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Tip-enhanced Raman spectroscopy – from early developments to recent advances

Tanja Deckert-Gaudig, D^a Atsushi Taguchi, D^b Satoshi Kawata^b and Volker Deckert *^{ac}

An analytical technique operating at the nanoscale must be flexible regarding variable experimental conditions while ideally also being highly specific, extremely sensitive, and spatially confined. In this respect, tip-enhanced Raman scattering (TERS) has been demonstrated to be ideally suited to, e.g., elucidating chemical reaction mechanisms, determining the distribution of components and identifying and localizing specific molecular structures at the nanometre scale. TERS combines the specificity of Raman spectroscopy with the high spatial resolution of scanning probe microscopies by utilizing plasmonic nanostructures to confine the incident electromagnetic field and increase it by many orders of magnitude. Consequently, molecular structure information in the optical near field that is inaccessible to other optical microscopy methods can be obtained. In this general review, the development of this still-young technique, from early experiments to recent achievements concerning inorganic, organic, and biological materials, is addressed. Accordingly, the technical developments necessary for stable and reliable AFM- and STM-based TERS experiments, together with the specific properties of the instruments under different conditions, are reviewed. The review also highlights selected experiments illustrating the capabilities of this emerging technique, the number of users of which has steadily increased since its inception in 2000. Finally, an assessment of the frontiers and new concepts of TERS, which aim towards rendering it a general and widely applicable technique that combines the highest possible lateral resolution and extreme sensitivity, is provided.

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^a Leibniz Institute of Photonic Technology, Jena, Germany

^b Department of Applied Physics, Osaka University, 2-1 Yamadaoka, Suita, Osaka, Japan

^c Physical Chemistry, University of Jena, Helmholtzweg 4, Jena, Germany. E-mail: volker.deckert@uni-jena.de



Tanja Deckert-Gaudig

Tanja Deckert-Gaudig studied chemistry at the University of Würzburg and received her PhD in organic chemistry in the group of Prof. S. Hünig in 1997. After her parental leave she switched to physical chemistry in 2002. Since then she has been working on Raman spectroscopy and scanning probe microscopy in the group of Prof. V. Deckert. After working at the Leibniz Institute of Analytical Sciences (ISAS) in Dortmund she joined the Leibniz Institute of

Photonic Technology (IPHT) in 2009. In 2011 she and her colleagues were awarded with the Thüringer Forschungspreis for their achievements in the field of Nanoscopy. Deckert-Gaudig's main focus is the structural and chemical analysis of biopolymers in particular amyloid fibrils using tip-enhanced Raman scattering (TERS).



Atsushi Taguchi

Atsushi Taguchi is an Assistant Professor of Applied Physics at Osaka University. He received his PhD in Mechanical Engineering in 2004 from Osaka University. In 2014, he joined the Applied Physics Department at Osaka University after postdoctoral positions at Osaka University and RIKEN where he worked for Professor Satoshi Kawata, and assistant professor at Tokyo University of Agriculture and Technology. His main research

interests include tip-enhanced Raman scattering (TERS) microscopy and he is a pioneer of TERS in deep-UV.

1. Introduction

In many scientific fields, miniaturization proceeds and thus necessitates analytical techniques operating on the same scale. Generally, in conventional Raman spectroscopy averaged information on the chemical composition of bulk samples is collected; however, structural and chemical heterogeneities on the molecular level - at least, those below the Abbe diffraction limit - remain elusive. Because of its specificity and extensive applications, Raman spectroscopy has many advantages over techniques that require sample labelling or elaborate sample preparation. Nevertheless, two obstacles need to be considered before Raman spectroscopy can be used for high-resolution applications: low sensitivity and diffraction-limited spatial resolution. The first drawback can be overcome using surfaceenhanced Raman scattering (SERS), which was discovered in the 1970s.¹⁻³ SERS increases the sensitivity of conventional Raman spectroscopy by many orders of magnitude while maintaining its specificity. The basic principle of SERS is a strongly enhanced electromagnetic field generated when rough noble metal surfaces or nanoparticles are irradiated with an appropriate wavelength of light. Notably, the excited/generated oscillations of the delocalized electrons at the metal surface, *i.e.*, surface plasmons, can be excited optimally only when the laser wavelength matches the plasmon resonance of the TERS tip.⁴⁻⁶ Although a single nanoparticle at the tip apex is responsible for the local signal enhancement, the plasmon resonance arises from the plasmon coupling of all adjacent nanoparticles illuminated. This effect in turn depends on the material, size, and geometry of the nanoparticles and can be tailored to the experimental conditions.^{7,8} The highly efficient electron-photon coupling leads to an enormous enhancement of the sensitivity of Raman spectroscopy, enabling the detection of even single molecules near the nanoparticles. For more details, see the respective review articles in this volume and, for instance, ref. 9 and 10 and the literature cited therein.

The remaining limitation of SERS - low spatial resolution can be overcome using tip-enhanced Raman scattering (TERS), in which Raman spectroscopy is combined with atomic force microscopy (AFM)¹¹ or scanning tunnelling microscopy (STM).¹² In a standard SERS experiment, all molecules within the laser focus are probed because generally many enhancing sites are present. By contrast, incorporating a single plasmonic particle using a scanning probe microscope into a Raman instrument reduces the area of enhanced molecules to a few nanometres. Hence, the key feature of TERS is a single plasmonically active scanning probe tip that serves simultaneously as a Raman signal enhancing unit and a topography scanner. The aforementioned matching of the laser wavelength and tip surface plasmon resonance certainly influences the selection of the tip material for TERS. The TERS results published thus far specify that in general, gold tips are used for excitations from 633 to 785 nm, whereas silver tips are used for experiments performed between 488 and 568 nm.

The first TERS experiments were performed by Stöckle *et al.* in 2000 using a back-reflection AFM-TERS setup and were promptly followed by reports from several other groups.^{13–15} Although all those studies were performed on transparent samples, opaque samples have since become accessible as well. As shown below, several illumination and detection geometries for AFM-TERS have been realized so far, and each has their own specific advantages.

In a second type of TERS setup, the Raman microscope is combined with an STM.⁴ Here, an etched gold or silver wire is



Satoshi Kawata

Satoshi Kawata received his PhD in Applied Physics in 1979 from Osaka University. He spent two years as a postdoctoral fellow at UC Irvine, and joined Osaka University as a faculty member in 1981. He was promoted to a full Professor in 1993, and then a Distinguished Professor in 2013. He has been also a Chief Scientist at RIKEN from 2002 to 2012, and a Team Leader till 2015. In 2007, he founded Photonics Advanced Research Center in Osaka Univer-

sity. Professor Kawata is now Professor Emeritus of Osaka University and Honorary Scientist of RIKEN. He served as the President of Japan Society of Applied Physics, President of Spectroscopical Society of Japan, and the Editor of Optics Communications. Professor Kawata is one of the pioneers of tip-enhanced Raman scattering microscopy and 3D two-photon nano-fabrication. He is a Fellow of OSA, SPIE, IOP, and JSAP.



Dr Volker Deckert is appointed as a Full-Professor for Physical Chemistry at the Friedrich-Schiller University in Jena and as a department head at the Leibniz Institute of Photonic Technology also in Jena. He obtained his Diploma and his PhD from the University of Würzburg (Germany), working in the field of Raman spectroscopy. He was a postdoc at the University of Tokyo and KAST, in Kawasaki, where he worked on non-linear and time-resolved laser

Volker Deckert

spectroscopy. During his habilitation at the ETH Zurich, he started working on near-field optical spectroscopy, a subject he pursued at his previous positions in Dresden and Dortmund and also at his current position in Jena.

used for topography scanning and simultaneous spectral acquisition. Ideally, in both AFM- and STM-TERS configurations, a highly intense evanescent field is generated at the apex by an individual single nanoparticle that interacts with the sample molecules. The tip-sample distance must be controlled accurately via a feedback mechanism not only for sensitive surface scanning but also for optimal and stable signal enhancement. Importantly, spectra are collected only from molecules directly localized beneath the tip apex. Consequently, the Raman signal enhancing field is confined to a few nanometres, and optimally, a spatial resolution in the (sub)nanometre region - independent from ambient conditions - can be reached in STM- and AFM-TERS.¹⁶⁻²¹ In fact, electromagnetic theory based on general particle sizes cannot explain this extraordinary resolution. Various theoretical approaches based on assumptions of the plasmonic particle dimensions are currently being pursued to explain experimental results, including quantum mechanical models describing the local dependence of tip-sample interactions²² and molecular cavity optomechanics incorporating nonlinear plasmonic contributions/response,²³ other nonlinear Raman effects,²⁴ atomistic features protruding from the apex,^{25,26} and the electric field gradient effect.²⁷

As already mentioned, TERS obtains detailed chemical information (*e.g.*, molecular structure and orientation, defects, purity, *etc.*) *via* the excitation of vibrational modes in a sample. Incorporating fluorescence spectroscopy,²⁸ coherent anti-Stokes Raman scattering,²⁹ photoluminescence^{30,31} or pulsed excitation³² enables complementary information (*e.g.*, electronic state) to be acquired from the probed sample. Because AFM and STM systems are not confined to merely detecting topography, modifying the imaging setup for TERS by combining it with for instance cyclic voltammetry³³⁻³⁶ enables simultaneous measurements of conductivity, potential, *etc.* Hence, the combined TERS/probe imaging concept allows thorough investigations of reaction mechanisms, molecular orientation, *etc.*

TERS is becoming increasingly popular for nanoscale structural analysis and is used to study samples in areas ranging from organic monolayers to 2D materials, catalysed chemical reactions, and biological applications. Concurrently, the underlying theory has been further developed to explain, *e.g.*, effects at the tip apex and the surprisingly high reported spatial resolution in AFM- and STM-TERS.

In Fig. 1, an overview of the different applications of TERS without explicitly including the different setups is shown. Importantly, the central tip can be either silver or gold coated.

Whereas only homemade setups were employed in the early stage of TERS, complete systems (including tips) have recently become commercially available, providing straightforward access to the technique.

In the following sections, instrumental and experimental developments will be covered, and selected results will be highlighted. The research goals and sample systems will be shown as becoming increasingly complex yet always targeting the detection of subtle molecular structures that would remain unattainable using conventional Raman measurements on a bulk sample.



Fig. 1 Overview of the different applications of TERS.

2. Technical development of different setups

2.a. AFM- and STM-TERS

Long before the first experimental realization of TERS, Wessel proposed the principle of the technique while investigating molecules in close proximity to a laser illuminated metallic tip.³⁷ Fifteen years later, early AFM-TERS experiments were performed with dyes and carbon materials using an inverted Raman microscope equipped with an AFM on top.13,15,38,39 Back-reflection geometry (see Fig. 2a) provides a high collection efficiency, particularly when using oil immersion objectives with a high numerical aperture (N.A.). To date, this geometry is still the most efficient optical setup used for TERS. Using high N.A. objectives for the excitation of tip plasmons is advantageous because it creates a stronger longitudinal field along the tip axis.^{40,41} The enhanced and localized field at the tip excites the Raman scattering of molecules beneath the tip. Focusing through a high N.A. (1.4-1.6) also generates a small laser focus, which in turn minimizes the far-field background contribution from the confocal volume of the sample. The signal enhancement can be further increased by using radially polarized laser light instead of linearly polarized light.42,43

Despite being very efficient, bottom-illumination setups are limited to transparent samples and substrates such as glass slides or mica sheets. Although some of the high collection efficiency is sacrificed, slightly modifying the tip holder, namely, by incorporating a dichroic mirror, renders opaque samples accessible.⁴⁴ Nevertheless, side-illumination^{14,45–51} and topillumination^{52–58} optics are more commonly used to investigate opaque samples. In a side-illumination configuration (see Fig. 2b), the light source illuminates the sample at an angle (45–80°)



Fig. 2 Different TERS setup geometries: (a) bottom illumination for working in back-reflection setups, (b) side illumination, and (c) top illumination.

from above the sample. Hence, long working distance objectives with a smaller N.A. (0.28–0.7) are required at the expense of the overall collection efficiency. Because of the angle between the sample and excitation beam, the focused spot in a side-illumination geometry becomes elliptical and larger and thus requires a higher laser power for equivalent results. In a comparative simulation, linearly polarized light was concluded to be highly effective for side-illumination, whereas radially polarized light was preferable for axial illumination (see also Section 3.b).⁴³ Nevertheless, some of the drawbacks of sideillumination can be compensated for by working in a gap-mode configuration (see Section 3.c).

For top-illumination (see Fig. 2c), shadowing effects from the tip must be considered, and generally, special cantilevers with a protruding tip are used to minimize shadowing. Compared to a side-illumination configuration, a top-illumination geometry has a smaller far-field contribution and can be better integrated into standard microscope systems.^{53–55}

A third possibility for investigating opaque sample is using a parabolic mirror (N.A. ~ 1). In addition to its high N.A., this system is intrinsically free of chromatic aberrations.^{31,59–64} While this geometry has many advantages and is used in many infrared-tip-enhanced systems, it still is not as common as transmission optic systems in the visible region, probably because of it is more challenging to integrate into standard AFM setups.

Whichever optical setup is chosen, the most crucial aspect of TERS is the actual field-enhancing tip. For an AFM-TERS system, cantilever tips are coated with either metal nanoparticles or a continuous metal film (2–10 nm), typically silver or gold. Massive silver and gold wires glued to standard AFM chips have also been reported.^{65–68} For shear-force and STMbased feedback systems, mostly thin Ag or Au wires are used. These aspects will be discussed in detail in the tip-related section below.

For AFM-TERS, in general, commercially available silicon or silicon nitride AFM cantilevers are used and directly coated with silver or gold prior to the actual experiment. Silver or gold are thermally evaporated in vacuum^{13,15,69–73} or sputtered.^{46,52,56,74} The evaporation process generally produces tips covered with silver island films,⁷⁵ whereas sputtering yields smoother films. After the sputtering or evaporation process, further sharpening of the tip with a focused-ion beam (FIB) is possible.⁷⁴ Currently, several companies offer ready-to-use TERS cantilever tips with different coatings and geometries, and these may be an alternative to homemade tips.

