Tech Tip

Keywords

- Raman
- Excitation wavelength
- Surface Enhanced Raman Spectroscopy (SERS)
- Autofluorescence

Techniques

- Raman spectroscopy
- Raman scattering

Applications

- Biomedical applications
- Authentication
- Chemical analysis
- Explosives detection
- Food integrity
- Materials analysis

Tech Tip: More Raman Wavelengths, More Choices

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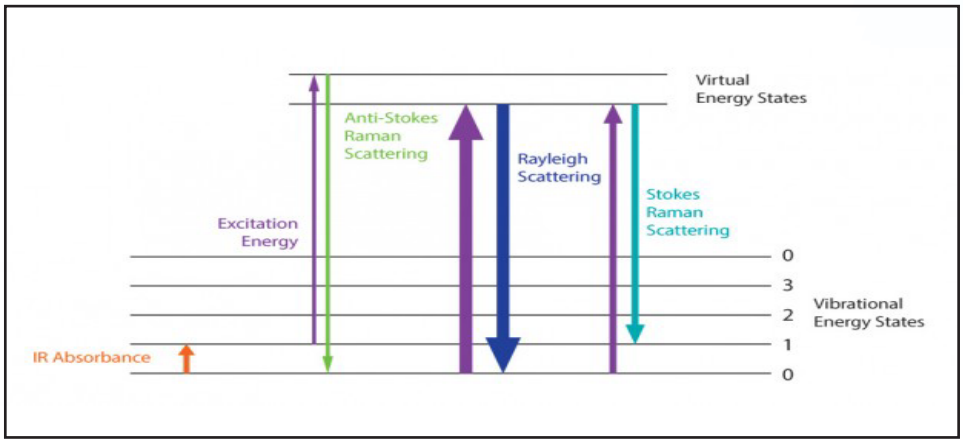
The Utility of Raman Spectroscopy

Raman spectroscopy has become a powerful tool for the analysis of materials – in the field, in the lab, and even in clinical settings. The ability to measure spectral fingerprints and compare them to a library of known substances allows identification of pharmaceutical ingredients at the loading dock and explosive materials in the field. In clinical applications, statistical analysis of Raman spectra enables detection of changes in genetic material, proteins and lipids, allowing its use to discriminate between healthy and unhealthy tissue or to detect changes at the cellular level.

Raman spectroscopy uses nonelastic scattering of laser light from a molecule to probe its molecular structure. Of every million photons bombarding the sample, one lone photon either gains or loses a small amount of energy, corresponding to a vibrational transition within the sample. As this happens again and again, a molecular fingerprint of the sample is gradually built up – one that can rival an FTIR spectrum, yet without the inconvenience of elaborate sample preparation or water interference. Even better, since the incident laser light is not being directly absorbed, it does not require a specific excitation laser. At least in theory …



Raman scattering from a molecule results in light of a slightly longer or shorter wavelength than the excitation laser, with the energy difference corresponding to a vibrational energy level transition within the molecule.

The Importance of Wavelength

Though any wavelength can be used to stimulate the Raman effect given enough incident intensity, some wavelengths are better than others, particularly for certain sample types. Ocean Optics offers a range of bundled modular Raman systems to suit a wide range of applications, and the guidance to help you select the right one for your application.

The probability of Raman scattering decreases rapidly as the excitation wavelength of the laser increases, scaling as $1/\lambda 4$. This means that increasing your laser wavelength merely from 532 nm to 638 nm will cost you half of your signal, and going to 785 nm drops it to nearly one-fifth! Though this might steer one to the use of shorter laser wavelengths, there is autofluorescence to be considered. Organic and biological samples tend to fluoresce when exposed to high laser intensities, creating a broad background that can obscure the Raman signal, which may degrade signal to noise and make Raman peaks difficult to resolve even when observed. Autofluorescence is strongest when the excitation laser energy corresponds to an electronic transition within the sample, and is typically greatest at visible laser wavelengths for organic materials. Polymers, pharmaceuticals, many synthetic materials and dyes are particularly subject to this effect.

For this reason, organic samples are often studied using red or NIR excitation wavelengths, where the benefits of reduced autofluorescence background easily compensate for the anticipated need for a longer acquisition time or increased laser intensity. In fact, autofluorescence can be almost completely eliminated in most samples by using 1064 nm laser excitation, as this energy is too low to excite an electronic transition in most materials. It can also be avoided by working at ultraviolet excitation wavelengths (200-250 nm), as the entire Raman spectrum of interest can be captured prior to the onset of autofluorescence at ~300 nm.

