

Tissue Raman Measurement at 1064nm

High signal and significantly reduced fluorescence using a dispersive 1064nm Raman spectrometer.

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Background

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 have led to a surge in Raman usage in a myriad of application areas, including pharmaceutical, biomedical, industrial, and forensic, among others. In all of these applications, however, there remains a struggle to extract useful Raman spectra from fluorescent and luminescent samples.

Fluorescence is much more likely and intense at short wavelengths where energies are more apt to cause an electronic transition. So, despite the lower Raman scattering cross-section (inversely proportional to λ^4), most users with potentially fluorescent samples long-ago switched to near-infrared wavelengths such as 785 or 830nm. These laser wavelengths push the detected signal to the spectral edge of silicon used in conventional multichannel CCD detectors, which are largely transparent above ~ 1050 nm. Yet even at these near-IR wavelengths, many substances still fluoresce, sometimes prohibiting Raman spectral acquisition.

For those users who require longer wavelengths such as 1064nm, the only available option has been FT-Raman, which is typically noisier 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 traditional dispersive Raman instruments with the fluorescence avoidance of traditional FT-Raman instruments. In addition, this dispersive geometry permits diffraction-limited optical performance, finally allowing confocal microscopic Raman at 1064nm.

Methods

A variety of animal tissues obtained from a local market were interrogated using two BaySpec benchtop systems: the RamSpec™ 785 and the RamSpec™ 1064. Both

systems utilized filtered fiber probes (InPhotonics RPB) designed for each respective wavelength, both with a 7.5mm focal length. Acquisition times were 30 seconds for both systems, unless otherwise noted. Power was set at 50mW for the 785nm measurements, and 150mW for the 1064nm measurements, unless otherwise noted.

Results

As noted previously, biological tissues are notorious for their difficulty in extracting clean Raman signal. Pigmented and porphyrin-rich tissues such as kidney, in particular, are often too fluorescent to be measured, even at 785nm; see Figure 1. But using 1064nm, a clear Raman spectrum relatively devoid of fluorescence background is generated using the same integration time. Additionally, because of the extended quantum efficiency of the InGaAs detector, high wavenumber features (C-H, O-H, and N-H stretching modes) are also simultaneously captured with the same laser. This is in contrast to high wavenumber Raman on a 785nm spectrometer, in which these bands present at the edge of Si detectability, thereby necessitating a second, shorter wavelength laser source (usually 671nm or 720nm)¹ and suitable filters and optics to accommodate both laser lines.

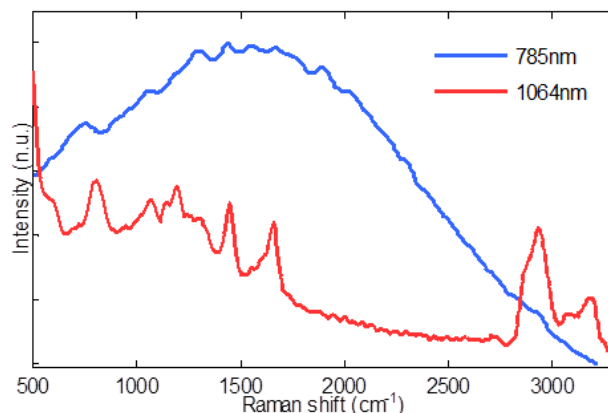


Figure 1: Kidney (porcine) is highly fluorescent at 785nm, preventing extraction of a usable Raman signal. At 1064nm, however, this fluorescence interference is largely avoided and clear Raman bands are evident.

In addition to the fluorescence of biological tissues, other major obstacles to clear Raman sampling is their inherently turbid optical environment and low Raman scattering cross-section. Even devoid of fluorescent interference, this noisiness and lack of signal generally require extended integration times, typically tens of seconds or more. To evaluate the necessary measurement times of the 1064nm Raman system for tissue, a number of spectra were obtained at various powers and integration times on a sample of porcine fat. As seen in Figure 2, high SNR spectra were readily obtained within 10 seconds, with only a slight decrease in SNR using integrations down to 2 seconds.

