Standard Operating Procedure
FT-Raman Spectroscopy

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Instrument Specifications:
Excitation 1064nm Nd:YAG diode pumped laser.
Spectral Range 3600-50cm⁻¹ (stokes) quartz broad-range beam splitter.
Germanium detector cooled by liquid nitrogen (3-5 day hold time).
Motorized xyz sample stage.
1. Purpose

The purpose of this document is to familiarize the user with the mode of function of the FT-Raman available at the facility, and to describe the sampling procedure.

2. Introduction

Raman spectroscopy is a spectroscopic technique that relies on the inelastic scattering of a laser beam on the sample surface, leading to the collection of dispersed light by a lens, which in-turn is sent through a monochromator, ultimately reaching a detector.

The inelastic scattering, also known as Raman scattering, is a type of photon scattering that occurs in approximately 1 in 10 million scattered photons. Whereas with elastic scattering the scattered photons have the same energy as the incident photons, inelastic scattering produces photons scattered by an excitation that typically causes scattered photons to have a lower energy than incident photons.

The Bruker® FT-Raman Spectrometer (MultiRAM) available at the facility employs a high sensitivity germanium diode detector, and uses liquid nitrogen for cooling to offer ultra-low signal detection for high sensitivity and minimal noise.

3. Scope

This instrument is used to determine vibrational information specific to chemical bonds as well as the symmetry of molecules of a compound.

It is also used to study changes in chemical bonding, such as when an enzyme is bound to its substrate.
4. Procedure

4.1. Preparation of sample disk.

1. Prepare a fine-ground powder from the analyte using a mortar and pestle.
2. Place a clean and dry sample disk on the sample holder (figure 2).
3. Place the upper piece on top of the sample holder (figure 3).
4. Add a spatula tip of the fine-ground sample and compress with the grinder (figure 3).
5. Use the edge of a spatula to flatten the top of the sample disk (sample should only be contained within the cup of the sample disk).

4.2. Initialization of the instrument.

N.B. Ensure the presence of sufficient liquid nitrogen in the cooling chamber of the instrument. The nitrogen in the chamber has a 3-5 day hold time and must always be replenished prior to initializing the instrument.

1. The FT-Raman laser is controlled by a key-operated power switch with two turn-settings: standby and on.
2. Upon the first turn, the ‘Line’ LED should be red, followed briefly by the ‘Warm-up’ LED (figure 6).
3. Once the instrument is warmed up, the ‘Warm-up’ LED will turn off and the ‘Standby’ LED will turn amber (Figure 7).
4. Proceed to turning the key to the second setting. The instrument is now ready for sample analysis (Figure 8)
4.3. Sample running.

1. Lift open the sample chamber (figure 9).
2. Secure the disk on the stage making sure the sample surface is facing the lens (figure 10).
3. Close the sample chamber.
4. Proceed to run OPUS software.
5. Click on ‘Acquire’.
6. In the dialogue box that appears, enter the sample description and adjust the Raman laser power (1-500mW), making sure to manually type in the laser value (setting and actual) in the ‘Sample form’ field (figure 11).
   
   N.B. ‘actual’ laser power is about 80% of the setting value.

7. Proceed to the ‘Measurement’ tab and click on ‘Spectrum’ in the ‘Show’ settings. This gives a preliminary view of the Raman spectrum.
8. Laser power can be adjusted on the scale to allow for maximum signal strength.
9. Use the ‘Forward’ and ‘Backward’ buttons to adjust the sample on the z-axis. This is used to maximize signal strength.

10. Once done with these manipulations, click ‘Sample Raman Spectrum’ and wait for the generation of the spectrum.

Figure 9 Open sample chamber.

Figure 10 Placement of sample disk on the stage.

Figure 11 Measurement dialogue box.
4.4. Measuring sample peaks.

1. With the sample spectrum open in OPUS, click on ‘Peak picking’.
2. In the dialogue box that appears, click ‘Start Interactive Mode’.
3. Adjust the horizontal line to be just under the smallest peak of interest.
4. The ‘Threshold’ scale on the left of the screen allows for the adjustment of sensitivity to peaks above the horizontal peak picking line (figure 12).
5. Save (or print) spectrum with added peak values.

![Interactive peak picking window for calcite sample](image)

**Figure 12** Interactive peak picking window for calcite sample.

5. Preparation of non-powder samples.

5.1. Liquid samples.

1. Place liquid sample inside the quartz liquid sample tube.
2. Secure inside sample chamber and place the rectangular mirror behind sample tube.
3. Run sample as per 4.3.4.

5.2. Macro or mineral samples.

1. Place macro or mineral rock sample inside the sample chamber.
2. Secure sample into position by ensuring that the surface of interest is facing the laser is as flat as possible (optimally the laser beam should form a normal to the surface).
3. Run sample as per 4.3.4.