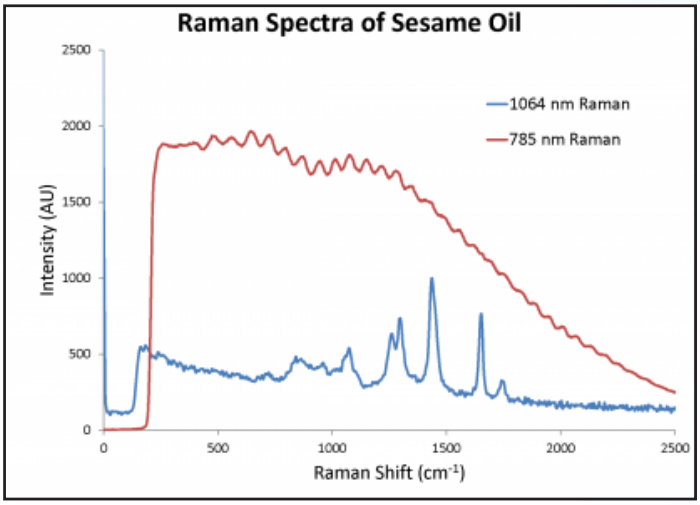
Benefits and Applications by Wavelength

Ocean Optics offers a variety of modular and turnkey solutions for Raman spectroscopy from the UV to NIR, including bundled systems for 532, 638, 785 and 1064 nm. Let's consider the benefits and applications typically studied by wavelength.

1064 nm excitation

This excitation wavelength has been gaining in popularity in recent years, due in large part to the minimal fluorescence generated, particularly for pigment-rich tissues and materials that can be troublesome even at shorter NIR wavelengths. Though the Raman signal is much weaker (6% of what would be predicted for 532 nm), the near-absence of fluorescence permits spectra to be obtained with a reasonable signal to noise ratio. Though the Fourier transform method (FT-Raman) is often used when working at 1064 nm excitation, sensitive diode-array spectrometers such as the NIRQuest can also offer adequate sensitivity and resolution for many applications. Care should be taken to avoid overheating of biological samples due to the longer wavelength illumination.

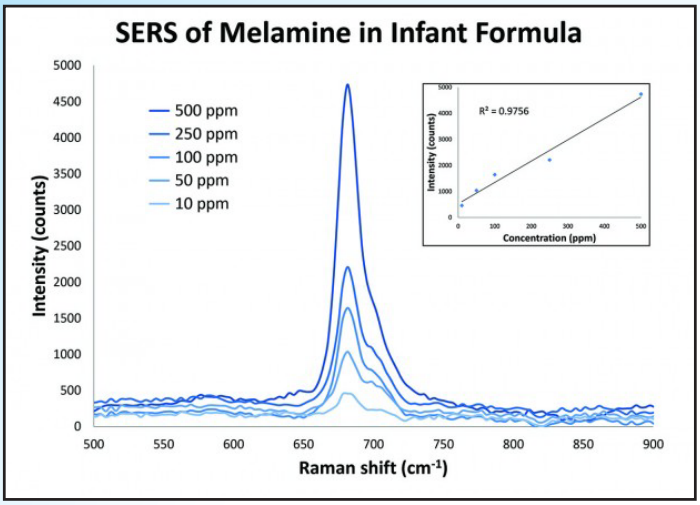
Applications: polymers, biological tissue, oils, edible oils, dyes, plant biomass, petrochemicals

