: The local field enhancement we measured was qualitatively in agreement with the theoretical predictions for single particles excited with directed electromagnetic waves. However, since different electromagnetic fields are involved in the surface plasmon excitation in near-field optical measurements, a generalisation to conventional, far-field optical experiments is restricted.

Influence of the SNOM tip on electromagnetic fields: The SNOM tip consists of a glass core surrounded by a metal layer of several tens of nanometer thickness. To bring the sub-wavelength sized optical source into the near-field, the tip has to approach the surface to within a few nm. The metal of the tip coating, therefore, is in very close proximity to the silver islands of the substrate, and can influence the electromagnetic field, which is responsible for the surface enhanced Raman scattering (Figure 4.25). Additional measurements with SNOM tips coated with different metals should be performed to deduce the effect of the SNOM tips on the resulting Raman signals.

![Figure 4.25](image)

Figure 4.25. SNOM Raman spectra taken on top of a silver particle and in the vicinity of the island. The bright circles in the shear-force image denote the estimated optical aperture. The corresponding outer circles depict the approximate dimensions of the metal coating of the tip. Although the upper spectra has been acquired off the silver island, the Raman spectrum is significantly enhanced. This can be due to a more complex surface plasmon of the combined metal-tip system. The influence of the tip on the Raman enhancement is difficult to predict in theory. The Raman spectra were acquired in 4x1 minute scans at an excitation wavelength of 488nm.
Conclusions

Raman measurements have been carried out on Rh6G coated, isolated silver islands on a glass substrate with 70nm lateral resolution. Suitable substrates were prepared by slow metal vapour deposition and a subsequent annealing step. The absolute dye concentration was determined by friction force microscopy. Using newly developed SNOM tips with high optical transmission, measuring times of around one minute per spectrum were achieved. Full Raman imaging of small regions with this resolution, therefore, can be carried out in a few hours. For quantitative analysis, several additional parameters have to be considered. Among them, the exact local dye concentration and photo-bleaching rate of the Raman scatterer are the most crucial. The registry of the optical and topographical image, morphological coupling, and sample drift can be controlled with advanced instrumentation, while the influence of the SNOM tip itself on the SERS measurement is currently less well understood. Absolute enhancement factors measured at distinct locations on top and in the vicinity of the silver islands were found to lie between 2'000 and 6'000, smaller than the overall EF as determined from far-field measurements (11'000). With few exceptions, the predictions for the electromagnetic enhancement of single silver protrusions qualitatively match our findings.
4.7 References


Enlightening the Nanoworld


Chapter 4 - Near-Field Raman Microscopy


Chapter 5

Nanoscale Laser Ablation & Mass Analysis
5.1 Near-Field Desorption

The previous chapters demonstrated the potential of SNOM for chemical analysis on the nanometer scale. The ability to overcome the Rayleigh criterion with near-field optics favours, in a similar manner, applications in fields such as photolithography and high-density data storage.

Several publications reported the use of SNOM as a tool for photolithography by exposing a photo-resist to a nanometer sized light source and subsequent development of the resist by plasma- or chemical-etching.1-8 Similarly, sub-wavelength optical data storage was performed by imaging and recording domains in magneto-optic materials,9-11 liquid crystals,12 or antimony thin films.13

In order to utilise SNOM as a universal tool for nanoscale lithography, a direct writing method is favoured, by which the surface material is ejected from the surface into the gas phase. As will be shown in this chapter, it is possible to desorb material under ambient conditions with very high resolution using appropriate SNOM tips. Besides patterning of surfaces,16-18 another, more challenging application is the collection of the desorbed sample for further analysis.19,20 This chapter shows that SNOM can be used as a “nano-sampling tool” for nanoscale laser ablation mass analysis.

5.2 Sample Preparation

For preliminary investigations, the two main prerequisites for the samples are: they should desorb at relatively low laser fluence, and they should exhibit a smooth surface, so that it is easy to detect the laser-induced surface modifications after illumination. Different classes of molecules show different characteristics when it comes to the formation of homogeneous, flat films. Therefore, a variety of “organic” substances were examined as test substrates, such as polymers, dyes, and other small organic molecules.

A series of commercially available polymers, listed in table 5.1, have been investigated in the desorption experiments. None of the examined polymers showed an absorption in the visible region, i.e. they were transparent or white, respectively.

Still, to achieve a low desorption threshold the photo-absorption of the sample can be tailored to the illuminating wavelength. To achieve this, dopants are commonly introduced as promoters to confer photosensitivity to the intrinsically non-absorbing host material. Subsequent to the absorption of the energy from the
laser pulse, the dopant may undergo ionisation, energy and/or electron transfer, radical formation, fragmentation, or intramolecular re-arrangements,\(^{21}\) in this way ejecting sample molecules into the gas phase.

The additive of choice was Rhodamine 6G \([M, 479g·mol^{-1}} from Fluka, Switzerland\]. The maximum absorption is at \(\lambda = 530\text{nm} \) \((e_{\text{max}} = 10.5·10^4\text{L·mol}^{-1}·\text{cm}^{-1})\),\(^{18}\) suitably close to the standard illumination wavelength of 532nm from the second harmonic of a Nd:YAG laser.

The additive can readily be doped into the base polymers, with the use of (heated) organic solvents (Chloroform, \(\text{CCl}_4\), \(\text{THF}\), dichloromethane, acetone, diethylether, methanol, or ethanol, respectively). The control of dopant concentration (typically 1:1'000 molar conc.), type of solvents, rate of solvent removal, etc. allow the adjustment of experimental conditions to form homogeneous and smooth films. In this way, a reproducible sample preparation with products easily recognisable by a complementary analysis technique (known mass spectra, etc.) is created. A detailed description of the polymer preparation can be found in Ref. [19]. After sample preparation, AFM images were obtained to characterise the surface roughness and homogeneity.

### Spin-Coating

Simply drop-coating of dissolved PEG 600\(^{\dagger}\) onto a microscopy glass cover slide resulted in a smooth polymer film. The film, however, was so soft \((T_{\text{m.p.}} \sim 20^\circ\text{C})\)

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\(^{\dagger}\) 600 denotes the nominal molecular mass of a polymeric chain.
that no AFM image of the surface could be acquired. A series of higher mass PEG oligomers have been evaluated by AFM, all showing a small surface roughness of less than 15nm (rms). A list of the examined PEGs can be found in table 5.1.

An improved way to apply a polymeric film onto a glass substrate is by spin coating a dissolved sample solution. To spin coat, the substrate is rotated at several

<table>
<thead>
<tr>
<th>Polymer</th>
<th>$M_r$ [g·mol$^{-1}$]</th>
<th>m. p. [$^\circ$C]</th>
<th>Suitability for AFM</th>
<th>Surface Roughness</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEG 300</td>
<td>285-315</td>
<td>-20 to -15</td>
<td>not suitable</td>
<td>[liquid]</td>
</tr>
<tr>
<td>PEG 600</td>
<td>570-630</td>
<td>17-23</td>
<td>not suitable</td>
<td>[liquid/gel]</td>
</tr>
<tr>
<td>PEG 1'000</td>
<td>900-1'100</td>
<td>37-40</td>
<td>non-contact only</td>
<td>very flat</td>
</tr>
<tr>
<td>PEG 1'500</td>
<td>1'400-1'600</td>
<td>45-50</td>
<td>non-contact only</td>
<td>very flat</td>
</tr>
<tr>
<td>PEG 2'000</td>
<td>1'900-2'200</td>
<td>50-52</td>
<td>just practicable</td>
<td>very flat</td>
</tr>
<tr>
<td>PEG 3'000</td>
<td>2'700-3'300</td>
<td>56-59</td>
<td>good</td>
<td>RMS&lt;5nm</td>
</tr>
<tr>
<td>PEG 6'000</td>
<td>5'000-7'000</td>
<td>60-63</td>
<td>good</td>
<td>RMS&lt;5nm</td>
</tr>
<tr>
<td>PEG 10'000</td>
<td>8'500-11'500</td>
<td>63-65</td>
<td>good</td>
<td>RMS&lt;10nm</td>
</tr>
<tr>
<td>PEG 20'000</td>
<td>16'000-24'000</td>
<td>63-66</td>
<td>good</td>
<td>RMS&lt;14nm</td>
</tr>
<tr>
<td>PEG 35'000</td>
<td>ca. 35'000</td>
<td>64-66</td>
<td>good</td>
<td>RMS&lt;12nm</td>
</tr>
</tbody>
</table>

Table 5.2. Polyethylene glycol oligomer thin films examined by AFM.

Figure 5.1. Selected PVC films doped with rhodamine 6G (1:1000) prepared by spin-coating: (a) no spinning; (b) a drop of 0.2ml sample solution (PVC:THF = 1:2, 10$^{-3}$M Rh6G) has been dried on a still substrate for 30s and then accelerated to 5000rpm for several seconds; (c) Several drops of the PVC solution have been applied on a substrate spinning at 5000rpm; (d) same as (c), but dried only for 10s.
hundred rpm while pipetting solution onto the surface. This causes the solution to spread radially in a homogeneous fashion due to the centrifugal forces on the solvent. In this way, the solvent evaporates much faster yielding a smooth polymeric thin film.

Depending on the experimental parameters, different surface morphologies are obtained. Figure 5.1 shows a selection of PVC films doped with rhodamine 6G prepared with various spin parameters. Obviously, the surface characteristics change drastically, even for small variances in the preparation process. Consequently, it has been found difficult to reproduce similar surfaces, even when employing identical experimental conditions.

The post-deposition treatment of the prepared films by annealing in a dry oven proved to be suitable for the creation of much smoother surfaces. Figure 5.2 shows a PVC film before and after heating to 120\(^\circ\)C and 200\(^\circ\)C, respectively. The polymer melts at temperatures below 200\(^\circ\)C, forming homogeneous and smooth surfaces. This post-deposition treatment, however, was not successful with other polymer matrices (PEG, PMMA, PS), either due to their decreased affinity to the rhodamine 6G dye (formation of dye agglomerates) or too high melting points (bubble formation due to evaporation of lower mass components).

![Figure 5.2. Spin-coated PVC film without (left, from figure 5.1d) and with post-deposition treatments (centre, right). The extremely small surface roughness seen in the right image was only achieved by heating the films to 200\(^\circ\)C.](image)

On the other hand, thin films of organic substances such as pure dyes (Rhodamine 6G, rhodamine B, coumarine, and brilliant cresyl blue) or anthracene were prepared by spin-coating without the addition of dopants. Generally, smooth films were obtained for the dyes, whereas rather rough and semi-crystalline surfaces resulted for anthracene.
Heat-Pressing

Another way to form extremely flat samples has been investigated by heat-pressing polymers onto freshly cleaved mica. Mica can be cleaved along its crystal planes forming an atomically flat substrate surface that is used as a template for the polymer films. A pneumatic press [TLP 10, Suter & Co Maschinenbau, Basel, Switzerland] with heat-controlled plates has been used for manufacturing of the sample specimens. Depending on the polymers, the heating was controlled from 140° to 220°C. An average pressure of 12 tons was applied over a ten minute period. After pressing, the temperature was slowly reduced to avoid cracking. Remaining mica was easily removed by an adhesive tape. Figures 5.3 and 5.4 show selected samples (40x12mm) with varying dopant concentrations (none, \(10^4\) M, \(10^3\) M). The AFM investigations show that only PVC and PMMA form both homogeneous and flat surfaces. Rhodamine 6G tends to agglomerate in the LDPE/PS and PS films, whereas HDPE and PVA do not show sufficiently smooth surfaces.

**Figure 5.3.** Photographs of selected PEG specimens prepared by heatpressing the pre-doped polymer: No additive (left), \(10^4\) concentration of rhodamine 6G (centre), and \(10^3\) concentration of rhodamine 6G (right).

