WHITE PAPER

Advantages of 1064nm Handheld Raman

Introduction to Raman Spectroscopy

Raman spectroscopy can be used to effectively and efficiently identify and distinguish different materials. The specificity of Raman spectroscopy comes from its being a vibrational technique. Any chemical or physical changes that will change molecular vibrations will change the Raman spectrum making Raman spectroscopy highly sensitive to chemical and some physical differences in materials. Most molecules have multiple molecular vibrations, and typical Raman spectra consist of multiple resolved peaks. Multiple narrow spectral peaks provide a characteristic spectrum for any given material which improves chemical specificity as compared to fluorescence, UV-visible absorbance, or near infrared absorbance spectra which have fewer and much broader peaks than Raman. The specificity of Raman and its relative ease of use, including the advent of small and portable instrumentation, have made Raman spectroscopy effective in applications where chemical identification is required.

In Raman spectroscopy a laser is focused at a sample and the light scattered by the sample is measured. This is different from techniques like infrared and UV-visible spectroscopy where the amount of light absorbed by the sample is measured. In Raman, the vast majority of light is scattered at the same wavelength as the incident laser light and only a very small portion of the light (approximately 1 out of every 10^6 to 10^8 photons) is scattered at a wavelength shifted from the incident laser wavelength. These shifted, or inelastically scattered, photons create the peaks in a Raman spectrum. The wavelength shifts, typically reported in wavenumbers (or cm^{-1}) correspond to the energy of a material's molecular vibrations. Since Raman peak positions depend on molecular vibrations, any changes that affect molecular vibrations will change Raman spectra. These can be changes in actual chemical structure, even small changes such as an acid vs. a base form of the same molecule, or changes in some physical characteristics such as crystallinity, polymorphism, and protein secondary and tertiary structure.

Along with its chemical specificity, ease of use has made Raman an accepted technique for applications in many industries, from pharmaceutical and biopharma, to safety and security, to foods, among others. Raman spectroscopy has become easier to use in recent years largely due to advances in instrumentation; it requires little or no sample preparation, is non-contact, and non-destructive. Water has relatively weak Raman peaks, unlike other vibrational spectroscopies such as near infrared (NIR) and Fourier transform infrared (FTIR), which allows Raman to measure samples that are hydrated or even in aqueous solutions. Measuring Raman scattering through a variety of containers and packaging materials is also possible. Raman intensity is proportional to concentration enabling semi-quantitative and in some cases quantitative measurements. Raman instrumentation has seen very significant advances in recent years; due to improvements in the components used in Raman spectrometers. Lasers, detectors, and electronics are continually getting smaller and more reliable, which has allowed for the development of small portable spectrometers that can collect Raman spectra in seconds to a few minutes, something that was difficult to imagine not very many years ago when a Raman spectrum took minutes to hours to collect. Instrument advances have also led to fast sample analysis at the point of use, eliminating the need to take samples to an analytical laboratory.

Another area where advances in instrument components have made an impact on Raman spectroscopy is in the choice of excitation wavelengths. Raman spectroscopy has traditionally been done with visible laser excitation wavelengths, such as 532 and 785nm. There are two reasons for this. First, the Raman cross section (or likelihood of a photon being Raman scattered) is higher at shorter excitation wavelengths. Second, the small portable lasers and detectors needed to build handheld Raman spectrometers at longer excitation wavelength, like 1064nm, have only become available in approximately the last decade and these components are still improving rapidly.[1] Raman peak positions are largely independent of the excitation wavelength. This is because Raman peak positions are measured as a difference from excitation wavelength, which corresponds to the vibrational energies of the molecules. For example, the Raman peak from the carbonate stretching vibration of calcium carbonate will have a peak at 1085 cm^{-1} whether 785nm or 1064nm excitation is used.[2,3] The Raman intensity, however, is inversely proportional to the excitation wavelength and, if all other things are equal, higher Raman intensities will be observed at shorter excitation wavelengths. To a first approximation, Raman intensity is proportional to $1/(\lambda^4)$ where λ is the excitation wavelength. For example, when comparing Raman cross sections between 785nm and 1064nm excitation, the cross section at 785nm is about 3.5 times higher. So if shorter excitation wavelengths result in more Raman signal, what is the advantage of going to longer excitation wavelength? The problem with shorter excitation wavelengths is fluorescence interference which increases background intensity. Fluorescence emission as well as Raman scattering can be excited by the laser and if present fluorescence has orders of magnitude

higher intensity than Raman scattering and can easily obscure the Raman signal. The presence (and intensity) of fluorescence will depend on the material that is being analyzed, but it does not have the specificity of Raman. Fewer materials fluoresce at longer excitation wavelengths than at shorter wavelengths, allowing longer excitation wavelengths, like 1064nm, to analyze a wider array of materials.[4] Sloping baselines such as those seen when a material fluoresces can be corrected for with baseline correction routines, but this only corrects the appearance of the spectra and does not remove fluorescence. Baseline correction can be helpful with low intensity fluorescence but strong fluorescence will decrease the dynamic range of the detector which is available to the Raman signal, limiting the signal to noise and thus the identification ability of the Raman spectrometer.

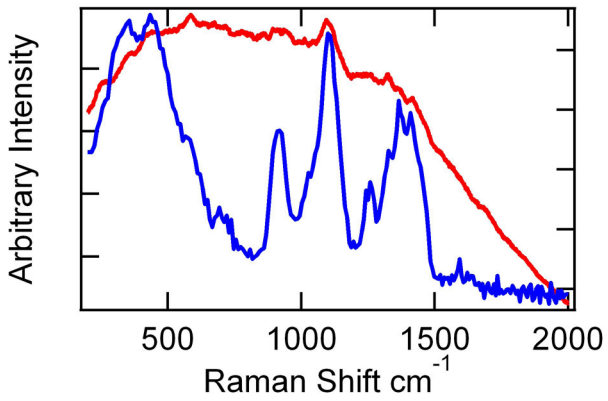

Figure 1. Raman spectra of sodium carboxymethyl cellulose collected with 785nm (red) and 1064nm (blue) laser excitation.

Rigaku Progeny

The Rigaku Progeny has been optimized as a high quality 1064nm excitation handheld Raman spectrometer. It is small, light, rugged, versatile and easy to use without compromising spectral quality. Each module, or major functional component, of the Progeny has been designed and built to meet these requirements. Components of a Raman spectrometer include the excitation source, sample illumination and collection optics, a spectrograph, a detector, as well as electronics and software for instrument control. Further details describing each of these modules and how they make the Progeny a high quality long wavelength handheld Raman spectrometer sized for single hand operation are provided below.

The excitation source in the Rigaku Progeny is a 1064nm high power wavelength stabilized diode laser. This Class IIIB laser provides the small size and high performance required in a handheld Raman instrument. The power is continuously adjustable from 30 to 490 mW. The illumination of the sample and collection of Raman photons use "fast" optics which have a wide relative aperture, providing a wide solid angle from which illumination and Raman collection occur. The wide relative aperture contributes to high spectral throughput. The Progeny focal position can be adjusted to maximize signal then locked in place to allow for analysis of samples through a variety of containers and at various sampling depths. After the scattered light is collected, it is passed through a very high efficiency filter to remove Rayleigh or elastically scattered photons.