In AFM-TERS, the tip-sample distance is force-controlled using a feedback-loop⁷⁶ mechanism and can be operated in contact mode^{47,56,70,77-82} or in dynamic mode (also known as intermittent contact mode, AC mode, or tapping mode). In general, the feedback signal can be detected through the deflection of a laser beam induced by the motion of a cantilever, which is proportional to the repulsive force applied to the tip.⁸³ Because avoiding interference of the Raman signal detection by the AFM laser is advantageous, infrared wavelengths above 800 nm are preferable. The majority of AFM-TERS setups utilize a dynamic AFM, in which the motion of the tip is temporally modulated by exploiting the mechanical resonance of the cantilever. The feedback signal is obtained by demodulating the sensor signal. Moreover, Si cantilevers and quartz tuning forks are the most popular transducers used. Because it is considered to be more "gentle" on the sample surface and tip, dynamic mode is preferred over contact mode and is well suited for analysing sensitive biomaterials. In dynamic mode, the tip oscillates vertically in the normal direction at close to its resonance frequency and at a minimum amplitude (<5–10 nm)^{56,84} to ensure that the tip constantly stays in the evanescent field. Notably, the intrinsic oscillatory motion of the tip in dynamic mode AFM can be used for studying the tip–sample distance dependence of the TERS signals.^{85,86}

As mentioned previously, AFM-TERS can also be operated in shear-force mode, in which an etched wire or a silver-coated optical fibre is mounted onto one prong of a tuning fork and oscillates laterally.^{6,30,39,50,87-94} The lateral motion of the tip leads to generation of a shear-force between the tip and sample. The oscillation amplitude of the tip is decreased under a constant excitation because of the damping effect as the tip approaches to the surface. This effect can be detected at a distance of up to 30 nm away from a sample surface, depending on the oscillation amplitude of the tip is held at a constant 2–5 nm distance from the sample *via* a feedback mechanism.^{6,30,92,96} With shear-force AFM, the tip-sample distance dependence of the TERS signals was found to decay within a length of 10 nm.^{91,97,98}

Generally, actual TERS experiments start by locating the tip in the laser focus, which can be accomplished by a tip scanner,^{69,76,87} a laser scanner^{54,56,57} or an objective scanner.⁹⁹ Once the tip has been positioned in the laser spot, only the sample is moved, leaving the optical alignment unchanged. Then, an overview topography image of the sample surface can be obtained by scanning the piezo-driven stage. Finally, the region of interest for TERS spectral acquisition is selected, and the spectra either are collected pixelwise during a topography scan for TERS imaging or are recorded at predefined points. Typically, a laser power in the µm-mW range is sufficient to acquire spectra at 0.1-30 s. To date, pixelwise data collection is recommended only for strong Raman scatterers or resonantly enhanced molecules because acquisition times must be kept short to exclude thermal drift issues when working under ambient conditions. TERS intensity maps show the distribution of the probed molecule by selecting marker bands from the complete spectrum. In the literature, TERS (AFM and STM) imaging has been reported for porphyrins,¹⁹ perylenes,¹⁰⁰ graphene,^{101–104} (single-walled) carbon nanotubes (CNTs),^{18,56,88,97,105–107} dyes,⁵⁴ polymers,¹⁰⁸ thiol monolayers,¹⁰⁹⁻¹¹¹ and dimercaptobenzene (DMAB).^{112,113} A pixelwise mapping of nanotapes formed of peptide fragments shows the distribution of the nanotapes on the substrates.¹¹⁴ However, as only one individual band of a single amino acid was detected, the intrinsic advantages of Raman band discrimination could not be used.115

STM-TERS was first introduced by Pettinger *et al.*^{4,116–118} and requires a conductive substrate, *e.g.*, indium tin oxide (ITO) or gold, which restricts the variety of samples severely. As mentioned

in the introduction, in such setups, etched gold or silver wires serve simultaneously as the probe and signal enhancer. In AFM-TERS, the feedback mechanism is force-controlled. By contrast, in STM-TERS, the bias current and voltage are the regulating parameters that ensure constant electron tunnelling and maintain the tip–sample distance *via* a feedback loop.^{109,118,119} Thus, to maintain the electron flow, the height of non-conductive samples is limited to a few nanometres. The optimal tip–sample distance is 1–2 nm^{4,97,109,118,120–123} because the TERS signal decays rapidly within 10 nm.^{5,85,97,121} Typically, the sharpness of the tip and better control of the tip–sample distance enables STM to provide a better drift control – particularly when operating in ultrahigh vacuum (UHV) and at cryogenic temperatures.

Using atomically flat gold or silver substrates for STM-TERS has the advantageous side effect of the "gap-mode", which further enhances the Raman signal.^{118,122-128} The gap-mode is not limited to STM and can also be exploited in AFM-TERS (see also Section 3.c).^{42,86,87,129-133} In this regard, homogenous gold films adapted from the established protocol for platinum surfaces¹³⁴ and modified procedures have been developed;^{4,110,117,118,124,128,134} however, 100 nm thick micrometresized gold crystals are also suitable.¹³⁵ Gold films attached to germanium¹³⁶ or micrometre-sized atomically flat silver or gold nanoplates with a height <20 nm are effective substrates for obtaining transparent gold surfaces.^{129,131,137} Notably, the surface flatness plays an important role: tiny kinks of only 2 nm can affect the signal enhancement perceptibly.¹³⁸⁻¹⁴⁰

In general, using smooth substrates for TERS experiments (*e.g.*, silver, gold, mica, HOPG) leads to a homogeneous molecule distribution with well-defined adsorption sites, 19,115,125,126,141 enabling, for example, detection of chemical reaction sites $^{131,142-144}$ or molecular orientation. 94,109,145

STM-TERS was further driven by the development of picosecond pulse excitation, which could facilitate dynamic processes with high lateral and high temporal resolution.³² All spectra recorded on a model compound, rhodamine 6G (Rh6G), showed signs of signal loss, which was attributed to the high pulse intensity and intense plasmonic enhancement in the gap between tip and substrate. Performing the measurements in UHV changed the results significantly.¹⁴⁶ Specifically, the signal decay decreased, and the observed fluctuations were comparable to those in normal UHV-TERS and were found to be sufficient to expand analysis in this field. Further aspects of UHV-TERS are discussed in the next section.

2.b. UHV-TERS

As already mentioned, STM-TERS can be conducted in a vacuum chamber at either room temperature (RT) or cryogenic temperatures.^{61,94,115,145,147–149} The advantage of the latter setup is obvious: the tip and sample are protected from environmental contamination, and the influence of a water layer between sample and tip is eliminated. As mentioned in the previous paragraph, TER spectra of Rh6G irradiated with a pulsed laser under ambient conditions showed a decaying signal, an observation that was attributed to the high pulse intensity and the

intense plasmonic enhancement in the gap between the tip and substrate.¹⁴⁶ Specifically, the signal decay decreased, and the observed fluctuations were comparable to those in continuously illuminated UHV-TERS and were found to be sufficient to expand analysis in this field.

In STM-TERS under UHV, the optical components can be positioned inside^{61,147} or outside^{115,148} the vacuum chamber. Both setups have their own characteristics, advantages and disadvantages. Locating the collection optics inside the chamber prevents the mirrors, filters and objective from being directly accessible. By contrast, mounting all optics outside the vacuum chamber provides more flexibility but requires a larger working distance objective with a low N.A. In a recently reported setup, only the objective was mounted inside the vacuum chamber, whereas all other parts remained outside.¹⁴⁹ Consequently, STM-TERS can gain new flexibility with respect to modifying beam polarization and wavelength. A further novelty of this specific setup is the inclusion of a third chamber for in situ molecular beam epitaxy sample preparation, which is intended to avoid contamination. However, this third chamber limits the choice of samples.

Working at cryogenic temperatures can minimize molecular mobility to such an extent that nanometre-sized domains, *e.g.*, of porphyrin derivative terraces, can be discerned in the topography, even when the distance is below 0.5 nm.¹⁷ Moreover, UHV can reduce photobleaching, as demonstrated with brilliant cresyl blue (BCB),¹⁵⁰ to improve the signal-to-noise ratio²⁸ and increase spatial resolution.¹¹⁵ Fig. 3a shows the results from a systematic study on Rh6G that compared STM-TERS measurements with SERS measurements. The data provide insight into the absolute number of measured molecules and the temperature dependence of the Raman bandwidth.¹⁴⁵ The SERS and TERS spectra of Rh6G recorded at RT (see Fig. 3a, spectrum a and b) and 19 K (LT) (Fig. 3a, spectrum c and d) were analysed by band fitting, and the obtained full width at half maximum (FWHM) values were compared.

The number of probed molecules beneath the tip apex is much smaller in TERS than in SERS, in which all molecules in the laser spot contribute to the signal. In the SERS experiments, Rh6G molecules were adsorbed onto rough nanoparticles, whereas for TERS, a flat silver surface was used. Thus, the observed adsorption geometry variations are smaller in TERS than in SERS resulting in the bandwidth narrowing from 20 cm^{-1} (SERS) to 17 cm^{-1} (TERS). When the working temperature was decreased to 19 K, the bandwidth further narrowed, and a value of 9 cm⁻¹ was estimated using Lorentzian band fitting analysis. At this low temperature, diffusion effects associated with varying molecule orientations were eliminated. The observed band position shifts $(\pm 20 \text{ cm}^{-1})$ in the LT-TERS experiments were associated with interactions of the sandwiched molecules in the tip-substrate gap. Similar observations were reported in a systematic temperature-dependent shear-force AFM-TERS study operated in a gap-mode configuration under vacuum on 1-2 malachite green molecules (see Fig. 3b).⁹⁴ The FWHM of selected bands in the TERS spectra were compared to those of the same bands in the conventional Raman spectra and were found to



Fig. 3 (a) Comparison of RT-SERS and STM-TERS measurements of Rh6G at RT and LT. (a) RT-SERS, (b) RT-TERS, (c) LT-SERS, (d) LT-TERS. The values above each band are FWHM values obtained by fitting with a Gaussian (SERS) or a Lorentzian (TERS) distribution using a least-squares approach. Spectra are background corrected and normalized by the maximum intensity in their respective composite fits. SERS spectra were recorded on silver over nanospheres substrates, and TERS spectra were recorded using STM-TERS. Reprinted with permission from ref. 145. Copyright 2014 American Chemical Society; (b) temperature-dependent spectra of malachite green recorded using AFM-TERS in a gap-mode configuration. Reprinted with permission from ref. 94. Copyright 2016 American Chemical Society.

be 2–8 cm⁻¹ narrower at 300 K and 6–10 cm⁻¹ narrower at 90 K. The authors ascribed these fluctuations to rotations and chemical changes around the few/single molecules. Consequently, working at cryogenic temperatures can give access to local dynamics with high sensitivity. At room temperature, the diffusion is too fast to distinguish single-molecule configurations and geometries, both of which remain inaccessible for many molecules. Instead, averaged information is obtained.

In a third independent approach, changes in the SERS and AFM-TERS (gap-mode configuration, RT) spectra of a thiophenol self-assembled monolayer (SAM) on a gold nanoplate were investigated *via* time-dependent experiments. Although vacuum and low temperature were not used, the experiments effectively support the aforementioned UHV LT-TERS experiments. Similar to those experiments, reduced FWHM values and larger band position alterations (6–9 cm⁻¹) compared to the those in the SERS spectra were observed.¹³²

STM measurements of a perylene diimide derivative absorbed onto a smooth silver crystal surface at ambient conditions in UHV indicated the presence of two phases formed by molecular diffusion.¹⁰⁰ The band intensity differences in the acquired TERS spectra indicated that in the condensed and the diffusion phases, the molecules had different geometries rather than different molecular structures. In TERS, the evanescent field is most effective along the main (*z*-)axis of the tip. Consequently, the in-plane modes of the planar perylene rings in the condensed phase are less enhanced than the out-of-plane modes of the perpendicular oriented rings in the diffusion phase.¹⁰⁰ The observation of suppressed in-plane modes was not only reported for STM-TERS but also for AFM-TERS conducted under ambient conditions on amino acids with an aromatic ring perpendicular to the tip main axis (see the gap-mode Section 3.c).¹³⁰

The above examples indicate the high potential of TERS operated at UHV and cryogenic temperatures for geometry studies on anchored molecules due to the elimination of thermal effects. Because of the high specificity of the technique, it is even possible to discern and localize subtle structural and orientation-related differences. Consequently, such a setup is well suited to investigate fundamental questions particularly regarding high-resolution aspects. For more specific information about UHV-TERS, we refer readers to a recently published review in ref. 151.

3. Intrinsic features of TERS

3.a. Enhancement factor and field decay at the tip apex

The fundamental signal enhancement mechanisms in SERS and TERS are similar and originate from electromagnetic and chemical interactions. Briefly, the electromagnetic mechanism enhances the signal intensity and dominates over the chemical mechanism, which can lead to alternating band positions. Because this issue also includes reviews on SERS and particularly on high-resolution TERS, the main concepts are only briefly addressed.

The highly localized signal enhancement at the tip apex is attributed to the large enhancement of the incident and



Fig. 4 Modelled distance dependence of the Raman enhancement factor and the FWHM of the TERS localization calculated for a 20 nm diameter silver sphere with a single atomic protrusion at the tip-sample gap. The marked points refer to an average enhancement or localization when the AFM is operating in dynamic mode with a tip oscillation amplitude of 10 nm. Adapted from ref. 25 published by the Royal Society of Chemistry.

scattered electric fields by coupling to the surface plasmons from the nanoparticles at the TERS tip. The excitation of localized surface plasmons is maximized when the incident laser wavelength matches the plasmon resonance wavelength.^{5,152} Finite-difference time-domain (FDTD) calculations showed that the dielectric environment, tip geometry, laser light polarization and angle strongly affect the efficiency of field enhancement. Additionally, the core material of coated tips was reported to affect the TERS signal enhancement (for details, see the tip preparation section).⁷⁰ Certainly, the nanoparticle shape, size and geometry similarly affect the enhancement factor.^{8,127} FDTD calculations, for instance, revealed highest values for nanoparticles with radii of 15–20 nm.

The highly localized field at the tip apex decays very rapid, as evidenced by Fig. 4. Consequently, only modes of molecules within a few nanometres can be efficiently excited and detected, as previously noted in 2003, when the total decay of the TERS signal was found to occur within 20 nm.⁵ In the following years, the range of enhancement was found to be even smaller (<8 nm),^{86,97,98,115,121} and 90–95% of the maximum signal enhancement decreased within 10 nm.¹³¹

In conclusion, the enhancement factor significantly depends on the tip material and, more importantly, on the tip-sample distance. Precise control of both parameters is essential for recording spectra with a stable and reproducible signal intensity. A third and similarly important factor is the polarization of the incident laser light and the tip-sample orientation, which will be surveyed in the next section.

3.b. Selection rules and polarization: why near-field Raman spectra differ from far-field Raman spectra

The highest field enhancement at the tip is generated when the external laser light is polarized along the tip axis (*e.g.*, p-polarization). Nevertheless, a certain electric field contribution perpendicular to the tip axis (s-polarization) always remains due to depolarization effects.^{97,153,154} Several approaches for optimally meeting this requirement have been developed. Generally, for a back scattering geometry, radially or linearly polarized light can be used for excitation. For radial polarization, the incident field is highest in the centre of the laser spot, whereas two lobes displaced from the centre show the highest field intensity for linear polarization.43,155 A detailed theoretical study by Maximiano et al. reported the development of a protocol describing the tip-enhanced Raman intensity as a function of the Raman polarizability tensor, excitation source polarization, tip-sample distance, and tip orientation. The highest electric field enhancement was found for vertically aligned tips in the field of a radially polarized laser beam.¹⁵⁵ These results were experimentally confirmed on an azobenzene thiol monolayer that was probed with radial and linear incident light polarization in a back-reflection geometry.¹³¹ The radially polarized excitation effected a 3 times greater signal intensity enhancement than linearly polarized excitation.

