APPLICATION NOTE

BaySpec

Pervasive Spectroscopy

Multi-wavelength Confocal Raman Microscope for Non-destructive Pharmaceutical Ingredient Analysis

Superior Fluorescence Avoidance at 1064nm

Background

The characterization of the active pharmaceutical ingredient (API) and its distribution and physical properties in commercial medicine is necessary in drug research and development process in the pharmaceutical industry. Among various analytical techniques are employed for this purpose, Raman spectroscopy is gaining more popularity due to its advantages as non-destructive, non-invasive, fast spectrum acquisition in seconds, high reproducibility and so on.

Owing to technological improvements spurred on by the telecommunications boom of the last decade, Raman spectroscopy has become much more accessible to users in all fields. The combination of improved technology and the technique's molecular sensitivity has led to a surge in Raman usage in a myriad of application areas, including pharmaceutical, biomedical, and forensic, etc. In all of these applications, however, there remains a struggle to extract useful Raman spectra from fluorescent and luminescent samples.

Fluorescence, a type of broad emission thousands times stronger than Raman effects, is very common in pharmaceutical samples such as API and final drug. It is much more likely and intense when illuminated by short wavelengths. 1064nm excitation, as a fundamental way to avoid the fluorescence, used to be only available in FT-Raman, which is typically more complicated and slower than dispersive Raman systems. But now, BaySpec's new dispersive 1064nm Raman spectrometer family of instruments offers users a turn-key solution that combines the speed, sensitivity, and rugged design of dispersive Raman instruments with same fluorescence avoidance of traditional FT-Raman. In addition, the dispersive geometry permits diffraction-limited optical performance, enabling confocal microscopic Raman configuration at 1064nm.

Results and Discussion

As the only Raman Microscope on the market today simultaneously equipped with three excitation sources from visible for near-infrared (532, 785, and 1064 nm), BaySpec's Nomadic™ Raman microscope (As in **Figure 1**) provides unprecedented flexibility and power to explore the fluorescence-free Raman spectra for chemical analysis.

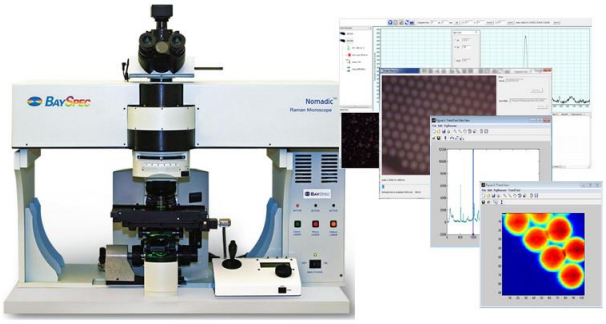

Figure 1: *BaySpec's Nomadic™ Raman microscope with multi-wavelength excitation (532, 785, and 1064 nm) and software interface for automatic Raman imaging.*

The Raman spectra of the Zantac® tablet collected through the coating by Raman Microscope with 532nm, 785nm and 1064nm excitation laser as in **Figure 2**. Even for 785nm, the fluorescent from the pigments in the coating dominated the whole spectrum and no distinguishable Raman features can be found. But with the advantages of superior fluorescence avoidance and minimal signal loss through the coating for 1064nm dispersive Raman, a clear Raman spectrum is generated.

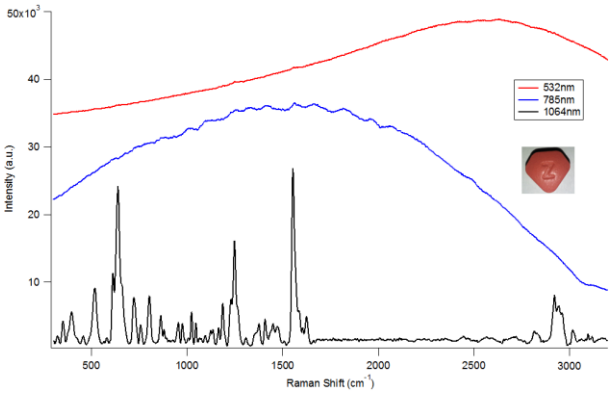

Figure 2: *Raman spectra of the Zantac® tablet collected through the coating with Nomadic™ Raman microscope excited by 532nm, 785nm and 1064nm laser.*

BaySpec, Inc., 1101 McKay Drive, San Jose, California 95131, USA. Tel: +1 (408) 512-5928 Fax: +1 (408) 512-5929
Web: www.bayspec.com Email: sales@bayspec.com

Equipped with highly efficient VPG® gratings and innovative, deep-cooled CCD or InGaAs detector, the BaySpec's Nomadic™ Raman microscope can provides Raman spectra of all three wavelengths with high optical efficiency, high single to noise ratio and high spectral resolution. For typical samples shown in the **Figure 3**, 1064nm Raman spectra of Acetaminophen, Aspirin and Caffeine were acquired on pure powder material. As the fundamental information for chemical identification, verification and Raman imaging, the unique Raman features of these three important pharmaceutical components can be easily distinguished.