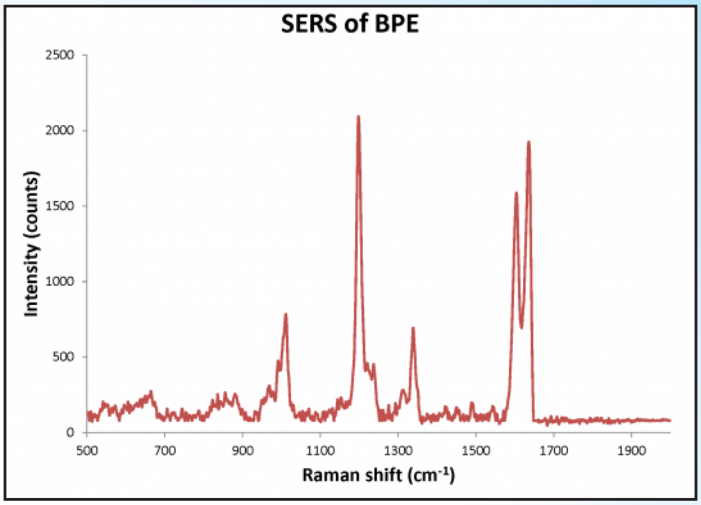


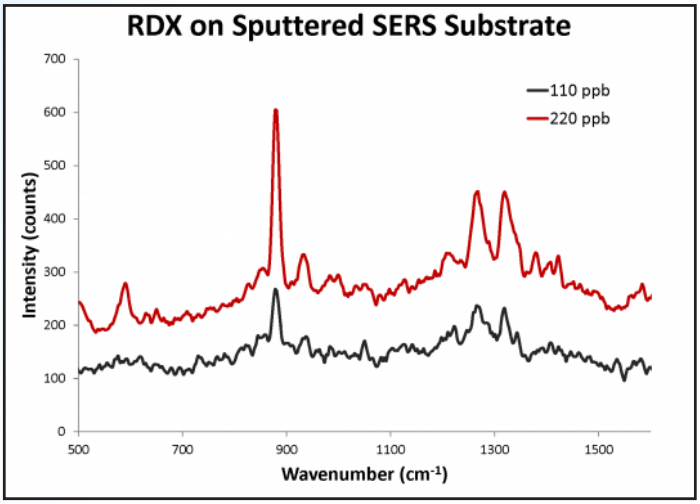
785 nm excitation

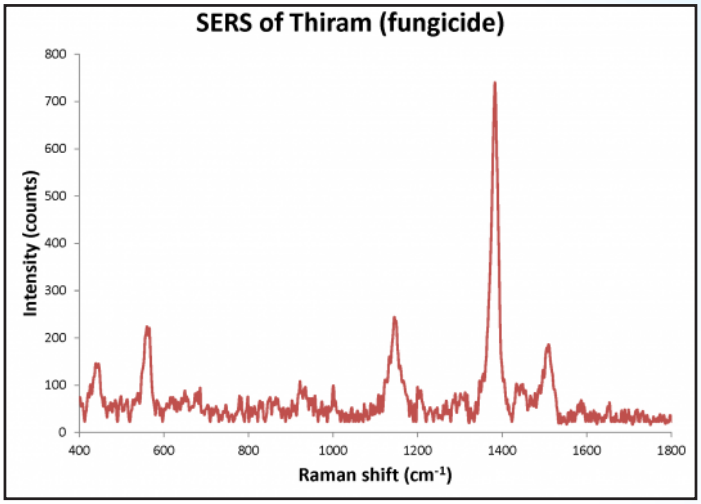
The most popular Raman excitation wavelength is 785 nm. Raman 785 nm systems yield excellent quality Raman spectra for most chemicals, with limited interference from fluorescence. These systems also offer very good spectral resolution, making 785 nm perhaps the preferred wavelength choice for general Raman spectroscopy of chemicals and organic materials

Applications: polymers, biological tissue, active pharmaceutical ingredients (APIs), art pigment identification, edible oils, petrochemicals, foods, explosives, sorting of dark plastics, through-bottle inspection, narcotics, SERS









638 nm excitation

Raman spectroscopy at 638 nm offers many of the same benefits as working at 785 nm, balancing signal level with fluorescence and offering very good resolution. This wavelength is often used for most biomedical applications, which need to balance the risk of sample damage with fluorescence generation. We have also found 638 nm to be an extremely versatile wavelength for generating high-quality SERS data for a wide range of analytes, from trace detection of explosives to pesticides, fungicides, and more.