A different situation is the gap-mode configuration, which was simulated using an FDTD approach. Particularly, the localized electric field at the tip apex for radially and linearly polarized light in different illumination geometries was compared.⁴³ The calculations revealed an additional enhancement compared to standard TERS experiments because of the tightly confined area at the tip apex. Furthermore, the results from Maximiano *et al.* could be confirmed.

Further polarization-dependent measurements of the incident laser light have been performed for linear, radial, and azimuthal polarization in a back-reflection geometry using the parabolic mirror setup.⁶⁰ Here, the backscattered light from an etched gold wire tip was collected. For radial polarization, the z-component of the scattered light dominated, whereas for linear and azimuthal polarization, the x- and y-components showed highest field intensity. Thus, the gold tip contributed to depolarization because of its roughness, which was similarly observed in side-illumination experiments.51,154,156,157 The degree of depolarization by a gold-coated tip was an objective of a study in which the backscattered light was investigated as a function of the polarization angle with respect to a horizontal (sample) surface. Focusing p-polarized light directly on the apex decreased the scattered light intensity along the tip axis compared to the contribution from 5-15% s-polarized light (orthogonal to the incident beam). This result was attributed to forbidden far-field Raman modes becoming allowed in TERS in such a process.157

Compared to the bottom-illumination geometry, an incident light polarization alignment along the tip axis is experimentally easier to realize by illuminating from the side.^{45,46,52,127} In such a side-illumination setup, the angle of the incoming light is highly critical and must be adjusted properly to minimize shadowing effects from the tip and large far-field contributions. Commonly, incident light angles between 45 and 80° relative to the tip axis are used.^{45,46,48,50,51,135,154} 3D-FDTD simulations of a gap-mode configuration predict a maximum field enhancement at angles in the range of 40–60° using p-polarized light.¹²⁷ The incident light angle dependence was experimentally demonstrated for a long chain (*trans*-)azobenzene thiol SAM adsorbed onto a gold surface. TERS spectra were consecutively recorded in a side-illumination geometry for different polarization states, *i.e.*, the transition from p-polarization (90°, electric field parallel to scattering plane) to s-polarization (0°).⁵¹ Interestingly, the maximum signal intensity at different positions on the sample was observed for angles slightly shifted from p-polarization. The molecules were concluded to have a different orientation at the probed positions (upright standing chains *versus* flat lying chains). A similar polarization-dependent behaviour was determined for single-walled CNTs (SWNTs) in a back-reflection geometry.^{158,159}

All the discussed theoretical and experimental results emphasize the importance of polarization control in all TERS setups. Depending on the molecule orientation with respect to tip and incident light polarization, the acquired spectra can strongly vary and deviate from conventional Raman spectra.

Because of the altered selection rules in TERS compared to far-field Raman spectroscopy, the appearance of "unexpected" bands (as well as the absence of well-known bands) has frequently been reported. In AFM-TERS, charge-transfer processes were hypothesized to be partly involved.¹⁶⁰ To explain these observations in STM-TERS, theoretical attempts based on the coupling of electric field intensity and electric field gradient nanogaps have also been reported.^{143,161}

As only a single nanoparticle enhances the signal intensity, averaging processes are less prominent, and (temporal) fluctuations are often observed and can render data assessment complex.^{122,132,162–166} To explain this phenomenon, several attempts that have considered molecular diffusions, changes in orientation, alternating tip-molecule interactions, and discrete signal loss have been made.^{122,139,145,160}

In back-reflection systems, where the electric field is aligned along the main (*z*-)axis of the tip, modes oriented parallel to the tip are preferably enhanced. Consequently, in-plane modes of flat lying molecules will be less (if at all) enhanced, and the corresponding bands may be missing, as demonstrated for amino acids,¹³⁰ graphene,¹⁰¹ copper phthalocyanine,¹¹⁵ anthracene moieties,¹⁶⁷ perfluoropentacene,³¹ perylene diimide¹⁰⁰ and nucleobases.¹⁶²

AFM-TERS spectra of flatly adsorbed aromatic ring-containing amino acids on smooth gold surfaces showed that the characteristic ring-breathing mode remained obscured when working in a back-reflection geometry.¹³⁰

Importantly, the assignment of the intense band at approximately 1000 cm⁻¹ to a ring-breathing mode for mono-, 1,3- and 1,3,5-substituted benzene derivatives, while quite common, is not the actual ring-breathing mode but the symmetric ringstretching mode.^{168,169} The close spectral vicinity of this band to the ring-breathing mode of benzene combined with its high intensity led to this widespread misunderstanding. Because this band does not appear for 1,2- and 1,4-disubstituted benzenes, it is a good indicator of the substitution pattern. Asher *et al.* had already demonstrated in 1986 that the correct position of the (less intense) ring-breathing mode of phenylalanine is at 790 cm⁻¹.



Fig. 5 Average SERS and TERS spectra of phenylalanine; top: SERS spectra on a Ag film, $\lambda = 533$ nm, $P = 940 \mu$ W, $t_{acq} = 0.5$ s; bottom: average spectrum of 6 AFM-TERS spectra of phenylalanine adsorbed on a smooth gold substrate recorded in a gap-mode configuration; the yellow bars highlight bands at 1007 cm⁻¹ and 793 cm⁻¹.

Fig. 5, which displays an average TERS spectrum of 6 phenylalanine spectra, shows that neither of the mentioned bands is present in the spectrum. By contrast, in an average SERS spectrum collected on a Ag island film, the characteristic band of phenylalanine at 1007 cm⁻¹ along with the ring-breathing mode at 793 cm⁻¹ is clearly visible (details on this experiment are provided in ref. 130).

In SERS, the orientation of molecules probed on rough nanoparticles is heterogeneously distributed, providing averaged information on all geometries of all molecules in the laser focus.

The selectivity of TERS to enhance out-of-plane modes rather than in-plane modes was further exploited in a recent study on organic semiconducting perfluoropentacene films. In this study, spectral changes were indicators of the different orientations of the molecules within a layer.³¹ For illumination, the authors used azimuthally or radially polarized doughnut mode laser beams for either exciting in-plane or out-of-plane modes. Additional information could be obtained by collecting the photoluminescence emission. In this manner terraces with ordered molecules could be distinguished from randomly aggregated areas.

For the sake of completeness, a different case of absent bands should be mentioned at the end of this section. The absence of the amide I band in the TERS (and SERS) spectra of protein samples has been observed frequently but is rather unexpected.^{170,171} Presumably, peptide bonds should interact with the tip in random orientations, and the above argument on in-plane modes cannot fully explain the experimental observations. Recent advances in this field assume that the size of the side chains plays a role in the suppression of amide I bands, but a final conclusion cannot be drawn yet. The reviewed publications highlight that the polarization of the incident laser light is as important as the tip–sample angle. In extreme cases, modes such as those oriented perpendicular to the tip might remain undetected. Because tip–sample interactions can influence the symmetry of a molecule, selections rules valid for conventional Raman spectroscopy could change, resulting in a detection of forbidden Raman modes. The situation becomes even more complex when the probed molecules are attached to a metallic substrate. This "gap-mode" configuration not only contributes to additional signal enhancement but also changes the electromagnetic situation in the tip–substrate cavity.

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3.c. Gap-mode configuration: metallic substrates for additional signal enhancement

STM-TERS intrinsically works in gap-mode configuration because the sample must be attached to a smooth metal substrate. The research reviewed in this section was performed on gold substrates, but other metals (*e.g.*, Pt, Cu, Ag, Si) are potential alternatives when gold is not desirable. Replacing gold, however, might be detrimental to conductivity and feedback stability.^{127,128} The scope of suitable substrates for gap-mode experiments was only recently expanded to include semiconducting molybdenum disulfide (MoS₂) films.¹⁷² However, it should be mentioned here that the characteristic Raman bands of MoS₂ are located in the 200–700 cm⁻¹ spectral region^{173,174} and thus may interfere with bands of the probed sample.

Opaque substrates cannot be used for AFM-TERS setups in a back-reflection geometry. As already mentioned in the setup development section, using transparent gold or silver nanoplates can overcome this problem while maintaining the optical advantages and the enhancement imparted by the gap.^{129,131,135,137} FDTD calculations for such experiments were in good agreement with the experimental results.⁴³

The first gap-mode TERS experiments were accomplished when STM was combined with Raman spectroscopy.^{4,116} In those experiments, the novel electromagnetic situation created by sandwiching the probed molecules between a gold film and a silver tip was not specifically mentioned. In subsequent studies on cyanide and malachite green on gold films, the signal enhancement was estimated to be up to 10^{6} ,^{118,175} and the substrate-tip gap was recognized to form an optical cavity where localized surface plasmons can be excited by laser illumination.¹⁷⁵

An independent preliminary theoretical model (not specifically for an STM-TERS configuration) has been developed to estimate the influence of the substrate on the spatial distribution and magnitude of the scattered electric field. In this respect, comparative finite element models (FEM) were constructed, and the effect on the electromagnetic field at the tip was investigated.¹⁷⁶ The results show that positioning a gold tip 2 nm above a glass slide enhances the electromagnetic field by a factor of 19 due to field confinement. Using a gold substrate confined the field even further and increased the enhancement beyond that of the glass substrate case by a factor of 10. Simultaneously, the spatial resolution increased from

13 nm to 8 nm. In this gap-mode configuration, the electric field enhancement was found to be strongly wavelength dependent, indicating resonance effects. This observation was explained by a strong Au-substrate–Ag-tip coupling. Notably, the dielectric properties of the sample itself influence the electromagnetic coupling in the substrate–sample–tip gap without affecting the spatial resolution significantly.¹⁷⁶

Substrate-dependent STM-TERS experiments demonstrated that the differing optical properties of gold and platinum resulted in slight band shifts and band intensity ratio changes in benzenethiol.¹²⁰ However, different molecule–substrate interactions could not be excluded.

As already mentioned in the introduction, the additional gap-mode enhancement can also be exploited in AFM-TERS experiments. This phenomenon was demonstrated in 2006 when buckminsterfullerene (C_{60}) attached to a silver film was investigated.¹⁷⁷

The unique electromagnetic situation in a metal substratemetal tip cavity was theoretically approached using FDTD calculations. The results illustrate the coupling of the surface plasmons from a silver film with the localized plasmons from a silver-coated AFM tip. Assuming a 4 nm gap between the metal surface and tip, the averaged intensity of the electromagnetic field was 20 times greater than that of the incident field. By contrast, the incident field was 5 times lower than the electromagnetic field when a glass substrate was used.⁴² In another theoretical approach, the situation of a gold tip 2 nm above a gold substrate was modelled, revealing a strong tip–substrate separation dependence.¹²⁷ Elevating the tip by 5 nm from the surface resulted in a 70% decay of the electric field enhancement and a subsequent 99% reduction in the TERS signal. At a distance of 20 nm, coupling effects could no longer be observed.

Generally, the signal enhancement becomes more effective when radially polarized light is used for excitation. Simulations were further developed and applied to measurements in different refractive indices, which are important for experiments in aqueous environments. Here, a clear redshift of the TERS plasmon resonance is expected.¹⁷⁸

In their STM-TERS experiments, Zhang et al. reported several morphological changes, particularly a heat-induced smoothing of the gold surface close to the measured position.¹³⁹ Interestingly, a flame-annealed crystalline gold surface turned out to be more stable under the same experimental conditions. For a deeper insight into the peculiarities of gap-mode experiments, studies concerning near-field photoluminescence were conducted. The collected photoluminescence spectra showed variations that could be correlated with topographic features. Consequently, those features modulated the plasmonic coupling by generating an additional field that can be approximated by a dipole. These results are consistent with those from Zhang et al., who described the effect of 2 nm protrusions from flat metal surfaces on the signal enhancement.^{138,139} 3D-FDTD simulations of such a system resulted in an extra enhancement of the electric field in the gap by \sim 30%, which was attributed to the lightning rod effect.¹²⁷ Thus, the field enhancement is affected by not only the geometrical confinement (determined by the tip curvature

and gap size), polarization, and incident light angle but also the metal film thickness.¹⁷⁹ Moreover, a resonant coupling of the tip's surface plasmons with their dipole mirror image in the gold surface, which is extremely sensitive to perturbations, must be considered.^{84,127,140}

All results reported in this section were achieved in a sideillumination geometry. Experiments in a gap-mode configuration and a top-illumination geometry have been reported as well and offer reduced background signal and lower laser power.⁵⁴

In summary, TERS in a gap-mode configuration creates an exceptional electromagnetic situation for molecules in the tipmetal substrate cavity. The electromagnetic field becomes more confined, and a higher spatial resolution and higher enhancement can be achieved. Smooth gold (or silver) substrates are an effective platform for a homogeneous immobilization of molecules, enabling a systematic probing of molecule orientation down to the single-molecule level. Because of the confinement, spectra can change significantly depending on the specific orientation of the probed molecules.

3.d. Molecule-tip orientation influences spectra

Although the fundamental principle of signal enhancement in SERS and TERS is based on the excitation of surface plasmons on rough metal nanostructures, there is another aspect that must not be neglected: in TERS, solely an individual/single nanoparticle or metal edge is interacting with the sample, which can have a considerable impact on the spectra, as outlined above. As mentioned previously, SERS generally obtains averaged chemical information of all molecules in the laser focus, whereas the orientation of the molecules with respect to the TERS tip plays an important role; additionally, a sitespecific probing of molecules was reported.^{17,20,79,145,163,164,180} In all these studies, spectral fluctuations were observed when the tip was slightly displaced from the analyte within the region of interest, which in essence reveals the high spatial resolution of TERS. Temporal fluctuations in Raman band intensities and vibrational energies were also observed in single-molecule experiments when spectra were continuously acquired from a single spot over time.^{122,132,165,180} These observations were mainly ascribed to molecular diffusion and orientation changes. All the experiments mentioned above were conducted in scanning probe feedback modes to keep the tip a certain distance from the sample. The situation changes when the tip constantly touches the sample, as performed in contact mode AFM-TERS experiments. Such a process can lead to pressureinduced modifications of the probed molecules and is a topic of special importance. Deforming a single adenine crystal by pressing the tip on the molecule induces clear band position and band intensity changes, which can be correlated with the probed molecular sites.^{77,79,164} Because of the direct tip-adenine contact, chemical interactions arise and can alter the symmetry of vibrational modes. Together with the highly localized electromagnetic field at the nanoparticle-coated tip apex, the polarizability of the molecule changes, which again can change the selection rules. Consequently, band intensity and position variations are detected.

For more details about the influence of molecule orientation on TERS spectra, we refer readers to the single molecule and SAM sections. In summary, the site specificity can be explained by the laterally confined field at a TERS tip, as this field exponentially decays within a few nanometres. These high-resolution aspects are thoroughly reviewed in a separate article in this issue and will be only briefly addressed in the next section.