**Figure 5.4.** (next page) Various polymers prepared by heat-pressing the pre-doped polymer: No additive (left), \(10^4\) concentration of rhodamine 6G (centre), and \(10^3\) concentration of rhodamine 6G (right). The photographs show the macroscopic homogeneity of the dye distribution. The AFM topography images taken from the heavily doped specimens depict the microscopic surface roughness. Note the varying image dimensions for the topographs.
Chapter 5 - Nanoscale Laser Ablation & Mass Analysis
Anthracene

Anthracene was chosen as a test substrate because it is a relatively small organic molecule, which should be easily detected and recognised by a mass spectrometer after laser-induced desorption. In contrast to the doped polymer systems, anthracene exhibits a strong absorption in the near UV region ($\lambda_{\text{max}} = 357 \text{ nm}$, $\varepsilon_{\text{max}} = 8'300$), sufficiently close to the third harmonic of the Nd:YAG laser (355 nm).

Unfortunately, heat-pressing of anthracene did not lead to microscopically flat surfaces (Figure 5.5). This finding can be explained from the fact that anthracene sublimes (rather than melts) at elevated temperatures under high pressures.\(^1\)

Figure 5.5. Photograph and AFM image of heat-pressed anthracene.

In addition, spin-coating of anthracene did not result in flat surfaces; mostly rough and semi-crystalline surfaces were obtained. The strong tendency of anthracene to form crystals, however, was used to grow larger structures: Spreading of a solution of anthracene in p-xylene on a cover glass and subsequent slow evaporation at 8°C for 20 minutes led to the formation of millimetre-sized crystals. The surface of those crystals were extremely flat with a surface roughness of less than a few nanometers.\(^16\)

Triazenes and Pentazenes

A way to circumvent the problem of samples having a low photo-absorption characteristic at the available wavelengths is to specifically design polymers suited for laser ablation. Thomas Lippert et al.\(^{22-30}\) have designed polymers with photo-

\(^{1}\) $T_{\text{sublimation}} (101'325 \text{ Pa}) = 226^\circ \text{C}$
labile groups in the polymer backbone, which decompose upon laser irradiation in the near ultra violet region. The absorption maximum can be tailored from 290 to 380nm by substitution of the chromophore, which is attached to a triazene (-N(R)-N=N-) or pentazene (-N=N-N(R)-N-N=N-) group (See figure 5.22 and graph 5.6, respectively for a molecular constitution).

Another advantage of polymers designed for UV photo-ablation, is that they decompose mainly to gaseous products. For this reason, little re-deposition of material is expected to deteriorate other sample locations. A photochemical mechanism is assumed to dominate the desorption process, while the liberated nitrogen acts as a driving gas promoting a clean ablation with minimal energy requirements. Therefore, such materials, often in polymeric form, are popular as high-performance resins for laser photolithography.

The triazene and pentazadiene polymers investigated in this work were kindly provided by Thomas Lippert in collaboration with the group of Alexander Wokaun (PSI Villigen, Switzerland) and the group of Oskar Nuyken (TU München, Germany). A detailed description of the absorption characteristics, molecular formula, mass spectra, etc. can be found in Ref. [19], [22], and [24]. The formation of flat films was successfully performed by spin-coating after dissolution in THF or chlorinated solvents (CH₂Cl₂, CHCl₃). Significant crystal formation was observed only for the pentazadiene monomer, which therefore could only be used for far-field investigations.

**Laser-Induced Desorption on a Nanometer Scale**

Laser-induced desorption on a nanometer scale was successfully accomplished for various types of samples (Figure 5.6 and 5.7). The pure dye films (Coumarine, rhodamine B, and rhodamine 6G (not shown)) and similarly the doped polymer films (PVB, PVC, and PVA/PVB (not shown) doped with Rh6G) could be ablated with laser wavelengths in the visible region (532nm). Furthermore, laser-induced surface ablation without sample re-deposition was achieved with anthracene and triazadiene specimens. Other samples were either too rough and/or inhomogeneous for scanning probe investigations or no holes were created and/or detected after pulsed laser illumination with SNOM. Samples denoted with an asterix (*) were imaged with the same SNOM tip used for desorption, whereas the others were imaged subsequently with a very sharp, high aspect ratio AFM tip. As a consequence, the morphological dimensions of those characterised by the shear-force feedback of the SNOM differ from reality due to topographic convolution of tip and sample. The holes, therefore, appear too small in the images, whereas surface elevations,
Figure 5.6. Scanning probe images of craters produced by pulsed laser light delivered through a SNOM probe. The actual dimensions may differ from the ones depicted due to topographic convolution with the scanning probe, especially for those marked with an asterix (*), since they were imaged by the shear-force feedback-mechanism of the SNOM rather than by AFM. The depth of the holes range from few nanometers to few tens of nanometers, with PVC doped with rhodamine 6G being the only exception. Coumarine has been imaged by Thomas Roth; Rhodamine B and PVB doped with rhodamine 6G (left panel) have been imaged by Bertrand Dutoit. Further, sub-wavelength sized holes have been created by SNOM with: Rhodamine 6G and PVA/PVB doped with rhodamine 6G (not shown).
probably, are much smaller in reality than shown in the pictures. Still, several examples of sub-wavelength sized craters were found, encouraging further investigations in the field of atmospheric nanoscale laser ablation with SNOM.

![Image of a 5x5 μm shear-force topographic image of an anthracene crystal surface modified by several laser pulses. The total topographic contrast is ~50 nm. The actual dimensions of the image may differ from the ones depicted due to morphological convolution of the sample with the SNOM probe. Measurements performed by Bertrand Dutoit.](image)

**Figure 5.7.** 5x5 μm shear-force topographic image of an anthracene crystal surface modified by several laser pulses. The total topographic contrast is ~50 nm. The actual dimensions of the image may differ from the ones depicted due to morphological convolution of the sample with the SNOM probe. Measurements performed by Bertrand Dutoit.

### 5.3 Mechanistic Aspects

#### Axial Expansion of Tip

Different mechanisms are possible for the creation of holes or craters by pulsed laser radiation transmitted through nanometer sized tip apertures. The simplest explanation is an axial expansion due to transient heating of the fibre tip. It can even be imagined that a small protrusion on the end of the tip can create indentations smaller than the overall tip diameter. A measured temperature coefficient of 100-
waxes with varying melting points onto SNOM probes. The temperature gradient has been evaluated from the position of the clearly visible phase change between the solid and liquid wax.\textsuperscript{17,36}

Due to these findings, transient heating effects of the chemically etched fibre tips occurring on a nanosecond time scale can account for melting holes into polystyrene (PS) and poly(vinylbutyral) (PVB, figure 5.6 bottom left) surfaces.\textsuperscript{17} Theoretical considerations show\textsuperscript{37,38} that a coated near-field optical probe is not only a source of optical radiation, but that the heated probe can also act as a thermal source, in which some of the heat from the probe is conducted across the air gap and into the sample. In addition, the heated probe can act as a “grey body” that emits electro-magnetic radiation, of which some is absorbed in the sample and converted to heat. However, in general it is found that the transmitted heat from the SNOM tip to the sample by thermal conduction is minimal compared to the optical power emitted by the probe.\textsuperscript{37,39,40}

The experiments performed by Zeisel et al. (Figure 5.6 bottom left)\textsuperscript{17} were repeated on the Lumina instrument (Figure 5.6 third from top in the right panel and 5.11), only that this time, the craters were imaged by AFM. The improved resolution reveals, that a significant amount of material has been ejected into the gas phase, and that the rim around the crater consists of re-deposited material rather than of melted polymer. Furthermore, the laser-induced heating and cooling through heat dissipation of the SNOM probe requires several tens of milliseconds.\textsuperscript{33,35} With a scan speed of several micrometers per second, the nanometer-sized holes had to be created within few microseconds, since no elongation of the craters are detectable. For these reasons, no empirical evidence is found to support the model of a heated SNOM tip melting holes in polymeric surfaces.

**Wavelength Dependence**

Theoretically, it is found that most of the power is conducted from the probe to the sample by electromagnetic radiation.\textsuperscript{18,37} Therefore, a photochemical or photothermal mechanism is expected to play the main role in laser-induced desorption with SNOM.

For experiments carried out with laser sources in the visible region, the photon energy is not sufficient for inducing chemical bond dissociation. More likely, the absorbed photon energy is converted to vibrational excitation, causing a rapid temperature increase in the sample that leads to thermal desorption of the molecules. To prove this assumption, a series of experiments have been carried out to study the influence of the incident laser wavelength on molecular thin films.\textsuperscript{18} Figure 5.8
shows the shear-force topography of a rhodamine B film recorded after attempts to ablate material from the film with pulsed laser [Nd:YAG pumped optical parametric oscillator, MOPO 730, Spectra Physics, Mountain View, CA] with a wavelength of at first 650nm (2.1μJ, left image) and then 532nm (1.4μJ, right image). No ablation was observed at the off-resonance wavelength of 650nm, whereas well defined 100nm wide and 10nm deep holes were created by the wavelength of 532nm, which is strongly absorbed by the sample. Moreover, the size of the observed indentations match the calculated predictions based on the absorbency of rhodamine B (ε_{max} = 10.7·10^{4}L·mol^{-1}·cm^{-1}) and the resulting near-field optical penetration depth of approximately 20nm.\(^{18}\)

In contrast, laser desorption processes with ultraviolet laser radiation can be explained by a purely photochemical ablation mechanism.\(^{21,22,26,29}\) For this reason, specially designed resins have been used to investigate surface ablation by SNOM. Due to the photo-labile groups in the triazene and pentazene molecules, the compounds decompose upon laser irradiation into gaseous products, which do not contaminate the sample surface or the optical set-up. Moreover, the absorption maximum can be tailored for specific wavelengths, making the resins highly sensitive at the employed radiation frequency, therefore, reducing the required energy for desorption. Figure 5.6 (right panel, second from top) depicts a triazene thin film after laser-induced ablation at 355nm, at a strong absorption band of the UV-photo-
resist. The sub-wavelength sized holes have sharp contours with no re-deposition of the ablated material. This sample which was finally chosen for nanoscale laser ablation mass analysis, will be discussed later in this chapter.

Transport of Material

The possibility of vaporising molecules from a surface is of great practical usefulness for analytical nano-sampling. The question of whether or not gas-phase material is released from the sample surface is also relevant for optical writing with SNOM technology, because re-deposited solid debris may seriously degrade the surface quality. Experiments carried out by Dutoit et al. show that rhodamine 6G is being vaporised by laser pulses delivered via uncoated SNOM probes, as detected by observing the fluorescence of re-deposited rhodamine on the tip (Figure 5.9).

Alternatively, the transport over distances of many micrometers was observed for several cases. Two selected results of such experiments are shown in figure 5.10. The images show the topographs of a PVB and a rhodamine 6G film before and after laser illumination, respectively. The surface modification can be interpreted by vaporisation and subsequent re-deposition of sample material. In the case of rhodamine 6G, the transport took place in a preferred direction, most likely determined by the asymmetric shape of the tip used. The transport of material occurred over several tens of micrometers.

Figure 5.9. Fluorescence spectra (excitation wavelength 514nm) recorded from the very end of a SNOM tip used for ablation. Upper graph: blank experiment. Several 0.5μJ laser pulses (λ = 532nm) were coupled into the fibre to create a large (10μm diameter) ablation crater on the surface of a rhodamine 6G film. Re-deposited molecules on the SNOM tip lead to a characteristic rhodamine spectrum when recorded from the end of the SNOM tip (lower graph). Image adapted from Ref. [18].
Figure 5.10. Transport of material over many micrometers after laser-induced nanoscale desorption by SNOM, imaged by AFM. The circles in the images before laser illumination denote the location of the succeeding nanoscale ablation. The total topographic contrast is 200nm for all images.

