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TM26 - Frequently asked questions (FAQ) WiRE™ 5

Introduction

This module offers a list of common questions and problems; offering possible solutions to them without having to delve into the manual or contact Renishaw for technical support.

Q1. Help! It's not working!

Whether new or experienced to their operation, the cure is nearly always very simple. Below are summarised a few of the common reasons why you may not be getting a spectrum.
If you are having trouble making the instrument or laser operate, check all of the following to ensure that the instrument and all accessories are powered up correctly.

- With the WiRE software closed, check that the instrument and accessories are plugged in and switched on.
- Ensure that the laser (and if necessary its power supply) is plugged in and switched on. Since there are many different types of laser, refer to its individual manual if you require further help with this.
- Check that the door of the instrument is securely closed and locked and that the interlock is operational.
- Check that the Class 1 enclosure door (if present) is securely closed.
- Start WiRE.

If all these operations have been checked, you are ready to capture your spectrum. With your sample under the microscope, and the WiRE™ software loaded and ready, check the following if you are still not getting a spectrum.

- Use a standard sample such as a silicon wafer. This is strong and sharp with the 1st order band located at 520 cm^{-1}.
- Ensure that your sample is loaded correctly under the microscope, that it is sharply in focus and that you are looking at the correct portion of the sample. It is often worth trying a different region within the sample because of the possibility of impurities giving unexpected results.
- Check all the settings in the measurement setup dialogue. If you have cosmic ray removal engaged, remember that this takes two additional, undisplayed spectra; this process may take some time depending on the scan time chosen so don't be concerned with the delay in spectral display.
- Check the laser spot is on the crosshair of the video. If not use the manual adjust for the bottom left beamsteer (Tools….Manual beamsteer) or perform a Laser autoalign.
- Check the correct lens set is within the instrument. The lens set is clearly labelled and the user is prompted on configuration change, where a change is necessary.
- Perform a CCD area auto align (inserting a silicon sample under the microscope for non-Reflex systems).

- Perform a slit auto align (search and optimise).

Now that this has helped you get a signal from your sample, you may find it is particularly noisy, if this is the case, try the suggestions below on how to improve signal-to-noise ratio and signal-to-background ratio.

Q2. Why do I keep getting random, sharp peaks in my spectra?

These are the result of cosmic rays. High-energy particles, passing through the CCD detector resulting in the generation of electrons which are, in turn, interpreted as signal by the camera. They are completely random in their time of occurrence and the position where they strike. Cosmic rays are very intense, resembling emission lines, and possessing a very small FWHM (< 1.5). To confirm the presence of a cosmic ray, immediately re-capture the data and you will notice a distinct absence of that feature. If however the line still exists, it is most likely a result of spectral contamination from room lights, etc. For further information, see 'I keep getting repeatable, sharp peaks in my spectra ...'

Cosmic rays become increasingly common with increasing exposure time. For long scans, where the presence of cosmic rays must be avoided, consider using the cosmic ray removal feature. This is an option in the experiment set-up window, when activated the spectrum is collected in triplicate (equivalent to 3 accumulations). The software uses the median value at each wavenumber value to ensure no cosmic ray features are seen.

Q3. I keep getting repeatable, sharp peaks in my spectra. What are they?

If you have repeated the scan and the spurious lines are still present in **exactly** the same place, the possibility of them being cosmic rays has been ruled out. Such sharp repeatable lines are usually due to emissions from fluorescent room lights or phosphorous in CRT monitors (figure 6.1). Using long working-distance objectives worsens the problem.

