University of Reading

# USE OF THE NXR FT RAMAN 9650 SPECTROPHOTOMETER.

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## PURPOSE

This SOP describes how to operate and use the NXR Fourier Transformation-Raman Module with a Nicolet™ Series spectrometer located in the CAF facility to collet FT-Raman data (3900-100 $cm^{-1}$ Raman shift range) according to the default method FT-Raman-NXR9600Series Raman-NXR-9600.exp. More specifically, the SOP describes how to use this instrument equipped with a HeNe laser, a 1064nm excitation laser, a InGaAs detector and a sample compartment with a 180 degree reflective sampling configuration and a beam focusing lens installed. For more information on the instrument please APPENDIX < Accessories.

## SCOPE

This SOP describes procedures to be followed by staff and students when using the NXR FT Raman 9650 Spectrophotometer with the aforementioned accessories (described in the purpose section) in the CAF facility.

## RISKS.

DO complete the CAF Blackboard safety test, official training and ONE run supervised by the CAF technician before using the equipment.

See the attached Risk assessment (R.A.) of the instrument.

You must have your own Risk assessment of the chemical products you are going to analyse.

Please if you want to analyse toxic, flammable, explosive, or longer term health hazards chemicals talk with the CAF technician before running your samples (check their MSDS). We may need to take extra safety measures.


## PROCEDURE.

PRECAUTIONS

- DO NOT spill solvents inside the cell compartment. Report any spills.
- DO NOT leave sample waste, glassware or other materials in the lab without properly disposing of them. In some cases, this may require that you bring waste back to your own lab.

- Please look for technical assistance if you face any instrument warning/error.
- Do not attempt to use if signage indicates that the instrument is out of use or is currently in use,
- This instrument (as a whole) is a Class 2 laser, thanks to an interlock the Raman laser light is contained in the sample area. The instrument is not hazardous when correctly used. Be careful, laser radiation is dangerous to eyes, never look at the laser radiation. For more information please check the R.A.

## INSTRUMENT STARTUP

- The **computer should be on** already. You just need to 'wake' it up and log in: (If somebody has signed out you will need to sig in to the PC domain)

  Username: user  Password: cafuser

  You can't log on to Rdg-home with your individual logon as the instrument can't be connected to the Internet due to safety issues (it is a Windows XP).

- **The instrument** (both the Nicolet XR and FT Raman module) **should be on** as indicated by the green light (power) in the spectrometer and front panels (Figure 1). If the power is off, look for technical assistance. There is a specific order to turn the instrument on and several tests that need to be done. The instrument has two lasers, a HeNe laser located in the spectrometer and a Raman (excitation) laser located in the Raman module. The HeNe laser is always on, but the Raman laser is only turned on when the sample is analysed.

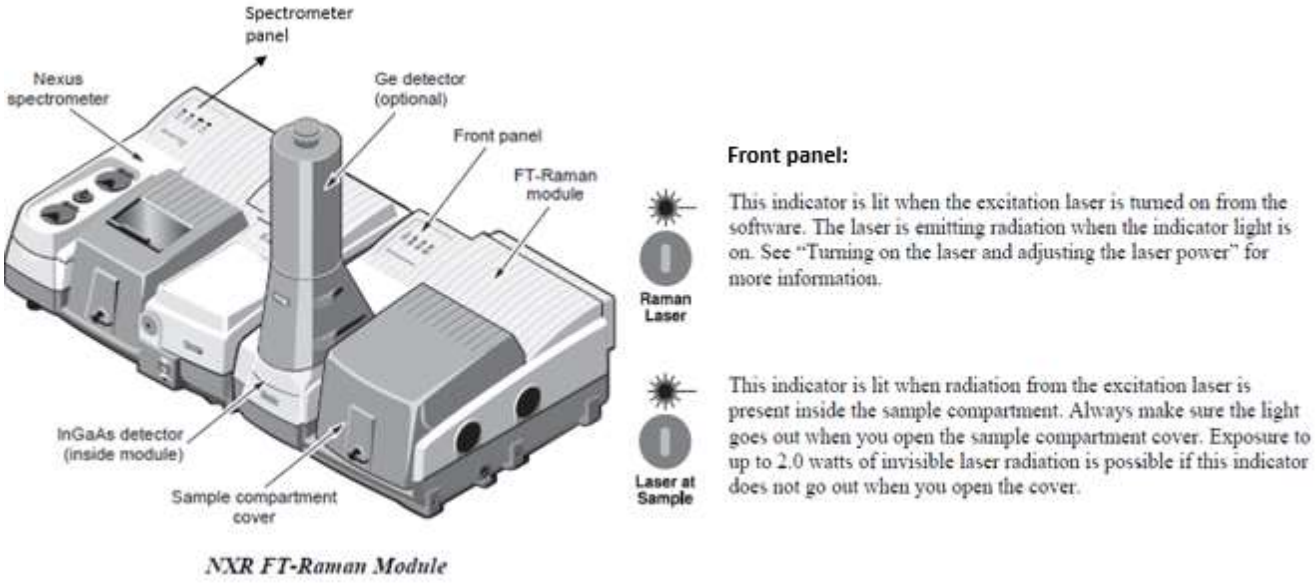



*Figure 1. Instrument main parts.*

- Double click on the 'OMNIC' icon on the desktop to initiate the software. You will be prompted to log into OMNIC with a username. The username is Pete. The 'P' is capitalised. The OMNIC window displays an empty spectrum.
- You will hear a noise as the **stage initialises.** Open the sample compartment covert, the stage must move, if not push it forwards a little bit.

SAMPLE PREPARATION AND SAMPLE LOADING.

First of all you have to verify the correct functioning of the instrument. To this end you have to analyse the $S_2$ standard (in the same sample container that you will use for your sample) and compare it with the previous $S_2$ result before analysing your sample. The following sections explain the procedure to analyse any sample and will specify the conditions to start with the $S_2$ sample (available in the white box close to the instrument).

**You can analyse** liquid and solid (powders, gels, films...) samples with the FT Raman (see the appendix for more information).

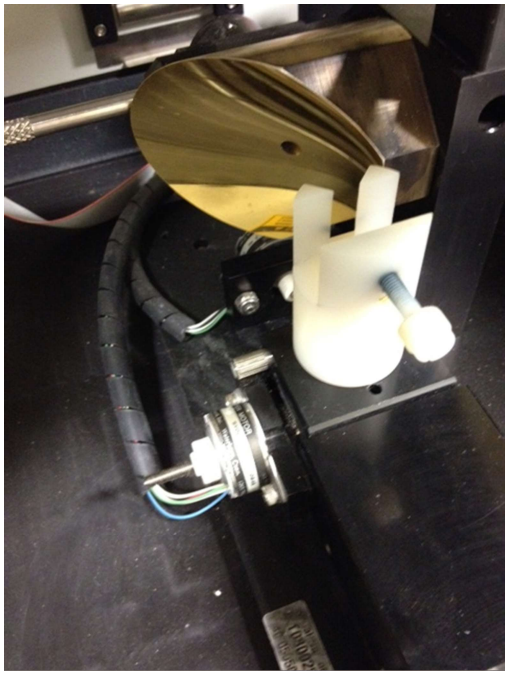


*Figure 2. Empty plastic holder for vials placed on the sample stage.*

- Select the appropriate **sample container** depending on the amount of available sample (see the appendix for more information). The amount needed will vary depending on your selection, but you usually only need to cover 0.5-1cm of the container. Cap the sample containers with a lid but don't tighten the lid too firmly to avoid high pressures.

