Confocal Raman Spectroscope

Basic Principles

Raman Spectroscopy is a non-destructive chemical analysis technique which provides detailed information about chemical structure, phase and polymorphy, crystallinity and molecular interactions. It is based upon the interaction of light with the chemical bonds within a material.

During a basic Raman Spectroscopy measurement, the sample is irradiated with photons of a specific wavelength (high intensity laser light source) and the sample molecules scatter the incident light (Figure 1).
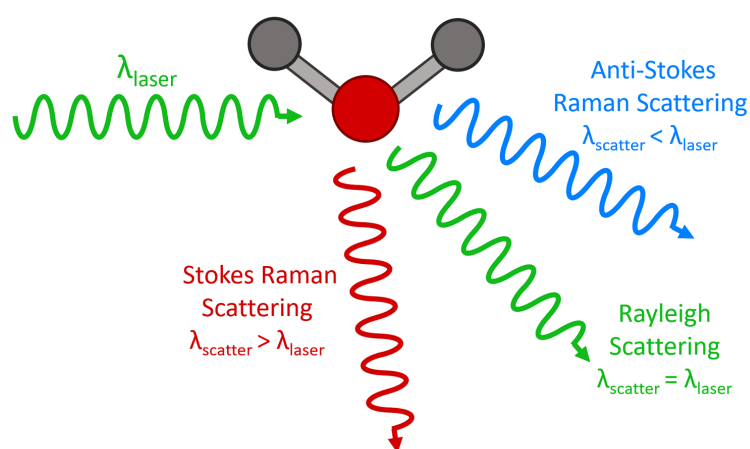

Figure 1: Raman light scattering

Most of the scattered light is at the same wavelength as the laser source a (Rayleigh Scatter from elastic collisions) while a small amount of light is scattered at wavelengths that are different from the source wavelength. This energy difference is due to inelastic collisions in which the photon interacts with the sample and is occurring due to a change in the rotational and vibrational energy of the molecule.
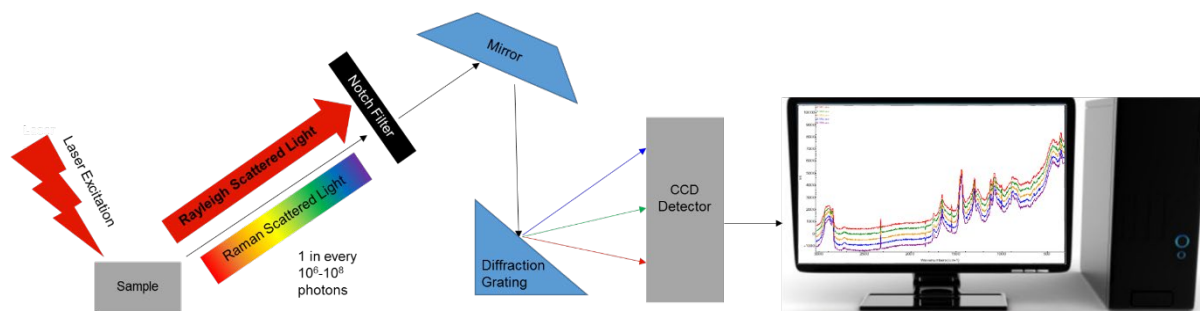

Figure 2: shows the basic instrumentation for a Raman spectrophotometer

A Raman spectrum features a number of peaks, showing the intensity and wavelength position of the Raman scattered light. Each peak corresponds to a specific molecular bond vibration, including individual bonds such as C-C, C=C, N-O, C-H etc., and groups of bonds such as benzene ring breathing mode, polymer chain vibrations, lattice modes, etc.