Applications: biomedical instrumentation, corrosion, pesticides, fungicides, SERS

SERS

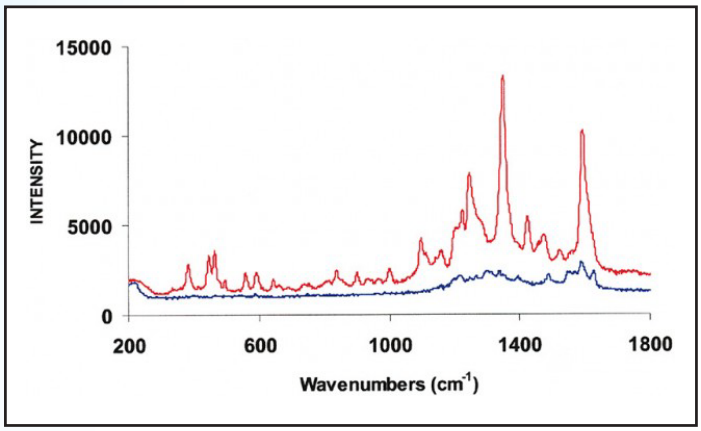
In Surface Enhanced Raman Spectroscopy (SERS), analytes are adsorbed on to a silver or gold surface prior to analysis, boosting the Raman signal intensity by millions of times. The use of solid state substrates for SERS allows ppb-level detection of chemical and biological materials in the field, as well as in pharmaceuticals, explosives and tags for anti-counterfeiting.

Most SERS substrates are fabricated using expensive lithography techniques and are not reusable, making cost a deterrent for mainstream applications. Ocean Optics inkjet-printed SERS substrates offer better performance at a fraction of the price, with peak intensity ratios repeatable to within 5%. Using SERS substrates is easy with our quick start guide.

532 nm excitation

This wavelength is the workhorse for inorganic materials, offering maximum signal for samples that do not suffer from autofluorescence. Often used for the study of carbon nanotubes, fullerenes and other carbon materials to avoid sample burning, 532 nm excitation is also good for resonance Raman experiments.

Applications: semiconductor materials, catalysts, polymers, minerals, carbon nanotubes and nanowires, temperature measurement, plasmon and superconducting gap excitation studies, nanowire composition, silicon crystallinity in solar cell manufacture, gemstone analysis and authentication



A 90:10 mixture of unmarked diesel-kerosene (blue trace) is easily distinguished from a mixture in which the kerosene diluting the diesel contains a marker (red trace).

Ultraviolet excitation

Though UV excitation and detection avoids the autofluorescence window entirely, it carries its own host of challenges. UV laser sources are generally lower in power and much more expensive than those at longer wavelengths, and require more expensive optical filters to block scattered laser light, which also may limit the starting wavelength of the detection range. The same Raman spectrum of 2500 cm-1 that spans ~190 nm when excited by 785 nm light is compressed into only ~16 nm when excited by 248 nm light, placing greater demands on the spectral resolution of the detection spectrometer. Though useful for fluorescence-prone samples like biomolecules and eye-sensitive applications like standoff Raman detection of explosives on the battlefield, UV excitation is impractical for many applications.

Applications: study of proteins, DNA and RNA, standoff explosives detection

Modular Raman Spectroscopy Systems

Take the guesswork out of creating a modular Raman spectroscopy system using the table below. Designed to operate as a system, the components selected for each excitation wavelength mate seamlessly to get you started taking Raman spectra more quickly. Remember to also purchase OceanView software and the appropriate laser safety glasses.

System	532 nm	638 nm	785 nm	1064 nm
Laser	LASER-532-IP-LAB	LASER-638-LAB-FC	LASER-785-LAB-ADJ	LASER-1064-LAB-ADJ-FC
Spectrometer	QE *Pro*, grating H6, 50 µm slit	QE *Pro*, grating H6, 50 µm slit	QE *Pro*, grating H36, 50 µm slit	NIRQuest512-1.9, grating NIR10, 50 µm slit
Probe	RIP-RPB-532-FC	RIP-RPB-638-FC-SMA	RIP-RPB-785-FC-SMA	RIP-RPB-1064-FC
Spectral Range	532-700 nm	638-799 nm	785-935 nm	1060-1446 nm
Spectral Resolution (FWHM)	0.43 nm	0.41 nm	0.48 nm	1.73 nm
Raman Range	150-4000 cm^{-1}	150-3150 cm^{-1}	150-2100 cm^{-1}	150-2480 cm^{-1}
Raman Resolution*	9-15 cm^{-1}	7-10 cm^{-1}	6-8 cm^{-1}	9-15 cm^{-1}

* Resolution in wavenumbers varies with the wavelength being measured. It is better close to the laser line, and lesser at longer Raman shifts.

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