3.e. Spatial resolution

The first subnanometre resolution experiment using TERS was realized in 2013 on porphyrins with STM-TERS under low-temperature high-vacuum conditions.¹⁹ Furthermore, STM-TERS experiments on CNTs with a resolution from 1.7 nm¹⁸ down to 0.7 nm can be accomplished at low temperature and UHV.²¹ In 2012, an AFM-TERS investigation on linear maps of proteins under ambient conditions decreased the spatial resolution limit into comparable dimensions.¹⁶ Subsequent studies in both research areas reproduced and emphasized these results.^{17,20} Naturally, the spatial resolution of TERS is the most intriguing feature of this near-field technique and is generally considered to be directly related to the diameter of the tip apex.^{38,75,97,150,181} Nevertheless, recent theoretical developments^{22,25–27} together with the abovementioned high-resolution experiments will lead to new insights.¹⁸²

The improvement of the spatial resolution in recent years can be credited to advances in the tip fabrication process, which will be reviewed in detail below.

4. The key feature of TERS: the tip

4.a. Tip design: different types of cantilever tips and etched tips

As mentioned above, signal enhancement by the tip is based on the excitation of surface plasmons at the tip apex. This excitation is generally triggered by light from the far field. Concurrently, Raman scattered light interacting with the plasmonic tip is detected by a far-field detector. This conversion process between the near field and far field is an intrinsic property of the TERS process. Therefore, the collection/scattering efficiency of a metal tip from/to the far field is an important factor to maximize TERS enhancement.

To couple with the far field, a plasmon must have a dipoletype mode distribution, which is accomplished by structuring a tip into a plasmon resonator. The structures shown in Fig. 6 are some examples of commonly used tip structures that have a dipolar-type plasmon mode distribution. In these structures, the external electric field induces an alternating electric charge across the structure, resulting in an oscillating dipole with a resonant frequency determined by the size and shape of the structure. This is similar to classical antenna theory, except for the size of the antenna and, of course, the damping at optical frequencies. In a classical radio-frequency antenna, the length of the antenna is an integer multiple of the half wavelength of the light, whereas in a plasmonic antenna, the length of the antenna is shorter than the half wavelength of the light,



Fig. 6 Commonly used metal tip structures for TERS in STM- and AFM based setups. (a) Antenna-structured tips, (b) sharpened tips.

which is due to the wavelength of the surface plasmon being shorter than that of the light propagating in the free space, as demonstrated by Smolyaninov.¹⁸³ A single sphere is the simplest form of a dipole scatterer.¹⁸⁴ Because the scattering cross section scales with the sixth power of the sphere diameter, reducing the sphere diameter to achieve better spatial localization results in a poor scattering efficiency. In this sense, a rod or truncated cone is better suited, as the dipole moment is larger because of its axial length. In all cases, the electric field is enhanced at the both ends of the structure. Although a finite length truncated cone, as shown in Fig. 6a, acts as a dipole scatterer, an infinitely long cone, as shown in Fig. 6b, does not have a net dipole moment, as discussed later.

Antenna-structured tips. As already mentioned, a standard method for tip preparation is the vacuum deposition of a plasmonically active metal film on dielectric AFM cantilever tips by thermal evaporation or sputtering.^{13,15,25,44,70,71,80} These tips are covered with silver/gold nanospheres with shapes approximated by spheres, as shown in Fig. 6a. The evaporation process yields a silver island film on the silicon tip, as shown in the scanning electron microscopy (SEM) images in Fig. 7a and b. The tip in Fig. 7a is a 25 nm thick silver layer deposited on a Si cantilever, and the tip in Fig. 7b is a 60 nm thick silver layer on a SiO₂ cantilever.⁸⁰

Electron beam evaporation¹⁸⁵ or sputtering techniques¹⁸⁶ have also been reported but are technically more complex. Since evaporation and, to a certain extent, sputtering result in a random distribution of nanoparticles on the tip, numerous approaches to introduce more diverse nanostructures have been reported to produce more reproducible tips. Ideally, the nanoparticles can be characterized and preselected prior to tip coating, therefore allowing the tailoring of a TERS tip to the experimental conditions. In the following paragraphs, several techniques for preparing effective TERS tips with a single particle at the tip apex are summarized. In Fig. 7a–d, selected SEM images of such nanoantennas are shown. Due to space limitations, only a few images from the large number provided in the literature can be displayed.

The single particle at the probe apex can be an electrochemically grown gold particle^{190,191} or chemically grown silver nanoparticle produced *via* electroless silver deposition,^{90,192,193} silver mirror reactions^{194,195} or photoinduced reduction.¹⁹⁶ The morphology of the deposited metal can be controlled by changing reaction parameters, such as solution concentration, applied potential and reaction time.



Fig. 7 SEM images of metal probes with various dipolar tip structures. (a) A 25 nm thick rough silver island film on a Si AFM tip used by the Deckert group; (b) a 60 nm rough silver island film on a SiO₂ AFM tip used by the Kawata group. Adapted with permission from ref. 80 from Wiley Materials 2012 John Wiley & Sons, Ltd. (c) Silver particle-coated AFM cantilever with patterned grating. Reproduced from ref. 187 with permission from the Royal Society of Chemistry; (d) single nanoparticle at the end of a metal-coated glass fibre produced by FIB milling. Adapted with permission from ref. 188 copyright 2007 American Chemical Society; (e) calculated scattering spectra of silver tips with spherical particles (dashed line) and a rough corrugated film (solid line). Reproduced from ref. 189 with permission from the Royal Society of Chemistry.

Okamoto *et al.* and Kalkbrenner *et al.* attached a gold nanoparticle on the end of an AFM probe and a glass fibre probe, respectively,^{197,198} and picked up a particle with an AFM probe that was precoated with a glue. Dielectrophoresis might be another option for the positioning of single silver or gold nanoparticles^{199–202} or even single crystalline nanowires.^{199,203}

Electron beam-induced deposition was reported to efficiently fabricate nanocones¹⁹³ or attach silver wires to AFM cantilevers.²⁰⁴ Finally, the immersion of cantilever tips in silver nanoparticle solutions containing spheres, rods,²⁰⁵ stars²⁰⁶ or cubes²⁰⁷ could also be an option.

Fig. 7d shows a tip with a rod-like particle shaped by FIB milling a metal-coated glass fibre apex.¹⁸⁸ This protrusion acts as a monopole-type antenna, equivalent to the rod antenna shown in the middle in Fig. 6a with a length two times greater than that of the monopole antenna. FIB milling was also successfully employed in the fabrication of truncated cones.²⁰⁸ Adjusting the length of the cone imparts control over the resonance wavelength. Bow tie-shaped dipole antennas are another example of using the FIB milling technique for TERS tip production.²⁰⁹ Here, a double tip was formed at the apex of silicon nitride AFM cantilevers, theoretically to further enhance the incident electromagnetic field. In principle, the sputtering technique provides smoother tips and can pattern etched silver and gold wires or silver-coated AFM cantilevers with a periodic grating,

providing access to TERS experiments using an illumination wavelength up to 800 nm.91,97,187 Fig. 7c shows the SEM image of tips consisting of a 40 nm silver layer deposited on a SiO₂ AFM cantilever and then patterned with a grating structure.¹⁸⁷ Varying the grating period enables optimization of the plasmon resonance to the excitation wavelength. When patterned wires are used, the tip is mounted onto an AFM tuning fork, and the grating is illuminated. Additionally, metallic pyramidal shapes can be structured in a similar manner to the wire tips.^{210,211} The generated surface plasmon polaritons propagate along the tip main axis and emit at the apex.96 Such remote excitation reduces the far-field background; however, the FIB fabrication process is time consuming and elaborate and is not yet applicable to produce large tip quantities. By contrast, template-stripping protocols have been developed for the mass production of tips in a single process on a standard silicon wafer with optimized pyramidal geometry²¹² or in a combined process with atomic layer lithography to yield wedges.²¹³ In addition to pyramids, nanoprisms can be shaped by an anglegrinding process, yielding sharp edges and vertices with inherently high signal enhancement.²¹⁴ A highly sophisticated TERS probe was designed with a "wedding-cake grating" that serves as a plasmonic antenna.²¹⁵ The curvature of the tip apex was determined to be approximately 4 nm, which exceeds the results of most other tip preparation procedures. Still, the preparation comprises multiple steps, rendering the procedure time consuming and elaborate.

All these results emphasize that various nanostructures can be used for a successful TERS experiment. Nevertheless, the most commonly employed AFM tips are produced *via* thermal silver evaporation. The surface of the silver film is not smooth but is covered with rough metal nanograins. The scattering spectrum of a molecule near the tip of a granular probe has been recently calculated¹⁸⁹ and, together with a scattering spectrum of a single silver particle at the tip of a glass probe, is shown in Fig. 7e. In both cases, a pronounced resonance peak is visible in the spectrum. This result demonstrates that probes covered with aggregated metal grains indeed act as a dipole scatterer.¹⁸⁹ The granular tip shows its resonance at 450 nm in the visible light region, whereas a 40 nm diameter silver particle tip has its resonance at 370 nm (UV region).

Silicon probes are commonly preferred over silicon nitride probes because the absorption maximum of attached nanoparticles redshifts as the refractive index of the dielectric material increases.⁷ Notably, the surface plasmon resonance is similarly strongly affected by the size, shape and aggregation of the nanoparticles.^{8,127,216,217} The resonance wavelength of granular tips can be controlled by changing the size of the grains or, alternatively, by changing the refractive index of the dielectric core on which silver is coated.^{218,219} Silver grains deposited on a SiO₂ (n = 1.5) cone show resonance in a blue spectral region, whereas those deposited on a Si (n = 4.4) cone show resonance in red spectral region.^{219,220} The use of shorter wavelengths is beneficial for Raman spectroscopy because the Raman scattering cross section is inversely proportional to the fourth power of the wavelength. The SiO₂ cones can be mass produced by simply oxidizing commercially available Si AFM probes^{80,219} or coating them with SiO_x or AlF_3 .²²¹

Etched metal tips. Another class of TERS probes are smoothly tapered massive metal tips, as shown in Fig. 8. Historically, this type of tip first appeared as a probe for scattering near-field scanning optical microscopy (s-NSOM).^{222,223} In one of the initial TERS experiments, a tip sharpened by the electrochemical etching of a gold wire was used,¹³ and this technique has become a well-established method in gold and silver wirebased TERS. Various modifications of the process have been reported over recent years to produce the smallest possible curvature radius of the tip apex with efficient electromagnetic field enhancement.^{30,87,114,122,126,224-231} The tip diameter can be decreased from 100-500 nm down to <10 nm. Another approach to AFM cantilever bases tips starts from gold or silver wires that are first flattened, then etched and finally glued to standard AFM chips.⁶⁶⁻⁶⁸ Once those tips become blunt, they can easily be re-etched to yield sharpened tips.⁶⁸

Nevertheless, because of variations in the etching parameters and metal wire crystal structure, the process is hardly reproducible. Furthermore, in the case of, for example, evaporated AFM cantilever probes, the enhancement cannot be predicted, and not all tips are active. In Fig. 8, a selection of SEM images of tapered TERS tips is shown. Only few examples of the antenna structure tips can be displayed due to limited space.

A SEM image of a silver tip tapered by electrochemical etching is shown in Fig. 8a.¹²² Fig. 8b shows the calculated scattering spectrum of a sharp smooth tip.¹⁸⁹ Unlike the scattering spectrum of dipolar tips shown in Fig. 7e, the scattering spectrum is featureless and does not show any resonant character. This means



Fig. 8 (a) SEM image of a sharply etched silver wire. Reprinted with permission from ref. 122. Copyright 2007 American Chemical Society. (b) Calculated scattering spectrum of a sharp smooth tip. Reproduced from ref. 189 with permission from the Royal Society of Chemistry. (c) TEM image of an etched conical gold tip produced by a DC-pulsed voltage etching procedure. Adapted from ref. 231 with permission from the Royal Society of Chemistry; (d) metallic tapered probe with a grating fabricated on the shaft. Reprinted with permission from ref. 96. Copyright 2007 American Chemical Society.

the sharp smooth tip is a less efficient scatterer compared to a tip with a dipolar resonant particle. The transmission electron microscopy (TEM) image in Fig. 8c shows a gold tip that was fabricated by DC-pulsed voltage electrochemical etching,²³¹ a new approach that was suggested to provide more reproducible apex radius curvatures than other etching procedures.

Importantly, surface plasmon polaritons propagate nonradiatively along a smooth metal film. Taking advantage of the wave guiding character of a smoothly tapered tip, Sanchez et al. proposed transmitting light energy along the tapered probe from the thicker side to the sharper tip end to create a near-field nanospot at the apex.²³² For this purpose, they fabricated a guartz microprism near the sidewall of the probe. This microprism acts as an Otto coupler by exciting surface plasmons on the probe wall. A grating can also act as a coupler (see Fig. 8d).⁹⁶ Upon propagation towards the tip apex, the phase velocity of the plasmon decreases and asymptotically approaches zero for an infinitely small tip radius.²³³ If the phase velocity reaches zero, then no light will be coupled out to the far-field, as suggested by superlensing experiments.²³⁴ In practice, every tip has a finite curvature radius. In this case, a part of the plasmon energy is scattered into the far field at the tip due to phase discontinuity. When the plasmon coupler is placed far apart from the tip, the tip plasmon can be excited without directly illuminating the tip part. This remote excitation of tip plasmon is useful for suppressing far-field signals that are otherwise interfering with the experiment.232,235 Background-free apertureless NSOM with smooth tapered tips and a remote grating coupler has been demonstrated.²³⁶ Similarly, a silver nanowire glued to an etched tungsten tip can be remotely excited and was used for TERS in shear-force mode.237

As previously mentioned, light scattering at a smooth tip apex is less efficient. The background-free excitation scheme has also been applied to TERS⁹¹ but used a metal substrate to take advantage of gap-mode enhancement.

Fig. 9 shows results obtained from comparative TERS experiments using various tips on a few-layer graphene sample.¹⁸⁹ The enhancement factor of the Raman scattering is ~ 5500 for discretely arranged particles (Fig. 9a), ~ 800 for a continuous rough surface (Fig. 9b), and ~ 230 for a fairly smooth tip (Fig. 9c). The highest enhancement is observed for aggregated grains with separation, indicating that separation between grains is important for effective signal enhancement.¹⁸⁹

Presumably, in contrast to coated cantilever probes, massive wire-based tips could not wear off due to nanoparticle loss. Nevertheless, even those tips were found to be frequently TERS inactive.^{39,227} The ability to produce a single crystalline silver apex by annealing the tip after the etching process could clearly improve this situation.²²⁷

In this section, the different TERS tip production schemes were reviewed. The most commonly used tips include antennastructured tips (coating blank probes with nanoparticles) and etched tips (etching wires). Although different approaches have been developed, obtaining tips with a reproducible enhancement factor remains a challenge. Developing a way to massfabricate tips that are inert to contamination would definitively



Fig. 9 Enhancement by silver probes with various shapes. (a) Discretely arranged grains on a tip. (b) Rough corrugated granular tip. (c) Smooth tip. The sample is graphene, and the excitation wavelength is 488 nm. Reproduced from ref. 189 with permission from the Royal Society of Chemistry.

refine the application of TERS. In the following section, recent advances in prolonging tip activity will be discussed.