Similarly, the transport of material can be seen from figure 5.11. The material has been deposited circularly around the desorption craters. From this figure, one can also estimate that only few large chunks of material are ejected. This too evidences that the material transport does not take place by mechanical dragging, but through vapourisation and re-deposition.

Finally, figure 5.12 demonstrates three consecutively acquired shear-force images of a rhodamine 6G doped PVB film, whereas several laser pulses ($\lambda = 532$nm) have been exposed to the surface via the SNOM probe on one line in the middle of

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1 The observed surface structures can be overestimated in size due to convolution with the imaging tip shape.
Figure 5.11. (above) AFM topographs of PVB doped with rhodamine 6G before and after laser ablation. Note, that the ejected material is re-deposited radially around the ablation crater. The deposited material, most likely, consists of small particles, which appear too big in the topography image due to topographic coupling with the imaging probe. The total topographic contrast is 200nm.

Figure 5.12. (left) Three subsequent scans of a PVB film doped with rhodamine 6G. In the middle of the second scan (arrows), a series of laser pulses (532nm, 10ns, 20Hz) have been instigated on the sample surface during imaging. From the lower part of the image (2) and image (3) a significant change in surface roughness is visible. No holes have been detected in the final scan (3.), possibly because they were smaller than the tip apex of the SNOM probe (200-300nm) that was imaging the surface. The homogeneity and size of the ejected particles suggest a evaporation-recondensation mechanism.
the second scan (arrows). An apparent change in the surface roughness becomes visible soon after the first laser pulse. The topographical contrast increases from few nanometers in the first image, to about 20nm for the second and third image. This implies that a significant amount of sample has been ejected from the centre of the observed image. Finally, the created holes could not be resolved in the third scan, probably because the dimensionality of the imaging SNOM tip was bigger than the created indents.

In conclusion, the coupling of pulsed laser radiation into suitable SNOM probes permits spatially resolved photo-thermal and photo-chemical ablation, as well as nano-structuring of polymers in the sub-wavelength dimension.

5.4 Interface to a Mass Spectrometer

Concept

Towards the goal of gaining molecular information on a nanometer scale, optical spectroscopy has been combined with scanning probe microscopy. However, in certain cases, vibrational, luminescence, or absorption spectroscopies are not suitable, especially, when investigating mixtures of unknown substances. Therefore, the need for a complementary analysis method arises. An excellent analytical method with a very high information content is mass spectrometry. While mass spectrometry generally provides no spatial resolution, nanoscale MS imaging is possible for elemental and molecular analysis using secondary ion mass spectrometry (SIMS). For investigation by SIMS and other methods with high spatial resolution such as Auger electron microscopy, samples have to be kept at high vacuum, which complicates both their manipulation and visual inspection during analysis, and is detrimental for biological samples. Similar problems were encountered by Kossakovski et al. who put an entire set-up for near-field laser ablation experiment in vacuum, in close proximity to an ion source and mass spectrometer.

![Figure 5.13. Schematic of the nanosampling set-up for the collection and transportation of near-field ablated material to a remote mass spectrometer.](image-url)
In the preceding paragraphs, nanometer-scale desorption under ambient conditions using pulsed laser radiation with SNOM, has been shown. With an interface that allows the collection and transportation of the desorbed material to a remote, complementary analyser, a new, sub-wavelength sized sampling source is created (Figure 5.13). For this task, an interface to transport the ablated material into a vacuum chamber for ionisation and subsequent mass characterisation was designed (Figure 5.14).

**Figure 5.14.** Schematic of the instrumentation involved for atmospheric nanoscale laser-induced mass analysis. The main components of the instruments are: The SNOM, the interface, the vacuum chamber including the ionisation source and mass spectrometer. See text for details.

**Instrumentation**

The interface consists of a metal tube with a tapered end positioned in close proximity to the ablation spot and the other end coupled to the vacuum chamber with a mass spectrometer. The driving force for the directed flow of the gas is the pressure difference between the vacuum chamber (10^{-4} to 10^{-7}mbar) and the
atmospheric pressure at the probe position. The gas flow rate through the metal nozzle can be altered by changing the tube geometry or by varying the final pressure at the inlet to the mass spectrometer.\textsuperscript{47-49}

Several metal tubes were manufactured mechanically by pulling and pressing a thin-walled, 1 cm outer diameter metal tube to form a short taper region with a very small opening of 20 to 80\,\mu m. Openings greater than 100\,\mu m created too high of a load for the vacuum pumps, whereas openings below 20\,\mu m were easily clogged by the desorbed material. The geometry of the tubing strongly influences the flow characteristics of the gas, and therefore, the transport efficiency. To reduce flow turbulence within the metal tubes, a perfectly circular, continuously expanding cone without kinks should be prepared. With decreasing inner tube diameters, this is an increasingly difficult task. For this reason, a stainless steel micro-capillary with a defined inner diameter [e.g. 20\,\mu m; Cat. No. 5-6713 from Supelco, Sigma-Aldrich Corp.] was expanded at one side and welded to a pre-tapered nozzle. One of the first suction tubes (length 20\,cm) is depicted in figure 5.15. Note that, although the outer shape of the tapered nozzle seems to be composed of different parts, the inner tubing should be perfectly even with no steps.

\textbf{Figure 5.15.} Photographs of one of the tapered metal capillaries (length 20\,cm) used for interfacing the near-field ablation in ambient conditions with a ionisation source and a mass spectrometer placed in high vacuum. The two clamps were used for final nozzle positioning and connection of the heating apparatus. The two insets show a magnified view of the tip end and aperture (ca. 20\,\mu m).
The vacuum chamber containing the ionisation source and the mass spectrometer consisted of a hollow aluminium cube (Ø 20cm) with a 15mm thick, transparent Plexiglas cover, which allowed to observe the processes within the recipient (Figure 5.14). The chamber was fitted directly onto an oil-diffusion pump [DIF 200; BPD08500/558 from Balzers], which was backed with a high volume rough pump [Duo 030A from Pfeiffer]. Pressures below $10^{-7}$mbar were achieved with closed metal nozzles. A detailed description of the utilised vacuum system can be found in Ref. [19].

The ion to neutral yield for irradiation experiments is usually very small, generally below 1:100000. Therefore, primarily neutral molecules are desorbed by laser-induced surface ablation (irradiances from $10^3$ to $10^5$W·cm$^{-2}$), and a post ionisation source has to be implemented between the interface and the mass spectrometer.

Initially, two resistively heated tungsten filaments were used for electron impact (EI) ionisation of the sample molecules. Due to oxygen that is sucked in along with the sample molecules to the ionisation source, the metal filaments corroded slowly, requiring the exchange of the filaments regularly (once a month). Although the ionisation efficiency proved to be sufficient for preliminary experiments, photo-ionisation by UV radiation should be employed for future experiments. For this reason, two opposing sapphire windows have already been fitted orthogonal to the sample beam at the height of the mass spectrometer inlet.

Mass analysis of the ions was performed by a Quadrupol Mass Spectrometer (QMS) [Spectra Int., Morgan Hill, CA]. The employed Faraday detector can detect ions in the mass range of 1 to 300Da at pressures below $10^{-4}$mbar. A Secondary Electron Multiplier (SEM) further increased the sensitivity of the detector by a factor of approximately a thousand. The commercial control unit of the QMS allowed the control of the EI-filaments and the entire mass spectrometer and allowed for easy data acquisition. A greatly improved time-resolution and higher sensitivity was achieved, however, by directly monitoring the analogue SEM output with a fast digital oscilloscope.

$^1$ Only for irradiances above $10^4$W·cm$^{-2}$ plasma formation occurs with ion yields approaching 100%. $^{51}$

$^{17}$ Specific values given in this and the following equations are for anthracene (bulk).
5.5 Far-Field Experiments

Sample Volume

One of the main problems in nanoscale laser ablation mass spectrometry lies in the small number of sample molecules available for mass analysis. Considering a crater diameter of 100 nm and a depth of 20 nm, a volume of less than 50,000 nm$^3$ is ejected into the gas phase. This volume corresponds to less than 300,000 molecules (0.5 amol)$^*$ in the case of anthracene or 60,000-70,000 molecules (0.1 amol)$^{**}$ in the case of Rhodamine 6G. From these molecules only very few are successfully transported, ionised and detected. For this reason, every component of the instrument has to be optimised to reduce the loss of gaseous sample. Obviously, the interface between the place of desorption and the vacuum chamber required considerable attention. Besides the geometrical shape of the suction tube, other parameters such as (i) the tilt angle of the metal tube in respect to the ionisation filaments and QMS, (ii) the distance between the nozzle outlet and the ionisation source (QMS), (iii) the tilt angle relative to the sample and SNOM probe, and (iv) the nozzle inlet-sample separation have been extensively investigated. Further, to promote the transport speed and efficiency, and to prevent clogging of the interface, the metal tube was heated.

Tilt Angle between Interface and QMS

The fitting of the metal tube to the vacuum chamber was performed by a thick polymer (Vitton) ring, thus, allowing for small displacements of the interface without the need to move the entire vacuum chamber. For a precise x-y-z control, a hydraulic displacement actuator [Nashirige, Nikon] was used for final nozzle alignment. A small angle of inclination of the interface, however, moves the tube outlet away from the filament and quadrupole inlet. To quantitatively study the dependency of the interface-QMS alignment, the nitrogen signal peak (m/z = 28) arising from the ambient air, which continuously is sucked in by the nozzle, was chosen as a measure.

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$^*$ Estimation based on crystallographic data.

$^{**}$ Estimated from bulk density considerations.
As expected, a maximum signal intensity was obtained for an on-axis alignment of the tube and interface (Graph 5.1). Small deviations of few degrees from this ideal arrangement lead to a significant signal reduction. A similar trend was found for a displacement to the side (horizontally rather than vertically). The dependence of the signal intensity for both directions follows a $\cos^2$ relation, possibly, due to a convolution of the wave-front form of the sample exiting the interface, the electron-field distribution, and the coupling efficiency into the quadrupole. A diversion of the tube outlet by $4^\circ$ horizontally and $4^\circ$ vertically resulted in a signal decrease of over 30%. For the later experiments, therefore, the interface - QMS was aligned rectilinear, sometimes requiring tilting the whole vacuum chamber by a few degrees.

**Graph 5.1.** *Influence of the relative angle between the interface and QMS axis. The fit follows a $\cos^2$ function.*
Nozzle Outlet – Ionisation Source Separation

In principle, a directed beam of molecules is expected to exit the nozzle outlet (effusive source). This can also be seen from the strong dependence of the tilt angle between the interface and QMS on the signal intensity. However, a significant expansion of the sample beam is expected when entering the vacuum chamber. Therefore, not only the direction of the beam in reference with the filament/QMS axis is important, but also the absolute separation of the outlet from the ionisation source is expected to play a considerable role. Again, optimal measuring conditions were fulfilled for parameters providing a maximal sample transport, i.e. when the interface outlet was as close to the ionisation source as possible (Graph 5.2).† The data points could best be fitted by an exponential function, showing the importance of this instrumental parameter.

Graph 5.2. Influence of the nozzle outlet - QMS separation. The signal intensity drops exponentially with increasing separation.
Tilt Angle of Sample

The sample is preferably tilted relative to the suction tube for two reasons: First, due to the geometrical shape of the tube taper, the sample has to be tilted by more than half of the nozzle’s cone angle to be able to position the inlet close to the sample surface (Figure 5.16). Secondly, a greater collection efficiency is expected for tilted samples that preferably eject the sample molecules towards the interface (Figure 5.17). On the other hand, the coupling of the optical near-field with the sample surface, in theory, is greatest for samples located perpendicular to the SNOM probe.