- Put your sample container (with the sample inside, first with the $S_2$) in the **sample holder**. There are several plastic holders for vials (Figure 2), but if you are using NMR and capillary tubes you need to ask for metallic sample holders to the CAF technician (see the appendix for more information). In the plastic holders for vials there are two black lines drawn on the front of the sample holder, which indicate the approximate area where the laser light will hit your sample. Ensure that enough sample is present to align with the black marks on the holder.

- **Wipe** the sample container (vial, capillary or NMR tube) with a soft tissue,

- Lift the instrument sample compartment cover and put the **sample holder with your sample on the stage**. The sample holder should be placed on the first two 'pins' of the sample plate (Figure 2). Placing the sample on the last two pins does not usually give the best results.

*CAUTION:*

To prevent any danger from the laser light always remember to keep the **sample compartment lid closed** while a sample is running, **and only open the lid** once the Raman laser has been turned off using the software. If the laser is off the Raman laser light in the front panel is off too. If the lid is closed both lights (Raman laser light and laser at sample light) will be on (Figure 1) .

The instrument has a safety interlocks in place. When you open the sample compartment lid the laser at sample light (front panel) will become off due to the interlocks, and no laser light will be in the sample chamber. However, before opening the lid it is always preferable to turn off the laser using the software

- Close the sample compartment lid.

SCAN SET-UP (Omnic software)

- In the software you will see the last user's set up. First of all, you need to go to the top right of the experiment window and check that the **system status is green and the experiment is:** FT-Raman-NXR9600Series Raman-NXR-9600.exp. This is the default method with the right configuration for FT-Raman analysis,

*CAUTION:*

**DO NOT change** sample configuration accessories or this default method without notifying it to technical support. This method has been optimized for the accessories and most common kind of samples analysed with this instrument.

- Just above the spectrum window are several icons. Select '***Expt Set'***. A new window will appear with different tabs. Users can only change Collect and Bench tab to carry out their experiments.

- Click on the '***collect'*** tab. The values present will be what the last user set during their experiment. Many of the parameters are set by the software and do not need to be changed. Please see below for the typical parameters.

  - **No. of scans:** 8 – 16 is the best starting point (higher value increases data collection time and S/N). However, if your sample does not burn/is not thermally instable, the most common value is 100/200 or few hundreds.

  - **Resolution:** The smaller the number the higher the resolution, the narrow the band you can distinguish. However, more scans are needed to get the same S/N (signal to noise) ratio, so more colleting time is needed. The smaller value means the closer the collection points are. A value of 4 is the default, 4-8 is typical for solid and liquids (higher value decreases data collection time), 16 can be used for polymers.

  - **Final format:** Shifted spectrum will provide the common 'Raman shifted' X axis

- **Correction:** None (under standard conditions)

  For $S_2$ standard put: 100 scans, resolution 8.

**Note:** None of the other parameters need to be adjusted including 'file handling'. You will save your spectra after it has been collected.

- Click on the '*Bench*' tab within the *'Expt Set'* window.

**Note:** You may receive a warning message when you select the Bench tab that reads – '*The aperture is too large to achieve the specified resolution. Set the optimum aperture automatically?*' This occurs when the aperture selected is 150 and resolution is lower than 8. You can choose to select *YES* or *NO*, However, you are recommended to select yes to allow the instrument to change to the optimum aperture value needed for the selected resolution.

- To the right of the live spectrum window you can adjust **other parameters** and values as follows:

  - Sample Compartment — Raman (do not change)
  - Detector — InGaAs (do not change)
  - Beamsplitter — $CaF_2$ (do not change)
  - Max range limit — 3900 (adjustable)
  - Min range limit — 100 (adjustable)
  - Gain 1 — 1.0 (do not change)
  - Optical velocity — 0.3165 (do not change)
  - Aperture (adjustable) — 150 (totally open) is the maximum and the default one. Used 150 for **$S_2$.** It is automatically adjusted by the instrument as a function of the resolution. Higher aperture means higher S/N. The aperture controls the width of the laser beam. Higher resolution means lower aperture and therefore, lower intensity.
  - Polarizer angle — Out (do not change)
  - White light — Off (it is only used for quantification)

- Rough ADJUSTMENT OF THE SAMPLE POSITION (with respect to the excitation laser and the rest of the optics): In the Raman Accessory/Stage control of the 'Bench' tab (Figure 6) you need to adjust, depending on your sample and sample container, the stage position to get one single red spot of the He-Ne laser on your sample (for glass vials: the spot must be between the two black lines of the sample holder where you have your sample). Setting the focus at 150 and the side to side at 150 are good starting points. The correct values are usually not far from 150. Set a new stage positon, open the sample compartment cover and check the He-Ne laser spot without moving the sample from the stage. Repeat this operation until you are happy with the adjustment. Don't use autofocus, the instrument could get blocked.

*The correct sample position is closely approximated by the focal point of the beam. When the visible He-Ne laser beam converges and strikes the sample, it forms red spots on the sample surface. These spots indicate the part of the sample that will be measured when the Raman laser is activated. By positioning the sample at the centre of the red spots, you can minimize the amount of fine adjustment that will be required later (*Figure 3*). Sometimes you can't get a single red spot but laser spots close to each other.*

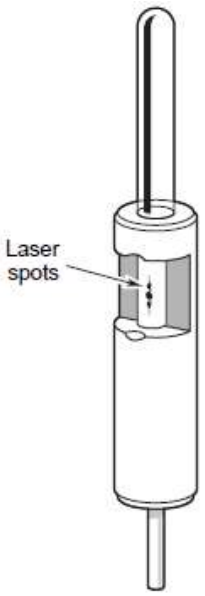



*Figure 3. He-Ne laser spots on a NMR sample tube, which is inside the metallic holder.*

*CAUTION:*

**To check the He-Ne laser spot on your sample,** open the sample compartment cover, put your head inside and look at the front face of the glass sample container from above. Indirect observation is not hazardous. However, do not stare into the He-Ne laser beam or its reflections. It can be dangerous.

Please be aware that at this point of the procedure the Raman laser is still off. You can't open the sample compartment cover if the Raman laser is on.

- Once happy with the rough position, close the sample compartment cover and **turn ON the** (Raman excitation) **laser** by selecting ON in the Raman source area of the 'Bench' tab ( Figure 4)

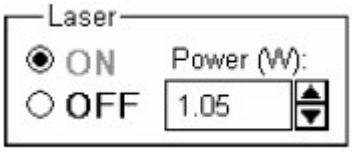



*Figure 4. Raman excitation control tab.*

- The **power should be set at 0.2 W** (1.5 for $S_2$) as a starting point for your sample, however this can be adjusted to get a good spectrum depending on your sample (do not exceed 1.5 W).

*You must use enough power to get a good S/N (signal to noise ratio) of the peaks of interest in your spectrum. The higher the power, the higher the S/N, but also the higher the possibility of thermal degradation of your samples. If you do not know the Raman peaks you are looking for, you can always start with 0.2W, check if you have clear peaks in the spectrum life window and if you are not happy increase the power another 0.5W until you see peaks, Once you see peaks, turn off the laser, open the sample cover (first check that the Raman laser light and laser at sample of the front panel are off) and check that your sample is not burnt or damaged. If the sample is fine you can increase the power until you reach 1.5W, which will be the maximum S/N.*

Note: This value is the power at the laser head. The laser power on the sample is always lower and it is shown in the bench life window. If your samples are easily burnt there are optional neutral density filters to reduce the power, look for technical assistance if you are interested (see the appendix for more information).