4.b. Tip preservation

Among the noble metals, silver tips provide the highest enhancement when irradiated with green laser light. However, they are susceptible to undesirable oxidation and enhancement decay within a few days when not stored under an inert atmosphere. In addition to chemical degradation, nanoparticle-coated AFM tips are mechanical feeble, and the nanoparticle at the tip apex can easily be lost when excessively large forces are applied.

A systematic study revealed that reducing the oxygen and moisture content (<1 ppm) of the atmosphere could significantly preserve tip activity for months.²³⁸ In particular, storing tips in a nitrogen glove box continuously being flushed with nitrogen was found to be highly efficient. To avoid rapid degradation, experiments should also be carried out under an inert atmosphere. Conversely, an appropriate coating with a robust and chemically inert layer can overcome these issues when Review Article

working at ambient conditions. In 2008, simulations revealed that a 5 nm SiO₂ layer might be suitable for tip protection but affects the plasmon resonance frequency and the field enhancement.²³⁹ Experimentally, SiO₂ was found to effectively protect metallized tips for TERS.^{82,240} Subsequent studies revealed that coating silver AFM tips with a 2–3 nm thick aluminum oxide (Al₂O₃) layer was more effective in degradation inhibition than coating with SiO₂ and did not significantly change in the optical properties of the tip.^{241,242} FDTD calculations provided opposing results for a silver tip coated with a 1 nm thick alumina layer. Here, the electric field was found to decrease by 30%, and destructive interferences from the excitation light and scattered electric field could not be excluded.⁴³

Chemically grown SiO₂ layers on silver were recently proposed as a pinhole-free and ultrathin (<3 nm) protective layer.²⁴³ The achieved enhancement with the chemically grown SiO₂ layer was comparable to the typical tip enhancement for TERS with fresh silver tips.²⁴³ Opilik *et al.* proposed electrochemical restoration of elemental silver after corrosion for tip lifetime prolongation.²⁴⁴ Using aluminum as an anode with a low reduction potential, they showed a successful recovery of tip enhancement. Another option is removing contaminants from gold wire tips by dipping them in concentrated sulfuric acid,⁴⁸ thus allowing different experiments to be performed using the very same tip.

Gold tips are resistant to corrosion but have low hardness and can easily be destroyed in AFM-TERS.⁸⁷ Moreover, because the adhesion of gold to silicon tips is weak, thin adhesion layer promoter (*e.g.*, Ge, Cr, Ti) is recommended.^{4,74,136,140,154,245,246}

Tips decorated with core-shell structures comprising a silver core surrounded by a gold shell were proposed to be very suitable for TERS experiments because the plasmonic properties can be tuned for the measurement conditions. The gold layer prolongs tip activity by shielding the silver from oxidation.²⁴⁷ Still, tip contamination by carbon during preparation and the elaborate synthetic procedure required cannot compensate for the mass production ability of this approach.

The highlighted results show that promising approaches for prolonging a TERS tip's activity have been suggested. In summary, a coating must have chemical inertness, mechanical robustness, no significant effect on the enhancement factor and optimally no Raman signals that interfere with the sample spectrum. Consequently, the appropriate coating ultimately depends on the experimental conditions.

5. Applications

As already mentioned, TERS can be applied to a broad range of materials, ranging from semiconductors and organic structures to materials for life science applications. Hence, we divided this section into three major parts: inorganic samples, organic samples, and biological samples. In a fourth section, topics that do not fit into these categories are summarized. At this time, the classifications chosen may seem arbitrary because the boundaries between the different compound classes are blurred. In any case, the diversity of investigated samples and molecules reflects the potential of TERS to characterize subtle structural variations in most samples (see also overview in Fig. 1).

5.a. Inorganic samples

5.a.I. Silicon and inorganic semiconductors. The use of TERS on inorganic samples is just beginning. In particular, the detection of nanoscale defects/impurities in crystalline siliconbased materials could be of interest in semiconductor industry. Furthermore, TERS is suitable for the characterization of gallium nitride (GaN) crystalline semiconductor films,²⁴⁸ GaN nanowires,⁴⁹ CdSe,²⁴⁹ silicon germanium (SiGe) nanowires²⁴⁵ and InN/GaN nanostructures.²⁵⁰ In addition to vibrational spectroscopy, photoluminescence spectroscopy was also demonstrated to provide complementary information.²⁴⁹

Similarly, local strain in silicon substrates, which remains obscured in far-field Raman spectra,^{47,74,251–253} is an important property in the semiconductor industry because it affects the mobility of electrons and holes, increases the speed of electronic devices and reduces power consumption.²⁵⁴

The main Si–Si mode at 520 cm⁻¹ is strongly polarized, and the band intensity is a good indicator of the optimum polarization of the incident beam along the tip main axis.⁴⁷ In this way, highly localized vibrational spectroscopies can detect local strains in Si crystals. Minimal changes in the structure can affect the band position and shape, enabling the distinction of strained and strain-free Si-based samples.²⁵²

5.a.II. Miscellaneous inorganic samples. LiNbO₃ nanocrystals were demonstrated to be well suited for comparing the crystalline symmetry and changing selection rules in TERS to those in far-field Raman spectroscopy.²⁵⁵ In BaTiO₃, ferroelectric domains can be identified and localized by detecting transverse optical phonon modes.⁵⁰

Tungsten diselenide (WSe₂)²⁵⁶ and molybdenum disulfide (MoS₂) are transition metal chalcogens and novel 2D materials with a graphene-like hexagonal structure comprising single Se–W–Se and S–Mo–S layers, respectively, that are tightly stacked by van der Waals forces. TERS studies on those materials are in an early stage, but several reports have already shown the promise of TERS in a gap-mode configuration for detecting edges and defects in single/few-layer MoS₂.^{172–174} Similar to graphene, the electronic properties of WSe₂ and MoS₂ strongly depend on the purity of the material, and TERS analysis can provide information on their optoelectronic behaviour.

Recently, TERS was also applied to investigate the composition of single atmospherically relevant aerosols. In particular, TERS provides access to the surface composition and offers important insight into the nucleation, ageing, and transformation processes.²⁵⁷

5.b. Organic samples

For TERS, applications involving organic sample analysis are by far the most common and range from single-molecule analysis to dynamic reactivity investigation. Analysing those samples has provided some of the most intriguing results because they demonstrate the full potential of TERS. Consequently, this section is the largest in this contribution.

5.b.I. Dyes and single molecules. In addition to dyes,^{4,15} SWNTs^{39,258} and $C_{60}^{13,177,259}$ have been favoured samples since the inception of TERS. In a review of the publications addressing dyes and SWNTs, it is important to note that under the reported experimental conditions, the spectra generally show a resonant Raman contribution. Consequently, the acquisition times can be kept short, and a good signal-to-noise ratio and potentially single-molecule sensitivity can be approached. The potential of TERS to analyse single molecules was experimentally realized for the first time with STM-TERS using malachite green^{87,180,260,261} and BCB.¹²² Characteristic observations in single-molecule TERS experiments include band position fluctuations (<10 cm⁻¹), as well as "blinking" (appearance and disappearance of spectra) or discrete signal loss, both were hypothesized to stem from molecules diffusing in and out of the field under the tip apex.^{87,122,150,165,166,180} Conversely, bands shifts and/or fluctuations do occur not concurrently, which renders the explanation of molecular diffusion doubtful.^{166,262} Particularly, a study on isotopically labelled Rh6G molecules gave insight into the contribution of diffusion effects to band position alterations.²⁶² The reported observations in this study were most likely due to subtle differences in the lifetime and energy of the excited states and the geometry of the molecule.

In another study, the contribution of charge-transfer interactions to the spectral fluctuations were examined. Coating the TERS tip with a <5 nm alumina layer enabled such processes to be excluded. Although the protective layer excludes direct silver-sample interactions, the fluctuations persisted, whereas the overall signal enhancement was not affected.¹⁶⁵

All these effects can be minimized when working at low temperatures.^{94,145} Insight into intramolecular interactions and dynamic processes on the single-molecule level could be achieved for malachite green at cryogenic temperatures because of the slowing down of molecular dynamics. Thus, suggestions for molecular orientations were presented.⁹⁴

When large molecules such as porphyrins are immobilized on a gold surface, they form domains comprising flat lying molecules that can be individually inspected using TERS.^{17,19,28} TERS spectra recorded under low-temperature and UHV conditions impressively reveal that scanning in 0.25 nm steps across the molecules allows not only derivatives to be determined based on their spectral fingerprints but also molecular sites.^{17,19} Complementary information on porphyrins substituted with four tertbutyl groups (structure see Fig. 10a) was obtained by integrating fluorescence spectroscopy with STM-TERS operated in UHV. Thus, the decay behaviour of the electronic excited states could be addressed.²⁸ Notably, fluorescence spectroscopic data were accessible only because of the presence of the four tert-butyl spacers at the porphyrin ring. These spacers lifted the central ring from the silver substrate and electronically decoupled the molecule from the substrate, thus preventing fluorescence quenching. However, molecule-substrate interactions still effected Raman signal enhancement and fluorescence spectra acquisition. Fig. 10b shows TERS spectra of a meso-tetrakis(3,5di-tert-butylphenyl)porphyrin (H2TBPP in Fig. 10a) monolayer on



Fig. 10 (a) Model of the orientation of a porphyrin molecule (H₂TBPP) on the silver substrate; (b) UHV-TERS spectra of a porphyrin monolayer on a smooth silver substrate. For excitation, $\lambda = 514.5$, 532, 561, 594 nm were used. Far-field spectra recorded with a retracted tip were subtracted from the TERS spectra. The black spectra correspond to TDDFT calculations assuming polarization along the tip main axis. Adapted with permission from ref. 28. Copyright 2015 American Chemical Society.

a silver substrate collected at different excitation wavelengths. Spectral differences between the different incident laser wavelengths were explained by the excitation of the molecule into different vibronic excited states, as confirmed by time-dependent density functional theory (TDDFT) simulations.

Theoretical considerations regarding synchronized TERS and tip-enhanced fluorescence spectroscopy were recently presented.²⁶³ Unsurprisingly, the tip-sample distance was found to strongly influence the enhancement factor. The proposed setup contained a combined two-laser system for separately exciting the Raman and the fluorescence signal. Accordingly, the acquisition of complementary spectroscopic data should become possible in a single experiment. Importantly, TERS data can be supplemented not only by additional spectroscopic techniques but also by, e.g., electric current flow measurements. This information becomes accessible in STM-TERS by simultaneously measuring the conductance state and the TERS spectra in the dry state (*i.e.*, fishing TERS).²⁶⁴ In both approaches, valuable information on the orientation of the probed molecules can be accumulated. For example, molecular level structural changes in 4,4'-bipyridine could be reversibly induced by altering the bias voltage precisely within a defined range. The observed splitting of bands was explained by the different bonding situations (and the corresponding electron transport) of the two pyridyl moieties with the substrate and the tip.

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Similarly, measuring the conductance can supply information on the tunnelling current in the substrate-tip gap in the presence of plasmons.²⁶⁵ For these specific experiments, the tunnelling current with and without laser light was measured by slightly modifying the conventional STM-TERS setup, namely, by chopping the laser. Combining the experimental results with theoretical calculations led to the conclusion that a plasmonassisted tunnelling current amplification occurred. Additionally, the current perturbation depended on the distance from the laser focus to the tip-sample gap.

5.b.II. Carbon-based materials. CNTs are essentially a rolled single graphene sheet with unique mechanical and electrical properties. Although CNTs can effectively be analysed by Raman spectroscopy, conventional Raman techniques cannot provide details on subtle structure variations or purity because of the lack of high-resolution capabilities. Therefore, AFM- and STM-TERS, both with and without a gap-mode configuration, is ideally suited for the detection and localization of minor irregularities in carbon sheets of SWNTs and multi-walled CNTs (MWNTs). The intensity ratio of the G-band (graphite-like mode, C=C stretch, ~ 1580 cm⁻¹) to the D-band (defect related, dispersive phonon mode, $\sim 1350 \text{ cm}^{-1}$)^{266,267} is a good indicator of the purity, strain fields and presence of defects in graphene and CNTs. 6,18,21,30,78,80,81,90,92,97,101,102,105,106,258,268,269 Depending on the spectral range, the second order 2D (or G') band (defect related, zone-boundary phonon mode, $\sim 2700 \text{ cm}^{-1}$) can also be considered. This band is generally detected in doublewalled CNTs and graphene layers and denotes interlayer interactions.^{103,266,269,270} The 2D:G-band ratio can similarly be used to determine the number of walls in MWNTs.²⁷⁰ Interestingly, investigating MWNTs using the gap-mode configuration is advantageous because their cross section is much lower than that of SWNTs.268

The radial ring-breathing mode ($\sim 100-500 \text{ cm}^{-1}$), which is highly sensitive to the excitation wavelength, can be resonantly enhanced and is often used for diameter studies of SWNTs.^{88,269,271}

The local chemical properties of CNTs have been extensively studied using TERS.^{6,97,105,106} The distribution of the localized defects along a single CNT was visualized at a spatial resolution of 15 nm using the intensity ratio of the D-band to the G-band.^{105,106} Mapping the D- and G-band intensity enabled a spatial resolution of 1.7 nm¹⁸ and, recently, even of 0.7 nm²¹ to be achieved using STM-TERS under UHV conditions. The spatial extent of defects and strain variation could be visualized with a subnanometre resolution using the D-band and the G-band, respectively. The TERS and near-field photoluminescence spectra of CNTs were also measured, and variations in chirality along the nanotube were distinguished.^{30,88,272}

The TERS image of a CNT shown in Fig. 11a was obtained using variations in Raman band position shifts.⁸¹ The band position shift enables the local strain in the deformed CNT to be deduced. The G-band spectrum of the CNT with respect to the axial position of the CNT is shown in the lower panel of Fig. 11a. The G-band peak from the highly deformed corner is significantly shifted to a higher frequency, as indicated by the yellow arrows (upper middle panel), whereas the corresponding peak from the straight part did not shift significantly (upper right panel). High frequency shifts were found at the bent corners, indicating that the nanotube structure is distorted by internal force.

Fig. 11b shows TERS spectra consecutively acquired in a shear-force setup along the main axis of an isolated SWNT.⁸⁸ The radial breathing mode 251 cm⁻¹, which indicates semiconducting property, was a main focus (spectra 11–14). Interestingly, this band shifted to 192 cm⁻¹ during the measurement (spectra 16–19), suggesting that the SWNT had a metallic character. Apparently, intramolecular junctions were detected based on the specific spectral signatures, which were induced by the molecule changing chirality.