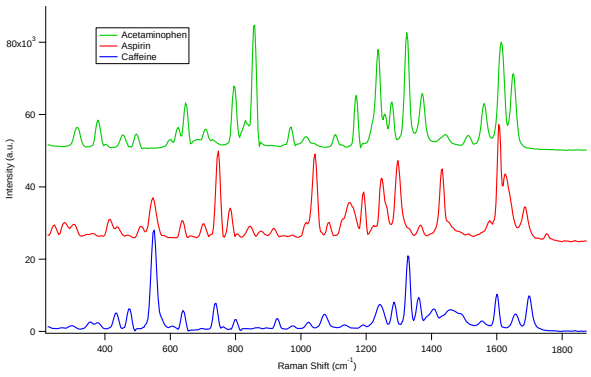

Figure 3: *Raman spectra of Acetaminophen, Aspirin and Caffeine acquired with 1064nm Raman microscope.*

In the pharmaceutical industry, it is critical to know the structure, polymorph and heterogeneous distribution of the API and excipients which can strong influence on drug dissolution and efficacy. Raman imaging provides the ability of mapping the chemical properties of pharmaceutical samples non-invasively. By coupling with an automated stage with high accuracy and repeatability, the confocal Raman microscope can reach a high special resolution of 0.5 µm. With software synchronized stage scan through X and Y directions and Raman spectra acquisition, Raman spectrum of each image point would be recorded and a Raman image would be automatically generated by integrating over a specific Raman peak or a region. In addition, the focal plane can be moved in the Z direction for performing depth profiling or real 3D Raman spectral imaging.

As an example, 1064nm Raman imaging of Excedrin® Extra Strength Tablet, which has Acetaminophen (250mg), Aspirin (250mg) and Caffeine (65mg) as its APIs, was executed within an area of 970 x 700 µm (97*70 pixels, bright-view image was shown in **Figure 4(B)**). As comparing the Raman features of the three major APIs, unique Raman features (**Figure 4(A)**) for each component were selected by markers with different colors to reconstruct the Raman image. As the color-coded Raman image in Figure 4(C), the distributions of three APIs in the scanned region were highlighted: the red region was indicated as Aspirin while the green and blue colors were where Acetaminophen and Caffeine located.

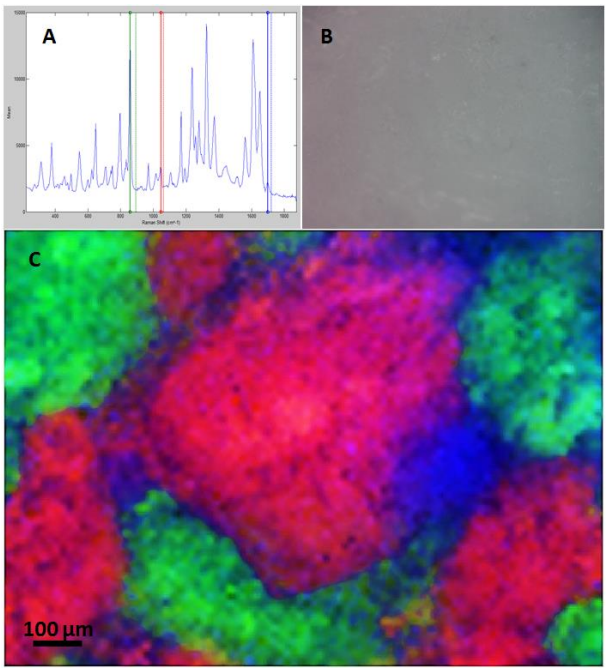

Figure 4: *Color-coded Raman image of Excedrin® Extra Strength Tablet for discovering the spatial distribution of the major components – Aspirin, Acetaminophen and Caffeine (C). (A) indicates the selected unique Raman features of different chemical constituents for Raman imaging and (B) is the bright-view image of scanning area.*

Conclusion

As shown above, multi-wavelength confocal Raman microscope is demonstrated as a viable new option and non-destructive method to chemical identification. Flexible selection on the excitation laser based on the sample properties can be achieved and the switching between different wavelengths is totally automatic and software controlled. Especially with 1064nm Raman, the Raman features could be efficiently revealed from the fluorescence without any signal processing. Moreover, the Raman imaging of the pharmaceutical tablets for the API distribution and uniformity analysis can lead to the success of drug development and manufacture.

Besides the multi-wavelength Raman measurement, BaySpec's Nomadic™ Raman microscope provides the options for fluorescence imaging and scattering measurement as well as various measurement accessories with flexibility, 96-well micro-plate Raman reader and fiber probe as example. The easy to use but powerful software interface has the abilities of library search, chemometrics analysis and automatic regional mapping, which makes the instrument suitable for every R&D, QA/QC and forensic lab.

References

1) Vankerisbilck, T., et al., *Trends Anal Chem,* **2002**, 21, 869-877.
2) Gendrin, C., et al., *J Pharmceut Biomed Anal*, **2008**, 49, 18-25.

BaySpec, Inc., 1101 McKay Drive, San Jose, California 95131, USA Tel: +1 (408) 512-5928 Fax: +1 (408) 512-5929
Web: www.bayspec.com Email: sales@bayspec.com