- **Fine adjustment of the sample position:** Now, once you have selected the power you are going to use, you must use the Raman stage control to get the best focus and side to side position. This position is the one that gives you the highest peak to peak (interferogram in the life window) or signal (spectrum) value (Figure 6). You only need to select the right/left and backwards/forwards arrows to move the stage. You can also write a number in the focus and side to side boxes. If you had previously done a good rough adjustment, the final focus and side to side values will be close to the original positions.

  *Below the life window you have a series of arrows that allow you to scale the spectrum/Interferogram life window . (yellow circle) (Figure 6),*

- After the fine adjustment of the sample you should get a **good sample** (first a $S_2$) **interferogram or spectrum** before proceeding (, Figure 6 and Figure 7). If you have a proper interferogram you will have high S/N peaks in the spectrum. Tick the box 'Spectrum' just below the spectrum window to view the continuous spectra produced by your sample (Figure 6). Now, only with $S_2$ (not with the sample) and once a good interferogram and intensity (ideal spectrum signal for S2: 1700-1900) has been achieved: To optimise the optics and get the maximum signal from your instrument in this analysis and in future (same session) analysis, go to diagnostic tab and select **reset bench**. Then, you can maximize the signal with the Accessory control arrows of the 'Bench' again. Do not select the align option in the Diagnostic tab, this should only be done by CAF technician with a special standard.

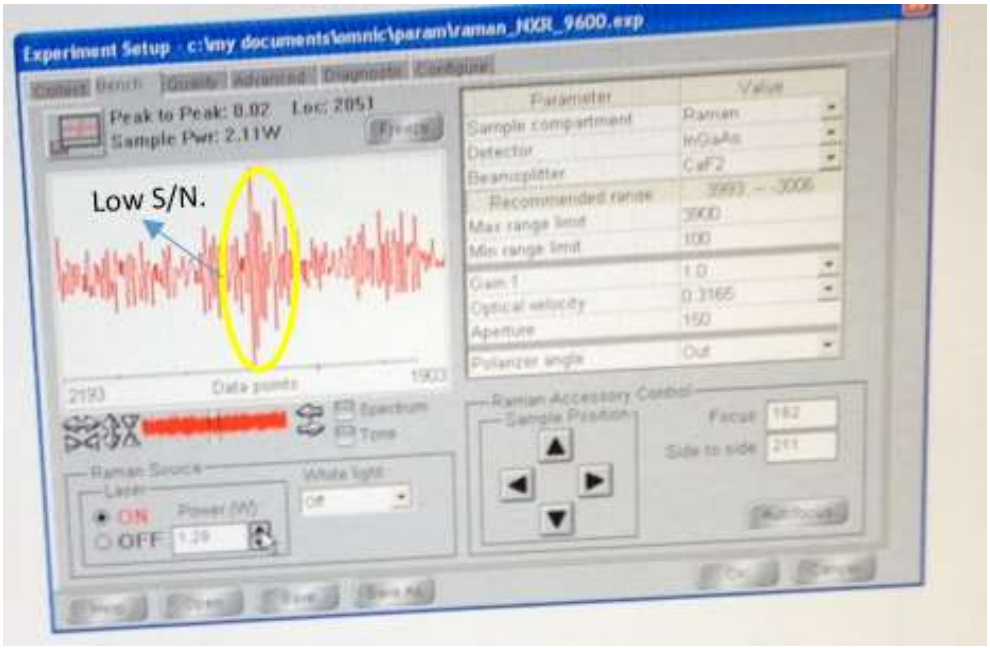



*Figure 5. Example of a bad sample FT Raman interferogram, whose S/N is very low.*

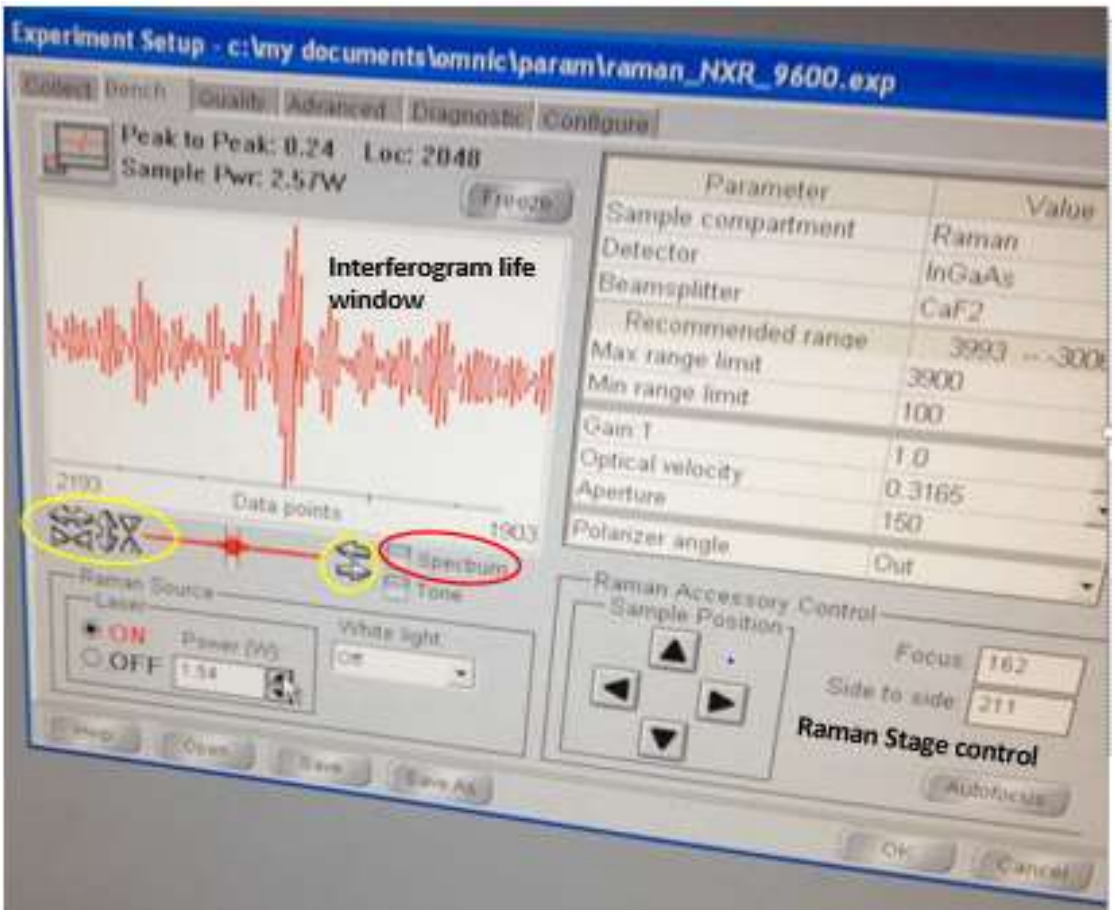



*Figure 6.Interferogram life window with a good sample FT Raman interferogram. Spectrum box in red circle and scale arrows in yellow circles.*

