Resonance Raman spectroscopy of amino acids and proteins

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We propose an experimental technique for measuring the resonance profiles of amino acids in the ultraviolet range. Recording Raman spectra in a broad excitation range demands a precise spectral calibration of the setup. An algorithm for postprocessing the single spectra is presented. The resonance profile indicates the optimal wavelength for obtaining the characteristic features of a molecule.

1 Introduction

Raman spectroscopy is a promising tool for investigating molecules in a nondestructive manner. The information ranges from molecular structure [1] to characterization [2] and identification of constituents [3]. Due to the small cross section of Raman scattering [4], an experimental setup with a high sensitivity is required. Resonance Raman spectroscopy (RRS) is able to generate a much higher Raman intensity and is applied when the excitation wavelength is in close vicinity to the absorption line of the molecule that is analyzed [5, 6]. The implementation of RRS demands no basic changes of the experimental setup compared to normal Raman spectroscopy. The enhancement factor compared to the latter can be as high as five orders of magnitude [4]. The absorption maxima of small biomolecules (e.g. amino acids) are located in the ultraviolet (UV) range [7, 8].

In order to find the excitation wavelength best fitting to the analyte, the wavelength dependent resonance enhancement (resonance profile) of that molecule is recorded. For this purpose, a tunable light source, a device for monitoring the applied energy, filters for blocking the Rayleigh scattered light, a spectrograph calibrated for a broad spectral range, and a suitable algorithm for postprocessing the raw spectra are required. We present a technique to obtain the resonance profiles of amino acids in the range from 244.8 nm to 266.0 nm.

2 Experimental setup

The setup used to measure the resonance profiles of amino acids is shown in Fig. 1. The excitation wavelength is delivered by an optical parametric oscillator (OPO, Ekspla PG122/UV). A beam splitter (B) made of fused silica reflects a small part of the light onto a photodiode (PD, Thorlabs DET10A/M) that tracks the amount of energy irradiated into the sample. The remaining light is focused by a lens (L1, f = 20 mm) in a cuvette (C) containing the sample. The second lens (L2, f = 20 mm) collects all scattered light and directs it through a long pass filter (LP, Semrock SEM-LP02-248RS-25, SEM-LP02-257RU-25, or SEM-LP02-266RU-25) that cuts off elastically scattered light. The Raman scattered light is focused by the last lens (L3, f = 50 mm) into a fiber (F, Thorlabs UM22-100) guiding it to a spectrograph (Andor Shamrock SR-500i-C-R with an Andor Newton DU940P-BU camera) for detection.

In order to image the Raman spectra onto the spectrograph at different excitation wavelengths, the grating of the spectrograph needs to be turned. Three grating positions were figured out for covering the spectral range where biomolecules show their characteristic features. According to this and the long pass filters used, there are three excitation wavelength bands ranging from 244.8 nm to 249.7 nm, from 252.4 nm to 257.6 nm, and from 260.3 nm to 266.0 nm. For recording resonance profiles, the single Raman spectra need to be comparable to each other in terms of relative wavenumbers and spectral intensities, making a precise calibration of the experimental setup essential.

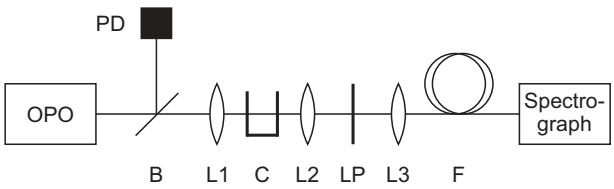

Fig. 1 *Experimental setup consisting of OPO: optical parametric oscillator, B: beam splitter, PD: photodiode, L: lens, C: cuvette, LP: long pass filter, F: fiber.*

3 Calibration

The wavelength dependent sensitivity of the experimental setup is taken into account by applying the device spectral response function (DSRF). The measured Raman spectra are divided by this function in order to correct them in terms of spectral intensities. The response function is obtained by emitting single pulses from the OPO at a specific wave-

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length. Their spectra are recorded and fitted with a Voigt profile to get the spectral response of the corresponding wavelength. This procedure was conducted for all wavelengths where Raman peaks are located.

4 Data postprocessing

The raw spectra measured with the experimental setup shown in Fig. 1 need to be postprocessed in order to make them comparable in terms of relative wavenumbers and Raman spectral intensities. In Fig. 2 the flow chart for data postprocessing is shown. Starting with a raw spectrum, the first step (1) removes any artifacts caused by cosmic rays. When the excitation wavelength approaches an absorption line of the analyte, a more prominent fluorescent background arises. The second step (2) calculates a baseline that is subtracted to correct the spectrum. The following step (3) accounts for the spectral properties of the experimental setup. Each data point of the spectrum is divided by the corresponding part of the DSRF. Step (4) compensates for the strong wavelength dependence of the scattering cross section and the actual energy that is irradiated into the sample. The last step (5) shifts the origin of the wavenumber axis to the position of the Rayleigh peak.

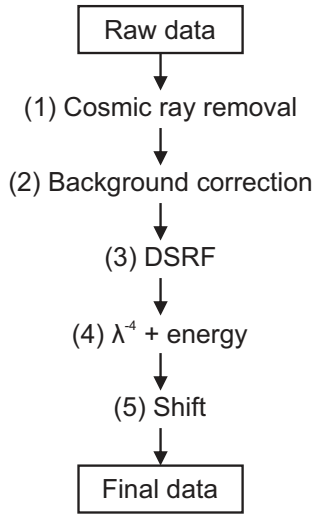

Fig. 2 *Flow chart for postprocessing of Raman spectra.*

5 Results

To illustrate the dependence of the Raman intensity on the excitation wavelength, an excitation-emission map (EEM) is recorded. The postprocessed raw spectra (cf. Fig. 2) are plotted as rows in a matrix. The Raman peaks appear as perpendicular lines. The increase of Raman intensity along these lines show the resonant enhancement in RRS. In Fig. 3 the characteristic EEM of proline is shown.

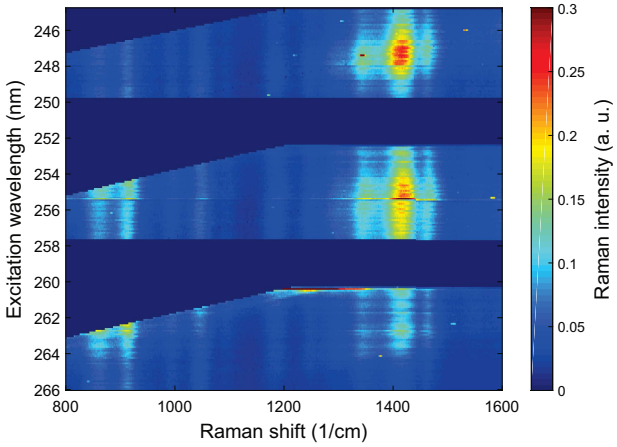

Fig. 3 *Excitation-emission map (EEM) of proline.*

6 Conclusion

In this study we presented an experimental setup combined with an algorithm for postprocessing Raman spectra for investigating the resonant enhancement in RRS. The excitation-emission map of proline suggests shorter excitation wavelengths being more efficient for obtaining the characteristic features of this molecule.

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