Photon Systems Application Note
Low Cost Deep UV Raman & Fluorescence Spectrometer & Methods

Abstract

Significant advantages have been demonstrated for detection and classification of materials using deep UV excited Raman spectroscopy, fluorescence spectroscopy of a combination of both. The four main advantages of deep UV excitation compared to near-UV, visible or near-IR counterparts include: 1) When a sample is excited between 220 and 250 nm, Raman emission occur within a fluorescence-free region of the spectrum, eliminating obscuration of weak Raman signals by fluorescence from target or surrounding materials within the excitation volume. 2) Because Raman and fluorescence occupy separate spectral regions, detection can be done simultaneously, providing a much wider set of information about a target. 3) Rayleigh law and resonance effects increase Raman signal strength and sensitivity of detection. 4) Penetration depth into many materials is very short in the deep UV, providing separation of a target material from its background or substrate.

Photon Systems has been developing miniature, low cost, narrow linewidth, deep UV lasers emitting at 224.3 nm and 248.6 nm and related components to enable customers to assemble inexpensive deep UV spectrometers and systems.

This Application Note provides a description of the advantages of deep UV Raman and fluorescence detection methods and provides a blueprint for the various ways you can create this capability in your own laboratory.

1. DEEP UV RAMAN AND LASER INDUCED NATIVE FLUORESCENCE

A broad perspective of the relationship between Raman and native fluorescence spectral regions is illustrated below in Fig. 1 along with the emission wavelength of typical lasers and the spectral range of their Raman range. It is commonly accepted practice to move to the near IR to avoid fluorescence from target molecules or surrounding materials within the exposure volume, but with excitation even as high as 830 nm, it has been shown that a large fraction of materials investigated exhibit major fluorescence interference[1] to the point that it completely obscures Raman emissions. Asher[2],[3] showed that natural materials did not fluoresce below a wavelength about 270nm, independent of the excitation wavelength. This was further proven in many subsequent publications such as Nelson[4], Sparrow[5], Wu[6], and many others. When excitation occurs below about 250nm, a fluorescence-free region exists above the laser wavelength in which to observe Raman spectra. This is not the case for lasers that provide excitation at longer wavelengths.

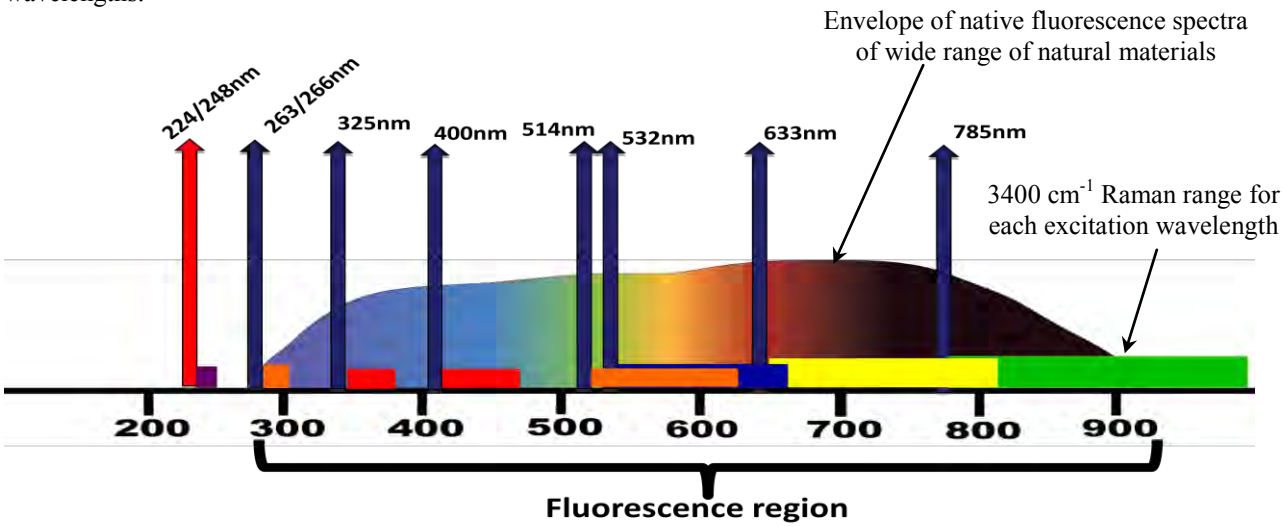

Figure 1. Broad relationship between Raman and native fluorescence spectral regions with emission wavelengths of typical lasers and their 4000 cm^{-1} Raman range.

