Experimental Laboratory 513.(010/011/014)

Raman Spectroscopy

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1 Outline

Raman spectroscopy is a powerful tool for the non-destructive investigation and characterization of all kinds of materials. It has a large range of applications, which span from fundamental science to *in situ* quality control in production lines. When a Raman spectrometer is combined with a confocal microscope (Raman microscope), it is even possible to visualize the distribution of materials on the micrometer scale.

The aim of this exercise is to give you a hands on experience of the possibilities, pit falls and limitations of Raman spectroscopy as well as the experimental setup and operation of a Raman microscope. Furthermore, it should give you an experience of the everyday challenges of academic research. For this reason a small research project is built around this laboratory exercise. You will start by measuring single spectra, 2D maps and 3D images of several standard samples to make yourself familiar with the instrument. After that you will be given a challenge, related to the research project, to be completed as best as you can with your newly acquired skills.

Students vs. Spectrometer

In our current laboratory exercise research project we are investigating the difference between similar grocery products (for example Red Bull, Race, Clever Energy Drink, ...). We want to know whether we can distinguish between these products by Raman spectroscopy and whether a distinction by means of spectroscopy is more accurate than a human evaluation (taste, smell, ...). For every laboratory exercise a variety of similar grocery products will be chosen. Your goal is to develop a spectroscopic model that is able to distinguish the products. You will then be given a set of unknown samples and try to distinguish them with and without the spectrometer. Which assessment is going to be more accurate?

2 Fundamentals

When light is scattered by a sample most of it is emitted at the same wavelength (energy) as the incident light. This is called Rayleigh scattering (elastic scattering). What happens on a molecular level (fig. 1) is that the photons are absorbed by the molecule, which goes from the ground state into a virtual energy state. After a very short time the excited molecule goes back into its ground state and emits a photon with the same energy in a different direction. However, some photons are emitted with a different energy than the incident light. This inelastic scattering is called Raman scattering. Raman scattering is possible because the molecule also has excited (vibrational) energy states. These excited states exist due to the vibrational modes of the molecule and there are two ways they can cause inelastic scattering. If the molecule was already in an excited state, when it absorbed the photon, and then goes back into the ground state, the photon gains the energy difference between the ground state and the excited state. This is called Anti-Stokes scattering (fig. 1). If the molecule was in the ground state, when it absorbed the photon, but then goes back into an excited state, the photon loses the energy difference between the ground state and the excited state. This is called Stokes scattering (fig. 1). Stokes scattering is usually measured in Raman spectroscopy, because it is much more likely than Anti-Stokes scattering, since there are more molecules in the ground state than in the excited states in the sample.

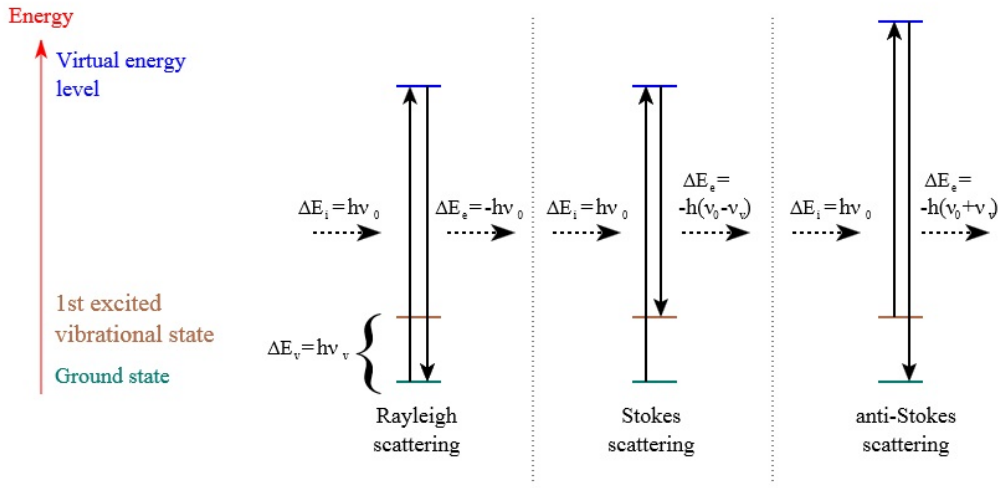

Figure 1: Sketch of the energy states during Rayleigh and Raman scattering

The main difficulty with Raman scattering is that only a tiny fraction (approximately 10^{-8}) of scattered photons are Raman scattered. Therefore, in Raman spectroscopy a laser is used to excite the molecule. The (monochromatic) Rayleigh scattering is then blocked by a filter and only the Raman scattering is measured by a spectrometer, which is equipped with a highly sensitive photon detector (fig. 2).