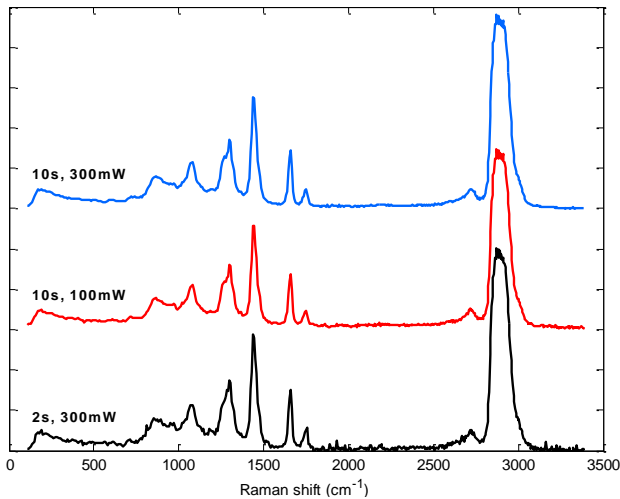


Figure 2: Raman spectra of fat (porcine) measured using various integration times and power at the sample show that high signal to noise ratio is possible in relatively short integration times, typical of those used in shorter wavelength tissue Raman studies.

In addition to allowing users the ability to measure Raman spectra from highly fluorescent samples, the dispersive 1064nm Raman systems also reduce the stringent sampling conditions necessary at shorter wavelengths. For example, 785nm Raman measurement through vials, cuvettes, or under cover slips often requires that these materials be made of fused silica, quartz, or calcium fluoride for their reduced fluorescence, all of which cost considerably more than bulk glass materials. However, as seen in Figure 3, even inexpensive glass sample vials can be used for 1064nm measurement of weak Raman scatterers such as chicken breast tissue.

Discussion

As demonstrated in the spectra presented here, dispersive Raman measurement at 1064nm offers a number of advantages over shorter wavelength options like 785nm. One of the lingering concerns about the use of 1064nm Raman is the reduced Raman scattering cross-section. As compared to 785nm Raman, this cross-section, indeed, reduces approximately $3.4\times$. However, according to permissible standards for human tissue (skin) exposure, the maximum power level that can be tolerated at 1064nm is approximately $3.4\times$

higher than the power permissible at 785nm.² So, even in photosensitive samples such as biological tissue, the physical reduction in Raman efficiency can be totally compensated by increased laser power.

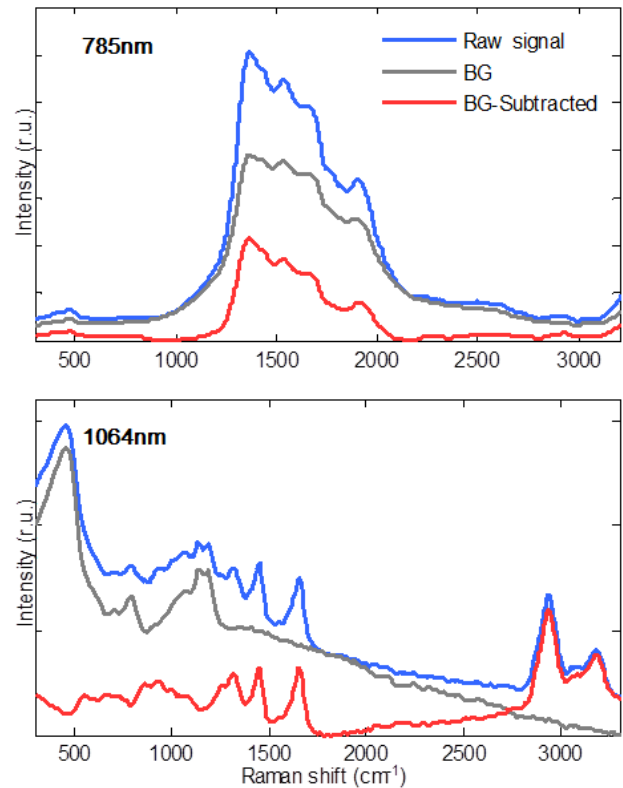


Figure 3: The effect of a glass container on chicken breast muscle Raman measurement at both 785 and 1064nm. The high background signal of the glass at 785nm pervades the measurement volume of the tissue and cannot be subtracted, leaving no resolvable Raman bands. At 1064nm, this background signal is much reduced and can be simply subtracted to reveal clear Raman bands.

Based on these experiments, 1064nm dispersive Raman will provide a viable new option for those users who are studying highly fluorescent tissues, desire to measure multiple samples in simple glass containers, or those users who are interested in simultaneous fingerprint and high wavenumber spectral acquisition. Future studies will certainly evidence further advantages over this approach, as compared to shorter wavelength (785 or 830nm) Raman approaches or FT-Raman systems.

References

- 1) Nijssen et al., J Biomed Opt, 12(3), p. 034004, 2007.
- 2) National Institute of Standards and Technology, Z136.1, p.76-77, 2007.