To deduce the dependence of the signal intensity on the inclination angle of the sample quantitatively, pulsed laser radiation ($\lambda = 355$nm) was focused ($\sigma > 40\mu m$) on a thick anthracene film providing a relatively large amount (compared to SNOM) of gaseous sample. A goniometer allowed the control of

---

Since any contact between the metal tubing and the resistively heated filaments (High voltage!) should be avoided, care has to be taken in the corresponding experiments.
the tilt angle of the sample surface without changing other parameters of the set-up (Graph 5.3). By increasing the tilt angle from 10° to 60°, the measured signal intensity at m/z = 178 increased notably, by over 650%.

Graph 5.3. Graph showing the influence of the relative angle between the sample and interface on the measured signal intensity.
Sample – Suction Tube Separation

As with the interface–ionisation source separation, the distance between the sample surface and the inlet of the suction tube proved to be important (Figure 5.18). In semi-quantitative measurements, maximum signal intensity was achieved for small nozzle-surface separations. The absolute signal intensity, however, also depended strongly on the sample type and laser flux which both influence the sample plume geometry and dimensions. For small micro-explosions, even small variations (micrometer range) in the nozzle–sample separations significantly changed the measurable signal intensity.

Heating of the Interface

To prevent sticking of the desorbed material onto the inner walls of the tubing the interface was heated externally in a controlled fashion by a heating tape [Omegalux HTWC102-006] that was strapped around the metal tube (Figure 5.19). The actual temperature at the metal surface was measured with a contact-thermocouple [Ebro TTX690]. With the reduction of the molecular adhesion to the metal of the interface, not only the transport efficiency was increased, but also a tremendous reduction of the delay time between desorption and detection was achieved. Moreover, for small aspiration-apertures (< 50μm) sizes, heating was necessary to prevent clogging of the interface inlet.

The absolute delay times were strongly sample dependent. A delay time of several seconds was measured for anthracene, which shows a great tendency to adsorb to metal at room temperature, whereas pentazadiene was detected only a few tens of milliseconds after desorption. However, both substances were transported much faster when the interface was heated (Graph 5.4). The retention time, signal rise time, and the peak width all shortened exponentially with increasing temperatures.
Almost no change was observed for temperatures above the boiling points of the samples where probably no adsorption takes place.

Graph 5.4. The transportation speed increases significantly when the suction tube is being heated. The graph shows the duration of the signal measured by the QMS with varying interface temperatures. The data points were fitted by a $1/\sqrt{T}$ function.

Far-Field Mass Spectra

Mass spectra were first obtained by focussing ($f_{\text{Lens}} = 5\text{cm}$) a pulsed laser beam (60ps, $\lambda = 355\text{nm}$, $P = 3\text{mW}$, $k_{\text{rep}} = 10\text{Hz}$) onto a flat anthracene, triazene polymer, and pentazadiene$^{22-27,29,30,36}$ surface, respectively. The ablation craters created by this arrangement were between 4 and 100$\mu$m in diameter (Figure 5.20), providing a sufficient amount of gaseous sample for preliminary measurements. A double tapered metal tube (Full taper angle $q = 6^\circ$ for the first 10mm, then $17^\circ$ for the next 54mm, then cylindrical for an additional 156mm) with a small circular opening of approximately 25$\mu$m at its apex, is placed in close proximity ($< 1\text{mm}$) to the desorption place. At the high vacuum end of the collection tube the pressure was $5\cdot10^{-7}\text{mbar}$. To prevent sticking of the desorbed material onto the inner walls of the
Figure 5.19. Photograph of the far-field desorption setup. The metallic interface is completely covered by the heating tape. The sample is positioned on a tiltable x-y-z translation stage. The laser source is focused onto the sample by a quartz lens (f=5 cm) from above.

Figure 5.20. Photograph of a PS specimen with holes created by far-field laser desorption. The diameters of the craters range from 4 μm to over 100 μm.
tubing, external heating of the tubing (260°C) was applied. Surface material transported via the tubing, finally, was ionised immediately after the tube-exit and then detected by the QMS.

Anthracene as well as the triazene polymers and pentazadiene were desorbed and detected successfully by the QMS. Overall signal intensity, response time, signal peak shape, and signal decay time were measured and analysed. A comparison of

Graph 5.5. Anthracene spectrum measured with the far-field set-up (top) and a reference spectrum from the NIST onllin database. The inset in the upper spectrum shows the time transient for the signal at m/z = 178. The inset in the lower spectrum depicts the absorption spectrum of anthracene around the used laser wavelength (355nm). The interface was heated to 260°C.
the acquired spectra with reference spectra\(^\dagger\) showed a good agreement (Graph 5.5). A detailed description of the fragmentation patterns, etc. can be found in Ref. [19].

The graphs 5.5 and 5.6 show time transients of the fragments at m/z = 178 (anthracene) and m/z = 91 (pentazadiene), respectively. Trendlines based on moving averaging of neighbouring data points have been added for clarity. The observed shape of the time transients are similar to those found for laser-induced ablation in vacuum.\(^{25}\) A theoretical model for the mechanistic functions are exploited in the next paragraph. For an exact fitting of the graphs, however, additional measurements with variable laser fluence will have to be performed.\(^{25-28,57,59}\)

\(^{\dagger}\) Anthracene: NIST on-line database http://webbook.nist.gov/chemistry/

\(^{\dagger\dagger}\) Triazenes, and pentazadiene: Reference spectra obtained by the MS-Service ETHZ (Trivid EI + Magnet BpM) and from Ref. [24].
Kinetics

One of the aims of the experiments carried out so far was to determine the important factors for an efficient transport of the desorbed molecules through the tapered tubing. The experiments showed a great dependence of the signal intensity on a variety of parameters. In order to provide a model for the course of the molecules in the interface, theoretical considerations were added to the empirical findings. A suitable model has to provide explanations for the measured retention times, rise times, peak widths, and decay times of the measured mass signals. Due to the complexity of the physical system a series of assumptions have to be made in order to obtain semi-quantitative values for the transport characteristics.

The kinetics involved in the particulate transport from the place of ablation to the ionisation source are not trivial. The time it takes for the sample molecules to reach the QMS depends on many factors, such as the sample material, desorption mechanism (thermal, photo-chemical, or photo-thermal), pressure gradient, interface geometry, etc.

The desorption mechanism and therefore the energy transfer from the laser pulse to the sample molecules is very much dependent on the sample and the illumination wavelength. In principle, one can differentiate between photo-ablation and thermal ablation. However, in practice most samples can be described as a mixture of both mechanisms. Considering that all absorbed light is converted completely to heat and neglecting phase transition and heat transfer, the temperature increase ($\Delta T$) at the sample surface can be calculated from:

$$\Delta T = \frac{E}{C \cdot d \cdot \rho}$$  \[1\]

$E$ Absorbed light energy per area of interest

$\rho$ Specific density $1.283$g·cm$^{-3}$†

$C$ Specific heat capacity $1.18$J·K$^{-1}$·g$^{-1}$ (solid)

$d$ Laser penetration depth

Neglecting multi-photon absorption, saturation, and plume attenuation, the laser penetration depth ($d$) can be deduced from:

$$d = \frac{1}{\sigma} \ln \left( \frac{1}{1 - r} \right)$$

† Values for anthracene (bulk)
\[ d = \frac{1}{\alpha} \cdot \log \left( \frac{F}{F_{th}} \right) \approx \frac{E}{\alpha \cdot I_0} \]  

\[ \alpha \quad \text{Absorption Coefficient} \]
\[ F \quad \text{Laser Fluence} \]
\[ F_{th} \quad \text{Threshold Fluence} \]
\[ I_0 \quad \text{Laser Intensity} \]

\[ 3^{,200} \text{cm}^{-1} (\lambda = 355 \text{nm}) \]
\[ <300 \mu \text{J/pulse} \]

\[ \Delta T = \frac{\alpha \cdot I_0}{C \cdot \rho} \]

Often, the translational temperature of the desorbed molecules is somewhat less than the calculated maximum surface temperature.\(^{62-65}\) Nevertheless, as a first approximation, the molecular speed can be estimated from the calculated temperature (700 to 1200K).\(^{21}\)

The individual velocities of the molecules are dispersed over a broad range of speeds, whereas the distribution broadens as the temperature increases (Figure 5.21). Further, lighter molecules have a broader distribution of speeds than heavy molecules. A measure for the speed distribution can be found following the Maxwell-Boltzmann function:\(^{77}\)

\[ f(v) = \frac{1}{2} \cdot \left( \frac{M}{R \cdot T} \right)^2 v^3 e^{-M v^2 / 2 R T} \]

**Figure 5.21.** Maxwell-Boltzmann distribution of speed for four different temperatures. Note that the distribution broadens as the temperature increases.
While random thermal energy is transferred into directed translational energy, internal energy distributions are cooled by post-desorption collisions. While the most probable velocity in the distribution increases with the distance from the desorption spot, the translational energy spread narrows or cools. Similar effects are observed for rotational and vibrational degrees of freedom. For readily cooled gases, the most probable speed approaches the mean speed value ($\overline{v}$), which can be calculated from:

$$\overline{v} = \sqrt{\frac{3 \cdot R \cdot T}{M}} \approx \sqrt{T}$$  \[5\]

Therefore, the initial speed of the molecules lies between 300 and 800 m/s, depending on the sample and initial laser power density. The fast release of nitrogen found for the triazenes and pentazadiene samples acts a “driving gas”, increasing the mean molecular speed to over 1 000 m/s.\textsuperscript{25,26,28,29}

The propagation radius ($R$) of a spherical blast wave originating from a laser desorption experiment can be calculated assuming the energy is instantaneously released into the gaseous product, and the explosion is originating from an infinite small location at the sample surface:\textsuperscript{26,67}

$$R = \xi_0 \cdot \left( \frac{E_0 \cdot t^2}{\rho_0} \right)^{1/5}$$  \[6\]

$\xi_0$ Constant from strong shock theory\textsuperscript{67}
$\rho_0$ Undisturbed atmospheric density
$E_0$ Energy released by the explosion
$t$ Elapsed time after explosion

This, however, is only valid when the spherical shock occurs in a surrounding atmosphere, of which the mass is significantly greater than the original explosive
mass. Since in the proposed set-up, the suction tube that collects the ejected material is located very close to the place of desorption, this condition is not fulfilled, and the ablated mass must be included in the analysis. It can be shown mathematically,\textsuperscript{26,29} that a modified model for the propagation radius has to be used when the observed blast waves are within an order of magnitude of the minimum propagation radius ($R_{\text{min}}$):

$$R_{\text{min}} = \sqrt{\frac{3 \cdot M_0}{2 \cdot \pi \cdot \rho_0}}$$  \hspace{1cm} [7]

$M_0$  \hspace{0.5cm} Original explosive mass

The “modified” propagation radius can be calculated from:

$$R = \frac{\left[\frac{3}{2} \cdot C_i \cdot \sqrt{E_0} \cdot t + (C_i \cdot t)^{2/3}\right]^{2/3} - C_z}{C_i} = \frac{\rho_s \cdot A \cdot \left[\frac{1}{\gamma - 1} + \frac{4}{\gamma + 1}\right]}{\gamma + 1} \quad C_z = \frac{\rho_s \cdot A \cdot d \cdot \left(\frac{2}{\gamma + 1}\right)^2}{8}$$  \hspace{1cm} [8]

$\gamma$  \hspace{0.5cm} Specific heat of air (1.4 J K$^{-1}$ mol$^{-1}$)

$A$  \hspace{0.5cm} Planar area ablated

$\rho_s$  \hspace{0.5cm} Solid sample density

$\rho_0$  \hspace{0.5cm} Air density (1.184 g cm$^{-3}$)

As a rule of thumb, blast waves generally expand to 1mm in less than 1μs and to 3mm within approximately 5μs.\textsuperscript{26} The time needed for the sample molecules to reach the nozzle inlet, therefore,

\textbf{Graph 5.7.} Total yield measured by a micro-balance after a laser pulse at $t=0$ on a ferulic acid film. The continuing loss of material from the film indicates a slow thermal desorption process. Adapted from Ref. [68].
plays only a minor role in the overall time-model. More importantly, thermal evaporation after laser illumination has to be included in the theoretical considerations. For many substances (e.g. anthracene), material is evaporating from the sample surface long after the laser-induced micro-explosion. The molecular yield can, for extreme cases, be subject to errors of up to 70% when collected only within the first second after the desorption process (Graph 5.7).\textsuperscript{68,69} Although no such behaviour has been found for the examined substances, no quantitative values were determined.