Fig. 11 (a) Frequency shift image of a CNT (upper left) TERS image with three frequencies denoted by colours: 1590 cm^{-1} (red), 1600 cm^{-1} (blue) and 1610 cm^{-1} (green). Frequency-shifted components (blue and green) are dominant in the bent corners. (middle and right) AFM images of bent and straight CNT (upper) and corresponding G-band spectra along the CNT (red line in the AFM image) (lower panel). Reproduced with permission from ref. 81. Copyright 2013 Macmillan Publishers. (b) TERS spectra recorded along the main axis of an SWNT. The focus is on the radial breathing mode at 251 cm^{-1} . Adapted from ref. 88 with permission of the American Chemical Society 2007.

An interesting effect on CNTs was investigated using AFM-TERS measurements in contact mode. Changing the applied force of the tip enabled the spectral changes resulting from pressure-induced deformation of SWNTs to be.²⁷³ The ringbreathing mode and the G-band were found to be more affected by pressing the SWNTs with the TERS tip than the D-band. Notably, the original band position and intensity were restored when the pressure was released from the nanotubes, indicating their elasticity and the reversibility of the deformation.

Finally, SWNTs were not only a subject of experimental TERS investigations but also of theoretical calculations. Cancado *et al.* proposed a theory to explain the TERS enhancement mechanism of SWNTs, which is not limited to 1D structures.²⁷⁴ They concluded that the enhancement factor depends on the enhancement abilities of the employed tip overall, not on the respective mode.

For sake of completeness, a work on MWNTs/styrenebutadiene rubber nanocomposites should be mentioned.²⁷⁵ The spectra of these nanocomposites led to the conclusion that the MWNTs significantly interacted with the phenyl rings of the polymer.

Another class of carbonaceous species that has been extensively studied using TERS is graphene.^{65,93,101-104,133,276-278} The spectral characteristics are similar to those of CNTs and provide information on edges, defects and purity. The obtained information about local strains in, for instance, single-layer graphene provides insight into the nanoscale defects of the material. This insight is important for understanding the electronic carrier transport properties of the material. One study reported that the sharp edges on single-layer graphene could be identified and localized by detecting the D-band, which was absent from the centre of the probed flake.¹⁰³ Because the D-band is highly sensitive to the polarization of the incident laser beam, the presence of edges and information on the orientation of graphene-like graphite flakes can be deduced based on changing band intensities.¹³³

TERS has also been applied to analyse local stress in graphene, which is inevitably induced during the epitaxial growth process of graphene on SiC substrates with silicon- and carbon-terminated faces. Here, large epitaxial graphene sheet areas can be observed. Interestingly, the physical and electronic properties of the graphene layers depend on the face on which they were grown: layers grown on the silicon face are homogeneous, whereas layers grown on the carbon face are characterize by having varying numbers of layers and twisted bilayers.^{278,279} Vantasin *et al.* have demonstrated that the presence of 6 nm high ridges in the topography of epitaxial graphene can be correlated with a shift of the G'-band to lower wavenumbers (indicating strain relaxation).²⁷⁸ Such subtle nanostructural changes remain obscured in far-field Raman spectra.

For sake of completeness, a work on graphene oxide should be mentioned. The D- to G-band ratio was reported to successfully localize disorder in voltage-induced graphene oxide and to indicate that defects were not induced by the reduction process.²⁸⁰

A study on explosive nanoparticles comprising TNT (2,4,6trinitrotoluene) and RDX (1,3,5-trinitroperhydro-1,3,5-triazine) should be included at the end of this section. This work is part of the carbon material section because the detonation products are nanodiamonds. In the Raman spectra of the nanoparticle agglomerates, either RDX or a mixture of TNT and RDX was detected. By contrast, the TERS spectra contained almost exclusively information on TNT. From these complementary far-and near-field spectroscopic measurements the existence of core–shell structures (RDX core, TNT shell) was deduced.²⁸¹ This kind of systematic analysis seems to be the most suitable for many other nanoparticles with a core that chemically differs from the shell.

5.b.III. Self-assembled monolayers (SAMs). The properties of a chemically modified surface correlates with the characteristics of the adsorbed molecules. In this respect, packing density, adhesion forces, molecule orientation, hydrophilicity, and hydrophobicity - to name only a few - are essential parameters. Moreover, the surface properties depend on temperature and pressure/vacuum, which influence the mobility of the molecules and the interactions of the interface with the environment. Because the phenomenon of domain formation in mixed SAMs is not fully understood, nanoscale characterization can provide valuable additional information. Generally, atomically flat gold substrates are used for the preparation of SAMs, and the respective thiol group-containing molecules are immobilized by sublimation, by incubation of the substrate in a millimolar solution, by drop casting of a preheated or template-stripped gold surface or by microcontact printing^{35,64,100,109,110,126,132,282} Notably, TERS experiments on SAMs on gold intrinsically work in a gap-mode configuration, independent from the scanning probe technique used. This additional enhancement of the signal is certainly welcome when weak Raman scatterers are probed.

Because of the firm bonding of thiol SAMs, these systems are ideally suited for systematic studies on molecular orientation and the influence of the tip-sample distance on signal intensities. Thus, the length of the thiol plays an important role in controlling the tip-sample distance.¹⁰⁹ The tip-substrate gap could be estimated at approximately 3 nm from the spectra of an azobenzene thiol composed of an alkyl chain and a benzene ring.109 Additional independent studies on an azobenzene derivative⁵¹ and bipyridine derivatives¹⁴¹ reported that these molecules can arrange into 2D ordered nanodomains. Insight into the ordered molecule orientation in an azobenzene containing SAM could be achieved using incident light polarization-dependent measurements. Furthermore, the molecular orientation was deduced by comparing the experimental results with theoretical models. For the azobenzene molecule, the N=N related mode was highly sensitive towards polarization angle changes, and the presence of domains with upright standing molecules and flat lying molecules was suggested.

Systematic time-dependent AFM-TERS experiments on a thiophenol monolayer were conducted to compare the observed spectral fluctuations to those from SERS experiments. The TERS spectra were found to show a larger band position variation than the corresponding SERS spectra (6–9 cm⁻¹ vs. 2 cm⁻¹).¹³² Moreover, the FWHM of the TERS bands was smaller than that

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of the corresponding SERS bands, indicating that the number of molecules contributing to the TERS signal intensity not only is low but also is minimal enough that orientational averaging is not applicable. Similar results were reported for single dye molecule measurements performed using STM-TERS under varying experimental conditions (see also the UHV-TERS Section 2.b).^{122,145,166,180} Both results indirectly suggest that the number of probed molecules in TERS is extremely low.

It is important to mention that a thiol monolayer being investigated under ambient conditions is still a dynamic system. Although the thiols form a covalent bond with the gold surface, they can move and even exchange sites. In this respect, a study in which a second thiol is incorporated into a SAM initially covered with a single thiol-containing molecule via prolonged immersion should be mentioned.²⁸³ In a recent study, binary SAMs have been intentionally prepared by the co-adsorption of phenyleneethynylene thiol and thiophenol from solutions with different concentration ratios.¹¹¹ The molecules form homogeneous SAMs on smooth gold surfaces when immersed independently but form domains when adsorbed from a premixed solution. Although the length of the molecules was quite different, no height differences between the domains were perceived in the STM topography image of the 1:1 mixed sample. By contrast, TERS maps generated from the marker band intensities of the thiols showed a clear contrast, indicating the presence of domains with dimensions of several $\mu m^2 - nm^2$. An earlier approach reported that an exchange of one thiol for another preferably occurs at defect sites on the gold substrate.²⁸⁴

Clearly separated regions containing 2-mercaptopyridine (2-PySH) and 4-mercaptopyridine (4-PySH) could be obtained using a microcontact printing technique.¹¹⁰ In this process, a gold surface was first patterned with 2-PySH, followed by immersion in a 4-PySH solution. The molecules were chosen because of the clear distinction between the two in terms of their marker bands (at 1000 cm⁻¹ and 1100 cm⁻¹, respectively) in the subsequent TERS experiment. Fig. 12a presents the distribution of 2-PySH across the probed sample area and shows clear distinction between the bright circles and darker surrounding fields. Fig. 12b displays the distribution of 4-PySH and the expected contrast inversion.

When a SAM-coated gold surface was densely covered by pyridinyl–biphenyl methanethiol molecules, restructuring was observed. In this work, the TERS data were supplemented by performing cyclic voltammetry on the bulk sample.³⁵ A defect-free monolayer was found to have formed after incubating the substrate in a thiol solution for 6 h. Prolonged immersion led to variations in the molecular packing structure. In the respective TERS spectra, a band shift of the characteristic aromatic ring mode at 1600 cm⁻¹ could be directly correlated with π - π interactions of adjacent molecules. The results indicated that intermolecular repulsive forces effected the observed SAM restructuring.

According to discussed results, SAMs are dynamic surfaces that are subjected to thermal influences, which lead to reorganization and molecular reorientation. Generally, averaged information of the molecular states is obtained, and processes such as configurational isomerization remain elusive.



Fig. 12 Distinction between gold patterned domains of 2-mercaptopyridine (2-PySH) and 4-mercaptopyridine (4-PySH) monolayers with AFM-TERS. (a) TERS map of 2-PySH from the marker band at 1000 cm⁻¹; (b) TERS map of 4-PySH from the marker band at 1100 cm⁻¹; (c) image of the ratio between the marker bands; (d) TERS spectra of 2-PySH (red) and 4-PySH (black). $\lambda = 632.8$ nm, $t_{acq} = 0.5$ s. Adapted from ref. 110 with permission of Beilstein Journal of Nanotechnology.

Because the mobility of a molecule is drastically reduced at low temperatures, isomeric changes can be studied. A recent low-temperature (80 K) UHV STM-TERS experiment demonstrated that the photoinduced trans-cis isomerization of azobenzylthiol derivatives can be monitored by reversible changes in two spectra in time-dependent measurements.⁶⁴ Anti-correlated changes in the spectra indicated isomerization around the N=N bond. Interestingly, isomerization could be photoinitiated only when trans-azobenzene molecules were picked up the tip, not when the molecules lay flatly on the gold substrate. Such a trans-cis isomerization is wavelength dependent and was not detected for the same molecule when the STM-TERS experiment was conducted under ambient conditions at 633 nm.²⁸⁵ This fact was the starting point of experimentally studying the TERS signal of the sandwiched molecule as a function of the tip-sample distance. The TERS signal was found to decrease faster than expected when the voltage was increased. According to the experimental data and a simple theoretical model, the authors concluded that the voltage-induced bending of the molecules in the tip-substrate gap was due to the increased electric field.285

The high sensitivity of TERS even facilitates the acquisition of anti-Stokes bands from a 1,2-benzenethiol monolayer under high-vacuum conditions.^{147,149} The temperature at the tip apex could be estimated from the Stokes to anti-Stokes band intensity ratio, which did not change during irradiation. Similar heating effects were detected in TERS experiments on a conductive polymer film.²⁸⁶ A temperature increase up to 100 K was evident, even at a low laser power of 1 mW.

5.b.IV. Chemical reactions. In 2012, the first monitoring of a chemical reaction catalysed by the tip in an AFM-TERS setup was reported¹⁴² and was well supported by similar experiments.²⁸⁷⁻²⁸⁹ In their study, van Schrojenstein Lantman et al. immobilized p-nitrothiophenol (p-NTP) on smooth gold nanoplates and performed TERS experiments in a gap-mode configuration, taking advantage of both the extra signal enhancement and homogeneous molecule orientation. At that time, the SERS experiments had already revealed the dimerization of pNTP to DMAB.²⁹⁰ Thus, the identification of the reaction products was straightforward and could be time-dependently visualized. To trigger the reaction, the system was illuminated with 532 nm light for 100-130 s (white bar in Fig. 13a), whereas a laser wavelength of 633 nm was used for spectral acquisition. Fig. 13a shows the time-dependent monitoring of the conversion of pNTP into DMAB. The reaction was monitored by the disappearance of the pNTP marker bands at 1335 cm⁻¹ and the appearance of a band at 1440 cm⁻¹, which is characteristic of the azo group in DMAB.

Fig. 13b displays two representative spectra of pNTP and DMAB. The marker bands are highlighted in grey. An intensity plot of the marker bands *versus* time clearly reveals the disappearance and appearance of the main bands (see Fig. 13c).

A similar reaction catalysed by the silver nanoparticle at the TERS tip was reported when *p*-aminothiophenol (pATP) was measured using STM-TERS under high-vacuum conditions¹⁴³ or using AFM-TERS.²⁹¹ Interestingly, when DMAB was irradiated under acidic or basic conditions, the reaction could be reversed, and pATP or pNTP could be identified.²⁸⁷ Conversely, coating the TERS tip with a thin layer alumina could suppress the reaction of pATP efficiently by separating the catalyst from the sample.¹¹² In general, the dimerization is wavelength dependent and is highly efficient when 532 nm light is used. Moreover, the polarization of the excitation laser beam could also affect the reaction.

In contrast to the transverse mode, radially polarized light surprisingly could prevent the reduction of pNTP.¹³¹ The required energy for initiating such plasmon-catalysed reactions is hypothesized to be supplied by "hot" electrons.²⁹²⁻²⁹⁶ Hot electrons were suggested to result from plasmon decay when TERS is operated in a gap-mode configuration. In such a process, the hot electrons provide enough energy to overcome the activation energy barrier necessary for the dimerization of two nitro groups or two amino groups (for pNTP and pATP, respectively). In this respect, thermal energy serving as a reaction trigger could be excluded.^{291,297} A pivotal requirement for the dimerization reaction is a homogeneously distributed reactant, which can be achieved by the aforementioned formation of a SAM on a smooth gold surface. The best strategy is using molecules possessing a terminal thiol group, which assures a firm attachment of the molecule to the gold surface via Au-S bond formation. During the immobilization process, the molecules form stable Au-S bonds with the gold substrate and adopt a highly ordered orientation.²⁸² The scope of chemical reactions that can be catalysed using TERS is not restricted to the formation of azo bonds (-N=N-). Indeed, many reaction can be catalysed by plasmons, providing the activation energy barrier can be overcome. Experiments on monomolecular sheets of anthracene derivatives¹⁶⁷ and the protonation of pyridine¹⁴⁴ are two further examples. The protonation reaction is visible by the appearance of an extra peak in the TERS spectra due to N⁺-H bond formation. Previously, similar observations had been reported for a 4,4'-bipyridine SAM; however, the results could not be explained at that time.126

In conclusion, photons are not directly involved in plasmoncatalysed chemical reactions but instead lead to the generation of hot electrons, which in turn serve as a reaction trigger. Nevertheless, environmental conditions such as the pH, irradiation wavelength and nanoparticle metal remain important reaction parameters.²⁹⁸

When electrochemistry is implemented into a TERS setup, a reaction can also be initiated by changing the potential between two electrodes. In this way, the redox behaviour of



Fig. 13 Dimerization of pNTP into DMAB monitored time-dependently using TERS. (a) TERS spectra at 633 nm shown before (above the white bar) and after (below the white bar) illumination at 532 nm for 100-130 s; (b) selected spectra from (a) before (black spectrum) and after (red spectrum) the illumination; (c) peak areas vs. time for the marker bands of pNTP at 1335 cm⁻¹ ((i) black dots) and for DMAB at 1440 cm⁻¹ ((ii) red dots). The grey bar in (c) indicates the period of illumination. Reprinted with permission from ref. 142 with permission of the Nature Publishing Group.

molecules can be studied at the nanoscale.^{33,34,36} Because this area is the topic on an entire review in this issue, we will only mention a few important experiments here. The first experiments in this field were performed on reversible redox systems using AFM-TERS³³ and STM-TERS.³⁴ In STM, the substrate and the wire tip are intrinsically conductive; thus, only a Pt wire (counter electrode) has to be integrated into the setup. To avoid interference from the tunnelling current and the current generated by the redox process at the tip, the tip needs to be insulated. (4'-(Pyridin-4-yl)biphenyl-4-yl)methanethiol (4-PBT) was used as model compound and could be reversibly protonated (oxidized) and deprotonated (reduced) according to the appearance/disappearance of the characteristic $-N^+$ -H mode by sweeping the potential.³⁴ In the AFM-TERS experiment, ITO was used as conductive substrate on which Nile Blue (NB) was adsorbed. The counter electrode was again a Pt wire, whereas the reference electrode comprised Ag/AgCl. In this specific experiment, the tip was operated in contact mode to ensure direct substrate-sample-tip interactions. Clear spectral differences allowed the differentiation of the oxidized and reduced species. The plotted steps in the correlated cyclic voltammograms led to the conclusion that at some points, only single molecules were probed.33 Both experimental approaches are very promising for investigating reaction mechanisms on the molecular level and may even enable the detection of intermediates and broaden the knowledge of reaction kinetics.