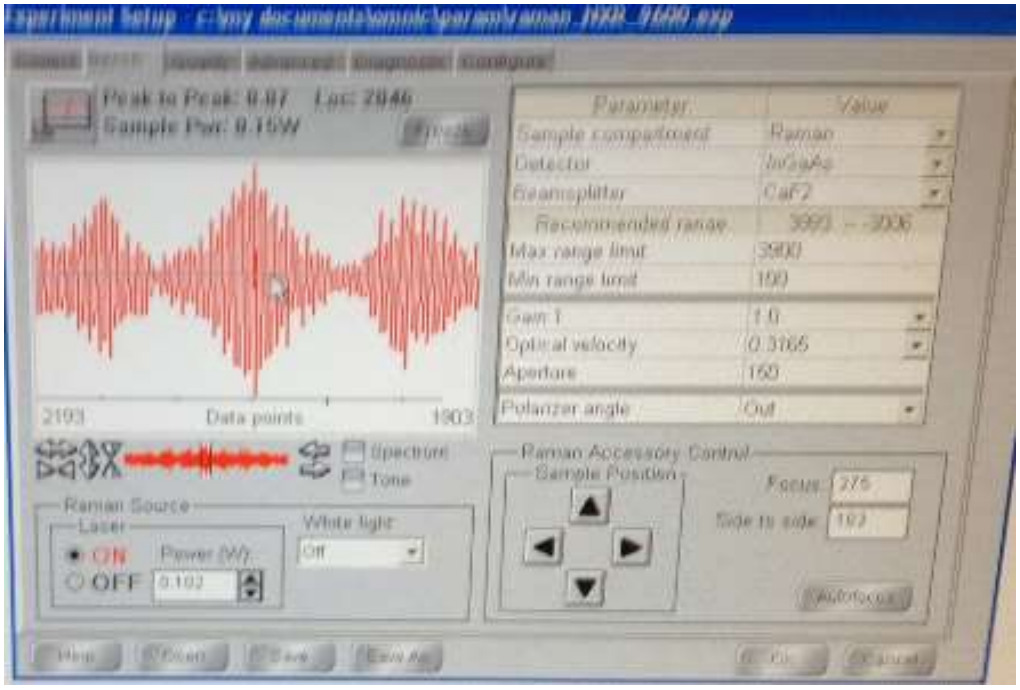


*Figure 7. Good life interferogram of $S_2$ before selecting reset bench.*

- Under normal operations you should **NOT** need to adjust any parameters in the **Quality, Advanced, Diagnostic or Configure tabs**. Look for technical assistance if you have any question about these parameters.

- Click **OK** to exit the 'Expt Set' tab to go to the next step.

## COLLECTING AND SAVING YOUR SPECTRA

- Just above the spectrum window are several icons (next to 'Expt Set') **select 'Col Smp'** to collect your data.

- A pop-up will prompt you to **name the spectra** of your sample (this does not need to be the name of the file). Press OK.

- A pop-up will now prompt you – '**Raman – please prepare to collect the Raman spectrum'**. Press OK. Then the spectrum of your sample will appear in the collection window.

- After the scans have completed another pop-up will prompt you to saying that data collection is complete. If you are happy with the spectrum you see you do not need to wait until all the scans are done, you stop the acquisition and close the first grey window, not the software (Figure 8, red circle).

  Once the data collection is ended you can choose to add the spectrum to the new window to proceed to the analysis and to save the file. However, if you are not happy with the result, you can select add more scans (not needed for sulphur), press okay and go to the left corner on the bottom, select more and write the number of additional scans you want to run. If you selected 100 and now you select 100 more, the instrument will have done a total of 200.

  For your $S_2$ analysis you only need to add the spectrum to the new window, no more scans needed.

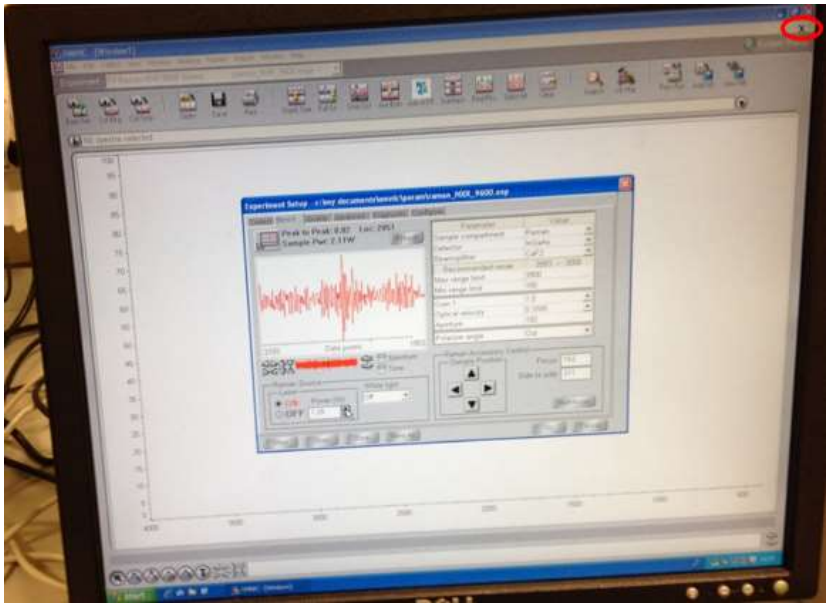


*Figure 8. FT Raman software window. You can see the close button of the experiment window in a red circle.*

- Once you are satisfied with your spectra and you have added the spectrum to the new window, you must go to the Exp Set < Bench < write **0.2 W in the Raman laser** tab and press enter. Now you have to select the **OFF** option and make sure that the Raman light and laser at sample light of the front panel is off. You have to do this for safety reasons and to prevent any damage on the sample and sample holder while you are analysing your data or unloading your sample.

- Click OK to exit the 'Expt Set' . **Now you can save the data file** by selecting the SAVE icon on the top menu and then continue with your data analysis. Save raw data in the following file formats: raw spectra (SPA, you can only open it with Omnic), csv text (Excel), TIF (picture). You can also edit, copy and paste in Word or Paint. Data path to save your data: C\My documents\Omnic\Spectra\Supervisor's folder\Your own folder.

  For your $S_2$ analysis you will need to compare your results (number and shape of peaks, $cm^{-1}$ and height of the peaks) with the historical value and fill in the Excel logbook before carrying on with your samples. Look for technical assistance to appraise your first $S_2$ analysis.

- **After having analysed your $S_2$ you can continue with your sample**, repeating the same procedure explained for $S_2$ but with your sample: sample preparation and sample loading, scan set-up, rough adjustment position, fine adjustment and collecting and saving your spectra. Regarding the rough adjustment, if you use the same kind of container you have used for sulphur you only need to check that the He-Ne laser is correctly focused on your sample. If you change the sample container you will need to run the $S_2$ again in the appropriate container to set up a correct adjustment of the sample position for this container. Sometimes, during a long sequence of analysis, you may need to redo the adjustment with sulphur and scan it to be sure that the instrument is still working well.

- You are advised to **save your data** into your individual data folder on the computer hard drive, but to also copy across to a USB stick. The instrument does not have an Internet connection.

- Please note that **data may be purged from the instrument** once a year (after back up) and therefore backing up your data onto your own computer regularly is advisable. For assistance on the **OMNIC data analysis** please see the software manual in the binder close to the instrument. Save the analysed spectra as a new file. It is important to keep the raw data. If you have more the one spectra, you can select the desired spectra in the instrument window (it will appear in red) and save only this selected spectra. You can also clear the undesired spectra, the main options are on the top bar.