The flux of matter ($J_{\text{matter}}$) describes the quantity of material (N) passing through a unit area per unit time:\textsuperscript{66}

\[
J_{\text{matter}} = -D \frac{dN}{dz} = \frac{\lambda \cdot \bar{e}}{3} \frac{dN}{dz}
\]

When both diffusion and convection are of similar importance, the total change of concentration in a region is the sum of the two effects and the generalised diffusion equation has to be considered:\textsuperscript{66}

\[
\frac{\partial c}{\partial t} = D \frac{\partial^2 c}{\partial x^2} - \nu \frac{\partial c}{\partial x}
\]

\[
D \quad \text{Diffusion constant}
\]
\[
\nu \quad \text{velocity}
\]
\[
c \quad \text{concentration}
\]

From the solution of the generalised diffusion equation for a point source in a large space of void and $J_{\text{matter}} \equiv \bar{c} \cdot p_{\text{vapour}}$ follows:\textsuperscript{66}

\[
p_{\text{vapour}} = \frac{n_0}{8 \cdot (\pi \cdot D \cdot t)^{3/2}} \cdot e^{-d^2/4D \cdot t}
\]

\[
n_0 \quad \text{Number of moles present}
\]
\[
d \quad \text{Distance from the source after a time } t
\]
The velocity profile of the gas in the metal tube can only be determined easily for laminar or molecular flows. To find out whether the gas flow in the interface is turbulent, laminar, or molecular, the Reynolds' number (Re) has to be determined:

\[ Re = \frac{\bar{v} \cdot \rho_g \cdot r_0}{\eta} \]  

Whereby the Reynolds' number is estimated to lie below 2’000 for the presented set-up, which accounts for a laminar flow (Turbulent flows are observed for Re > 4000). However, since a pressure gradient (rather than a constant pressure)
exists in the suction tube, and the theoretical considerations account only for ideal (symmetrical, no steps, defect free) capillaries, and no definitive statement can be made with the available data. Nevertheless, assuming a laminar flow, the velocity profile within the capillary, can be estimated from:\(^\text{48}\)

\[
v(r) = 2 \cdot v \cdot \left(1 - \left(\frac{r}{r_0}\right)^2\right)
\]

\[\text{Distance from the capillary centre}\]

From this one would conclude that almost all molecules would be transported through the centre of the interface with no contact with the metal walls. Although, the short transmission times measured (a few milliseconds for 20cm transportation length) could be explained with this finding, one would not expect any dependence on heating the suction tube if this were in fact true.

Another way to look at the problem is to imagine the aperture at the nozzle inlet as an effusive source. After the gaseous plume has passed the small aperture at the nozzle, it expands rapidly due to the rapid decrease in pressure. The rate of effusion (Graham’s Law of Effusion) is:\(^\text{66,70}\)

\[
k_{\text{effusion}} = Z_w \cdot A_0
\]

\[\text{Area of the hole through which the gas evaporates}\]

With \(Z_w\): collisions with walls and surfaces per unit area per unit time:

\[
Z_w = \frac{p}{\sqrt{2 \cdot \pi \cdot m \cdot k \cdot T}}
\]

\[\text{Pressure of the gas at the tube opening}\]

\[\text{Temperature at the tube opening}\]

\[\text{Evaporated mass}\]

From \[17\] and \[18\]:

\[
k_{\text{effusion}} = \frac{p \cdot A_0}{\sqrt{2 \cdot \pi \cdot m \cdot k \cdot T}} = \frac{p \cdot A_0 \cdot N_A}{\sqrt{2 \cdot \pi \cdot M \cdot R \cdot T}}
\]
It is expected from molecular beam theory (effusive sources)\(^{70}\) that the majority of the gas species will collide with the capillary wall shortly after passing the nozzle inlet. The probability that a molecule sticks to a surface (Sticking coefficient) is:\(^{71}\)

\[
s_n = \left[ 1 + \frac{v_d}{v_a \cdot e^{(E_a - E_d) / k \cdot T}} \right]^{-1}
\]

\(v_d\) (generally unknown) pre-exponential factor  
\(v_a\) (generally unknown) pre-exponential factor  
\(E_a\) Energy barrier to chemisorption  
\(E_d\) Energy barrier to desorption

Therefore, the probability of a molecule to adsorb to a surface decreases as the surface temperature increases (for the usual case \(E_a < E_d\)). The actual rate of adsorption (Chemisorption) can be calculated with:\(^{71}\)

\[
k = \frac{N \cdot c \cdot \sigma^0 \cdot e^{(-E_a / R \cdot T)}}{\sqrt{2 \cdot \pi \cdot M \cdot R \cdot T}}
\]

\(N\) Number of molecules  
\(c\) Heat capacity  
\(\sigma^0\) Fraction of available space (Langmuir)  
\(-E_a^*\) Activation energy for covalent binding of molecule to surface

The average time of stay (\(t\)) of the molecule on the capillary surface can be calculated from:\(^{72}\)

\[
\tau = \tau_0 \cdot e^{(-Q / R \cdot T)}
\]

\(\tau_0\) 10\(^{-12}\) to 10\(^{-13}\) sec  
\(Q\) Interaction energy (Energy of adsorption)
Knudsen’s accommodation coefficient ($\alpha$) determines what temperature the molecules have after desorbing from the capillary surface:

$$\alpha = \frac{T_3 - T_1}{T_2 - T_1}$$

[22]

- $T_1$: Temperature of the gas molecule before it strikes the surface
- $T_2$: Temperature of the surface
- $T_3$: Temperature of the molecule leaving the surface

If the sample molecules stay longer than several vibration periods (> ns), it becomes reasonable to consider that adsorption has occurred; temperature equilibration between the molecule and the surface is approached ($\alpha = 1$) and, on desorption, the molecules leave the surface in a direction that is independent of that of their arrival. Table 5.2 lists some numbers for $Q$ and their corresponding stay times.

<table>
<thead>
<tr>
<th>Q [kcal·mol$^{-1}$]</th>
<th>$\tau$ (25°C)</th>
<th>$\alpha$</th>
</tr>
</thead>
<tbody>
<tr>
<td>No adsorption, Specular reflection</td>
<td>0.1</td>
<td>$10^{-13}$ sec</td>
</tr>
<tr>
<td>Region of physical adsorption</td>
<td>1.5-10</td>
<td>$10^{-12}$-$10^{-7}$ sec</td>
</tr>
<tr>
<td>Region of chemisorption</td>
<td>&gt;20</td>
<td>100-$10^{17}$ sec</td>
</tr>
</tbody>
</table>

Table 5.3. Depending on the type of adsorption, the average time of stay ($\tau$) and Knudsen’s accommodation coefficient ($\alpha$) change. From Ref. [72].

The observed time transients for desorbed sample molecules narrowed as predicted from the effusive theory when heating the interface. For this reason, the molecules are expected to travel through the capillary in a zigzag fashion with numerous collisions with the wall. Ideally, the transport behaviour, therefore, can be described as the diffusion of a gas supported by a convective flow towards the
vacuum chamber. The Stokes-Einstein equation describing the diffusion of a perfect gas is:

$$D = \frac{\lambda^2}{2 \cdot \tau} = \frac{\lambda \cdot \bar{v}}{3} = \frac{\lambda}{3} \cdot \frac{8 \cdot R \cdot T}{\pi \cdot M}$$ \[23\]

| \(\lambda\) | Mean free path |
| \(\tau\) | Residual time of the molecules at the surface |

Therefore, and from [18] and [21] the time it takes to transport the molecules from one end of the interface to the other (\(\Delta t\)) decreases with increasing temperature (T):

$$\Delta t \propto \frac{1}{\sqrt{T}}$$ \[24\]

This relationship resembles the empirical findings (Graph 5.4) nicely.

### 5.6 Near-Field Optical Mass Spectrometry

**Near-Field Experiments**

Figure 5.22 demonstrates that the sensitivity of this set-up is sufficiently high for individual near-field ablation events to be detected. The sample chosen for this experiment was Bis(p-phenyl-triazeno-N,N-diethyl)-ether (See inset). For this experiment, the QMS was set at m/z = 28, the mass of nitrogen, which is produced upon laser photo-decomposition of the triazene dimer. To reduce the background signal from nitrogen in the atmosphere and to reduce the burning of the filament through ambient oxygen, the sample was flooded with argon (m/z = 40). The desorption was performed by the SNOM probe depicted in figure 5.23. From this, the aperture size is estimated to be about 170nm wide in diameter, in good agreement with the measured crater diameters of approximately 200nm. The 60ps laser pulse was estimated to yield a local energy of approximately 2nJ below the SNOM tip at a wavelength of 355nm. The sample was held at a tilt angle of approximately 60°, allowing the capillary orifice to be positioned very close (< 5µm) to the SNOM
approach, first proposed by Jersch and Dickmann, permits the desorption of material with a resolution below 10nm, by focussing laser radiation in the near-field of a metallic probe (See also Chapter 6).\textsuperscript{73-76}

Nevertheless, even with rather simple instrumentation, the feasibility of molecular nano-sampling mass spectrometry was demonstrated. The experiments can be conducted in air with minimal to no sample preparation, providing topographical data, optionally in combination with spectroscopic information (Fluorescence, Raman, etc.) of the sample surface. This method is thus expected to become a useful tool in nano-analytical science and technology.
5.7 References


Chapter 6

Tip Enhanced Raman Spectroscopy (TERS)
6.1 Classification

Aperture SNOM

The prevailing method for nanoscale imaging and spectroscopy under ambient conditions is SNOM. The most delicate component in SNOM is the nanoscopic tip itself. Extensive research has advanced the optical and morphological properties of the probe, allowing for high resolution and high sensitivity surface analysis by optical spectroscopy under ambient conditions. Several fundamental and technological problems, however, hinder further improvement of the aperture probe, and therefore SNOM:

- SNOM probes have a low damage threshold that limits the achievable optical near-field intensities. This results in prolonged acquisition and, in general, makes non-linear excitation spectroscopy (e.g. two photon excitation fluorescence, CARS, etc.) difficult with SNOM.

- Decreasing aperture sizes lead to exceedingly small near-field intensities.

- The ultimate resolving power is limited fundamentally by the skin depth of the coating material.

- Current aperture probes are susceptible to artefacts due to a miss-registry of topographic and optical centre of the probe and the relatively large dimensions of the opaque screen forming the sub-wavelength aperture.

- Mass production of high-quality SNOM tips is difficult. The manual fabrication of probes is expensive, and further, lowers the reproducibility of the resulting probes.

- The required wave-guiding property of the probe restricts the applicable range of wavelengths in an experiment. The use of wavelengths from UV to IR is almost impossible with one single optical fibre due to the lack of suitable materials.