5.c. Biological samples

Interestingly, despite the increased complexity of biological samples, TERS investigations of life science-related compounds have contributed to high-resolution analytical aspects of the technique. Moreover, several very fundamental aspects regarding TERS were initially discovered while studying these types of samples.

5.c.I. Cells, bacteria and viruses. In 2006, TERS was used to investigate biological samples, namely, *epidermidis* cells, where different surface composites could be identified without the need for labelling.²⁹⁹ After this early study, which represented the first example of TERS analysis driven by general life scientific questions rather than clearly specified physical or chemical targets, more TERS investigations on this subject were quickly published.

Preliminary studies on biologically relevant model systems such as calcium alginate fibres (biofilm) and lipid mixtures were considered suitable for establishing a database allowing the classification of these components in complex biological samples.^{300–302} The studies convincingly show that even small volumes of weak Raman scatterers can be identified using TERS. In this respect, attention should be paid to measurements on bacterial membranes, in which TERS measurements on soft biological samples were demonstrated to often bear the risk of tip contamination, thus stressing the importance of control experiments.³⁰³ Because of the fragile texture, small fragments can be easily picked up – even when working in dynamic mode. An undeniable indicator of tip contamination from biological samples is consecutively collected TERS spectra having a striking similarity. Budich *et al.* particularly noted that reference/ control measurements on the bare substrate are recommended to rule out tip contamination. In principal, tip contamination can be associated with not only picking up sample molecules but also the formation of carbonaceous species.³⁰⁴ Naturally, these carbon species influence TERS experiments as well and must be excluded because a large carbon-related background can obscure sample signals.³⁰¹ Carbon is most often generated from an organic sample burning/degrading and can often be avoided by reducing the laser power or by irradiating at a longer wavelength.

In a more detailed study, TERS was employed to collect spectra from equidistant measuring points on cell surfaces. The results showed that the spectral fingerprint of proteins and lipids can be used to classify these molecules and image their distribution accordingly.^{305,306} In contrast to far-field Raman spectroscopy, in which lipids can be identified by bands at approximately 3000 cm^{-1} , this spectral region is often not very prominent in TERS (or SERS). When the laser wavelength matches the absorption maximum of the nanoparticles, the excited surface plasmons at the TERS tip work most efficiently only within a small wavenumber region (for instance, 532-488 nm for silver tips and 633–785 nm for gold tips). Thus, bands above 2500 cm^{-1} often do not experience a strong signal enhancement. Consequently, many experiments focus on the spectral fingerprint region. Despite these challenges, Opilik et al. demonstrated that the higher wavenumber region can be accessed and that C-H bands could be detected on lipid bilayers.³⁰²

Importantly, TERS analysis of cell membranes can become complicated if micrometre-sized areas are probed using a step size of a few nanometres. The high spatial resolution therefore leads to a rapid increase in the number of data points and associated spectra. Conversely, if the distance between the measuring points is set too large (*e.g.*, at 100 nm and above), undersampling occurs.³⁰⁵ Thus, limiting data acquisition to a specific region of interest that can be preselected from a topography image seems more reasonable. In general, for large TERS datasets multivariate data analyses such as N-FINDR,³⁰⁵ Principal Component Analysis (PCA),^{307–309} and Linear Discriminant Analysis (LDA)³¹⁰ were successfully applied for evaluation.

Because the penetration depth of TERS is limited to a few nanometres, buried components remain undetected in the spectra – unless they are resonantly enhanced.³¹¹ This surface specificity enables the identification of molecular changes on cell membranes, which were previously inaccessible without sample degradation. Experiments on wound healing processed cells demonstrate that complementing established biological analyses with the near-field results can provide a more comprehensive image of the sample.³¹² To investigate intracellular compartments, cell sections must be prepared. In this manner, Wood *et al.* used TERS to access haemozoin crystals protruding from malaria infected cell sections.³⁰⁸ The ability to localize this compound was considered very promising for further studies, in which antimalarial drugs, which preferably bind to haemozoin, should be traced.

Additional protein-related TERS studies have focused on understanding the adsorption behaviour of proteins on specific surfaces (*i.e.*, phosphoprotein osteopontin on oxalate crystals¹⁸⁵) or the chemical interaction and localization of protein–ligand systems. Preliminary studies on biotin–streptavidin systems demonstrated promising approaches in this regard.^{313,314} These studies were expanded to a cell model, which was treated with ligand-functionalized gold nanoparticles to bind them to specific receptors. TERS spectra were recorded pixelwise during topography scanning and were found to be highly enhanced when approaching a receptor–ligand–gold nanoparticle.^{315,316}

As already mentioned, cell membrane analysis with TERS is realistic only when specific areas can be selected; otherwise, undersampling must be taken into account.

The situation is different for viruses with diameters below 500 nm. TERS has a clear advantage over established biological methods for the characterization of these infectious species: ideally, a single particle is sufficient for identifying the strain. Several TERS studies have been aimed at distinguishing between viral strains using only their TERS spectra.^{252,310,317} The obtained data were either manually processed or processed using automated procedures. In an early example of this approach, viruses with and without a lipid envelope could clearly be discriminated.³¹⁰

5.c.II. Protein-based material. The first proteins probed with TERS were large systems in which the protein was directly bound to a haem moiety. The TERS spectra of cytochrome c and haemoglobin were found to contain contributions not only from the resonantly enhanced haem moiety but also from the bound protein.^{318,319} Comparatively, in the far-field Raman spectrum of these haem-containing proteins, the weak amide bands are obscured by the dominating bands of the resonantly enhanced haem moiety. Because of the high specificity of TERS, even different oxidation states of the central iron cation could be identified based on the results of an earlier work on haemozoin.^{308,311,319} Furthermore, the spectra changed when the tip was laterally displaced on the molecule, allowing information on the orientation of the molecule on the substrate to be estimated. The resonant Raman contribution of cytochrome c is large enough to detect the compound even when buried in the membrane of the mitochondria, where the evanescent field at the tip apex is supposed to have almost decayed.³¹¹

In addition to haem-containing proteins, glycosylated proteins play an important role in metabolism and are mainly characterized using conventional Raman spectroscopy or Raman optical activity. In general, these compounds have fairly small Raman scattering cross sections and less distinctive features than other biomolecules. Nevertheless, recent TERS experiments have demonstrated the successful identification of glycosylated sites on single mucins³²⁰ and ribonucleases.³⁰⁹

The systematic development of TERS for neat protein analysis began with single amino acids^{129,130,321} and a short peptide³²² and, in 2012, led to detailed studies on complex insulin aggregates, *i.e.*, amyloid fibrils.¹⁶ In this specific work, distinct spectral changes could be observed within the step size of the experiment, indicating a nanometre spatial resolution with AFM-TERS - even at ambient conditions. The studies were expanded to provide a more detailed insight into the surface composition of amyloid fibrils.^{20,57,114,252,323-331} Following the study of aggregated peptides/proteins, TERS became a technique of high medical relevance. Because the exact mechanism of amyloid fibril formation cannot yet be elucidated using standard techniques, a new label-free structural analysis on the nanoscale is a new promising approach to broaden the knowledge of these pathogenic aggregates. Systematic characterization of insulin amyloid fibrils generated at different pH values revealed that these fibrils differ not only in their morphology but also in their secondary structure composition, namely, the abundance of α -helix/unordered structures and β -sheet structures.^{325,327} An even more detailed insight into the surface composition of amyloid fibrils was accomplished by measuring along the main axis of single fibrils with a step size matching the estimated spatial resolution.²⁰ Fitting the spectra enabled the distribution of selected amino acids within in the investigated area to be imaged and local information on the hydrophilicity/hydrophobicity to be deduced. In this work, multivariate data analysis was demonstrated to be applicable not only to large TERS datasets with contributions from chemically distinct components, such as proteins and lipids,^{305,310} but also to only proteins. Apparently, the spatial resolution and sensitivity of TERS enables the probing of an amyloid fibril at the subprotein level without the need of a gap-mode configuration.²⁰ Fig. 14 presents a selection of images from the mapping experiment. The path of the tip on the fibril in Fig. 14a is sketched on the topography image of a single fibril, and Fig. 14b shows a visualization of the distributions of the amide I and amide III bands based on a band fitting analysis of the respective marker bands. The maps correspond very well to those generated using multivariate data analysis (see Fig. 14c).

To visualize the distribution of amino acids, another approach involving STM-TERS was introduced.¹¹⁴ Here, the presence of phenylalanine could be imaged on nanotapes from beta-amyloid (1–40) fragments and was visualized during topography scanning. Because the spectra showed only the characteristic mode of phenylalanine, no additional information on the secondary structure could be extracted from the dataset. Phenylalanine contains an aromatic ring with delocalized electrons and should be more conducive to electron transport than nonaromatic amino acids, which would most likely act as a resistor.¹⁰⁹ Consequently, only phenylalanine could be detected when the tip and substrate were separated by the 5 nm thick nanotapes. Nevertheless, the results show a clear contrast between the substrate and protein structures.

All the above reviewed experiments on amyloid fibrils were aimed at classifying amino acids or conformations on amyloid fibril surfaces and were conducted on aggregates that were grown from the neat protein/peptide solution (*i.e.*, without any additives). Because amyloidogenic structures can be found both on and in cells, studying interactions with lipid membranes is of high interest. Preliminary TERS experiments addressing this issue were performed on human islet precursor protein fibrils that were generated in the presence of lipid bilayers.



Fig. 14 AFM-TERS mapping experiment on a single amyloid insulin fibril without a gap-mode configuration. (a) Topography of characterized fibrils illustrating the path of the tip during the measurement; (b) and (c) maps showing the distribution of the amide I and amide III bands. The maps were generated using band fitting analysis (b) and N-FINDR analysis (c). Adapted from ref. 20 with permission from the Nature Publishing Group.

Essentially, the collected TERS spectra contained lipid contributions and led to the conclusion that lipids were incorporated on the fibrils.³²⁸

More elaborate studies aimed at determining the effect of potential inhibitors on the fibrillation process of insulin. Various small and large organic molecules were chosen, and the isolated aggregates were characterized thoroughly.³³⁰ Although the morphology of the investigated aggregates appeared similar to their morphology in AFM images, the overall secondary structure determined using far- and near-field Raman spectroscopy were clearly different.

5.c.III. Single- and double-stranded DNA. Preliminary experiments on DNA-related material were performed on nucleobases^{69,79,125} and nucleotides.⁶⁹ As expected, the TERS spectra of nucleobases shown in the work by Domke et al. resembled SERS spectra rather than far-field Raman spectra.¹²⁵ By contrast, the TERS spectra recorded by Rasmussen et al. resembled standard Raman spectra more closely than SERS spectra, and neither band width broadening nor band position shifts were observed.⁶⁹ The authors concluded that chemical tip-nucleobase interactions were not operative in the TERS experiment. Most likely, the silver nanoparticle was not directly positioned at the tip apex but instead was lopsided, preventing direct contact. The authors estimated that charge-transfer process-based chemical interactions cannot occur when the tip and sample are separated by 1 nm or more.^{165,332} Those results point towards an aspect that must not be neglected when interpreting TERS spectra: the chemical enhancement not only contributes to the overall signal intensity by a factor of *ca.* 100^{160} but can also shift the band position notably by $\pm 5 \text{ cm}^{-1}$, as systematically examined in comparative SERS and TERS studies on adsorbed thiophenol.132

Measurements on more complex nucleobase containing samples were performed on homopolymer RNA and DNA single strands and revealed that the spectra change when moving stepwise along the strands.^{163,333} Because of the specificity of TERS, assigning different nucleobases on the DNA molecules^{333,334} or deducing the hydrogen bonding situation between adenine and thymine was possible.³³⁵ Furthermore, in this specific work, the specificity and sensitivity of TERS enabled an estimation of the molecule-tip orientation based on subtle differences in the spectra of adenine–thymine base pairs.

In the future, the highly localized spectra acquisition could be exploited to identify defects in DNA/RNA strands or specific interaction zones. The spatial resolution at ambient conditions was only recently found to be high enough to distinguish single bases in single-stranded DNA (ssDNA) comprising adenine and cytosine using AFM-TERS.¹⁶²

A mandatory requirement for such measurements is a consistent immobilization of the DNA *via* the backbone on the substrate. This immobilization can be achieved by pretreating the employed mica substrates with solutions containing either Mg²⁺ or Ni²⁺ cations.¹⁶³ For double-stranded DNA, which has twice the height of ssDNA, silanized glass was reported as an alternative.³³⁶ Consistent with an earlier report,³³³ all nucleobases could be assigned based on their spectral fingerprints. Additional signals of the ribose-phosphate backbone could be identified and was considered as a marker for the differentiation of dsDNA³³⁶ from ssDNA. The capability of TERS to facilitate the detection of subtle differences in chemical structures was clarified once more in a work reporting on dsDNA breaks that were induced *via* UV-C light irradiation.³³⁷ In Fig. 15a, the different potential cleavage sites are indicated by arrows in the structures on the left side. During the TERS measurements, three spectral classes were distinguished using principal component analysis (PCA) and visual inspection.