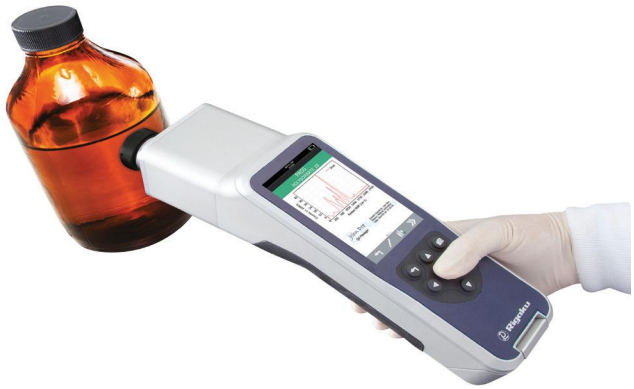

Figure 2. Photo of the handheld Progeny Raman analyzer.

Rayleigh scattering is a potential source of significant stray light and must be eliminated before light enters the spectrograph.

Design and implementation of the spectrograph is another critical piece of a handheld Raman spectrometer. The wavelength separation element in the Progeny is a high throughput transmission volume phase grating (VPG) that has been optimized for near-infrared wavelengths. The use of the VPG plays a major role in providing the needed features to make a high quality, small and rugged Raman spectrometer. The VPG provides excellent spectral efficiency (>90%), good resolution, lower cost and a shorter optical path, when compared to other spectrographs. In addition, it allows the instrument to have no moving parts which improves ruggedness and portability.

Silicon CCD detectors have long been an option for detection in the visible region of the spectrum but these quickly loose sensitivity in the near-infrared region. More recent improvements in near-infrared detectors have been required for handheld Raman spectrometers in this region of the spectrum to be made. Raman detection in the near-infrared no longer requires liquid nitrogen cooling but now can be done with reasonably priced, miniaturized, solid state thermoelectrically cooled detector elements. The detector in the Progeny is an indium gallium arsenide (InGaAs) thermoelectrically cooled array detector which allows for portable and sensitive Raman measurements in a spectral region that minimizes fluorescence interference. This reduction in fluorescence interference broadens the number of materials that can be analyzed by Raman. The optics, spectrograph, and detector work together to provide high quality spectra over a wavelength range from 200 to 2500 cm^{-1} with a resolution of 8 - 11 cm^{-1} as determined by measuring calcite according to the ASTM E2529-06 guide.[5]

Progeny instrument control is done using state of the art electronics which provide the necessary combination of high performance and small size required for a handheld Raman spectrometer. The entire instrument control can be done from the handheld Progeny which has a user screen size and software operation similar to that found on many smart phones. All the Progeny software functionality can be quickly and easily accessed from the main screen shown in Figure 3.

The software has been designed to provide different levels of access depending on the user login privileges. This allows it to be very flexible or very simple depending on the level of access assigned to a given user. For high access level users the software will provide for

Figure 3. Intuitive, modern and workflow driven software.

flexible data acquisition, spectral processing, and library searching so that these may be optimized to best analyze the user's samples. Wavenumber and intensity calibration is done based on ASTM standards. Adjustable data acquisition parameters that can be optimized for method development include: laser power, exposure time, spectral averaging, dark background subtraction, auto adjust exposure time and auto save data. Spectral processing options include peak search, and baseline correction.
Spectral libraries can be built directly on the instrument by the user or they can be factory libraries. Library searching can be done using a variety of methods including correlation algorithms, wavelet searching and mixture analysis. Search methods may be done over the entire spectral range or by selecting a smaller region of the spectrum to search. The software has been designed so that all of the data acquisition, spectral processing and library search parameters may be saved together for future use. This allows a user who has a high access level to the software to develop sets of instrument and data analysis parameters that can be saved for later use by themselves or lower level instrument operators.

Software privileges are under control of a local administrator. Customizable access can be flexible and powerful or simple and straight forward depending on experience and needs of the end user. An analytical development specialist can be given full access to method development tools and workflow improvements, in order to optimize measurements and workflows. On the other hand, an operator can be given access only to simple method selection and measurement tasks to help avoid mistakes. Additionally, the software has been designed to guide users through various tasks including library and applications development, as well as data collection of single and batch measurements. The instrument includes a camera that can read barcodes and collect and store photographs of the sample to be saved with measured data. This capability permits integration with existing sample tracking systems and better sample/measurement verification. Measured spectra and results may be saved in non-editable and editable encrypted formats allowing for both a secure data trail and easy data access.

Saved spectra may be reopened for further viewing, zooming and peak picking are supported. Data and results may also be printed to customizable .pdf reports as well as saved in formats that can be reopened. Data may be synced with an external computer via WiFi and USB.

The Progeny 1064nm Raman has been designed to meet stringent environmental and regulatory requirements. The instrument is IP-68 certified, which requires it to be sealed for dust, liquid and be liquid submersible. It is also MIL-810-G certified durable and drop resistant and can be wiped down with isopropyl alcohol, bleach and dilute sodium hydroxide. It is CE certified and meets 21 CFR part 1040, FDA requirements for light emitting products. The software and data security are 21 CFR part 11 compliant and the instrument meets the specification for Raman instruments outlined in the United States Pharmacopeia Chapter <1120> and European Pharmacopeia Chapter 2.2.48.

The Progeny has an optional docking station which easily performs a number of tasks. It will charge batteries both one in the instrument and an additional spare battery. It will automatically sync data to an external computer when docked. The Progeny docking station compliments the spectrometer as do specially designed sampling accessories which allow for accurate, reproducible and easy sample positioning.

Applications of Handheld Raman Spectroscopy

Pharmaceutical and Biopharmaceutical Applications of Raman Spectroscopy

Excipients

Raman spectroscopy generates unique fingerprints for many materials. Portable Raman instruments permit pharmaceutical companies to improve manufacturing efficiency, facilitating the verification and expedient release of raw materials at the loading dock rather than relying on lengthy and expensive laboratory based testing. While API drug compounds are usually easy to identify with Raman, many of the common excipients used in pharmaceutical manufacturing fluoresce to some degree at commonly used Raman excitation wavelengths. Fortunately, with 1064nm excitation Raman spectroscopy the range of materials that can be verified is much greater than those that can be identified with the more common handheld Raman laser excitation at 785nm. The following examples demonstrate how a 1064nm Raman excitation can improve capability for material release testing for pharmaceutical manufacturing.

Gelatin, microcrystalline cellulose, and hydroxypropylmethyl cellulose (HPMC) are among commonly used excipients in solid dose pharmaceutical manufacturing. These materials are used as bulking agents, binders, lubricants, coatings and often release modifiers (HPMC) in many solid-dose formulations. These materials present a challenge for Raman spectroscopy because they have a relatively weak Raman signal with some fluorescence background at 785nm excitation. Although this background interference is not always insurmountable for release verification, 1064nm Raman yields a cleaner, more consistent spectrum.

Comparisons of 785nm (in red) and 1064nm Raman spectra (in blue) for these material are shown in Figure 4 to Figure 6.

Identification with handheld Raman spectroscopy is done by collecting reference or library spectra from known materials and saving them to a library. Unknown samples are measured and then compared with the spectra in a library to determine which of the known spectra is the closest match to the unknown. If the match is good enough, then the unknown material is identified or verified. Different mathematical algorithms can be used to determine which library spectrum is the best match and Progeny supports a correlation algorithm, proprietary wavelet searching, and advanced mixtures analysis. Table 1 provides a library search example for the identification of six different excipients. In this case, each excipient was measured six times and compared to a custom made library. It can be seen from the table that each material was identified correctly every time.

In the cellulose examples shown, some Raman peaks are visible with 785nm excitation, but 1064nm excitation gives cleaner spectra.