- The conservation of the state of polarisation is not always satisfactory and usually wavelength dependent.

- Probe tips are fragile and, consequently, require elaborate feedback control and vibrational damping.
• The absorption of light in the metal coating causes significant heating. This can present a problem when examining delicate specimens (e.g. biological applications).

The above limitations impede the investigation of many interesting systems. For biological systems, the possibility to image surface domains with near-molecular resolution would be a big step forward. In order to observe specific cell structures or even single proteins an, optical resolution of below 10 nm is desirable.1-4

Aperture-Less SNOM

Catalysed by the desire to overcome the technological limitations inherent to aperture SNOM, a new class of near-field optical imaging has emerged. In contrast to conventional SNOM, the light is not guided through a tapered fibre with a sub-wavelength sized aperture at its apex, but is focused onto a sharp metallic tip from outside. The tip locally interacts with the electromagnetic fields at the sample surface and the perturbed optical near-field is scattered in all directions and can be detected in the far-field. To distinguish between the incident radiation and the near-field scattered light, either modulation techniques or the illumination in total internal reflection was employed. Until recently, the response to the perturbation has been detected at the same frequency of the incident radiation only, consequently, complicating the deconvolution of the optical contrast from the strong topographic coupling.5-14 Further, as in pure intensity sensitive SNOM (Chapter 3.2) only limited chemical information on the sample surface can be retrieved in this way.

Focussing of Laser Radiation in the Near-field of a Tip

The tip can also be used as a local excitation source providing spectroscopic response.15-22 Corresponding to the SERS theory (Chapter 4.3) the incident electromagnetic field is strongly enhanced at a metallic particle. In short, the enhancement arises from a high surface charge density at the tip that is induced by the incident light polarised along the tip axis.9,23-26 The field enhancement (several thousand times compared to the incident field) can further be increased when exciting the conduction electrons within the metal protrusion resonantly. In this way a surface plasmon is created and increases the local field intensity by several orders of magnitude. Since the enhanced field mainly consists of non-propagating (evanescent)

1 The aperture-less probe acts as a Mie-Rayleigh particle, diffracting the evanescent waves lying on the sample surface.
components, it is highly localised at the tip end.\textsuperscript{15,16,23,27-29} The interaction of the enhanced field with the sample surface, therefore, is locally confined, allowing to assign any spectroscopic changes in the detected far-field signal to the region under the tip.

With such a system, it is difficult to perform fluorescence spectroscopy due to the immense background fluorescence originating from the illuminated area within the laser focus. Nevertheless, phase sensitive detection systems allow the small signal changes arising from the tip-sample interaction to be observed. Some groups have proposed to oscillate the tip perpendicular to the sample surface and to subtract the spectra with the tip close to the sample from the ones where the tip retracted from the surface.\textsuperscript{6,29,30} Less interference from background fluorescence exists for non-linear absorption spectroscopy techniques (e.g. two-photon excitation fluorescence)\textsuperscript{15} or for techniques, where illumination can be performed at non-absorbing frequency regimes of the sample (e.g. Raman spectroscopy)\textsuperscript{31,32}.

Raman spectroscopy and imaging are well suited for identifying the molecular composition of complex materials because they provide a wealth of chemical information, which can be obtained under ambient conditions. The feasibility of using a metal tip for local Raman enhancement to obtain chemical information on a nanometer scale has been studied experimentally. The spectroscopic results and their theoretical implications are discussed in the following section.

**Tip Enhanced Raman Spectroscopy**

The concept of "Tip Enhanced Raman Spectroscopy" (TERS) can alternatively be viewed as a modification of the frequently utilised Surface Enhanced Raman Spectroscopy (SERS, Figure 6.1).

In SERS, the sample must be deposited as a thin layer onto a rough noble metal film, an electrode, or colloids. Unfortunately, the SERS enhancement varies across the sample and depends critically on the substrate preparation. This severely limits its applicability and renders quantitative measurements almost impossible. To overcome these shortcomings, the sample has to be separated from the metal support. This offers two main advantages: First, no sample preparation is needed to allow the observation of surfaces in their natural conformation. Secondly, any sample surface, even of bulky specimens, can be investigated. In addition, the position of the separated SERS substrate is swapped with that of the sample, visualising the top-down approach utilised in TERS. This can be considered as if all SERS particles are blocked (or removed) leaving only one single particle exposed. Light illuminating the surface will therefore excite strong fields only at the exact location of the probe.
Chapter 6 - Tip Enhanced Raman Spectroscopy (TERS)

TERS

Scan single particle over substrate.

Detect light scattered from Ag particle.

Block all but one silver particles.

Inverse SERS

Separate sample and Ag substrate.

Figure 6.1. Tip Enhanced Raman Spectroscopy (TERS).

In the final step, the sample is moved relative to the particle while the tip is scanned over the sample using focused laser light from below or above the sample. The rough metal film used in SERS is replaced with a sharp metal tip. This approach of Surface Enhanced Raman Spectroscopy (SERS) as the top-down approach provides a localized and mobile surface-enhanced Raman scattering probe that shows identical enhancement at every sample location, allowing for quantitative SERS measurements. Furthermore, this approach provides excellent lateral resolution and topographic information is obtained simultaneously and can be directly correlated with the spectroscopic data. This approach provides a localized view of the sample from the side.

Tip Enhanced Raman Spectroscopy (TERS)
Figure 6.2. Electron micrographs of a pyramidal shaped contact (left) and a non-symmetric non-contact (right) AFM probe coated with ca. 10nm silver. The rough silver structure on the tip surfaces is not resolved in these images.

6.2 Instrumentation

TERS Tips Fabrication/Characterisation

The production of single particle plasmon probes for scanning probe microscopy is straightforward. A thin silver film (ca. 10nm) is evaporated onto commercial AFM tips. Evaporation conditions for the fabrication of highly active SERS substrates, like slow evaporation rates, high chamber pressures, etc. (Chapter 4) have been employed resulting in a rough silver film consisting of distinct (mostly separated) metal islands. For preliminary experiments, various contact and non-contact tips have been investigated (Figure 6.2). In principle, any of the investigated probes can be utilised for TERS, since the optical and topographical resolution mostly is a function of the subsequently applied metal particle. Note that the probability that a specific tip has a suitable particle at its apex is statistical. Therefore, blunt probes have a higher chance of having a particle at the tip end (rather than on

\[ Usually, 10-15\text{nm silver was deposited at a rate of }0.05\text{nm/s under an argon atmosphere of }10^{-\text{mbar}}. \]

The evaporation source-AFM tip separation was ca. 10cm.\]
the side of the apex). Figure 6.3 shows a typical TERS probe created by metallising a micro-fabricated AFM tip with silver. The grain at the tip apex is estimated to be about 50nm in diameter. The silver film consisting of separated silver islands can be seen more clearly in the zoom of the side of the tip taper. The sizes of these islands are even smaller than the one on the apex, most probably due to different conditions at the (heated) tip apex and the (tilted) tip side during evaporation.

Instead of metallising an AFM cantilever, the SERS active probes can also be made directly by electrochemical etching of metal wires. A thin wire is partially immersed into an appropriate electrolyte and a voltage is applied between the wire and the electrolyte via a counter electrode in form of a ring surrounding the metal wire. This method produces two tips: the wire section outside and inside the electrolyte, where the latter usually is thinned along its entire section and, therefore, is not used. Especially for gold tips, this method worked very

Figure 6.3. Silver coated AFM probe used for TERS. The silver grain at the tip apex has an estimated diameter of 50nm. The silver grains at the side of the tip (bottom right) are even smaller (ca. 20nm).
well. The gold tips used were generally very sharp, with a tip apex diameter below 20 nm as determined by SEM (Figure 6.4). Although such metal probes potentially showed much sharper tips, they were less robust as can be seen in figure 6.5. The feedback mechanism failed for this tip, resulting in a gradual impingement with the sample surface during scanning. The resulting characteristic coil was found for several tips examined by electron microscopy after the TERS experiments.

Another possibility for creating very sharp gold tips was investigated by pushing a (non-metallised) contact-mode AFM tip into a gold surface using a relatively high load force in the feedback mechanism. On retraction from the surface, a short and very thin gold strip is often pulled from the surface, sticking on the apex of the tip. Subsequent AFM imaging proved that the resulting imaging resolution of the initially blunt tips improved significantly (Figure 6.6). However, the gold wire at the tip apex is not firmly attached and is often lost during scanning. Modification of the AFM tip surface (e.g. by covalently attaching sulphur groups to the AFM tip end) prior to metallisation could help to improve the stability of the otherwise superior system.

**Figure 6.6.** AFM image of a gold surface acquired with sharpened contact tip. The initially blunt probe was not able to resolve the small particles (not shown). Sharpening was performed simply by indenting the tip into the surface (few nanometers). On retraction, a thin piece of gold is pulled from the surface forming the new, sharper tip apex. From topography resolution considerations, the attached gold feature is several tens of nanometers long and less than 10 nm wide.

**Optical Set-Up**

The optical set-up used for the TERS experiments was basically the same as the one used for Raman microscopy (Chapter 4.2). For technical reasons, the illumination of the tip was performed in a back-scattering configuration from below the sample (Figure 6.7). A high numerical aperture oil-immersion microscope objective was used for illumination and detection. For this reason it was assumed that all electromagnetic vector states are present to some extent at the laser focus allowing the creation of the required surface plasmon for field-enhancement. The control of
Figure 6.7. Detection and illumination pathways used for the TERS experiments. The laser light was focussed onto the tip through an oil-immersion microscope objective from below. Raman signals typically were only detected when the tip was in contact with the sample.
polarisation in the illumination and detection pathway, nevertheless, was shown to be important for obtaining high TERS signal intensities (Chapter 5.5). The Lumina instrument was used as a scanning platform. The tip-sample separation was controlled by contact AFM (metallised AFM tips) or by the shear-force feedback (tapered metal wires fixed on tuning forks). In all experiments, the 488nm line of the argon ion lasers was used. The collected light was filtered by holographic notch filters and optionally by a notch filter for the diode laser in case of the AFM feedback mode. The detection was performed by a cooled avalanche photodiode [SPL C4777 with APD S5343 from Hamamatsu, Schüpfen, Switzerland] for integrated reflectivity measurements, or by the Raman spectrometer for spectrally resolved measurements.

Due to the very large enhancement at the tip apex, no oscillation of the tip in combination with modulated amplification was necessary to detect the optical response of the tip interacting with the sample. Spectra with the tip retracted from the surface were used for background calibration (a). A significant increase in Raman intensity could be measured directly when approaching the tip to the sample surface (b).

**Laser Focus – Tip Alignment**

The alignment of the TERS tip with the laser focus is a difficult task and presents, probably, the greatest technical difficulty. Reasons for this are the extremely small dimensions of the laser focus (ca. 300nm) and the tip apex (below 50nm), which cannot be observed directly with an optical microscope.

A possible approach is to match the obtained topographic and integrated optical image (reflection). The relative tip position could be adjusted after each scan with micrometer screws. This method was not very reproducible, mainly due to the limited control over the relative tip position with the available coarse positioning system. Often more than twenty scans were necessary prior to the acquisition of the tip enhanced Raman spectra. During this procedure, the tip shape was sometimes changed,† probably when a silver particle broke off or the silver was weared by the excessive scanning.

A more practical way of aligning the tip with the laser focus was found by scanning the tip over the sample and laser focus in an initial step (Figure 6.8). If the integrated reflectivity is measured, such a scan yields an optical map of the AFM cantilever.