Deep UV optical sensors for detecting and classifying or identifying CBE materials UV have several advantages over sensor operating in the near-UV, visible, or near-IR. These advantages are summarized as:

1. Clear Raman spectra with no obscuration of weak Raman spectra by native fluorescence or alteration of the fluorescence spectra by major C-H and O-H Raman bands. Raman and fluorescence are truly independent and orthogonal measurements.
2. The ability to simultaneously detect Raman and native fluorescence emissions from target materials with no possible confusion due to overlapping spectra regions.
3. Much higher sensitivity due to Rayleigh law and resonance Raman signal enhancements, providing much lower limits of detection of CBE agents
4. Simplification of Raman spectra due to resonance effects, enabling the use of Raman marker bands in the chemometric method
5. Short depth of penetration into target materials allowing discrimination against background materials.
6. Solar blind detection of Raman and fluorescence because of short operating wavelength, and gated detection
7. Non-contact, non-destructive, no sample handling
8. Reagentless
9. Reduced eye hazard (DHHS/CDRH Class I based on single data sample, Class IIIb based on repetitive sampling)
10. Longer depth of focus without the need to focus the sensor

2. EXAMPLES OF DEEP UV RAMAN AND NATIVE FLUORESCENCE SPECTRA TAKEN WITH AN INEXPENSIVE SPECTROMETER AND PHOTON SYSTEMS DEEP UV LASERS

Following are a few examples of deep UV excited Raman and fluorescence spectra taken on a variety of material which illustrate the advantages described above. First is an example of the simultaneous Raman and fluorescence spectra of a few materials to illustrate the separation of Raman and fluorescence emission spectral regions with excitation at 248.6 nm. In these examples, fluorescence emission is weak because it is caused by trace organic impurities in the chemical tested. Normally, fluorescence emission is so strong, especially in organic materials, that the Raman portion is very weak and would not normally be detectable except that it occurs below the spectral region of fluorescence.