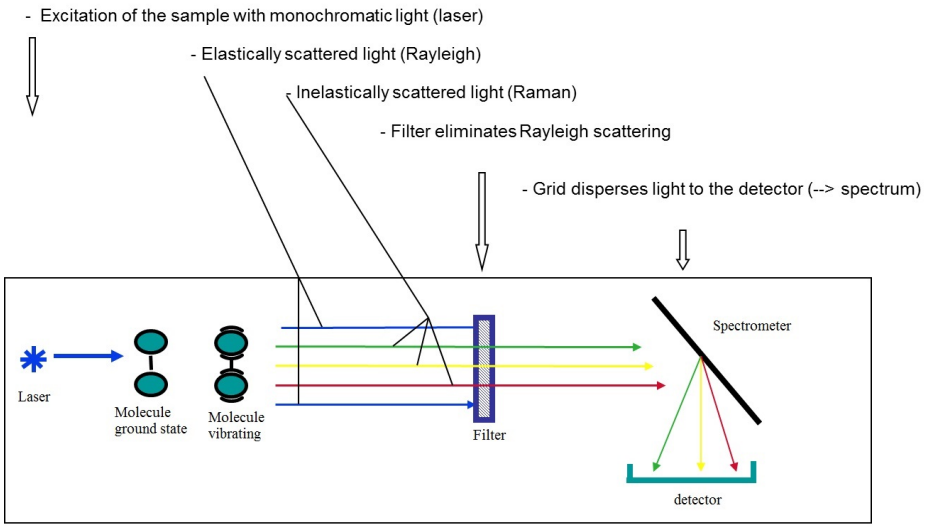

Figure 2: Sketch of the stages of a Raman spectrometer

The spectrum is usually not plotted in terms of the total energy of the photons, which is dependent on the laser wavelength, but rather as the energy shift of photons relative to the laser energy. The energy (Raman) shift is equal to the energy of the vibrational states of the molecule and independent of the laser wavelength. By convention the unit used to plot the Raman shift is the wavenumber (cm^{-1}).

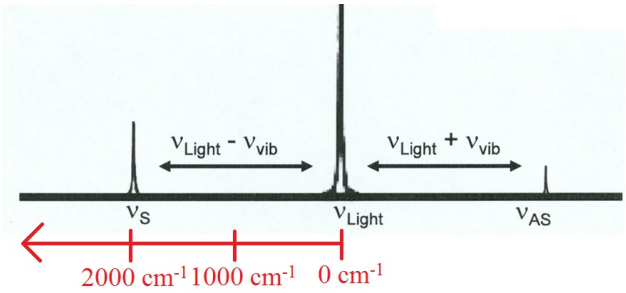

Figure 3: Sketch of the way a Raman spectrum is plotted

The energies of the vibrational states contain a lot of information about the sample such as the chemical composition, the stress/strain stated, crystal symmetries and crystal quality (fig. 4). However, in most cases the chemical composition is investigated by spectroscopy.

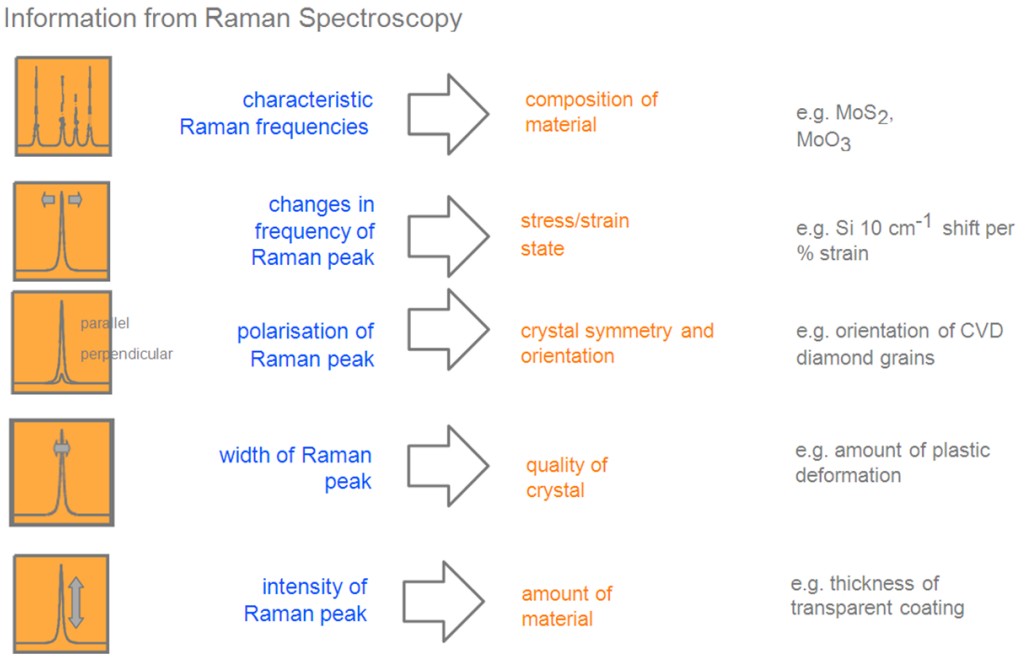

Figure 4: Information accessible by Raman spectroscopy

The dependence of the energies of the vibrational states on the chemical composition can be illustrated by the simple model of the harmonic oscillator. If we imagine an oscillating mass (atom, or molecule fragment) that is connected by a spring (chemical bond) and assume a harmonic oscillator, the energy states of this system can immediately be derived.

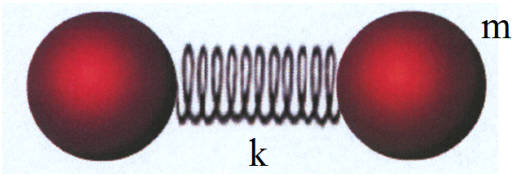

Figure 5: Harmonic oscillator; m is the mass of an atom or molecule fragment and the spring constant k is proportional to the strength of the chemical bonds

For this harmonic oscillator the energy levels are given by:

$$\omega = \sqrt{\frac{k}{m}} \qquad E_v = (v + \tfrac{1}{2}) * \hbar * \omega \qquad v = 0, 1, 2, ...$$

For the 1. excited state ($v = 1$) we have as a rule of thumb that the position of a Raman band is proportional to the square root of the bond strength divided by the mass of the oscillating atom. This is a good guideline for quick assessments, but obviously a real spectrum is much more complicated. Another thing that influences the energy of the Raman band is the vibrational mode. There are two major kinds of vibrations, namely stretching and deformation (bending), which are illustrated in figure 6. Stretching changes the length of the chemical bond and usually requires more energy than deformation.