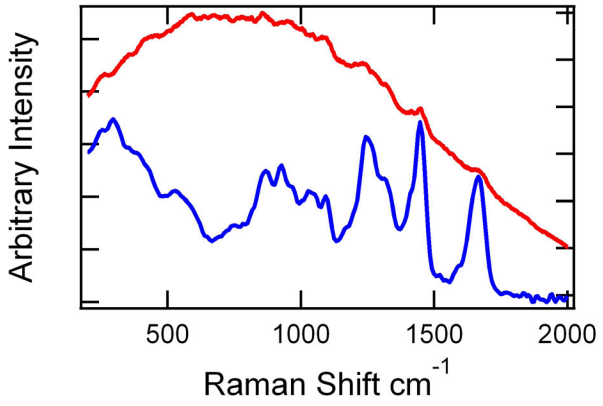

Figure 4. Raman spectra of gelatin collected with 785nm (red) and 1064nm (blue) excitation.

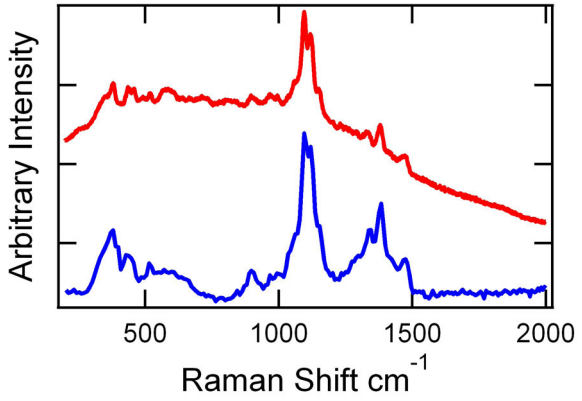

Figure 5. Raman spectra of microcrystalline cellulose collected with 785nm (red) and 1064nm (blue) excitation.

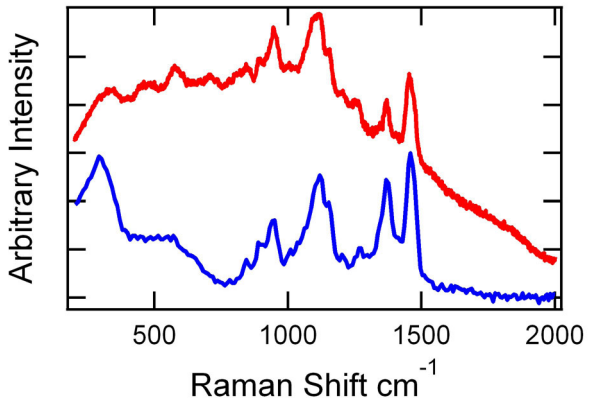

Figure 6. Raman spectra of hydroxylpropylmethyl cellulose collected with 785nm (red) and 1064nm (blue) excitation.

Identifying Test Materials

ID / Real	MCC	Methyl Cellulose	HPMC	CAP	PTFE	Brown Sugar
MCC	6					
Methyl Cellulose		6				
HPMC			6			
CAP				6		
PTFE					6	
Brown Sugar						6

Table 1

However for other materials, like many cell culture media, the fluorescence background interference at 785nm or lower excitation wavelength can make it impossible to characterize materials.

Cell Culture Media

Biopharmaceutics is a growing area of the pharmaceutical industry, and cell culture is a key process. As with other raw material inspection, the specificity, ease-of-use and portability of Raman is attractive for cell culture media inspections. However, fluorescence has been a common problem with Raman measurements on cell culture media. The advent of the 1064nm handheld spectrometer has alleviated this fluorescence difficulty. The spectra shown in Figure 7 are from a Minimum Essential Medium formulated by Harry Eagle which is one of the most commonly used synthetic cell culture media. The Raman spectra collected at 785nm and 1064nm excitation are shown below. The 785nm spectrum is dominated by fluorescence making it impossible to obtain reliable and specific information about the sample. In contrast, the 1064nm spectra show clear Raman peaks that can be used to reliably identify the media.

Figure 8 shows the Raman spectra from two cell culture media, both of which are versions of a minimum essential medium developed by Harry Eagle. The media have only small differences in their components. Clear differences can be seen in these Raman spectra and when a correlation analysis was performed, these two media could be accurately and reliably distinguished.

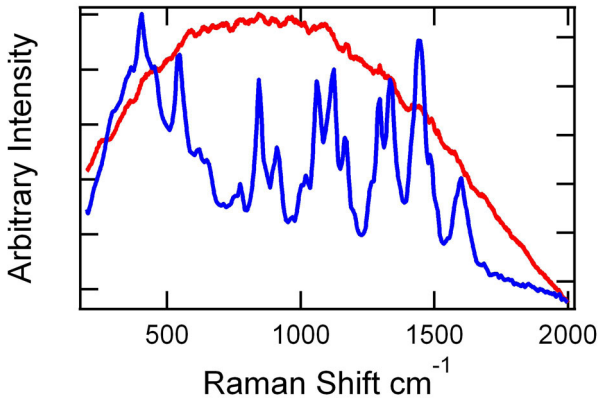

Figure 7. Comparison of 785nm (red) and 1064nm (blue) excitation Raman spectra of cell culture medium.

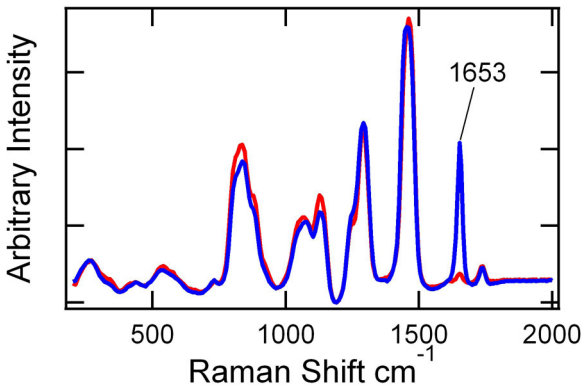

Figure 9. Raman spectra collected with 1064nm laser excitation of polysorbate 20 (red) and 80 (blue) measured through amber glass bottles.

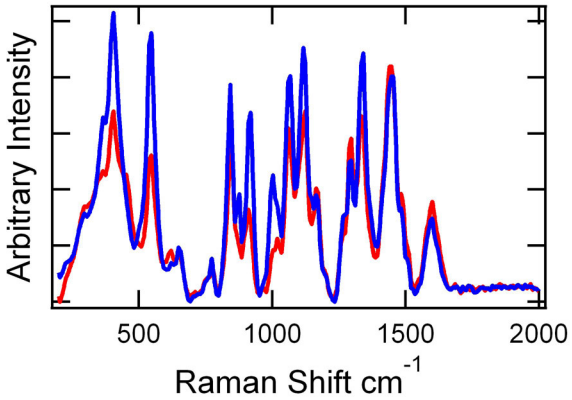

Figure 8. Raman spectra collected with 1064nm laser excitation of two slightly different cell culture media.

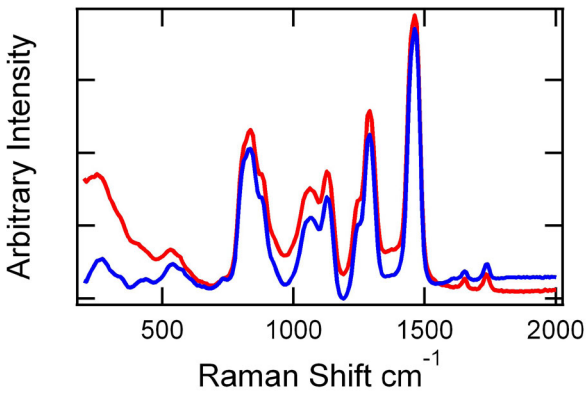

Figure 10. Polysobate 20 Raman spectra collected with 1064nm excitation showing baseline corrected spectrum (blue) and uncorrected spectrum (red).