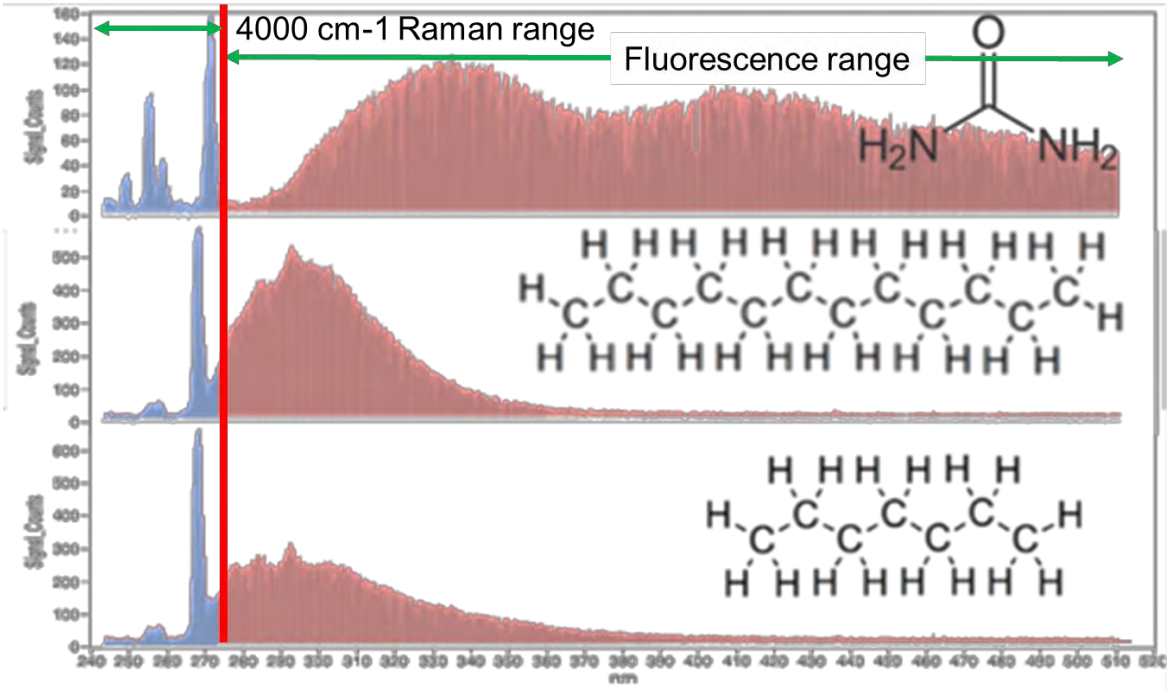

Figure 2. Examples of the separation of Raman and fluorescence emission regions with 248.6 nm excitation.

Another example, shown below, is of histidine, where the spectrum on the left shows both the Raman and fluorescence spectral regions and the spectrum on the right is of the Raman region only.

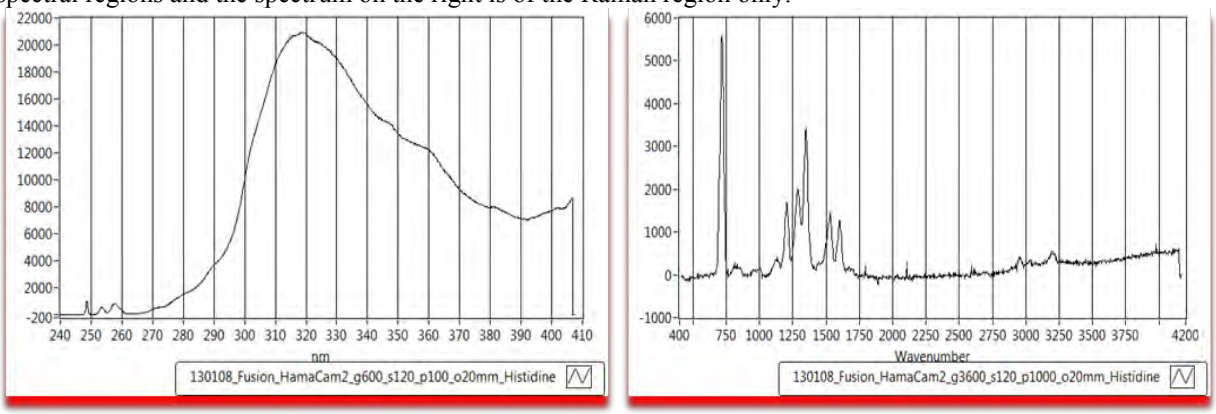

Figure 3. Example of combined Raman & fluorescence (left) and Raman spectra alone (right) of histidine with 248.6 nm excitation.

Another example, shown below, is of glycine, where the spectrum on the left shows both the Raman and fluorescence spectral regions and the spectrum on the right is of the Raman region only.

