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Latest instrumental developments and bioanalytical applications in tip-enhanced Raman spectroscopy

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ABSTRACT

This review intends to provide an overview about the state-of-the art and the latest instrumental developments of tip-enhanced Raman spectroscopy (TERS), particularly addressing selected applications for biological samples. Additionally, a practical guideline of "How to conduct a TERS experiment?" is presented, in order to understand, motivate and practice TERS experiments. Consequently, this review aims to set a basis for further instrumental developments, especially addressing TERS in liquid for prospective applications towards a larger variety of samples.

1. Introduction

Accessing the nanoworld is of major interest to understand fundamental properties and sample behavior down to molecular units. Investigating the nanoscale with optical techniques, requires overcoming the optical diffraction limit. The lateral resolution of an optical microscope, the well-known Abbe diffraction limit [1] is defined by:

$$d = \frac{\lambda}{2NA}$$

Accordingly, depending on the wavelength (λ) and numerical aperture (NA) of the microscope objective, an optimal resolution (d) close to half of the wavelength can be achieved in air. Since then, instrumental development to improve the spatial resolution made progress. In 1928 Synge proposed a radical different approach, essentially the scanning near-field microscope (SNOM) [2], by employing a scanning aperture smaller than the incident wavelength, to overcome the Abbe diffraction limit and access the so-called near-field optical regime. Realization, however, required several decades. Raman spectroscopy gathered high interest for nanomaterial characterization, but as optical technique limited in spatial resolution and by low sensitivity. The second issue was successfully addressed in the 1970s by applying surface-enhanced Raman scattering (SERS), by drastically increasing the sensitivity of conventional Raman spectroscopy [3,4]. Stimulated by the invention of the scanning tunneling microscope (STM) [5], Wessel proposed in 1985 the concept of a scanning SERS setup. This approach combines the benefits from SERS and provides a solution for the spatial resolution issue by utilizing a sharp metallic tip [6]. Consequently, this nowadays called tip-enhanced Raman spectroscopy (TERS) provides with its plasmonically active tip an enhanced Raman signal with high sensitivity and exceptional spatial resolution [7,8]. The necessary step from the idea to experimental realization was shown in 2000 by Stöckle et al. [9], and concurrently confirmed by three different research groups [10–12]. Experimentally, a TERS setup is straight forward: an excitation laser is focused onto a metal or metallized plasmonic active tip, with set polarization along the tip axis generating an enhanced Raman signal, which is subsequently detected. The highly localized signal is mainly based on electromagnetic (EM) enhancement, but also chemical enhancement is not negligible [13]. The EM enhancement results from a combination of localized surface plasmon resonance (LSPR) and antenna effects. Localized surface plasmons are collective electron oscillations at a nanoparticle metal surface excited by

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light. The signal enhancement at the tip apex further increases when the plasmon resonance wavelength matches those of the excitation laser. Hitherto, TERS went through several further instrumentational developments, which are highlighted in the following sections, particularly reviewing latest results for bio-analytical applications.

2. Technical aspects and key components of a TERS setup

TERS combines two well-established techniques: Raman spectroscopy and scanning probe microscopy (SPM). This instrumental combination needs to fulfill three basic requirements: the incoming laser beam needs to be focused onto the tip, the tip has to be in close proximity and in a stable feedback condition with respect to the sample surface and finally, the backscattered Raman signal needs to be detected efficiently. Therefore, the following sections will discuss optical geometries, feedback modes and polarization control and emphasize the key element for a successful measurement – the plasmonically active tip.

2.1. Optical geometries

For an optimized optical geometry of a TERS setup a simple rule applies: it depends on the sample. Different samples require different illumination and detection geometries to obtain a maximum signal, which is mainly related to intrinsic properties of transparent or opaque samples. The schematic diagrams of five different illumination–collection configurations are shown in Fig. 1a–e.

2.1.1. Bottom illumination configuration

The illumination and collection from below the sample was the method of choice for the first TERS experiments and is also called transmission or epi-illumination TERS mode [9,11,12]. In this configuration (Fig. 1a), the SPM tip is located on top of a sample placed on an inverted optical microscope. The excitation laser passes an objective with high numerical aperture (typically $1.3 < NA < 1.6$) and illuminates tip and sample in transmission. The enhanced Raman signal is collected through the same objective. As sample substrates, thin glass slides or mica sheets are employed. High NA oil immersion objectives increase the illumination and detection efficiency correlating with a reduced background, reduced acquisition times and a satisfactory signal-to-noise ratio (SNR). However, this configuration is limited to transparent samples and substrates. To enable access to opaque specimen in this geometry, a special approach by guiding light around the specimen to the tip with a single dichroic mirror right on top of the AFM tip holder has been demonstrated [14]. In combination with a long-working distance objective, this setup enables access to opaque samples without further modification and leaves room for further development.