SHUTDOWN PROCEDURE

- Set the **laser** to the minimum power (**0.2**), press enter and turn off the laser by returning to the 'Expt Set' icon and the 'Bench' tab and selecting laser OFF. **Make sure** that the Raman light and laser at sample light of the front panel is **off.** The
- Lift the sample compartment lid and remove your sample from the sample holder. Return the sample holder to the compartment and shut the lid.
- Fill in the **Excel Logbook** (Desktop).
- **Close** the OMNIC software and log off the computer.
- Leave the instrument and the PC **on.**

**Technical support:** Dr Pedro Rivas scs16o@reading.ac.uk

## APPENDIX.

## THEORY.

The Raman scattering technique is a vibrational molecular spectroscopy which derives from an inelastic light scattering process. With Raman spectroscopy, a laser photon is scattered by a sample molecule and loses (or gains) energy during the process. The amount of energy lost is seen as a change in energy (wavelength) of the irradiating photon. We are interested in how much the scattered light differs from the incident light, so the spectrum is normally plotted against the difference between the two-the Raman shift (Stokes spectrum). This energy loss is characteristic for a particular bond in the molecule. Raman can best be thought of as producing a precise spectral fingerprint, unique to a molecule or indeed and individual molecular structure. In this respect it is similar to the more commonly found FT-IR spectroscopy. However, unlike FT-IR, there are a distinct number of advantages when using Raman:

• Raman can be used to analyse aqueous solutions since it does not suffer from the large water absorption effects found with FT techniques.

• The intensity of spectral features in solution is directly proportional to the concentration of the particular species

• Raman requires little or no sample preparation. It does not need the use of Nujol, or KBr matrices and is largely unaffected y sample cell materials such as glass.

• The use of a Raman microscope such as the Renishaw Raman instrument provides very high level of spatial resolution and depth discrimination, not found with the FT methods of analysis (such as these instruments).

• It can provide information on physical characteristics such as crystalline phase and orientation, polymorphic forms, and intrinsic stress.

Please check the additional documents available in the instrument binder for more detailed information.

## HELP MENU.

For Raman: Enter in the Omnic software and select < Raman tab (at the top) < Raman help (Figure 9).

For the Software: This is different to the Help tab, which is for the Omnic software and for sampling techniques.

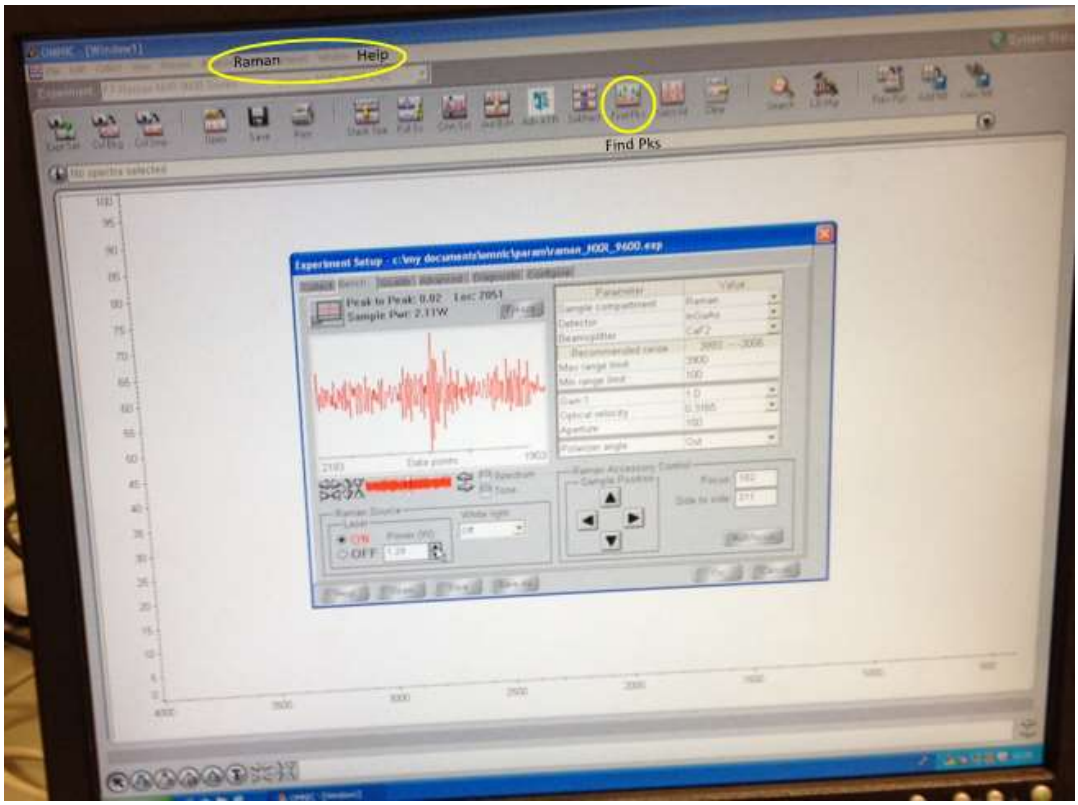



*Figure 9. Situation of the Raman. Help and Find Peak (Pks) menus.*

## SAMPLES.

Solid (films, gels, powders…) and liquid samples can be analysed on FT Raman.

Please follow the **next recommendations** before analysing your samples:

Surface analysis of bulk samples: With this FT Raman you only analyse the surface of the sample. Moreover, you only get the Raman spectra of the small area excited by the Raman laser. Therefore, this instrument is not ideal to characterize heterogeneous samples.

Thermal behaviour: One of the most important is understanding the impact that excitation laser power has on your samples before running the analysis. It is particularly important to have precise control over excitation laser power. The impact of laser power is two fold.

First it is important to be aware that with some materials it is possible to damage or alter the sample with the excitation laser. This damage can be very obvious in extreme cases where the laser burns a dark hole in the sample; in other cases the damage can be more subtle and if care has not been taken to avoid damaging the sample, it may result in spectra that do not represent the true sample. Figure 10 provides an example of one such situation with a sample of $C_{60}$ fullerene. $C_{60}$ begins to breakdown into other structures probably amorphous carbon (1600-1400 $cm^{-1}$ approx.), with as little as 0.5 mW of laser energy applied to the sample.

The second way that laser power can impact samples of carbon nanomaterials is by changing the temperature of the sample. For example, the Raman spectra of many carbon nanomaterials can be very sensitive to even small temperature changes. Most carbon nanomaterials are black and will absorb significant amounts of visible light (heat). As the temperature increases because of laser excitation the bonds become somewhat looser, which results in a lower vibrational energy and hence a significant shifting of the G-band to lower wavenumbers (Figure 11) which affect fine Raman characterization.