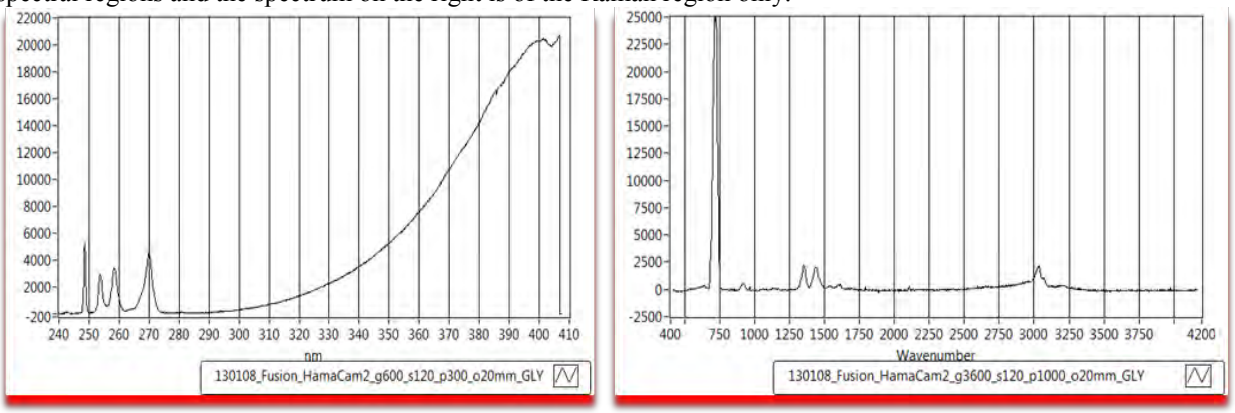

Figure 4. Example of combined Raman & fluorescence (left) and Raman spectra alone (right) of glycene with 248.6 nm excitation.

A final example, shown below, is of cyclohexane, where the spectrum on the left shows both the Raman and fluorescence spectral regions and the spectrum on the right is of the Raman region only.

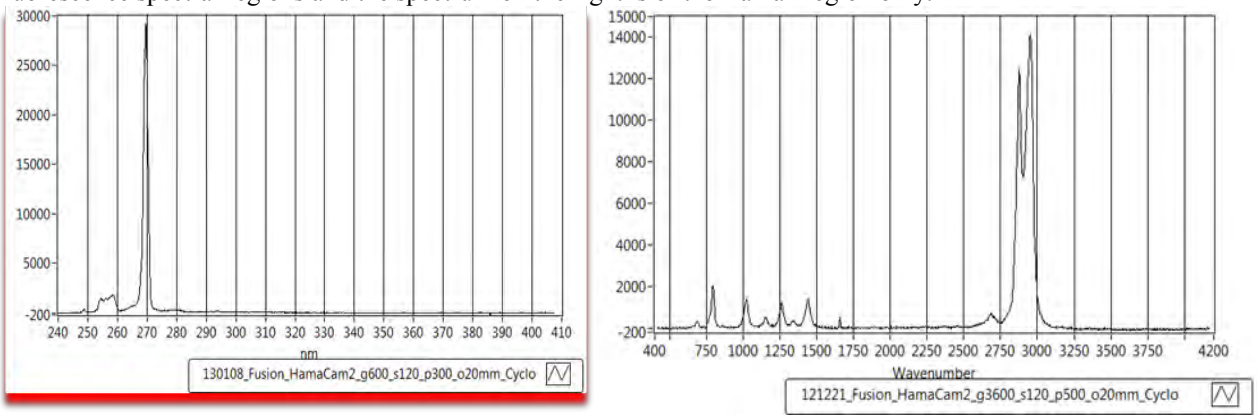

Figure 5. Example of combined Raman & fluorescence (left) and Raman spectra alone (right) of cyclohexane with 248.6 nm excitation.

3. AN INEXPENSIVE DEEP UV RAMAN & FLUORESCENCE SPECTROMETER SYSTEM

Below, in Fig. 6, is a schematic of the Raman system used to take the data shown in Figs 2 through 5 above.