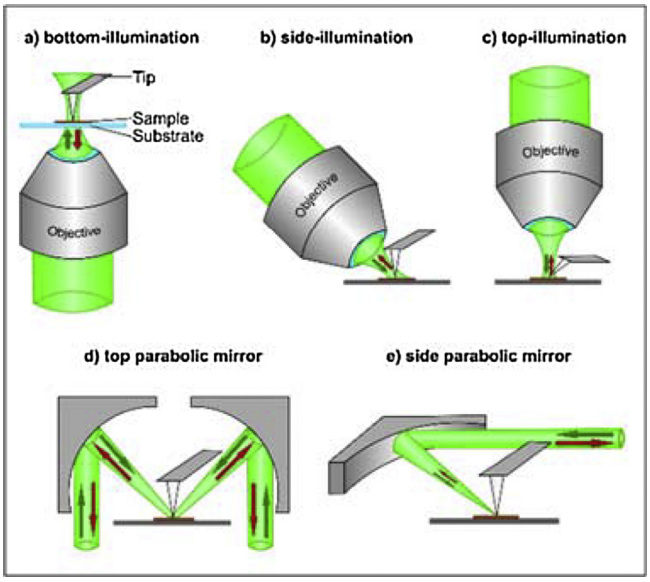

Fig. 1. TERS configurations based on a) bottom-illumination, b) side-illumination, c) top-illumination and d), e) parabolic mirror configuration. Partly adapted from Ref. [16].

2.1.2. Side illumination configuration

Commonly used for opaque samples are side-illumination configurations (Fig. 1b). First applied in 2001 as side-illumination and bottom detection. Instrumental development proceeded quickly and several setups with side-illumination and detection followed, see also cited literature in Refs. [15,16]. Here, the incident light passes a long-working distance objective ($0.28 < NA < 0.7$), which is located at the side of the SPM scanning head. Usually, the light is focused on the tip apex at an angle of 45°–80°. Due to the angle between incoming light and tip, the focal spot becomes elliptical and relatively large, consequently a higher laser power is required to obtain comparable results. The larger spot size also leads to stronger far-field background contributions, resulting in a lower SNR. The collection efficiency for the detection through the same objective is limited by the smaller angle, which can be compensated by top detection with high NA objectives. For sample support, no restrictions apply.

2.1.3. Top illumination configuration

Another well-established configuration is provided by top-illumination and signal collection, also known as reflection mode (Fig. 1c). It combines advantages of bottom and side-illumination configurations and allows to work on both – transparent and opaque samples. In this configuration, long-working distance objectives are required, resulting in a limited NA. The main challenge is the shadowing by the TERS tip. When the incoming light is focused on the tip apex, a certain amount of the excitation light and also the signal is blocked. The key parameter is the NA of the focusing element in order to “see around the tip”. Two major focusing elements fulfill this criterion: the parabolic mirror and special long working distance objectives with high NA. The latter one offers easy handling and is commonly employed for top illumination configurations. Generally limited to a NA of 0.45, this leads to comparatively weaker signal enhancements due to shadowing and generally larger background signals as the excitation spot is larger. Further improvement was recently achieved with 0.7 NA objectives, providing the opportunity to conduct full TERS mappings with acquisition times lower than 1s/pixel [17].

2.1.4. Parabolic mirror configuration

An interesting alternative to “see around the tip” while maintaining maximum collection efficiency, is the parabolic mirror (PM) configuration (Fig. 1d), applicable to transparent or opaque samples. Besides, PMs are intrinsically aberration free. PM setups can be designed in two ways. One approach employs the PM for excitation and collection by locating an on-axis PM (NA~1) on top of the sample. The center of the PM is equipped with an axial hole in order to access the sample with the tip. Thus, the PM is symmetrically illuminated from below and focusses the laser light as a diffraction limited spot on the tip. The Raman signal is collected via the same path back. A second, recently developed approach applies a PM from the side. The excitation light passes an off-axis PM and is