Information provided by Raman Spectroscopy

Raman spectroscopy is both qualitative and quantitative

The general spectrum profile (peak position and relative peak intensity) provides a unique chemical fingerprint which can be used to identify a material, and distinguish it from others. Often the actual spectrum is quite complex, so comprehensive Raman spectral libraries can be searched to find a match, and thus provide a chemical identification.
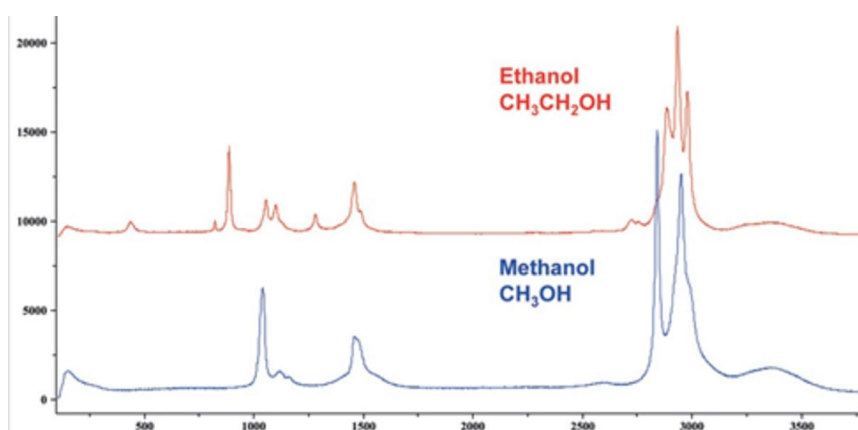

Figure 3: Raman spectra of ethanol and methanol showing the significant spectral differences.

The intensity of peaks observed in a spectrum is directly proportional to concentration of the species from which it was generated. Typically, a calibration procedure will be used to determine the relationship between peak intensity and concentration, and then routine measurements can be made to analyse for concentration. With mixtures, relative peak intensities provide information about the relative concentration of the components, while absolute peak intensities can be used for absolute concentration information.
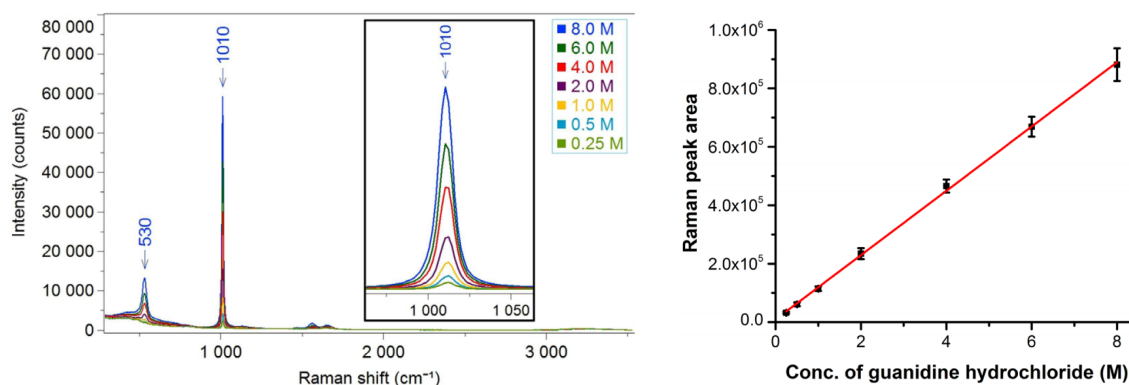
Figure 4: Raman spectra from 0.25, 0.50, 1.0, 2.0, 4.0, 6.0, and 8.0 M guanidine hydrochloride (left). Calibration plot for 1010 cm-1 peak area relation to guanidine hydrochloride concentration (right).

Quantitative analysis of more complex samples may require peak deconvolution, multivariate analysis and chemometrics in order to distil out the important information from the data.

Raman spectroscopy for microscopic imaging and analysis

Raman spectroscopy can be used for microscopic analysis, with a spatial resolution in the order of 0.1-1 µm. Such analysis is possible using a confocal Raman microscope.

A Raman spectrometer coupled to an optical microscope allows for high magnification visualization of a sample and Raman analysis with a microscopic laser spot. A true confocal Raman microscope can be used for the analysis of micron size particles or surface features. It can even be used for the analysis of different layers in a multi-layered sample, contaminants and features beneath the surface of a transparent sample (e.g., impurities within glass, and fluid/gas inclusions in minerals).

Motorized mapping stages allow Raman spectral images to be generated, which contain many thousands of Raman spectra acquired from different positions on the sample. False colour images can be created based on the Raman spectrum that can be overlaid onto microscopy images. These can be tailored to show the distribution of individual chemical components and variation in other effects such as phase, polymorphism, stress/strain, and crystallinity.

Type of samples analysed with Raman
Raman can be used to analyse many different samples. Some typical examples of where Raman is used today:

- Art and archaeology – characterization of pigments, ceramics and gemstones

- Carbon materials – structure and purity, defect/disorder characterization
- Chemistry – structure, purity, and chemical characterisation, reaction monitoring, calibration and quantification of analytes, phase transition temperatures etc.
- Geology – mineral identification and distribution, fluid/gas inclusions and phase transitions
- Life sciences – single cells and tissue imaging, drug interactions, disease diagnosis
- Pharmaceuticals – content uniformity and component distribution in formulations
- Semiconductors – purity, alloy composition, intrinsic stress/strain

The technique is considered as non-destructive, however, photo-degradation of samples can be a severe problem in Raman spectroscopy. Constant illumination of a substrate can heat up the sample and eventually cause burning or evaporation of the sample or matrix.

