Tienta Sciences

Application Guide AG-004
Raman Detection of DNA

Introduction

Raman spectroscopy is proven to be useful in the study of DNA isolated from herring sperm, calf thymus, *Micrococcus luteus*, *Clostridium perfringens*, and *Escherichia coli*. (1-3). Ultimately, several studies were carried on DNA in fibers, solution and films (4-5).

While Raman spectroscopy is very discriminative and sensitive, a drawback could be observed with isolated DNA samples. Indeed, the isolation of genomic DNA, through extraction with commercially available DNA purification kits, results in samples that are too dilute for direct Raman spectroscopy measurements when using standard substrates such as glass. This problem can be overcome using the Tienta Sciences SpectRIM™ slide.

This application guide demonstrates how, even dilute, solutions of DNA can be studied by using the SpectRIM slide and describes the Drop Coated Deposition Raman (DCDR) technique invented in the laboratory of Ben-Amotz et al. (6).

Raman Instrumentation

Any moderate to high resolution Raman system can be used with this application guide.

Data described in this application guide were collected with a conventional 90° optical configuration sampling geometry Raman system 633. A 1.4 W argon ion laser was used at 514nm. The power at the sample was 250mW. The SpectRIM slide loaded with sample was positioned so the specularly reflected laser beam did not enter the collection optics. Data was acquired using a 30 second accumulation scan with a total of 85 scans per sample.

Materials and Reagents

- SpectRIM slide
- DNA sample 5 to 30 µg/ml in water
- Micropipette

Preparation of DNA Solution

The DNA sample used in this application guide was collected from *Bacillus megaterium.* Cells were lysed using lysozyme and detergents were used to aid in the dissociation of the protein-DNA complexes. Proteins were cleaved and nucleases removed by Proteinase K digestion.

DNA was then separated from cellular components and isolated using the GenElute™ Bacterial Genomic Miniprep Kit.

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

Procedure

A 200 to 1000 µl sample of DNA was deposited on the SpectRIM slide then allowed to dry for 6 to 8 hours at room temperature to produce a DNA spot of 1-3 mm diameter. The spotted slide was stored in a desiccator to prevent contamination. Smaller sample deposits of 2 to 10 µl were realized for micro-Raman measurements and required under an hour of drying time.

Results and Discussion

Figure 1 shows the white light optical image of *Bacillus megaterium* DNA deposited on the SpectRIM slide. The 400 µl deposition at 20 µg/ml resulted in a circle with a diameter of approximately 2 mm.

Figure 1. White light optical image of the *Bacillus megaterium* DNA deposit on the SpectRIM slide.

The image was obtained using a Nikon Light optical microscope with a Sony CCD high-resolution video camera

Figure 2 shows the Raman spectra obtained from the deposit of *Bacillus megaterium* DNA. The Raman instrument was equipped with a 514 nm laser and 1800 grooves/mm grating, providing a spectral resolution of 1 cm^{-1} over the region 3800-150 cm^{-1}. Laser power at the sample was 250 mW. Exposure time was 30 seconds, with 85 scans collected, each spectrum consisting of a total of five frames.

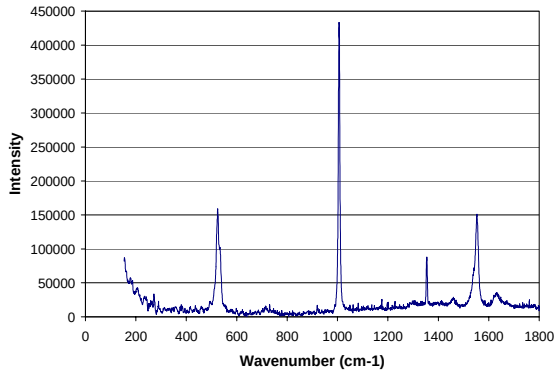

Figure 2. Raman spectrum of a deposit (8 µg) of *Bacillus megaterium* on the SpectRIM slide.

The present work demonstrates that DCDR may be used to obtain high-quality normal (non-enhanced) Raman spectra from quantities of DNA normally extracted from commercial kits. The resulting spectra are highly reproducible.

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

This Application Guide was produced jointly by Tienta Sciences, Inc. and Ms. Connie Gryniewicz and Dr. Clifton Merrow at the University of Missouri, Rolla.

If you have any questions, please contact Tienta Sciences at

Phone (317) 274-0690
Fax (317) 278-8190
Web: WWW.TIENTASCIENCES.COM

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Tienta Sciences, Inc.
351 West 10th St.
Indianapolis, Indiana 46202
Phone: (317) 274-0690
Fax: (317) 278-8190
www.tientasciences.com

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