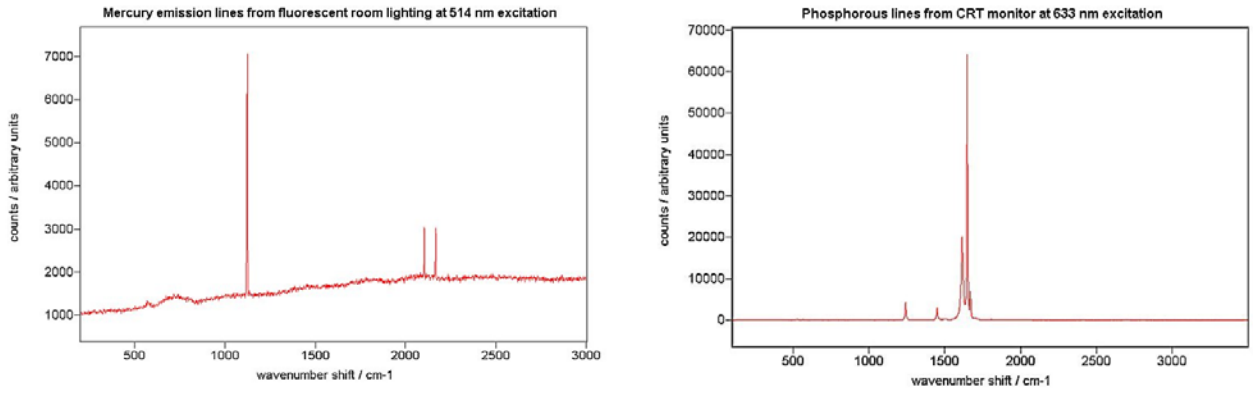

Fig. 1. Fluorescent room lights (left), monitor phosphorus lines (right)

Fluorescent lights result in spectral contamination from mercury emission lines; simply turn off all fluorescent lighting in the room and work under the minimum incandescent light. The room should be as dark as practicable. Similarly, a thorough effort must be made to exclude sunlight from the room since spectral aberrations will results from the numerous emission lines of 'white' light.

Phosphorous lines are often present due to the phosphour coating in all CRT monitors; if such lines are a problem, turn the monitor off or reduce the contrast until the screen is darker.
It is important to remember that emission lines are always present at the same position on an absolute wavenumber scale and will therefore be seen to move on a scale of Raman shift when using different laser wavelength. These lines are more prominent when using collection optics of longer working distance.

Q4. Why do some of my spectra give such an intense background signal that masks the Raman information?

A high background in a Raman spectrum is the result of sample fluorescence (or phosphorescence); an intrinsic property of the material of the sample. Unfortunately, this is an unavoidable consequence of laser irradiation and in many cases the fluorescence is stronger than the Raman signal. Despite fluorescence being an unavoidable side effect, steps can be taken to minimise or irradicate the problem.

- **Change laser wavelength:** the approach that will have a most significant effect for highly fluorescent samples. In general, fluorescence is worse with visible lasers, and moving to a laser in the UV or NIR is likely to cure or reduce the problem. Renishaw manufacture and can supply a wide-range of lasers from UV through to NIR.

- **Quenching:** possible with some samples. By leaving laser light incident on the sample for a period of time before acquiring a Raman spectrum, it is sometimes possible to quench (reduce) the fluorescent background, enhancing the Raman features. The period of time required is sample dependent but normally some effect is observed in seconds to minutes. It is worth noting however, that quenching is exponential and therefore the greatest effect will be seen initially. Cycle the spectrum to see this affect occur with a live update.

- **Confocal mode:** by acquiring data from the small sample volume that is strongly irradiated by the laser, the fluorescence may be greatly reduced. This approach may also be beneficial where the sample being investigated is contained within a substrate that is strongly fluorescent, for example, a sample confined within a fluorescent matrix.

If there is too much ambient light in the room, either fluorescent or incandescent, it is possible this may cause unnecessary background signal in your spectra. It is best to work with lighting at a minimum, however, if this is not possible, for example, if the instrument room is used by other people, consider the use of a Renishaw enclosure. This prevents stray light from entering the instrument and further minimises exposure to the laser beam. The enclosure is available in either Class I, or Class 3b, laser safety forms.

Q5. Why is my signal so weak and / or why do I get such a poor signal-to-noise ratio?

If the signal is weak, first check that the sample is correctly placed under the microscope and sharply in focus; you could also try moving to a different sample point. Check that the instrument is set up for Regular mode and check the laser power setting; if the power is less than 100%, try increasing it to improve the signal. If the spectrum is very noisy, this may be improved by increasing the scan time or number of accumulations.

- Increasing the scan time allows the CCD to acquire more Raman signal, enhancing the features over the extraneous noise. This method is ideal if both the background and Raman signal are low, however, if either of these is intense, then increasing the scan time increases the chance of saturating the CCD.

- Accumulating the data takes a number of identical scans and co-adds them together, enhancing weak Raman features from the random background noise and improving the signal-to-noise ratio.

Careful adjustment of these two parameters allows the maximum possible exposure without saturation and will improve the signal-to-noise ratio. It is worth bearing in mind that the signal-to-noise ratio is proportional to the square root of the number of accumulations; 4 accumulations provides a two-fold improvement in the signal-to-noise ratio.

Another factor as important as the signal-to-noise ratio is the signal-to-background ratio; these two ratios are intimately linked. If the background component is high, it will mask the Raman signal and contribute noise to the system. See also **'Why do some of my spectra give such an intense background signal which masks the Raman information?'** for further details.

Q6. How can I stop my sample from being damaged by the laser?