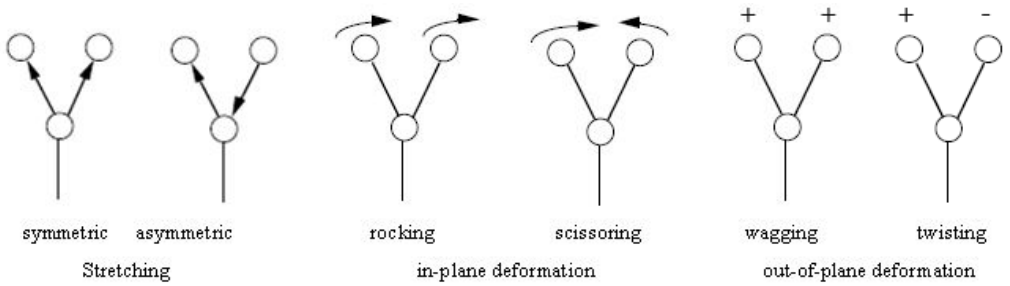

Figure 6: Sketch of vibrational modes

3 Interpretation of spectra

The most important information that can be obtained using Raman spectroscopy is the chemical composition of the sample. An example of the Raman spectrum of cellulose in an embedding compound is shown in figure 7. Every band in the spectrum is associated with the vibration of a chemical bond in cellulose or the embedding compound. All bands are marked as either cellulose (C) or embedding compound (E). Additionally, for the cellulose bands the chemical bond causing the band is identified.

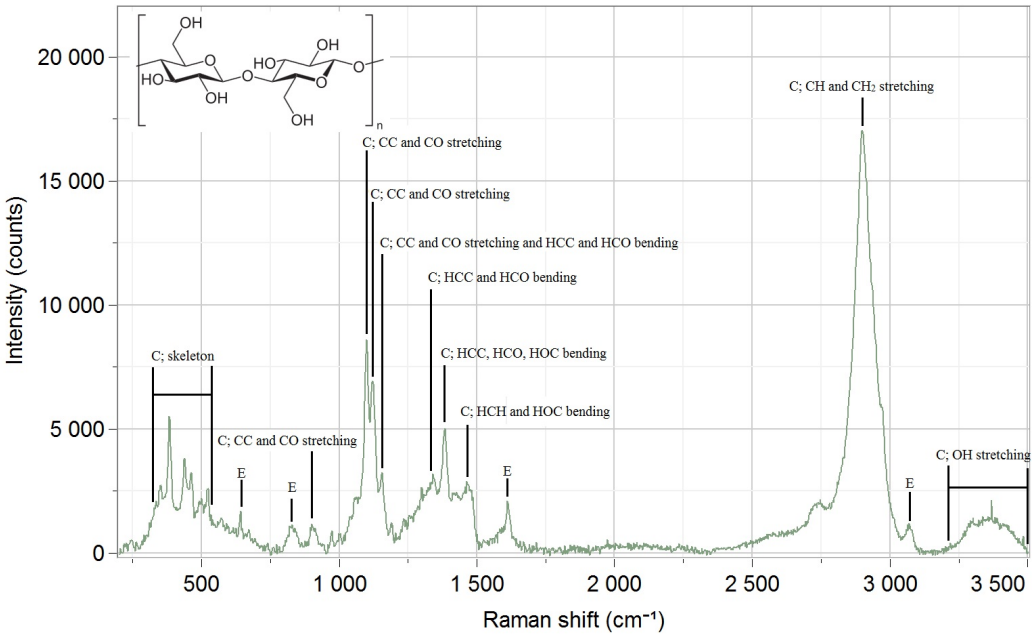

Figure 7: Raman spectrum of cellulose and an embedding compound with band assignment

An interpretation of the spectrum like this is the ideal case. However, it is very difficult to do and requires prior knowledge of the sample. Therefore, in most cases a simpler method of interpretation is chosen. Other methods of spectral interpretation are (in ascending order of accuracy):

- Identifying H- and C-bonds using the harmonic oscillator approximation
- Identifying functional groups by looking for group bands
- Identifying the chemical composition by comparing to a known reference

Identifying H- and C-bonds using the harmonic oscillator approximation

Due to the approximate dependence of the energy on the mass of the atom and the bond strength, some bonds are easily identified, namely X-H-bonds, where X stands for another atom. Because H is by far the lightest element, X-H-bonds are found at higher energies than anything else ($2800 - 4000\ cm^{-1}$). Furthermore, triple bonds ($2100 - 2300\ cm^{-1}$) are significantly stronger than double bonds ($1500 - 1800\ cm^{-1}$). Double bonds are again stronger than single bonds ($< 1500\ cm^{-1}$). X-H-bonds, triple bonds and double bonds can be distinguished right away, because of this separation in terms of energy. The single bond range is also known as the "finger print" as it shows fairly complex band structures that are unique for every molecule. The spectrum of an unknown substance is shown in figure 8, with the spectral ranges from the rule of thumb indicated below the spectrum. We can immediately tell that there are C-H and C= bonds, but no N-H or O-H and no triple bonds. This might be a common polymer.