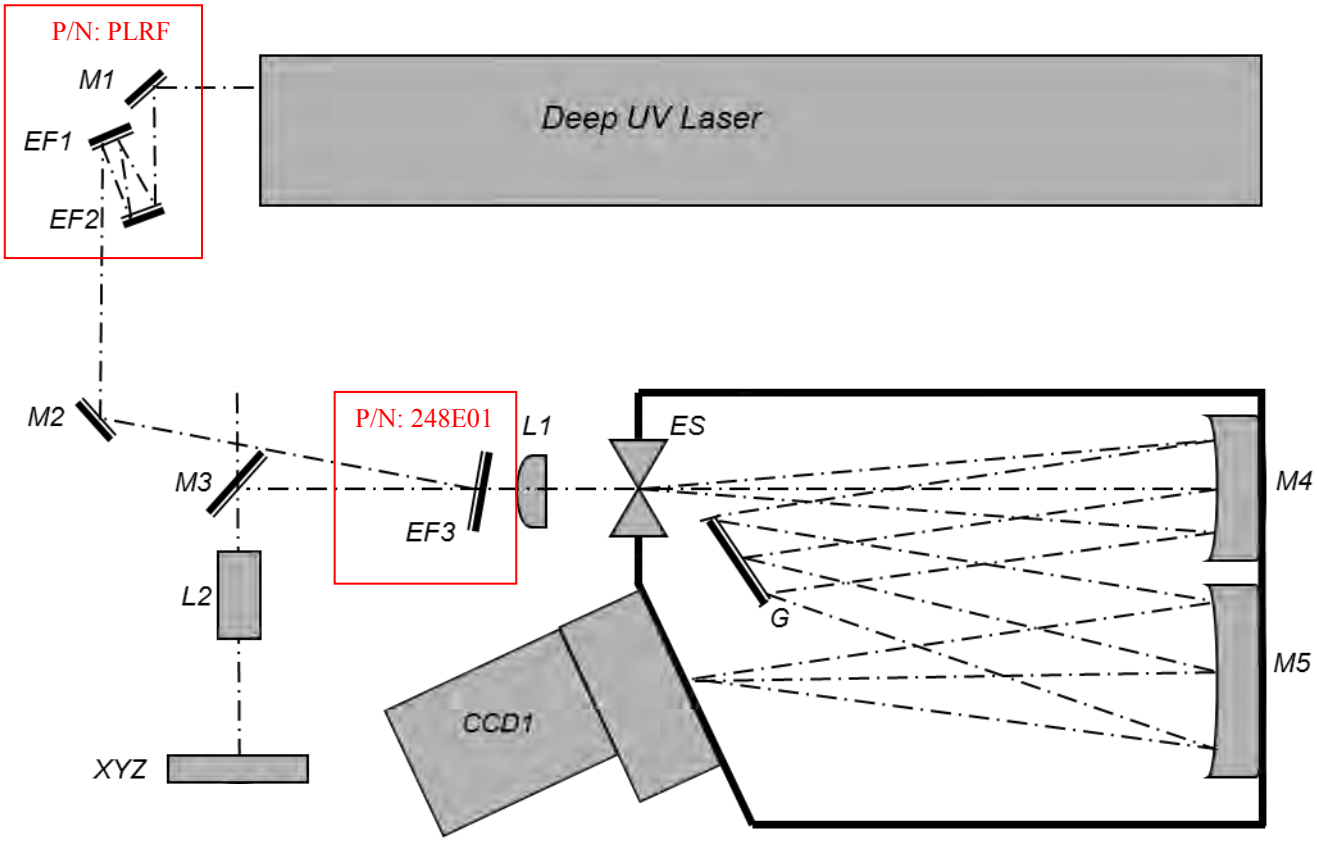

Figure 6. Schematic for inexpensive deep UV Raman and fluorescence spectrometer.

A description of the elements of the system in Fig. 6 are described as:

1. Deep UV laser from Photon Systems emitting at 224.3nm (HeAg) or 248.6nm (NeCu),
2. M1, M2, M3 =deep UV enhanced aluminized mirrors
3. EF1=EF2=EF3=laser line edge filter,
4. L2=UV microscope objective lens,
5. XYZ translation stage to hold sample,
6. L1=spectrograph focusing lens,
7. ES=spectrograph entrance slit with M4, M5 and G internal to spectrograph
8. CCD1= deep UV, back thinned, back illuminated CCD array detector with 2 stage or more TE cooler.

For the system illustrated in Fig. 6, most of the optical components are commercially available from several sources. The key components are the deep UV laser, the spectrometer with deep UV optics and grating/s, and the deep UV detectors.

For the data shown above in Figs. 2 through 5, a 248.6 nm NeCu deep UV (DUV) laser from Photon Systems was employed. This laser has power consumption about 5W. The laser transition is CW but the laser is operated in a long pulse mode with pulse width typically between 50 us and 100us. This provides a soft pulse to sample materials and ensures that little sample damage can occur, compared to Q-switched lasers, where pulse width is of the order of 1 ns. Typically the laser is operated at a pulse repetition rate of 5 Hz to 20 Hz. The number of pulses used to obtain a Raman

spectra range from a single pulse to many pulses, depending on the Raman scattering cross-section of the sample material, concentration, and desired signal to noise ratio in the spectra.