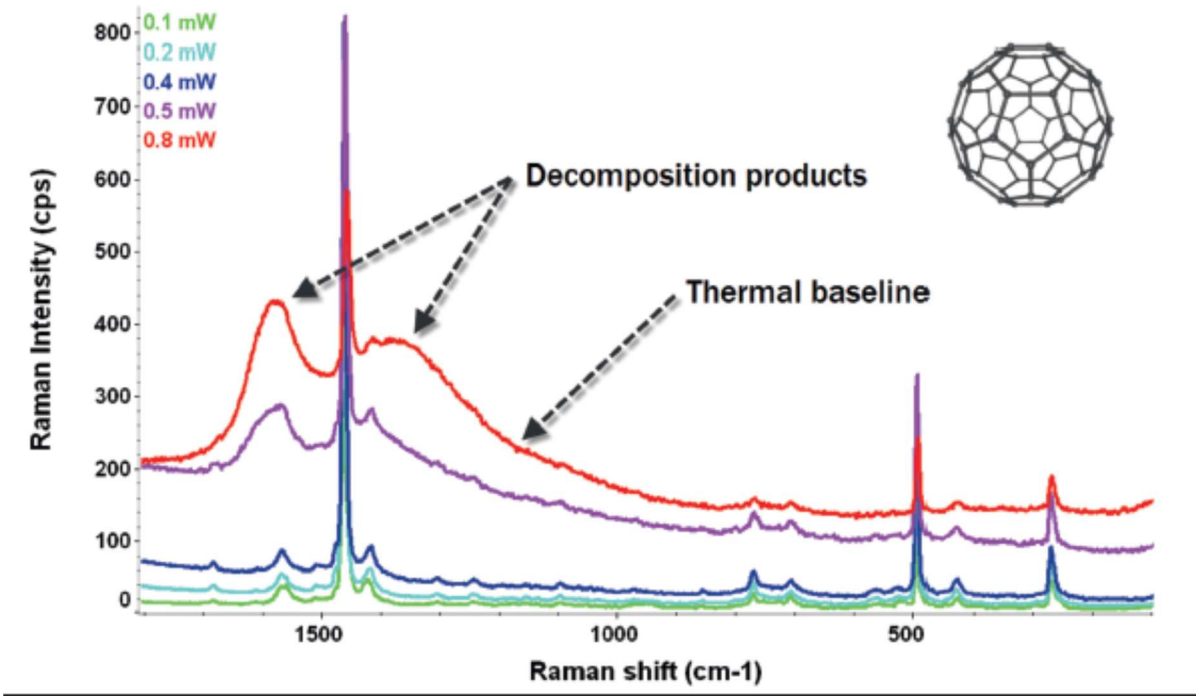



*Figure 10. Effect of increasing laser power on C60 (532 nm excitation laser). (Thermo Scientific Application Note 51948).*

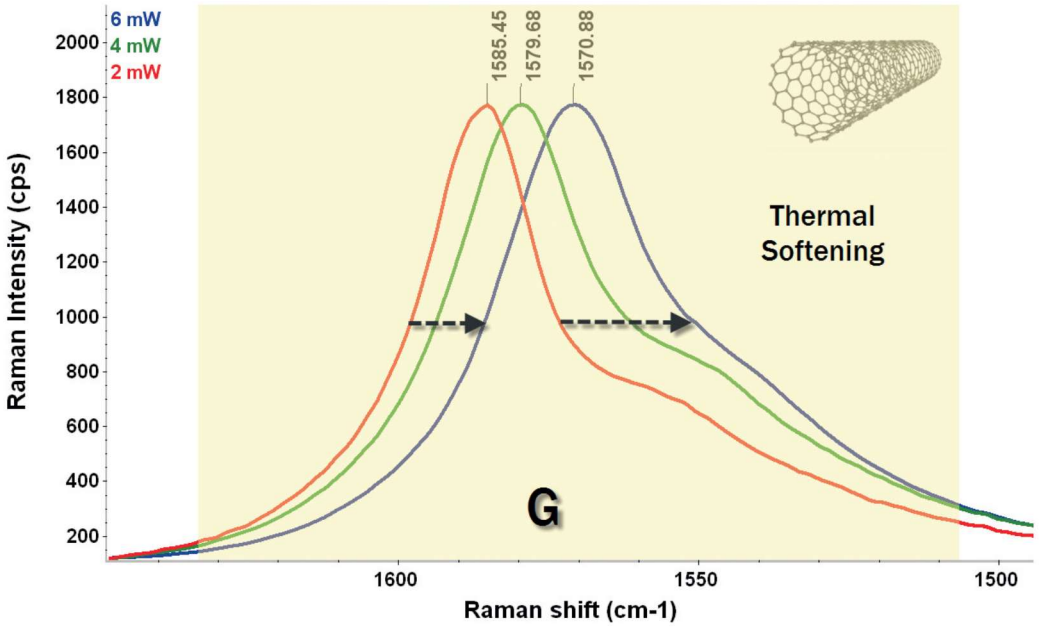



*Figure 11. Effect of thermal softening with increasing laser power on single wall carbon nanotubes (780 nm excitation laser). (Thermo Scientific Application Note 51948).*

Due to these impacts of laser power, you have to exercise some caution as it is possible that surface modifications to these materials may not be as laser tolerant as the base materials. Please follow the next recommendations to avoid these side effects:

- Knowing the melting point and decomposition temperatures of your sample is always helpful.

- When beginning to characterize new materials or materials with new modifications, the analyst should always start with very low laser power and gradually increase it. You can also test a small area of one sample to check for laser tolerance.

- If you have enough sample you can collect spectra at different laser powers to identify how much power can be applied before you begin to see damage in the Raman spectrum.

- For sensitive samples it is better to keep the laser power low and avoid generating a lot of heat. The sample will dissipate the heat more efficiently thereby stabilizing spectral variations.

- Never leave a sample unattended with the laser on.

- If you need to carry out several hundreds of scans of your sample, make sure you are not burning your sample: turn off the laser, open the sample cover (first check that the Raman laser light of the front panel is off) and check that your sample is not burnt or damaged.

- Do not overtighten the sample container, it could explode.

Solvents: Water is an ideal solvent for Raman analysis, since its signal is very low, in contrast to organic solvents. When analysed dissolved samples, first of all, you are advised to analyse a blank (solvent) before analysing your sample. On another matter, if your sample is not dissolved, you also must be aware of any possible solvent residue present in your sample. This could affect the flammability of your sample and also could give a Raman signal interfering with your sample. Ideally, there should not be any solvent residue

from the synthesis in your sample, unless your sample can be affected when removing it. If this is the case, you can analyse a solvent as a blank to remove its Raman signal.

Due to the optics and the current configuration of the instrument, this instrument is not ideal for the analysis of samples with a weak Raman signal, such as aqueous samples.

Mixtures: you are recommended to analyse the mixture and the compounds separately. If you do not have these compounds you should find the Raman spectra of these compounds.

Polymeric drug delivery systems: It is important to analyse the polymer and the drug separately too.

CONTAINERS.

Sample Containers: Glass containers are the preferred ones. However, they should be clear. You must avoid any glass containers with labels (printed-like some GC vials- or written labels) since they could produce a Raman signal. Furthermore, glass itself has a weak signal so you must pay attention during the adjustment process of the sample position, the He-Ne laser spot should be centred in your sample. If the sample does not cover the spot you could get a Raman signal from the glass vial or even from the sample holder.

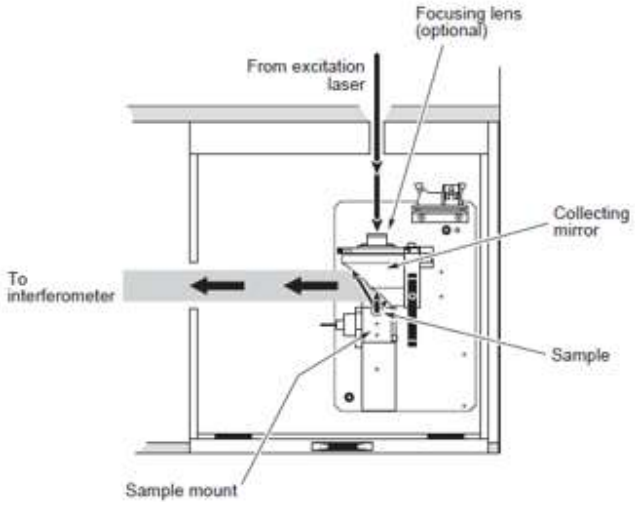
The default containers available on our FT Raman are glass NMR tubes, capillary tubes and a range of glass scintillation vials (various sizes can be accommodated in the standard set-up). Ask for the recommended dimensions to the CAF technician, the vials must enter into the sample holder. Select the appropriate sample container depending on the amount of available sample, all can be used for solids and liquids. The glass vials are the easiest ones to use. Capillary tubes are not recommended for viscous samples.