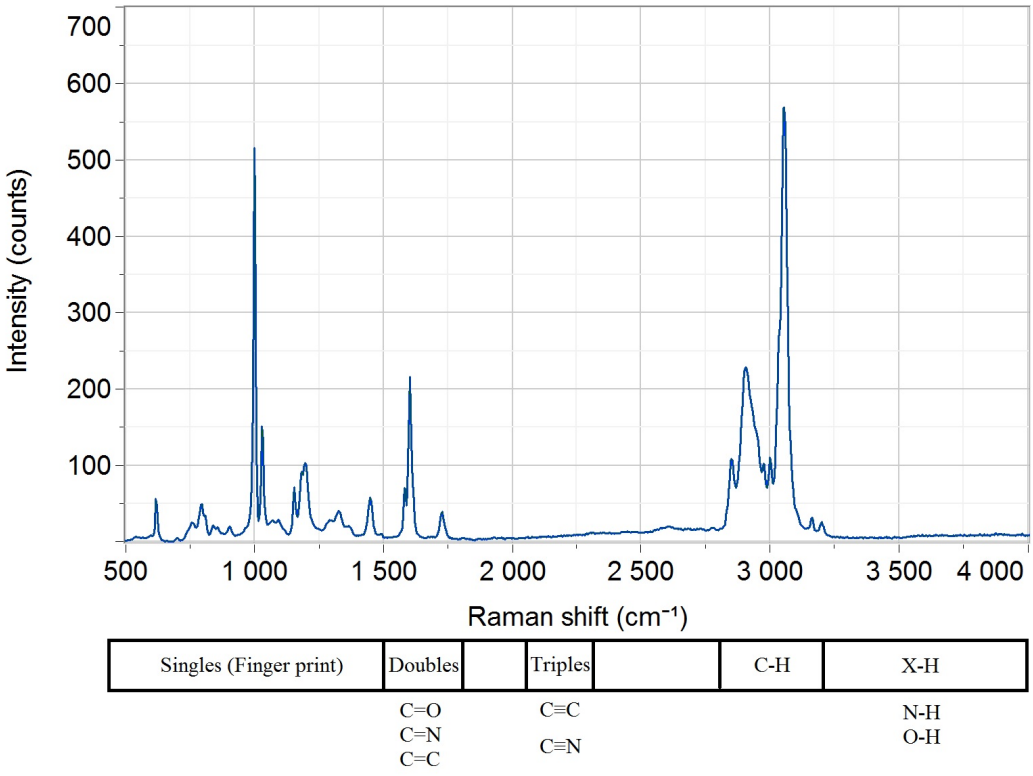

Figure 8: Unknown Spectrum with "rule of thumb" spectral ranges

Identifying functional groups by looking for group bands

Specific functional groups such as aliphatic, aromatic, carbonate, etc. have characteristic bands that appear somewhat independent of the rest of the molecule. The next step after identifying the H-bonds and C-bonds is to look for groups of bands that are associated with functional groups. Unfortunately, there are no simple rules for this procedure and identifying functional groups is a tedious procedure that requires experience as well as the aid of a reference book or software. Figure 9 shows again the unknown spectrum with two functional groups and their expected band positions. This could be polystyrene.

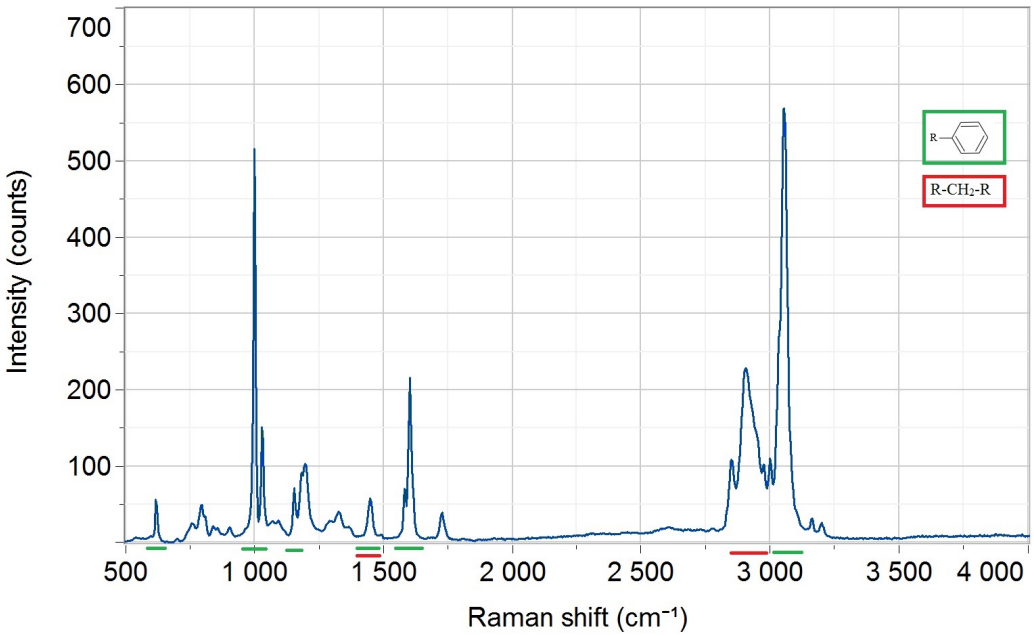

Figure 9: Unknown Spectrum with possible functional groups

Identifying the chemical composition by comparing to a known reference

A easy and reliable way to identify the chemical composition is to simply compare the spectrum to that of a known reference. The obvious drawback is that either some prior idea of what the sample might be is necessary or a database with a large number of high quality spectra must be available. If we compare the unknown spectrum with that of polystyrene, it is immediately obvious that it is in fact polystyrene.