Analysis of solids, liquids and gases
Raman spectra can be acquired from nearly all samples which contain true molecular bonding. This means that solids, powders, slurries, liquids, gels and gases can be analysed using Raman spectroscopy.

Although gases can be analysed using Raman spectroscopy, the concentration of molecules in a gas is typically very low, so the measurement is often more challenging. Usually specialized equipment such as higher powered lasers and long path length sample cells are necessary. In some cases where gas pressures are high (such as gas inclusions in minerals) standard Raman instrumentation can easily be used.

Analysis from a mixture of materials
The Raman spectrum from a material will contain Raman information about all of the molecules which are within the analysis volume of the system. Thus, if there is a mixture of molecules, the Raman spectrum will contain peaks representing all of the different molecules. If the components are known, the relative peak intensities can be used to generate quantitative information about the mixture's composition. In case of complex matrixes, chemometrics and multivariate methods might also be employed to build quantitative methods.

Raman Spectroscope Horiba Labram Evolution Confocal Raman Spectrocope

Labram HR Evoloution Raman Spectroscope located in 302-930B

Microscope and sample stage

The Labram HR Evolution is a fully integrated confocal Raman microscope instrument incorporating an adjustable confocal pinhole aperture ranging from several μm to 1200 μm. The instrument is equipped with revolving turret equipped with following 5x, 50x, 100x and NIR achromatic objectives and is fitted with high throughput achromatic coupling optics optimized to work from UV to NIR with maximum efficiency.

The instrument is fitted with a motorised/manual XYZ stage with step resolution of 0.1 μm. The stage control can be automated for Z height adjustment at optimum laser focus.

Temperature control

The Raman lab has a Linkam temperature controlled cryostat/stage that can be fitted into the sampling space. This equipment can allow for temperature control down to liquid nitrogen temperature -196 °C and up to 400 °C.

Lasers

The instrument has three laser options that can be selected for your experiments and each can be applied to the sample using a range of power settings. The Rayleigh scattering from each laser line is removed with an edge filter. This means we cannot observe anti-stokes Raman signals with the current configuration.

1. Air-cooled 532 nm Nd:Yag laser lase (532 nm / 100 mW / 1MHz)
2. 785 nm laser including air-cooled intracavity regulated laser diode with point source for maximal confocal performance (785 nm/ 100 mW)
3. 1064 nm laser kit including air-cooled Nd: Yag laser (1064 nm / 500 mW).

When choosing a laser for excitation and the power settings you will operate under, consider resonance effects, fluorescence and the likelihood of sample photodegradation.

For example, in cases where samples strongly fluoresce with visible excitation, NIR excitation can allow production a high quality Raman spectrum. However, since Raman scattering efficiency is proportional to λ^{-4} , where λ is the laser wavelength, Raman scattering using a 785 nm laser source is almost a factor of 5 times less efficient than with a 532 nm laser source.

Gratings

The instrument is fitted with an 800 mm focal length flat field monochromator with three grating options: 1800 gr/mm, 600 gr/mm (500) and 600 gr/mm (750) that can be used to vary the spectral resolution and coverage. Higher resolution spectra can be obtained with increased groove density of the grating. **Gratings can only be changed by the Technologist and not by general users.** Please contact Cherie if you want to change the grating

Detectors

The instrument is fitted with two detectors:

1. Multichannel air-cooled (-60 °C) CCD array detector for UV-VIS-NIR offering a spectral range of 200 to 1050 nm. The quantum efficiency is low for wavelengths longer than 800 nm
2. Liquid nitrogen cooled InGaAs line detector (-110 °C) ideal for low-light-level measurements in the NIR spectral region from 800–1700 nm.

Software

Labspec 6 software is used to control the instrument and data acquisition and can be used for data manipulation and processing. LabSpec 6 has a suite multivariate analysis functions and you can export your data for use in other software.

Labspec 6 is a licenced software, you can install the basic operations on your device for free. If you need the more advanced features of Labspec 6 you can access the full software by VPN. Talk to the Raman technologist to arrange access.