APPLICATION NOTE

Pervasive Spectroscopy

1064 nm Dispersive Raman Systems in Biofuel and Plant Research

Raman now works on highly fluorescent plant-based samples without sample preparation.

Raman spectroscopy is a non-invasive, highly sensitive technology to quantitatively probe and analyze chemical compositions and structures. It requires essentially no sample preparation. However, in the past it did not find much usefulness on plant-based samples, due to the high level of photosynthetic pigments in those samples. Their fluorescent background can easily overwhelm any Raman signals in all visible wavelengths. This issue is now resolved by BaySpec, Inc.'s complete line of 1064 nm excitation dispersive Raman systems that offer maximum reduction in fluorescence interference.

For example, as we strive for reducing greenhouse gas emissions and energy security, biofuels (both cellulosic and algal based) are becoming a current focus of government funding, research efforts, and many industries. Intensive R&D effort is the key to make the new-generation of biofuels economical and widely available. Traditionally, these efforts are largely based on wet chemistry methods which are not efficient because they are very slow and they need spend large amounts of samples. Some fluorescence based optical methods allow in-situ analysis but only work on very limited samples. Raman spectroscopy would be ideal for high-throughput and real-time analysis. However, traditional Raman instruments based on visible and NIR (e.g., 785 or even 830 nm) lasers induce strong fluorescent background from plant-based samples and render the method useless.

Only now, BaySpec's 1064 nm Raman systems offer the means to minimize interference from fluorescence and unmask Raman spectra for those highly fluorescent samples. We use corn stover and microalgae as an example. Both are popular plant biomass sought for new-generation biofuels. Corn biomass is a feedstock for cellulosic ethanol. Microalgae can efficiently produce high level of lipids which then can be converted into biodiesel. Due to their abundance of pigments, only 1064 nm Raman systems can produces their Raman spectra.

BaySpec's ***Agility***™ transportable Raman spectrometer is used in these studies. ***Agility***™ delivers high sensitivity and repeatability in an affordable, ruggedized, battery-operated package, available in 532, 785, and 1064 nm with single or dual band options. Its integrated sample compartment and Raman probe options allow the utmost flexibility, via its quick-change, auto-aligning sample holders (**Figure 1**).

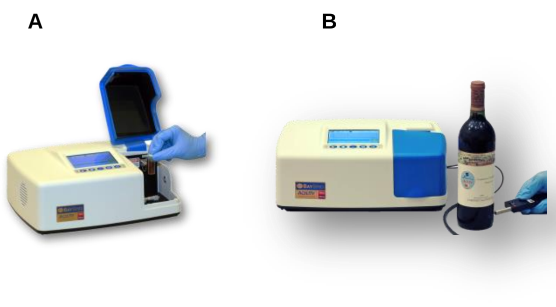

Figure 1. (A) ***Agility***™ *transportable Raman spectrometer with cuvette holder. (B)****Agility***™ *with fiber optic Raman probe enables real-time in situ probing.*

The ***Agility***™ employs a highly efficient volume phase grating (VPG®) as the spectral dispersion element and a cooled CCD or InGaAs array detector, thereby providing high-speed parallel processing and continuous spectral measurement. High reliability (MIL STD 810 shock and vibration) is achieved through a rugged mechanical design with no moving parts. Periodic calibration is not required, but can be performed automatically. Convenient USB interface for outputting data or control via external computer is provided, along with integrated WiFi (optional) for remote control of all system functions. The included ***Agile20/20***™ software platform allows full control of spectral acquisition as well as library search functionality.

The ***Agility***™ series offers users the most versatile sampling options available, with a number of Quick-

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Change inserts that can be rapidly exchanged within the base system. These inserts maintain the precise optical alignment necessary to ensure high-quality spectral acquisition, and accommodate a number of sample types. These options include a vial/cuvette holder for liquids and powders, a fiber adapter for attachment of a remote fiber probe, a solid sample insert with upright or inverted configuration, and a pill holder for liquid and solid capsules.

The corn stover measurement was performed using an ***Agility***™ 785/1064 dual-band. The enriched photosynthetic pigments in the biomass, when excited by 785 nm laser, produce high-level of fluorescence that masks any usable Raman signal. Only 1064 nm excitation produces high quality, signature-rich Raman spectrum for the biomass. The spectrum can be used for quantitative study of the biomass (Figure 2).

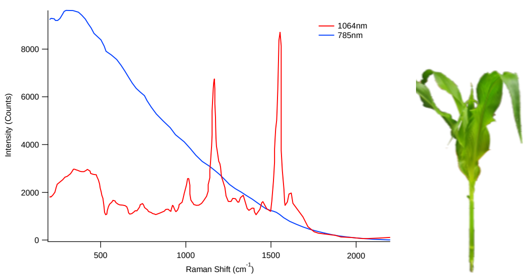

Figure 2: Raman spectra of corn stalk measured with 785 nm and 1064nm excitation. Only 1064 nm excitation produces high quality, signature-rich Raman spectrum.

In **Figure 3**, grown microalgae cultures have been analyzed using the ***Agility***™ 1064 nm system with in situ Raman probes. The 1064 nm Rama reveals important Raman peaks related to microalgae's composition and physiology changes.

Based on these experiments, the 1064 nm dispersive Raman is a viable new option for users who are studying highly fluorescent samples such as plants and biofuels. Samples in native state can be simultaneous measured. Future studies will certainly evidence further advantages of this approach, as compared to shorter wavelength (e.g., 785 nm) Raman or FT-Raman systems.

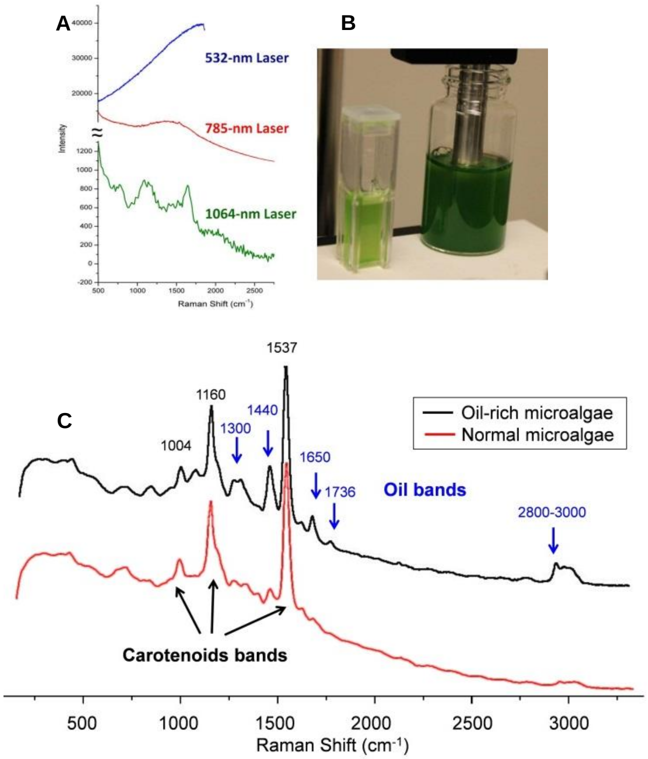

***Figure 3**. (**A**) Only 1064 nm laser can produce microalgae's Raman spectra. Visible lasers excite high fluorescence which overwhelms Raman signals. (**B**) Native microalgae cultures can be tested without any preparation with a dip-in probe. (**C**) Raman spectra of microalgae cultures grown in different conditions reveal their compositional difference.*