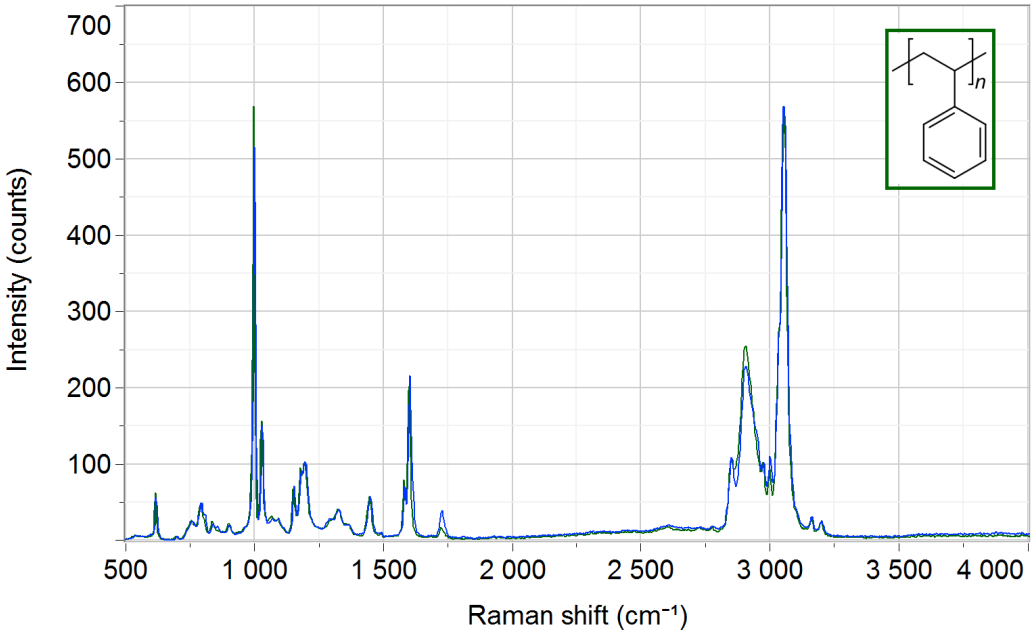

Figure 10: Unknown Spectrum (blue) compared to the spectrum of polystyrene (green)

4 Choosing the laser

Despite the fact that the spectrum itself (frequency shift) is independent of the laser wavelength, the choice of the right laser is vitally important when doing Raman spectroscopy. The following factors have to be considered when choosing the laser wavelength:

- Fluorescence
- Band Intensity
- Spectral resolution and range
- Spatial resolution

Fluorescence
A major problem in Raman spectroscopy is fluorescent. Since fluorescence has in general far more intensity, it can completely mask the Raman spectrum. Often fluorescence can be avoided by choosing the right laser wavelength, because it always occurs at the same wavelengths regardless of the wavelength of the excitation (assuming it has at least enough energy to excite fluorescence), whereas the same Raman spectrum can be measured at different wavelengths depending on the laser wavelength (fig. 11). As fluorescence can make the measurement impossible avoiding it is the most important factor to be considered when choosing the laser wavelength.

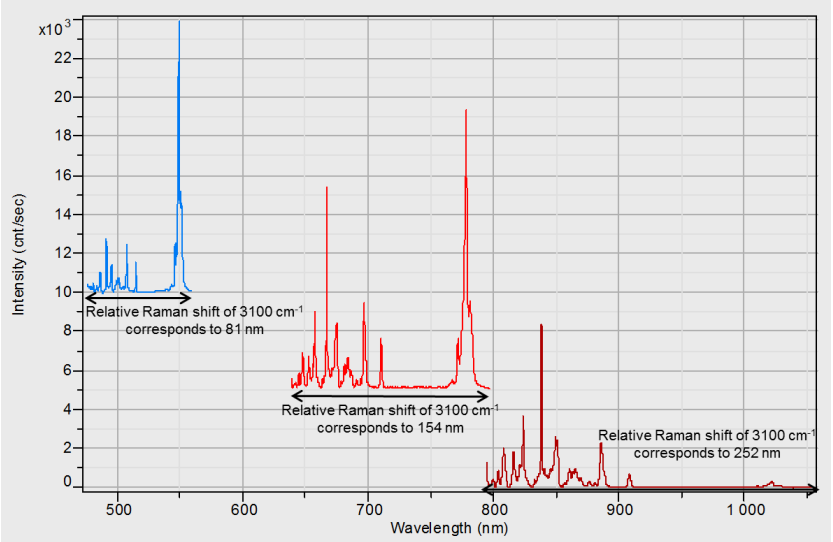

Figure 11: The same Raman spectrum recorded at different wavelengths

Band Intensity

Since the amount of scattered light is strongly dependent on the wavelength ($I_{Rayleigh} \propto \lambda^{-4}$), a smaller laser wavelength will yield significantly higher band intensities. This is especially important for spectral maps, where the acquisition time per spectrum is an important issue, as well as heat sensitive samples that require small laser powers. The relative gain of Raman signal as a function of the wavelength is shown in figure 12.