† Visible from small features in the topographic image, which represent a convolution of the scanning probe shape with the surface protrusion.
itself (Figure 6.9). The AFM cantilever and tip base can clearly be detected in the optical images. A zoom into the tip region sometimes allowed to exactly locate the apex (Figure 6.10a, b and d), but most often resulted in a complex reflection pattern of the irregular tip surface (Figure 6.10c). The piezo actuators in the tip head could be used to position the tip on the (virtual) tip apex of reflection image with very high precision. In a the following step, the sample is scanned relative to the pre-aligned tip and focus. Since only one high voltage stage was initially available, this procedure could not be fully tested. In principle, however, this method of tip-focus alignment offers many advantages, like the determination of the place of maximum enhancement on the tip, by selectively changing the tip position on the reflection image. An electronic switchboard to change from tip to sample scanning mode without changing the tip position relative to the laser focus (i.e. without changing the voltages applied to the piezo actuators in the tip head) has already been designed, and will be employed in future investigations.

Figure 6.8. An image of the tip and cantilever can be acquired by raster scanning the probe over a homogeneous sample while keeping the laser focus fixed.

Figure 6.9. Optical map acquired by scanning a TERS tip over a homogeneous sample surface. The inset show the simultaneously acquired topograph ($\Delta z < 10\text{nm}$).
Figure 6.10. Selected optical maps of TERS probes. The tip apex cannot always be resolved, since the intensity of the measured reflectivity does not always correlate with the tip morphology.

5.3 TERS Investigations

TERS Spectra

A Raman signal increase of more than 30 times was obtained when the metallised AFM-tip was brought into contact with a thin layer of brilliant cresyl blue (BCB) (Figure 6.11b) compared to measurements with the tip retracted from the sample (Figure 6.11a). Since only a tiny fraction of the illuminated area provides this enhancement (see insets), we can estimate an enhancement factor of more than 2'000 in this case, based on an illuminated area of 300nm in diameter and a tip diameter of less than 50nm. In an independent control experiment, normal silicon nitride AFM probes were used rather than a metallised AFM tip. No signal enhancement was observed in this case. Figure 6.11c demonstrates the scanning capabilities of the set-up by cross-sectional Raman mapping of the boundary of the

\[ \text{Compare with chapter 2.7: "Optical Resolution (Diffraction Limited)"} \]
Figure 6.11. Tip enhanced Raman spectra of brilliant cresyl blue (BCB) dispersed on a glass support measured with a silver-coated AFM probe. The two Raman spectra in the upper part were measured with the tip retracted from the sample (a) and with the tip in contact with the sample (b) at the position marked #2 in the topography image (c). Contour plots of 25 Raman spectra measured across a sample boundary recorded at the positions marked in (c) with the tip retracted (d) and in contact (e) with the sample. (Acquisition time: 60s per spectrum).
BCB film to correlate the chemical composition with the topographic image. As can be clearly seen in the contour plots in Figure 6.11d and 6.11e the BCB Raman signature at 1655 cm$^{-1}$ reappears at the lateral position corresponding to an island (lines 10–12), proving that it is also composed of BCB. The increase of signal intensity is only present if the SERS tip is in contact with the sample. The rise and fall of the BCB signal also indicates that no sample has been picked up by the tip. Note, that in 6.11d, even very small background signals (i.e. glass, immersion oil, etc.) lead to many contour lines, resulting in stripes in the contour plot.

A tapered gold wire mounted on a tuning fork was used for enhanced Raman spectroscopy on a C$_{60}$ thin film that was drop-coated onto a glass substrate (Figure 6.12). The C$_{60}$ Raman signal was easily detected in the presence of the gold tip, whereas virtually no signal was observed without the tip. From signal-to-noise considerations, the signal increase was estimated to be at least 40. Assuming a tip diameter of 20nm (Figure 6.4) and a laser spot diameter of 300nm, the tip-induced enhancement was estimated to be larger than 40,000 in this case. Artefacts from

![Figure 6.12. Raman spectra of C$_{60}$ measured with a tuning fork set-up. Trace (a), gold tip in contact with the sample, trace (b), tip retracted. Circles mark C$_{60}$ normal modes, stars mark C$_{60}$ modes due to charge transfer with the gold tip. The crosses mark background Raman bands (i.e. immersion oil). (Acquisition time: 200s per spectrum)](image)

The rather conservative estimation is based on the assumption, that the Raman signal intensity for the case where the tip is retracted from the surface is just as big as the noise level and therefore cannot be detected in the spectra. The real enhancement, therefore, can be significantly higher than 40'000 in this case.
adsorbed $\text{C}_6\text{O}_6$ molecules on the tip itself were ruled out by repeatedly collecting data on a blank part of the substrate, where no Raman signal was detected.

**TERS Mechanism**

The origin of the SERS effect, although still controversial, may be explained by at least two mechanisms: electromagnetic and chemical effects (Chapter 4.3). The electromagnetic effect is either due to the increase of the electrostatic field in the vicinity of large curvatures or edges (lightning rod effect) or due to the excitation of surface plasmon-polaritons by the incoming electromagnetic waves. These electrodynamic resonances are coupled modes of electromagnetic waves and collective electron density fluctuations that are bound to the surface. Both effects significantly increase the surface electromagnetic field compared to that of the incoming radiation field. The chemical effect can result from an increased polarisability of the adsorbate due to charge-transfer or bond formation with the metal.

The main peaks in figure 6.12 (marked with stars) correspond to bands known from SERS spectra of $\text{C}_6\text{O}_6$ on gold. The enhancement and the line shift compared to bulk measurements can be attributed to a chemical effect caused by a charge-transfer interaction between the sample and the gold. In our case, the $\text{C}_6\text{O}_6$ molecules are assumed to chemically interact with the tip in a similar way. Interestingly, the two peaks near 1440 cm$^{-1}$ have shoulders shifted to the blue (marked with circles in figure 6.12). These shoulders coincide with bands known from bulk $\text{C}_6\text{O}_6$ Raman spectra. They can be assigned to purely electromagnetically enhanced signals of underlying or neighbouring molecules, further away from the probe. The coexistence of both shifted and unaltered bands indicates that both electromagnetic and chemical effects are present in the tip-enhanced Raman spectra of $\text{C}_6\text{O}_6$. Since the electromagnetic and chemical effects have different interaction ranges, appropriate experiments (e.g. by introducing spacer molecules) will allow distinguishing between the two classes of enhancement.

### 6.4 Resolving Power

An additional and important advantage of the SERS-active probe is its small size, usually below 50nm in diameter. The enhancement, therefore, stems from an interaction area that is well below the diffraction limit of the incident laser light. The lateral optical resolution of this method is consequently determined by the size of the probe in the same way as the topographic resolution. As demonstrated in
Figure 6.13. Ultraflat homogeneous BCB sample measured in tip-scan mode: In contrast to figure 6.11, the sample spot remains in the laser focus, while the tip is scanned (see schematic in inset of (d)). (a) Reflectivity image. The fringes are due to an optical (continued on next page) interference between the cantilever and sample surface (the
Figure 6.14. Three dimensional representation of the Raman map in figure 3.13 (inset). The localised origin of the Raman scattering is assumed to have a Gaussian-like shape.

Figure 6.14c-e, the correlation of sample morphology and optical data is therefore straightforward. In addition, combination of this technique with the multitude of different scanning probe microscopies to obtain detailed and specific information on the sample is also possible.

To confirm the lateral resolution, the tip-scanning mode of the instrument can be employed. In such an experiment, the metallised AFM tip was scanned while leaving the laser focus and the sample, a homogeneous thin layer of BCB, aligned (see cantilever is not parallel to the sample surface). (b) Zoomed reflectivity image of the tip region. Raman spectra were measured along the line in figure (b) at the marked positions; the intensity of the main Raman band between 1630 and 1680 cm\(^{-1}\) is displayed colour coded in (c). Note that the tip locations in (b) are not equiedistant. For a linear representation, see figure 6.14. (d) Two selected overview Raman spectra measured with the tip inside (position #15) and outside (position #8) of the laser focus. (Acquisition time: 60s per spectrum).
Because no sample inhomogeneities were detectable by AFM measurements, the observed signal variations can only be caused by the SERS tip. As has been noted earlier, the measured integrated reflectivity yields an optical map of the AFM cantilever itself (Figure 6.13a). For the Raman experiments, only the tip apex (Figure 6.13b) is relevant. The Raman active region of the tip can be measured by deconvoluting the laser spot from the observed spot size using a Raman line scan (Figure 6.13c and Figure 6.14). Note that the relative Raman intensity does not correlate with the reflectivity in the integrated optical image. The selected Raman spectra shown in Figure 6.13d represent data collected at two positions of the tip inside (15) and outside (8) of the laser spot. The full width at half maximum of the intensity distributions for the Raman scattered light was measured to be 1.2 μm. Note, that the observable Raman scattering stems from the convoluted area of laser focus on the sample surface (ca. 300 nm) and the dimensions of the TERS tip, therefore, the actual confinement of the field enhancement is much smaller. A three dimensional representation of the Raman signal are depicted in Figure 6.14. By reducing the tip size or when making use of short-range chemical effects, a resolution of only few nanometers can be expected.

6.5 Polarisation Dependence

Theoretical models show that the conduction electrons (i.e. surface plasmon) in an asymmetrically shaped metal particle are efficiently excited only by the incident laser radiation having the appropriate states of polarisation. In theory, the enhancement for ideal, conical tips is only induced for incident light polarised along the tip axis, whereas no field enhancement is expected for incident light with polarisation perpendicular to the tip axis. Obviously, the hand-made TERS tips all exhibit an asymmetry of some kind. Therefore, the resulting Raman enhancement is expected to be a function of state of polarisation of the incident laser radiation.

Figure 6.15 shows two TERS spectra acquired from a thin BCB layer on glass with a metallised AFM tip. The only difference between the two spectra is the polarisation orientation in the sample plane: The spectra were produced with linearly polarised light, incident at 0° and 90° respectively. The detection was in both cases performed normal to the incident polarisation (see insets). To compensate for the different collection and detection sensitivity of the instrumental set-up for different polarisation directions, the spectra were normalised using the sample independent glass bands (<550 cm⁻¹) in the Raman spectra. Therefore, assuming a statistical
distribution of the surface molecules, the resulting differences in the signal intensities are a direct function of the particular field intensity at the metal tip.

To keep the illumination area small, the illumination with an oil-immersion objective from below is preferred over an illumination from the side of the tip. To still induce high field intensities at the tip apex, an illumination with an appropriate laser mode is necessary. Calculations by Novotny et al. reveal that a Hermite-Gaussian (1,0) mode should be used for excitation, since suitable electromagnetic vectors present in the incident radiation allow the formation of high surface charge densities at the tip apex. Besides modifying the laser cavity, the proposed Hermite-Gauss (1,0) can be produced by scrambling the laser mode with the use of an optical fibre and subsequent spatial filtering or by bisectioning a fundamental Gaussian beam at the edge of a $180^\circ$ phase plate.

The reasons why a significant enhancement still is found for most TERS tips, even when illuminating with a "improper" state of polarisation are the following: First, due to the high numerical aperture of the microscope objective used (N.A. 1.4), a great part of the incident radiation is illuminating the sample with a non-perpendicular angle. Some of the electromagnetic waves present, therefore, always have an appropriate orientation for field enhancement. Secondly, the theoretical considerations hold only for perfectly symmetric metal tips. In practice, rather complex metal features account for the immense field enhancement. Olivier Martin
Figure 6.16. Reflectivity images of two selected TERS tips (a and b) acquired with linearised light in 0° and 90°, respectively. To date, the origin of the apparent change in the reflectivity pattern cannot fully be explained.

and coworkers have found significant variances for non-regular particles in theory. For example, triangular particles exhibit a very complex behaviour at optical wavelengths, with multiple resonances. Thirdly, for technical reasons, it is extremely difficult to approach the metal tip perfectly perpendicular with respect to the focal plane. The naturally induced asymmetry yields in an off-axis illumination of the tip, allowing for high near-field intensities even with a zero order laser mode illumination.

