Tip-enhanced Raman Spectroscopy - Bio-Molecule Identification on the Nanometer Scale

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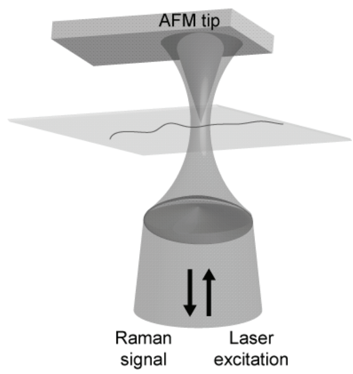
Tip enhanced Raman scattering (TERS) is a recent application of the well established surface enhanced Raman spetroscopy (SERS) technique. TERS combines in a unique way the advantages of the field enhancing properties of SERS and the high lateral resolution capabilities of near-field optical microscopes. The technique has been conceptually predicted by Wessel [1] 1995 but it lasted 15 years until the first experimental verification using a silver coated AFM tip on a brilliant cresyl blue substrate[2].

Fig 1 shows the basic setup of a back reflection TERS instrument. The sample is placed in the optical near field of a metal protrusion that enhances the electromagnetic field. The active part can either be a sharp edge of a metal wire or a small nano particle. There are also other optical arrangements that use side-illumination/collection modes, however, if the sample is transparent as it is the case for most biological samples, the crucial advantage of the back-refection setup is its high collection efficiency.

TERS is always useful, when the key challenge is to obtain molecular information on a nanometre scale. Potential applications range from industrial questions (semiconductors, polymers) to basic research. Of specific interest in the semi conductor field is for instance the detection of stress in silicon.



More related to organic chemistry is the investigation of carbon nanotubes using TERS. Lateral resolution down to 30nm has been demonstrated and a clear distinction of different single walled carbon nanotubes by means of a combination of Raman detection and simultaneous fluorescence detection is feasible.

Biochemistry and cell biology are other fields of research where molecular information with a lateral resolution beyond the diffraction limit of visible light is required. The applications here range from the chemical analysis of smallest amounts of biological active substances to the identification of cell wall compounds in vivo.

With respect to the analysis of biomolecules, the aspect of a direct and label-free sequencing of nucleic acid or amino acid based polymers is probably the most intriguing. TERS investigations of nano crystals of single nucleobases already proved the possibility to distinguish between the four bases[3]. Recent experiments on a single RNA strand immobilized on mica also demonstrate the potential of single base identification when the tip is scanned along the strand. Such a single base would be required for a direct sequencing method.[4] This principle can then be applied to the sequencing of peptides and proteins in a general fashion. A major issue is the immobilization of the molecules to the surface. Novel transparent gold plates provide a smooth enough surface for single DNA investigations while even further enhance the electromagnetic field. Thus compensating for the transmission losses.

Many compounds or active sites in a cell are much smaller than the diffraction limit. While the progress in single cell Raman spectroscopy is very impressive, the detected spectra are very complicated because they naturally represent the mixture of all the chemicals in the cell. With a high enough spatial resolution combined with a sufficient enhancement of the Raman signals, not only a mapping of the cell would be possible with higher resolution, also the spectra should become much more simple and easier to assign to specific compounds. shows the example of a TERS experiment on a living *S. Epidermidis* cell. The signal-to-noise ratio between “tip-in-contact” and “tip-retracted” was about 60:1 compared with the area difference of tip and laser spot, and also taking several other measurement parameters into account an overall enhancement of 6 to 10 orders of magnitude can be estimated. Such enhancement factors can be used to further decrease acquisition times considerably.[5] This also necessary because with the small volumes involved with TERS, the diffusion of certain cell compartments or even single molecules can not be neglected anymore. This effect was already characterised earlier, when in the course of a TERS sequence experiment at a fixed position on a cell wall, the spectral features fluctuated. The observed changes could be assigned to all major expected cell wall compounds.[6]

The high resolution capabilities have been also applied to the investigation of Malaria infected blood cells, where the distinction between hemoglobin and hemozoin, the major product of the parasite and a potential target for anti-malaria drugs was demonstrated.

TERS is presently the only tool that can be used to molecularly assign compounds on the nanometre scale. But not only the applications are attractive, also the investigation of novel physical effects that arise when single objects are investigated on such a small length scale is very interesting. Small molecules will move or rotate, the influence of the surface must be considered, and effects of the TERS tip and the sample must be taken into account and might even open new possibilities for sample identification

1. J. E. Wessel, "Surface-enhanced optical microscopy," J. Opt. Soc. Am. B **2**(9), 1538 (1985).
2. R. M. Stöckle, Y. D. Suh, V. Deckert, and R. Zenobi, "Nanoscale chemical analysis by tip-enhanced Raman spectroscopy," Chemical Physics Letters **318**(1-3), 131-136 (2000).
3. A. Rasmussen and V. Deckert, "Surface- and tip-enhanced Raman scattering of DNA components," J. Raman Spectrosc. **37**(1-3), 311-317 (2006).
4. E. Bailo and V. Deckert, "Tip-Enhanced Raman Spectroscopy of Single RNA Strands: Towards a Novel Direct-Sequencing Method," Angew. Chem. Int. Ed. **47**(9), 1658-1661 (2008).
5. C. Budich, U. Neugebauer, J. Popp, and V. Deckert, "Cell Wall Investigations Utilizing Tip-Enhanced Raman Scattering" J. Microsc. in press.
6. U. Neugebauer, U. Schmid, K. Baumann, W. Ziebuhr, S. Kozitskaya, V. Deckert, M. Schmitt, and J. Popp, "Towards a Detailed Understanding of Bacterial Metabolism - Spectroscopic Characterization of Staphylococcus Epidermidis," ChemPhysChem **8**(1), 124-137 (2007).