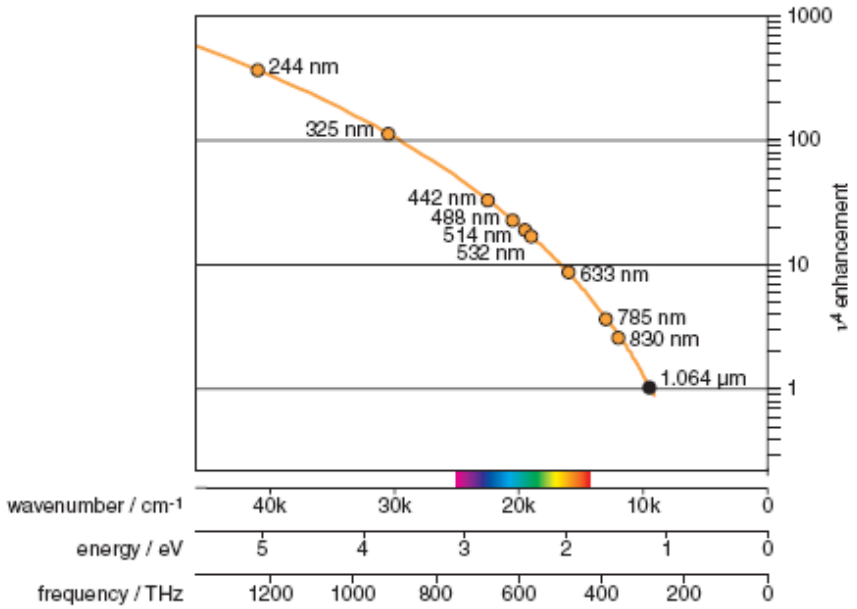

Figure 12: Effect of the laser wavelength on the Raman intensities; the data is normalized to a excitation at 1.064 μm

Spectral resolution and range

Both the spectral range that can be covered in one measurement and the spectral resolution depend on the laser wavelength. The spectrometer is able to cover the same wavelength range with the same resolution at every wavelength, but, as is illustrated in figure 11, the same Raman (energy) shift translates to different wavelength shifts depending on the laser wavelength. This is because of the inverse proportionality between energy and wavelength. Therefore, shorter laser wavelengths can cover larger spectral ranges with one measurement at the expense of spectral resolution, as is illustrated in figure 13.

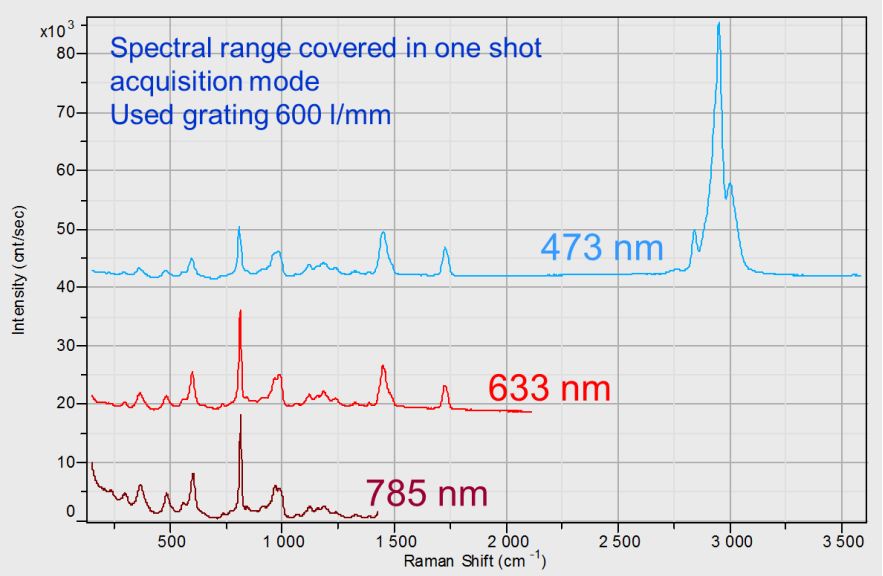

Figure 13: Spectral range covered at different laser wavelengths

Spatial resolution

For Raman microscopes the spatial resolution depends on the laser wavelength because of the diffraction limit and is approximately given by:

$$Spotsize \approx \frac{1.22*\lambda}{NA} \qquad NA...numerical\ aperture\ of\ the\ lens$$

5 Experimental setup and parameters

When a Raman spectrometer is combined with a microscope (Raman microscope), spectra can be measured with high spatial resolution. It is also possible to acquire spectral images (chapter 6) of any sample that can be imaged in a light microscope, including depth profiles and 3D-maps of transparent samples using a confocal microscopy. A sketch of a Raman microscope is shown in figure 14. The Laser source and spectrometer are simply attached to a microscope. This way spectra can be collected with high lateral resolution from the focal point of the microscope. A confocal setup can be used as well to limit the scattering volume in the vertical direction.