The laser spot incident on the sample has a high power density. This is especially true of UV systems and those with high laser powers. Unfortunately, some samples are susceptible to thermal or photo degradation. The resulting spectrum will contain features caused by modification, not natively present in the sample (for example, broad amorphous carbon bands around 1500 cm^{-1}). Often, viewing the white light image before and after acquisition will indicate a clearly altered region of sample (Figure 2) where the laser was incident.

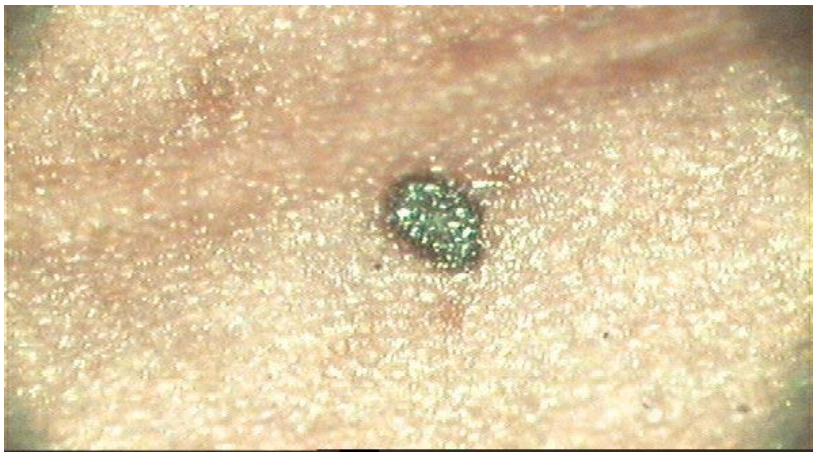

Figure 2. Laser induced sample damage

To prevent damage, it is prudent to start initial analysis with low laser powers, especially when using NIR and UV systems, from here, the power can be increased, balancing sample damage prevention with the needs for a strong signal.

If reducing the laser power to very low levels (<1%) still results in sample damage, use of a **line focus** accessory may help. The line focus reduces the laser power density by spreading the laser power out over a greater area. This increases the number of Raman scatterers and the resulting Raman signal is therefore significantly higher than conventional methods.

Conventional methods include:

- Using a lower magnification objective to reduce the power density at the sample (this produces a larger spot size but also produces less Raman singal as the numerical aperture is significantly lower)

- Defocusing the laser spot using the beam expander.

Line focus is a superior option for faster data collection as it not only reduces the power density, but also optimises the throughput in the spectrometer (unlike the beam expander spot defocus method).

Q7. I can't fit my sample on the stage because its a liquid / powder / very large! What can I do?

While Renishaw Raman instruments provide an excellent way of analysing samples with very little preparation, some samples can't simply be placed on a microscope slide. It is possible to place samples with heights of up to 50 mm directly onto the stage. **Renishaw's macro-sampling kit** provides an excellent way of dealing with problem samples such as powders, liquid or samples which are large and can't be easily placed on the stage while still requiring no sample preparation.

Large samples which maynot fit under the microscope can be analysed using a **flexible sampling arm**. This enables Raman analysis external to the microscope and enclosure. As the arm is direct coupled it has all the resolution and throughput benefits of the inVia – unlike a fibre probe coupling.

Fibre probes are available and are ideal for distant Raman analysis, and integration within other instruments.

Q8. How can I stop my sample from moving around on the microscope stage?

It is important that samples can be constrained so they do not move during analysis. This is even more important when using Raman mapping methods. Flat samples such as polymer films need to be held flat so the laser focus does not change during analysis or depth profiling. Other samples need to be held in XY so they do not slide or shift during fast mapping experiments. The **high speed encoded stage accessory kit** (HSES) enables samples of all types to be firmly held in place to prevent experiments having to be repeated.

Q8. I'd like do be able to examine my samples under different pressures, is there a way I can do this?

The diamond anvil cell, available from Renishaw, enables you to analyse your samples under high pressure.

Q9. I want to be able to perform polarisation measurements. Is this possible?

A polariser and half-wave plate set for each wavelength may be purchased from Renishaw. These enable you to examine the molecular symmetry of your sample and assist in assigning bands to vibrations within the molecule. Motorised laser polarisation control is also available.

Q10. I've noticed that placing my sample in different orientations gives a different spectrum. Why is this?

This is caused by the laser being incident on different crystal planes within the sample. Using a quarter-wave plate can help to remove these orientation effects by scrambling (circularly polarising) the Raman/laser light. This method is often of use to confirm relative intensity Raman information is not sample orientation induced (e.g. for highly ordered systems such as polymers or single crystals).