Ideally you should fill the container with your sample until you cover 0.5-1cm of the container (enough for the He-Ne laser spot). If you have enough sample it is always preferable to add a little bit more than the minimum amount to dissipate the heat and to more easily align the laser.

Sample holders: you have plastic holders for glass vials and metallic containers for NMR tubes and capillary tubes. If you have enough sample the first option is a glass vial placed on a plastic holder. For transparent samples, like some aqueous samples, the best option is to fill a glass NMR tube or a capillary tube with your sample and to put the tube in a Raman metallic holder. The metallic holder will dissipate the heat and will prevent the plastic holder from burning. Moreover, metallic bonds are not Raman active, as opposed to the plastic container. You could get the signal from the plastic holder and not from your sample if you use this holder instead of using the metallic holder. Also remember that the laser must not hit plastic folders since they could be easily burnt.

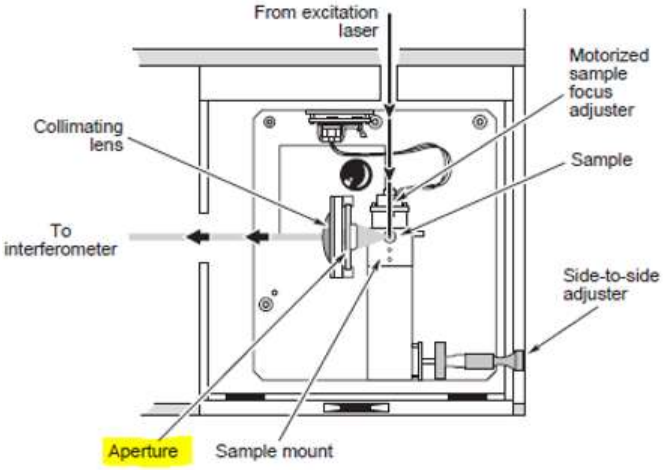
ACCESSORIES. We have different accessories that can be used with the instrument. However, before using them you have to look for technical assistance since they will need to be installed. A new SOP and R.A will be needed when using an accessory.

*SAMPLING CONFIGURATIONS: there are three available for this instrument:




- A 180 degree reflective configuration, this is the one currently installed. It is used for samples that require maximum optical efficiency. This configuration collects the most energy.

*Figure 12. 180 degree reflective sampling configuration.*




- 90 degree refractive sampling configuration. It comes with a removable aperture for limiting the angle of radiation of the beam that reaches the lens. Spectra collected using the aperture are closer to the theoretical results, which are based on an angle of radiation of 90 degrees.

*Figure 13. 90 degree refractive sampling configuration (top view).*

- Bottle holder sampling configuration. It is available for measuring powder or liquid samples contained in a glass bottles.

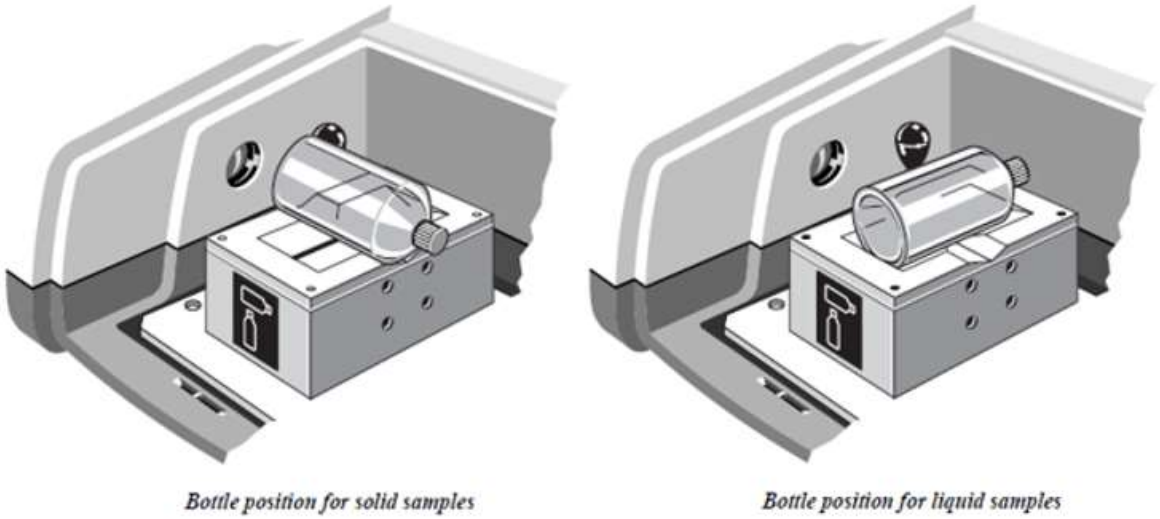



*Figure 14. Bottle holder sampling configuration.*

* NEUTRAL DENSITY FILTERS: they allow you to reduce the laser power entering the sample compartment. This may be useful when you have a heat-sensitive (usually dark) sample.

* POLARIZATION ROTATOR: You can use it to rotate the polarized excitation laser beam from its perpendicular orientation to a horizontal one.

* BEAM FOCUSING LENS: You can use them with a sampling configurations to focus the normally defocused laser beam onto a smaller are of the sample surface. This lens are currently installed and align in the instrument.

* POLARIZER OPTION: It can be installed under the main cover of the module. This allows you to polarize the beam emitted by the sample before it enters the interferometer.

* SAMPLE SPINNER**,** It could be used to decrease the laser intensity on a particular point of the sample surface. However, according to the instrument engineer, this is not a very useful accessory.

## DATA ANALYSIS.

The data displayed in the spectrum is only the Raman shift (Stokes spectrum). You don't see the Raylegih dispersion nor the Antistokes spectrum. The Raman shift error for our interferometer is ≤1cm$^{-1}$. The intensity of the peaks mainly depends on the instrument optics (laser, power, number of scans), the sample polarizability (the higher the polarizability the higher the intensity) and the sample concentration amongst other factors.

The basic data analysis you can carry out with the Omnic software is finding peaks above a specified height. There is no need of background correction in Raman analysis. You can also overlay and stack several spectrum in the same view and change the layout. There are many other functionalities too, like reprocessing spectra, which is important when comparing spectra (to change units, for quantifications, to match resolution and other parameters, to change spectral range, to improve the data...). Please look for technician assistance when using these other functionalities.

### FINDING PEAKS ABOVE A SPECIFIED HEIGHT.

You can use Find Peaks option in the Analyse menu or the tab in the commands bar (Figure 15). This command find peaks in the displayed spectral region or in the selected region. The command searches for peaks whose value exceed a specified threshold value and then labels them with their X values (Raman shift, wavenumber cm$^{-1}$). By adjusting the threshold and the sensitivity values you can find the spectral features you are interested in without labelling noise and other unimportant features. Follow the next steps (Refer to Figure 15).