Polysorbates

Polysorbates are widely used in pharmaceuticals, foods and cosmetics, commonly as emulsifiers and wetting agents. Polysorbates are light and air sensitive and typically shipped in sealed amber bottles. Therefore, it is highly advantageous to be able to perform measurements without opening original bottles. The spectra shown in Figure 9 are of polysorbate 20 and 80 measured in original amber glass bottles with 1064nm excitation. Similar spectra were collected on a 785nm excitation Raman spectrometer but the fluorescence was so strong that it was difficult to see the Raman peaks (data not shown). As can be seen from the spectra in Figure 9, the 1064nm handheld Raman spectrometer collects clear spectra and can distinguish these materials through the original packaging. These two spectra have only small differences with the exception of the peak at 1653 cm^{-1}. The Rigaku Progeny software has the ability to quickly report peak positions using a peak picking function. This 1653 cm^{-1} peak is indicative of the carbon-carbon double bond stretching of the monooleate group in polysorbate 80.

Other data processing such as spectral subtraction and baseline correction are easily performed with the flexible software. An example of baseline correction is shown in Figure 10. Fluorescence is the major contributor to sloping baselines and simply performing a baseline correction will not remove the effects of strong fluorescence, although it can improve the spectral appearance when only low levels of fluorescence are present. For this reason actual removal of strong fluorescence with 1064nm excitation is important, even when baseline correction is available.

Dyes

Dyes are another class of materials that benefit greatly from inspection with 1064nm Raman. FD&C Blue #2 and FD&C Yellow #6 are examples of dyes used in some pharmaceutical coatings that exhibit fluorescence at lower excitation wavelengths, yet these same two materials give very nice easily identifiable Raman spectra at 1064nm excitation. FD&C Yellow #6 has very strong fluorescence at 532nm excitation that is somewhat reduced at 785nm excitation, and basically disappears to give a very clean spectrum with 1064nm excitation, see Figure 11. FD&C Blue #2 has even stronger fluorescence than the yellow dye and it is impossible to distinguish any Raman peaks until the 1064nm excitation is used, Figure 12. These dyes are commonly used in foods and cosmetics as well.

Finished Pharmaceutical Products Identification

In addition to being used as an analyzer to verify incoming raw materials, 1064nm Raman can be used to confirm the identity of in-process and finished pharmaceutical products. Formulated dosages such as white, over the counter (OTC) analgesic tablets can yield identifying spectra easily using either 785nm or 1064nm Raman.

Spectra of Excedrin® measured using both excitation wavelengths can be seen in Figure 13. Bands from key active pharmaceutical ingredients (API) aspirin, caffeine, and acetaminophen are clearly observable using either wavelength. However, a dark green gelatin capsule containing the same three API compounds yields overwhelming fluorescence background at 785nm excitation. In contrast identifying Raman information is present in the 1064nm spectrum and peaks specific to each API are observed, Figure 14.

The spectral resolution of 1064nm handheld dispersive instruments is more than adequate to discriminate between similar compounds including API polymorphs and API salt forms. Spectra of ranitidine polymorphs are shown in Figure 15 and ibuprofen free acid vs ibuprofen sodium salt is shown in Figure 16. Spectra in Figures 15 and 16 were acquired at 1064nm excitation.

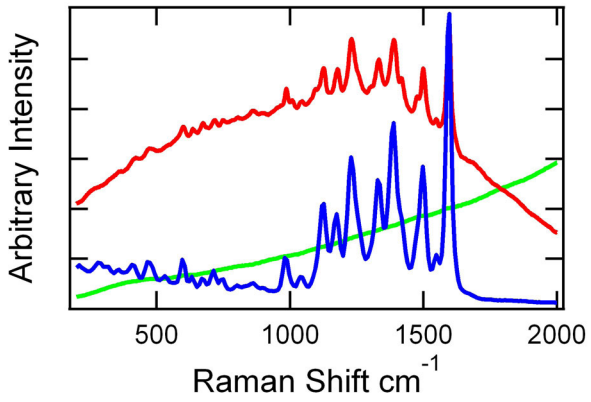

Figure 11. FD&C Yellow #6 measured using 532nm (green), 785nm (red) and 1064nm (blue) Raman systems. Reduced fluorescence is seen at higher excitation wavelengths.

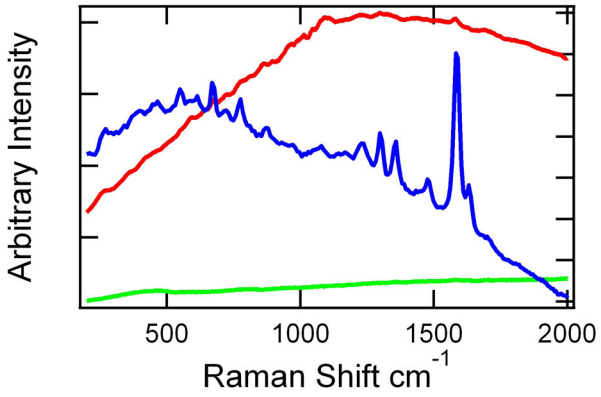

Figure 12. FD&C Blue #2 measured using 532nm (green), 785nm (red) and 1064nm (blue) Raman systems. The material is identifiable at the 1064nm excitation but impossible to identify at shorter wavelength excitation due to fluorescence background interference.

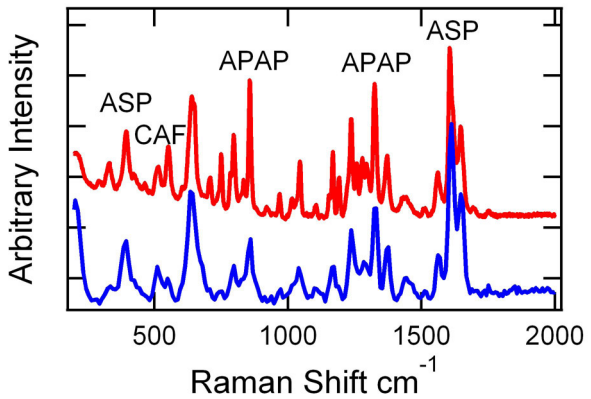

Figure 13. Comparison of 785nm (red) and 1064nm (blue) Raman spectra for a coated, Excedrin® Extra Strength tablet. Raman bands for key APIs are labeled: aspirin (ASP), acetaminophen (APAP) and caffeine (CAF).

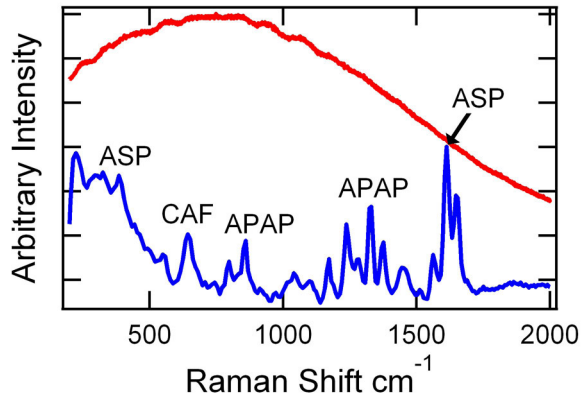

Figure 14. Comparison of 785 and 1064nm Raman spectra for a CVS Pharmacy brand dark green analgesic gelatin capsule. Raman bands for key APIs are labeled: aspirin (ASP), acetaminophen (APAP) and caffeine (CAF).

