

Surface-enhanced Raman Scattering for the Assessment of Biomolecule-Nanoparticle-Interactions

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Introduction

Within the working group of Prof. Zellner at the Institute of Physical Chemistry of the University Duisburg-Essen, the effects of nanoscale particles in the biological environment are investigated. In order to elucidate the molecular and mechanistic details of such bio-nano interactions, surface-enhanced Raman spectroscopy (SERS) is used as one of the state-of-the-art methods. In general, the SERS effect of nanoscopic materials is due to their localized surface plasmon resonances and, hence, an increased electro-magnetic field in the near of the surface is generated which amplifies the scattering cross-sections of adsorbed Raman reporters. The evanescent character of the enhancing field allows conclusions about surface functionalization as well as adsorption geometries of biomolecules adsorbed onto the nanoscale surfaces. SERS substrates such as nanoscopic copper, silver, gold and some other heavy metals provide particularly high enhancement factors. However, light adsorption of SERS substrates produces not only Raman enhancement but also local heating, which can result in a thermal decomposition of adsorbed molecules. Therefore, we have designed and deployed a Raman setup which allows simultaneous detection of the local temperature.

Experimental

Our Raman microscope setup consists of a 532 nm cw, diode-pumped solid-state laser which is reflected into a microscope objective (10x, 0.2 NA), and is then used to stimulate the emission of Raman light within the sample. The backscattered Raman light is collected throughout the same objective, passed through two notch filters (Kaiser Optical Systems, holographic super notch plus) and is then fed into the spectrometer module via a specially made-up multimode fiber. This fibre produces a slit image over a holographic grating (1800 gr/mm) on a sensitive EMCCD-Camera (Luca-R DL604, Andor Technology, 1004 x 1002 pixels), providing single photon detection sensitivity and unrestrained quantum efficiency of about 65%.

Results

In order to estimate the local sample temperature we chose cystein as a Raman reporter. The corresponding temperature information is contained in the line intensities of Stokes and anti-Stokes regions of the Raman spectrum. These intensities were investigated using dif-

Application Note

ferent laser powers. Due to the challenging simultaneous detection of Stokes and anti-Stokes scattered light the use of a highly sensitive EMCCD camera such as Luca-R is indispensable.

The derived Raman spectra as obtained with an acquisition time of 40 s and with performing 5 accumulations are shown in Figure 1. The recorded spectra are plotted without any corrections. One single frame of a 40 s accumulation time is inserted in the top of the chart.

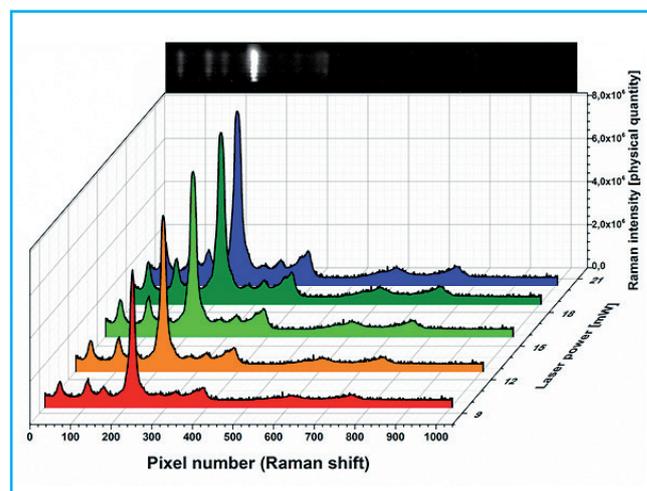


Figure 1. "Waterfall" diagram of Raman spectra of bulk cysteine derived with increasing laser power (9.5, 12.7, 15.8, 18.7 and 20.4 mW)

The rejected and therefore invisible Rayleigh line is centered at approx. pixel 520 (correlated to 0 cm^{-1}) on the EMCCD chip. The Stokes region (0–480 pixel) and the anti-Stokes region (560–1004 pixel) are shown on the left hand side and right hand side of the spectrum, respectively. The Stokes region is lower in energy compared to the exciting light due to vibronic transitions, and thus it contains information about the molecular structure of the Raman scatterer. The higher energy anti-Stokes photons contain the same information. The intensity ratio of anti-Stokes and Stokes scattering (I_{AS}/I_S) of the same vibration mode is the result of the temperature-dependent population probability of the vibrational energy levels. If a Boltzmann distribution of vibrational levels is considered, the temperature of the sample can be calculated as follows:

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$$T = \frac{-h\nu_{vib}}{k_B \cdot \ln\left(\frac{I_{AS}}{I_S}\right)} \quad (1)$$

In this equation h denotes for Planck constant, ν_{vib} is the vibration frequency and k_B the Boltzmann constant. Applying this equation, the highly intensive SS stretching frequency of bulk cystine, are used (approx. 220 pixel in the Stokes and 760 pixel in the Anti-Stokes region, corresponding to wavenumbers of $+498.5 \text{ cm}^{-1}$ and -498.5 cm^{-1}).

It is found that for low laser powers there is little deviation of the local sample temperature from the ambient temperature. However, as the laser intensity is increased the local temperature rises. Figure 2 shows the laser power dependent temperature, calculated from the Raman-spectra according to equation (1):

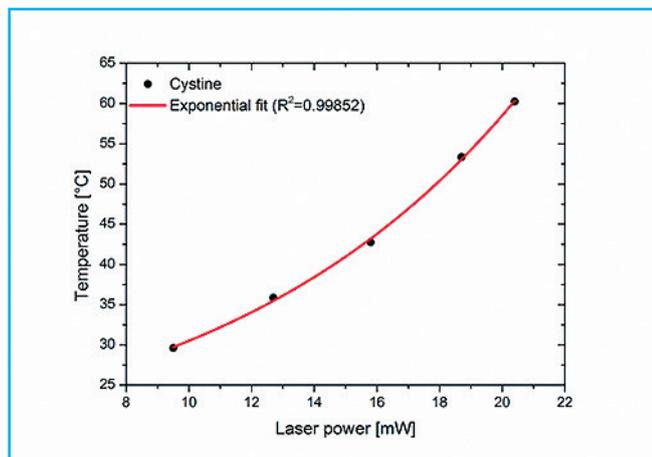


Figure 2. Local temperatures of bulk cystine calculated with Raman intensity ratios from fig. 1 assuming Boltzmann distributed vibrational states of the SS bond stretching frequency.

As can be seen from this figure 2 the local temperature in the focal volume of a microscope objective depends exponentially on the laser power. Therefore, the Raman reporter cystine is significantly heated by laser light exposure, although it is a white solid and hence its light absorption in the visible range should be small. But these small light absorptions are sufficient for the observed temperature increase because of the high volume power density as obtained with the microscope objective.

Application Note

In summary, these results show how local temperatures can be derived from Raman spectroscopic measurements when Stokes and Anti-Stokes regions are simultaneously detected. This procedure is very helpful in interpreting experimental results from SERS as well as other Raman techniques. More importantly, it has been shown in this work that the EMCCD camera Luca-R DL604 is not only suitable for fluorescence microscopy but also for detection of low-light anti-Stokes scattering.

References

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