1. Select the spectrum whose peaks you want to find and label.

2. Display the spectral region in which you want to find peaks, or use the region tool to select a smaller region.

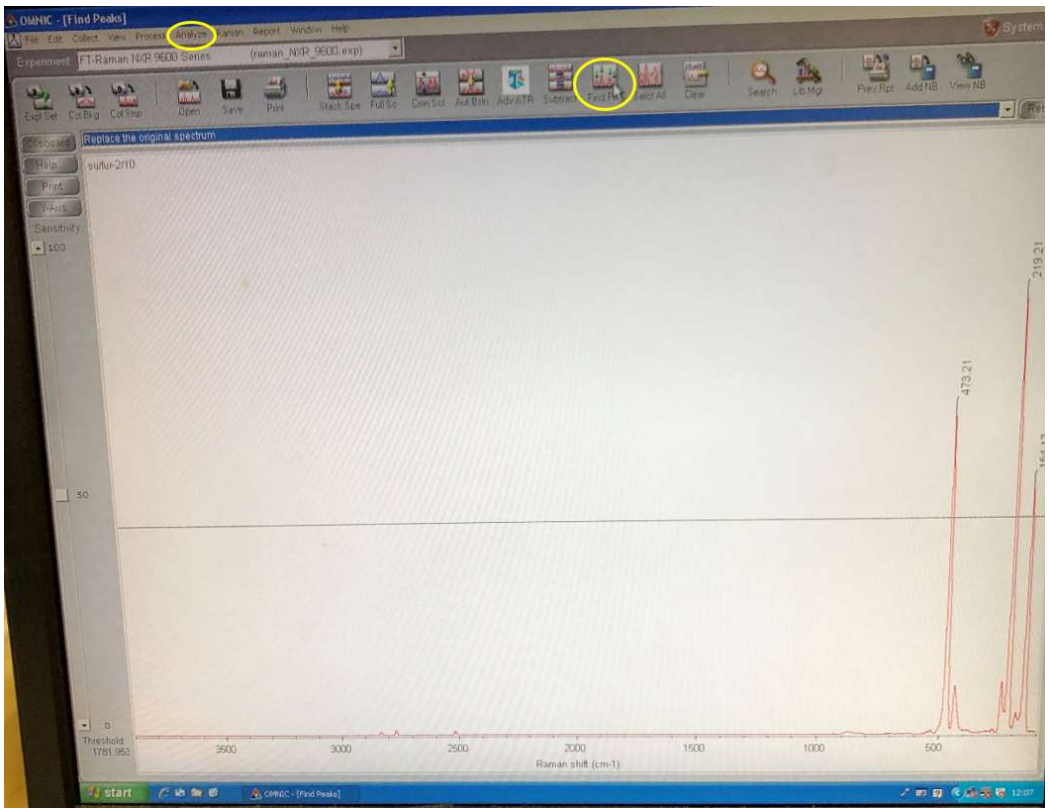



*Figure 15. Find Peaks window of a $S_2$ spectrum. Find Peaks option and the Analyse menu in yellow circles. Threshold line visible.*

3. Choose Find Peaks from the Analyse menu or the tab in the commands bar

The find Peaks window appears. The peaks above the Y (intensity) default threshold value are labelled with their X values. The threshold is indicated graphically by the horizontal line that runs through the spectrum. The exact threshold value appears in the Threshold box at the left bottom.

4. If you want to change the threshold value click a point above or below the threshold line. Set the threshold value equal to or slightly less than the Y value of the smallest peak you want to find.

5. To change the sensitivity setting, use the Sensitivity scroll bar (on the left of the window). The current setting is displayed to the right of the scroll bar. When you finish scrolling, any additional found peaks are labelled and peaks that are no longer found lose their labels.

The sensitivity determines how readily Find Peaks finds shoulders on peaks and small peaks in the baseline. Unlike the threshold value, which merely specifies the Y value above which peaks are found, sensitivity takes into account the relative size of adjacent spectral features. It is not a percentage of the peaks label above the threshold value. If you use a low sensitivity setting, a shoulder will be considered as part of the larger peak and a small peak will be considered to be part of the noise in the baseline, neither feature will be found. At a higher sensitivity setting, the shoulder and the small peak will be found and labelled as peaks.

If the sensitivity is too high, noise and other unimportant features above the threshold will be labelled as peaks. Use a combination of sensitivity and threshold settings that lets you find just the special features/peaks you are interested in. See example in Figure 16.

6. To display or remove the Y-axis from the window, choose Y-axis.

You can use the annotation tool to edit the text of a peak label.

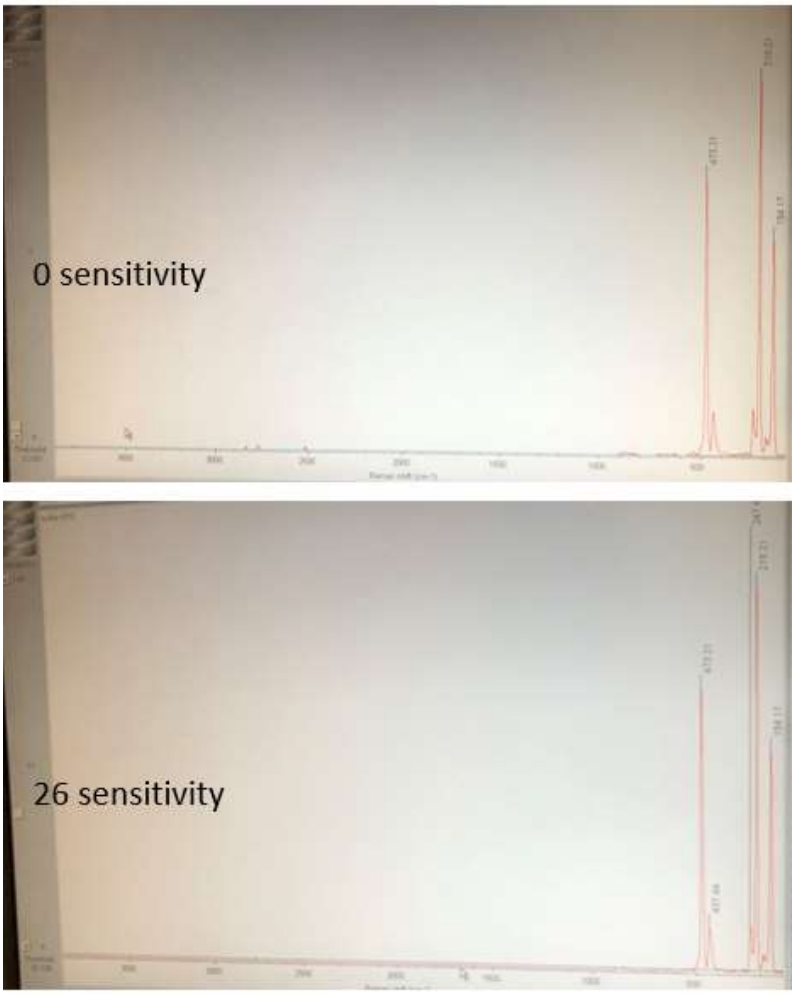



*Figure 16. Example of how sensitivity value affect the labelling process in a S2 spectrum. Same threshold (at the bottom of the spectrum) in both cases.*

7. To print the spectrum in Pdf and a table of the labelled peaks below it, choose Print. The peaks are listed in the table in order of wavenumber position or intensity, depending on the selected Process options (available through Options in the Edit menu).

8. To copy a table of the labelled peaks to the Windows Clipboard, choose Clipboard. You can then paste the table into a Word document or spreadsheet program.

9. To replace the original spectrum with the labelled result spectrum or add the result to a spectral window, select the desired option from the window selection box at the top of the Find Peaks window and then choose Add or Replace. It is better to save the replaced spectrum in a new file to keep the raw data

If you close the window without doing anything, the results are added to the log. Then if you save the spectrum the annotations are saved in a table and can be viewed with the Annotation button.

**REFERENCES.**

- NXR FT-Raman Spectrometer User's Guide. Copyright C 2003 by Thermo Electron Corporation, Madison, Wi 53711.

- OMNIC User's Guide. Version 7.3. Copyright C 2006 by Thermo Electron Corporation, Madison, Wi 53711.

- Thermo Scientific Application Note 51948).