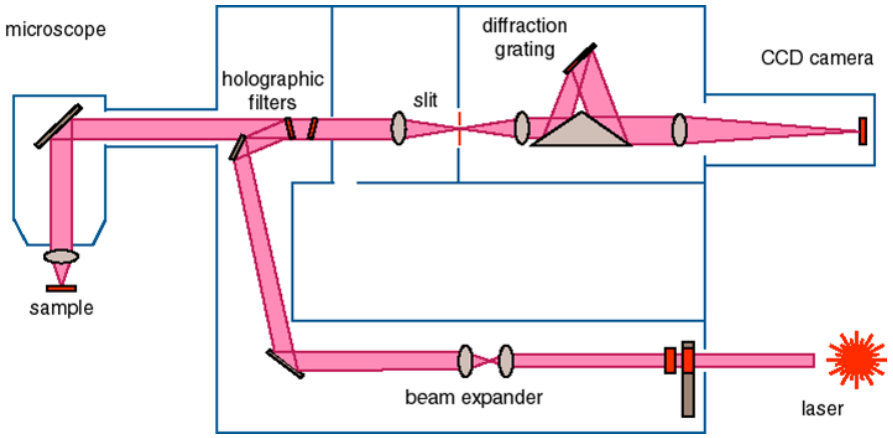

Figure 14: Sketch of a Raman microscope

Using a Raman microscope, a lot of experimental parameters have to be considered related to the microscope, the spectrometer and the laser source. The most important parameters are summarized below:

- Laser wavelength
- Laser power
- Grating spacing
- Slit size
- Acquisition time
- Number of accumulation
- Objective magnification

- Pinhole size
- Spot size

For maps only:

- Laser scanning or stage scanning
- Step size

Laser wavelength
The laser wavelength is the most important parameter and influences almost anything. It has been discussed in its own chapter (chapter 4).

Laser power
A higher laser power increases the intensity of the Raman scattering and sometimes also suppresses fluorescence. Thus, the laser power should be chosen as high as possible without damaging the sample.

Grating spacing
The spacing of the grating influences the spectral range that can be covered with one measurement and the spectral resolution. A grating with more lines per mm (l/mm) will generate a spectrum with higher resolution at the cost of covering less spectral range. It is worth mentioning that increasing the spectral resolution always decreases the intensity and will usually make higher acquisition times necessary.

Slit size
Decreasing the slit size increases the spectral resolution; though the spacing of the grating has a far bigger influence. The slit size can be used to fine tune the spectral resolution.

Acquisition time
The acquisition time determines the signal to noise ratio of the spectrum. A higher acquisition time produces a better signal to noise ratio at the cost of a longer measurement. It is important to note that the acquisition time cannot be increased indefinitely, because the detector will eventually saturate.

Number of accumulation
Instead of increasing the acquisition time several spectra can be accumulated to increase the signal to noise ratio. In general it is less time consuming to increase the acquisition time than to accumulate spectra. However, accumulating spectra is the only way to generate a better signal to noise ratio, if

the acquisition time cannot be increased anymore. Accumulating at least 2 spectra is also usually done to avoid spikes from cosmic radiation. These are sharps spikes that sometimes occur when a high energy particle from cosmic radiation happens to hit the detector during the measurement. They can also be remove by post processing of a single spectrum but using a filter, based on the accumulation of 2 spectra, is by far the cleaner approach.

Objective
The magnification of the objective defines the spatial resolution.

Pinhole size
The size of the pinhole also influences the spatial resolution, especially in the vertical direction. Making the pinhole smaller will improve the spatial resolution at the cost of intensity.

Spot size
It is possible to artificially increase the size of the laser spot or change its shape by moving the laser around much faster than the acquisition time. This is very convenient when the average spectrum of a larger particle or area is of interest.

Laser scanning or stage scanning
When acquiring a spectral map either the laser spot or the sample has to be moved. The laser spot can only be moved over a limited distance (depending on the objective $\approx 10 - 100\ \mu m$), whereas moving the sample can introduce artifacts in the image, due to drifts and vibration.

Step size
The step size is the distance between the positions where spectra are acquired when measuring a spectral image. The spatial resolution of a spectral image is usually defined by the step size rather than the optical resolution of the microscope due to time constrains (see chapter 6).

6 Spectral Images

A spectral image is simply an image with a spectrum in every pixel, as is illustrated in figure 15. Usually the spectra in a spectral image are evaluated by integration over band intensities or some more sophisticated spectral model, in order to create a color map of the distribution of chemical components in the sample. Other than light microscope images, which can be recorded as a whole by a camera, spectral images usually have to be measured pixel wise.