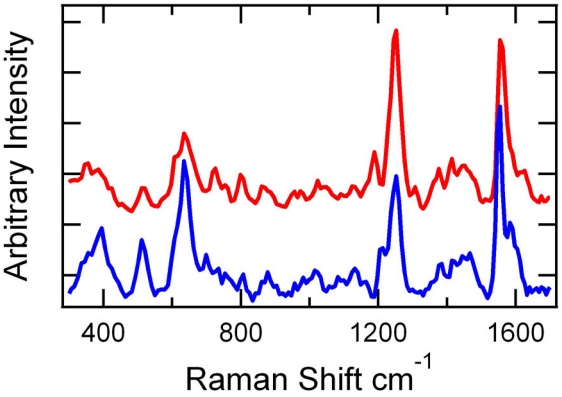

Figure 15. Raman spectra collected with 1064nm excitation of two different polymorphic forms of ranitidine in Zantac® (blue) and a CVS Pharmacy brand Acid Reducer (red).

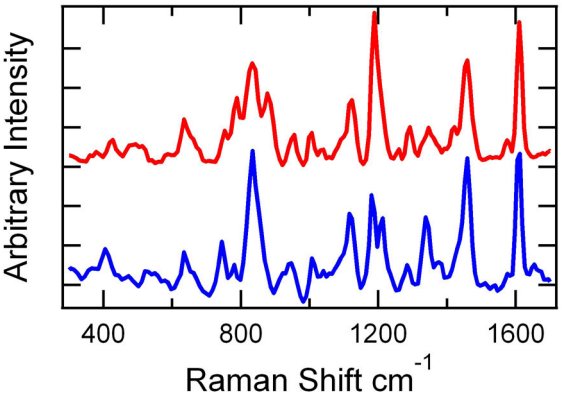

Figure 16. Raman spectra collected with 1064nm excitation can differentiate between ibuprofen free acid and the Na salt of ibuprofen in tablet cores of Advil® (acid) and New Advil® (Na salt).

Counterfeit Pharmaceutical Identification

Because Raman spectra are typically so unique, the technology can be used by pharmaceutical investigators to determine whether suspicious drugs are counterfeit or authentic. Portable Raman is especially well suited to address this problem because materials can be analyzed in the field vs. having to wait on lab results to determine drug authenticity. Figure 17 shows the spectra for a genuine capsule of the weight loss medication Alli® measured with both 785nm and 1064nm excitation. The spectrum collected with 785nm excitation shows significant fluorescence as observed in the broad y-axis curvature. This fluorescence makes it difficult to see the sharper Raman peaks that are present on top of this curvature. Similar results were seen when authentic Viagra® was measured at both 785nm and 1064nm excitation, data not shown. In Figure 18, authentic Alli® (60 mg orlistat) spectral data was overlaid directly with the counterfeit spectral data. The differences are clearly visible between the authentic and counterfeit samples measured with 1064nm excitation. Figure 19 highlights the difference between spectra from authentic and counterfeit erectile dysfunction drug Viagra® acquired at 1064nm. Spectra of coated and uncoated Viagra® acquired at 1064nm are presented in Figure 20. In the coated tablet, spectral features of the Opadry Blue coating as well as spectral components from excipients and the API in the tablet core are visible. Spectra from counterfeit and authentic Cialis measured in the field are presented in Figure 21. These spectra were collected with a 1064nm handheld spectrometer and clear differences are seen between the authentic and counterfeit medications.

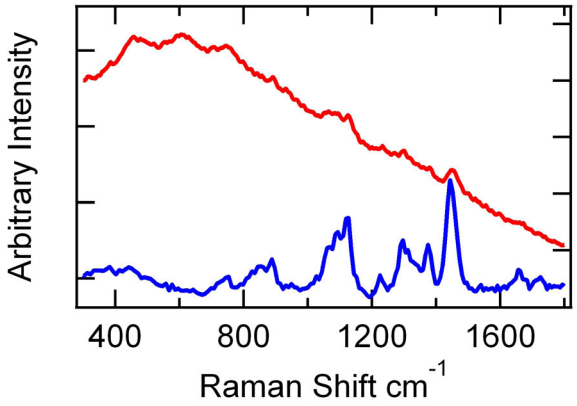

Figure 17. Authentic Alli® spectra acquired at 785nm (red) and 1064nm (blue) excitation.

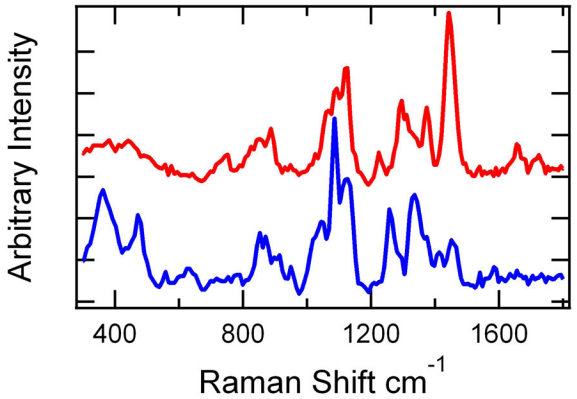

Figure 18. Authentic (red) and counterfeit (blue) Alli® capsule blend scanned using 1064nm excitation.

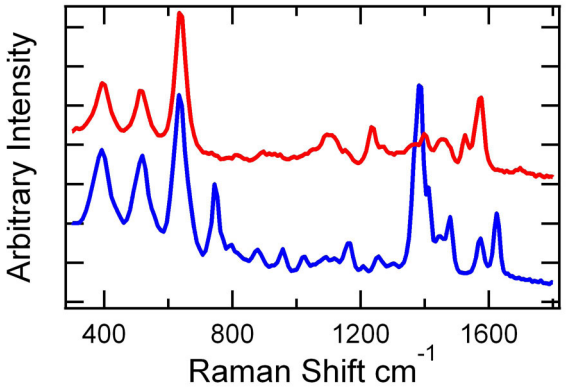

Figure 19. Authentic (red) and counterfeit (blue) Viagra® spectra acquired 1064nm.

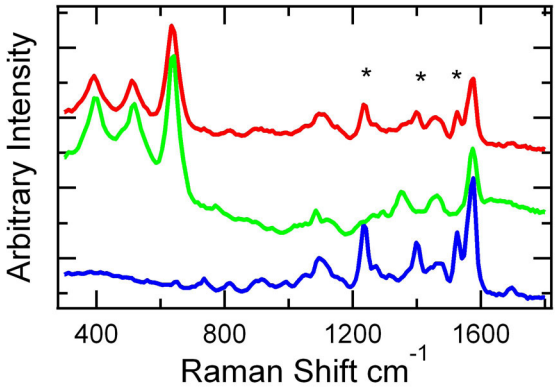

Figure 20. Raman spectra collected at 1064nm excitation of a Viagra® 100 mg coated tablet (red), Opadry Blue coating material (green) and an uncoated tablet (blue). API bands are labeled with an "*".

