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A Princeton Instrument CCD is an ideal detector for ultrasensitive confocal micro-Raman chemical identification, quantification and imaging. Here we demonstrate a user-friendly system for proteomic analysis of fmol quantities derived from μL volumes of μM protein solutions; a regime in which Raman spectroscopy was previously considered to be impossible – the results tell a different story. Not only can high quality spectra be obtained from such samples, but they can be used to distinguish subtle chemical differences such as single amino acid substitutions and post-translational modifications (1–4). Princeton Instruments, in a joint effort with SpectraCode, Inc. can provide a complete micro-Raman system solution to take such spectral measurements.

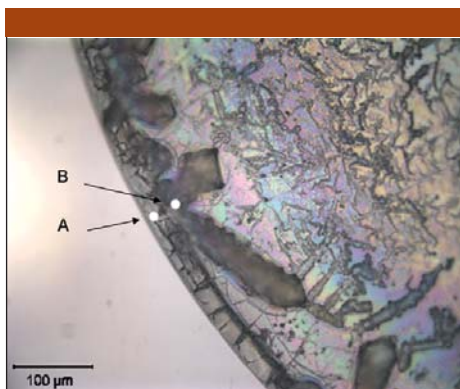


Figure 1. Microscope image of slide after 5 μL droplet containing 20 μM lysozyme and 2 mM PBS (phosphate) buffer was deposited.

System Description

The following confocal micro-Raman system was designed by Yanan Jiang of SpectraCode Inc. and built at Purdue University for proteomic research. The Raman system includes a 300 mm focal length imaging spectrograph (Acton Research SpectraPro 2300i with a 600 g/mm grating) and liquid nitrogen cooled CCD detector (Princeton Instruments Spec-10:100BR/LN, back-illuminated, deep-depletion, LN cooled CCD camera). It is equipped with a 632.8 nm He-Ne excitation laser, which is first bandpass filtered to reject plasma lines, and then reflected by a holographic filter. The laser beam is then coupled to a microscope and focused using a 100X objective lens to produce a spot size of about $\sim 1 \mu\text{m}$ diameter and a power of 6 mW at the sample. The back-scattering light is collected using the same objective, passed through the same holographic filter and an additional holographic filter. A 25 mm diameter lens with 75 mm focal length is used to focus the Raman scattered light onto 7 x 50 mm core-diameter collection fiber bundle which is circular at the collection end and linear at the entrance slit of the spectrograph. A spatial filter placed between the two optical holographic filters in the collection path. This spatial filter consists of a pinhole with diameter of 50 μm situated between two 75 mm focal length lenses separated by a pinhole, such that light is collimated before and after the lenses.

Representative Results

The results shown the figure were obtained using the recently developed drop coating deposition Raman (DCDR) (1–4) method, in which a small volume of a dilute solution of protein (and buffer) is deposited on a slide, consisting of a highly polished stainless steel coated with a nm Teflon coating (5). In particular, a 5 μL droplet containing 20 μM lysozyme and 2 mM PBS (phosphate) buffer was deposited on the slide to produce the concentrated evaporation residue shown in the micrograph. Note that in Figure 1, the protein and buffer segregate, as the protein forms a “coffee ring” around the edge of the $\sim 1 \text{ mm}$ diameter deposition region. Thus, the Raman spectra taken from different

Confocal Micro-Raman Spectroscopy and Proteomic Applications

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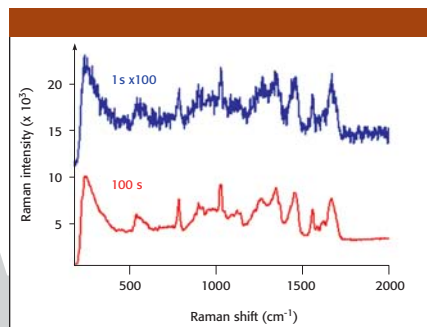


Figure 2. Raman spectra of pure lysozyme; 100 second integration vs. 1 second integration.

points contain either pure lysozyme, as shown in Figure 2, or only phosphate salt, as shown on Figure 3. This method may also be used to remove fluorescent impurities from various other sorts of solid compounds of research, pharmaceutical or biochemical interest. Even with a laser power of only 15 mW it is possible to obtain clearly identifiable Raman spectra in only 1 s or super high-quality spectra in 100 s.

References

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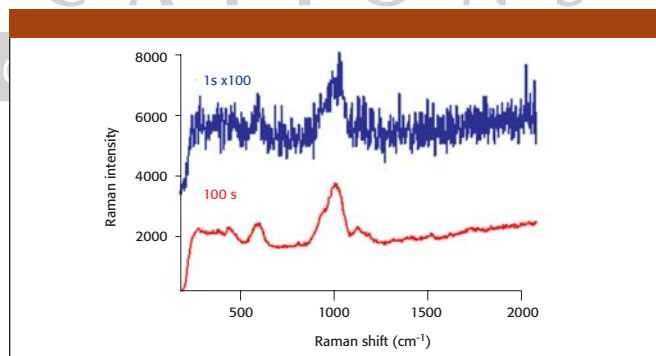


Figure 3. Raman spectra of phosphate salt; 100 second integration vs. 1 second integration.

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