The second most important component of the system in Fig. 6 is the spectrometer. The data taken in Fig. 2 was taken with a commercial Avantes spectrometer. The data in Figs 2 through 5 were taken on a ¼ m Oriel spectrometer (MS260i) fitted with a 3600 g/mm grating for Raman data and a 600 g/mm grating for simultaneous Raman and fluorescence data. Both gratings were blazed at 250 nm. There are many suitable commercial spectrometers on the market. You can use your own spectrometer if you have one. What is important is that the optics are suitable for use in the deep UV. The better the efficiency of the mirrors and grating, the better the results, with lower number of laser pulses per spectra and higher quality spectra.

The third most important component of the system is the detector. The detector employed for the above spectra was a two-stage cooled, back thinned, back illuminated 1024 by 64 element CCD array camera from Hamamatsu (C7042) with S7032-1006. This is a relative inexpensive camera and detector for use in the deep UV. More expensive cameras, operating at lower temperatures, will yield better results with higher signal to noise. The best spectra we have taken employ either a liquid nitrogen or 4-stage TE cooled, back thinned, back illuminated detector from E2V, Andor, Horiba, or others. A single photodiode or PMT detector can also be used when the spectrograph is used at a monochromator and the spectra are generated using a series of laser pulse, one or more for each spectral increment.

To obtain good Raman spectra it is important that the optical components used are designed for operation in the deep UV. At the output end of the laser we employ a Plasma Line Rejection Filter (PLRF) to reject all emissions from the laser except the laser line. This is essential since the laser emits low energy radiation due to plasma light generated in the laser. Photon Systems sell this filter as an accessory at low cost.

An Edge Filter (EF3) is used to reflect the laser beam into the optical path toward the sample and collect Raman and fluorescence emissions from the sample, blocking Rayleigh scattered light from the sample at the laser wavelength. This filter is very important and typically is employed so the angle between the incoming laser beam and the optical axis from the sample to the spectrometer entrance slit is shallow, typically less than 15 degrees, although 10 degrees is better. These filters are available from Photon Systems (248E01) or from Semrock, or other sources.

The final key component is a good quality lens to focus Raman and fluorescence energy into the spectrometer (L1) and to focus the laser beam onto the sample and collect emitted radiation from the sample (L2). In the data shown in Fig. 2 through 5 we used a simple 20 mm focal lens for L2 and a 50 mm focal length lens for L1. Both lenses were simple plano-convex fused silica lenses which are far from perfect due to chromatic and spherical aberrations. Reflective optics would provide much better spectra but are hard to find in high quality surface finish and roughness needed for UV.

Several publications illustrate the use of Photon Systems lasers for used in resonance Raman spectroscopy in the deep UV. One recentl examples of this is illustrated below. Two figures below were taken from a paper by Masashi Unno, et. al. from Saga University, Saga, Japan, Japan Science and Technology Agency, Saitama, Japan, and Tokyo Institute of Technology, Yokohama, Japan [7]