The first indication of the detected molecular damage was the morphology changing from supercoiled to a more relaxed and linear state. The acquired complete spectral dataset could be divided in three types: first, spectra without phosphate bands, as shown in Fig. 15a; second, spectra with a phosphate contribution and ribose bands (see Fig. 15b); and third, spectra with phosphate bands but without a ribose contribution (Fig. 15c). Detecting terminal groups and thoroughly assigning the bands led to the conclusion that the structural defects occurred at the 3'- and 5'-bonds of the desoxyribose residue.

An even more complex sample is plasmid-embedded DNA, which possesses extra curls compared to dsDNA. A direct comparison of the TERS data of both species revealed that spectra of plasmid-embedded DNA show high band intensities, as more nucleobases contributed to the signal.¹³⁷ Notably, to identify the nucleobases the authors did not consider the spectral range where the characteristic ring-breathing modes were expected but instead focused on bands at higher wavenumbers. As already mentioned, a defined orientation of DNA/RNA on the substrate is highly important for TERS on DNA. This defined orientation can be achieved by either modifying the mica surface, which is generally used for DNA immobilization, or using smooth gold surfaces. Consequently, either the nucleobases point upwards for ssDNA/RNA,^{163,333} or the phosphate-sugar backbone face the tip for dsDNA.^{336–339} However, the conformation of DNA is also susceptible to environmental changes.



Fig. 15 TERS of dsDNA: identification and localization of UV light-induced potential breaks. (a–c) Potential cleavage sites indicated by arrows along with corresponding spectra and the assignment of ribose residues (blue) and phosphate groups (purple). The dsDNA was fixed on mica. Adapted with permission from ref. 337 from Wiley Materials 2014 John Wiley & Sons, Ltd.

In this respect, a detailed study was performed on fragmented model DNA.³³⁹ According to the topographic information obtained using AFM, a partial transition from the B-conformation to the A-conformation occurred when Mg²⁺ or Mn²⁺ ions (for mica surface charge conversion) were added to the DNA solution prior adsorption.³³⁹ The TERS spectra eventually allowed the regions of transition being determined as the end of the strands rather than the middle parts. By contrast, silanization of mica prior DNA deposition did not alter the native DNA B-conformation.

At the end of the application section, the results should be reconciled. In particular, systematic single-molecule and monolayer studies have revealed the impressive potential of TERS. The observed spectral fluctuations in AFM- and STM-TERS indicate the sensitivity of these techniques for changing detection sites and the influence of temperature and vacuum. In STM-TERS, temperature and vacuum can be employed to eliminate thermal drift and contamination in order to increase reproducibility and control environmental conditions. Due to the (sub-)nanometre resolution of TERS, subtle structural changes indicating defects, reaction centres and molecule (re)orientation that would remain concealed using conventional Raman spectroscopy can be visualized. The development of this emerging nearfield technique is constantly being progressed by integrating other techniques. Still, instrumental challenges/limitations must overcome to further broaden the scope of the applications of the technique, as outlined in the last section.

6. Frontiers in TERS

In this section, special conditions for TERS are discussed to indicate the current frontiers of this method. In particular, the main target of the experiments here is the development of novel techniques related to "classical" TERS instead of the characterization of a specific material.

6.a. Tip-enhanced CARS

Nonlinear optical responses of samples increase the spatial resolution of optical microscopy because the effective point-spread function of imaging system is squared or cubed depending on the order of the nonlinearity.^{340–344} This is also valid for the tipenhanced field. Nonlinear signal emission under the tip provides narrower spatial distribution than the intensity distribution of the excitation field.

Coherent anti-Stokes Raman scattering (CARS) is a nonlinear Raman process that amplifies anti-Stokes Raman scattering intensity. The energy diagram of the CARS process is illustrated in Fig. 16a. For CARS excitation, two narrow-band lasers with different frequencies, namely, pump and Stokes lasers with frequencies ω_p and ω_s , respectively, are used. When the two lasers are superimposed, a beat wave is generated at the difference frequency ($\omega_p - \omega_s$). If the beat frequency is equal to a molecular vibration frequency Ω , and the phase matching conditions are fulfilled, then the CARS process is resonantly enhanced, and the resultant anti-Stokes signal is emitted at the frequency of $\omega_{as} = \omega_p + \Omega$. Fig. 16b shows a tip-enhanced CARS



Fig. 16 (a) Energy diagram of CARS. $\omega_{\rm p}$, $\omega_{\rm s}$, $\omega_{\rm as}$ and Ω denote angular frequencies of the pump light, Stokes Raman scattering, anti-Stokes Raman scattering, and molecular vibration, respectively. (b) Tip-enhanced CARS image of a DNA network at a frequency of 1337 cm⁻¹, which is on-resonant with adenine. The CARS intensity profile along the dashed line is shown in the figure. Adapted with permission from ref. 29. Copyright 2004 American Physical Society.

image of DNA double helix filaments comprising adeninethymine base pairs.²⁹ The excitation lasers were tuned at $\omega_p = 786.8$ nm and $\omega_s = 879.3$ nm in wavelength, which is in resonance at the vibrational frequency of 1337 cm⁻¹ and thus corresponds to a characteristic Raman mode of adenine. The CARS signal disappeared when the laser wavelength was detuned from the resonant condition of the sample.²⁹ The intensity profile along the line indicated in the figure shows a spatial resolution of 15 nm, which surpasses the typical spatial resolution obtained using spontaneous (linear) Raman scattering in a comparable configuration.²⁹ Because both the pump and Stokes frequencies were fixed in Fig. 16b, the tip-enhanced CARS image represents a single Raman vibrational frequency. To acquire a full-spectral image in a multiplex fashion, one approach is to substitute a narrow-band Stokes beam ω_s with a broadband light source.345,346 Tip-enhanced broadband CARS images of CNTs have been demonstrated using a supercontinuum light source generated by a photonic crystal fibre.³⁴⁷

Stimulated Raman scattering (SRS) is another type of nonlinear coherent Raman scattering. SRS is free from the nonresonant background that is problematic in CARS.³⁴⁸ SRS was recently combined with TERS,³⁴⁹ and a spatial resolution of 6 nm and an enhancement factor of nine orders of magnitudes were reported.

Tip-enhanced CARS requires two (three) pulsed lasers synchronized in time and space at the very end of the tip, both of which are technically demanding. In contrast to conventional Raman spectroscopy, instead of full spectra, specific vibrational modes within the probed area are sampled. Consequently, the laser system has to be tuned to each specific band of the probed molecule separately. Tip-CARS is very specific, but the current approaches are still demanding.

6.b. DUV resonant TERS

Deep-ultraviolet (DUV) Raman spectroscopy is a powerful option to collect specific chemical information from molecules. In particular, DUV Raman is of interest for bio-related materials, almost all of which have a resonance in this spectral region. For example, nucleobases and aromatic acids show characteristic absorption bands in the DUV below 300 nm. Notably, the intrinsically small Raman scattering cross section is significantly increased when molecules are irradiated with a wavelength close to the molecular absorption band. Thus, in resonant Raman scattering, the signal intensity can be enhanced in extreme cases by even eight orders of magnitude.³⁵⁰ To date, the instrumentation remains a major challenge: the laser sources in the required wavelength range not only are more expensive but also needs the remaining optical setup for this spectral range to be "UV ready". When the N.A. is high, apochromatic microscope objectives with properties close to their visible region counterparts are desired.

For DUV-TERS measurements, a further obstacle has to be considered: silver and gold, which are commonly used for TERS tips, behave as absorptive dielectrics, not as plasmonic materials. Aluminum and indium could be used as alternatives and have dielectric functions with the real part smaller than -2 at wavelengths longer than 140 nm³⁵¹ and 170 nm,³⁵² respectively, indicating that these two elements can support a localized (dipolar) mode of surface plasmon resonance at these DUV wavelengths. Comparatively, the dielectric function of silver does not have a value smaller than -2 at the wavelength shorter than 350 nm, rendering it unsuitable for DUV-TERS measurements. The localized plasmon of aluminum nanoparticles has been studied in the UV^{353,354} and DUV³⁵⁵⁻³⁵⁷ regions. A theoretical study of Al-coated silicon cantilever probes has demonstrated that the instantly formed Al₂O₃ layer notably decreases the enhancement ability of the tip in the DUV.358 Because aluminum and indium have smaller values in their imaginary part of dielectric functions for shorter wavelengths, absorption losses in these metals become reasonably small in the DUV wavelength range. Furthermore, as SERS with DUV excitation has been reported on aluminum films,73,359 aluminum nanoparticles³⁶⁰ and indium islands,³⁶¹ TERS should also be possible using these substrates.

For the first TERS experiments in the UV, aluminum grains with a diameter of ~20 nm were grown on a Si AFM cantilever *via* vapour deposition under vacuum⁷³ and were used to investigate adenine nanocrystals. Using an engaged aluminum tip enhanced the Raman spectrum by a factor of ~ 1.3×10^3 . Such resonance enhancement can be particularly important when the wavelength can be switched between distinct electronic resonances. In this way, a resonance TERS imaging of cell compounds will provide a much more conclusive picture. In addition to the mentioned instrumental requirements that a TERS-DUV setup must fulfil, UV-induced degradation of organic samples must be taken into account. However, a simple technique to overcome this problem has been introduced only recently.³⁶² The addition of lanthanide ions to the sample leads to an energy transfer from the excited molecules to the lanthanides and drastically reduces the probability of dissociation. If this method can be applied as a general approach, then UV-TERS will be able to handle experiments in the visible region.

6.c. TERS in liquids

TERS analysis in aqueous media is still in its infancy but has a large potential to study, for instance, biological samples in their native environment. TERS in the liquid phase involves several issues that must be considered carefully: precise optical alignment and focusing of the laser on the tip and efficient signal collection. The main challenge is most likely to maintain the TERS tip activity and prevent silver nanoparticle degradation, contamination and ablation. One promising attempt is coating a silver tip with a thin SAM, as demonstrated for ethylthiolate, or covering a tip with SiO_r in an AFM-TERS setup.³⁶³ Of course, any coating will be at the expense of the enhancement factor; however, the tip's lifetime can be prolonged notably. In another approach, a tungsten wire was coated with alumina and then with silver.³⁶⁴ The alumina layer was intended to increase the adhesion of the silver layer. Thus, the stability of the TERS tip in aqueous environments should be increased. These tips enabled lipid bilayers of different lipids to be characterized in water. Recently, an electrochemical reaction was performed using a gold tip mounted in an AFM-TERS setup. Here, no tip protection was mentioned, indicating the stability of a goldcoated AFM cantilever under such conditions.33

A completely different approach to AFM-TERS in liquids is presented in a work in which the TERS probe was coated with the molecules of interest.³⁶⁵ The chosen molecules had either a basic ($-NH_2$ in *p*-aminothiophenol) or an acidic (-COOH in *p*-mercaptobenzoic acid) terminal group and were anchored to an etched silver tip wire *via* a thiolate group. A glass substrate was modified with a silane layer with amino groups pointing upwards and served as the sample. The topography was scanned using the functionalized tips, and TERS spectra in liquid were collected simultaneously. The tip–sample interaction induced reactions that could be identified in the TERS spectra by the corresponding modes. Further tip–sample surface dependent measurements revealed that the tips could serve as nanoscale pH sensors.

TERS in liquids was performed not only using AFM setups but also using STM setups. A recent study demonstrated that the side-illumination technique can be applied to the measurement of molecular monolayers of malachite green and thiophenol on gold in aqueous solution.⁵⁸ For STM-TERS measurements in organic liquids, overlaying the probed SAM with a 2–20 μ m layer of hexadecane was also reported to be quite efficient.³⁶⁶ In such an assembly, extra insulation (to avoid current leakage), as demonstrated for an electrochemical TERS experiment,³⁴ was not necessary.

6.d. Beyond 2D-3D intracellular nano Raman imaging

One of the recent trends in optical microscopy is fast 3D imaging, which is especially important for obtaining biologically significant information from living samples. Recent developments in nonlinear coherent Raman microscopy, such as CARS and SRS, have enabled 3D and video-rate imaging of living tissues without sectioning or fixation.^{367,368} However, TERS microscopy is basically a surface imaging technique and has a relatively slow imaging speed.

Recently, 3D and nanoresolution Raman microscopy has been demonstrated through the use of a plasmonic particle.³⁶⁹ In this method, gold nanoparticles are introduced in a living cell *via* endocytosis and used as a SERS antenna.³⁷⁰ The position of the gold nanoparticles is monitored in three dimensions using dark-field optics; simultaneously, the enhanced Raman signal from the gold nanoparticle is measured. The spatial resolution of this microscopy technique is determined by the positioning accuracy of the gold nanoparticle, which is ~ 10 nm laterally and ~ 30 nm vertically, while the temporal interval of successive Raman measurements is 50–100 ms.^{371,372}

Huang *et al.* investigated the 3D particle trajectory of a gold nanoparticle (80 nm in diameter) in a macrophage cell.³⁷³ The particle trajectory and the simultaneously obtained local Raman spectra show significant changes with respect to the spatial location during the measurement. In particular, random motion and the stationary location of the particle were found to correspond to different spectra. Consequently, this technique potentially allows interactions between nanoparticles and biomolecules involved in intracellular transport functions to be investigated.

7. Outlook

The intention of this review is to cover the development and applications of TERS from its inception in 2000 to its current state. In particular, considerable progress has been made in the past ten years regarding TERS instrument stability, tip production, lateral resolution, and theory. Clearly, the technique is accepted and used for applications in various fields. Thanks to commercially available setups, TERS is no longer only for "experts" but rather is open to everyone. A thorough understanding of the high spatial resolution of TERS was lacking, and a clear comprehension of the high-resolution capabilities of TERS could be suggested only recently. In addition, a deeper insight into the effect of incident light polarization and tip–sample orientation on the TERS spectra can elucidate the differences between near- and far-field Raman spectra.

Currently, the majority of TERS experiments are performed at ambient conditions, rendering thermal drift an unavoidable issue. A transfer to low temperatures could overcome this problem and has already been realized in STM-based studies; however, only one group thus far has employed this strategy using an AFM-based setup.

Because of the increasing stability of modern setups, the amount of data in a single experiment is steadily increasing, which necessitates a simultaneous development of appropriate data evaluation methods. In this respect, robust automated methods would be advantageous to avoid time-consuming manual spectral interpretation.

Recent studies concerning biological materials reveal that sample preparation and data interpretation rather than the actual TERS experiments are the primary limiting factors. These issues especially apply to measurements recorded under native conditions. As TERS is a surface-sensitive technique, materials such as cells or tissues have to be pretreated to render internal compartments accessible without interference from the embedding media or buffer.

In general, a reliable prediction about the future development of a technique is risky. Considering the continuous competition between fluorescence, infrared and Raman spectroscopy-based techniques over many decades, any of those spectroscopic method being satisfied with the *status quo* is difficult to imagine. Surely, this is also true for TERS, and it will be very interesting to follow, for instance, where the physical limit of resolution will finally end up or whether time-resolved systems can actually combine ultrahigh lateral and temporal resolution to analyse, for example, a single working protein.

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