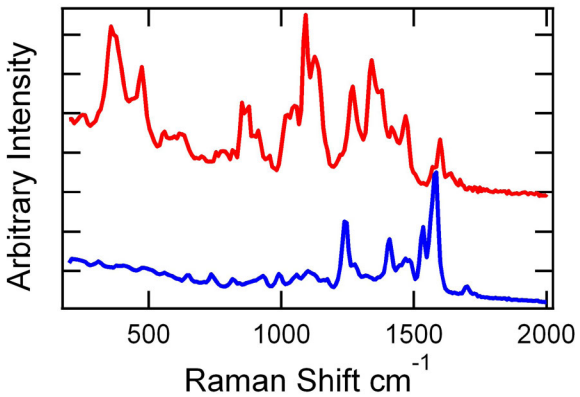

Figure 21. Raman spectra of authentic (red) and counterfeit (blue) Cialis® collected at 1064nm excitation.

Safety and Security Applications of Handheld Raman Spectroscopy

Narcotics

As drug abuse and illegal trafficking of controlled substances continues to be a global problem, security personnel and forensic teams require the ability to perform rapid and often on-the-spot identification to effectively remove these increasingly dangerous substances from circulation. As we have discussed, Raman spectroscopy can provide detailed and specific chemical information about a variety of samples. It is non-destructive, requires no sample preparation and delivers actionable results in seconds.

A prominent challenge when identifying narcotics is caused by the impurities typically found in street samples. These impurities often cause fluorescence which interferes with the Raman signal and reduces accuracy of results for many real world samples. Because of the prominence of fluorescence in real world narcotic samples, its elimination with use of a 1064nm laser excitation for the Raman measurements is especially important.

Here we review the results of four controlled substances analyzed with Rigaku handheld Raman spectrometer using 1064nm laser excitation. All spectra were measured in 10 seconds or less and show clear Raman peaks for each of the narcotics. Figure 22 shows spectra of two types of amphetamines, 3, 4-methylenedioxymethamphetamine (MDMA or Molly) and 1-Phenyl-2-methylaminopropane (methamphetamine). These are both similar types of narcotics but as can be seen the Raman spectra are quite different. Two even more closely related narcotics are shown in Figure 23, cocaine hydrochloride and crack cocaine (the free base form). Here the differences in the Raman spectra are more subtle but still present highlighting the specificity of Raman spectroscopy. The specificity of Raman allows narcotics to quickly be identified and distinguished from one another and from other materials.

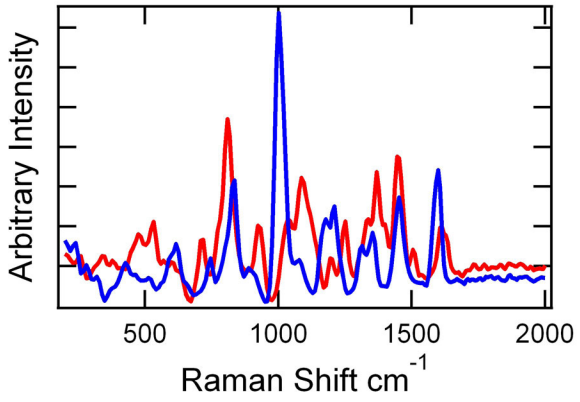

Figure 22. Raman spectra of two types of amphetamines, 3, 4-methylenedioxymethamphetamine (MDMA or Molly, red spectrum) and 1-Phenyl-2-methylaminopropane (methamphetamine, blue spectrum) collected with 1064nm excitation.

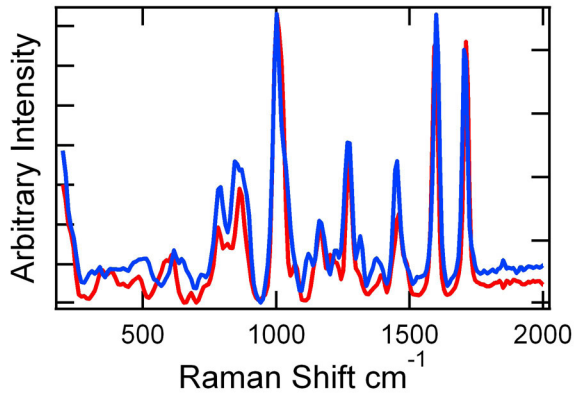

Figure 23. Raman spectra of cocaine HCl (red) and crack cocaine (blue) collected with 1064nm excitation.

Explosives

It has been documented that Raman spectroscopy is an effective technique for measuring explosive materials. However, many materials and precursors show significant fluorescence when Raman spectrometers with 532nm or 785nm excitation are used. Fluorescence interference reduces the signal to background noise ratio, can significantly increase the acquisition time, and reduces the number of peaks available for explosives identification.[6]

Raman measurements made with 1064nm excitation can eliminate fluorescence interference resulting in spectra that provide more reliable material identification. Figure 24 shows spectra from three different common explosives and high energy materials collected with a handheld Rigaku 1064nm excitation Raman spectrometer. Collection times were less than five seconds. As seen in the figure the spectra are quite clear and distinctive allowing them to be used for fast reliable identification of the materials at the point of use.

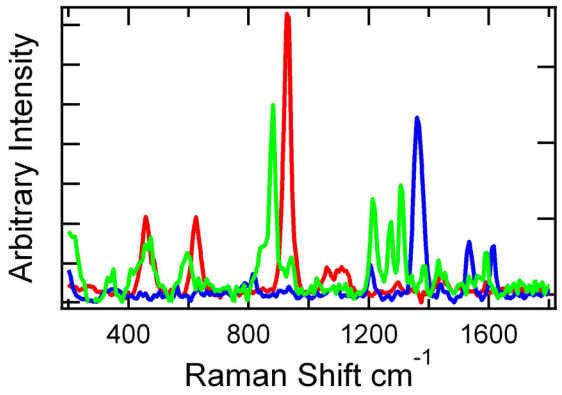

Figure 24. Raman s pectra of ammonium perchlorate (red), composition C (green) and trinitrotoluene (blue) collected with 1064nm excitation.

Dietary Supplements, Foods, and Food Additives Applications of Handheld Raman Spectroscopy

Due to a much reduced likelihood of fluorescence background interference, 1064nm based Raman has expanded possible applications for Raman spectroscopy in the areas of nutritional products and food science. Raman spectroscopy can be applied to dietary supplements, foods, and food additives for material inspection, process and product monitoring and counterfeit identification.

To comply with stringent regulations, dietary supplement manufacturers the world over are seeking more effective ways to perform materials verification throughout the production process. The widespread proliferation of Raman analyzers in the pharmaceutical industry is creating a paradigm shift in quality control methods that can influence and benefit the supplement industry. Figure 25 and Figure 26 are Raman spectra of highly colored nutritional products; turmeric and green tea extract. Both of these materials have been used to promote health in eastern traditional medicine and their use is increasing in popularity in the west.

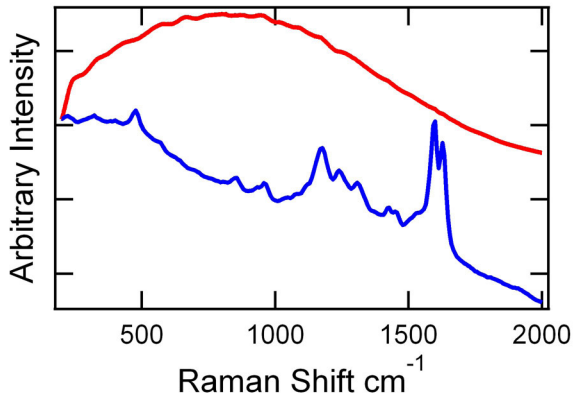

Figure 25. Raman spectra of turmeric measured at 785nm (red) and 1064nm (blue) excitation. Curcumin bands are visible at 1064nm excitation but are obscured by fluorescence at 785nm excitation.

