Tienta Sciences

Application Guide AG-001
Raman Detection of Proteomic Analytes

Introduction:

Raman spectroscopy can be a powerful probe of structure and function in biochemical systems.[2-5] Recently, Dor Ben-Amotz described the technique of Drop Coating Deposition Raman (DCDR) for obtaining Raman spectra of protein solutions[1]. In the application guide described here, Raman spectra can be obtained from the manual deposition of uL volumes of uM protein solutions. This application guide demonstrates the method for detecting down to 5 pmol ($1X10^{-12}$ moles) of material.

Although the pre-concentration (solvent evaporation) process produces protein deposits that are in a solid-like state, they appear to remain well hydrated.[1] Protein deposits on non-wetting substrates often accumulate in a circular ring formed during the evaporation process once the protein reaches its saturation concentration.

This well-known "coffee ring" effect[6] further localizes the deposited protein and thus facilitates additional detection limit improvement.

Raman Instrumentation

This application requires a Micro-Raman Instrument with a CCD detector equipped with a high magnification objective (e.g. 100X).

Materials and Reagents

- Tienta SpectRIM Slide
- lysozyme (MW 14,388)
- water (18.2 MΩ cm^{-1})
- Micropipette capable of delivering 1 to 10 uL of liquid

Preparation of 10uM Protein Solution

Prepare a convenient volume (< 1 mL) of a 10 uM solution of Lysozyme by dissolving Lysozyme in distilled or deionized water at a concentration of .144 mg/mL.

Note: Care should be taken to avoid fluorescent contaminants from water, glassware, and other laboratory equipment.

Procedure

Deposit volumes of 5 and 10 uL of the 10 uM Lysozyme solution onto the metal surface (substrate) of Tienta SpectRIM Slide. Be careful not to touch the pipette tip to the surface of the substrate.

Allow the spots to dry for at least 20 minutes. The width of the outer protein ring scales approximately with protein concentration, and results in deposits with diameters of 2-4mm and rings of approximately 10-150 um in width. No spectral changes should be observed after drying periods as long as several weeks. Note: The drying times and deposition appearance are dependent on the temperature and humidity conditions.

Focus on an area of the outer ring and collect the Raman Spectrum. Use a low illumination power to avoid burning the sample.

Results and Discussion

Figure 1 shows the white light optical image of a portion of a 10 uL deposit of lysozyme. The deposition resulted in a circle of a ~4 mm diameter.

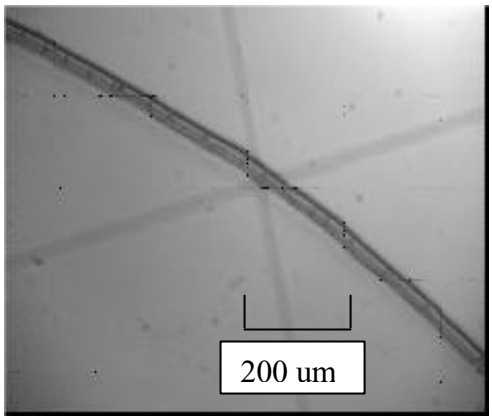

Figure 1. White light optical image of portion of lysozyme ring.

Figure 2 shows the Raman spectra obtained from the outer ring of the lysozyme deposit. The

lysozyme spectrum shown in Fig. 2 has a comparable signal-to-noise ratio to that reported by Carey et. al.[7] and Pelletier and Altkorn[8], which were obtained from solutions of higher protein concentration. Furthermore, the present results were obtained without any solvent or substrate background subtraction.

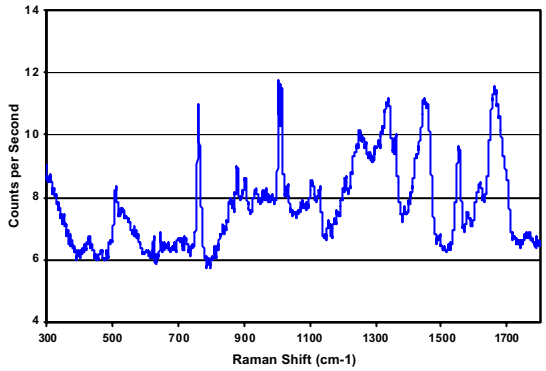

Figure 2. Raman Microscope spectrum of lysozyme ring deposited on the Tienta SpectRIM™ slide recorded on a JY LabRAM (100x objective, 600 g/mm grating, 633nm laser, 8 mw @ sample, continuous Kiefer scanning mode, 20x3 sec).

The present work demonstrates that DCDR may be used to obtain high-quality normal (non-enhanced) Raman spectra from picomole quantities of protein. Ben-Amotz has reported spectra from femtomole quantities by using an ink-jet microprinter[1]. The resulting spectra are highly reproducible and essentially identical to those obtained from higher concentration protein solutions. The spectra are independent of laser intensity and remain unchanged over the period of weeks.

Results may vary depending on instrumentation, illumination wavelength integration time and the thickness and purity of the sample.

References

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This Application Guide was produced jointly by Tienta Sciences, Inc., Jobin Yvon, Inc. and Dr. Dor Ben-Amotz of Purdue University.

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Part Number AG-001-01 2/25/2004