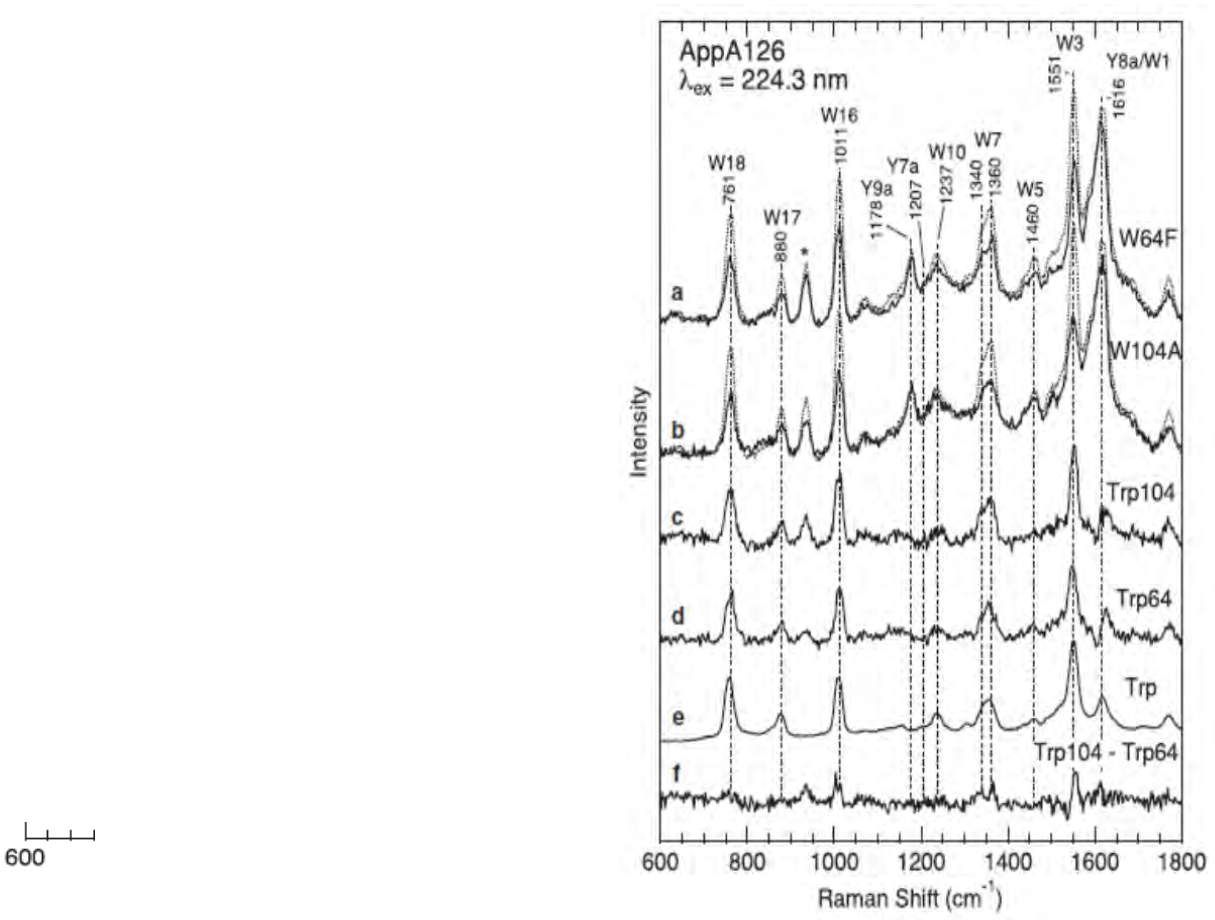

Figure 7. UV resonance Raman spectra of WT AppA126 compared to aqueous aromatic amino acids (left) and with different mutants and aqueous aromatic amino acids (right) [7]

If you have further questions, please contact our representative in Japan, Pneum.

REFERENCES

[1] Frosch, T., et.al. UV Raman Imaging, A promising tool. Anal. Chem. Vol.79, No.3, (Feb. 1, 2007)

[2] Asher, S.A., and C.R. Johnson, "Raman Spectroscopy of a Coal Liquid Shows That Fluorescence Interference Is Minimized with Ultraviolet Excitation", Science, 225, 311-313, 20 July (1984).

[3] Asher, S.A. and C.R. Johnson, "UV Resonance Raman Excitation Profile Through the $^1B_2$ State of Benzene", J. Phys. Chem. Vol. 89, pp. 1375-1379 (1985).

[4] Nelson, W.H., R. Manoharan and J.F. Sperry, "UV Resonance Raman Studies of Bacteria", App. Spect. Reviews, 27 (1), pp67-124, (1992)

[5] Sparrow, M.C., J.F. Jackovitz, C.H. Munro, W.F. Hug, and S.A. Asher, "A New 224nm Hollow Cathode UV Laser Raman Spectrometer", App. Spect., Vol. 55, No. 1, Jan (2001).

[6] Wu, M., M.Ray, K.H.Fung, M.W. Ruckman, D. Harder, and A.J. Sedlacek, "Stand-off Detection of Chemicals by UV Raman Spectroscopy", App. Spect., Vol.54, No.6, pp. 800-806 (2000).

[7] Unno, M. et.al. Structural Refinement of a Key Tryptophan Residue in the BLUF Photoreceptor AppA by Ultraviolet Resonance Raman Spectroscopy, Biophysical Journal, Vol. 98, pp 1949-1956, May 2010.