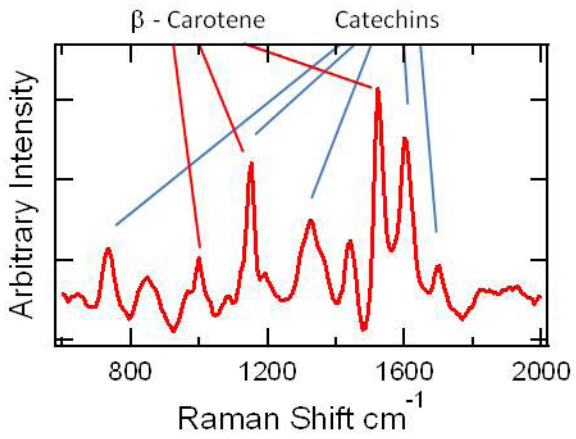

Figure 26. Raman spectrum of green tea extract. Peaks from nutritionally important phenolic antioxidant flavonoid compounds called catechins are labeled along with β–carotene. The 785nm spectra were completely obscured with fluorescence and are not shown.

Foods are another area handheld Raman may be used to assess product quality. Raman at 1064nm excitation can be used to differentiate edible oils, based on degree of saturation. This can be seen in Figure 27 which shows significant differences between some cooking oils. These are primarily due to differences in the percent of saturated and unsaturated fatty acids. The Raman peaks at approximately 1666 cm^{-1} and 1276 cm^{-1} are from unsaturated fatty acids and the peaks at approximately 1444 cm^{-1} and 1302 cm^{-1} are from saturated fatty acids. Spectra from eight different oils were added to a Rigaku user library; seven of the eight oils could be consistently identified.

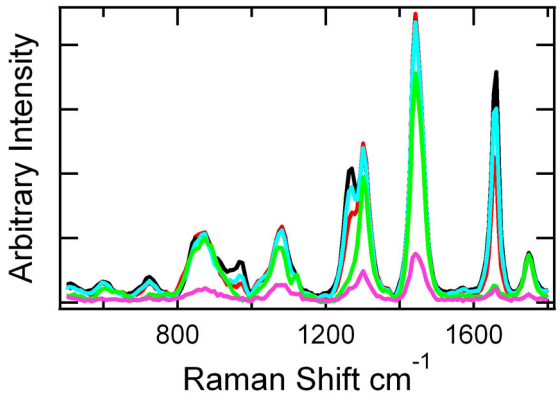

Figure 27. Normalized Raman spectra from several different edible oils, all measured using 1064nm excitation. Oils are differentiated based on fatty acid content. Spectra correspond to the following oils, olive (red), soybean (black), canola (blue), coconut (green), and palm (pink).

In a preliminary study, 1064nm Raman was used to differentiate honey sources. Raman spectra of synthetic and naturally sourced honey were collected. Spectral data were then exported to an external license free chemometrics package called R for principle component analysis (PCA). Preliminary PCA results, plotted in Figure 28 indicate that it may be feasible to source honey based on standards of known materials.

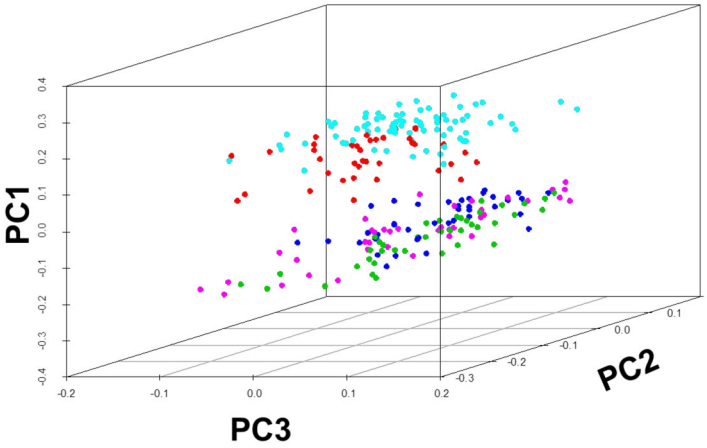

Figure 28. Discrimination between synthetic (cyan) and natural honey (blue, green and violet) using PCA. Preliminary data indicates it may be possible to differentiate different sources of honey based on 1064nm Raman.

Methanol in Drinking Alcohol

Methanol occurs naturally in food, notably in fresh fruits and vegetables and their juices. Natural methanol is also present in minute amounts in many alcoholic drinks with no immediate health threat. An alcoholic beverage with less than 2% methanol is considered to be safe for human consumption.[7] However, if methanol is present in high concentrations in alcoholic products it can cause severe health problems or even death.[8] Counterfeit alcohol, especially improperly distilled alcoholic beverages, may contain dangerous levels of methanol and injuries and deaths are attributed to methanol poisoning every year.

Raman spectroscopy has the specificity to distinguish ethanol and methanol and since Raman intensity is proportional to concentration it can also be used to determine the concentration of methanol present. Raman measurements were performed using a Rigaku 1064nm excitation portable Raman device. In order to obtain accurate concentration measurements, calibration standard spectra were collected, and chemometric models were built.

Figure 29 shows 1064nm excitation Raman spectra of different known concentrations of methanol spiked into red wine. The 1064nm excitation was used because it can analyze light colored and dark colored drinks. Dark colored drinks, such as whiskey and red wine, fluoresce strongly at shorter excitation wavelengths making their analysis difficult or impossible with 785nm excitation systems. Detection of methanol in red wines was tested by adding different concentrations of methanol to various red wines. The acquired Raman spectra were divided in two groups: spectra from samples with a methanol concentration lower than 2% (group "safe") and spectra from samples having a methanol concentration greater than 2% (group "toxic"). The model was constructed using the Raman spectra of the sweet red wine, whereas the prediction was made on the Raman spectra recorded from the dry red wine. Chemometric analysis was done with R, a license free program, using a support vector machines (SVM) algorithm. The identification results obtained using SVM analysis have an accuracy of 100% (see Table 2) , demonstrating that discrimination between samples with less than 2% methanol (safe beverages) and samples with more than 2% methanol (toxic beverages) is doable using a portable Raman device.

		Real	
		Less than 2%	More than 2%
Predicted	Less than 2%	20	0
	More than 2%	0	40

Table 2. Identification outcome using SVM on samples with less than 2% methanol ("safe") and samples with more than 2% methanol ("toxic")

To demonstrate that Raman spectroscopy can be used to detect methanol in strong alcoholic drinks, we have investigated a system simulating a 40% alcoholic drink, where alcohol was a mixture of ethanol and methanol in water. To test the reproducibility of the results, five sets of independent measurements were performed. The first three sets were used to create a statistical model using partial least squares regression (PLSR) while the last two sets were used for predictions. Figure 30 shows the PLSR model is able to correctly predict the concentration for samples having significantly lower concentrations of methanol than the approximately 5% which can be seen without chemometric analysis.

Raman measurements can be performed directly through original bottles, without the need of sample extraction, as shown in Figure 31. However, the limit of detection of methanol in various beverages is expected to increase. In addition, the content of some thick dark colored bottles might be difficult to investigate without sample extraction.