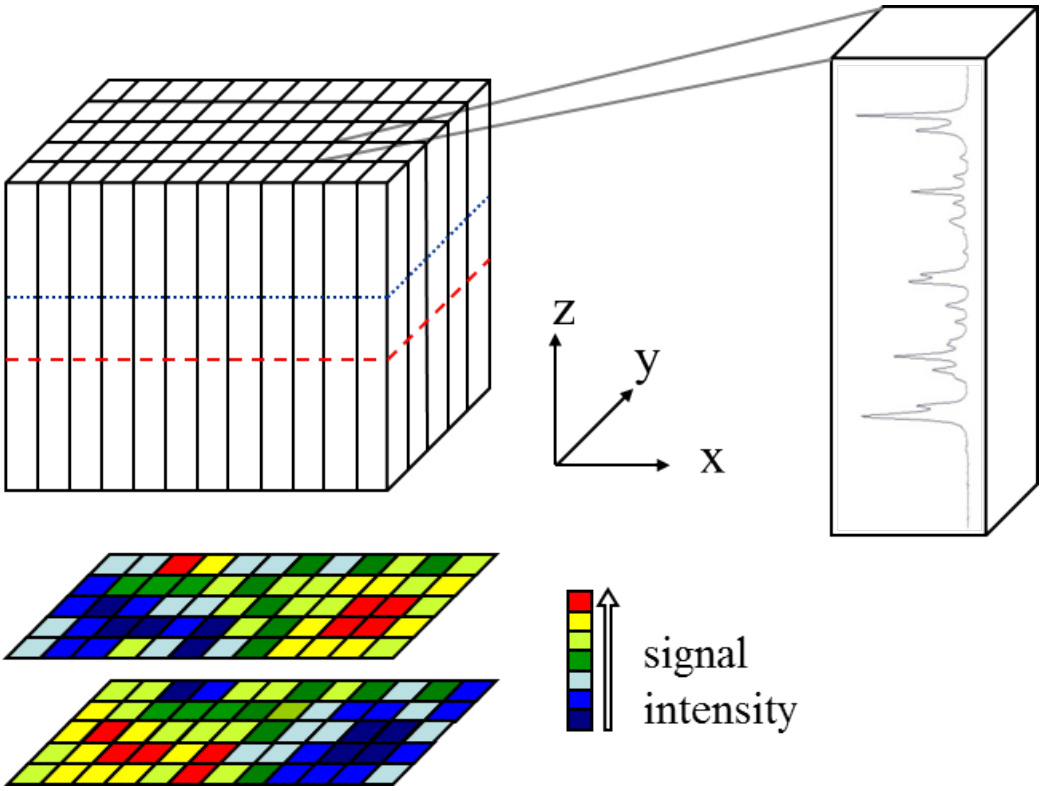

Figure 15: Sketch of a spectral image and two color maps created from it showing different chemical components

The main difficulty with spectral imaging is that a lot of spectra have to be recorded. This means that there basically always has to be a trade-off between the quality of the spectra and the quality of the image. For example measuring a 50×50 μm sample at maximum spatial resolution would require around 50000 spectra to be recorded. Recording a spectrum with very high spectral resolution and good band intensities would require at least 10 seconds, even for a sample that is ideal for Raman spectroscopy. This measurement would take 500000 seconds, which is about 6 days. As an example

of what such a trade-off might look like, a 3D-spectral image of a layered polymer system is shown in figure 16. In this case, spectra with sufficient quality to distinguish the different components could be recoded with a measurement time of 1 second per spectrum. A bad lateral resolution ($Step\ size \approx 7\ \mu m$) was chosen, because it was already known that the sample is a somewhat regular layered structure, whereas the almost best possible depth resolution ($Step\ size \approx 1\ \mu m$) was chosen because the thicknesses of the layers were the main point of interest. The measurement took about 15 hours.

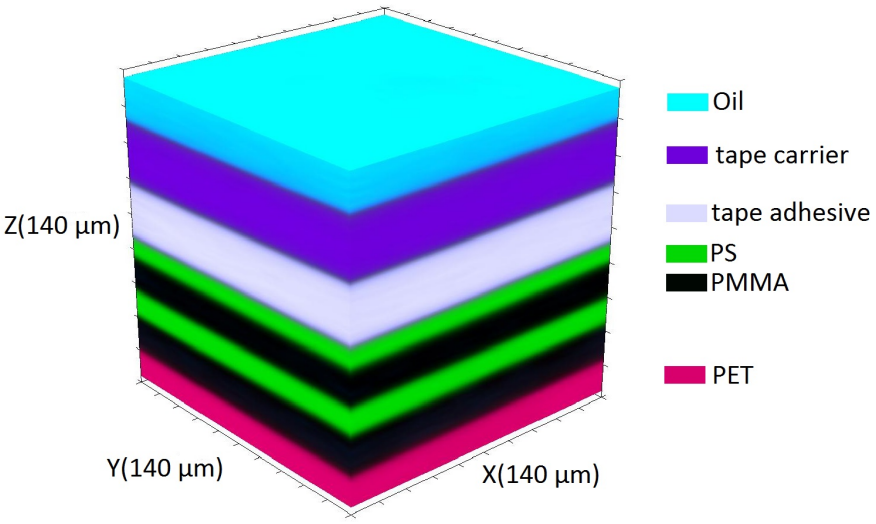

Figure 16: Example of a 3D-spectral image of layered polymers; for this measurement bad lateral resolution and medium spectral quality were chosen to ensure high depth resolution in a reasonable measurement time

7 Summary

After reading this introduction you should know...

- what Raman scattering is
- how it is measured
- what exactly is plotted on an axis labeled Raman shift (cm^{-1})
- why Raman spectroscopy is useful
- what is meant by "harmonic oscillator approximation" and "rule of thumb spectral range"
- 3 different ways to approach an unknown spectrum
- what the most important thing is when choosing the laser wavelength
- what else to consider when choosing the laser wavelength
- what a Raman microscope is
- a list of experimental parameters relevant to Raman microscopy
- what a spectral image is
- which trade-off a spectral image requires

...and if you don't know these things by now you'll have to read the introduction again, read the books suggested below or have a friend google them and explain them to you.

8 Literature

1. Ferraro, John R.; Nakamoto, Kazuo and Brown, Chris W. "Introductory Raman Spectroscopy", Elsevier Inc. (2003)
2. Long, Derek Albert. "The Raman Effect - A Unified Treatment of the Theory of Raman Scattering by Molecules", John Wiley & Sons (2002)
3. Grasselli, Jeanette G. and Bulkin Bernard J. "Analytical Raman Spectroscopy", John Wiley & Sons (1991)