The dependence of the illumination mode and polarisation on the resulting field intensity and distribution can also be seen in the integrated optical images (Figure
Excitation at $0^\circ$  
(Detection $90^\circ$)  

Figure 6.17. Tip scan over the laser focus with illumination with $0^\circ$ and $90^\circ$ polarisation. Note that the maximum enhancement is only slightly reduced in the latter case. However, the location of maximum enhancement shifted slightly on changing the plane of polarisation of the incident radiation.

6.16). Besides the apparent differences in the patterns as seen from a change in the intensity distribution, the absolute response also changes. Note that the optical images in figure 6.16 have not been normalised, and therefore, do not account for differences in the collection and detection sensitivity on different polarisation states. Why a change in polarisation should have this great an effect also on the reflectivity image is not clear.

The line section discussed in figure 6.13 has been scanned a second time with an incident polarisation turned by $90^\circ$ compared to the initial scan (Figure 6.17). The polariser in the detection pathway has been rotated accordingly for cross-polarised detection. In this case, the maximum Raman enhancement is only slightly reduced compared to the illumination at $0^\circ$. Surprisingly though, a shift in the location of maximum enhancement is apparent. This can be only partially explained by a displacement of the focal point resulting from changes (rotation of polariser, waveplates, etc.) in the illumination pathway. More likely, the intensity distribution of the induced near-field at the tip changed with the modification of the incident state of polarisation. Further evidence for this can be found from the transformed contour of the measured field. Although less absolute enhancement is found for illumination at $90^\circ$, the resolving power would probably be even higher than estimated for the illumination at $0^\circ$. 

excitation at $0^\circ$ and $90^\circ$
6.6 TERS Imaging

There have been several reports on Raman imaging at a lateral resolution around 100nm using aperture near-field optical tips.\textsuperscript{40-48} However, there are a number of limitations with the aperture SNOM approach, such as the low transmission of SNOM tips, often combined with a low damage threshold (~5-10mW). For so-

![Integrated Optical Images](image1)

*Figure 6.18. Forward (centre left) and backward (centre right) scan TERS images acquired by raster-scanning the TERS probe over a homogeneous ($\Delta z = 10$nm) sample surface (top right). The region of interest was found by zooming into the tip region of the reflectivity images (top panel). In a subsequent scan, Raman spectra like the ones depicted in the bottom panel were acquired by integrating the TERS signal over periods of four seconds during scanning. The resulting pixel resolution is circa 142nm. The intensity of the Raman band at 1660cm\textsuperscript{-1} was used for colour coding. Yellow denotes high Raman intensity, black stands for spectra with almost no Raman bands of BCB (see bottom panel). Raman scattering was observed only when the TERS probe was in the laser focus, nicely mapping the intensity distribution of the incident electromagnetic field.*
TERS. As in previous experiments, the tip has been raster scanned over the fixed sample and laser focus. The top panels show the resulting reflectivity images with different zoom factors. The place where the tip apex is expected has been raster scanned a second time with a very slow scan speed of 35nm/s (ca. 0.007Hz). The Raman signal was thereby accumulated over short periods of 4 seconds yielding 35 spectra (pixels) per scan line. For the representation of the Raman intensity distribution over the entire scan range, the normalised integrated area of a Lorenz-fit of the 1660cm\(^{-1}\) band was chosen as a measure (See spectra in the bottom panel): Bright, yellow pixels stand for intense Raman spectra, whereas dark pixels represent spectra with no or only very little Raman signal. Obviously, the Raman enhancement does not correlate with topographic features of the very flat sample surface (\(\Delta z < 20\text{nm}\), see top right). No correlation between the reflection and the TERS image is observable, either. The TERS images acquired in the forward and backward scan direction correspond to each other nicely within the experimental error. The variation of the different spectra can only originate from the change of the relative TERS tip position. The distinct elevation in the three dimensional representation of the forward scan image (Figure 6.19), therefore, can be attributed to the intensity profile of the laser focus incident at the sample surface. This preliminary experiment shows the potential of sensitive Raman imaging with ultra-high lateral resolution (pixel dimension in this case is 142nm). In future experiments, this type of investigation can, for instance, be employed to find the optimal alignment between tip and laser focus before imaging the specimen surface with the sample scanning mode.
The surface enhancement in TERS imaging always originates from the same metal particle at the tip apex, and hence, is identical at all sample positions. Quantitative SERS measurements can therefore be performed. The laborious substrate preparation becomes unnecessary and imaging even of fragile bio-samples becomes possible in their natural form. This has possible implications for surface analysis, since, for the first time, Raman spectroscopy can be performed with sub-wavelength resolution without the use of especially prepared substrates, hence, providing detailed vibrational information on “native” surface molecules.

### 6.7 Outlook

An interesting perspective for tip-enhanced Raman scattering might be to study the origin of the SERS effect. Because only a single particle is used for the enhancement, the laser wavelength can be tuned to exactly match the corresponding plasmon frequency. Further, there have been efforts to separate the electromagnetic and chemical effects in SERS using specially prepared SERS substrates. With the method described here, it is possible to measure the Raman enhancement as a function of tip-to-sample distance ranging from a few angstroms to several nanometers directly. Hence, the tip-enhanced Raman scattering mechanism can be varied between long range (electromagnetic) and short range (chemical) enhancement regimes under otherwise identical experimental conditions. Further the controlled variation of incident wavelength, tip material, tip shape, laser mode and polarisation will give further evidence on the exact enhancement mechanism involved in SERS and the surface plasmon theory in general.

The demonstrated aperture-less SNOM method combines high-resolution scanning probe microscopy with surface enhanced Raman spectroscopy. This alliance provides a method for “nanoscopy” without the drawbacks of the individual techniques. Figure 6.20 shows plasmid DNA strands on activated mica imaged with high resolution AFM in air and buffer solution, respectively. Their topography has been imaged with a lateral resolution below 5nm using ultra sharp tips [Digital Instruments, Inc.]. The images acquired in air clearly show the nucleation centres of DNA on the modified mica as bright spots. The distance between two successive turns of a helical doubly stranded DNA is 3.4nm and the width of the chain is

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1 Freshly cleaved mica was treated with aminopropyltriethoxysilane (APTES), which renders the surface positively charged and thus is capable of binding to a variety of bio-molecules, such as DNA. APTES is commercially available from BioForce Laboratory, Inc.
2.0nm wide (Figure 6.20). A full turn of the helix accounts for approximately 10 base pairs. Assuming the confinement of the field-enhancement to scale with the tip dimension, or making use of the short-range chemical enhancement, TERS imaging with the above mentioned AFM set-up would, in principle, allow for sequencing a single DNA-strand by deconvoluting spectra of adjacent locations of succeeding base pairs. Moreover, with highly evolved scanning tunneling microscopy instruments, surface imaging with near-Angstrom resolution is possible. This shows the future potential for direct DNA sequencing of single strands using advanced TERS. Similarly, high-resolution chemical imaging without the compulsion of elaborate sample preparation will find use in many other research fields, like the semiconductor industry, cell-biology, or catalysis.

Figure 6.20. AFM images of plasmid DNA on activated mica. Single DNA strands can be imaged with very high resolution in both air and buffer solution. Imaging was performed at the BioZentrum in Basel on a MultiMode Nanoscope [Digital Instruments] with ultra sharp AFM probes in tapping (non-contact) mode. The right panel sketches the model of DNA and its characteristic dimensions.
6.8 References


APPENDIX
A. Important Facts in Photonics

A lens system can be defined via the following parameters:

- \( F \) Focal point
- \( f \) Focal length
- \( \phi \) (Effective) lens diameter
- \( n_D \) Refractive index
- \( \Theta \) = \( \tan(\phi / 2f) \) (Object angle)
- \( f_{\text{number}} = f/# = f / \phi \)
- \( \text{N.A.} = n_D \cdot \sin(\Theta) \) (Numerical Aperture)
  - \( = n_D \cdot [\phi / 2f] \)
  - \( = n_D / 2f_{\text{number}} \)

For Gaussian beams, the beam dimensions in the focal point are approximately:

- \( \Delta x \approx 0.61 \cdot \lambda / \text{N.A.} \) (Airy disk diameter; Reyleigh criterion)
- \( \approx 0.5 \cdot \lambda / \text{N.A.} \) (Airy disk diameter; Abbé barrier)
- \( \Delta z \approx (8 \cdot \lambda / \pi) \cdot (f/#)^3 \) (Depth of focus)
  - \( \approx \lambda / 2 \cdot \text{N.A.}^2 \)

The optimal diameter of an aperture for spatial filtering can be calculated from:

- \( D_{\text{opt}} = 2 \cdot \lambda \cdot (f/#) \)

Rules of Thumb

- The minimum linewidth of feature size that can be viewed with the unaided human eye is roughly 75 to 125\( \mu \)m.
- The visual acuity limit is about one minute of arc, or two minutes for comfortable long-duration viewing.
- The naked eye can detect a lack of flatness with a radius of curvature up to about 10’000x the length of the surface being viewed.
B. Metal Coating Thickness

The thickness of the metal-layers that have to be applied around the sides of the probe to confine the light to the sub-wavelength sized the aperture can be estimated from the geometrical parameters of the evaporation and the absolute rate of metal deposition (E) measured on a plate parallel to the evaporation source. Assuming the frequency of rotation to be bigger than the total length of evaporation (t), the resulting coating thickness can be calculated from:

\[ D = E \cdot t \cdot \frac{1}{\pi} \int_{0}^{\pi/2} \sin[\alpha] \cdot \cos[\theta] \cdot \arctan \left( \frac{r \cdot \sin[\alpha]}{l + r \cdot \cos[\alpha]} \right) d\alpha \]

\[ 2\tau = \text{fibre thickness} \]

\[ l = \text{length of the taper region} \]

A general solution of the above integral has not been found. Alternatively, one can use the projection of a still tip to the orthogonal plane of the evaporation direction:

\[ A = \frac{r^2 \cdot \cos[\theta]}{\tan[\frac{\alpha}{2}]} \]

(Approximation good for small \( \alpha \) and \( \Theta \))

The resulting thickness on this projection after metallisation has then to be evenly distributed over the whole cone area. For a rotating tip, the coating thickness, therefore, can be estimated from:

\[ D = \frac{E \cdot t}{\pi} \cdot \cos[\theta] \cdot \cos[\frac{\alpha}{2}] \]

(See graph below for a figurative description)
C. Tip Holder/Etching Assembly
D. Curriculum Vitae

Personal Details

• Date of Birth: January 10, 1974
• Place of Birth: Zürich, Switzerland
• Citizen of: Rotmonten SG
• Nationality: Swiss
• Languages: Swiss German (mother tongue), English (fluent), German (fluent), French (basics)
• Contact: raoul@zhol.ch

Education

1997 - 2000 PhD at the Swiss Federal Institute of Technology Zürich, Dept. IV [Chemistry] (ZH)
1993 - 1995 Swiss Federal Institute of Technology Zürich, Dept. IV
1980 - 1987 Primary Schools in Switzerland (Benglen (ZH), Zumikon (ZH), Schwellbrunn (AR), Zollikerberg (ZH)), Nepal (Lincoln School, Kathmandu), and Indonesia (Bandung Int. School)

Qualifications

• M Sc. in Chemistry (University of Kent at Canterbury, U.K.), Distinction
• 2. Vordiplom in Chemistry (ETHZ, Dept. IV, CH)
• 1. Vordiplom in Chemistry (ETHZ, Dept. IV, CH)
• Matura Typus C (MNG - ZH, CH)