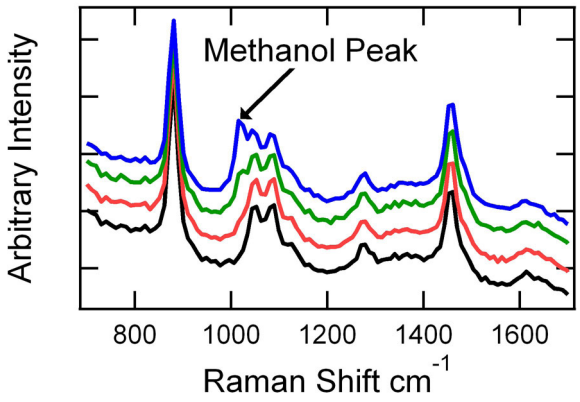

Figure 29 . Raman spectra, collected with 1064nm excitation, of different concentrations of methanol in sweet red wine. The percent methanol in the above spectra are 5% (blue), 3% (green), 1% (red), 0% (black).

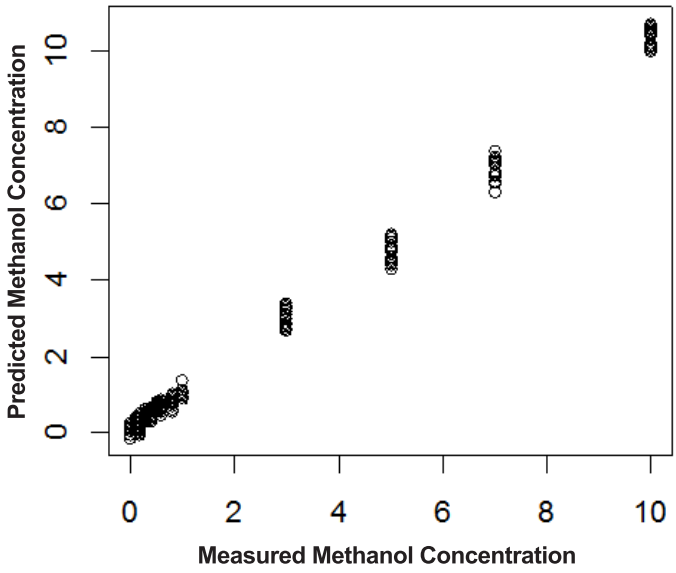

Figure 30. Prediction of the amount of methanol in strong (40%) alcoholic drinks.

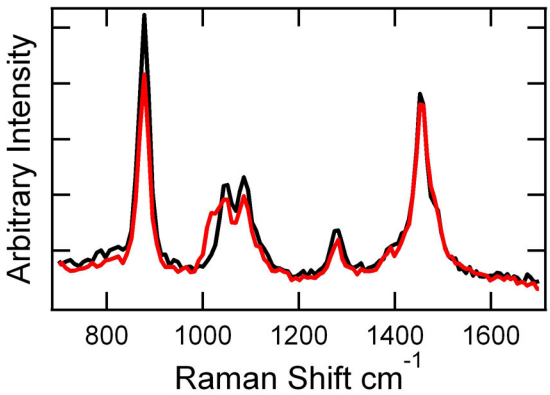

Figure 31. Raman spectra of un-spiked (black) and 9% methanol spiked (red) whisky. The measurements were performed through the original bottle.

Art and Archeology Applications of Handheld Raman Spectroscopy

The non-destructive nature of Raman is critical to its use in art and archeology. In addition, handheld spectrometers such as the Progeny are important because many works may not be removed from museums or archeological sites. Fluorescence interference and sample heating are more likely to be a problem with colored objects. Raman at 1064nm reduces the likelihood of fluorescence interference and state of the art software for Progeny permits control of the laser output to avoid heating and potential damage. The top and bottom Raman spectra in Figure 32 are respectively from a stone on the binding of the manuscript known as Liber Evangeliorum and an emerald. By spectral comparison this stone in the manuscript binding can be identified as an emerald. These spectra were measured with a 1064nm Rigaku handheld spectrometer to avoid strong fluorescence that is emitted from emeralds measured at shorter excitation wavelengths. A second example is shown in Figure 33 where a handheld Raman spectrometer was used to distinguish ancient Roman ivory from medieval ivory in two different artifacts without removing the artifacts from the museums.

Conclusion

Rigaku's Progeny 1064nm handheld Raman is well suited for a variety of application needs. Users now have the power to identify more materials using a rugged device that is easy to use and provides customizable settings, enabling lab-quality analysis at any time and in any place.

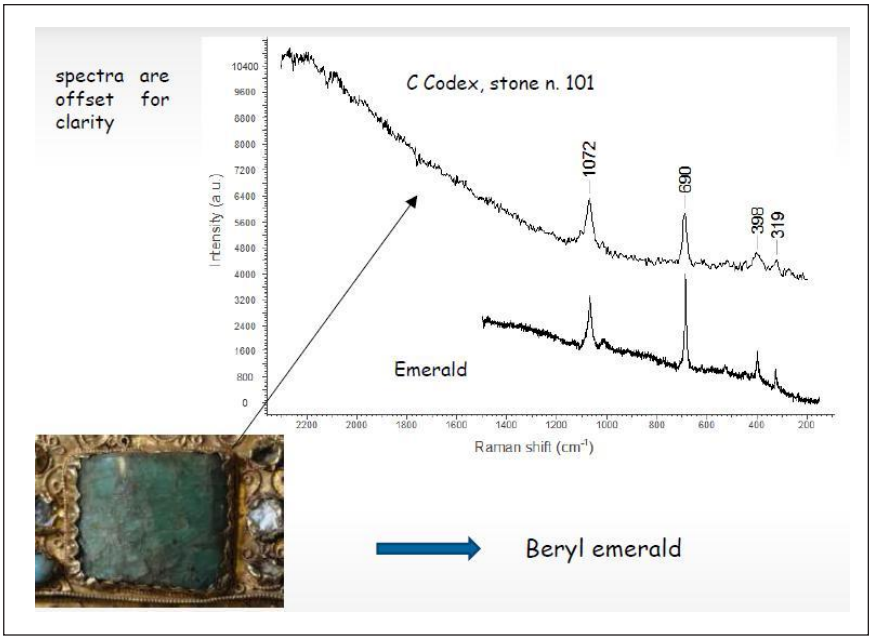

Figure 32. Identifying an emerald in the binding of the manuscript known as Liber Evangeliorum or C Codex from the Vercelli Museum of Cathedral treasure, XI century AD.

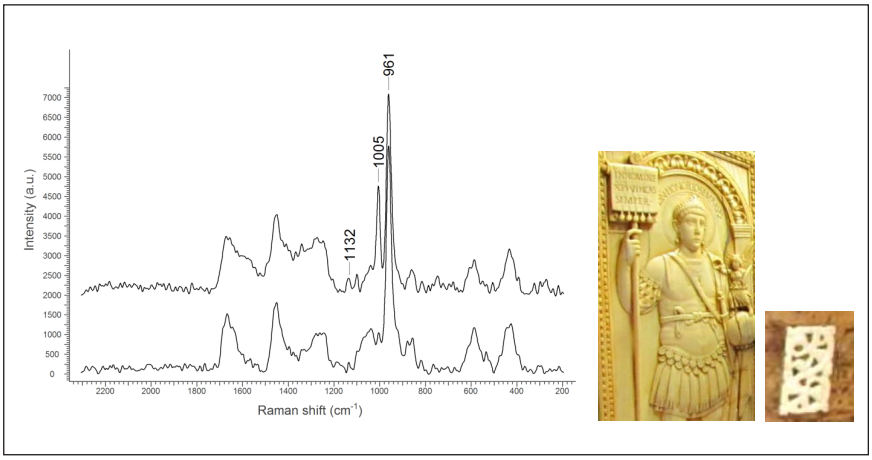

Figure 33. Raman spectra of (lower trace) V century A.S. Roman ivory diptych (most probably made of elephant ivory) and (upper trace) medieval ivory box.

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